Prodrugs as Drug Delivery Systems IV: *N*-Mannich Bases as Potential Novel Prodrugs for Amides, Ureides, Amines, and Other NH-Acidic Compounds

HANS BUNDGAARD ˣ **and MARIANNE JOHANSEN**

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Abstract □ The hydrolysis kinetics of a series of *N*-Mannich bases of carboxamides, thioamides, and other NH-acidic compounds were studied to assess their suitability as prodrugs for various drugs. The pH–rate profiles for the compounds were determined at 37° and were accounted for by assuming the spontaneous decomposition of both free and protonated Mannich bases. The reaction rate for the free base increased sharply with increasing steric effects of the amine component of the *N*-Mannich bases and also with increasing acidity of the amide component. *N*-Mannich bases may be potentially useful prodrugs for NH-acidic compounds such as various amides, imides, and ureides and for amines.

Keyphrases □ Prodrugs—*N*-Mannich bases of amides, ureides, amines, and imides, hydrolysis kinetics □ Hydrolysis kinetics—*N*-Mannich base prodrugs of amides, ureides, amines, and imides □ Amides—*N*-Mannich base derivatives as prodrugs, hydrolysis kinetics □ Amines—*N*-Mannich base derivatives as prodrugs, hydrolysis kinetics □ Imides—*N*-Mannich base derivatives as prodrugs, hydrolysis kinetics □ Ureides—*N*-Mannich base derivatives as prodrugs, hydrolysis kinetics □ *N*-Mannich bases—derivatives of amides, amines, imides, and ureides, potential as prodrugs, hydrolysis kinetics

Bioreversible derivatization of drug substances to produce prodrugs with altered physiochemical properties can improve substantially both drug efficacy and safety (1–3). Chemical transformation of active drug substances into inactive derivatives *per se*, which convert to the parent compounds within the body, may be relatively simple for drug substances possessing groups (*e.g.*, hydroxyl or carboxyl) that are bioreversibly esterifiable. However, for many drug molecules, no apparently readily derivatizable functional groups or entities are present.

BACKGROUND

As a part of studies (4–6) to identify potentially useful bioreversible derivatives of difficult-to-derive chemical entities and drug molecules, it was found that *N*-Mannich bases may be potentially useful prodrugs for NH-acidic compounds such as various amides, imides, and ureides and for amines.

N-Mannich bases of amides, imides, and various other NH-acidic compounds are known (7–10), and several drug substances bearing an NH-acidic group have been modified by *N*-aminomethylation and tested as potential medicinal agents, *e.g.*, hydantoins (11, 12), barbituric acids (13), sulfonamides (14), and succinimides (15, 16). However, almost no information on the stability and reactivity of such derivatives in aqueous solution is available. A recent study (17) of the degradation kinetics of rolitetracycline, a highly water-soluble *N*-Mannich base formed by condensation of the carboxamide group in tetracycline with formaldehyde and pyrrolidine (18), showed that the compound readily decomposed in aqueous solution to yield tetracycline, formaldehyde, and pyrrolidine quantitatively. The degradation half-life at pH 7.4 and 35° was 43 min, indicating the potential of *N*-Mannich bases as prodrugs, at least for carboxamides.

Based on this observation, numerous *N*-Mannich bases of various amides and other NH-acidic compounds were prepared. The decomposition kinetics of the compounds were studied in aqueous solution over a broad pH range to elucidate their stability and to assess their suitability as prodrugs for various drugs.

EXPERIMENTAL

The compounds (Table I) were prepared by heating formaldehyde, the amine (or the amine hydrochloride), and the amide in water–ethanol solutions according to standard literature procedures (7, 9, 10, 19). Several compounds were obtained as the hydrochloride (19).

All kinetic experiments were carried out in aqueous buffer solutions at 37.0 ± 0.1°. Hydrochloric acid, acetate, phosphate, borate, and carbonate buffers were used; the total buffer concentration was 0.1 *M* except in experiments where buffer effects were studied specifically. A constant ionic strength ($\mu = 0.5$) was maintained for each buffer by adding a calculated amount of potassium chloride. The initial concentration of the *N*-Mannich bases in the buffers was in the 10^{-4}–5×10^{-3} *M* range.

In rapid reactions, decomposition was followed spectrophotometrically[1] by recording absorbance changes at a wavelength where the absorptions of substrate and products differed maximally. Slower reactions were followed by measuring the amount of formaldehyde released using a colorimetric procedure described previously (20). The rate constants were determined from plots of log $(A_t - A_\infty)$ or log $(A_\infty - A_t)$ *versus* time, where A_t and A_∞ are the absorbance readings at time t and infinity, respectively.

RESULTS AND DISCUSSION

Decomposition Kinetics—At constant pH and temperature, the decomposition rates of the compounds studied followed strict first-order kinetics; the reactions went to completion in all kinetic runs, as revealed by the formation of formaldehyde and parent amide in stoichiometric amounts. Since the degradation rates were unaffected by variations (0.02–0.5 *M*) in the buffer concentrations used to maintain a constant pH, no general acid–base catalysis was apparent.

The influence of pH on the degradation rate for some compounds is shown in Fig. 1, where the logarithm of the observed apparent first-order rate constants, k_{obs}, was plotted against pH. The pH–rate profiles for the other compounds listed in Table I have a similar shape as those shown in Fig. 1. These pH dependences of k_{obs} can be accounted for by assuming spontaneous decomposition of the Mannich bases, B, and their conjugate acids, BH^+, as shown in Schemes I and II:

$$B \xrightarrow{k_1} \text{products}$$

Scheme I

$$BH^+ \xrightarrow{k_2} \text{products}$$

Scheme II

With $[B]_T$ as the total concentration of *N*-Mannich base (*i.e.*, $[B]_T = [B] + [BH^+]$), the overall rate equation is:

$$k_{obs}[B]_T = k_1[B] + k_2[BH^+] \qquad \text{(Eq. 1)}$$

where k_1 and k_2 are the apparent first-order rate constants for the spontaneous degradation of B and BH^+, respectively.

By introducing into Eq. 1 the identities:

$$[B] = \frac{K_a}{a_H + K_a}[B]_T \qquad \text{(Eq. 2)}$$

and:

$$[BH^+] = \frac{a_H}{a_H + K_a}[B]_T \qquad \text{(Eq. 3)}$$

[1] Zeiss PMQ II equipped with a thermostated cell compartment.

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Table I—Rate Data for Decomposition of Various *N*-Mannich Bases in Aqueous Solution (μ = 0.5; 37°) [a]

Compound	k_1, min^{-1}	$k_2 \times 10^4$, min^{-1}	pKa[b]	$t_{1/2}$[c], min
N-(Diethylaminomethyl)benzamide	0.52	1.1	7.7	4.0
N-(Ethylaminomethyl)benzamide	0.0084	0.9	7.5	190
N-(Methylaminomethyl)benzamide	0.0026	0.4	7.5	600
N-(Benzylaminomethyl)benzamide	0.0020	3.0	6.4	380
N-(Cyclohexylaminomethyl)benzamide	0.026	1.1	7.6	70
N-(Piperidinomethyl)benzamide	0.051	0.4	7.8	47
N-(Morpholinomethyl)benzamide	0.0005	6	5.6	1400
N-(Isobutylaminomethyl)benzamide	0.019	1.0	7.5	82
N-(Piperidinomethyl)niacinamide	0.17	1.0	7.5	8
N-(Morpholinomethyl)trichloroacetamide	0.72	ND	4.1	1.0
N-(Piperidinomethyl)trichloroacetamide	35	ND	6.5	0.02
N-(Piperidinomethyl)thiobenzamide	13	ND	6.6	0.06
N-(Morpholinomethyl)-*p*-toluenesulfonamide	ND[d]	1.5×10^4	ND[d]	<0.02
N-(Morpholinomethyl)-*N'*-acetylthiourea	0.91	ND	4.7	0.8
N-(Piperidinomethyl)methylurea	ND	ND	ND	5

[a] ND = not determined. [b] Kinetically determined values; for some compounds, pKa values also were determined titrimetrically and agreed with the kinetically obtained values. [c] Hydrolysis half-lives at pH 7.4 and 37°. [d] $k_1K_a = 1.9 \times 10^{-2}$ *M*/min.

where K_a is the apparent ionization constant of the protonated *N*-Mannich bases and a_H is the hydrogen-ion activity, the expression for k_{obs} is:

$$k_{obs} = \frac{k_1K_a}{a_H + K_a} + \frac{k_2a_H}{a_H + K_a} \qquad \text{(Eq. 4)}$$

The lines in Fig. 1 were constructed from Eq. 4 and the rate constants and pKa values given in Table I. Although Eq. 4 and, consequently, Schemes I and II adequately describe the observed degradation kinetics, other kinetically equivalent reactions can account for the observed k_{obs}–pH relationship.

Structural Effects on Reaction Rate—The rate data in Table I show that the structure of the amine component in the *N*-Mannich bases dramatically affected the rate constant, k_1 and, accordingly, the decomposition rate of the compounds in weakly acidic to basic aqueous solutions (*cf.*, the half-lives at pH 7.4). This result was predominantly a steric effect of the alkyl groups on the amine nitrogen. For some *N*-Mannich bases of benzamide, an excellent linear correlation existed between log k_1 and Charton's steric parameter, ν (21), for alkylamino groups (Fig. 2). The only deviation observed was for the benzylamine derivative and may have been due to the lower pKa value for the corresponding Mannich base as compared with the other compounds (Table I).

The data also show that for the same amine component, the *N*-Mannich base decomposition rate increased sharply with increasing acidity of the parent amide. The k_1 values of the piperidine derivatives increased in the order benzamide < niacinamide < thiobenzamide < trichloroacetamide, which is also the order of amide acidity. The difference in acidity among these amides was reflected in the pKa values for the Mannich bases with piperidine (Table I), and a linear correlation can be shown to exist between log k_1 and the pKa values for these *N*-Mannich base derivatives. For the most acidic amide of the compounds studied, *p*-toluenesulfonamide (pKa 10.3), k_1 was too high to be determined. A high k_1 value also was observed for morpholine derivatives of other acidic compounds such as succinimide, phenytoin, and barbital.

Structural factors other than steric effects and stability of leaving groups also may influence the reactivity of *N*-Mannich bases. Urea and thiourea Mannich bases were more reactive than expected on the basis of their acidity. Salicylamide derivatives degraded markedly faster in neutral solution than did the corresponding benzamide derivatives. The degradation half-lives of salicylamide *N*-Mannich bases with morpholine and α-alanine at pH 7.4 and 37° were 38 and 17 min, respectively [*cf.*, 1400 min for *N*-(morpholinomethyl)benzamide].

Reaction Mechanism—Although insufficient experimental evidence

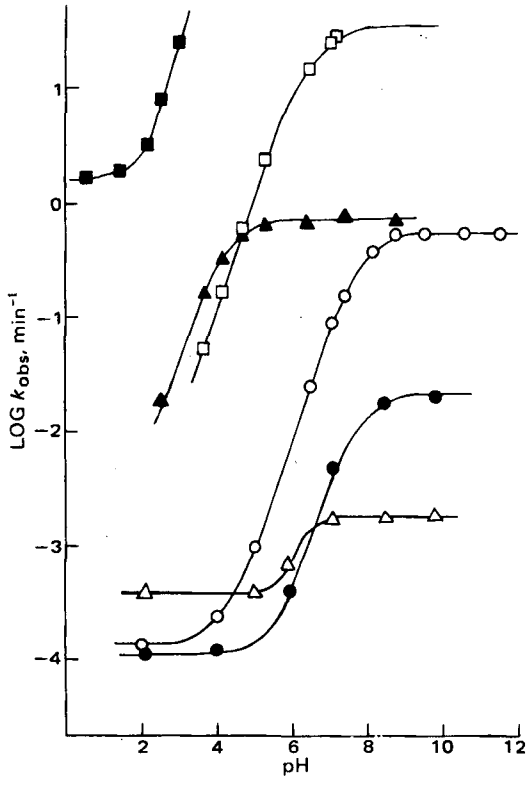


Figure 1—*The pH–rate profiles for the decomposition of various* N-*Mannich bases in aqueous solution at 37°. Key:* △, N-(*benzylaminomethyl*)*benzamide;* ●, N-(*isobutylaminomethyl*)*benzamide;* ○, N-(*diethylaminomethyl*)*benzamide;* ▲, N-(*morpholinomethyl*)*trichloroacetamide;* □, N-(*piperidinomethyl*)*trichloroacetamide; and* ■, N-(*morpholinomethyl*)-p-*toluenesulfonamide.*

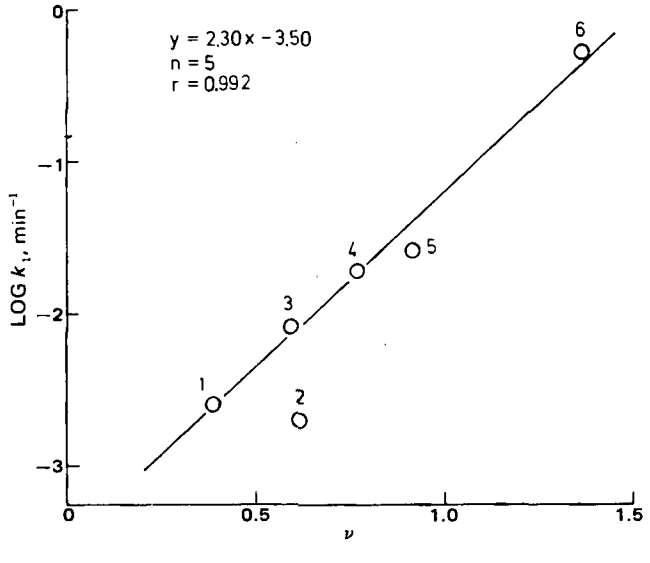
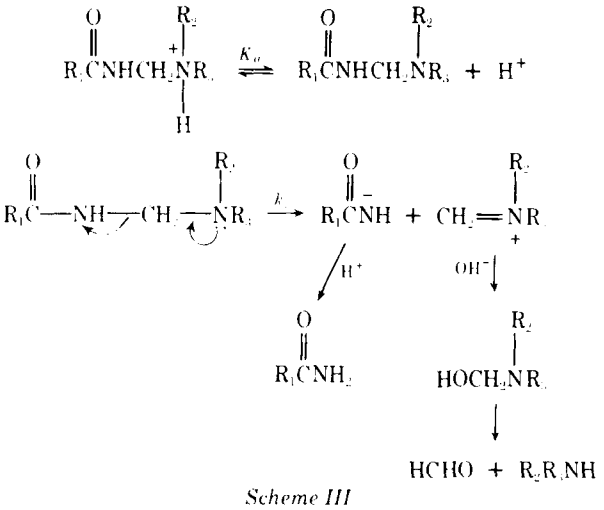


Figure 2—*Plot of log* k_1 *against Charton's steric substituent parameter for alkylamino groups for* N-*Mannich bases of benzamide* ($C_6H_5CONHCH_2R$). *Key: 1, R = methylamino; 2, R = benzylamino; 3, R = ethylamino; 4, R = isobutylamino; 5, R = cyclohexylamino; and 6, R = diethylamino. Compound 2 was excluded from the correlation plot.*



Scheme III

exists for a firm mechanistic scheme for the degradation, a plausible mechanism that agrees with the observed data is given in Scheme III. The rate-determining step involves unimolecular N–C bond cleavage with formation of an amide anion and an immonium cation. In subsequent fast steps, a solvent molecule transfers a proton to the amide anion and a hydroxide ion to the immonium ion, giving carbinolamine, which rapidly dissociates (22, 23) to formaldehyde and amine. In accord with this mechanism, the large steric effects exhibited by the amine alkyl groups may be explained as hindrance of amine solvation (*cf.*, 24), resulting in increasing nucleophilicity with increasing steric hindrance and, hence, increasing the lability of the N–C bond. The possible formation of *N*-(hydroxymethyl)amides in a rate-determining step does not seem likely since these compounds degraded much slower than the corresponding *N*-Mannich bases in separate experiments.

CONCLUSION

The present study shows that *N*-Mannich bases can be considered as potentially useful prodrugs of amides and various other compounds containing acidic NH-groups. By appropriate selection of the amine component, it should be feasible to obtain prodrugs with varying physicochemical properties such as the cleavage rate, aqueous solubility, and lipophilicity. Transformation of an amide into an *N*-Mannich base introduces a readily ionizable moiety, which may allow the preparation of derivatives with greatly increased aqueous solubilities at slightly acidic pH values where the stability is sufficiently high. For example, morpholine derivatives possess good aqueous solubility[2].

Finally, *N*-Mannich bases also can be considered as prodrug candidates for primary and secondary amines. The pKa of amines decreases considerably by *N*-amidomethylation. [With benzamide, the decrease is ~3 pKa units (*cf.*, Table I)]. Therefore, a potentially useful purpose for transforming amino compounds into *N*-Mannich base transport forms would be to increase the lipophilicity of the masked amines at physiological pH by depressing the ionization of the conjugate acid forms.

[2] H. Bundgaard and M. Johansen, unpublished results.

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