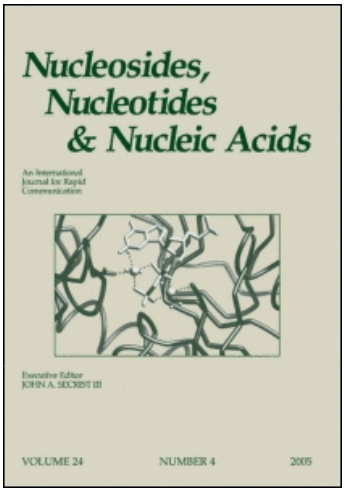


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Determination of the Tautomeric Populations of the Y-Base

C. Glemarec[a]; G. Remaud[a]; J. Chattopadhyaya[a]
[a] Department of Bioorganic Chemistry, Box 581, Biomedical Center, University of Uppsala, Uppsala, Sweden

To cite this Article Glemarec, C. , Remaud, G. and Chattopadhyaya, J.(1989) 'Determination of the Tautomeric Populations of the Y-Base', Nucleosides, Nucleotides and Nucleic Acids, 8: 8, 1513 — 1517
To link to this Article: DOI: 10.1080/07328318908048858
URL: http://dx.doi.org/10.1080/07328318908048858

NUCLEOSIDES & NUCLEOTIDES, 8(8), 1513-1517 (1989)

DETERMINATION OF THE TAUTOMERIC POPULATIONS OF THE Y-BASE

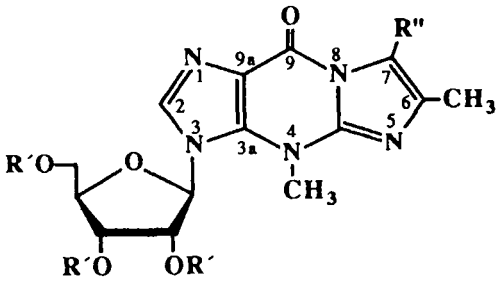
C. Glemarec, G. Remaud & J. Chattopadhyaya*

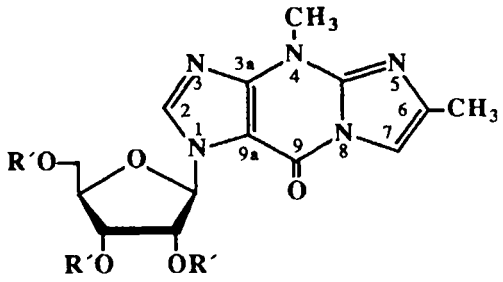
Department of Bioorganic Chemistry, Box 581, Biomedical Center, University of Uppsala, S-751 23 Uppsala, Sweden

Abstract: *Determination of tautomeric population of the Y-base in 3 by ^{13}C-NMR spectroscopy has shown that its most thermodynamically preferred tautomeric form is the N^1-H tautomer (~ 95 %) which explains its chemical reactivity in glycosylation reaction and also in the facile, lewis acid promoted isomerization of wyosine-2',3',5'-O-triacetate 5 to its N^1- isomer 4. It is likely that the consequence of occurrence of natural wyosine in the thermodynamically unfavoured N^3-form is its unusual lability across the glycosyl bond under mild acidic conditions.*

Y-nucleoside (wyosine) **1** and its 7-substituted congeners **2a - c** occur naturally, adjacent to the 3'-anticodon loop of yeast phenylalanine tRNA [$tRNA^{Phe}$][1,2]. Their glycosyl bonds are extremely labile[3] and the loss of Y-base **3** from $tRNA^{Phe}$ by mild acidic treatment causes it to loose its codon recognition property[3]. Following two chemical observations have prompted us to study the tautomerism of Y-base **3**: (1) the glycosylation of the Y-base by 1',2',3',5'-tetra-O-acetyl-β-D-pentofuranose under acidic conditions gave exclusively the N^1-isomer of wyosine **4**; no trace of isomeric Y-nucleoside **5** was detectable[4,5]; and (2) a treatment of wyosine-triacetate **5**[12] with anhydrous $AlCl_3$ in dry CH_2Cl_2 gave exclusively the N^1-isomer **4**, while the N^1-isomer **4** was found to be completely stable under the latter condition[6]. We reasoned that these specific chemical behaviours might be possibly understood in terms of the thermodynamic stabilities of the preferred tautomer of the Y-base[13] in **3**. Our attempts to study this prototropic tautomerism of the imidazole moiety of Y-base in **3** by ^{15}N-NMR spectroscopy have been unsuccessful primarily because of the fact that the expected NMR time-weighted average absorptions of N^1 and/or N^3 could not be observed due to N^1-H $\rightleftarrows$ N^3-H prototropism of Y-base in **3**. This prompted us to examine this problem by measurement of ^{13}C chemical shifts of C3a and C9a, and the vicinal $^3J_{C9a,H2}$ and $^3J_{C3a,H2}$ coupling

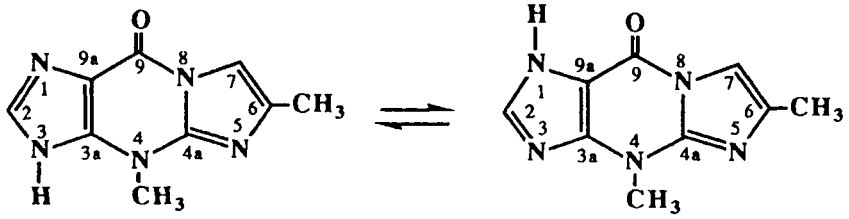
constants of Y-base in solution and determine the population of predominant tautomeric forms in **3**. At ambient temperature, the rate of tautomeric proton exchange in **3** is faster than the NMR time scale and, therefore, the weighted average of the contributing tautomeric structures in **3** leads to single chemical shift for each carbon. The calculation of tautomeric populations, hence, requires[7,9,10] the determination of the ^{13}C chemical shifts C3a and C9a, and the vicinal $^{3}J_{C9a,H2}$ & $^{3}J_{C3a,H2}$ coupling constants of each tautomeric form blocked specifically in the N^1- and N^3- such as pairs **1** & **6**, and their triacetates **4** & **5**[12,14].





4 : R′ = Acetyl
6 : R′ = H

1 : R′ = H, R" = H
2a : R′= H, R" = $CH_2CH_2CH(NHCO_2Me)CO_2Me$
2b : R′= H, R" = $CH_2CH(O_2H)CH(NHCO_2Me)CO_2Me$
2c : R′= H, R" = $CH_2CH(OH)CH(NHCO_2Me)CO_2Me$
5 : R′ = Acetyl, R" = H



3

In the first method, the tautomeric population is determined by the difference of the ^{13}C chemical shift of the C3a and C9a between compounds **1** & **6**, and **4** & **5**[12,14]. In the second method, the decrease of the vicinal three bond $^{3}J_{C9a,H2}$ and $^{3}J_{C3a,H2}$ coupling constants through a pyridine- *versus* a pyrrole-type nitrogen atom is used[12,14]. The following equations were used to calculate the % N^1-H tautomer from either C3a or C9a chemical shifts[7]:

$$[\%\ N^1\text{-}H]_{C3a} = \frac{(\delta C3a)_{Y\text{-}base} - [(\delta C3a)_{N3} - \alpha]}{(\delta C3a)_{N1} - \beta] - [(\delta C3a)_{N3} - \alpha]} \quad \ldots\ldots \quad \text{eqn. 1}$$

Table 1: Calculation of % of N^1-H tautomer using the ^{13}C chemical shifts of C3a & C9a and [$^3J_{C3a,H2}$] and $^3J_{C9a,H2}$] in compounds **1** & **3-6**.

Compound	δ(C3a)* [eqn. 1]	$\%N^1$-H**	[$^3J_{C3a,H2}$]# [eqn. 3]	$\%N^1$-H§	δ(C9a) [eqn. 2]	$\%N^1$-H	[$^3J_{C9a,H2}$]# [eqn. 4]	$\%N^1$-H§
Y -base **3**	141.8		7.2		105.2		4.3	
N^3-isomer **1**	140.0	100%	4.2	97%	115.8	99%	12.1	98%
N^1-isomer **6**	142.6		7.7		104.8		4.1	
N^1-isomer **4**	142.6	100%	+	+	104.3	94%	+	+
N^3-isomer **5**	140.1		+	+	115.7		+	+

\+ Coupling constants could not be obtained with sufficient accuracy.
* 0.1 Hz resolution.
** Error limit of ~10% is expected due to approximation made in the corrective factor (see refs. 7, 8 & 9).
\# 0.05 Hz resolution.
§ error limit of ~4% is expected (see ref. 9).

$$[\%\ N^1\text{-}H]_{C9a} = \frac{(\delta C9a)_{Y\text{-}base} - [(\delta C9a)_{N3} - \beta]}{(\delta C9a)_{N1} - \alpha] - [(\delta C3a)_{N3} - \beta]} \quad \ldots\ \text{eqn. 2}$$

The correction of the chemical shifts of the bridgehead carbons, C3a & C9a, by factors α & β (-0.3 and -0.8 ppm, respectively) due to the replacement of the proton in the Y-base **3** by the β-D-ribofuranosyl groups in **1** & **6**, and **4** & **5** have been defined[8]. The calculation of the % N^1-H tautomer has been achieved using the three bond vicinal $[^3J_{C3a,H2}]$ & $[^3J_{C9a,H2}]$ of the Y-base and **1** & **6**, and **4** & **5** [eqns. 3 &4][9,10]:

$$[^3J_{C3a,H2}]_{Y\text{-}base} = \chi\,[(J_{C3a,H2})_{N1} - \Delta] + (1-\chi)[(J_{C3a,H2})_{N3}] \quad \ldots\ \text{eqn. 3}$$

$$[^3J_{C9a,H2}]_{Y\text{-}base} = (J_{C9a,H2})_{N1} + (1-\chi)[(J_{C9a,H2})_{N3} - \Delta] \quad \ldots\ \text{eqn. 4}$$

where χ denotes population of the N^1–H tautomer and Δ is the corrective term (0.4 Hz) representing the α effect of the bridgehead carbon. The results of these studies are presented in table 1[7,9,10].

This ^{13}C-NMR study is also supported by our recent fluorescence measurements on wyosine and its derivatives which show that the spectral emission of the Y-base and the N^1-blocked derivative **4** have predominant amplitude of fluorescence decay times of 7.01 ns (100%) & 10.12 ns (96%), respectively, while the wyosine-triacetate **5** show the predominant amplitude of fluorescence decay time of 0.53 ns (95%) which clearly show the *electronic similarity* of the Y-base with that of the N^1-blocked derivative **4**[11].

These studies together confirm that the Y-base in **3** mainly exists (~ 95 %) in the thermodynamically preferred N^1-H tautomeric form. The consequences of this preferred form of Y-base are its regioselective glycosylation at N^1, facile isomerization of **5** → **4** and the unusual instability of the glycosyl bond of **5** under mild acidic conditions.

Acknowledgements

Authors thank Swedish Board for Technical Development (STU), Swedish Natural Science Research Council (NFR) for generous financial support and Wallenbergstiftelsen for funds for the purchase of 270 and 500 MHz NMR spectrometers

References & Notes

1. (a) U. L. Rajbhandary, S. H. Chang, A. Stuart, R. D. Faulkner, R. M. Hoskinson and H. G. Khorana, *Proc. Natl. Acad. Sci. U.S.A.*, **57**, 751 (1967); (b) U. L. Rajbhandary, R. D. Fulkner and A. Stuart, *J. Biol. Chem.*, **243**, 575 (1968); (c) K. Nakanishi, N. Furutachi, M. Funamizu, D. Grunberger and I. B. Weinstein, *J. Am. Chem. Soc.*, **92**, 7617 (1970).

2. (a) Special issue on tRNA, *Acc. Chem. Res.*, **10** (11), 385 (1977); (b) S. M. Hecht, *Tetrahedron*, **33**, 1671 (1977).

3. (a) R. Thiebe and H. G. Zachau, *Eur. J. Biochem.*, **5**, 546 (1968); (b) H. Kasai, M. Goto, S. Takamura, T. Goto and S. Matsura, *Tetrahedron Lett.*, 2725 (1971); (c) K. Nakanishi, S. Blobstein, M. Funamizu, G. van Lear, D. Grunberger, K. Lanks and I. B. Weinstein, *Nature New Biol.*, **234**, 107 (1971); (d) C. B. Reese and N. Whittall, *Nucl. Acid Res.*, **3** (12), 3439 (1976).

4. S.H. Blobstein, R. Gebert, D. Grunberger, K. Nakanishi and B. Weinstein *Arch. of Biochem. & Biophys.*, **167**, 668-673 (1975).

5. H. Kasai, M. Goto, K. Ikeda, M. Zama, Y. Mizuno, S. Takemura, S. Matsuura, T. Sugimoto and T. Goto, *Biochemistry*, **15**, 898-904 (1976).

6. C. Glemarec, J-C Wu, G. Remaud, H. Bazin, M. Oivanen, H. Lönnberg and J. Chattopadhyaya, *Tetrahedron*, **44**, 1273-1290 (1988).

7. M.T. Chenon, R.J. Pugmire, D.M. Grant, R.P. Panzica and L.B. Townsend, *J. Am. Chem. Soc.*, **97**, 4636 (1975).

8. M.T. Chenon, R.J. Pugmire, D.M. Grant, R.P. Panzica and L.B. Townsend, *J. Am. Chem. Soc.*, **97**, 4627 (1975).

9. J. Riand, M.T. Chenon, C-C. Tzeng, and R.P. Panzica, *J. Chem. Soc. Perkin. Trans. II*, 931 (1986).

10. M.Schumacher and H. Gunther, *J. Am. Chem. Soc.*, **104**, 4167-4173 (1982).

11. T.M. Nordlund, R. Rigler, C. Glemarec, J-C. Wu, H. Bazin, G. Remaud and J. Chattopdhyaya, *Nucleosides & Nucleotides*, **7** (5,6), 805-811 (1988).

12. H. Bazin, X-X. Zhou, C. Glemarec and J. Chattopadhyaya, *Tetrahedron Lett.*, **28**, 3275 (1987).

13. Compound **3** was obtained by depurination of wyosine **1** in 80% acetic acid at room temperature.

14. ^{13}C-NMR spectra were recorded in dimethylsulfoxide-d_6 solution (0.06 M) at ~30 ^{0}C on a Jeol GX 270 spectrometer at 67.8 MHz. The ^{13}C chemical shifts were measured using an inverse gate proton noise decoupled mode. The [$^3J_{C3a,H2}$], [$^3J_{C9a,H2}$] coupling constants were determined using a ^{1}H selective decoupling method.

Received February 24, 1989.