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PRO-DRUGS AS DRUG DELIVERY SYSTEMS XIX. BIOREVERSIBLE DERIVATIZATION OF AROMATIC AMINES BY FORMATION OF N-MANNICH BASES WITH SUCCINIMIDE **

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SUMMARY

The kinetics of decomposition of various N-Mannich bases derived from succinimide or 5,5-dimethylhydantoin and a series of primary aromatic amines in aqueous solution at 37°C was studied to assess their suitability as pro-drugs for such amino compounds. The pH–rate profile for each compound showed a sigmoid shape and could be accounted for by assuming spontaneous decomposition of unprotonated Mannich base. The reaction rate increased markedly with increasing amine basicity. For the succinimide Mannich bases the half-lives of decomposition at pH 7.4 and 37°C were found to range from 0.9 min for the *p*-toluidine derivative to 4 h for derivatives of procaine and benzocaine. The results suggested the potential utility of such N-Mannich bases as pro-drug candidates for drugs containing a primary aromatic amino group, e.g. with the aim of protecting such drugs against metabolic inactivation by N-acetylation.

INTRODUCTION

In previous studies (Bundgaard and Johansen, 1980a, b and c, 1981; Johansen and Bundgaard, 1980a and b, 1981), the concept of N-aminomethylation of amides, imides, hydantoins, urea derivatives and various other NH-acidic compounds as a potentially useful means of obtaining pro-drug forms of such not easily derivatizable chemical entities were explored. Such N-Mannich bases were shown to decompose quantitatively to the parent compounds (for an amide Mannich base, see Scheme 1) in aqueous solution at rates highly dependent on pH of the medium and on structural factors including amide

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acidity, amine basicity and steric effects of the amine substituents.

$$R - CONHCH_2NR_1R_2 + H_2O \rightarrow R - CONH_2 + CH_2O + R_1R_2NH$$

Scheme 1

Besides cleavage rate, large modifications in other physicochemical properties such as aqueous solubility, intrinsic dissolution rate and lipophilicity for the parent amide-type compounds can be achieved by the appropriate selection of the amine component and by the proper choice of salt form (Johansen and Bundgaard, 1980a and b; Bundgaard and Johansen, 1980c).

In addition to being potential pro-drugs for amide-type compounds, N-Mannich bases may also be considered as pro-drug candidates for primary and secondary amines in which case the amide component would act as a transport group. A prerequisite for fulfilling this purpose is that the derivatives are converted to the parent amines within the body at a sufficiently high rate and recently, salicylamide was shown to be a promising transport group candidate for various aliphatic amines due to a high cleavage rate of the corresponding N-Mannich bases at pH 7.4 and 37°C (Johansen and Bundgaard, 1980b).

To further explore the possibilities of N-Mannich bases as potential transport and delivery forms for drugs containing primary or secondary amino groups, these studies have been extended to include primary aromatic amines. This functional group is contained in several drug substances but only a few types of aromatic (or aliphatic) amine pro-drug derivatives have hitherto been described (Eckert et al., 1970; Digenis and Swintosky, 1975). Due to the weak basicity of aromatic amines, N-Mannich bases with carboxamides may be too stable to function as bioreversible derivatives (Bundgaard and Johansen, 1980b). Therefore, more acidic amide-type transport groups may be needed, such as imides or hydantoins. In the present paper the kinetics of decomposition of a number of N-Mannich bases derived from succinimide or 5,5-dimethylhydantoin and various aromatic amines (Fig. 1) are described along with some suggestions on the potential utility of the derivatives as pro-drug forms.

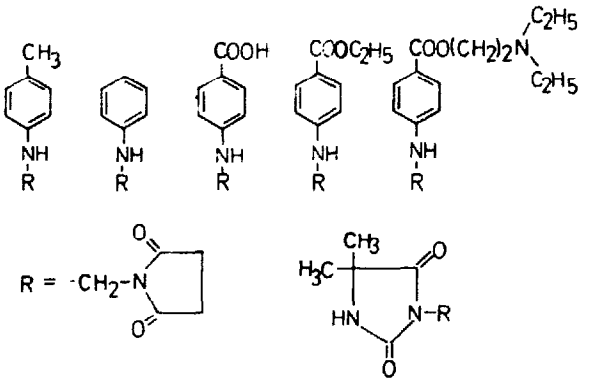


Fig. 1. Chemical structures of tested N-Mannich bases derived from succinimide or 5,5-dimethylhydantoin and various primary aromatic amines.

MATERIALS AND METHODS

Chemicals

The N-Mannich bases of succinimide were prepared by refluxing equimolar amounts of succinimide, primary aromatic amine and formaldehyde (used in the form of a 37% aqueous solution) in 95% ethanol for one hour according to the procedure described by Winstead et al. (1962): N-(anilinomethyl)succinimide, m.p. 172–173°C (from ethanol); N-(*p*-toluidinomethyl)succinimide, m.p. 140–141°C (from ethanol); N-(succinimidomethyl)-*p*-aminobenzoic acid, m.p. 220–222°C (from ethanol); ethyl N-(succinimidomethyl)-*p*-aminobenzoate, m.p. 137–138°C (from ethanol–petroleum ether); 2-diethylaminoethyl N-(succinimidomethyl)-*p*-aminobenzoate, m.p. 108–110°C (from ethanol–petroleum ether). The melting points of these derivatives, except for the latter compound not hitherto described, were in agreement with those reported by Winstead et al. (1962). The structure of the new procaine derivative was confirmed by elemental and IR analysis as well as by molecular weight determination by measuring the amount of formaldehyde released upon hydrolysis (Johansen and Bundgaard, 1979). 3-(N-*p*-toluidinomethyl)-5,5-dimethylhydantoin was prepared in a similar way, as the succinimide derivatives, m.p. 159–162°C (from ethanol), rep. m.p. 163–165°C (Horiki, 1976).

Buffer substances and all other chemicals used were of reagent grade.

Apparatus

Ultraviolet and visible spectral measurements were performed with a Zeiss PMQ II spectrophotometer and a Perkin-Elmer 124 recording spectrophotometer, using 1-cm cuvettes. Readings of pH were carried out on a Radiometer Type PHM 26 meter at the temperature of study. Melting points were taken on a capillary melting point apparatus and are uncorrected.

Kinetic measurements

All rate studies were performed in aqueous buffer solutions at 37.0 ± 0.2°C. Hydrochloric acid, formate, acetate and phosphate were used as buffers; the total buffer concentration was generally 0.1 M and a constant ionic strength (μ) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride.

For rapid reactions the decomposition was followed spectrophotometrically by recording the decrease in absorbance at 245 nm (the derivatives of aniline and *p*-toluidine). Reactions were performed in 2.5-ml aliquot portions of buffer solutions in a thermostatted quartz cuvette and were initiated by adding 15–25 μl of a stock solution of the compound in acetonitrile to give a final concentration of about 4×10^{-4} M. Rate constants were determined from plots of $\log(A_t - A_\infty)$ against time, where A_t and A_∞ are the absorbance readings at time t and at infinity, respectively.

Other reactions were monitored by measuring the amount of formaldehyde released during decomposition of the N-Mannich bases using a previously described modification (Johansen and Bundgaard, 1979) of the colorimetric method of Sawicki et al. (1961). Stock solutions of the compounds in acetonitrile were added to the buffer solutions (1 : 25), pre-heated at 37°C, to give a concentration of about 10^{-4}–10^{-3} M (dependent on the solubility of the derivatives). The solutions were kept in a water-bath of 37°C and at

appropriate intervals 500-μl samples were withdrawn and analyzed for formaldehyde as described previously (Johansen and Bundgaard, 1979). Pseudo-first-order rate constants were calculated from the slopes of linear plots of $\log(A_\infty - A_t)$ against time, where A_∞ and A_t are the absorbance readings (at 625 nm) at infinity and at time t, respectively.

RESULTS AND DISCUSSION

Kinetics of decomposition

The kinetics of breakdown of the N-arylaminomethylated succinimide and 5,5-dimethylhydantoin derivatives was studied in aqueous solution at 37°C over the pH range 0.4–8. Under the experimental conditions used all reactions proceeded to completion as revealed by the formation of formaldehyde in stoichiometric amounts. Furthermore, for compounds whose decomposition was monitored by direct ultraviolet spectral measurements the spectra of the completed reaction solutions superimposed exactly those of the parent components. At constant pH and temperature the reactions displayed strict first-order kinetics over more than 4 half-lives.

The rates of decomposition were found to be independent of buffer concentration from 0.02 to 0.1 M. Such lack of significant general acid–base catalysis was also noticed for the decomposition of N-Mannich bases of various carboxamides with aliphatic amines (Bundgaard and Johansen, 1980b; Johansen and Bundgaard, 1980b).

The influence of pH on the degradation rate for some N-Mannich bases is shown in Figs. 2 and 3, where the logarithms of the observed apparent first-order rate constants (k_{obs}) are plotted against pH. The sigmoidal shape of the pH–rate profiles is similar to that previously observed for the decomposition of N-Mannich bases of various carboxamides, thioamides, sulphonamides and ureides with primary or secondary aliphatic

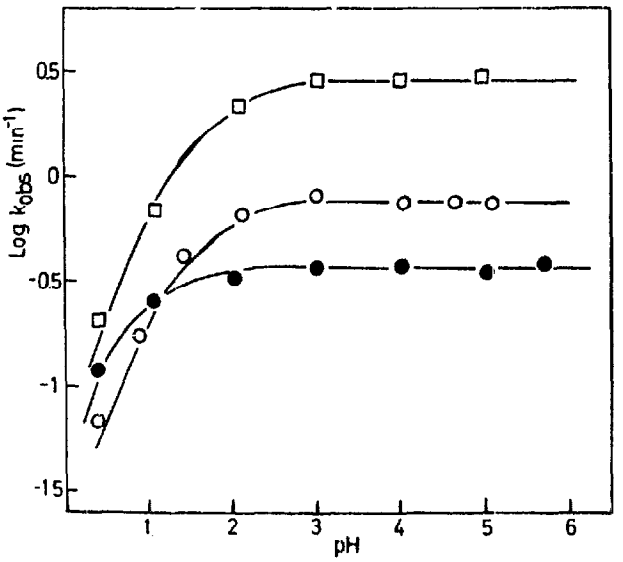


Fig. 2. The pH–rate profiles for the decomposition of N-(anilinomethyl)succinimide (•), N-(*p*-toluidinomethyl)succinimide (○) and 3-(*p*-toluidinomethyl)-5,5-dimethylhydantoin (□) in aqueous solution at 37°C.

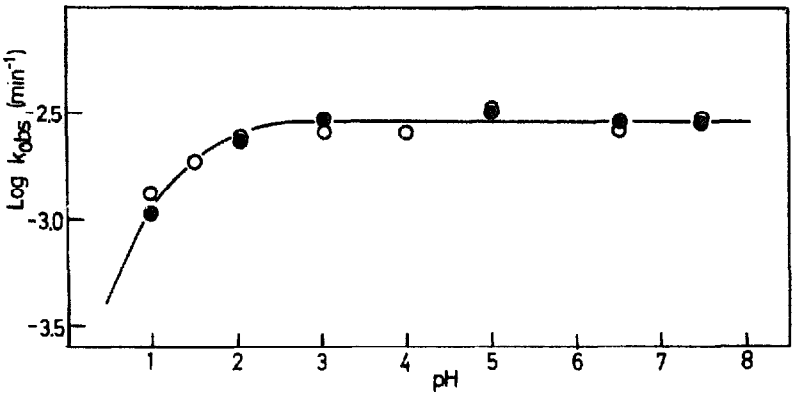


Fig. 3. The pH–rate profiles for the decomposition of the succinimide N-Mannich bases of benzocaine (•) and procaine (o) in aqueous solution at 37°C.

amines (Bundgaard and Johansen, 1980a and b), and the dependence of k_{obs} on pH can be accounted for by assuming spontaneous decomposition of the unprotonated Mannich base:

$$k_{obs} = \frac{k_1 K_a}{a_H + K_a} \tag{1}$$

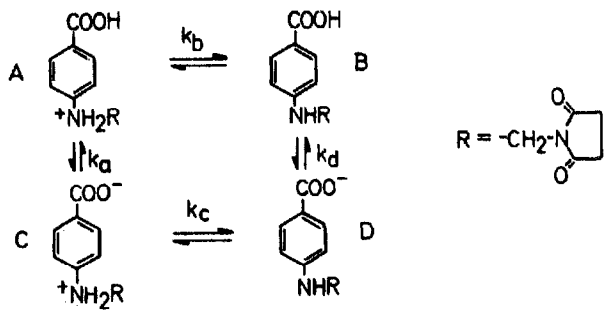
where K_a is the apparent ionization constant of the protonated N-Mannich base, a_H is the hydrogen ion activity and k_1 is an apparent first-order rate constant for spontaneous degradation of unprotonated Mannich base.

Rearrangement of Eqn. 1 gives:

$$k_{obs}a_H = -K_a k_{obs} + k_1 K_a \tag{2}$$

Plotting the rate data according to this equation ($k_{obs}a_H$ vs k_{obs}) afforded the values of k_1 and K_a listed in Table 1. The solid curves in Figs. 2 and 3 were constructed from Eqn. 1 and these values and the good fit observed demonstrate that Eqn. 1 adequately describes the degradation kinetics.

The pH–rate profile for the degradation of the N-Mannich base derived from succinimide and *p*-aminobenzoic acid is shown in Fig. 4. In the pH range studied the derivative can exist in 4 different forms. These are shown in Scheme 2, in which k_a, k_b, k_c and



Scheme 2

TABLE 1

IONIZATION CONSTANTS AND RATE CONSTANTS FOR THE DECOMPOSITION OF VARIOUS N-MANNICH BASES IN AQUEOUS SOLUTION (μ = 0.5) AT 37°C

Compound	k_1 (min^{-1})	pK_a (N-Mannich base)	pK_a [a] (parent amine)
3-(N-*p*-toluidinomethyl)-5,5-dimethylhydantoin	2.85	1.60	5.07 [b]
N-Succinimidomethyl derivatives of:			
p-Toluidine	0.76	1.55	5.07 [b]
Aniline	0.36	1.10	4.60 [b]
p-Aminobenzoate anion	0.032	(pK_{II} 4.70) [d]	3.72 [c]
p-Aminobenzoic acid	0.0028	(pk_I 1.25) [d]	2.44 [c]
Procaine	0.0029	1.25	2.45 [b]
Benzocaine	0.0029	1.25	2.38 [c]

[a] At 20–25 °C.

[b] From Perrin (1965).

[c] From Schulman et al. (1978).

[d] pK_{II} and pK_I are the macroscopic ionization constants for the carboxylic and amino groups, respectively.

k_d are microscopic ionization constants, describing the interconversion of the 4 species denoted A, B, C and D. The macroscopic ionization constants K_I (for the protonated amino function) and K_{II} (for the carboxyl group) are related to the microscopic ionization constants as shown in Eqns. 3–5:

$$K_I = k_a + k_b \quad (3)$$

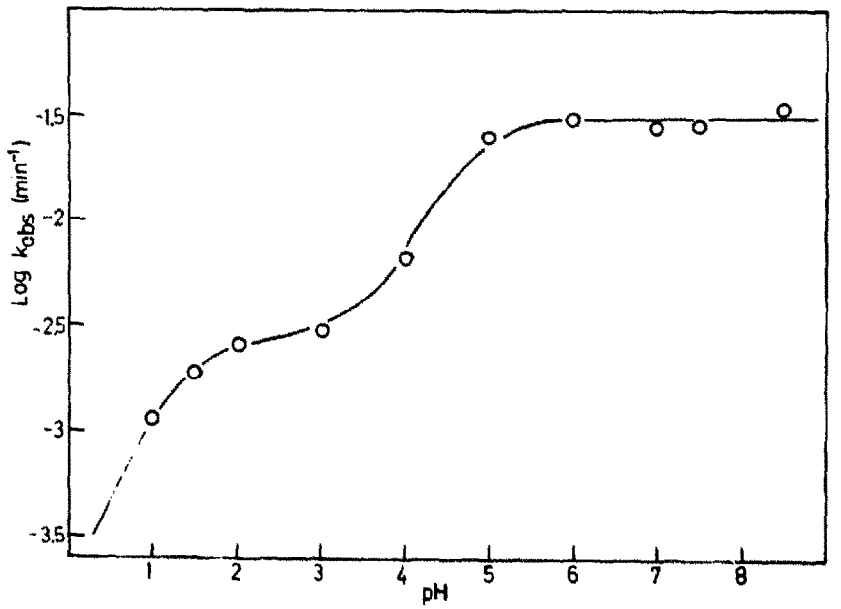


Fig. 4. The pH–rate profile for the decomposition of the succinimide N-Mannich base of *p*-aminobenzoic acid in aqueous solution at 37°C.

$$1/K_{II} = 1/k_c + 1/k_d \tag{4}$$

$$K_I K_{II} = k_a k_c = k_b k_d \tag{5}$$

The pH–rate profile in Fig. 4 indicates that the overall decomposition can be accounted for in terms of spontaneous degradation of the neutral (B) and/or the zwitterionic (C) forms and of the anionic species (D). The species B and C are related by a constant of proportionality which is independent on pH:

$$\frac{[B]}{[C]} = \frac{k_c}{k_d} = \frac{k_b}{k_a} \tag{6}$$

Thus, the suggested reaction scheme is:

$$(B) \text{ and/or } (C) \xrightarrow{k_1} \text{products} \tag{7}$$

$$(D) \xrightarrow{k_1'} \text{products} \tag{8}$$

and the equation for k_{obs} is accordingly:

$$k_{obs} = k_1(f_B + f_C) + k_1' f_D \tag{9}$$

where f is the fraction of the N-Mannich base present as the specified ionic form at the pH of measurement, and k_1 and k_1' are apparent first-order rate constants. The rate constant, k_1', and the ionization constant, K_{II}, were calculated from the rate data at pH > 4 and k_1 and K_I from the remaining data along with the following expression for the fractions of (B) and (C):

$$(f_B + f_C) = \frac{K_I a_H}{a_H^2 + K_I a_H + K_I K_{II}} \tag{10}$$

Eqn. 10 was derived from Eqns. 3–5 and the equations defining the microscopic ionization constants (e.g. $k_a = a_H[C]/[A]$). The derived constants are listed in Table 1. The solid curve in Fig. 4 was constructed from Eqn. 9 and the good agreement observed between the calculated and experimental data demonstrates that the reaction scheme of Eqns. 7 and 8 adequately describes the degradation kinetics.

The proposed kinetic scheme for the reactions of the N-Mannich bases derived from aromatic amines is similar to that previously shown to account for the decomposition of N-Mannich bases of amides and aliphatic amines (Bundgaard and Johansen, 1980a and b) except for the lack of reactions involving the protonated form of the compounds. Such reactions may possibly be observed in more strongly acidic solutions than those covered by the investigation.

According to the previous studies cited, a most plausible mechanism for the degradation of the N-Mannich bases involves as rate-determining step a unimolecular N–C bond cleavage with formation of an imide anion and an immonium cation. In subsequent fast steps, a solvent molecule transfers a proton to the imide anion and a hydroxide ion to the

immonium ion, giving methylolamine, which rapidly dissociates to formaldehyde and amine.

Structural effects on decomposition rate

The structural effects on the decomposition rate of N-Mannich bases derived from amide-type compounds and aliphatic amines have previously been shown to involve steric effects and basicity of the amine component and acidity of the amide-type component (Bundgaard and Johansen, 1980a and b). The rates of the reactions of unprotonated Mannich bases were accelerated strongly by increasing acidity of the parent amide-type compound and by increasing steric effects within the amine substituent. Although insufficient data were available an indication was obtained for increasing reaction rate with increasing basicity of the amine component (Bundgaard and Johansen, 1980b).

The present results allow a surer description of the effect of amine basicity on reaction rate since the compounds studied primarily differ in this respect, the steric effects within the amine components at the reaction center being practically constant. As can be seen from the data in Table 1, the rate constants k_1 increase strongly with increasing basicity of the parent amine and as demonstrated in Fig. 5 an excellent linear correlation exists between $\log k_1$ and pK_a of the amines. The regression equation ($r = 0.977$) for the N-(arylaminomethyl)succinimide derivatives is given by Eqn. 11:

$$\log k_1 = 0.93\ pK_a - 4.81\ (n = 6) \qquad (11)$$

This dependency of reaction rate on amine basicity is consistent with the proposed reaction mechanism since the rate-determining N–C bond cleavage step should be facili-

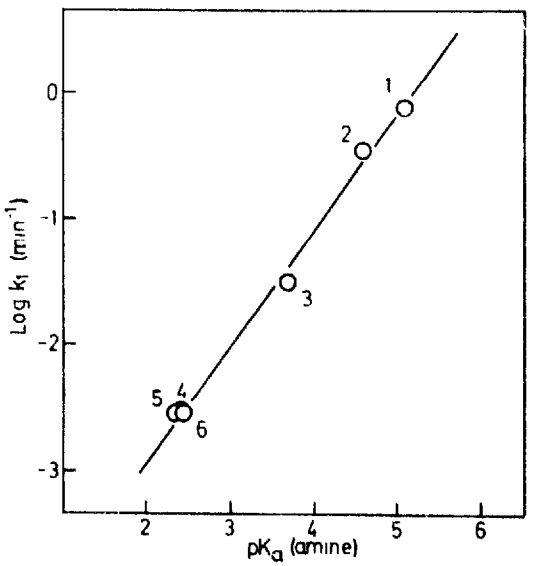


Fig. 5. Plot of the logarithm of the rate constant, k_1, for decomposition of various succinimide N-Mannich bases vs the pK_a of the parent aromatic amine. The numbers refer to the following amines: (1) *p*-toluidine; (2) aniline; (3) *p*-aminobenzoate anion; (4) *p*-aminobenzoic acid; (5) benzocaine; and (6) procaine.

tated with increasing amine nucleophilicity, i.e. with increasing pK_a for the amine.

The 8-fold greater reactivity of the *p*-toluidine derivative of 5,5-dimethylhydantoin as compared with that of succinimide (Table 1) is in accord with the relationship referred to above between reaction rate and acidity of parent amide-type compound, the hydantoin being more acidic than the imide. The pK_a value for succinimide is 9.62 (Walton and Schilt, 1952) while that for 5,5-dimethylhydantoin is 9.19 (Zief and Edsall, 1937). The N-Mannich base of phenytoin and aniline was prepared as described by Winstead et al. (1965) and found to be so labile that no rate data could be obtained. This high reactivity is in harmony with the relative high acidity of phenytoin (pK_a 8.3) (Agarwall and Blake, 1968). The compound was previously tested for various biological effects (Winstead et al., 1965), but it was not recognized that the compound is easily decomposed. N-Mannich bases of succinimide and phenytoin with morpholine (pK_a 8.3) or piperidine (pK_a 11.1) were previously shown to be so labile that no rate data could be obtained (Bundgaard and Johansen, 1980b). This fact is now readily conceivable on the basis of the relationship between rate and amine basicity described by Eqn. 11.

N-Mannich bases as pro-drug candidates for aromatic amines

The present study shows that N-Mannich bases can be considered as potential pro-drug forms for primary aromatic amines. With a relatively acidic amide-type transport group such as succinimide the derivatives release the parent amines at appropriately high rates, the actual values being highly dependent on the pK_a of the amines. At pH 7.40 and 37°C the half-lives of decomposition for the various derivatives were found to be 0.9 min (*p*-toluidine), 1.9 min (aniline), 22 min (*p*-aminobenzoic acid) and 4.0 h (procaine and benzocaine). As for other N-Mannich bases (Johansen and Bundgaard, 1981) these rate values may be assumed to be valid also in vivo.

The modification of the amines obtained through N-succinimidomethylation may profoundly effect the physicochemical properties such as lipophilicity and hence the delivery characteristics of the amines. For example, the basicity of the amines is lowered upon the derivatization corresponding to 1.2–3.5 pK_a units as can be seen from Table 1.

One potential objective for transient derivatizing primary amino groups in drugs may be to obtain protection against metabolic inactivation by N-acetylation. Several drugs including *p*-aminobenzoic acid, *p*-aminosalicylic acid, procainamide and various sulphonamides have been shown to undergo first-pass metabolism or, to a certain extent, metabolism in extrahepatic tissues due to acetylation of the primary amino group (e.g. Drucker et al., 1964; Wagner et al., 1973; Bloedow and Hayton, 1976; Mandelbaum-Shavit and Blondheim, 1981). Such first-pass metabolism which gives rise to decreased or highly variable systemic bioavailability upon oral administration may possibly be circumvented or depressed by protecting temporarily the vulnerable aromatic amino groups by N-imidomethylation.

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