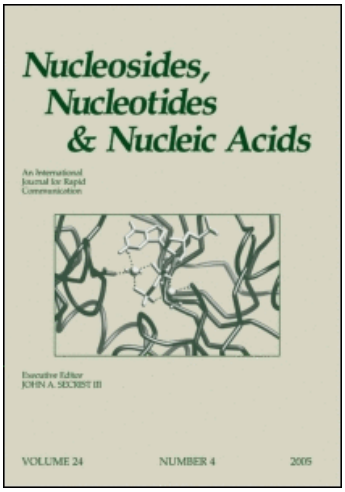

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Sonawane, Kailas D. and Tewari, Ravindra(2008) 'Conformational Preferences of Hypermodified Nucleoside Lysidine (k^2C) Occurring at "Wobble" Position in Anticodon Loop of $tRNA^{Ile'}$, Nucleosides, Nucleotides and Nucleic Acids, 27: 10, 1158 — 1174
To link to this Article: DOI: 10.1080/15257770802341475
URL: http://dx.doi.org/10.1080/15257770802341475

Nucleosides, Nucleotides and Nucleic Acids, 27:1158–1174, 2008

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770802341475

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CONFORMATIONAL PREFERENCES OF HYPERMODIFIED NUCLEOSIDE LYSIDINE (k^2C) OCCURRING AT "WOBBLE" POSITION IN ANTICODON LOOP OF $tRNA^{Ile}$

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□ *Conformational preferences of hypermodified nucleoside, 4-amino-2-(N^6-lysino)-1-(β-D-ribofuranosyl) pyrimidinium (Lysidine or 2-lysyl cytidine), usually designated as k^2C, have been investigated theoretically by the quantum chemical perturbative configuration interaction with localized orbitals (PCILO) method. The zwitterionic, non-zwitterionic, neutral, and tautomeric forms have been studied. Automated geometry optimization using molecular mechanics force field (MMFF), semi-empirical quantum chemical PM3, and ab initio molecular orbital Hartree-Fock SCF quantum mechanical calculations have also been made to compare the salient features. The predicted most stable conformations of zwitterionic, non-zwitterionic, neutral, and tautomeric form are such that in each of these molecules the orientation of lysidine moiety (R) is* trans *to the N(1) of cytidine. The preferred base orientation is* anti *($\chi = 3^\circ$) and the lysine substituent folds back toward the ribose ring. This results in hydrogen bonding between the carboxyl oxygen O(12a) of lysine moiety and the 2′-hydroxyl group of ribose sugar. In all these four forms of lysidine O(12a)...H-C(9) and O(12b)...H-N(11) interactions provide stability to respective stable conformers. Watson-Crick base pairing of lysidine with A is feasible only with the tautomeric form of usual* anti *oriented lysidine. This can help in recognition of AUA codon besides in avoiding misrecognition of AUG.*

Keywords Hypermodified; lysidine; k^2C; stable conformers

INTRODUCTION

Hypermodified nucleoside lysidine 4-amino-2-(N^6-lysino)-1-(β-D-ribofuranosyl) pyrimidinium, designated as k^2C, naturally occurs in the first "wobble" position of anticodon loop of *Mycoplasma capricolum* $tRNA^{Ile}$[1] and *E. coli* $tRNA^{Ile}_2$,[2,3] *Bacillus subtilus*,[4] and potato mitochondrial $tRNA^{Ile}$.[5] This nucleoside is derived in cells through post transcriptional

Received June 22,2007; accepted June 26,2008.
Senior Research Fellowship to K. D. Sonawane from CSIR, India.
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enzymatic modification from cytidine by substitution of the oxygen atom in position 2 with ε-nitrogen atom of L-lysine. Modification of cytidine to lysidine involves condensation of lysine to the pyrimidine ring of cytidine. In *E. coli* two isoleucine tRNA species are observed, $tRNA^{Ile}_{major}$ (or $tRNA^{Ile}_{1}$), which can recognize the AUU and AUC codons,[6] while $tRNA^{Ile}_{minor}$ (or $tRNA^{Ile}_{2}$) recognizes the codon AUA only.[2] The lysidine modification prevents the misrecognition of AUG as isoleucine and that of AUA as methionine. The modification from C to k^2C also changes the aminoacylation identity of the tRNA from methionine to isoleucine.[7] Thus, the codon and amino acid specificity of tRNA can be converted by a single post-transcriptional modification of the first anticodon position from cytidine to lysidine. This may also be considered an alternative way of RNA editing in bacterial $tRNA^{Ile}$.[8] The lysidine modification in the first position of anticodon loop of isoleucine tRNA can be recognized by isoleucyl-tRNA synthetase from *E. coli*.[9] Extensively modified nucleosides also occur naturally at anticodon 3′-adjacent position in several tRNAs[10–12] and help define the reading frame for codon-anticodon interactions. Anticodon 3′-adjacent modifications may also optimize the strength of codon-anticodon interactions,[13,14] thus enabling smooth in-phase protein biosynthesis, although wide variations in codon-anticodon base pair sequences occur. The conformational preferences of hydrophilic substituents i^6A, ms^2i^6A, *cis*-, or *trans* isomers of zeatin (io^6A) and its 2-methylthio derivatives[15,16] and effects of protonation induced conformational transitions[17–20] have also been studied. AMBER force field parameters for the naturally occurring modified nucleosides in RNA have recently been reported.[21]

The chemical structure of novel modified nucleoside lysidine was determined by NMR spectroscopy, mass spectroscopy and by chemical analysis.[22] The extensive mutant analyses based on the atomic crystal structure provide the structural basis for the mechanism of lysidine formation by tRNA (Ile)-lysidine-synthetase (TilS).[23] The present study on conformational preferences of zwitterionic, non-zwitterionic, neutral, and tautomeric forms of lysidine has been undertaken to understand the structural and functional significance of hypermodified nucleoside lysidine.

In order to understand the specific recognition of the codon AUA by $tRNA^{Ile}_{minor}$ two base pairing schemes between lysidine, k^2C and A may be considered.[9,22] In case the lysine substituent R in N(2)HR of lysidine orients *cis* to N(1) in the pyrimidine, then N(2)H may pair as hydrogen bond donor to N(1) of Adenine. Also the N(3) in lysidine may accept a hydrogen bond from N(6)H of Adenine. In this way A may be recognized by lysidine if lysine orientation is *cis* to N(1). In case lysine substituent orients *trans* to N(1) then only tautomeric form of lysidine (having N(3)H and N(4)H) can provide [22] the matching hydrogen bond donor–acceptor

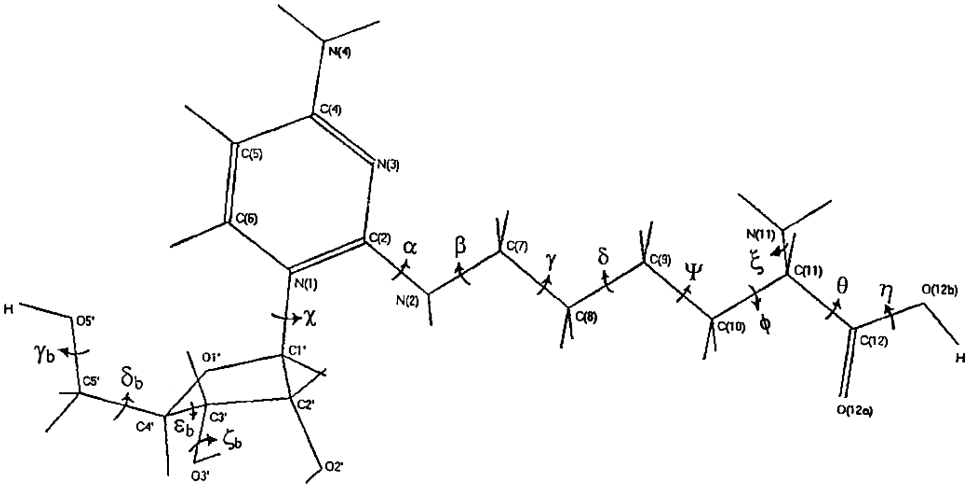

FIGURE 1 Atom numbering and various torsion angles in lysidine (non-zwitterionic form).

pattern to base pair with N(1) and HN(6)H respectively of adenine and recognize A. Thus, it is of interest to study whether the lysine moiety preferably orients *trans* or *cis*.

NOMENCLATURE, CONVENTION, AND PROCEDURE

The atom numbering and identification of the torsion angles (which specify the internal rotation around the various acyclic chemical bonds) are depicted in (Figure 1). The torsion angle α [N(1)C(2)N(2)C(7)] denotes the rotation of C(7) around bond C(2)-N(2) and is measured in the right hand sense of rotation, with reference to the eclipsed orientation of N(1)C(2) and N(2)C(7) bonds. Likewise, the following chemical bonds sequences along the main extension of the substituent define the subsequent torsion angles β [C(2)N(2)C(7)C(8)], γ [N(2)C(7)C(8)C(9)], δ [C(7)C(8)C(9)C(10)], Ψ [C(8)C(9)C(10)C(11)], ϕ [C(9)C(10)C(11)C(12)], ξ [C(10)C(11)N(11)H], θ [C(10)C(11)C(12)O(12b)], and η [C(11)C(12)O(12b)H].

The ribose-backbone torsion angles take values as specified in the Holbrook $tRNA^{Phe}$ model.[24] The torsion angles in the ribose-phosphate backbone are distinguished by the subscript b to refer to the backbone. These backbone torsion angles retain the nomenclature and the values for the 34th nucleoside as in the tRNA model[24] referring likewise to the right hand sense of rotation around the central bond, measured from the eclipsed position of the outer bonds γ_b[H-O5′-C5′-C4′], δ_b[O5′-C5′-C4′-C3′], ε_b[C5′-C4′-C3′-O3′], and ζ_b[C4′-C3′-O3′-H]. The glycosyl torsion angle χ[O1′-C1′-N1-C6] is held anti $\chi = 3^\circ$ and the ribose ring puckering is C_3′-endo, similar to the wobble nucleoside at 34th position in tRNA model.

The quantum chemical perturbative configuration interaction with localized orbitals PCILO method[25–27] has been utilized for the energy calculations of the various molecular conformations. The appropriately selected bond lengths and bond angle values for various forms of lysidine molecule have been used in the conformational energy calculations. In the case of hydrogen atoms, the standard values from the reference source data,[28] suited for the particular bonding environment have been used. The automated geometry optimization calculations using MMFF[29] method has been used to compare the salient features. Likewise full geometry optimization calculations are carried out to compare the salient features using ab initio (molecular orbital Hartree-fock SCF) quantum mechanical energy calculations using 3–21G* basis set (PC Spartan Pro[30] version 6.0.6).

RESULTS AND DISCUSSION

Zwitterionic Form of Lysidine

In conventional depiction of the zwitterionic form of lysidine a positive charge is present on the N(1) of cytidine as well as at N(11) of lysine moiety and the carboxyl group has a negative charge. The PCILO preferred most stable base substituent orientation for zwitterionic form of hypermodified nucleoside lysidine is shown in Figure 2. The torsion angles describing the base substituent orientation are ($\alpha = 180^\circ$, $\beta = 180^\circ$, $\gamma = 30^\circ$, $\delta = 60^\circ$, $\Psi = 180^\circ$, $\phi = 60^\circ$, $\xi = 330^\circ$, and $\theta = 150^\circ$). The preferred orientation of lysine moiety is such that the amino acid carboxyl end folds back towards the ribose ring. The carboxyl oxygen O(12a) of lysine moiety forms hydrogen bond with the 2′-hydroxyl group of ribose sugar ring. The carboxyl oxygen O(12a) also interacts with N(2) and C(9) of lysine moiety. The preferred structure is stabilized by intramolecular interactions between O(12a)...HO2′, O(12a)...HN(2), O(12a)...HC(9), and O(12b)...HN(11) as indicated in (Table 1). Allowing the glycosyl angle χ to change freely validates the initial choice of the glycosyl torsion angle for lysidine-based on the Holbrook model $\chi = 3^\circ$, as energetically preferred stable conformation as well. The orientation of lysine moiety is *trans* to the N(1) of cytidine. The modified nucleoside lysidine does not allow Watson-Crick base pairing with G, due to the presence of bulky R group in place of hydrogen bond acceptor O(2) of cytidine. Besides this steric factor, in lysidine, the presence of hydrogen bond donor N(2)HR group in place of the usual hydrogen bond acceptor O(2) of cytidine marks a qualitative change causing mismatch in base pairing with G.

Higher energy (3.0 kcal/mol) alternative conformation (Figure 3), is arrived by flipping to $\theta = 330^\circ$ as shown in Table 2. This structure is stabilized by intramolecular interactions between O(12b)...HO2′, O(12b)...HN(2),

TABLE 1 Hydrogen bonding—Geometrical parameters, for the preferred conformations in various forms of lysidine

Atoms involved (1-2-3)	Distance atom pair1–2 (A°)	Distance atom pair 2–3 (A°)	Angle 1-2-3 (deg.)	Figure reference
O(12a)...H-O2′	1.67	0.96	173.4	2, 4, 5
O(12a)...H-N(2)	2.59	1.09	157.8	2, 4, 5
N(2)...H-C(9)	2.43	1.09	94.4	2, 4, 5, 7
N(2)...H-C(10)	2.66	1.09	110.4	2, 4, 5
O(12a)...H-C(9)	2.02	1.09	131.8	2, 4, 5
O(12b)...H-N(11)	1.83	1.01	118.5	2
O(12b)...H-N(11)	1.95	1.01	109.3	4, 5
O(12a)...H-N(2)	2.62	1.09	158.6	7
O(12a)...H-O2′	1.58	0.96	149.2	7
O(12a)...H-C(9)	1.87	1.09	147.6	7
O(12b)...H-N(11)	1.73	1.01	121.8	7

O(12b)...HC(9), O(12b)...HN(11) as shown in Table 3. In this alternative conformation (Figure 3), one of the interesting feature is that O(12b) interacts with O2′H, N(2) and C(9), in place of O(12a) in the most stable conformation (Figure 2).

Starting with the PCILO preferred structure in Figure 2, the full geometry optimization carried out by HF-SCF method results in torsion angles $\alpha = 175°$, $\beta = 101°$, $\gamma = 59°$, $\delta = 67°$, $\psi = 218°$, $\phi = 75°$, $\xi = 4°$, $\theta = 114°$, and $\chi = 20°$. Whereas the torsion angles α and δ differ to a

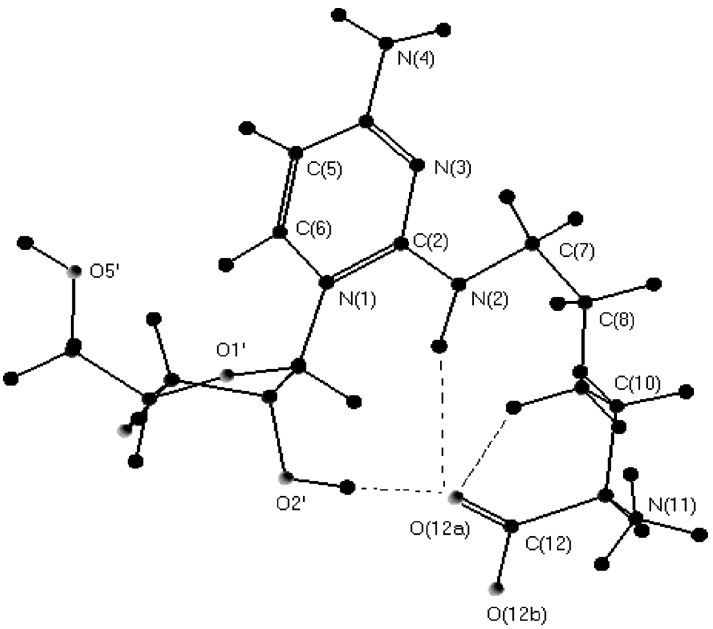

FIGURE 2 PCILO most stable structure for zwitterionic form of lysidine.

TABLE 2 Alternative stable conformation for various forms of lysidine

Torsion angle	Rel. Eng. (kcal/mol)	Figure ref.
$\alpha = 180°, \beta = 180°, \gamma = 30°, \delta = 60°, \Psi = 180°, \phi = 60°, \xi = 330°, \theta = 150°, \chi = 3°$	0.0	2
$\alpha = 180°, \beta = 180°, \gamma = 30°, \delta = 60°, \Psi = 180°, \phi = 60°, \xi = 330°, \theta = 330°, \chi = 3°$	3.0	3
$\alpha = 180°, \beta = 180°, \gamma = 30°, \delta = 60°, \Psi = 180°, \phi = 60°, \xi = 30°, \theta = 150°, \eta = 180°, \chi = 3°$	0.0	5
$\alpha = 180°, \beta = 180°, \gamma = 180°, \delta = 60°, \Psi = 60°, \phi = 60°, \xi = 30°, \theta = 60°, \eta = 180°, \chi = 3°$	3.7	6
$\alpha = 180°, \beta = 180°, \gamma = 180°, \delta = 60°, \Psi = 180°, \phi = 300°, \xi = 30°, \theta = 60°, \eta = 180°, \chi = 3°$	4.4	
$\alpha = 180°, \beta = 180°, \gamma = 30°, \delta = 180°, \Psi = 150°, \phi = 0°, \xi = 0°, \theta = 120°, \chi = 3°$	0.0	7
$\alpha = 180°, \beta = 90°, \gamma = 180°, \delta = 180°, \Psi = 300°, \phi = 0°, \xi = 0°, \theta = 120°, \chi = 3°$	2.9	8
$\alpha = 180°, \beta = 180°, \gamma = 30°, \delta = 180°, \Psi = 150°, \phi = 0°, \xi = 0°, \theta = 300°, \chi = 3°$	4.6	

TABLE 3 Alternative stable conformations for various forms of lysidine

Name of Molecule (lysidine) [figure no.]	Torsion angles	Geometrical parameters for hydrogen bonding: Atoms involved (1–2-3)	Dist. Atom pair (1–2)	Dist. Atom pair (2–3)	angle (deg.) (1-2-3)
1) Zwitterion form:	$\alpha = 180°, \beta = 180°, \gamma = 30°, \delta = 60°, \psi = 180°, \phi = 60°, \xi = 330°, \theta = 330°, \chi = 3°$	O(12b)...HO2′	1.65	0.96	171.3
[3]	(Rel. Engergy = 3.0 kcal/mol)	O(12b)...HN(2)	2.42	1.09	158.2
		O(12b)...HC(9)	1.82	1.09	135.2
		O(12b)...HN(11)	1.97	1.01	112.2
2) Neutral form:	$\alpha = 180°, \iota = 180°, \gamma = 180°, \delta = 60°, \psi = 60°, \phi = 60°, \xi = 30°, \theta = 60°, \eta = 180°, \chi = 3°$	O(12a)..HN(11)	2.78	1.01	82.2
[6]	(Rel. Engergy = 3.7 kcal/mol)				
3) Tautomer form:	$\alpha = 180°, \beta = 90°, \gamma = 180°, \delta = 180°, \psi = 300°, \phi = 0°, \xi = 0°, \theta = 120°, \chi = 3°$	O(12a)..HN(3)	2.94	1.09	137.3
[8]	(Rel. Engergy = 2.9 kcal/mol)	O(12b)..HN(3)	1.73	1.09	121.8
		O(12a)..HC(8)	2.46	1.09	115.2
		O(12b)..HC(8)	2.46	1.09	149.2
		O(12a)..HC(9)	2.26	1.09	111.4
		N(3)...HC(7)	2.40	1.09	98.2

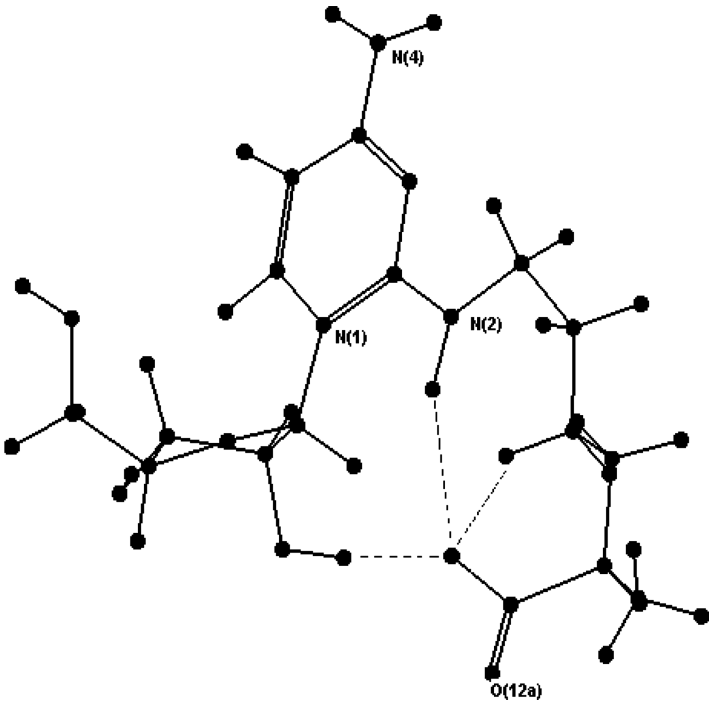

FIGURE 3 Alternative structure of zwitterionic form of lysidine with $\theta = 330^\circ$.

minor extent from the preferred PCILO values, larger variations are found for torsion angles β, γ, ψ, ϕ, ξ, θ, and χ. Geometry optimization through semi-empirical quantum chemical (PM3) method results in torsion angles $\alpha = 183^\circ$, $\beta = 91^\circ$, $\gamma = 63^\circ$, $\delta = 60^\circ$, $\psi = 215^\circ$, $\phi = 65^\circ$, $\xi = 357^\circ$, $\theta = 122^\circ$, and $\chi = 36^\circ$. The torsion angles α, δ and ϕ have minor variations but β, γ, ψ, ξ, θ, and χ show larger difference from the preferred PCILO structure. Results of geometry optimization through MMFF method show $\alpha = 165^\circ$, $\beta = 171^\circ$, $\gamma = 58^\circ$, $\delta = 54^\circ$, $\psi = 220^\circ$, $\phi = 72^\circ$, $\xi = 337^\circ$, $\theta = 125^\circ$, and $\chi = -3^\circ$. Except for γ which differs by 28°, ψ by 40°, θ by 25° from the preferred PCILO value, the other optimized torsion angle values have smaller differences within the range of ($\pm 20^\circ$). Hydrogen bonding parameters after the optimization are in Table 4.

Non-Zwitterionic Form of Lysidine

The conventional non-zwitterionic form of lysidine is represented with a positive charge on N(1) of cytidine, however, the atom N(11) and the substituent amino acid terminal carboxyl group remain neutral. The most stable conformation predicted using PCILO for non-zwitterionic lysidine nucleoside is depicted in Figure 4. The set of torsion angles for the preferred orientation of the base substituent are ($\alpha = 180^\circ$, $\beta = 180^\circ$, $\gamma = 30^\circ$, $\delta =$

TABLE 4 Parameters for hydrogen bonding, from geometry optimization of PCILO conformation, using various methods

	PM3		MMFF		HF-SCF		
Method used Atoms 1-2-3	r12	∠123	r12	∠123	r12	∠123	Figure ref.
O(12a)—HO2′	1.77	174.4	1.72	155.9	1.72	154.2	2
O(12a)—HN(2)	1.77	170.8	1.62	177.0	2.22	163.1	2
O(12a)—HO2′	1.82	149.8	1.95	151.9	1.77	156.6	4
O(12a)—HN(2)	2.73	156.5	2.34	157.8	2.48	157.2	4
O(12a)—HC(9)	2.52	118.0	2.42	118.3	2.53	114.5	4
O(12a)—H-O2′	1.77	174.4	1.66	163.2	1.78	155.9	7
O(12a)—HN(2)	1.77	159.7	1.56	164.0	1.88	162.6	7
O(12b)—HN(11)	1.79	121.6	1.79	121.6	1.66	128.2	7

60°, $\Psi = 180°$, $\phi = 60°$, $\xi = 30°$, $\theta = 150°$, $\eta = 150°$). The lysine moiety folds back toward the ribose ring. The carboxyl oxygen O(12a) of lysine moiety forms hydrogen bond with O2′H of ribose sugar ring. Likewise, the carboxyl oxygen O(12a) is well placed to interact also with N(2) and C(9) of lysine moiety. The lysine moiety has orientation similar to that shown in fig.2. The structure is stabilized by intramolecular interactions between O(12a)...HO2′, O(12a)...HN(2), O(12a)...HC(9) and O(12b)...HN(11). Geometric parameters for hydrogen bonding of these are included in Table 1. Starting from the PCILO preferred conformation (Figure 4), automated geometry optimization through HF-SCF method yields $\alpha = 173°$, $\beta = 94°$, $\gamma = 56°$, $\delta = 63°$, $\psi = 196°$, $\phi = 70°$, $\xi = 43°$, $\theta = 181°$, $\eta = 185°$, and $\chi = 20°$. The torsion angles α, δ, ψ, ϕ, ξ and χ differ within $\pm 20°$ but γ differs by 26°, θ by 29° and η by 35° as compared to PCILO preferred values. The torsion angle β shows a larger difference. Results of geometry optimization through semi-empirical quantum chemical (PM3) method are $\alpha = 186°$, $\beta = 82°$, $\gamma = 62°$, $\delta = 55°$, $\psi = 194°$, $\phi = 66°$, $\xi = 47°$, $\theta = 189°$, $\eta = 183°$, and $\chi = 38°$. The torsion angles α, δ, ψ, ϕ, and ξ differ within $\pm 20°$, whereas γ differs by 32°, θ by 39° and η by 33° as compared to PCILO preferred values. The torsion angle β shows a larger difference similar to that described using HF-SCF optimization. Full geometry optimization by MMFF method results in $\alpha = 170°$, $\beta = 170°$, $\gamma = 48°$, $\delta = 54°$, $\psi = 192°$, $\phi = 67°$, $\xi = 67°$, $\theta = 177°$, $\eta = 181°$, and $\chi = 5°$. The torsion angle α, β, γ, δ, ψ, ϕ, χ show minor differences, where as torsion angle ξ differs by 37°, θ by 27° and η by 31° from the PCILO values. Key hydrogen bonding parameters after the optimization are in Table 4.

Allowing the glycosyl torsion angle to change freely over the entire range (0–360°) validates the initial glycosyl torsion angle choice $\chi = 3°$, based on the Holbrook model. The orientation of the lysine moiety is *trans* to the N(1) of cytidine. In this conformation N(2)H is not suitably oriented

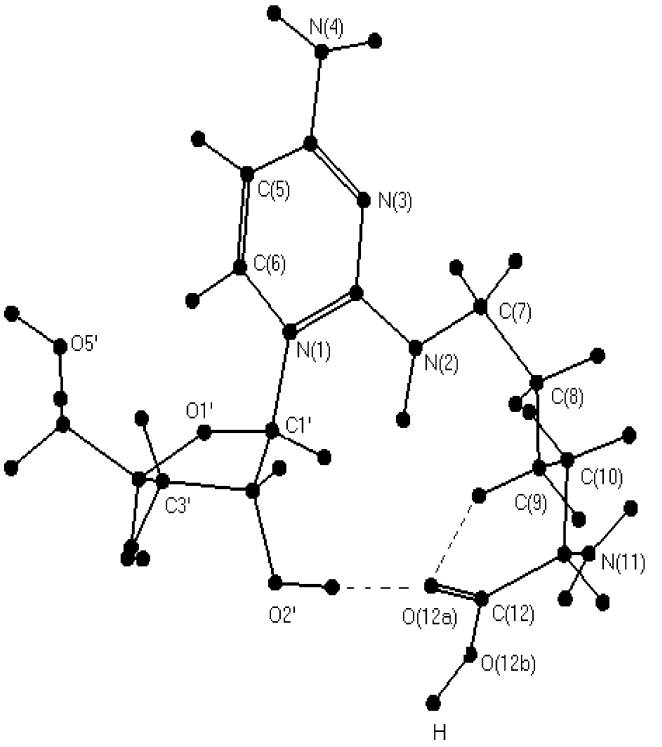

FIGURE 4 PCILO most stable structure for non-zwitterionic lysidine.

to enable pairing with N(1) of 'A.' Due to mismatch of hydrogen bond donor and acceptor groups with 'G'—besides the steric hindrance due to long lysine substituents, canonical base pairing with 'G' is excluded.

Lysidine in Neutral Form

The PCILO predicted most stable structure, of neutral lysidine is depicted in Figure 5. The preferred torsion angle values describing the base substituent orientation in lysidine are ($\alpha = 180°$, $\beta = 180°$, $\gamma = 30°$, $\delta = 60°$, $\Psi = 180°$, $\phi = 60°$, $\xi = 30°$, $\theta = 150°$, $\eta = 180°$). The lysine moiety folds back towards the ribose ring. The carboxyl oxygen O(12a) of lysine moiety forms hydrogen bond with O2′H of ribose sugar ring. The carboxyl oxygen O(12a) is also involved in hydrogen bonding with N(2) and C(9) of lysine moiety. The structure is stabilized by intramolecular interaction between O(12a)...HO2′, O(12a)...HN(2), O(12a)...HC(9) and O(12b)...HN(11). The geometrical parameters for hydrogen bonding of these are included in Table 1. The orientation of lysine moiety is similar to the preferred structures shown in Figures 2 and 4.

Starting from the most stable structure shown in Figure 5, the results of full geometry optimization have been obtained by using HF-SCF and MMFF

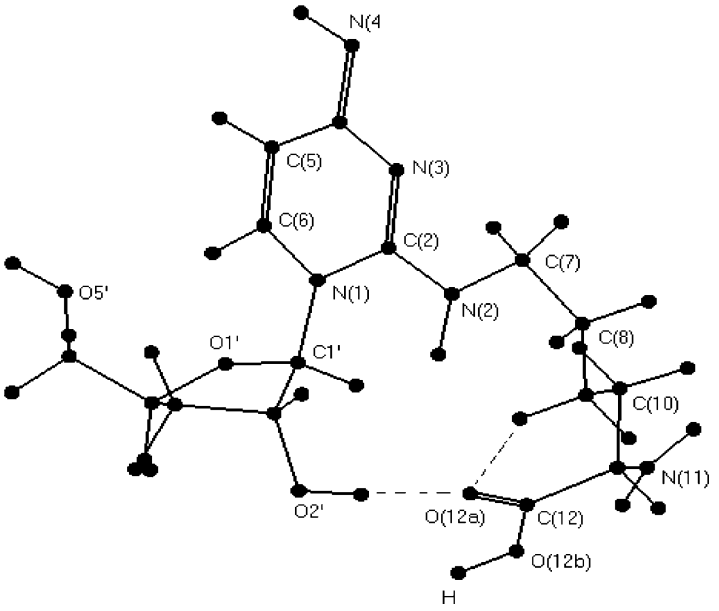

FIGURE 5 PCILO most stable structure for neutral lysidine.

methods. The optimized torsion angle values by HF-SCF method are $\alpha = 173°$, $\beta = 83°$, $\gamma = 53°$, $\delta = 64°$, $\psi = 193°$, $\phi = 72°$, $\theta = 182°$, $\xi = 42°$, $\eta = 184°$, and $\chi = 24°$. The torsion angle values for α, δ, ψ, ϕ, ξ, and η differ within 20° of the PCILO most stable values but torsion angle β shows larger difference and γ, θ, and χ differ within 30°. The results of optimized geometry by PM3 method are $\alpha = 177°$, $\beta = 104°$, $\gamma = 59°$, $\delta = 56°$, $\psi = 197°$, $\phi = 64°$, $\theta = 228°$, $\xi = 67°$, $\eta = 183°$, and $\chi = 27°$. The torsion angle values for α, δ, ψ, ϕ, and η show variation within 20° as compared to PCILO preferred values, whereas, the torsion angles γ, and χ are within 30°. The torsion angles β and θ show greater difference. The optimized geometry using MMFF method has $\alpha = 172°$, $\beta = 163°$, $\gamma = 51°$, $\delta = 54°$, $\psi = 187°$, $\phi = 71°$, $\theta = 193°$, $\xi = 72°$, $\eta = 181°$, and $\chi = 14°$. The optimized torsion angles α, δ, ψ, ϕ, χ are similar ($\pm 15°$) to the preferred structure in Figure 5. The torsion angle β differs by 27°, γ by 21°, ξ by 42°, and θ by 17° from the PCILO preferred values.

Higher energy (3.7 kcal/mol) PCILO alternative stable conformation (Figure 6), in Table 2, may be realized by flipping to $\gamma = 180°$, $\Psi = 60°$, $\theta = 60°$. Interaction of carboxyl oxygen O(12a) with O2′H is absent in this alternative structure. Another higher energy (4.4 kcal/mol) alternative conformation may be arrived by flipping of torsion angles (γ, ϕ, θ) to $\gamma = 180°$, $\phi = 300°$, and $\theta = 60°$.

The orientation of lysine remains *trans* to the N(1) atom of cytidine. In neutral lysidine, the third position of the pyrimidine ring lacks a hydrogen

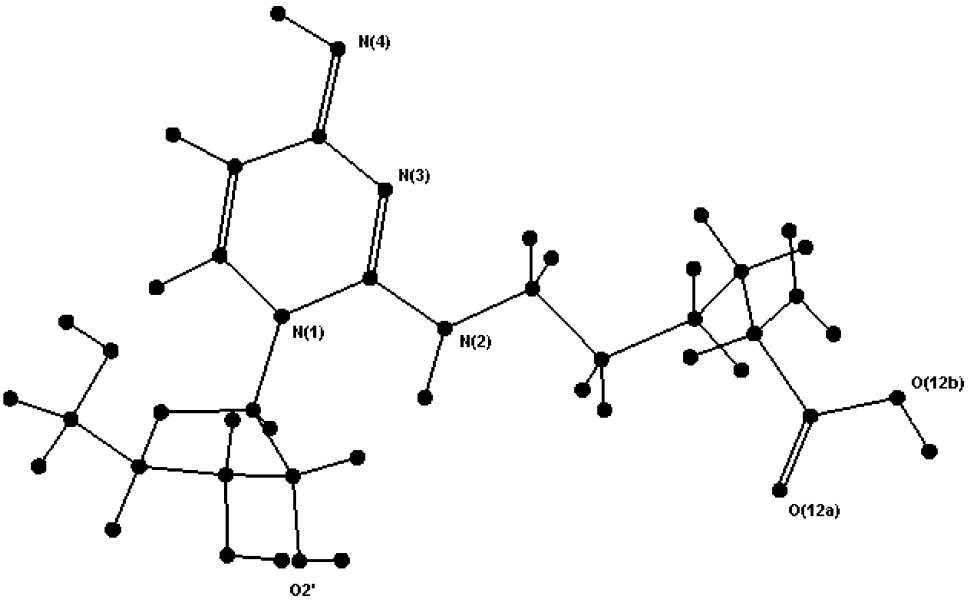

FIGURE 6 Alternative structure of neutral form of lysidine with $\gamma = 180°$, $\psi = 60°$, $\theta = 60°$.

atom to serve as a hydrogen bond donor to allow for pairing with N(1) of A. Likewise, the HN(4)H in cytidine lacks an acceptor to match the HN(6)H donor in A. Thus the neutral form of lysidine molecule can not form Watson-Crick base pairing with A.

If in case the lysine substituent R in N(2)HR of lysidine could orient *cis* to N(1) in the pyrimidine, then N(2)H may pair as hydrogen bond donor to N(1) of Adenine. Also the N(3) in lysidine may accept a hydrogen bond from N(6)H of adenine. In this way A may be recognized by lysidine, when lysine orientation is *cis* to N(1).

In case lysine substituent orients *trans* to N(1) then only tautomeric form of lysidine (having N(3)H and N(4)H) can provide the matching hydrogen bond donor–acceptor pattern to base pair with N(1) and HN(6)H respectively of adenine & recognize A. Thus, it is of interest to find whether the lysine moiety in tautomeric form of lysidine preferably orients *trans* or *cis*.

Lysidine (Tautomer Form)

The lysidine in tautomer form has –NHR group in position 2, -NH hydrogen bond donor group in position 3, and hydrogen bond acceptor group = NH in position 4. The PCILO predicted preferred structure of lysidine (Tautomer form) with zwitterion and positive charge on N(1) with *anti* oriented base ($\chi = 3°$) is shown in Figure 7. The set of preferred torsion angle values for the favored substituent orientation are ($\alpha = 180°$, $\beta = 180°$,

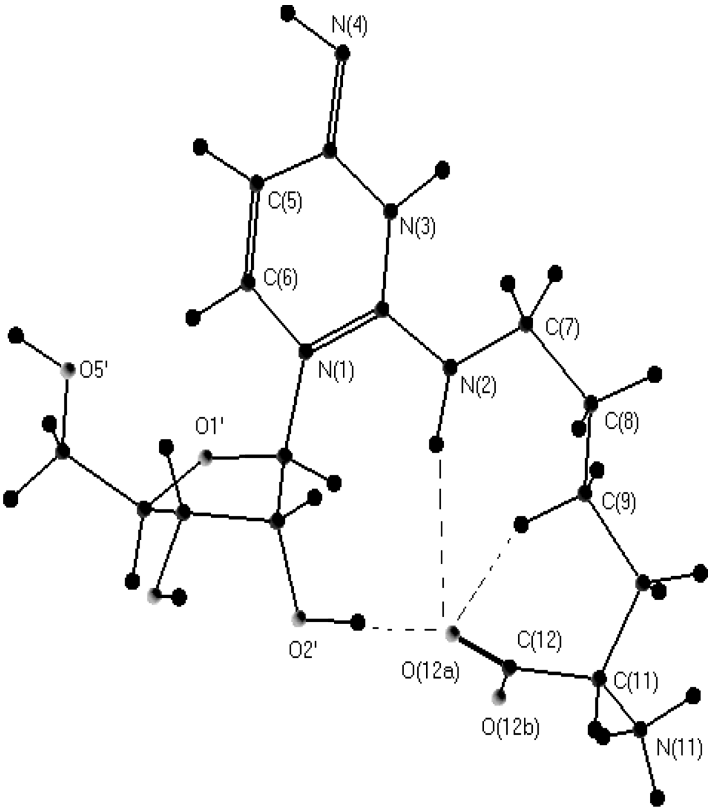

FIGURE 7 PCILO favored structure for lysidine (tautomer form).

$\gamma = 30°$, $\delta = 180°$, $\Psi = 150°$, $\phi = 0°$, $\xi = 0°$, $\theta = 120°$, $\chi = 3°$). The structure is stabilized by intramolecular interactions between O(12a)...HO2′, O(12a)...HN(2), O(12a)...HC(9), O(12b)...HN(11). Hydrogen bonding geometrical parameters for these are included in Table 1. The preferred orientation of lysine substituent here can be related to that of Figures 2, 4, and 5. Whereas lysidine (zwitterion form) in Figure 2 has ($\alpha = 180°$, $\beta = 180°$, $\gamma = 30°$, $\delta = 60°$, $\Psi = 180°$, $\phi = 60°$, $\xi = 330°$, $\theta = 150°$, $\chi = 3°$), but lysidine in tautomer form in fig. 7 has ($\alpha = 180°$, $\beta = 180°$, $\gamma = 30°$, $\delta = 180°$, $\Psi = 150°$, $\phi = 0°$, $\xi = 0°$, $\theta = 120°$, $\chi = 3°$). Among these torsion angles, α, β, and γ take similar values but δ, ψ, ϕ, ξ, and θ have greater variation. Notably the torsion angle ($\delta = 180°$) has quite different value in Figure 7, as compared to ($\delta = 60°$, $\theta = 150°$) in Figure 2. Consequently a closer interaction occurs between O(12a)...HC(9) and O(12a)...HO2′ (Table 1). It may also be noted that values of ($\phi = 0°$, $\xi = 0°$, $\theta = 120°$), as in Figure 7, differ from ($\phi = 60°$, $\xi = 330°$, and $\theta = 150°$), as in Figure 2. These changes result in closer interaction between O(12b)...HN(11) in (Figure 7; also noted in Table 1).

Starting from PCILO preferred structure (Figure 7), results of full geometry optimization by using HF-SCF method are $\alpha = 182°$, $\beta = 183°$, $\gamma = 76°$, $\delta = 210°$, $\psi = 106°$, $\phi = 325°$, $\xi = 2°$, $\theta = 116°$, and $\chi = 16°$. The torsion angle γ differs by more than 46°, δ differs by 30°, Ψ differs by 44°, and ϕ differs by more than 35°. The other torsion angles have minor differences. The geometry optimized torsion angles by PM3 method are $\alpha = 184°$, $\beta = 100°$, $\gamma = 105°$, $\delta = 235°$, $\psi = 111°$, $\phi = 312°$, $\xi = 356°$, $\theta = 122°$, and $\chi = 36°$. The torsion angles α, ξ, θ differ within 5°, but the torsion angle ψ differs by 39°, ϕ differs by 48° and χ differs by 33° from the preferred PCILO values. Comparatively, the torsion angles β, γ, δ, ψ show larger differences. These may also be compared with the results of MMFF geometry optimization yielding $\alpha = 185°$, $\beta = 177°$, $\gamma = 82°$, $\delta = 218°$, $\psi = 103°$, $\phi = 325°$, $\xi = 333°$, $\theta = 130°$, and $\chi = 9°$. The MMFF optimized values differ by more than 50° for γ, by more than 40° for δ, and by more than 77° for Ψ, but the other torsion angles have smaller differences. Hydrogen bonding parameters after the optimization are included in Table 4.

Higher energy (2.9 kcal/mol) PCILO alternative conformation (Figure 8) is realized with torsion angles $\beta = 90°$, $\gamma = 180°$ and is also included in (Table 2). The intramolecular hydrogen bonding between O(12b)...HN(3) and O(12a)...HC(9) may provide additional stability (Table 3). Thus interaction between O(12b)...HN(3) is an alternative to O(12a)...HO2′. The next higher energy conformation 4.6 kcal/mol may be realized by flipping to ($\theta = 300°$). This conformation is similar to Figure 7, except that atom O(12b) replaces O(12a) for hydrogen bonding interactions with HO2′. Interaction of the base C(6)H with backbone O5′ [O5′...HC(6)] may additionally contribute to stability.

In the anticodon loop of tRNAs, *anti* base orientation is common for wobble nucleosides.[11,24,31,37–39] Post-transcriptional modification enzyme that converts C_{34} to lysidine k^2C_{34} has been identified and its mechanism with specific recognition reported.[23,32–34] Multi-function requirements[35–38,40] on tRNA to serve as a suitable substrate for aminoacylation at the acceptor arm 3′-terminal, while also allowing codon recognition by anticodon for enabling protein biosynthesis through ribosomal binding and translocation from A to P sites as well, can strictly limit permissible tRNA conformations. The severe constraints in the anticodon loop with bulky lysine substituent—at the wobble position—can further strongly constrain the conventional *anti* glycosyl orientation.

In Figure 7 -NHR is in position 2, >NH is in position 3, and = NH is in position 4. These, hydrogen bond donor–acceptor sites in lysidine do not complement with the sites in G. However, matching hydrogen bond donor–acceptor sites with 'A' is possible (Figure 9). Thus, with the tautomer form of lysidine the selective recognition of codon AUA is permissible while avoiding the mis-recognition of AUG.

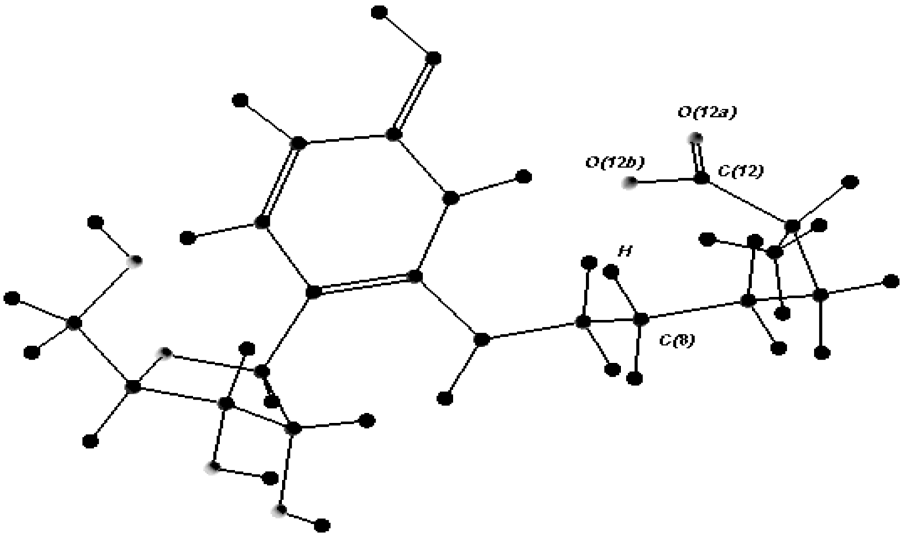

FIGURE 8 Alternative structure for lysidine tautomer with $\beta = 90°$, and $\gamma = 180°$.

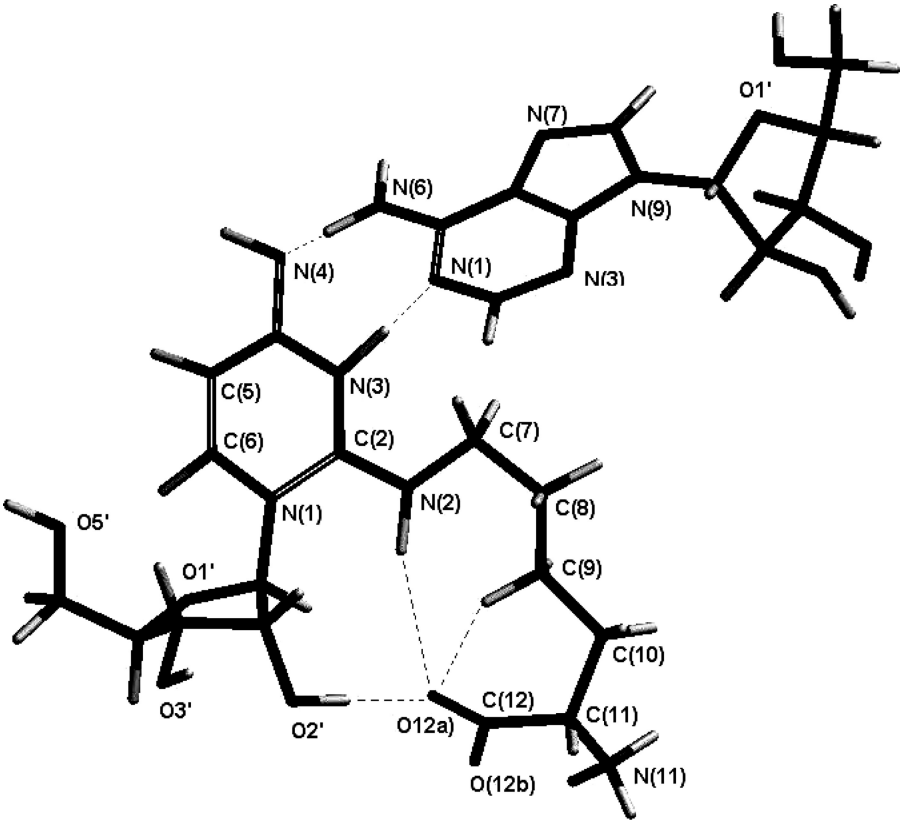

FIGURE 9 The scheme for recognition of A by lysidine tautomer, that is, $k^2C : A$.

CONCLUSION

In conclusion, the orientation of bulky lysine moiety (R) is located *trans* to the N(1) atom of cytidine in the various forms of lysidine, prohibiting Watson Crick base pairing with 'G.' Lysidine with zwitterion, non-zwitterion, and in the neutral form is unable to recognize 'A' from codons due to lack of matching hydrogen bond donor group at position 3, and hydrogen bond acceptor group at position 4 in the pyrimidine. These hydrogen bond donor–acceptor situations are not met in the above discussed three forms of lysidine (see Figures 2–6). Only tautomer form of lysidine (Figure 7) may provide compatible hydrogen bond donor–acceptor sites to enable base pairing with 'A' (Figure 9) and may thus recognize the codon AUA instead of AUG.

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