Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron Letters 46 (2005) 3079–3083
Tetrahedron Letters

General-buffer catalysis of the reaction of *N*-(hydroxymethyl)benzamide: a new pathway for the aqueous reaction of carbinolamides

Amanda G. Mennenga, Amy L. Johnson and Richard W. Nagorski*

Department of Chemistry, Illinois State University, Normal, IL 61790-4160, USA

Received 4 January 2005; revised 1 March 2005; accepted 1 March 2005
Available online 18 March 2005

Abstract—The second-order rate constants for the general-buffer catalyzed breakdown of *N*-(hydroxymethyl)benzamide (**1**) in water at 25 °C, $I = 1.0$ (KCl) by pivalic, acetic, chloroacetic, and dichloroacetic acid were determined by initial rates. The observed rate increased with increasing amounts of the acidic form of the buffer and a Brønsted correlation of $\alpha = 0.35$ was determined. The results presented here, represent the first evidence for a general-buffer catalytic mechanism for the aqueous reaction of **1** and for carbinolamides in general.

Carbonyl derivatives, such as acetals,[1–3] acylals,[4–6] and hemiacetals[7–9] play important roles in both synthetic and biological chemistry. Mechanistic studies of the aqueous reaction and methods of catalyzing this reaction have been crucial in providing both an understanding of their intrinsic reactivity and insight into the biological systems in which they are utilized.[1–5] The broad nature of the types of functionality found within these structurally similar groups are reflected in the diverse mechanisms by which they react. For example, both acetals and acylals were thought to react exclusively via specific-acid catalyzed mechanisms, however, examples of acetals were found, which reacted by general-acid mechanisms.[10–14] In addition, acylals have been proposed to react through ester hydrolysis mechanisms under circumstances where the electron-donating ability of the alkoxy group was diminished due to the presence of electron-withdrawing groups.[4,5] The focus of the study described here was on the general-buffer catalysis of the aqueous reaction of carbinolamides, which were previously thought to react only via specific-acid catalysis in the acidic region of their pH-rate profiles.[15–21]

Carbinolamides are biological intermediates, which are enzymatically generated and cleaved during the biosynthesis of peptide hormones.[22–27] In general, carbinolamides are known to react via both hydronium ion and hydroxide-catalyzed routes.[15–21]

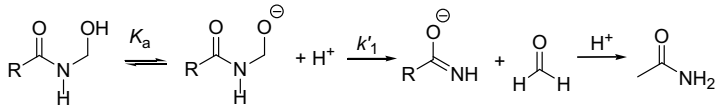
(a) *Hydroxide-catalyzed mechanism*: Overall, a specific-base catalyzed mechanism, which involves deprotonation of the hydroxyl group followed by rate-limiting breakdown of the alkoxide to form aldehyde and the anionic amide (Scheme 1).[15–21]
(b) *Hydronium ion-catalyzed mechanism*: This reaction is thought to occur via specific-acid catalyzed protonation of the amide portion of the carbinolamide,[20] followed by rate-limiting breakdown to the amide and protonated aldehyde (Scheme 2).[16,18,20] (A water reaction (k_{HOH}) must be included in the rate expression to explain the appearance of the pH-rate profile, see Eq. 1.)[16,18,20]

$$k_{obsd} = k_H[H^+] + k_{HOH} + k_1 \frac{K_a[HO^-]}{K_w + K_a[HO^-]} \qquad (1)$$

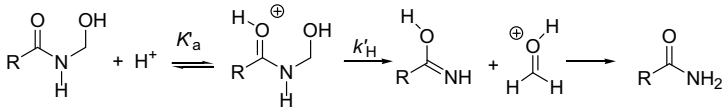
The dichotomy between the currently accepted mechanisms and the existence of an enzyme, which catalyzes the breakdown of carbinolamides was puzzling when the general-acid/general-base catalytic schemes, often associated with enzymatic systems, was considered.[28,29] If the lyase portion of PAM (peptidylglycine α-amidating

Keywords: Carbinolamide; *N*-(Hydroxymethyl)benzamide; Buffer catalysis.
* Corresponding author. Tel.: +1 309 438 8978; fax: +1 309 438 5538; e-mail: rnagor@ilstu.edu

0040-4039/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2005.03.011



Scheme 1. Specific-base catalyzed reaction.



Scheme 2. Specific-acid catalyzed mechanism.

monooxygenase, EC 1.14.17.3)[22–27] utilizes a general-base/acid catalytic method to cleave the carbinolamide intermediates (such a mechanism has been suggested),[26,30] why has general-catalysis of the aqueous reaction of carbinolamides not been previously observed? Herein, we provide the first evidence for general-buffer catalysis of the aqueous reaction of a carbinolamide (*N*-(hydroxymethyl)benzamide, **1**), as a pathway for its breakdown in the acidic and water-catalyzed regions of its pH-rate profile.

$$\frac{A_{car}}{(A_{car} + (1.8)(A_{amide}))} = -k_{obsd}\, t \qquad (2)$$

N-(Hydroxymethyl)benzamide (**1**) was synthesized and purified as previously reported.[20,31] The conversion of **1** into benzamide (**2**) and formaldehyde was followed in H_2O, $I = 1.0$ (KCl), at 25 °C in the presence of varying concentrations of pivalate, acetate, chloroacetate, or dichloroacetate buffer, as a function of pH. A typical experiment involved the injection of a volume of concentrated stock solution of **1**, dissolved in CH_3CN, into a thermally equilibrated aqueous solution, yielding a final substrate concentration of 5×10^{-5}–1×10^{-4} M.[20] The progress of the reactions were monitored by following the disappearance of the starting material and the appearance of amide, using HPLC.[32] As a result of the sluggish nature of these reactions,[20] an initial rates method[28] was employed where the disappearance of the first 3–4% of **1** and the appearance of **2** was followed. The observed rate of the reaction (k_{obsd}) was determined from the linear plot of the fraction of **1** remaining versus time (s) according to Eq 2, where A_{car} was the observed area of the peak of **1**, A_{amide} was the area of the peak for the amide product and a correction factor of 1.8 was used that statistically corrected the observed area of **2** for differences in the molar absorptivities between **1** and **2**, at the wavelength at which reaction progress was followed.[32]

Previous investigations, performed at low buffer concentrations ($[B_{tot}] \leqslant 0.05$ M), did not report any buffer catalysis, leading to the conclusion that the acid-promoted reaction occurred via a specific-acid catalyzed mechanism.[20] Further investigations at higher buffer concentrations have shown that as the [buffer] was increased, at the same pH, there was an increase in k_{obsd} for the reaction of **1**. A plot of k_{obsd} versus the total concentration of chloroacetic acid (see Fig. 1) showed a linear correlation between [buffer] and k_{obsd} for the reaction of **1** in H_2O, at 25 °C, $I = 1.0$ (KCl). From Figure 1, as the pH of the solution decreased and, therefore, the concentration of the acidic form of the buffer rose, there was an associated rise in the slope of the correlation, yielding an initial conclusion that the acidic form of the buffer was catalyzing the reaction of **1**.

The *y*-intercepts, of the plots in Figure 1, yielded the rate of the buffer independent reaction at that pH, and those values were in good agreement with previous studies of the aqueous reaction of **1** (see Table 1).[20] The second-order rate constant for the reaction of **1** (k_{HA}), as a function of the acidic form of the buffer, was obtained by plotting the second-order rates (k'_{HA}) from Figure 1 (k_{obsd} vs [total buffer]) versus the fraction of the acidic form of the buffer and was found to be $k_{HA} = 2.0 \times 10^{-7}\ M^{-1}\ s^{-1}$ for chloroacetic acid (see Supplemental materials for plot). Similar studies to those described above were performed with acetic acid, dichloroacetic acid, and pivalic acid.[33] In all cases, a linear correlation between the [buffer] and k_{obsd} was found and *y*-intercepts correlated with the calculated values from previous studies[20] (plots of k_{obsd} vs [buffer] available in the Supplemental material).

Shown in Table 1 are the second-order rate constants (k_{HA}), determined as a function of the acidic form of the buffer, for the aqueous reaction of **1** at 25 °C,

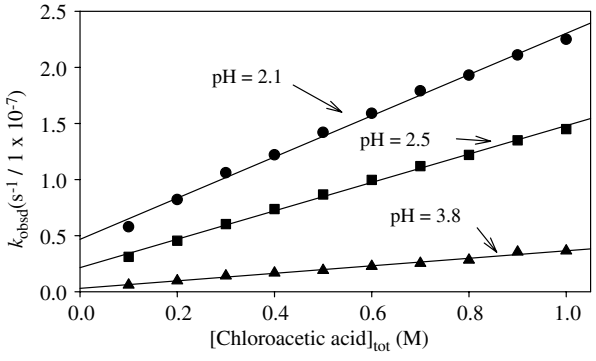


Figure 1. Effect of increasing concentrations of chloroacetic acid on the rate of the aqueous reaction (k_{obsd}) of *N*-(hydroxymethyl)benzamide in H_2O at 25 °C, $I = 1.0$ (KCl) at pH's = 2.1, 2.5, and 3.8.

Table 1. Second-order rate constants for the buffer-catalyzed breakdown of *N*-(hydroxymethyl)benzamide (**1**) and the experimental and calculated rates for the buffer-independent reaction in H_2O, at 25 °C, $I = 1.0$ (KCl)

Buffer	pK_a[a]	pH[b]	k_{HA} (M^{-1} s^{-1})	k_{bkg}[e] (s^{-1})	k_{bkg}^{calc}[f]
CH_3CO_2H	4.76	5.61		$(2.5 \pm 0.2) \times 10^{-9}$	3.2×10^{-9}
		4.44	$(5.2 \pm 0.5) \times 10^{-8}$[c]	$(1.4 \pm 0.6) \times 10^{-9}$	2.1×10^{-9}
		3.80		$(3.4 \pm 0.4) \times 10^{-9}$	2.9×10^{-9}
$ClCH_2CO_2H$	2.86	3.81		$(3.0 \pm 0.4) \times 10^{-9}$	2.9×10^{-9}
		2.51	$(2.0 \pm 0.3) \times 10^{-7}$[c]	$(2.2 \pm 0.1) \times 10^{-8}$	2.4×10^{-8}
		2.10		$(4.7 \pm 0.3) \times 10^{-8}$	6.0×10^{-8}
Cl_2CHCO_2H	1.29	1.50	$(2.0 \pm 0.1) \times 10^{-6}$[d]	$(1.9 \pm 0.2) \times 10^{-7}$	2.3×10^{-7}
$(CH_3)_3CCO_2H$	5.05	4.92	$(4.1 \pm 0.3) \times 10^{-8}$[d]	$(2.0 \pm 0.3) \times 10^{-9}$	2.1×10^{-9}

[a] pK_a values from Ref. 45b.
[b] pH of the solution in which the experiment was performed.
[c] Calculated by plotting k'_{HA} for the buffer-catalyzed reaction versus fraction of the buffer in the acidic form.
[d] Calculated from the slope of the k_{obsd} versus fraction acidic form of the buffer.
[e] The *y*-intercept values of the plots of k_{obsd} versus [buffer].
[f] Calculated using Eq. 1 (see Ref. 20).

$I = 1.0$ (KCl). From Table 1, as the acidity of the buffer increases, its catalytic effectiveness also increases. This observation can be more easily observed in Figure 2 where the Brønsted correlation between the acidity of the buffer catalyst was plotted versus $\log k_{HA}$, and resulted in an $\alpha = 0.35$. A point for H_3O^+ has also been included in the plot (rate for H_3O^+-catalyzed reaction was determined previously, $k_H = 7.3 \times 10^{-6}$ M^{-1} s^{-1})[20] and showed a good correlation with the acetate buffers indicating a consistent mechanism between the buffer-catalyzed reactions and the hydronium-ion catalyzed reaction. Even more interesting was the observation that the second-order rate for the water-catalyzed reaction ($k_{HOH} = 3.1 \times 10^{-11}$ M^{-1} s^{-1})[20,34] of **1** correlated well with the buffer and hydronium ion points. This observation suggested that the water-catalyzed reaction, a difficult reaction to study due to the slow reaction rates,[35]

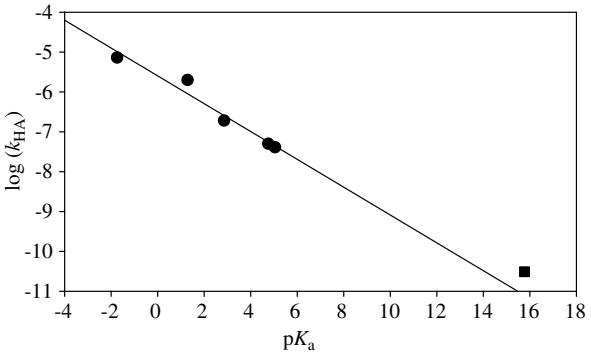
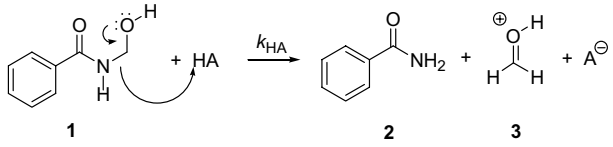


Figure 2. Brønsted plot of the $\log k_{HA}$ (second-order rate of buffer catalyzed reaction of *N*-(hydroxymethyl)benzamide in H_2O at 25 °C, $I = 1.0$ (KCl)) versus the pK_a for the acid catalysts used to catalyze the reaction.



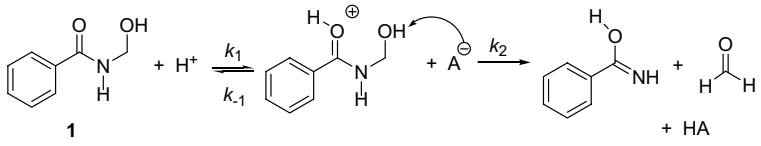
Scheme 3. General-acid catalyzed mechanism.

reacts via the same mechanism as the acetate and hydronium ion-catalyzed reactions.

The catalytic effect of carboxylate buffers can be interpreted in two ways:[36]

(a) *General-acid catalysis* (Scheme 3): The rate determining step of the reaction involves proton transfer from an H^+ source to the amide portion of **1** as breakdown into **2** and **3** occurs. In Scheme 3, proton transfer to the nitrogen has been shown as the carbinolamide cleaves, however an equally valid mechanism could be written wherein proton transfer occurs at the carbonyl oxygen of the amide.[37] The mechanism (Scheme 3) correlates with the suggested mechanisms for the general-acid catalyzed breakdown of acetals and would lead to an overall rate expression for the reaction of carbinolamides shown in Eq. 3.

(b) *Specific-acid followed by general-base* (Scheme 4): Here, the amide portion of the carbinolamide is protonated, followed by rate-limiting general-base catalyzed deprotonation of the hydroxyl group to produce formaldehyde and a tautomeric form of benzamide.[20,37] Such a mechanism yields the same acid dependence as in Scheme 3 (see Eqs. 3 and 4, where K_a^{HA} is the K_a of the buffer catalyst and $k_2[A^-]$ can be dropped from the denominator of Eq. 4 as it is assumed to be slow compared to k_{-1}).



Scheme 4. Specific-acid followed by general-base.

Based on the results presented here, both of the mechanisms discussed above are feasible. The general-acid catalyzed mechanism could be criticized on the basis of the generation of protonated formaldehyde (**3**). Mechanistic studies of the reaction of acetals have shown that general-acid catalysis only occurred in those cases where a stable carbocation was created.[1–3] Under the conditions of the studies described here, **3** would not be stable enough to exist in solution[38–44] and, therefore, the general-acid mechanism suggested in Scheme 3 would not be possible. However, the mobility of the proton on **3** versus the alkylated version in acetal studies could avoid the generation of this unstable intermediate.[45]

$$k_{\text{obsd}} = k_{\text{H}}[\text{H}^+] + k_{\text{HA}}[\text{HA}] + k_{\text{HOH}} + k_{\text{HO}}\frac{K_{\text{a}}[\text{HO}^-]}{K_{\text{w}} + K_{\text{a}}[\text{HO}^-]} \quad (3)$$

$$k_{\text{HA}}[\text{HA}] = \frac{k_1 k_2[\text{H}^+][\text{A}^-]}{k_{-1} + k_2[\text{A}^-]} = \frac{k_1 k_2 K_{\text{a}}^{\text{HA}}[\text{HA}]}{k_{-1} + k_2[\text{A}^-]} = \frac{k_1 k_2 K_{\text{a}}^{\text{HA}}[\text{HA}]}{k_{-1}} \quad (4)$$

Further evidence supporting a mechanism such as that shown in Scheme 4 comes from buffer catalysis studies of the aqueous reaction of hemiacetals formed from formaldehyde and a variety of alcohols (pK_{a}'s ranging from 12.4–16).[8] Cross-interaction coefficients generated by correlating structure/reactivity studies and Brønsted α's (α-values varied between 0.28 and 0.36 for formaldehyde hemiacetals) led to the conclusion that, under acidic conditions, the hemiacetals studied reacted via a buffer-catalyzed mechanism similar to that shown in Scheme 4.[8] The structural relationship of these hemiacetals to **1** and the similarity of the Brønsted α-values to that determined for **1** provide support for the reaction of **1** occurring via mechanism like that shown in Scheme 4. Although no conclusion, with respect to the mechanism of the catalysis by carboxylate buffers, is possible based on the results presented here, cross-interaction coefficient studies should lead to a resolution of this kinetic ambiguity.[7–9]

In conclusion, it has been shown that carboxylate buffers catalyze the aqueous reaction of *N*-(hydroxymethyl)benzamide (**1**). Previous studies,[15–21] focused on the aqueous breakdown of carbinolamides, failed to note any catalytic effect by buffers, which, for **1**, was probably due to the low total [buffer][20] used in those studies and the modest rate enhancements observed for the catalysts in general. It can be argued that the catalytic effect of acetate derivatives are as a result of general-acid catalysis or specific-acid followed by general-base catalysis with further studies necessary to resolve this issue. Also, the correlation of the second-order rate constants in the Brønsted plot suggests that the buffer, hydronium ion, and water-catalyzed reactions occur via a consistent mechanism, which is quite remarkable when the broad range in their pK_{a}'s are considered. The results of these studies have provided the first evidence that the aqueous reaction of carbinolamides can occur via a general acid/base mechanism. This observation could provide insight into the mechanism by which PAM catalyzes the breakdown of the carbinolamide intermediates generated in peptide hormone synthesis.

Acknowledgments

This work was supported by an award from Research Corporation and the donors to the Petroleum Research Fund, administered by the American Chemical Society.

Supplementary data

The plots of the change in the k_{obsd} versus the change in [buffer] for acetic acid, dichloroacetic acid, and pivalic acid. Also plots of the k'_{HA} plotted versus the fraction of buffer in the acidic form are available free of charge via the Internet. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tetlet.2005.03.011.

References and notes

1. Dunn, B.; Bruice, T. C. *Adv. Enzymol.* **1973**, *37*, 1.
2. Cordes, E. H.; Bull, H. G. *Chem. Rev.* **1974**, *74*, 581–603.
3. Fife, T. H. *Acc. Chem. Res.* **1972**, *5*, 264–272.
4. Fife, T. H. *Adv. Phys. Org. Chem.* **1975**, *11*, 108.
5. Capon, B. *Chem. Rev.* **1969**, *69*, 407.
6. Hall, C. D.; Le, V. T. *J. Chem. Soc., Perkin Trans. 2* **1998**, 1483–1488.
7. Engall, K. M.; McClelland, R. A.; Sørensen, P. E. *Can. J. Chem.* **1999**, *77*, 978–989.
8. Funderburk, L. H.; Aldwin, L.; Jencks, W. P. *J. Am. Chem. Soc.* **1978**, *100*, 5444–5459.
9. Sørensen, P. E.; Jencks, W. P. *J. Am. Chem. Soc.* **1987**, *109*, 4675–4690.
10. Fife, T. H.; Jao, L. K. *J. Am. Chem. Soc.* **1968**, *90*, 4081.
11. Anderson, E.; Capon, B. *J. Chem. Soc. B* **1969**, 1033.
12. Anderson, E.; Fife, T. H. *J. Am. Chem. Soc.* **1969**, *91*, 7163–7166.
13. Fife, T. H.; Brod, L. H. *J. Am. Chem. Soc.* **1970**, *92*, 1681.
14. Capon, B.; Nimmo, K. *J. Chem. Soc., Perkin Trans. 2* **1975**, 1113.
15. Bundgaard, H. In *Design of Prodrugs*; Bundgaard, H., Ed.; Elsevier: Amsterdam, 1985; pp 1–92.
16. Bundgaard, H.; Buur, A. *Int. J. Pharm.* **1987**, *37*, 185–194.
17. Bundgaard, H.; Johansen, M. *Int. J. Pharm.* **1980**, *5*, 67–77.
18. Bundgaard, H.; Johansen, M. *Int. J. Pharm.* **1984**, *22*, 45–56.
19. Johansen, M.; Bundgaard, H. *Arch. Pharm. Chem. Sci. Edn.* **1979**, *7*, 175–192.
20. Tenn, W. J.; French, N. L.; Nagorski, R. W. *Org. Lett.* **2001**, *3*, 75–78.
21. Zaugg, H. E.; Martin, W. B. *Org. React.* **1965**, *14*, 52–269.
22. Kulathila, R.; Merkler, K. A.; Merkler, D. J. *Nat. Prod. Rep.* **1999**, *16*, 16.
23. Eipper, B. A.; Perkins, S. N.; Husten, E. J.; Johnson, R. C.; Keutmann, H. T.; Mains, R. E. *J. Biol. Chem.* **1991**, *266*, 7827–7833.
24. Eipper, B. A.; Stoffers, D. A.; Mains, R. E. *Annu. Rev. Neurosci.* **1992**, *15*, 57–85.

25. Eipper, B. A.; Mains, R. E. *Annu. Rev. Physiol.* **1988**, *50*, 333–344.
26. Kolhekar, A. S.; Bell, J.; Shiozaki, E. N.; Jin, L. X.; Keutmann, H. T.; Hand, T. A.; Mains, R. E.; Eipper, B. A *Biochemistry* **2002**, *41*, 12384–12394.
27. Bell, J.; Ash, D. E.; Snyder, L. M.; Kulathila, R.; Blackburn, N. J.; Merkler, D. J. *Biochemistry* **1997**, *36*, 16239–16246.
28. Jencks, W. P. *Catalysis in Chemistry an Enzymology*; Dover Publications: Mineola, NY, 1975.
29. Fersht, A. *Enzyme Structure and Mechanism*, 2nd ed.; W.H. Freeman & Company: New York, 1985.
30. Mounier, C. E.; Shi, J.; Sirimanne, S. R.; Chen, B. H.; Moore, A. B.; Gillwoznichak, M. M.; Ping, D. S.; May, S. W. *J. Biol. Chem.* **1997**, *272*, 5016–5023.
31. Nair, B. R.; Francis, J. D. *J. Chromatogr.* **1980**, *195*, 158–161.
32. Solvent system used to separate **1** from **2** via HPLC was 90% 70/30 water/methanol and 10% methanol. The chromatograms were generated by following the reactions at 254 nm.
33. Acetic acid studies were performed at three different pH's while those performed in the presence of dichloroacetic acid and pivalic acid were investigated at only one pH.
34. Second-order rate for the water reaction was calculated by dividing pseudo-first rate (see Ref. 20) by the concentration of water (1.7×10^{-9} s^{-1}/55.5 M).
35. The half-life of the water-catalyzed reaction is ~13 years.
36. Lowry, T. H.; Richardson, K. S. *Mechanism and Theory in Organic Chemistry*; Harper & Row: New York, 1987.
37. Arguments for protonation on oxygen or nitrogen could be made based on acid-catalyzed amide hydrolysis studies (see Ref. 20).
38. Young, P. R.; Jencks, W. P. *J. Am. Chem. Soc.* **1977**, *99*, 8238–8248.
39. Craze, G.-A.; Kirby, A. J.; Osborne, R. *J. Chem. Soc., Perkin Trans. 2* **1978**, 357–368.
40. Knier, B. L.; Jencks, W. P. *J. Am. Chem. Soc.* **1980**, *102*, 6789–6798.
41. Jencks, W. P. *Acc. Chem. Res.* **1980**, *13*, 161–169.
42. Jencks, W. P. *Chem. Soc. Rev.* **1981**, *10*, 345–375.
43. Amyes, T. L.; Jencks, W. P. *J. Am. Chem. Soc.* **1988**, *110*, 3677–3679.
44. Amyes, T. L.; Jencks, W. P. *J. Am. Chem. Soc.* **1989**, *111*, 7900–7909.
45. (a) The pK_a of protonated formaldehyde should be ~−1.74; (b) *CRC Handbook of Biochemistry and Molecular Biology*; Fasman, G. D., Ed., 3rd ed.; CRC: Cleveland, USA, 1976.