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An investigation of the reactions of 2,2-dichloromethylbenzimidazole with primary amines has led to the synthesis of 2-(*N*-*t*-butylformimidoyl)benzimidazole, 2-*N*-(2-phenylethyl)formimdoylbenzimidazole, and 1-(ethoxycarbonylmethyl)-2-(*N*-*t*-butylformimidoyl)benzimidazole. Under mild alkali conditions, 2,2-dichloromethylbenzimidazole was rapidly converted into a dimer, namely, 6*H*,13*H*-pyrazino[1,2-*a*:4,5-*a*']bisbenzimidazole-6,13-diol.

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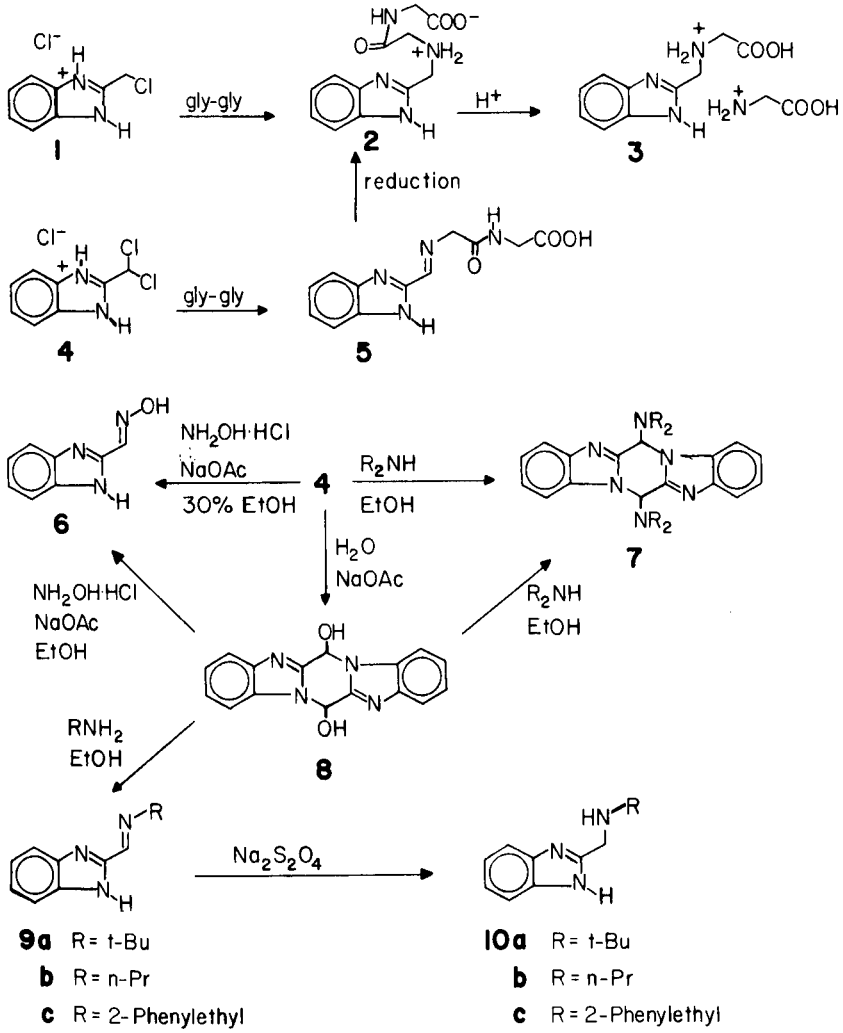
The Edman degradation is a most useful chemical process for protein sequence analysis. In principle, the degradation could be repeated indefinitely, however, the acid-catalyzed second step of the degradation which affords a phenylthiohydantoin can also lead to side reactions and, therefore, difficult analytical problems (1). For sometime, we have been of the opinion that certain benzimidazole derivatives of peptides might catalyze the cleavage of the peptide bond under mild alkaline conditions and circumvent these serious acid-catalyzed side reactions.

Our initial investigation involved a study of the interaction of 2-chloromethylbenzimidazole (**1**) (2) with amino acids and peptides (1 → 2 → 3). Although we were successful in developing a qualitative sequencing procedure with this reagent, all attempts to develop a quantitative procedure have been unsuccessful (3). This study did prove, however, that the benzimidazole group readily cleaves peptides under mild alkaline treatment, and thus our attention was shifted to another benzimidazole namely, 2,2-dichloromethylbenzimidazole (**4**). This compound has



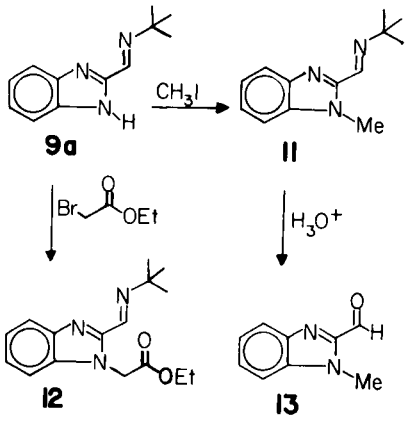
been studied in some detail by Hensel (4). This investigator has shown that 2,2-dichloromethylbenzimidazole readily reacts with hydroxylamine to give benzimidazole-2-carboxaldehyde oxime (**6**), and with secondary amines to give pentacyclic compounds of type **7** (5). It was felt that conditions similar to Hensel's could be employed to synthesize **5**. However, when the reaction of amino acids or peptides and **4** was carried out in 30% ethanol buffered to *p*H 6, the amino acid or peptide was recovered and the only product was 6*H*,13*H*-pyrazino[1,2-*a*:4,5-*a*′]bisbenzimidazole-6,13-diol (**8**). In aqueous solutions buffered at *p*H 6 or greater, the hydrolysis of **4** to the dimer **8** is a very rapid reaction and **8** appears to be an intermidiate in the conversion of **4** into compounds **6** and **7**. In support of this contention, compound **6** has been prepared directly from **8** under these conditions.

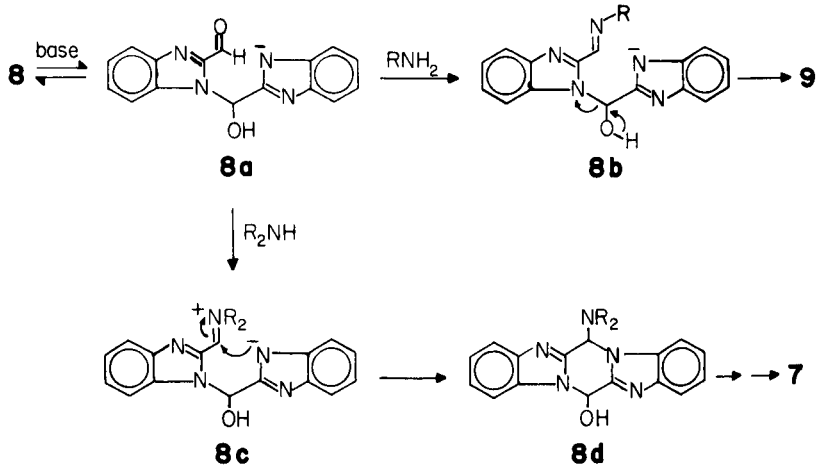
Having failed to synthesize **5** directly from **4**, it was decided that the reactions of simple primary amines with **8** should be investigated before studying peptides and amino acids. It was found that 2-(*N*-alkylformimidoyl)benzimidazoles (**9**) are easily prepared by refluxing **8** and the amine in ethanol (6). The presence of an imine proton absorption in the nmr spectrum of these compounds confirms that they are monomers. For example, the spectrum of 2-(*N*-*t*-butylformimidoyl)benzimidazole (**9a**) shows a singlet at 8.39 δ, a typical imine proton absroption. Compounds fo type **9** are easily reduced to 2-(*N*-alkylaminoemthyl)-benzimidazoles (**10**) with sodium dithionite. The nmr spectra of these compounds show methylene proton absorptions at about 4.1 δ, and, of course, no imine proton signals. These compounds apparently exist as hydrates because their nmr spectra contains broad peaks in the region from 4.5 to 5.6 δ. For example, the spectrum of **10a** shows a broad signal centered at 4.55 δ which disappears after deuterium oxide exchange.

Further evidence for this structural assignment was obtained by alkylation studies. The benzimidazole derivative **9a** reacts with methyl iodide in the presence of potassium carbonate to yield 1-methyl-2-(*N*-*t*-butylformimidoyl)benzimidazole (**11**); on mild acid hydrolysis **11** yielded 1-methylbenzimidazole-2-carboxaldehyde (**13**). Alkylation with ethyl bromacetate was also accomplished with the preparation of 1-(ethyl carboxymethyl)-2-(*N*-*t*-butylformimidoyl)benximidazole (**12**).

It appears that the formation of the Schiff base **9** probably is produced *via* the following:

This mechanism becomes plausible as the nmr spectrum of **8** in sodium deuteroxide shows a formyl proton absorption at 9.39 δ. However, the integration for this signal corresponds to less than one proton when compared to the integration of the signals from the aromatic protons of the benzimidazole ring. Therefore, it seems likely that in basic solution, **8** exists in equilibrium with the half open form **8a**. Only after condensation with the primary amine **8b**, does the dimer open to the monomer **9**.





The smooth condensation between the dimer and priamry amines gave us some hope that a similar condensation with peptides might be successful. However, that has not been the case. All attempts to condense the dimer with amino acids or peptides have been unsuccessful. Phenylalanine, for example, reacts with the dimer to yield an amorphous, labile substance of a molecular weight 420 ± 5. The nmr spectrum of this substance shows no imine proton absorption. A similar compound was produced when glycylglycine was reacted with the dimer. Furthermore, glycine was not released from this substance under either Edman or alkaline hydrolytic conditions. At the present time, we have been unable to assign structures to these derivatives because of their instability. Both compounds appear to be extremely air-sensitive, and thus far we have been unable to analyze them.

EXPERIMENTAL

General

^{1}H nmr spectra were recorded on a Varian A-60-A NMR spectrometer using tetramethylsilane as an internal standard. Melting points were determined in open capillary tubes on a Mel-Temp apparatus and are uncorrected. Elemental analyses were performed by Mr. R. Seab of the Louisiana State University Department of Chemistry.

2,2-Dichloromethylbenzimidazole Hydrochloride (**4**) and 6*H*, 13*H*-pyrazino[1,2-*a*:4,5-*a'*]bisbenzimidazole-6,13-diol (**8**) were prepared by the method of Hensel (4).

Glycylglycine and 2,2-Dichloromethylbenzimidazole Reaction.

Glycylglycine (0.132 g., mmole) and 2,2-dichlorobenzimidazole hydrochloride (0.238 g., 1 mmole) were dissolved in 30% ethanol and stirred at room temperature. A saturated sodium acetate solution was then added to the mixture until the *p*H was adjusted to 6. At this time, the reaction mixture was allowed to stir for 12 hours. At the end of this time, a yellow substance had precipitated from the reaction mixture. Thsi precipitate (0.110 g.) was collected and after purification was shown to be the dimer **8** by direct comparison of its ir spectrum with the spectrum of an authentic sample. Paper chromatographic analysis of the filtrate revealed only the presence of unreacted glycylglycine.

Benzimidazole-2-carboxaldehyde Oxime (**6**).

The dimer **8** (0.584 g., 2 mmoles), hydroxylamine hydrochloride (0.280 g., 4 mmoles), and sodium acetate (0.8 g.) were mixed in 25 ml. of 95% ethanol. The reaction mixture was refluxed for two hours, cooled to room temperature, and filtered. The filtrate was collected and the solvent was removed. The resulting crude, tan oxime, was dissolved in 30 ml. of methanol and decolorized with charcoal. The methanol was removed, and the resulting white solid was dried under vacuum for three hours to yield 0.583 g. of oxime **6** (90%, m.p. 283-284°, lit. (4) m.p. 287-288°): nmr (deuteriochloroform-DMSO-d_6): δ 5.22 (very broad 2, 1H), 7.05-7.80 (m, 4H), 8.22 (s, 1H).

2-(*N*-*t*-Butylformimidoyl)benzimidazole (**9a**).

The dimer **8** (5.82 g., 0.02 mole) and *t*-butylamine (10 ml.) were mixed in 250 ml. of absolute ethanol. The mixture was refluxed for three hours, and then the ethanol and excess amine were removed. The crude tan product was dissolved in 250 ml. of hot ethyl acetate and was decolorized with charcoal. The ethyl acetate was partially removed until white crystals began to form. The mixture was placed in a freezer to complete crystallization, and was then filtered. The fine white needles were washed twice with petroleum ether and were allowed to air dry to give 6.41 g. of **9a** (80%, m.p. 219-222° dec.; nmr (deuteriochloroform-DMSO-d_6): δ 1.33 (s, 9H), 7.06-7.78 (m, 4H), 8.39 (s, 1H).

Anal. Calcd for $C_{12}H_{15}N_3$: C, 71.61; H, 7.51; N, 20.88. Found: C, 71.64; H, 7.45; N, 21.05.

2-(*N*-Propylformimidoyl)benzimidazole (**9b**).

This compound was prepared from **8** and *n*-propylamine by a method similar to that for **9a** in 79% yield. The product was recrystallized once from dichloromethane and ethyl acetate, m.p. 151-152°; nmr (deuteriochloroform-DMSO-d_6): δ 0.98 (t, 3 H), 1.76 (sextet, 2 H), 3.68 (t, 2 H), 7.08-7.88 (m, 4 H), 8.38 (m, 1 H).

Anal. Calcd for $C_{11}H_{13}N_3$: C, 70.56; H, 7.00. Found: C, 70.52; H, 7.26.

2-(*N*-(2-Phenylethyl)formimidoyl)benzimidazole (**9c**).

This compound was prepared from **8** and 2-phenylethylamine in 78% yield by a method similar to that for **9a**. The product was recrystallized from dichloromethane and ethyl acetate, m.p. 195-196°; nmr (deuteriochloroform-DMSO-d_6): δ 3.03 (t, 2 H), 3.98 (t, 2 H), 7.07-7.82 (m, 9 H), 8.28 (m, 1 H).

Anal. Calcd for $C_{16}H_{15}N_3$: C, 77.08; H, 6.06; N, 16.85 Found: C, 76.42; H, 6.24; N, 16.79.

2-(*N*-*t*-Butylaminomethyl)benzimidazole (**10a**).

A solution of 0.87 g. (5 mmoles) of sodium dithionite in 20 ml. fo water was added to a solution of 0.201 g. (1 mmole) of **9a** in 30 ml. of ethanol. The resulting was stirred overnight. The ethanol was then removed, on the remaining aqueous mixture was extracted three times with 25 ml. portions of chloroform. The chloroform layer was dried over sodium sulfate and the solvent removed to yield 0.179 g. of **10a** monohydrate (81%, m.p. 138-140° recrystallized from methanol and ethyl acetate); nmr (deuteriochloroform-DMSO-d_6): δ 1.23 (s, 9 H), 4.10 (s, 2 H), 4.55 (very broad s, 3 H), 7.03-7.72 (m, 4 H). The dihydrochloride salt of **10a** was prepared by saturating a solution of **10a** monohydrate in ethanol with dry hydrogen chloride gas. The salt was precipitated with ether and washed twice with ether, m.p. 218-220°.

Anal. Calcd for $C_{12}H_{19}Cl_2N_3H_2O$: C, 52.36; H, 6.90. Found: C, 52.66; H, 6.95.

1-(Ethoxycarbonylmethyl)-2-(*N*-*t*-butylformimidoyl)benzimidazole (**12**).

Ethyl bromacetate (0.44 ml., 4 mmoles) and **9a** (0.802 g., 4 mmoles) and potassium carbonate (0.6 g.) were mixed in 70 ml. of ethyl acetate and refluxed 20 hours. The solvent was then removed. Petroleum ether (100 ml.) was added to this material, and as much of the solid as possible was dissolved. The resulting mixture was filtered, the filtrate collected, and the solvent removed to yield 0.754 g. of bright yellow, crude **12** (66%, m.p. 95-97°). This material may be recrystallized from dichloromethane and ethyl acetate to give white needles, m.p. 107-108°; nmr (deuteriochloroform): δ 0.17-1.38 (m, 12 H), 4.18 (quartet, 2 H), 5.43 (s, 2 H), 7.22-8.00 (m, 4 H), 8.48 (s, 1 H).

Anal. Calcd for $C_{16}H_{21}N_3O_2$: C, 66.88; H, 7.37; N, 14.62. Found: C, 66.74; H, 7.58; N, 14.61.

1-Methylbenzimidazole-2-carboxaldehyde (**13**).

Methyl iodide (0.65 ml., 10 mmoles), **9a** (0.504 g., 2.5 mmoles), and potassium carbonate (0.4 g.) were mixed in 70 ml. of ethyl acetate and refluxed 44 hours. The mixture was then filtered and

the filtrate set aside. The remaining precipitate was dissolved in water. This aqueous solution was extracted three times with 30 ml. portions of chloroform. The chloroform and ethyl acetate solutions were combined and the solvent removed. The remaining material, crude 1-emthyl-2-(*N*-*t*-butylformimidoyl)benzimidazole (**11**) (7), was mixed in 60 ml. of water. One ml. of concentrated hydrochloric acid was added and the mixture was stirred under nitrogen for two hours. The *p*H of the solution was adjusted to about 5, and the solution was extracted with five 50 ml. protions of chloroform. The chloroform layers were combined and dried over sodium sulfate, and the solvent was removed to yield 0.235 g. of **13** (59%, m.p. 105-106° (recrystallized from ethyl acetate), lit. (8) m.p. 110°.

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(5) Hensel prepared compounds of type **7** where R was diethylamino, dipropylamino, dibutylamino, and peperidino.

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(7) Compound **11** has not been isolated in pure form, but the crude material has the following nmr spectrum: nmr (deuteriochloroform): 1.29 (s, 9H), 4.14 (s, 3H), 7.13-7.75 (m, 4H), 8.50 (s, 1H).

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