Unified View of Carbon-Bound Hydrogen Exchange of H(2) in Imidazoles and H(8) in Purine Nucleosides and Their Metal Ion Complexes[1]

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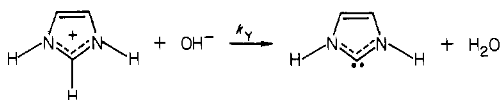
Abstract: The apparent contradiction in the common observation that bound metal ions inhibit H(2) exchange in imidazoles and catalyze H(8) exchange in the imidazole ring of purines is resolved. The specific second-order rate constant for hydroxide ion attack on protonated imidazole rings to yield an ylide is compared with that for attack on metalated rings. For both the bis(3-methylhistidine) complex of Pd(II) and the inosine and guanosine complexes of Pt(II) at N(7), the specific second-order rate constant for carbon-bound hydrogen exchange is 10^5 times faster for protonated than for metalated rings. The factor is more than 10^2 times greater than that expected from the basicity of the adjacent nitrogen. The many times greater basicity of N(1) or N(3) in imidazoles compared to N(7) in nucleic bases results in a greater concentration product of protonated species and hydroxide ion in neutral solutions. Coordination of a metal ion at N(7) of a nucleic base places a positive charge where a proton does not appear until quite low pH and results in catalysis of exchange in neutral solutions. In contrast, the much more basic imidazoles partially protonate even in neutral solutions and the greater polarizing power of the proton over a metal ion reduces the apparent relative role of the metal ion.

Exchange of the H(2) hydrogen of imidazole rings observed in NMR investigations has aided identification of histidyl residues in proteins. The more slowly exchanging H(2) protons are assigned to the less solvent-accessible protein side chains.[3] Ready exchange of the analogous H(8) hydrogen occurs in purines[4-8] and has led to the identification of this hydrogen in NMR spectra of adenosine, guanosine, inosine, and derivatives.

Metal ions bound at N(7) are well-known to promote H(8) exchange in purine derivatives such as inosine.[8-11] In contrast, metal ions inhibit H(2) exchange in $(NH_3)_5Co^{III}$-coordinated imidazole[12] and in the complexed histidyl side chains of superoxide dismutases.[13] The H(8) hydrogen in purines forms part of a fused benzimidazole-type ring system with nitrogens in the 1 and 3 positions of the six-membered ring. The H(8) hydrogen of purines occupies a position in the five-membered ring analogous to the H(2) hydrogen in imidazoles. Why then is the apparent carbon-bound hydrogen exchange promoted in purines but inhibited in imidazoles by bound metal ions? This paper compares the effects of bound Pd(II) or Pt(II) on the exchange rates of H(2) in 3-methylhistidine and H(8) in inosine or inosine monophosphate. We report the first example of H(2) exchange in a complexed imidazole derivative and thus can compare its rate with that of the free ligand.

Several groups of workers have shown that H(2) exchange occurs via OH^- attack on protonated imidazoles to yield an ylide.[14,15]



The observed reaction rate, $k_y[ImH^+][OH^-]$, increases with pH until the pK_a of deprotonation from a nitrogen, above which the rate levels off to a plateau. The observed first-order rate constant is given by $k_{obsd} = k_yK_w/(K_a + [H^+])$, which at pH > p$K_a$ reduces to the constant k_yK_w/K_a. To form a plateau in the pH-rate profile for H^+ exchange, OH^- attacks on imidazole ring bearing one more proton than occurs as the predominant species in the plateau region. Though perhaps self-evident in a simple imidazole derivative, generalization of this statement to cases where several microspecies occur will allow us to write immediately the relevant exchange producing reaction. Analysis of the pH-rate profile yields pK_a values for the predominant species, not necessarily the reactive species.

The greater the acidity of H(2) in imidazoles, the greater should be the ease of its abstraction by OH^- as measured by k_y. The H(2) acidity is so low that it is difficult to determine by potentiometric methods. Acidity constants, K_a, are known, however, for loss of a proton from a neighboring nitrogen in many imidazoles. For imidazole itself, pK_a = 7.1.[14] We anticipate some correspondence between ease of abstraction of H(2) and acidity at a nitrogen in imidazoles. The specific second-order rate constant, k_y, for H(2) exchange in 12 nitro- and fluoroimidazoles has been determined at 50 °C in D_2O, and their pK_a values in H_2O reported at 25 °C range from −0.6 to +7.7.[16] We have constructed a plot of log k_y vs. pK_a for these 12 cases and have found that the points fall near a straight line of slope −0.58 ± 0.05.

For the analogous H(8) exchange in purines, plateaus are also observed. Detritiation experiments have been reported for many purine derivatives at 85 °C and pK_a values at N(7) inferred from the pH-rate profile. The same group has performed studies on imidazoles under a similar conditions. We have plotted the logarithm of the reported specific second-order rate constant vs. pK_a ($1.2 \leq pK_a \leq 5.9$) for 12 compounds: inosine, 1-methylinosine, guanosine, 1-methylguanosine,[17] 9-methylhypoxanthine,[18] benzimidazole,[19] histamine, histidine, glycylhistidine, glycylhistidylglycine, *N*-acetylhistidine, and β-alanylhistidine.[20] The resulting plot of purine and imidazole derivatives follows a single straight line of slope −0.80 ± 0.03. If instead of the 85 °C pK_a values,

(1) This research was supported by grants from the National Science Foundation and from the National Cancer Institute (Grant CA-14832).
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Table I. H(8) Exchange in Inosine at 61 °C

pH^a	$-\log k_{obsd}$, s^{-1}
Free Inosine (Ino)	
5.52	4.62
6.04	4.70
$dienPtIno^{2+}$	
4.97	5.29
5.44	4.83
5.98	4.38
$enPtIno_2^{2+}$	
5.20	5.04
5.61	4.61
6.06	4.27

[a] Measured room-temperature pH in D_2O.

room-temperature values are used, the slope is less negative and for the same 12 compounds becomes −0.59 ± 0.03. This value agrees well with that found for the nitro- and fluoroimidazoles. The nearly identical slopes for purines and imidazoles suggest that to a first approximation, for these free ligands, the specific second-order rate constant for carbon-bound hydrogen exchange may be estimated from the acidity of a proton bound to a neighboring nitrogen. The analysis does not include results for adenosine derivatives.[21,22] We have estimated $pK_a \simeq 0.4$ for N(7) protonation in neutral adenosine.[23] Use of this value places derived second-order rate constants for adenosine and derivatives on the same straight line.

Experimental Section

The 2:1 chelate of 3-methylhistidine (more accurately 1-methylhistidine[24]) and Pd(II) was prepared by mixing L-3-methylhistidine and K_2PdCl_4 in a 2:1 mole ratio. Potentiometric titrations confirmed complete 2:1 complex formation. Stock solutions of $dienPt(H_2O)^{2+}$ at 0.1 M were prepared by suspending [dienPtCl]Cl in D_2O, adding 1.98 equiv of $AgNO_3$, and stirring overnight. The precipitate was filtered and the solution used directly. Stock solutions of 0.05 M $enPt(H_2O)_2^{2+}$ were prepared similarly but a longer reaction time of at least 2 days was allowed. Samples for exchange were prepared by mixing $dienPt^{2+}$ and $enPt^{2+}$ solutions with 1 and 2 equiv of nucleoside, respectively. The solutions were stirred for several hours to complete coordination. Buffers of 0.2 M maleic acid or succinic acid were used. The ionic strength was controlled at 0.8 M with $NaNO_3$.

For all kinetic experiments the concentration of ligand was 0.06–0.12 M. First-order exchange rates were measured by following the disappearance of carbon-bound protons in D_2O on a Varian EM 390 (90 MHz) proton magnetic resonance spectrometer. H(4) in 3-methylhistidine and H(1′) in the nucleosides were used as internal controls. These hydrogens do not exchange under our conditions. Even when the kinetic results refer to elevated temperatures, all pH meter readings were taken at room temperature. Reported pH values are pH meter readings. From extrapolation of handbook values for D_2O to 61 °C, we obtain $pK_w = 13.8$.

Results

Table I gives the observed first-order rate constants at 61 °C for replacement of the H(8) hydrogen by deuterium in inosine and two metal ion complexes. Except for the last entry, the rate constants for each pH represent the average of two parallel experiments. For the free ligand the two pH values of 5.52 and 6.04 occur in a plateau region for H(8) exchange.[18] The average value in Table I is $\log k_{obsd} = -4.66$.

The tridentate diethylenetriamine (dien) complex of strongly tetragonal Pt(II) binds to inosine in a unidentate mode. In an equimolar solution in the pH 5–6 region the predominant binding mode is M_7BH_1, where $dienPt^{2+}$ is at N(7) of inosine and N(1) remains protonated.[23] Similarly, the ethylenediamine (en) complex

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Table II. H(8) Exchange in Guanosine at 61 °C

pH^a	$-\log k_{obsd}$, s^{-1}
$dienPtGuo^{2+}$	
5.74	5.51
5.94	5.28
6.27	4.96
$enPtGuo_2^{2+}$	
5.62	5.60
6.05	5.22
6.11	5.14

[a] Measured room-temperature pH in D_2O.

Table III. H(2) Exchange in 3-Methylhistidine at 61 °C

pH^a	$-\log k_{obsd}$, s^{-1}
Free Ligand	
6.90	4.09
7.08	4.10
7.47	4.07
7.75	4.11
10.8	3.41
PdL_2	
11.3	4.40
11.7	3.95
11.7	3.99
12.0	3.63

[a] Measured room-temperature pH in D_2O.

$enPt^{2+}$ binds two inosine ligands at N(7) with N(1) still protonated. Table I shows that for both the $dienPt^{2+}$ and $enPt^{2+}$ complexes H(8) exchange in inosine is nearly first order in OH^-, and the observed first-order rate constants for the two complexes show no significant difference. For both Pt(II) complexes of inosine from pH 5.0 to pH 6.1, $\log k_{obsd} - pH = -10.27 \pm 0.06$.

The two Pt(II) complexes of guanosine behave similarly to those of inosine, and the results appear in Table II. Each entry is the average of two determinations. Again there is no significant difference between the $dienPtGuo^{2+}$ and $enPtGuo^{2+}$ complexes from pH 5.7 to pH 6.3, where $\log k_{obsd} - pH = -11.24 \pm 0.02$ at 61 °C. Insolubility of guanosine prevents a direct rate comparison with the free ligand by the NMR method. A 25 °C value of $\log k_{obsd} - pH = -8.57$ for detritiation in the $enPtGuo^{2+}$ complex[25] corresponds to about a 10^4 times faster rate than ours when the temperature difference is included. We cannot account for this difference. The proton magnetic resonance method we have used measured directly the loss of H(8) peak intensity upon its replacement by deuterium.

As already portrayed graphically, the pH–rate profile for H(2) exchange on the imidazole ring of histidine shows an increasing rate to about pH 6–8 (depending upon temperature), where the neutral plateau appears, followed by another rate increase to pH 9–11, where the basic plateau begins.[20,26] Since the kinetic analysis is simpler, we sought data in the two plateau regions. The first four pH values in Table III tabulate observed first-order rate constants for H(2) exchange in free ligand 3-methylhistidine in the neutral plateau region. For the first four pH values in Table III the average $\log k_{obsd} = -4.09$ (s^{-1}) at 61 °C.

Between pH 8 and pH 10 the observed first-order rate constants increase slightly with pH, reaching a second (basic) plateau by pH 10.8. At the basic plateau $\log k_{obsd} = -3.41$ (s^{-1}) at 61 °C. This value is 0.68 log units greater than the average in the neutral plateau. Thus the observed first-order rate constant is about 4.8 times greater in the basic plateau.

The 2:1 complex of 3-methylhistidine and Pd^{2+} forms fully in the pH 11.3–12.0 region. Each ligand chelates via the amino

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nitrogen and the (pyridine) imidazole nitrogen in a six-membered ring. The results for the last four pH values in Table III show that the observed first-order rate constants for the complex increase with pH over the pH 11.3–12.0 range. The average value of log k_{obsd} – pH = –15.67 ± 0.03. Thus the exchange rate is proportional to the OH^- concentration. This result assures us that the exchange rate of the complex is being measured because in the same pH region the observed exchange rate of the free ligand remains independent of pH.

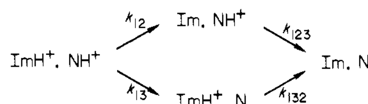
Discussion

H(8) exchange in inosine in the pH 5.5 plateau region is due to OH^- attack on N(7)-protonated species, $^+H_7BH_1$. Since the neutral species, BH_1, predominates in the pH 5.5 region, the observed first-order rate constant is given by $k_{obsd} = k_+K_w/K_1$, where k_+ is the specific second-order rate constant for OH^- attack on $^+H_7BH_1$. Rearrangement yields log k_+ = log k_{obsd} – pK_1 + pK_w = –4.66 – 1.0 + 13.8 = 8.1. The pK_1 value for N(7) protonation is an estimate based on a value of 1.34 for inosine monophosphate at 34 °C in D_2O and allowance for the acidifying influences of loss of the phosphate and increase of temperature.[23]

In both Pt(II) complexes of inosine in the pH 5–6 region H(8) exchange occurs by OH^- attack on the N(7)-coordinated ligand so that $k_{obsd} = k[OH^-] = kK_w/[H^+]$, where k is the specific second-order rate constant. In the Results we have already determined that log k_{obsd} – pH = –10.27. The expression for the specific second-order rate constant yields log k = log k_{obsd} – pH + pK_w = –10.27 + 13.8 = 3.5.

We now have our first direct comparison of the specific second-order rate constants for OH^- attack on N(7)-protonated and N(7)-platinated inosine. Hydroxide ion attack proceeds $10^{4.6}$ times faster on the protonated ligand than on the complex. Thus, in contrast to the observed rate comparison, metalation at N(7) of a purine base promotes deprotonation at C(8) far less effectively than does a proton at N(7).

H(2) exchange in free ligand 3-methylhistidine exhibits both neutral and basic plateau regions for the observed first-order rate constant. We can account for both plateaus and the 0.68 log unit greater observed first-order rate constant in the basic plateau by considering the microspecies present in solution. At all pH > 6 values considered here, the carboxylate group is completely ionized. Designating the imidazole group as Im and ImH^+ in its deprotonated and protonated forms and the amino group similarly as N and NH^+, we may write for the two pathways of deprotonation



The four acidity microconstants (on the arrows) are defined in the usual way. Due to the properties of a cyclic system, $k_{12}k_{123} = k_{13}k_{132} = K_2K_3$, where K_2 and K_3 are the second and third macroacidity constants determined from titration. We also have

$$K_2 = k_{12} + k_{13}$$

and

$$K_3^{-1} = k_{123}^{-1} + k_{132}^{-1}$$

For H(2) exchange to occur via an ylide intermediate, the imidazole ring must be positively charged. Therefore, for hydroxide ion attack at H(2) in 3-methylhistidine

$$\text{rate} = k_+[ImH^+, NH^+][OH^-] + k_0[ImH^+, N][OH^-] \quad (1)$$

where k_+ and k_0 are microscopic specific second-order rate constants. The total ligand concentration equals the sum of the concentrations of all four microspecies. By division of the rate by this total ligand concentration and from the definitions of acidity constants, it may be shown that the observed first-order rate constant is given by

$$k_{obsd} = \frac{k_+K_w + k_0k_{13}K_w/[H^+]}{[H^+] + K_2 + K_2K_3/[H^+]}$$

In the region of the neutral plateau the first term in the numerator and middle terms in the denominator dominate so

$$k'_{obsd} = k_+K_w/K_2 \quad (2)$$

For the basic plateau region the last terms in the fraction prevail and

$$k''_{obsd} = k_0k_{13}K_w/K_2K_3 = k_0K_w/k_{132}$$

The average k_{obsd} between the two plateaus occurs at pH ≃ pK_3. The equations for the observed first-order rate constants in the two plateau regions are of the same form. That for the neutral plateau involves the specific second-order rate constant for OH^- attack on ImH^+, NH^+ and the microacidity constant for imidazolium deprotonation from this species, k_{12}. It has been estimated that in histidine the ratio $[Im, NH^+]/[ImH^+, N]$ = 50.[14] Therefore, we have $k_{12} \simeq K_2$ (and also $k_{123} \simeq K_3$). The equation for the observed first-order constant in the basic plateau involves the specific second-order rate constant for OH^- attack on ImH^+, N and the microacidity constant for imidazolium deprotonation from this species of one less positive charge, k_{132}. Not directly measurable, this constant needs to be estimated.

Alternatively, the rate in the region of the basic plateau might have been formulated in terms of the predominant neutral species present as

$$\text{rate} = k_N[Im, NH^+][OH^-]$$

from which

$$k''_{obsd} = k_NK_w/k_{123} \simeq k_NK_w/K_3$$

The macroscopic acidity constant $K_3 \simeq k_{123}$, the microscopic constant determined from titration of the ammonium group. The advantage of this formulation is that it is in terms of the predominant species present in solution; the disadvantage is that k_N refers to OH^- attack on Im, NH^+, which is not the most reactive species. More accurately, we write $k''_{obsd} = k_0K_w/k_{132} + k_NK_w/K_3$, the sum of the two previous equations. For practical purposes the minor ImH^+, N species reacts so much more readily than the major Im, NH^+, species of the same net charge, which does not yield an ylide intermediate, that only the k_0K_w/k_{132} term contributes to k''_{obsd}.

For 3-methylhistidine the ratio of observed first-order rate constants in the neutral and basic plateaus is given by

$$1/4.8 = k'_{obsd}/k''_{obsd} = k_+k_{132}/k_0K_2 \quad (3)$$

Separation of the pH–rate profile into two plateaus depends upon this ratio being different from unity. We can show that the slope of a plot of the log of the specific second-order constant vs. pK_a for deprotonation from the imidazolium group is given by

$$\frac{\log (k_+/k_0)}{pk_{12} - pk_{132}} = \frac{\log (k'_{obsd}/k''_{obsd})}{pk_{12} - pk_{132}} - 1$$

For histidine the difference in the two imidazolium pk values is –1.3.[14] Application of this value to 3-methylhistidine yields a slope of –0.48. This value nears that found for the 12 substituted imidazoles in the introduction and provides strong support for the mechanism proposed in eq 1 for H(2) exchange in histidines.

Since both K_2 and k_{132} refer to imidazolium deprotonations, their ratio remains relatively temperature independent, and the difference pk_{132} – pk_{12} = 1.3 is the same as in histidine at room temperature.[14] We then obtain from eq 3 k_+/k_0 = 4.2. Thus hydroxide ion attacks H(2) in ImH^+, NH^+ about 4 times faster than in the species ImH^+, N of 1.3 log units greater pK_a for imidazolium deprotonation.

The specific second-order rate constant, k_+, for OH^- attack on diprotonated 3-methylhistidine may be evaluated by rearranging eq 2 to give log k_+ = log k'_{obsd} – pK_2 + pK_w = –4.1 – 6.2 + 13.8 = 3.5 (s^{-1} M^{-1}). The log k'_{obsd} value comes from Table III and the pK_2 value from our determination of pK_2 = 5.72 at 61 °C in H_2O to which a factor is added for the D_2O solvent.[27] From the

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previous paragraph we obtain for the specific second-order rate constant for OH^- attack on 3-methylhistidine protonated only on the imidazole ring log k_0 = 2.9 (s^{-1} M^{-1}).

For the Pd(II) complex of 3-methylhistidine the observed first-order rate constant for H(2) exchange is given by

$$k_{obsd} = k_c[OH^-] = k_cK_w/[H^+]$$

From the average log k_{obsd} − pH = −15.67 in Table III at 61 °C we calculate for the specific second-order rate constant for OH^- attack on complexed 3-methylhistidine log k_c = −1.9 (s^{-1} M^{-1}).

The specific second-order rate constants for OH^- attack on severals forms of 3-methylhistidine may now be compared. Slowest is the chelated ligand in PdL_2, with log k_c = −1.9 (s^{-1} M^{-1}). The two rate constants for the protonated imidazole ligand are log k_0 = 2.9 for the minor tautomer with only the imidazole ring protonated and log k_+ = 3.5 for the major diprotonated species in weakly acidic solutions. Thus the rate constants for OH^- attack on the protonated imidazole ligand are $10^{4.8}$ and $10^{5.4}$ times greater than that for the Pd(II) complex. These ratios are similar to that of $10^{4.6}$ found for N(7)-protonated inosine and its complex with Pt(II) at N(7). Thus both imidazole and purine derivatives behave similarly.

The magnitude of $\sim 10^5$ found for the ratio of the specific second-order rate constants for OH^- attack on protonated and metalated ligand exceeds that inferred from consideration of pK_a values at the imidazole nitrogen. Because of the methyl group in 3-methylhistidine, we cannot measure the acidity of a proton at that position and turn our attention to histidine. In enPd-(histidine) the imidazole nitrogen deprotonation across the ring from the chelated Pd(II) occurs with pK_a = 10.8.[28] When a proton replaces the Pd(II), the imidazole deprotonation in histidine occurs with pK_a = 6.1.[14] Applying the slope of −0.58 from the introduction to this difference of 4.7 log units in pK_a, we anticipate a specific second-order rate constant about $10^{2.7}$ times greater in the ligand than in the complex. That the calculated value is more than 10^2 times greater indicates that the Pd(II) complex falls more than 2 log units below the expected log k in a plot of log (second-order rate constant) vs. pK_a. That H(2) exchange was not observed in an imidazole complex of Co(III)[12] suggests that metal ions generally inhibit exchange more than anticipated on the basis of the pK_a of nitrogen-bound hydrogen in the complexed imidazole ring.

In both cases examined, inosine and 3-methylhistidine, the specific second-order rate constant for exchange occurs about 10^5 times faster in the protonated ligand than in the metal ion complexed ligand. (It is still less in the neutral ligand, which cannot form an ylide.) How then is the observation referred to in the introduction that metals promote H(8) exchange in nucleic bases and inhibit H(2) exchange in imidazoles consistent with the above conclusion? The resolution lies in the substantially different pK_a values at N(1) or N(3) in imidazoles and N(7) in nucleic bases. In our examples with $pK_a \simeq 6$ at N(1) in 3-methylhistidine, there is an appreciable amount of protonated species even in basic solutions so that the concentration product of N(1)-protonated species and $[OH^-]$ is relatively high. In contrast, for inosine with pK_a = 1.0 for N(7) protonation, the concentration product of N(7)-protonated species and $[OH^-]$ is much lower. Complexation of metal ions to inosine or another purine base at N(7) places a positive charge where a proton does not occur until quite low pH. In these instances the metal ion functions as a superacid catalyst in neutral solutions. In contrast, the much more basic imidazoles partially protonate even in neutral solutions, and the much greater polarizing power of the proton over a metal ion[29] reduces the apparent relative role of the metal ion.

Registry No. Ino, 58-63-9; $dienPtIno^{2+}$, 69215-32-3; $enPtIno_2^{2+}$, 80263-30-5; $dienPtGuo^{2+}$, 69667-81-8; $enPtGuo_2^{2+}$, 72950-44-8; 3-methylhistidine, 332-80-9; $Pd(3\text{-methylhistidine})_2$, 80263-31-6.

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2-Amino-8-methyladenosine 5′-Monophosphate Dihydrate. A Nucleotide with Syn C4′-Exo Conformation and "Triple-Stranded" Packing

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Abstract: The preparation and crystal structure of 2-amino-8-methyladenosine 5′-monophosphate dihydrate, $C_{11}H_{14}N_6O_7P\cdot 2H_2O$, are described. The space group is $R3$, with a = 20.192 (1) Å and c = 11.0849 (7) Å. The final R factor was 2.3%, using counter data corrected for absorption and extinction, and the resulting esd's are the smallest yet reported for an X-ray structure of a nucleotide. The nucleotide molecules adopt the unusual syn C4′-exo conformation, and the extensively hydrogen-bonded components of the crystals are organized into crystallographic helices which, in turn, form triangular groupings reminiscent of the early Pauling and Corey model for the nucleic acids. The extensive involvement of water bridges in the packing is of interest. The observation of the syn C4′-exo conformation is relevant to modeling studies of polynucleotides and may indicate that the allowable ranges of sugar conformation are broader than previously suspected.

Mutual rotation of the base and sugar of nucleotides about the glycosidic bond has received extensive investigation in recent years.[1-14] X-ray crystallographic studies have shown a high preponderance of the anti conformation,[3] although, especially among purine nucleosides, a number of syn structures have been

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