then kept at room temperature for 24 hr. The mixture was added to 100 ml of water and extracted with ether. The ether extracts were washed with 10% hydrochloric acid, water, and saturated salt solution. The ether solution was dried over anhydrous magnesium sulfate and the ether was evaporated to give 1.1 g of pyran aldehyde, ir 5.8, 6.0 and 6.2 μ, which was used directly in the next step.

The crude aldehyde was stirred in an open flask for *ca.* 40 hr. The mixture was dissolved in ether, and the ether solution was extracted with sodium bicarbonate solution. The basic extract was acidified with 5% hydrochloric acid and extracted with ether. The ether was removed to leave 0.57 g (48%) of crude acid, which was recrystallized from chloroform: mp 199–200°; ir 5.8 μ (s), 6.0 (m), 6.15 (m), and 6.25 (m); nmr (dilute solution $CDCl_3$) 2.22 (s, 3, C=CCH_3), 1.43 (s, 6, >$C(CH_3)_2$) and 1.08 ppm (d, 3, >$CHCH_3$). The mass spectrum displayed important ions at *m/e* 236 (2%), 221 (100%), 203 (3%), 43 (13%).

Anal. Calcd for $C_{14}H_{20}O_3$: C, 71.16; H, 8.53. Found: C, 70.44; H, 8.37.

A suspension of 100 mg of acid VIII in ether was treated with an ethereal solution of diazomethane. The solvent was evaporated affording an oil whose infrared spectrum was identical with that of the methyl ester of III.

Pyran IX.—A mixture of 0.4 g of acid VIII and 0.4 g of copper powder was heated at 220–300° resulting in the distillation of 0.2 g of liquid: ir 5.8 μ (m) and 6.0 (w); λ_{max}^{MeOH} 211 mμ (log ϵ 3.30) and 2.29 (sh) (3.14); nmr (CCl_4) δ 4.27 (q, 1, J = 1 Hz, =CH), 1.70 (d, 3, J = 1 Hz, *cis*-CH_3C=CH), 1.05 (s, 6, >$C(CH_3)_2$), and 1.02 ppm (d, 3, >$CHCH_3$). The mass spectrum exhibited important ions at *m/e* 192 (4%), 177 (100%), 135 (9%), 43 (11%).

Anal. Calcd for $C_{13}H_{20}O$: C, 81.20; H, 10.48. Found: C, 81.42; H, 10.55.

Registry No.—Pulegone, 15932-80-6; ethyl acetoacetate, 141-97-9; III, 18600-02-7; III (methyl ester), 18588-64-2; XI (methyl ester), 18588-65-5; reduction product of III, 18588-66-4; VI, 18588-67-5; VIII, 18588-68-6; IX, 18588-69-7; XI, 18588-73-3.

The Synthesis of Some Fluoronitrobenzimidazoles and Their Reactivities toward Peptide Nucleophiles

KENNETH L. KIRK AND LOUIS A. COHEN

Laboratory of Chemistry, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland 20014

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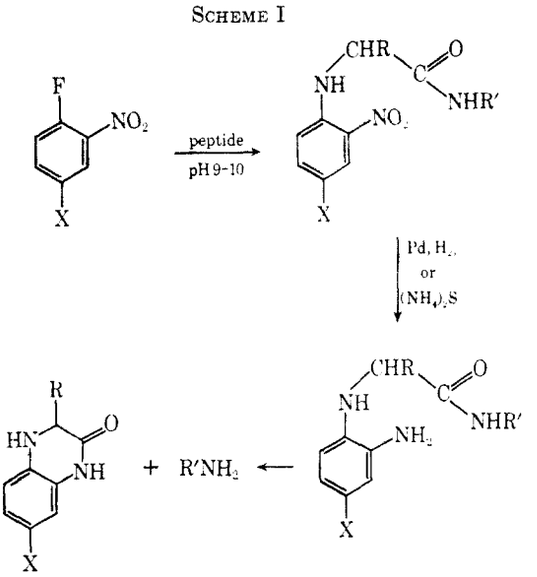
A series of mononitro- and dinitro-4-fluorobenzimidazoles has been prepared using 2-fluoroacetanilide as starting point. Assignment of position to the nitro groups is based on analyses of nmr hydrogen–fluorine coupling constants. Orientation in nitration is controlled by the fluorine atom rather than by the fused imidazole ring, except where steric factors intercede. At 25°, 5,7-dinitro-4-fluorobenzimidazole is 84 times as reactive as 2,4-dinitrofluorobenzene toward a peptide nucleophile. The enhanced reactivity is attributed primarily to the ability of the fused imidazole ring to participate in stabilization of the Meisenheimer adduct. The corresponding benzimidazole anion, as well as a series of mononitrofluorobenzimidazoles, are unreactive under the same conditions.

A series of investigations in this laboratory on the tertiary structure of proteins[1] created a need for methods for the quantitative determination of N-terminal amino acids in mixtures of polypeptides. Since neither the fluorodinitrobenzene[2] nor the phenyl isothiocyanate[2] method satisfactorily fulfilled our needs for quantitation, efforts were initiated several years ago to develop an alternative procedure.

Of the various possibilities considered, the approach first described by Holley and Holley[3] seemed attractive, primarily by virtue of the mild conditions under which peptide cleavage could be effected. In this method, the peptide is coupled with 1-fluoro-2-nitro-4-X-benzene, in which X = nitro or another electronegative, fluorine-activating substituent. The 2-nitro group of the peptide derivative is subsequently reduced, either catalytically[3] or with sulfide ion,[4] to provide an amino group as a favorably placed nucleophile for intramolecular attack on the amide bond of the N-terminal residue (Scheme I).

SCHEME I



It is obvious that the availability of a reagent with a preexisting nucleophile at position 2 would simplify

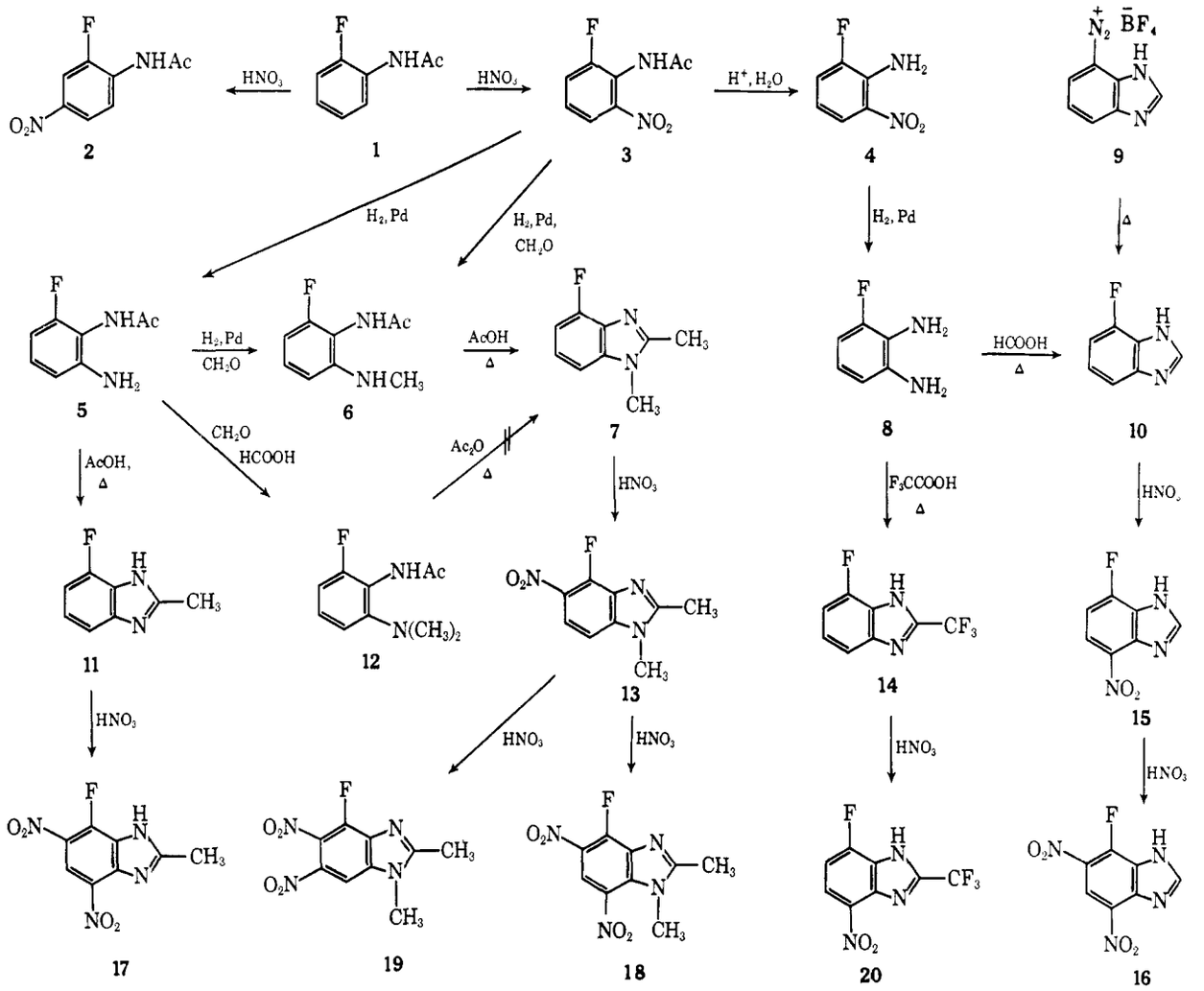
(1) L. Farber and L. A. Cohen, *Biochemistry*, **5**, 1027 (1966); J. G. Wilson and L. A. Cohen, *J. Amer. Chem. Soc.*, **85**, 564 (1963); S. Isoe and L. A. Cohen, *Arch. Biochem. Biophys.*, **127**, 522 (1968).

(2) J. L. Bailey, "Techniques in Protein Chemistry," Elsevier Publishing Co., New York, N. Y., 1962, Chapter 6.

(3) R. W. Holley and A. D. Holley, *J. Amer. Chem. Soc.*, **74**, 5445 (1962).

(4) E. Scoffone, E. Vianello, and A. Lorenzini, *Gazz. Chim. Ital.*, **87**, 354 (1957).

SCHEME II



the procedure measurably, by eliminating the need for a reduction step. Unfortunately, almost any functional group, which might be a reasonable candidate for the role of intramolecular participant, has the undesired effect of deactivating the reagent by electron release into the benzene ring. Guided by the demonstrated nucleophilicity of imidazoles toward activated esters[5] and by the hope that the aromatic character of the imidazole ring would counteract the electron-releasing ability of anilino nitrogen, we prepared a series of dinitro-4-fluorobenzimidazoles for study as bifunctional peptide reagents. The present report deals with the synthetic procedures, structural assignments and kinetics of fluorine replacement. The following paper[6] describes the results of rate studies on the intramolecularly catalyzed hydrolysis of the benzimidazole derivatives of various peptides.

4-Fluorobenzimidazole (**10**) has been prepared in low yield by small-scale pyrolysis of the corresponding diazonium fluoroborate, **9** (Scheme II).[7] Since efforts to expand the reaction scale resulted in even lower yields, an alternative route was developed. Controlled nitration of 2-fluoroacetanilide (**1**) provides a mixture of the *o*- (**3**) and *p*-mononitro (**2**) derivatives, from which 2-fluoro-6-nitroacetanilide (**3**) is readily separated by virtue of its greater alkali solubility.[8] Acid-catalyzed deacetylation of **3** to 2-fluoro-6-nitroaniline (**4**), followed by catalytic hydrogenation, led to 3-fluoro-*o*-phenylenediamine (**8**). When heated in formic acid solution, **8** was converted into 4-fluorobenzimidazole (**10**). Although the over-all yield, at this point, is 25%, large-scale synthesis offers no particular difficulties. Nitration of **10**, under relatively mild conditions, led to the 7-nitro derivative (**15**) while, under more vigorous conditions, the 5,7-dinitro derivative (**16**) was obtained. In either case, no isomeric nitration products were detected. It is noteworthy that benzimidazole, itself, is mononitrated exclusively at C-5; upon further nitration, a mixture of the 5,6-dinitro (54%) and of the 5,7- (or 4,6-) dinitro (21%) isomers is obtained.[9] Evidently, the directive influence of the fluorine atom takes precedence over that of the imidazole ring. Assignment of structure to

(5) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. I, W. A. Benjamin, Inc., New York, N. Y., 1966, Chapter 1.
(6) K. L. Kirk and L. A. Cohen, *J. Org. Chem.*, **34**, 390 (1969).
(7) E. C. Fisher and M. M. Joullié, *J. Org. Chem.*, **23**, 1944 (1958).
(8) B. M. Wepster and P. E. Verkade, *Rec. Trav. Chim.*, **68**, 77 (1949).
(9) G. E. Ficker and D. J. Fry, *J. Chem. Soc.*, 736 (1963).

TABLE I

NUCLEAR MAGNETIC RESONANCE SPECTRAL DATA FOR FLUORONITROBENZIMIDAZOLES[a]

Compd	$\delta_{1\text{-}CH_3}$, ppm	$\delta_{2\text{-}H}$, ppm	$\delta_{2\text{-}CH_3}$, ppm	$\delta_{5\text{-}H}$, ppm	$\delta_{6\text{-}H}$, ppm	$\delta_{7\text{-}H}$, ppm	$J_{HH(ortho)}$, Hz	$J_{HF(ortho)}$, Hz	$J_{HF(meta)}$, Hz	$J_{HF(para)}$ Hz
13	4.18		3.10		8.50 (q)	7.75 (q)	9		6	1
15		9.52		7.67 (q)	8.72 (q)		9	9	4	
20				7.42 (q)	8.60 (q)		9	9	4	
16		9.70			9.30 (d)				6	
17			3.23		9.22 (d)				6	
18	4.30		3.18		9.15 (d)				6	
19	4.30		3.14			8.58 (d)				1.8

[a] Spectra were measured in trifluoroacetic acid as solvent (approximately 0.02 *M*) with tetramethylsilane as internal reference.

these and other nitrobenzimidazoles is based, largely, on analyses of hydrogen–fluorine coupling constants in their nuclear magnetic resonance (nmr) spectra, as discussed below.

By direct hydrogenation of **3**, 2-amino-6-fluoroacetanilide (**5**) was obtained, which was converted into 2-methyl-4-fluorobenzimidazole (**11**) by heating its solution in acetic acid. The product was nitrated directly to 5,7-dinitro-4-fluoro-2-methylbenzimidazole (**17**); no effort was made to prepare a mononitro derivative.

The N-methyl compound, **6**, was obtained by catalytic hydrogenation of **5** in the presence of a small excess of formaldehyde. Direct conversion of **3** into **6** could also be effected by the same technique, although the yield was less satisfactory. By heating a solution of **6** in acetic acid, 1,2-dimethyl-4-fluorobenzimidazole (**7**) was obtained. In earlier experiments, **5** was converted into its N,N-dimethyl derivative, **12**; the conversion of **12** into **7** by boiling its solution in acetic anhydride was attempted. Although there have been several reports on the formation of 1,2-dimethylbenzimidazoles by heating 2-dimethylaminoanilines with acetic anhydride,[10] such conversion could not be effected in the present case. Mononitration of **7** led to the 5-nitro rather than the 7-nitro derivative, evidently the result of steric interference by the 1-methyl group. Upon further nitration of the product, **13**, a mixture of the 5,6-dinitro (**19**) and the 5,7-dinitro (**18**) isomers was obtained, the latter predominating. Separation of the isomers could be effected by column chromatography.

Finally, **8** was converted into 4-fluoro-2-trifluoromethylbenzimidazole (**14**) by heating its solution in trifluoroacetic acid. Nitration of **14** led to the 7-nitro derivative, **20**.

The positions of nitro groups in the mono- and dinitro derivatives were assigned by analysis of aromatic hydrogen–hydrogen and hydrogen–fluorine coupling constants (Table I). The values obtained for the latter ($J_{HF(ortho)}$ = 9 Hz, $J_{HF(meta)}$ = 4–6 Hz, and $J_{HF(para)}$ = 1–2 Hz) are consistent with those observed in other series of fluoroaromatic compounds.[11] It would appear that 4-fluorobenzimidazoles nitrate preferentially at C-7 (**15** and **20**). *ortho* nitration, in the case of **13**, is probably the result of steric interference at C-7 by the 1-methyl group; such interference is readily observed upon examination of space-filling models. The steric effect is evidently sufficient to encourage the formation of the 5,6-dinitro isomer (**19**), in addition to the desired 5,7-dinitro isomer (**18**). The 5,7-dinitro compounds, **16** and **17**, were obtained free of isomeric products.

The imidazole protons in **16** and **17** are fairly acidic. The marked shifts in visible absorption resulting from ionization (Figure 1) permit spectroscopic determination of their dissociation constants. Compound **16** was found to have a pK_a value of 7.97 and compound **17**, 8.18. Clearly, such acidity is the result of resonance stabilization of the negative charge by the nitro substituents (**21**), as well as by the imidazole ring.

The fused imidazole ring has a marked activating effect on aromatic nucleophilic displacement reactions. As may be seen from Table II, the reactivities of **16**, **17**, and **18** extend from 20 to almost 100 times that of

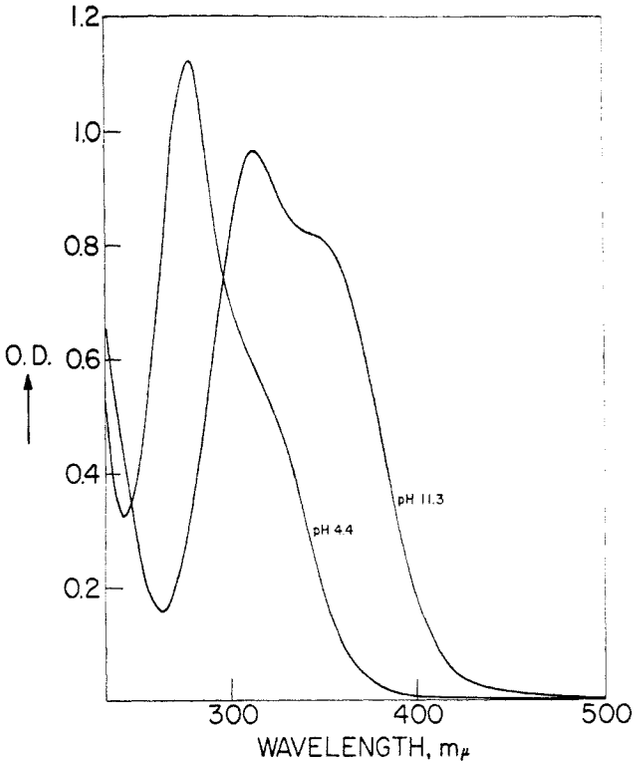
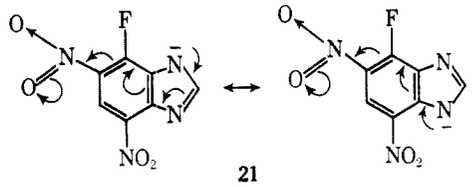


Figure 1.—The uv spectra of 2-methyl-4-fluoro-5,7-dinitrobenzimidazole (**17**) in neutral and alkaline media.

(10) K. Hofman, "Imidazole and Its Derivatives," Interscience Publishers, New York, N. Y., 1953, p 263.

(11) J. A. Pople, W. G. Schneider and H. J. Bernstein, "High Resolution Nuclear Magnetic Resonance," McGraw-Hill Book Co., Inc., New York, N. Y., 1959, p 324.



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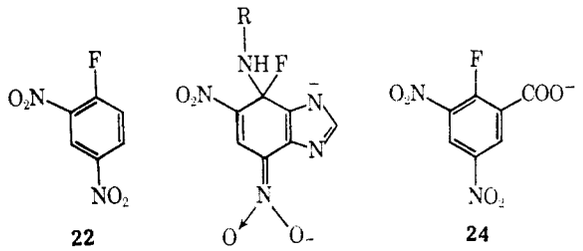
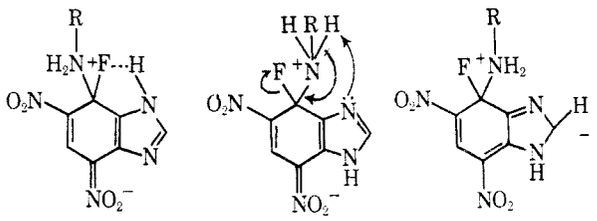
2,4-dinitrofluorobenzene (**22**) toward alanylglycine. The order of reactivities is consistent with the expected electron-releasing effects of methyl substitution in the imidazole ring. The rate of reaction, in the case of **17**,[12] shows a maximum near pH 8, since the protonated peptide is unreactive at low pH and the ionized benzimidazole is unreactive at high pH. The lack of reactivity of the benzimidazole anion may be attributed either to resonance-coupled deactivation of the benzene ring by the negative charge, inability of the Meisenheimer complex (**23**) to accommodate two negative charges, or electrostatic repulsion between the imidazole anion and the negatively charged dipeptide. We have not attempted to distinguish between the two former possibilities; however, the latter explanation is discounted by the observation that 3,5-dinitro-2-fluorobenzoate anion (**24**)[13] is significantly more reactive than 2,4-dinitrofluorobenzene toward alanylglycine (Table II).



22 23 24

Two factors, in addition to electronic activation, must be considered as potential contributors to the reactivites of **16**, **17**, and **18**. The first depends on the ability of the reagent to associate with a peptide by hydrogen bonding of the amide group to the imidazole nitrogen prior to nucleophilic attack and, thus, provide the reaction with intramolecular characteristics. To evaluate the importance of this factor, rate data were obtained for a simple amine as nucleophile. Compound **17** is approximately 18 times as reactive as **22** toward methylamine and 38 times as reactive toward alanylglycine (Table II). The enhancement factor of 2 may be the result of hydrogen bonding or may be due to the difference in nucleophilic power of the two amines. At any rate, the factor is too small to be given an important role.

The second pathway for intramolecular assistance depends on hydrogen bonding between the imidazole NH and the departing fluorine atom (**25**) or on intramolecular proton removal by the imidazole nitrogen acting as a general base (**26**).[14] The former possibility may be excluded by the fact that **18**, which should not participate in such a mechanism, shows



25 26 27

an order of reactivity consistent with its structure, and the latter pathway is rendered unlikely by the very weak basicity of the imidazole nitrogen ($pK < 0$) in these compounds. We conclude that the reactivity of the dinitrofluorobenzimidazole system is due solely, or very largely, to electronic activation of the benzene ring.[15] Such activation may be visualized as resulting from the availability of an additional canonical form for the anion hybrid (**27**).

The mononitro derivatives show essentially no reactivity toward alanylglycine under the conditions employed, although they are reactive toward the more nucleophilic mercaptans.[16] No effort was made to effect dinitration of the 2-trifluoromethylbenzimidazole (**14**), since the mononitro compound (**20**) was already extensively ionized at neutral pH ($pK_a = 5.4$). As expected, the 5,6-dinitro isomer (**19**) was considerably less reactive than the 5,7-dinitro compound (**18**).

TABLE II

RATES OF REACTION OF ACTIVE FLUORINE COMPOUNDS WITH ALANYLGLYCINE[a]

Compound	pH	k_{obsd}, sec^{-1}	k_2,[b] M^{-1} sec^{-1}	k_2',[c] M^{-1} sec^{-1}	Relative rates
22	7.95	2.92×10^{-5}	6.52×10^{-3}	0.0176	1
16[d]	7.95	2.84×10^{-3}	2.81×10^{-1}	1.48	84.2
17[e]	7.95	1.52×10^{-3}	1.53×10^{-1}	0.656	37.3
17	6.32	8.90×10^{-5}	8.90×10^{-3}	0.664	37.7
17	10.15[f]	6.29×10^{-5}	6.92×10^{-3}	0.660	37.5
18	7.95	1.47×10^{-3}	1.47×10^{-1}	0.397	22.5
19	7.95	1.80×10^{-5}	1.80×10^{-3}	0.00485	0.28
24	7.95	2.41×10^{-3}	2.25×10^{-1}	0.607	34.5
22[g]	8.90[h]	4.10×10^{-4}	4.10×10^{-2}	2.20	125
17[g]	8.90[h]	1.17×10^{-3}	1.10×10^{-1}	39.2	2230

[a] See Experimental Section. [b] $k_2 = k_{obsd}/[alagly]$. [c] $k_2' = k_2$ divided by fraction of peptide present as free base and by fraction of benzimidazole present in neutral form (where applicable). [d] pK_a (NH → N$^-$) = 7.97. [e] pK_a = 8.18. [f] In carbonate buffer (0.2 *M*). [g] Reaction with methylamine as nucleophile. [h] In borate buffer (0.2 *M*).

(12) Although its pH dependence was not investigated, **16** would be expected to show a similar rate profile.

(13) K. L. Kirk and L. A. Cohen, manuscript in preparation.

(14) B. Capon, M. J. Perkins, and C. W. Rees, "Organic Reaction Mechanisms 1965," Interscience Publishers, New York, N. Y., 1966, Chapter 6.

(15) A lack of proper data prevents evaluation of the inductive contribution of the fused imidazole ring toward activation of the fluorine.

(16) By comparison, 2,4-dinitrofluorobenzene is at least 20,000 times as reactive as 2- or 4-nitrofluorobenzene toward methoxide ion [C. W. L. Bevan and G. C. Bye, *J. Chem. Soc.*, 3091 (1954)].

Experimental Section[17]

2-Fluoro-6-nitroacetanilide (3).—Nitration of 2-fluoroacetanilide was performed on a 1-mol scale, following the published small-scale procedure.[8] Yields of purified material (30–35%) were comparable with those reported. Attempts to increase the yield of the desired *ortho* isomer, by varying reaction time or the strength of nitric acid used, were unsuccessful.

2-Fluoro-6-nitroaniline (4).—A suspension of 10 g (0.05 mol) of 2-fluoro-6-nitroacetanilide (**3**) in 50 ml of 2 *N* hydrochloric acid was heated at reflux for 3 hr. The reaction mixture was cooled, neutralized with sodium carbonate solution, and chilled. The product was obtained as orange needles, 7.5 g (95%), mp 72.5–74°. The amine has also been obtained by base-catalyzed deacylation of the acetanilide, mp 75–76°.[8]

3-Fluoro-*o*-phenylenediamine (8).—The crude nitroaniline (**4**, 7.5 g, 0.048 mol) was hydrogenated in ethanol solution, using palladium-on-charcoal catalyst. The theoretical amount of hydrogen was absorbed rapidly at room temperature and atmospheric pressure. Following removal of catalyst and solvent, a reddish oil remained which crystallized upon storage. The product was purified by vacuum sublimation: 5.8 g (95%), mp 40–41°.

Anal. Calcd for $C_6H_7FN_2$: C, 57.13; H, 5.59; F, 15.06; N, 22.21. Found: C, 57.04; H, 5.53; F, 15.02; N, 22.53.

4- (or 7-) Fluorobenzimidazole (10).—A solution of 1.0 g (0.08 mol) of 3-fluoro-*o*-phenylenediamine (**8**) in 50 ml of 90% formic acid was heated at reflux for 2 hr. Following removal of the solvent, the residue was purified by vacuum sublimation and crystallization from aqueous ethanol to give 0.86 g (80%) of colorless product, mp 188–190° (lit.[7] mp 180–185°).

The product was found to be identical with a sample prepared by pyrolysis of the diazonium fluoroborate (**9**), by mixture melting point and by comparison of nmr spectra. The nmr spectrum (DMSO-d_6) showed a one-proton singlet at 8.2 (C-2) and an aromatic multiplet at 6.7–7.5 ppm (3 H).

4- (or 7-) Fluoro-2-trifluoromethylbenzimidazole (14).—A solution of 1.0 g (0.08 mol) of 3-fluoro-*o*-phenylenediamine (**8**) in 25 ml of trifluoroacetic acid was heated at reflux for 2 hr. Following removal of the solvent, the residue was dissolved in ethanol and the solution was decolorized with Norit (hot). After filtration and evaporation of the solvent, the residue was purified by vacuum sublimation to give 1.36 g (83%) of colorless material (mp 183–187°). A portion was crystallized from water and resublimed: mp 188–189°.

Anal. Calcd for $C_8H_4F_4N_2$: C, 47.07; H, 1.98; F, 37.23; N, 13.73. Found: C, 47.18; H, 2.09; F, 37.25; N, 14.04.

The nmr spectrum (DMSO-d_6) showed only an aromatic multiplet at 6.9–7.7 ppm.

2-Amino-6-fluoroacetanilide (5).—A solution of 10 g (0.05 mol) of 2-fluoro-6-nitroacetanilide (**3**) in 100 ml of ethanol was hydrogenated, using 10% palladium on charcoal. Reduction was complete in 3 hr at room temperature and atmospheric pressure. Following removal of catalyst and solvent, the colorless product (8.4 g, 99%) was crystallized from water and sublimed: mp 154–155°.

Anal. Calcd for $C_8H_9FN_2O$: C, 57.13; H, 5.39; F, 11.30; N, 16.66. Found: C, 56.87; H, 5.58; F, 11.73; N, 16.83.

The ir spectrum (KBr) showed amide absorption at 1675 cm^{-1}. The nmr spectrum (acetone-d_6) exhibited a methyl singlet at 2.12 (3 H) and an aromatic multiplet at 6.2–7.2 ppm (3 H).

4- (or 7-) Fluoro-2-methylbenzimidazole (11).—A solution of 0.50 g (3 mmol) of 2-amino-6-fluoroacetanilide (**5**) in 20 ml of acetic acid was heated at reflux for 3 hr. Following removal of solvent, the product was purified by sublimation: 0.45 g (100%), mp 210–212°.

Anal. Calcd for $C_8H_7FN_2$: C, 63.99; H, 4.70; F, 12.65; N, 18.66. Found: C, 63.82; H, 4.76; F, 13.14; N, 18.62.

The ir spectrum was devoid of carbonyl absorption. The nmr spectrum (DMSO-d_6) showed a methyl singlet at 2.54 (3 H) and an aromatic multiplet at 6.7–7.4 ppm (3 H).

2-Fluoro-6-N-methylaminoacetanilide (6). A.—To a solution of 5.0 g (0.03 mol) of 2-amino-6-fluoroacetanilide (**5**) in 50 ml of ethanol was added 2.6 g of 37% formaldehyde solution (approximately 0.032 mol of formaldehyde) and the mixture was hydrogenated using 10% palladium on charcoal. After 36 hr at room temperature and atmospheric pressure, 90% of the theoretical amount of hydrogen had been consumed. Following removal of catalyst and solvent, 5.4 g of crystalline material was obtained. The product was recrystallized from water or ether and sublimed: mp 122–123°.

Anal. Calcd for $C_9H_{11}FN_2O$: C, 59.33; H, 6.09; F, 10.43; N, 15.38. Found: C, 59.63; H, 6.15; F, 10.36; N, 15.41.

The ir spectrum (KBr) showed amide absorption at 1665 cm^{-1}. The nmr spectrum ($CDCl_3$) showed a C-methyl singlet at 2.12 (3 H), an N-methyl singlet at 2.88 (3 H), broad absorption (NH?) at 4.1–4.4 (1 H), and an aromatic multiplet at 6.3–7.7 ppm (3 H).

B.—To a solution of 3.0 g (0.015 mol) of 2-fluoro-6-nitroacetanilide (**3**) in 50 ml of ethanol was added 1.4 g of 37% formaldehyde solution (approximately 0.017 mol of formaldehyde and the mixture hydrogenated as above. After 2 days, catalyst and solvent were removed, leaving an oil which crystallized slowly upon storage. Thin layer chromatography showed the material to be a mixture of **5** and **6**. After two recrystallizations from water and sublimation, a low yield (0.8 g) of the desired N-methyl compound was obtained.

1,2-Dimethyl-4-fluorobenzimidazole (7).—A solution of 1.0 g (5.5 mmol) of 2-fluoro-6-N-methylaminoacetanilide (**6**) in 25 ml of acetic acid was heated at reflux for 12 hr. Following removal of solvent, 0.88 g (98%) of colorless, crystalline material was obtained. The product was recrystallized from water and sublimed: mp 144–146°.

Anal. Calcd for $C_9H_9FN_2$: C, 65.84; H, 5.53; F, 11.57; N, 17.06. Found: C, 65.73; H, 5.57; F, 11.58; N, 16.82.

The ir spectrum wss devoid of carbonyl absorption. The nmr spectrum (DMSO-d_6) showed a C-methyl singlet at 2.58 (3 H), an N-methyl singlet at 3.68 (3 H), and aromatic multiplet at 6.7–7.4 ppm (3 H).

2-N,N-Dimethylamino-6-fluoroacetanilide (12).—To a solution of 5.0 g (0.03 mol) of 2-amino-6-fluoroacetanilide (**5**) in 25 ml of formic acid was added 10 ml of formaldehyde solution (37%) and the mixture was heated at reflux for 6 hr. Following removal of solvent, the colorless residue was crystallized from water to give 4.6 g (79%) of dialkylated product, mp 132–135°.

Anal. Calcd for $C_{10}H_{13}FN_2O$: C, 61.21; H, 6.68; F, 9.68; N, 14.27. Found: C, 60.94; H, 6.43; F, 9.78; N, 14.32.

The nmr spectrum (acetone-d_6) showed a C-methyl singlet at 2.10 (3 H), an N-methyl singlet at 2.70 (6 H), and an aromatic multiplet at 6.6–7.4 ppm (3 H).

Several attempts were made to convert **12** into **7** by boiling its solution in acetic anhydride for periods up to 24 hr.[10] In each case, **12** was recovered almost quantitatively.

4- (or 7-) Fluoro-7- (or 4-) nitrobenzimidazole (15).—A solution of 0.25 g of 4- (or 7-) fluorobenzimidazole (**10**) in 1 ml of concentrated nitric acid was added dropwise to 1 ml of concentrated sulfuric acid with stirring and ice cooling. After storage for 3 hr at 25°, the solution was poured onto ice and the pH of the mixture was adjusted to 5 with saturated sodium bicarbonate solution. The yellow-white solid was collected and dried. Following purification by sublimation, 0.20 g of product, mp 240–247° dec, was obtained, *m/e* 181.03000 (calculated for $C_7H_4FN_3O_2$, 181.03035). Because of difficulty in crystallization of this and other fluoronitrobenzimidazoles, no attempt was made to purify them for combustion analysis. In each case, the composition was ascertained on the basis of parent peaks in the mass spectrum and homogeneity demonstrated by nmr spectra. The uv spectrum (ethanol) showed λ_{max} 316 mμ (ϵ 7870). For nmr data, see Table I.

1,2-Dimethyl-4-fluoro-5-nitrobenzimidazole (13).—Mononitration of 1,2-dimethyl-4-fluorobenzimidazole (**7**) was effected by following the general procedure given above. The product was obtained in 88% yield: mp 170–172° (benzene), *m/e* 209.05766 (calculated for $C_9H_8FN_3O_2$, 209.06165). The uv spectrum (ethanol) gave λ_{max} 300 mμ (ϵ 8870). For nmr data, see Table I.

4- (or 7-) Fluoro-7-(or 4-) nitro-2-trifluoromethylbenzimidazole (20).—Mononitration of 4-fluoro-2-trifluoromethylbenzimidazole (**14**) yielded 80% of a pale yellow product which was purified by sublimation: mp 120–122°, *m/e* 249.00621 (calculated for $C_8H_3F_4N_3O_2$, 249.02252). The uv spectrum gave $\lambda_{max}^{pH\ 1}$ 316 mμ (ϵ 9700), $\lambda_{max}^{pH\ 6.3}$ 353 mμ (ϵ 7950), and $\lambda_{max}^{pH\ 11}$ 335 mμ (ϵ 8750). On

(17) All melting points were determined on a Kofler block and are uncorrected. Ultraviolet (uv) spectra were measured using a Cary recording spectrophotometer, Model 14, nmr spectra with a Varian A-60 spectrometer, and infrared (μ) spectra with a Perkin-Elmer Infracord spectrophotometer. High resolution mass spectra were measured on an Hitachi double-focusing spectrometer, Model RMU-6E. Microanalyses were performed by Dr. W. C. Alford and his associates of this institute.

the basis of spectral data obtained at several pH values, the pK_a (NH → N^-) was estimated to be 5.4.

5,7- (or 4,6-) Dinitro-4- (or 7-) fluorobenzimidazole (16).—To a solution of 0.47 g of 4-fluorobenzimidazole (**10**) in 5 ml of concentrated sulfuric acid was added 16 ml of fuming nitric acid (*d* 1.50) and the mixture was heated at 120° (open condenser) for 12 hr. The mixture was cooled and poured onto ice to give a clear yellow solution. Upon neutralization to pH 6 with saturated sodium bicarbonate solution, a pale yellow solid separated: 0.57 g, mp 200° dec, *m/e* 226.01529 (calculated for $C_7H_3FN_4O_4$, 226.01543). On the basis of its nmr spectrum, the dinitro derivative was found to be free of the mononitro compound. The uv spectrum showed $\lambda_{max}^{pH\ 1}$ 275 mμ (ε 16,100), $\lambda_{max}^{pH\ 7.9}$ 300 mμ (ε 11,700), and $\lambda_{max}^{pH\ 10.7}$ 305 mμ (ε 13,700) and 345 (11,000). From spectral data, the pK_a (NH → N^-) was calculated to be 7.97.[18] For nmr data, see Table I.

5,7- (or 4,6-) Dinitro-4- (or 7-) fluoro-2-methylbenzimidazole (17).—Nitration of 4-fluoro-2-methylbenzimidazole (**11**) was carried out as described above and heating was continued for 20 hr at 120°. The pale yellow product was obtained in quantitative yield: mp *ca.* 250° dec, *m/e* 240.03176 (calculated for $C_8H_5FN_4O_4$, 240.03108). The uv spectral data showed $\lambda_{max}^{pH\ 1}$ 276 mμ (ε 14,600), $\lambda_{max}^{pH\ 7.9}$ 282 mμ (ε 13,400), and $\lambda_{max}^{pH\ 11.7}$ 310 mμ (ε 14,600). From spectral data, the pK_a (NH → N^-) was calculated to be 8.18.[18] Spectra of **17** in neutral and alkaline media are reproduced in Figure 1. For nmr data, see Table I.

1,2-Dimethyl-5,7-dinitro-4-fluorobenzimidazole (18) and 1,2-Dimethyl-5,6-dinitro-4-fluorobenzimidazole (19).—The product obtained by analogous nitration of 1,2-dimethyl-4-fluorobenzimidazole (**7**) was found, by thin layer chromatography [silica gel, tetrahydrofuran–chloroform (1:1)] to be composed of two isomers. Chromatography of 200 mg of the mixture on a column of silicic acid (Merck) and elution with tetrahydrofuran–chloroform (1:1) led to the recovery of 120 mg of the 5,7-dinitro isomer (**18**), which was recrystallized from benzene and further purified by sublimation: mp 99–100°, *m/e* 254.04658 (calulated for $C_9H_7FN_4O_4$, 254.04672). The uv spectrum gave $\lambda_{max}^{pH\ 7.9}$ 280 mμ (ε 13,100).

The slower moving 5,6-dinitro isomer (**19**) was recovered from the column by elution with the same solvent mixture (20 mg) and recrystallized from benzene: mp 164–168° dec, *m/e* 254.04457. The uv spectrum showed $\lambda_{max}^{pH\ 7.9}$ 238 mμ (ε 13,600) and 330 (4600).

Further nitration of the mononitro derivative (**13**) led, in a similar manner, to a mixture of the dinitro isomers. In addition to their spectral differences, the isomers may be differentiated on thin layer plates by their rates of reaction with ammonia vapor. The 5,7 isomer rapidly forms an intense, deep yellow spot, whereas the 5,6 isomer requires at least 1 hr for reaction with ammonia.

Reaction of Dinitrofluorobenzimidazoles with Alanylglycine.—To a solution of 50 mg (0.34 mmol) of L-alanylglycine in 5 ml of 0.2 *M* sodium bicarbonate–carbonate buffer (pH 9) was added, in one portion, 50 mg (0.22 mmol) of 5,7-dinitro-4-fluoro-2-methylbenzimidazole (**17**). The mixture was heated at 50° for 30 min, cooled, and acidified to pH 3. The yellow derivative was collected, dried (62 mg, 82%), and crystallized from aqueous ethanol: mp 220–230° dec. The uv spectrum (in 2 *M* KCl–10% ethanol) gave λ_{max} 415 mμ (ε 4680), 370 (8550), 305 (8200), and 228 (9700).[19]

Anal. Calcd for $C_{13}H_{14}N_6O_7$: C, 52.63; H, 3.85; N, 22.95. Found: C, 42.82; H, 3.77; N, 23.04.

The **alanylglycine** derivative of **5,7-dinitro-4-fluorobenzimidazole (16)** was prepared in a similar manner: 74% yield, mp 225–240° dec (aqueous ethanol). The uv spectrum (in 2 *M* KCl–0.02 *N* HCl–10% ethanol) gave λ_{max} 415 mμ (ε 9500), 362 (11,200), 295 (7800), and 228 (7900).

Anal. Calcd for $C_{12}H_{12}N_6O_7$: C, 40.91; H, 3.44; N, 23.86. Found: C, 40.87; H, 3.25; N, 23.92.

The **alanylglycine** derivative of **1,2-dimethyl-5,7-dinitro-4-fluorobenzimidazole (18)** was obtained in 80% yield: mp 195–200° dec (aqueous ethanol). The uv spectrum (in 2 *M* KCl–10% ethanol) showed λ_{max} 425 mμ (sh) (ε 4800), 375 (8350), 250 (9400), and 230 (9150).

Anal. Calcd for $C_{14}H_{16}N_6O_7$: C, 44.21; H, 4.24; N, 22.10. Found: C, 44.39; H, 4.54; N, 21.68.

The dipeptide derivatives were shown to be homogeneous by thin layer chromatography on silica gel GF, using chloroform–*t*-amyl alcohol–acetic acid (70:30:5) as developing agent.

Kinetic Runs with Alanylglycine.—Reaction mixtures were prepared by adding 0.50 ml of an ethanol solution of the appropriate dinitrofluorobenzimidazole (0.08–0.09 m*M*) to 4.5 ml of a solution of alanylglycine (in 0.2 *M* phosphate buffer, pH 7.95). The final concentration of reagent was approximately 0.1 m*M* and that of alanylglycine, 10 m*M*. Samples were maintained at 25° and the progress of the reaction followed by the increase in absorption at 410 mμ. Comparative rates for reaction of alanylglycine with 2,4-dinitrofluorobenzene were followed at 370 mμ. Pseudo-first-order rate constants were obtained from the slope of the plot of $O.D._\infty/(O.D._\infty - O.D._t)$ *vs.* time. Reactions were carried to completion, infinity values and final spectra being in agreement with those obtained from samples of the purified dipeptide derivatives.

Registry No.—**5,** 18645-85-7; **6,** 18645-86-8; **7,** 18645-87-9; **8,** 18645-88-0; **11,** 18645-89-1; **12,** 18645-90-4; **13,** 18645-91-5; **14,** 18645-92-6; **15,** 18645-93-7; **16,** 18645-94-8; alanylglycine derivative of **16,** 18645-99-3; **17,** 18645-95-9; alanylglycine derivative of **17,** 18646-00-9; **18,** 18645-96-0; alanylglycine derivative of **18,** 18646-01-0; **19,** 18645-97-1; **20,** 18645-98-2.

(18) Spectral pK_a values were measured at 25° in 0.2 *M* phosphate buffer containing 10% ethanol.

(19) The uv spectra of peptide derivatives are reproduced in the following paper.[6]