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STRUCTURE AND REACTIVITY OF WYOSINE (Y-NUCLEOSIDE) AND ITS DERIVATIVES. CHEMICAL, KINETIC AND SPECTROSCOPIC STUDIES

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Summary: Wyosine-triacete **4** undergoes electrophilic formylation, iodination and bromination reactions exclusively at C^7 while 2',3',5'-tris-O-(t-butyldimethylsilyl)wyosine (**6**), under a base-induced deuterium exchange reaction, forms a C^2-deuterio derivative **14**. This clearly shows that the "right" imidazole in wyosine (**1**) and in its triacetate **4** is much more π-electron-rich (electrophilic) than the "left" imidazole part. pK_a measurements and ^{15}N-NMR spectroscopic studies of wyosine (**1**), its triacetate **4** and several of its C^7-substituted derivatives shows that the preferential site (ca. 80%) of protonation in compound **4** is N^5 and the N^1 is only 20% protonated. A C^7-electron-withdrawing substituent (-CHO), as in **7**, however, promotes protonation mainly at N^1. The N^1-ribosylated isomer **15**, prepared by the acid-catalyzed isomerization of **4**, is more basic (pK_a 3.10) than wyosine-triacetate **4** (pK_a 2.36) which has been corroborated by the differrence of their ^{15}N shifts between protonated and neutral species ($\Delta\delta$). Furthermore, acidic depurination studies particularly with N^1-ribosylated isomer **15** and its comparison with that of wyosine-triacetate **4** have shown that the latter undergoes depurination reaction only 7 times faster; it may therefore be assumed that the unusually enhanced rate of acidic hydrolysis of glycosidic bond in wyosine (**1**) and in its triacetate **4** is not primarily due to steric acceleration by its N^4-methyl group, but due to electronic factors. Metal ion binding studies, using ^{15}N-NMR spectroscopy, suggest that Mg^{2+} ion does not bind to the Y-base but to its phosphodiester function in the anticodon loop of $tRNA^{Phe}$.

The hypermodified, fluorescent Y-nucleoside (wyosine) **1** and its 7-substituted congeners **2** and **3** occur naturally, adjacent to the 3'-end of the anticodon loop of yeast phenylalanine tRNA ($tRNA^{Phe}$)[1,2]. Their glycosidic bonds are extremely acid-labile[3]. The excision of the Y-base by mild acid treatment almost completely removes the ability of $tRNA^{Phe}$ to show codon recognition property which is necessary for the protein biosynthesis[3]. These Y-nucleosides **1** to **3** have been the subject of many studies due to their distinctive physical[4,5] and chemical[6-9] properties as well as biological interests[1,2,3a].

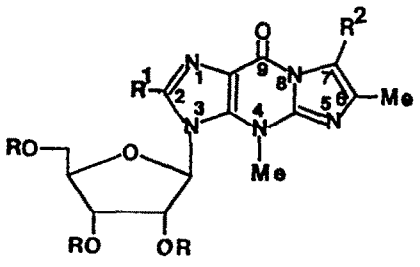
The unique site of methylation of Y bases in compounds **1**, **2** and **3** at N^4, and their extreme acid-labilities have posed a considerable challenge for their high yielding synthesis for almost last 20 years[1-3,6-9]. This problem has been recently addressed in this laboratory and has been realized in a one-step synthesis of wyosine-triacetate **4** from N^4-desmethylwyosine-triacetate **5b** in 74% yield[10]. Since this work has made wyosine (**1**) and its triacetate **4** available in a large amount, we decided to explore its chemical reactivity and electronic structure by ^{15}N-NMR spectroscopy in order to understand why such a hypermodified aglycone is so

important[1-5] in a molecule of $tRNA^{Phe}$ for its codon recognition - a step that is necessary in protein biosynthesis of phenylalanine residues in yeast cells. We have therefore first explored the nature of electrophilic reactivities of the tricyclic aglycone in wyosine-triacetate 4 and have thus attempted to ascertain the relative electrophilicities and the bascities of its "left" and the "right" imidazole moieties. Subsequently, ^{15}N-NMR spectroscopy has been used to provide confirmatory evidence regarding the importance of various basic centers in Y-nucleoside and its derivatives to explain their chemical reactivites and the major sites of protonations which predominantly contribute in the gross pK_a of these molecules.

RESULTS & DISCUSSIONS

Chemistry

Wyosine-2',3',5'-tri-O-acetate (4) was subjected to a Vilsmeier formylation reaction[11a] which exclusively took place at C^7 to give the corresponding 7-formyl derivative 7 (83%). The C^7-formyl group in 7 also could be suitably protected either as its 1,3-dithiane[11b] derivative 8a (82%) or as a cyanotrimethylsilyl adduct[11c] 9 (96%). Compounds 8 and 9 were prepared keeping in view that either of these compounds could easily give stable carbanions[11], which should readily react with a suitable electrophile to form a new carbon-carbon bond in order to synthes ize other C^7 aminoacid substituted congeners 2 and 3. 7-formylwyosine derivative 7 was selectively reduced to give 7-hydroxymethyl derivative 10 (76%) by $NaBH_4$ in dry THF solution[11d]. The conversion of C^7-formyl in 7 to C^7-hydroxymethyl function in 10 opened the possibility of its functionalization with an appropriate leaving group (*e.g.* a tosylate) which then can be employed in a nucleophilic displacement reaction to form a new carbon-carbon bond at C^7 for the synthesis of congeners 2 and 3. We have subsequently carried out other specific electrophilic substitution reactions at C^7, namely bromination and iodination, in order to show the over whelmingly dominating electrophilic character of the "right" imidazole ring over the "left" in the wyosine-triacetate 4. Thus the bromination of 4 with bromodime thylsulfonium bromide[12] gave the 7-bromowyosine derivative 11 in 62% yield and an electrophilic iodination reaction with silver trifluoroacetate and iodine[13] gave the 7-iodo derivative 12 in 89% yield. No C^2 substituted product was found to have been formed in any one of the above electrophilic formylation or halogenation reactions. In view of above electrophilic reactions at C^7, we envisaged that the generation of a carbanion, under the influence of a strong base, should take place at the C^2 since the "left" fused imidazole was deemed to be relatively more electron-deficient. In order to establish this, wyosine-triacetate 4 in dry THF solution was subjected to the treatment of n-butyllithium at -78 °C, quenched with methyl iodide, followed by a standard work up and then a treatment of crude products in dry pyridine solution with an excess of acetic anhydride gave a product which was isolated in 47% yield. This was spectroscopically characterized as to be 2-C-acetyl-2',3',5'-tri-O-acetyl wyosine (13). It was presumably formed by an intermolecular nucleophilic attack of the carbanion generated at C^2 on to the carbonyl of the sugar acetate function of the second molecule of wyosine-tri acetate since 13 was also isolated (51%) when the reaction was quenched with CH_3CO_2D instead of CH_3I. An unambiguous evidence for the formation of a carbanion specifically at C^2 of 2',3',5'-tri-O-(t-butyldi methylsilyl) [TBDMS] wyosine (6) was however obtained upon its treatment in THF solution with n-butyllithium at -78 °C followed by a quenching with D_2O. The compound that was isolated in a quantitative yield was C^2-deuterio-tris-(TBDMS)-wyosine 14, thus establishing that



1 : R = R^1 = R^2 = H

2 : R = R^1 = H ; R^2 = *aminoacid**

3 : R = R^1 = H ; R^2 = *aminoacid*+

4 : R = Ac ; R^1 = R^2 = H

6 : R = TBDMS ; R^1 = R^2 = H

7 : R = Ac ; R^1 = H ; R^2 = CHO

8a: R = Ac ; R^1 = H ; R^2 = $CH(S\text{-}CH)_2$

8b: R = TBDMS ; R^1 = H ; R^2 = $CH(S\text{-}CH)_2$

9 : R = Ac ; R^1 = H ; R^2 = $CH(CN)SiMe_3$

10 : R = Ac ; R^1 = H ; R^2 = CH_2OH

11 : R = Ac ; R^1 = H ; R^2 = Br

12 : R = Ac ; R^1 = H ; R^2 = I

13 : R = R^1 = Ac ; R^2 = H

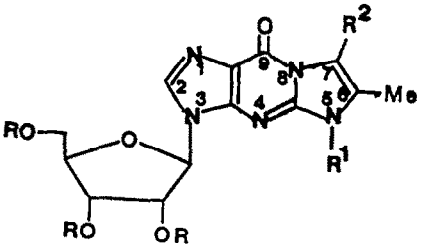
14 : R = TBDMS ; R^1 = ^2H ; R^2 = H

16 : R = R^1 = H ; R^2 = Me

17 : R = Ac ; R^1 = H ; R^2 = Me

* $CH_2CH_2CH(NHCO_2Me)CO_2Me$

\+ $CH_2CH(OH)CH(NHCO_2Me)CO_2Me$



5a : R = R^1 = R^2 = H

5b : R = Ac ; R^1 = R^2 = H

5c : R = R^1 = H ; R^2 = Me

5d : R = Ac ; R^1 = H ; R^2 = Me

5e : R = TBDMS ; R^1 = R^2 = H

5f : R = R^1 = Ac ; R^2 = H

18a : R = R^2 = H ; R^1 = Me

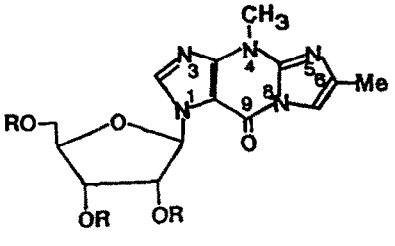
18b : R = Ac ; R^1 = Me ; R^2 = H

18c : R = H ; R^1 = R^2 = Me

18d : R = Ac ; R^1 = R^2 = Me

19 : R = Ac ; R^1 = Me ; R^2 = CHO

20 : R = Ac ; R^1 = Me ; R^2 = I



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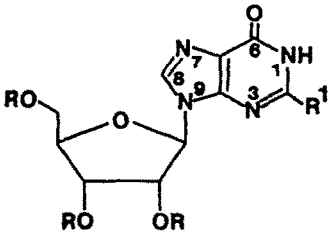
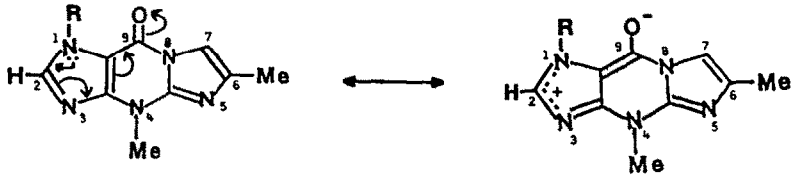


21 : R = H ; R^1 = NH_2

22 : R = Ac ; R^1 = NH_2

23 : R = H ; R^1 = H

24 : R = Ac ; R^1 = H



(A) (B)

R = 9- β- D-(2',3',5'-tri-O-acetyl)ribofuranosyl

the generation of a carbanion on the masked C^2 formyl functions of compounds 8 or 9, would be quite nonselective. Therefore, compounds 8 and 9 would be synthetic ally less useful as intermediates for a hitherto unreported synthesis of congeners 2 and 3.

During the course of this investigation on the reactivity of wyosine-triacetate 4, we subjected it to the treatment of a lewis acid, anhydrous aluminium trichloride, in dichloromethane solution at ca. 0 °C in order to explore its stability. What we found in this reaction is that the compound 4 was transformed quantitatively to a new higher R_f compound on the t.l.c which was isolated (74%) and spectroscopically characterized as to be 4,9-dihydro-4,6-dimethyl-9-oxo-1-(2',3',5'-tri-O-acetyl -β-D-ribofuranosyl)imidazo[1,2-a]purine (15) - the N^1 isomer of wyosine-triacetate 4.

The electrophilic reactivity of the "right" imidazole ring in the N^5-methyl isomer 18b is very much less than wyosine-triacetate 4. Thus the C^7-formylation of 4 → 7 was complete within 45 min at 0 °C in 83% yield while a corresponding reaction of 18b, under an identical condition, gave 19 in only 11% yield (cf. experimental section for an optimized condition for preparation of 19). Similarly, the C^7-iodination of 4 → 12 (89%) was complete within 20 min in 20 °C, but a correspond ing reaction of 18b → 20 (51%) required at least 3 h under an identical condition. These suggest that the "right" imidazole of 4 is more aromatic than that of 18b which can be understood from the consideration that the latter with two pyrrole type nitrogens obviously delocalize their π-electrons much less readily and is therefore less basic (pK_a 1.63), than compound 4 (pK_a 2.36) with one of sp^2 and sp^3 type nitrogens. The enhanced aromatic character of the "right" imidazole in 4 as compared to that of 18b is also evident in its distinctive downfield ^{13}C-NMR shifts [δ_{C^6} , $\delta_{C^6\text{-}CH_3}$ & δ_{C^7} in 4 absorb at 138.1, 14.25 & 106.4 ppm, respectively, as compared to the corresponding absorptions for 18b at 126.8, 10.7 & 103.2 ppm] in $CDCl_3$.

Protonation study by ^{15}N-NMR spectroscopy

Having established that the "right" imidazole part in wyosine-triacetate 4 is the most reactive moiety in electrophilic reactions, and therefore is most electron-rich, while its "left" imidazole part generates a carbanion quite readily because of its electron-deficient nature, we set out to establish the exact sites of protonation in 4 and in its other analogues by ^{15}N-NMR spectroscopy[14] and then correlate them with their pK_a data. ^{15}N-NMR spectroscopy has proved to be a very powerful tool to determine both the site and the magnitude of protonation[15-17]. The degree of protonation (δΔ) indicates the potential reactivity of a given nitrogen to electrophiles[16]. Therefore we studied the changes of ^{15}N chemical shifts of 4 and 17 both in neutral solutions and also upon their protonations with CF_3COOH (TFA) in order to obtain Δδ values for all nitrogens in these molecules. Such studies were aimed to understand the primary and secondary sites of protonations in 4 and 17 which may in turn be used to address why Y-nucleosides are so extreme ly labile accross their glycosidic bonds[3-8,26] under very mild acidic conditions. Parent compounds 1 and 16 could not, however, be studied in any detail both because of their poor solubilities and stabilities in acidic medium. Although the protection of the hydroxyl functions of the sugar moiety as acetates is known to stabilize the glycosidic linkages[9] in 4 and 17, yet they are considerably susceptible to depurination reaction even under a mild acidic condition.

Consequently, we have used a ^1H decoupled INEPT pulse sequence[18,22] where the parameters have been set at their optimum values. The ^{15}N chemical shifts of 4, 17, 18a-d and 19 and other tricyclic nucleosides in neutral solutions in CH_2Cl_2 and/or DMSO and also upon protonation with TFA are presented in tables 1 and 2. The magnitude of the ^{15}N shifts ($\Delta\delta$) upon protonation is larger[17] in CH_2Cl_2 probably due to its poorer solvating properties. However, the relative ratios of various protonated species in a specific nucleoside does not change in any significant way upon changing solvents.

The main conclusion which can be drawn from these studies is that the primary site of protonation in both compounds 4 and 17 is N^5 as shown by its $\Delta\delta$ shifts of ca. 40 ppm in compound 4 and ca. 46 ppm in compound 17 while their N^1-nitrogens shift upfield by only ca. 2.5 ppm and ca. 1.5 ppm, respectively, in CH_2Cl_2. These $\Delta\delta$ shifts of N^5 versus N^1 nitrogens as measures of their respective bascities should be carefully considered in view of the fact that the derivatization of the sugar hydroxyl functions by electron-withdrawing groups such as acetates in nucleosides can yield drastic changes in the bascities (vide infra) of the imidazole ring in the purine moiety (table 3). However, the studies with 4 and 17 clearly show, with their respective $\Delta\delta$ shifts of N^1 and N^5 and their ratios, that (i) the first site of protonation is N^5, and (ii) the amount of N^1 protonated species perhaps do not exceed 20% of that of N^5 nitrogen as estimated from the data in tables 2 and 3. It is however difficult to extrapolate these data for $\Delta\delta$ shifts of N^5 and N^1 in wyosine (1) and in its C^7-methyl congener 16.

In N^4-desmethyl wyosine 5a, its N^6-methyl derivatives 18a-d and in other analogues 5b-d, it is the N^1 which is exclusively protonated (table 2). The relatively low chemical shifts for N^5 in these derivatives suggest that it is not a pure sp^3 hybridized nitrogen[14] and its lone pair is, therefore, partly delocalized in the imidazole ring. The chemical shifts of N^5-nitrogens in these compounds also remain unchanged upon addition of 1 equiv. of TFA which explains its poorer reactivity. On the other hand, the competition for protonation between N^1 and N^5 nitrogens in wyosine derivatives 4, 6 and 17, as shown by their $\Delta\delta$ shifts, reveal that the "right" imidazole ring is considerably more basic than the "left" imidazole. These observations are consistent with the chemoselective electrophilic formylation and halogenation reactions at C^7 (vide supra). This result is also consistent with the fact that 2'-deoxy-1-deazawyosine shows almost a similar pk of protonation (3.75) as wyosine itself[19]. In their work[19], the authors attempted to explain this similarity by attributing to their common protonation sites as to be the carbonyl group (C^9) which, we reasoned, should be directly observable by ^{13}C-NMR shift of carbonyl-carbon (C^9) upon protonation with TFA. This was attempted in this present investigation but no such ^{13}C shift was observed. Thus the present ^{15}N data and the similarity in the pk_a of wyosine and 1-deaza-2'-deoxywyosine are probably better explained by assuming that their comparable bascities are mainly due to their "right" imidazole parts and the N^5 nitrogen is also the preferred site of protonation in the latter. This, however, needs to be unequivocally established by ^{15}N-NMR studies.

The resonance signal of N^4 in 4 and 17 moves very slightly downfield upon protonation, suggesting that the lone pair of N^4 does not stabilize the N^5 protonated imidazole ring by delocalization. In an earlier study[21] with adenosine and guanosine derivatives, it has been found that the downfield shift of an exocyclic sp^3 nitrogen is proportional to the degree of protonation of a conjugated endocyclic sp^2 hybridized nitrogen. Since the protonation studies with compounds 4 and

17 show that the delocalization of the lone pair of N^4 is not favoured and therefore the planarity of the central pyrimidine ring and the imidazole ring containing N^6 is not completely achieved in CH_2Cl_2 or DMSO solutions which is consistent with the fact that the $^2J_{N^8,H^7}$ in 4 is 4.3 Hz[22] as compared to $^2J_{N^3,H^8}$ of 3.3 Hz in *planar* ethenocytidine[15,23]. This observation is comparable with that of the central nitrogen atom in flavins in the 1,5-dihydro state. The ^{15}N NMR studies[24,25] of flavin derivatives have also shown that the delocalization of the lone pair of the central sp^3 hybridized nitrogen can indeed reflect the coplanar ity of the molecule in a solvent under consideration.

Bascity of wyosine and its derivatives

The pK_a values of wyosine, its triacetate and some of the 7-substituted derivatives have been measured (table 4) in this work in order to correlate their electronic properties to those of $\Delta\delta$ shifts obtained by ^{15}N-NMR spectroscopy. The pK_a of wyosine is reported by Itaya et al.[26] is 3.06 (ionic strength 1.0 mol dm^{-3}) which is 0.4 units larger than that of the result under our measurement condition (table 4). The difference of this magnitude is however, expected because of the difference of ionic strengths[27]. As expected, an electropositive C^7-methyl group, as in 17 (pK_a 2.85) (table 4), slightly increases the bascity of the wyosine-triacetate 4 (pK_a 2.36), consistent with the $\Delta\delta$ shift of 46.3 ppm for the N^5 in the former and $\Delta\delta$ of 39.9 ppm for the latter in CH_2Cl_2 (table 1). On the other hand, the electronegative C^7-formyl function as in compound 7 (pK_a -0.3) reduces the pK_a of wyosine-triacetate 4 quite corsiderably. This reduction of overall bascity of compound 7 due to the C^7-formyl group is also reflected by $\Delta\delta$ = 1.0 ppm for N^6 nitrogen and $\Delta\delta$ = 6.6 ppm for N^1 nitrogen as compared to that of wyosine-triacetate 4 ($\Delta\delta$ = 39.9 ppm for N^6 and 2.4 ppm for N^1 in CH_2Cl_2). Interestingly, a similar behaviour was noted[20] due to the electron-withdrawing effect of N^6-benzoyl group in N^6-benzoyl-2′-deoxyadenosine in which ca. 30% $(N^1H)^+$ and 70% $(N^7H)^+$ species were found in a protonation experiment as compared to 100% $(N^1H)^+$ in 2′-deoxyadenosine. This preferred site of protonation at N^1 in 2′-deoxyadenosine is also profoundly reflected in its relatively higher stability in acidic medium as compared to N^6-benzoyl-2′-deoxyadenosine[20]. The influence of C^7-bromo in compound 11 is unexpectedly small in view of the fact that the difference of pK_a units between purine-riboside and the 6-chloropurine-riboside is more than 2 log units[28]. A possible explanation is that the inductive effect of C^7-Br is not transmitted as efficiently to the N^1 position as to the adjacent N^6 position. Thus the relative distribution of the site of protonation is changed and, therefore, the influence of C^7-Br on the gross pK_a value is smaller than could be expected. An alternative explanation for a smaller effect of C^7-Br on the gross pK_a is that the inductive effect of -Br is partly compensated by its ability to donate electrons mesomerically. The effect of C^7-CHO group on the pK_a of wyosine-tri acetate 4 is more complex because of the possibility of both a keto ⇌ enol tautomerism of the formyl group and also due to its inductive effect in compound 7. It is likely that as regards the relative inductive effect of C^7-CHO and C^7-Br, the former is probably more electronegative as evident in the pK_as of 7 and 11 respectively. The influence of the C^7-methyl group (~0.5 log units) is very similar to the effect of a methyl substituent in purines[27,29]. However, with compounds 18a-d, the influence of methyl groups on pK_a is slightly smaller (table

4). It may also be noted that the acetates of the sugar moiety reduce the basicity by 0.3-0.4 log units (compare 1 versus 4 & 16 versus 17) which could not be verified by $\Delta\delta$ shifts by ^{15}N-NMR because of extreme lability of compounds 1 and 16 in acidic medium. This difference is, however, comparable to those found between guanosine ($\delta\Delta$ = 46 ppm for N^7 in DMSO) and 2', 3', 5'-tri-O-acetyl guanosine ($\Delta\delta$ = 26 ppm for N^7 in DMSO) and in several other pairs of sugar-acetylated and non-acetylated nucleosides shown in tables 2 and 3. A comparison of the electron-withdrawing effect of sugar-acetates in compound 4 (pK_a 2.36, $\Delta\delta$ = 39.9 ppm for N^5 and 2.4 ppm for N^1 in CH_2Cl_2) with that of the sugar-TBDMS in compound 6 (pK_a 1.10, $\Delta\delta$ = 32.0 ppm for N^5 and 3.3 ppm for N^1 in CH_2Cl_2) on the overall pK_a of the aglycone in DMSO-water solution shows that their effects are not quite similar, TBDMS groups have clearly a better electron-withdrawing function in the sugar moiety than the corresponding acetates. A comparison of the pK_a of wyosine-tri acetate 4 (2.36) with that of its N^1 isomer 15 (3.10) shows that the latter is more basic by ca. 0.7 log units. This enhanced basicity of the aglycone in 15 can be particularly attributed to the enhanced bascity of its "right" imidazole part. This has been corroborated by the magnitude of ^{15}N shifts between protonated and neutral species of 15 ($\Delta\delta$ = 45.7 ppm for N^5 and 0.0 ppm for N^1 in CH_2Cl_2) as compared to compound 4 ($\Delta\delta$ = 39.9 ppm for N^5 and 2.4 ppm for N^1 in CH_2Cl_2). This is probably due to the fact that the "left" imidazole of the N^3 isomer 4 can relatively deactivate the π-electron rich "right" imidazole part more effectively by an inductive effect through the C^9-carbonyl function than that is possible in the N^1 isomer 15. A study of the pair of canonical structures (A and B) in scheme 1 for the N^1 isomer 15 reveal that its sp^2 hybridized C^2 of the "left" imidazole is in direct conjugation with the C^9-carbonyl which is clearly not possible in the wyosine-triacetate 4. It is tempting to suggest that this activation of the C^9-carbonyl in 15 may itself be responsible for an enhanced basic character of its "right" imidazole part and may also effectively contribute in cancelling the electron-withdrawing effect of the "left" imidazole in 4 on to its "right" imidazole part. The experimental evidence of participation of a canonical structure such as B (scheme 1) for the N^1 isomer 15 as compared to 4 can be found in their spectroscopic properties [δ values for ^{13}C & ^{1}H are in DMSO-d_6 and $CDCl_3$ solutions respectively]: (1) the C^2 [δ_C = 142. 3 ppm] and H^2 [δ_H = 8.07 ppm] are more deshielded in 15 than the corresponding absorptions for 4 [δ_C = 135.2 ppm, and δ_H = 7.73 ppm] due to an increased electron deficiency of "left" imidazole in the former; (2) interestingly, the resulting higher electron density of the "central" ring in 15, due to the resonating structure B (scheme 1), is also notably reflected in an enhanced shielding of its N^4-methyl group (δ_C = 31.2 ppm and δ_H = 3.97 ppm) as compared to the corresponding absorption (δ_C = 33.8 ppm, δ_H = 4.19 ppm) in the N^3 isomer 4, a contribution of structure B also clearly deshields the N^4 nitrogen (δ_N = 283.0 ppm) by ca. 6 ppm in 15 as compared to 4 (δ_N = 290.3 ppm in CH_2Cl_2); (3) the enhanced ionic character of the C^9-carbonyl group, due to favourable conjugation in 15, also results in its shielding (δ_C = 149.9 ppm) as compared to the C^9-carbonyl absorption in 4 (δ_C = 151.4 ppm); (4) because of the increase of the ionic character of the C^9-carbonyl in 15, the lone-pair of its N^8 is more delocalized in its "right" imidazole part causing an enhancement of its aromatic character as evident from 5.8 ppm downfield shift of its N^5 (δ_{N^5} = -158.2 ppm in 4 & -152.4 ppm in 15). The resonance effect of the ionic form B of 15 is also seen in its IR stretching vibration mode ($\upsilon^{CCl_4}_{C=O}$

1689 cm^{-1}) which has a less carbonyl stretching character than found in wyosine-triacetate 4 ($\upsilon_{C=O}^{CCl_4}$ 1701 cm^{-1}), establishing that the carbonyl-carbon (C^9) is more electron-rich in the latter.

Kinetics of the cleavage of the N-glycosidic bond in wyosine and its derivatives

Table 4 records the second-order rate constants for the hydrolysis of the N-glycosidic bond of the wyosine nucleosides studied. The rate constant obtained with wyosine (1) is in a complete agreement with the value of Itaya and Harada[26]. Acetylation of hydroxyl groups of the sugar moiety retards the hydrolysis by two orders of magnitude, as can be seen by comparing the reactivities of 1 and 16 to those of 4 and 17. A rate-retardation of this magnitude is expected on the basis of the mechanism suggested[26,30] for the hydrolysis of wyosine nucleosides, viz rate-limiting release of the protonated base moiety with concomitant formation of the free wyosine base and a resonance stabilized ribofuranosyl carbonium ion. As strongly electron-withdrawing substituents, acetyl groups reduce the electron density at the anomeric carbon, and hence destabilize the developing carbonium ion intermediate. The effect of the adjacent 2'-O-acetyl group is naturally largest. For comparison, slightly less electronegative 2'-chloro group (σ_m = 0.39 for $-OCOCH_3$ and 0.37 for -Cl)[31] has been shown[32] to retard the acid-catalyzed hydrolysis of methyl β-D-glucofuranoside by a factor of 20.

C^7-Substituents exert only a moderate influence on the hydrolytic stability of 4, in consistence with the suggested mechanism. Electronegative groups, for example, significantly reduce the basicity of the base moiety, as indicated above, but at the same time they make the wyosine base as a better leaving-group by increasing the polarity of the N-glycosidic bond. As in the hydrolysis of purine nucleosides[33,34], these two influences almost completely cancel each other. Except for the hydrolysis of the C^7-Br compound 11, the pH-rate profiles were observed to be linear, indicating that the second-order rate constants for the partial reactions via mono- and di-protonated substrates are almost equal, analogous to the hydrolysis of purine nucleosides[34]. With 7-bromowyosine derivative (11) the rate-profile is linear at high oxonium ion concentrations only ($[H^+] > 0.5$ mol dm^{-3}). In less acidic solutions nucleophilic attacks on the monoprotonated wyosine ring appear to compete with the hydrolysis of the N-glycosidic bond, resulting in a large positive deviation. The situation may in this respect be compared to the acidic hydrolysis of unsubstituted 9-(β-D-ribofuranosyl)purine[35].

The data in Table 4 also reveal that wyosine-triacetate 4 undergoes acidic hydrolysis 7 times more rapidly than its N^1-ribosylated counterpart 15. One may tentatively assume that the hydrolysis of 15 is not accelerated sterically as much as that of 4. The 9-oxo group in 15 is considerably smaller than N^4-methyl in 4 (E_s = -0.55 for OH and -1.24 for CH_3)[36] and hence the steric compression in the initial state is larger with 4 than with 15. However, the relatively small reactivity difference lends some support to the recent suggestion of Czarnik et al.[37], according to which the exceptionally high rate of the acidic hydrolysis of N^3-alkylated purine nucleosides, i.e. the structural analogues of wyosine nucleosides, could not be accounted for by steric acceleration only.

Binding of metal ion to wyosine and its precursors

The importance of metal ion interactions with nucleic acid components is now well recognized[39] because these phenomena are related to the toxic properties or anti-

tumor activity of metal ion-nucleoside or nucleotide conjugates. The "hard" metal ions[52] such as Mg^{2+}, Ca^{2+}, Ba^{2+} complex preferentially at oxygenated sites of nucleosides[53], therefore, ^{15}N NMR is not suitable for these kind of studies[40]. On the other hand, soft[52] metal ions such as Hg^{2+}, Pd^{2+}, Ga^{3+} or medium soft[52] metal ion such as Zn^{2+} bind usually to nitrogens in a nucleoside[41]. A large change in ^{15}N chemical shifts have been observed in the ^{15}N spectra of nucleosides upon addition of $HgCl_2$ and $Zn(NO_3)_2$ furnishing information regarding the site-specific metal ion binding to a particular nitrogen atom(s) in a molecule[42,43]. In order to compare the possible metal ion binding site(s) of wyosine and its derivatives with those of guanosine and desmethylwyosine derivatives, we herein report the effect of Hg^{2+} and Zn^{2+} on the ^{15}N resonances. Hg^{2+} and Zn^{2+} have been chosen because of their great affinities for endocyclic nitrogens of nucleobases[41]. ^{15}N-NMR studies have been carried out in DMSO solutions since relatively high concentrations of both metal ion and nucleoside can be achieved in this medium. The results were compared with the protonation studies in the same solvent. As mentioned in the introduction, the unstability of wyosine (1) has limited its study in the present context.

(i) Studies with guanosine, inosine and their triacetates.

The N^7 is the binding site of Hg^{2+} and Zn^{2+} in guanosine, inosine and their triacetates, although the pronounced upfield shifts of N^7 is larger with Zn^{2+} than Hg^{2+} in the triacetate 22. An almost equal shift of N^7 of guanosine (21) has been found[42] upon complexation with Hg^{2+} and Zn^{2+}. As found in the protonation studies (vide supra), the acetate functions of the sugar moiety also reduced the complexation ability of N^7 in 22 and 24. However, it seems that Zn^{2+} is insensi tive to this effect since the N^7 shift in 22 with 1 equiv. of Zn^{2+} is very close to that of Zn^{2+}-guanosine complex[42], while with 1 equiv. of Hg^{2+} the N^7 shift is reduced by ca. 40% in 22. The greatest affinity of Zn^{2+} to complex with N^7 of purines has been demonstrated[29] with adenosine to which Zn^{2+} has N^7 as the binding site while Hg^{2+} complexes with its N^1 which is also its protonation site[21]. Therefore, Hg^{2+} behaves like H^+ while Zn^{2+} which is "harder" [52] than Hg^{2+} goes to a site(s) where electrostatic interactions are more favoured. The N^3 moves slightly upfield but remains to be a minor binding site. Zn^{2+} and Hg^{2+} complex to N^7 of inosine-triacetate (24) as strongly ($\Delta\delta$ ca. 3.5 ppm) as found upon its protonation. Surprisingly, the strength of Hg^{2+} complexation with inosine-tri acetate is comparable to that of inosine (24)[43] but Zn^{2+}, however, complexes with it rather poorly. This perhaps indicates that the N^7 of inosine is comparatively a softer base that that of guanosine.

(ii) Studies with N^4-Desmethylwyosine and its triacetates.

The site of protonation of N^4-desmethylwyosine 5a, by TFA in DMSO, is the N^1 nitro gen with a magnitude almost equivalent of that of guanosine, $\Delta\delta$ = 41 ppm and 46 ppm, respectively. It is interesting to note that Hg^{2+} behaves as H^+, as found in other studies (vide supra), and produces a similar upfield ^{15}N chemical shifts. Zn^{2+} has, however, much less affinity to N^1 in 5a or 5b than in 21 or 22. On the other hand, the N^1 shift with 1 equiv. of $Zn(NO_3)_2$ is only 3 ppm in 5b and 9.5 ppm for 5a. While Hg^{2+}, with 0.5 equiv. of $HgCl_2$, induced a shift of 5 ppm for 5b (4 ppm for the protonation with 0.5 equiv. of TFA) and 5 ppm for 5a (11.5 ppm for 5a with 1.0 equiv. of Hg^{2+}). Therefore, the nature of complexation of Hg^{2+} with 5a and 5b is comparable to that of complexation of Zn^{2+} with guanosine-triacetate (22), however, the complexation of Zn^{2+} with 5a is not as efficient as with 21.

(iii) Studies with wyosine and wyosine-triacetate.

The N^1 in wyosine (1) moves only by ca. 1 ppm with 1 equiv. of $Zn(NO_3)_2$ while the N^6 of 1 and 4 binds to Hg^{2+} very slightly, producing only 1.5 and 2.3 ppm shifts respectively with 0.5 equiv. of $HgCl_2$, denoting that the N^6 of 1 and 4 are indeed very soft basic centers. This also indicates that such poor strengths of these complexations with these medium soft (Zn^{2+}) and soft (Hg^{2+}) metal ions[52] rule out any possibilty of formimg a complex with a hard metal ion[53] such as Mg^{2+}. These observations suggest that the nucleophilicity of the N^6 nitrogen in wyosine is relatively poorer than that of N^7 in guanosine or N^1 of N^4-desmethylwyosine and hence the interaction is presumably not thermodynamically feasible. Since the binding sites of nucleosides are usually those of the nucleotides, it is therefore unlikely that Hg^{2+}, Zn^{2+} or Mg^{2+} would at all bind to the Y-base of a wyosine-phosphate derivative. Our present study therefore substantiates low-resolution X-ray diffraction data[50,51] of $tRNA^{Phe}$ that the role of wyosine at the 3'-end of the yeast $tRNA^{Phe}$ loop does not involve any binding with Mg^{2+} which is most probably coordinated to phosphate 37 in the anticodon loop[4], as would be expected from its properties as a hard metal ion[53]. This study therefore reinforces the theory[4,5] that wyosine-nucleotide may act as a regulator of the hydrophobicity inside the anticodon loop.

Conclusions

The aromatic character of the "right" imidazole ring in N^4-methyl isomer 4 and N^6-methyl isomer 18b controls the reactivities of their respective tricylic aglycones. Both protonation and binding to metal ions in compound 18b take place at N^1 but the C^7 is its site for electrophilic reactions (in guanosine, site of protonation and ion-binding is N^7, while electrophilic reactions take place at C^2). On the other hand, in wyosine-triacetate 4, both the preponderant protonation site (N^6) and the site for electrophilic reaction (C^7) is in the "right" imidazole ring. Clearly, the aromatic character of the "right" imidazoles in 4 and 18b are determined by the π-electron delocalizing abilities of their nitrogen atoms; N^4 methylation in 4 completely locks the N^6 nitrogen in sp^2 hybridized state which contributes to the enhanced bascity of its "right" imidazole ring as compared to compound 18b. In comparison with purine nucleosides, the overall reactivity of wyosine-triacetate 4 seems to be much poorer. This work has shown that the 4 does not bind to any metal ions and it is also shown in the present work that the 4 is a poor proton-acceptor in an acidic medium (compare for example $\Delta\delta^{15}N$ shifts in DMSO of 4 [N^1 ~ 0.2 ppm and N^6 ~ 11.5 ppm] with those of 5b [N^1 ~ 10 ppm], 18b [N^1 ~ 12 ppm, N^6 ~ -0.5 ppm], 18d [N^1 ~ 12.5 ppm, N^1 ~ -0.8 ppm], 22 [N^7 ~ 27 ppm] and 24 [N^7 ~ 4 ppm]). On the other hand, it has been reported[49] that Y-base gives a rigid conformation of the anticodon loop[50,51] due to its strong stacking properties with the 3'-neighbouring base and recent data[4,5], along with our present metal ions binding studies, also suggest that Mg^{2+} ion binds to its phosphate and thus directs the 3'-stack $\rightleftharpoons$ 5'-stack equilibrium. Thus its poor basicity and its enhanced stacking ability and hydrophobicity, than any other purine base[49], makes it an ideal candidate for giving the $tRNA^{Phe}$ anticodon loop a conformation such as to provide an unusually high affinity (codon-anticodon interaction) to bind to complementary mRNA sequence in ribosomal complex for phenylalanine biosynthesis in yeast cells.

EXPERIMENTAL

^{1}H-NMR spectra at 90 MHz and ^{13}C NMR at 23.7 MHz were recorded with Jeol FX 90Q instrument. Tetramethylsilane was used as the internal standard and the chemical shifts are reported in ppm (δ scale). UV absorption spectra were recorded with a Varian-Cary 2200 instrument and Jeol DX 303 instrument was used for recording the mass spectra. IR absorption was recorded with Perkin-Elmer 298 spetrometer. Thin-layer chromatography (t.l.c.) was performed on Merck precoated $60F_{254}$ plates. Merck Kieselgel G was used for short column chromatography.

^{15}N NMR. ^{15}N chemical shift determinations were made on a Jeol GX 270 spectrometer at 27.4 MHz. All ^{15}N NMR spectra were performed relative to $CH_3{}^{15}NO_2$ in CD_3NO_2 in a capillary. The assignments of ^{15}N resonances were done by fully proton decoupled condition (NOE) or under an inverse gated proton-noise decoupled mode (without NOE) or using INEPT pulse sequences[18,22,44-47]. Routinely, 16 K data points were used for the acquisition, zero filled to 32 K and Fourier transformed with a broadening factor of 1-3 Hz. The samples were washed by an EDTA solution in order to remove metallic impurities and dissolved in distilled DMSO or CH_2Cl_2. The accuracy of the ^{15}N chemical shift is estimated to be within 0.1 ppm. A negative value for the chemical shift denotes an upfield shift.

Kinetic measurements: Kinetic measurements were carried out by the HPLC technique described in detail previously[34].

Acidity constants: These were determined spectrophotometrically (Cary 17D as described earlier[38].

Preparation of 4,9-dihydro-4,6-dimethyl-9-oxo-1-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)imidazo [1,2-a]purine (15).

To a solution of wyosine-triacetate (115 mg, 0.25 mmol) in dichloromethane (5 ml) anhydrous aluminium chloride (66 mg, 0.5 mmol) was added at 0 °C. After 0.5 h, the ice-water bath was removed and the stirring was continued overnight at room temperature. The reaction mixture was poured into cold saturated sodium bicarbonate solution (30 ml) and then extracted with dichloromethane (2 x 30 ml). The organic phase was dried with $MgSO_4$ and evaporated. The residue was purified by preparative TLC to give the title compound, yield 85 mg (74%). UV (MeOH): UV (MeOH): λ_{max} = 312 nm (ϵ = 4.400), 260 nm (ϵ = 4.800), 231 nm (ϵ = 22.500) [pH 7]; 312 nm (ϵ = 4.400), 260 nm (ϵ = 4.800), 231 nm (ϵ = 22.500) [pH 13]; 285 nm (ϵ = 5.870), 234 nm (ϵ = 26.300) [pH 2]; MS (FAB$^+$): calc. for $(M+H)^+$ 462.1625, found 462.1585. $[\alpha]_D^{20}$ +31.7 (c 0.09, MeOH). ^{1}H-NMR ($CDCl_3$): 8.06 (s, 1H) H2; 7.37 (d, $^4J_{H-CH_3}$ = 1.2 Hz, 1H) H-7; 6.44 (d, $J_{1',2'}$ = 4.4 Hz, 1H) H-1'; 5.74 (dd, $J_{2',3'}$ = 4.2 Hz, 1H) H-2'; 5.47 (m, 1H) H-3'; 4.44 (m, 3H) H-4',5',5''; 3.97 (s, 3H) N-CH_3, 2.35 (d, 3H) 6-CH_3; 2.16, 2.12 (9H) 3 x OAc. ^{13}C-NMR ($CDCl_3$): 149.4 (C-9); 149.0 (C-4a); 142.3 (C-3a); 140.3 (d, J_{CH} = 212 Hz) C-2; 138.4 (C-6); 105.4 (d, J_{CH} = 197 Hz) C-7; 104.9 (C-9a); 88.5 (d, J_{CH} = 171 Hz) C-1'; 79.7 (d, J_{CH} = 151 Hz) C-4'; 74.0 (d, J_{CH} = 160 Hz) C-2'; 69.3 (d, J_{CH} = 155 Hz) C-3'; 62.4 (t, J_{CH} = 148 Hz) C-5'; 31.0 (N-CH_3); 14.1 (6-CH_3).

Preparation of 2',3',5'-tri-O-(t-butyldimethylsilyl) [TBDMS] wyosine (6)

Wyosine-triacetate (180 mg, 0.39 mmol) in methanolic ammonia (10 ml) was stirred at room temperature for 3 h. The solvent was removed and the residue was dried by coevaporations with toluene. The solid was dissolved in dimethylformamide (3 ml) and then imidazole (238 mg, 3.51 mmol) and t-butyldimethylsilyl chloride (315 mg, 2.34 mmol) was added. The stirring was continued at room temperature overnight. The mixture was poured into a solution of cold sodium bicarbonate (30 ml) which was extracted with dichloromethane (3 x 30 ml). The organic phase was evaporated and coevaporated with toluene to dryness. The residue was purified on silica gel column, yield 232 mg (88%). UV (MeOH): λ_{max} = 289 nm (ϵ = 6.800), 234 nm (ϵ = 29.500) [pH 7]; 290 nm (ϵ = 7.100), 234 nm (ϵ = 33.300) [pH 13]; 270 nm (ϵ = 10.900), 226 nm (ϵ = 31.100) [pH 2]. MS (FAB$^+$): calc. for $(M+H)^+$ 678.3903, found 678.3932. $[\alpha]_D^{20}$ -19.9 (c 0.091, MeOH). ^{1}H-NMR ($CDCl_3$): 7.98 (s, 1H); 7.48 (d, $^4J_{H^7-CH_3}$ = 1 Hz, 1H) H-7; 6.27 (d, $J_{1',2'}$ = 7.6 Hz, 1H) H-1'; 4.4 (m, 1H) H-4'; 4.22-4.17 (m, 5H) H-2',3', N-CH_3; 3.86 (m, 2H) H-5',5''; 2.35 (d, 3H) 6-CH_3; 0.98, 0.76 (m, 27H) 3 x t-butyl; 0.17 (m, 18H) 6 x CH_3. ^{13}C-NMR ($CDCl_3$): 151.9 (C-9); 142.3 (C-4a); 139.3 (C-3a); 137.6 (C-6); 134.3 (d, J_{CH} = 215 Hz) C-2; 116.2 (C-9a); 106.4 (d, J_{CH} = 195 Hz); 87.1 (d, J_{CH} = 151 Hz) C-1', C-4'; 78.0 (d, J_{CH} = 144 Hz) C-2'; 73.0 (d, J_{CH} = 155 Hz) C-3'; 63.1 (t, J_{CH} = 142 Hz) C-5'; 34.4 (N-CH_3); 14.0 (6-CH_3).

Preparation of 2',3',5'-tri-O-acetyl-7-formyl wyosine (7)

Phosphorus oxychloride (746 µl, 8 mmol) was added to anhydrous dimethylformamide (1.5 ml) at 0 °C, which was then kept at room temperature for 15 min. This reagent was dropped into a solution of wyosine-triacetate (461 mg, 1 mmol) in anhydrous dimethylformamide (3 ml) in ice-water bath. Stirring was continued for 45 min and then the mixture was poured into a cold solution of sodium bicarbonate (30 ml) and extracted with dichloromethane (3 x 30 ml). Solvent was removed under vaccum and coevaporated with toluene to dryness. The residue was purified on a silica gel column, yield 404 mg (83%). UV (MeOH): λ_{max} = 318 nm (ε = 18.900), 232 nm (ε = 25.800) [pH 7]; 322 nm (ε = 12.600) 234 nm (ε = 23.500) [pH 13]; 318 nm (ε = 12.400) 282 nm (ε = 7.500), 231 nm (ε = 27.100) [pH 2]. MS (FAB^+): calc. for $(M+H)^+$490.1574, found 490.1559; $[\alpha]_D^{20}$ -25.7 (c 0.105, MeOH). ^{1}H-NMR ($CDCl_3$): 10.8 (s, 1H) -CHO; 7.79 (s, 1H) H-2; 6.28 (d, $J_{1',2'}$ = 5.9 Hz, 1H) H-1'; 5.92 (dd, $J_{2',3'}$ = 5.8 Hz, 1H) H-2'; 5.50 (dd, $J_{3',4'}$ = 4 Hz, 1H) H-3'; 4.54 (m, 1H) H-4'; 4.31 (d, 2H) H-5',5''; 4.25 (s, 3H) NCH_3; 2.61 (s, 3H) 6-CH_3; 2.19, 2.10 (9H) 3 x OAc. ^{13}C-NMR ($CDCl_3$): 182.2 (d, J_{CH} = 188 Hz) CHO; 153.0 (C-9); 150.4 (C-4a); 143.9 (C-3a); 138.9 (C-6); 134.7 (d, J_{CH} = 217 Hz) C-2; 125.8 (C-9) 86.3 (d, J_{CH} = 165 Hz) C-1'; 81.1 (d, J_{CH} = 153 Hz) C-4'; 72.8 (d, J_{CH} = 154 Hz) C-2'; 70.6 (d, J_{CH} = 153 Hz) C-3'; 62.7 (t, J_{CH} = 147 Hz) C-5'; 34.3 (4-NCH_3); 15.0 (6-CH_3).

Preparation of 2',3',5'-tri-O-acetyl-7-(1,3-dithane-2-yl)wyosine (8a)

To a solution of 7-formylwyosine-triacetate (250 mg, 0.51 mmol) in 1,2-ethanedi thiol (2.0 ml, 19.0 mmol) was added seven drops saturated solution of hydrogen chloride in acetic acid at 0 °C. After 10 min the ice-water bath was removed and the stir-ring was continued at room temperature for 1 h. The mixture was poured into a cold solution of sodium bicarbonate which was extracted with dichloro methane (3 x 20 ml). The organic phase was evaporated and coevaporated with toluene to dryness. The residue was purified by a silica gel column, yield 235 mg (82%). UV (MeOH): λ_{max} = 300 nm (ε = 5.200), 282 nm (ε = 5.200), 242 nm (ε = 24.500) [pH 7]; 300 nm (ε = 5.200), 242 nm (ε = 25.100) [pH 13]; 278 nm (ε = 8.400), 232 nm (ε = 20.600) [pH 2]. MS (FAB^+): calc. for $(M+H)^+$ 566.1379, found 566.1387. $[\alpha]_D^{20}$ -26.6 (c 0.092, MeOH). ^{1}H-NMR ($CDCl_3$): 7.71 (s, 1H) H-2; 7.41 (s, 1H) H-C[SCH_2]$_2$ at C^7; 6.23 (d, $J_{1',2'}$ = 6.1 Hz, 1H) H-1'; 5.84 (dd, $J_{2',3'}$ = 4.6 Hz, 1H) H-2'; 5.47 (dd, $J_{3',4'}$ = 3.6 Hz, 1H) H-3'; 4.50 (m, 1H) H-4'; 4.30 (m, 2H) H-5',5''; 4.13 (s, 3H) N-CH_3; 3.41 (m, 4H) -S-CH_2CH_2-S-; 2.51 (s, 3H) 6-CH_3; 2.17, 2.14, 2.10 (3s, 3 x 3H) 3 x OAc. ^{13}C-NMR ($CDCl_3$): 154.2 (C-9); 142.2 (C-4a); 139.1 (C-3a); 138.4 (C-6); 133.4 (d, J_{CH} = 213 Hz) C-2; 119.9 (C-9a); 109.1 (C-7); 85.7 (d, J_{CH} = 170 Hz) C-1'; 80.8 (d, J_{CH} = 153 Hz) C-4'; 72.6 (d, J_{CH} = 154 Hz) C-2'; 70.5 (d, J_{CH} = 157 Hz) C-3'; 62.6 (t, J_{CH} = 150 Hz) C-5'; 45.5 (d, J_{CH} = 161 Hz) H-C[SCH_2]$_2$; 39.8 (m, -S-CH_2CH_2-S-); 33.7 (N-CH_3); 15.2 (6-CH_3).

Preparation of 2',3',5'-tri-O-(t-butyldimethylsilyl) [TBDMS]-7-(1,3-dithane-2-yl)-wyosine(8b)

Compound (8a) (110 mg, 0.19 mmol) in methanolic ammonia (10 ml) was stirred at room temperature for 3 h. The solvent was removed and the residue was dried by coevaporations with toluene. The solid was dissolved in dimethylformamide (1 ml) and then imidazole (95 mg, 1.4 mmol) and t-butyldimethylsilyl chloride (150 mg, 1 mmol) was added. The stirring was continued at room temperature overnight. The mixture was poured into a solution of cold sodium bicarbonate (20 ml) which was extracted with dichloromethane (3 x 20 ml). The organic layer was evaporated and coevaporated with toluene to dryness. The residue was purified on silica gel column, yield 105 mg (65%). UV (MeOH): λ_{max} = 298 nm (ε = 6.500), 279 nm (ε = 6.500), 242 nm (ε = 30.200) [pH 2]; 298 nm (ε = 5.900), 279 nm (ε = 6.500), 242 nm (ε = 27.000) [pH 13]; 280 nm (ε = 9.500), 234 nm (ε = 25.700) [pH 2]. MS (FAB^+): calc. for $(M+H)^+$ 782.3657, found 782.3734. $[\alpha]_D^{20}$ -26.0 (c 0.096, MeOH). ^{1}H-NMR ($CDCl_3$): 7.93 (s, 1H) H-2; 7.55 (s, 1H) H-C[SCH_2]$_2$; 6.22 (d, $J_{1',2'}$ = 7.5 Hz) H-1'; 4.43 (m, 1H) H-4'; 4.18-4.11 (m, 5H) H-2',3', N-CH_3; 3.84 (m, 2H) H-5',5''; 3.45 (m, 4H) -S-CH_2CH_2-S-; 2.55 (s, 3H) 6-CH_3: 0.97, 0.78 (27H) 3 x t-butyl; 0.16 (18H) 6 x CH_3. ^{13}C-NMR ($CDCl_3$): 154.5 (C-9); 142.4 (C-4a); 139.0 (C-3a); 138.2 (C-6); 134.2 (d, J_{CH} = 224 Hz) C-2; 119.7 (C-9a); 116.7 (C-7); 86.9 (d, J_{CH} = 155 Hz) C-4'; 86.9 (d, J_{CH} = 160 Hz) C-1'; 77.8 (d, J_{CH} = 142 Hz) C-2'; 73.1 (d, J_{CH} = 155 Hz) C-3'; 63.2 (t, J_{CH} = 150 Hz) C-5'; 45.6 (-C-[S-CH_2]$_2$); 39.8 (-C-[S-CH_2]$_2$) 34.2 (N-CH_3); 15.2 (6-CH_3).

Preparation of cyanosilylated adduct of 2',3',5'-tri-O-acetyl-7-formylwyosine (9)

To a solution of 7-formylwyosine-triacetate (100 mg, 0.20 mmol) in dichloromethane (8 ml) trimethylsilyl cyanide (52 µl, 0.71 mmol) and one crystal of zinc iodide were added. The stirring was continued overnight at room temperature. Solvent was removed under vaccum and the foam was dissolved in dichloromethane (50 ml) which was washed with water (2 x 20 ml). The organic phase was dried with $MgSO_4$ and evaporated to obtain desired compound, yield 115 mg (96%). UV (MeOH): λ_{max} = 295 nm (ϵ = 6.800), 239 nm (ϵ = 26.000), [pH 7]; 322 nm (ϵ = 14.000), 230 nm (ϵ = 17.000) [pH 13]; 320 nm (ϵ = 6.800), 250 nm (ϵ = 9.400), 277 nm (ϵ = 16.500) [pH 2]. MS (FAB^+): calc. for M^+ 588.2000, found 588.2045. $[\alpha]_D^{20}$ -25,9 (c 0.093, MeOH). ^{1}H-NMR ($CDCl_3$): 7.78 (s, 1H) H-2; 7.18 (s, 1H) H-C(CN)TMS; 6.24 (d, $J_{1',2'}$ = 5.9 Hz, 1H) H-1'; 5.85 (m, 1H) H-2'; 5.50 (m, 1H) C-3'; 4.51 (m, 1H) H-4'; 4.31 (m, 2H) H-5',5''; 4.16 (s, 3H) N-CH_3; 2.50 (s, 3H) 6-CH_3; 2.17, 2.14, 2.10 (3s, 3 x 3H) 3 x OAc. ^{13}C-NMR ($CDCl_3$): 152.4 (C-9); 142.5 (C-4a); 140.2 (C-3a); 139.3 (C-6); 133.8 (d, J_{CH} = 217 Hz) C-2; 118.3 (C-9a); 118.0 (C-7); 85.7 (d, J_{CH} = 165 Hz) C-1'; 80.9 (d, J_{CH} = 152 Hz) C-4'; 72.4 (d, J_{CH} = 160 Hz) C-2'; 70.4 (d, J_{CH} = 162 Hz) C-3'; 62.5 (t, J_{CH} = 151 Hz) C-5'; 54.4 (d, J_{CH} = 153 Hz) H-C(CN)TMS; 33.7 (4-NCH_3); 13.8 (6-CH_3).

Preparation of 2',3',5'-tri-O-acetyl-7-hydroxylmethyl wyosine (10)

To a solution of 7-formylwyosine-triacetate (100 mg, 0.2 mmol) in dry tetrahydrofuran (2 ml) sodium borohydride (3.7 mg, 0.1 mmol) was added at 0 °C. The stirring was continued for 2 h and then the mixture was poured into a cold solution of ammonium chloride (10 ml) which was extracted with dichloromethane (2 x 20 ml). The organic phase was dried with $MgSO_4$ and the residue was purified with preparative TLC, yield 70 mg (70%). UV(MeOH): λ_{max} = 292 nm (ϵ = 6.300), 238 nm (ϵ = 25.200) [pH 7]; 292 nm (ϵ = 7.100), 235 nm (ϵ = 28.300) [pH 13]; 272 nm (ϵ = 10.500), 228 nm (ϵ = 23.400) [pH 2]. MS (FAB^+): calc. for M^+ 491.1653, found 491.1615. $[\alpha]_D^{20}$ -24,8 (c 0.131, MeOH). ^{1}H-NMR ($CDCl_3$): 7.72 (s, 1H) H-2; 6.26 (d, $J_{1',2'}$ = 5.7 Hz, 1H) H-1'; 5.87 (dd, $J_{2',3'}$ = 5.9 Hz; 1H) H-2'; 5.48 (dd, $J_{3',4'}$ = 3.7 Hz, 1H) H-3'; 4.77 (broad, 2H) -CH_2OH; 4.51 (m. 1H) H-4'; 4.30 (d, 2H) H-5',5''; 4.15 (s, 3H) N-CH_3; 2.24 (s, 3H) 6-CH_3; 2.16, 2.09 (9H) 3 x OAc. ^{13}C-NMR ($CDCl_3$): 154.2 (C-9); 142.5 (C-4a); 139.6 (C-3a); 135.7 (C-6); 133.9 (d, J_{CH} = 214 Hz) C-2; 122.1 (C-7); 116.8 (C-9a); 85.7 (d, J_{CH} = 168 Hz) C-1'; 80.7 (d, J_{CH} = 153 Hz) C-4'; 72.5 (d, J_{CH} = 155 Hz) C-2'; 70.2 (d, J_{CH} = 162 Hz) C-3'; 62.4 (t, J_{CH} = 151 Hz) C-5'; 53.7 (C^7-CH_2OH); 33.7 (4-NCH_3); 12.2 (6-CH_3).

Preparation of 2',3',5'-tri-O-acetyl-7-bromowyosine (11)

To a solution of wyosine-triacetate (150 mg, 0.32 mmol) in dichloromethane (2 ml) bromodimethylsulfonium bromide (85 mg, 0.38 mmol) in dichloromethane (2 ml) was added dropwise at room temperature under Argon. Stirring was continued for 1 h then the mixture was poured into a ice-cold sodium bicarbonate solution (20 ml) which was extracted with dichloromethane (2 x 20 ml). The organic layer was dried with $MgSO_4$. After evaporation the residue was purified on a silica gel column, yield 107 mg (62%). UV (MeOH): λ_{max} = 296 nm (ϵ = 6.600), 240 nm (ϵ = 29.600) [pH 7]; 296 nm (ϵ = 6.300), 239 nm (ϵ = 29.200) [pH 13]; 277 nm (ϵ = 7.800), 236 nm (ϵ = 26.900) [pH 2]; MS (FAB^+): calc. for M^+ 539.0652, found 539.0654. $[\alpha]_D^{20}$ -21.1 (c -0,114, MeOH). ^{1}H-NMR ($CDCl_3$): 7.62 (s, 1H) H-2; 6.26 (d, $J_{1',2'}$ = 6.1 Hz, 1H) H-1'; 5.93 (dd, $J_{2',3'}$ = 5.6 Hz, 1H) H-2'; 5.48 (dd, $J_{3',4'}$ = 2.7 Hz, 1H) H-3'; 4.50 (m, 1H) H-4'; 4.30 (m, 2H) H-5',5''; 4.13 (s, 3H) NCH_3; 2.21 (s, 3H) 6-CH_3; 2.20, 2.18, 2.10 (3s, 3 x 3H) 3 x OAc. ^{13}C-NMR ($CDCl_3$): 152.3 (C-9); 142.4 (C-4a); 138.4 (C-3a); 137.6 (C-7); 133.4 (d, J_{CH} = 217 Hz) C-2; 116.3 (C-4a); 85.8 (d, J_{CH} = 166 Hz) C-1'; 80.7 (d, J_{CH} = 153 HZ) C-4'; 72.4 (d, J_{CH} = 151 Hz) C-2'; 70.5 (d, J_{CH} = 160 Hz) C-3'; 62.6 (t, J_{CH} = 150 Hz) C-5'; 33.5 (4-NCH_3); 12.7 (6-CH_3).

Preparation of 2',3',5'-tri-O-acetyl-7-iodo-wyosine (12)

To a suspension of wyosine-triacetate (230 mg, 0.5 mmol), silver trifluoroacetate (110 mg, 0.5 mmol) in dichloromethane (8 ml), a solution of iodine (127 mg, 0.5 mmol) in dichloromethane (6 ml) was added dropwise. After 10 min, the mixture was poured into a ice-cold sodium bicarbonate solution. Filterred, and the aqueous phase was extracted with dichloromethane (2 x 20 ml). The organic phase was washed with saturated $Na_2S_2O_3$ solution (20 ml), water (20 ml) and then dried on $MgSO_4$. After evaporation the residue was purified on a silica gel column. Yield 260 mg (89%). UV (MeOH): λ_{max} = 300 nm (ϵ = 5.000), 240 nm (ϵ = 22.500) [pH 7], 292 nm (ϵ = 5.500), 232 nm (ϵ = 27.000) [pH 13]; 270 nm (ϵ = 9.000), 226 nm (ϵ = 37.000)

[pH 2]. MS (FAB^+): calc. for $(M)^+$ 587.0513, found 587.0493. $[\alpha]_D^{20}$ -15.7 (c 0.108, MeOH). ^{1}H-NMR ($CDCl_3$): 7.66 (s, 1H) H-2; 6.22 (d, $J_{1',2'}$ = 5.9 Hz, 1H) H-1'; 5.89 (dd, $J_{2',3'}$ = 5.3 Hz, 1H) H-2'; 5.48 (dd, $J_{3',4'}$ = 3.8 Hz, 1H) H-3'; 4.50 (m, $J_{4',5'}$ = 3.0 Hz, 1H) H-4'; 4.31 (d, 2H) H-5', H-5''; 4.14 (s, 3H) N-CH_3; 2.28 (s, 3H) 6-CH_3; 2.18, 2.10 (2s, 3 x 3H) 3 x OAc. ^{13}C-NMR ($CDCl_3$): 152.3 (C-9); 144.2 (C-4a); 143.6 (C-3a); 138.7 (C-6); 133.4 (d, J_{CH} = 222 Hz) C-2; 116.4 (C-9a); 111.6 (C-7); 85.8 (d, J_{CH} = 165 Hz) C-1'; 80.7 (d, J_{CH} = 153 Hz) C-4'; 72.4 (d, J_{CH} = 156 Hz) C-2'; 70.6 (d, J_{CH} = 157 Hz) C-3'; 62.6 (t, J_{CH} = 151 Hz) C-5'; 33.9 (N-CH_3); 14.3 (6-CH_3).

Synthesis of 2-acetyl-2',3'-5'-O-triacetyl wyosine (13)

Method 1:
To the solution of 2',3',5'-O-triacetylwyosine (100 mg, 0.22 mmol) in dry THF (4 ml) at -70 °C, was added n-butyllithium (0.17 ml of 2.6 M solution in hexane, 0.43 mmol). After stirring at -70 °C for 70 min, methyl iodide (0.05 ml 0.52 mmol) was added to the reaction mixture. The reaction was kept at -70 °C for another 20 min and then poured into saturated sodium hydrogen carbonate solution (20 ml) and extracted with dichloromethane (2 x 20 ml). The organic layer was washed with water once, and dried *in vacuo*. After coevaporation with pyridine for two times, the residue was dissolved in pyridine (3 ml). To the solution was added acetic anhydride (0.2 ml, 2 mmol). After reaction for 1.5 h at room temperature, the reaction mixture was worked up in the usual way. The product was purified by short silica gel column chromatography. Yield 51 mg (47%). UV (MeOH): λ_{max} = 278 nm (ϵ = 21.400), 327 (ϵ = 13.600) [pH 7]; 261 nm (ϵ = 15.200), 278 (sh), 302 (ϵ = 14.500) [pH 2]; 241 nm (18.900), 263 nm (ϵ = 14.800), 299 nm (ϵ = 18.700), 343 nm (ϵ = 12.800) [pH 13]. MS (FAB^+). calc. for $(M+H)^+$ 504.1653 (14%), found 504.2078; m/z 244 (41.3%) [2-acetyl-wyosine + H]$^+$ and m/z 259 (13%) (2',3',5'-triacetylpentose). ^{1}H-NMR ($CDCl_3$): 7.40 (d, J = 1.2 Hz, 1H) H-7; 6.30 (d, J = 4.9 Hz, 1H) H-1'; 5.72 (m, 2H) H-2' and H-3'; 4.39 (m, 3H) H-4' and H-5',5''; 4.14 (s, 3H) N-CH_3; 2.75 (s, 3H) 2-acetyl; 2.32 (d, J = 1.2 Hz, 3H) 6-CH_3; 2.13, 2.11, 2.07 (3s, 3 x 3H) 3 x OAc. ^{13}C-NMR ($CDCl_3$): δ = 190.2 (2-CO); 151.6 (C-9); 143.1 (C-4a)*, 142.6 (C-3a)*; 142.4 (C-2)*; 138.4 (C-6); 115.9 (C-9a); 107.0 (d, J_{CH} = 197 Hz) C-7; 88.7 (d, J_{CH} = 161 Hz) C-1'; 80.4 (d, J_{CH} = 150 Hz) C-4'; 73.2 (d, J_{CH} = 160 Hz) C-2'; 69.1 (d, J_{CH} = 159 Hz) C-3'; 62.4 (t, J_{CH} = 151 Hz) C-5'; 36.3 (N-CH_3) 27.4 (2-acetyl CH_3); 14.1 (6-CH_3). [**These assignments may interchange.*]

Method 2
The 2',3',5'-O-triacetylwyosine (46.2 mg, 0.1 mmol) was dissolved in dry THF (2 ml), and treated with n-butyllithium (0.04 ml, 0.11 mmol) in the similar way as described in Method 1. After 1 h, a solution of CH_3COOD (0.1 ml, 1 mmol) in deuterium oxide (0.5 ml) was added. The reaction was kept at -70 °C for another 10 min and then worked up in the usual way. The organic layer was dried and acetylated with acetic anhydride (0.1 ml, 1 mmol) in pyridine (1.5 ml) as described in *method 1*. The product was purified by short silica gel column chromatography. Yield: 26 mg (51%). The spectroscopic properties [^{1}H-NMR, ^{13}C-NMR and UV] of this product was identical to the ones obtained for product isolated by method 1.

Preparation of 2-C-deuterio-2',3',5'-tri-O-(t-butyldimethylsilyl)wyosine (14)

To the solution of 2',3',5'-tri-O-(t-butyldimethylsilyl)wyosine (97 mg, 0.14 mmol) in dry THF (3 ml) at -70 °C, was added n-butyllithium (0.1 ml of 2.6 M solution in hexane, 0,26 mmol). After stirring at -70 °C for 1 h, deuterium oxide (0.5 ml, 25 mmol, 99.9 atom % D atom) was added to the reaction mixture. The reaction was kept at -70 °C for another 20 min and then poured into saturated ammonium chloride solution (20 ml) and extracted with dichloromethane (2 x 20 ml). The organic layer was washed with water (20 ml) and dried with $MgSO_4$. After evaporation pure product was obtained, yield 96 mg (100%). ^{1}H-NMR ($CDCl_3$): 7.48 (d, $^4J_{H-CH_3}$ = 1 Hz, 1H) H-2; 6.27 (d, $J_{1',2'}$ = 7.3 Hz, 1H) H-1'; 4.42 (m, 1H) H-4'; 4.24 (m, 2H) H-2', H-3'; 4.17 (s, 3H) N-CH_3; 3.86 (m, 2H) H-5, H-5''; 2.35 (d, $^4J_{H-CH_3}$ = 1 Hz, 3H) 6-CH_3; 0.98, 0.76 (m, 27H) 3 x t-butyl; 0.17 (m, 18H) 6 x CH_3; MS (FAB^+): calc. for $(M+H)^+$ 679.3965, found 679.3979.

Preparation of 5,9-dihydro-5,6-dimethyl-7-formyl-9-oxo-3-(2',3',5'-O-triacetyl-β-D-ribofuranosyl)imidazo[1,2-a]purine (19)

Phosphorus oxychloride (158 µl, 1.7 mmol) was added to anhydrous dimethylformamide (0.5 ml) at 0 °C, which was then kept at room temperature for 15 min. This reagent was dropped into a solution of N^5-methyl isomer of wyosine-triacetate (80 mg,

Table 1: ^{15}N chemical shifts[a] of wyosine derivatives in neutral and acidic DMSO (T = 30 °C) and/or CH_2Cl_2 (T = 25 °C) solutions.

Compound	TFA (equiv.)	N^1	N^3	N^4	N^5	N^8
4[b]	0	-130.5	-220.6	-290.3	-158.2	-192.1
	1	-132.9	-217.7	-287.9	-198.1	-192.9
4[c]	0	-129.6	-218.0	-287.4	-156.6	-191.2
	1	-129.8	-217.4	-286.7	-168.0	-191.5
6[d]	0	-130.3	-219.0	-291.4	-159.3	-192.2
	1	-133.6	-216.3	-289.8	-191.3	-192.9
6[e]	0	-129.1	-216.7	-288.8	-156.9	-191.6
	1	-129.5	-216.1	-288.4	-167.6	-191.8
7[d]	0	-130.7	-219.0	-285.7	-153.7	-198.0
	1	-137.3	-218.2	-285.2	-154.7	-198.1
17[b]	0	-130.0	-221.2	-291.8	-162.2	-194.0
	1	-131.6	-217.9	-289.5	-208.5	-194.5
15[d]	0	-213.7	-151.3	-283.0	-152.4	-191.9
	1	-212.5	-151.4	-281.9	-198.1	-192.4
15[c]	0	-212.9	-149.6	-281.6	-150.1	-191.3
	1	-211.8	-149.2	-279.9	-174.6	-192.1

[a] in ppm with respect to $CH_3{}^{15}NO_2$ in a capillary. [b] ~0.5 M in CH_2Cl_2. [c] ~0.15 M in DMSO. [d] ~0.25 M in CH_2Cl_2. [e] ~0.25 M in DMSO.

0.17 mmol) in anhydrous diemthylformamide (2 ml) in ice-water bath. Stirring was continued for 1 h at 0 °C and another 4 h at room temperature. The mixture was poured into a cold solution of sodium bicarbonate (20 ml) and extracted with dichloromethane (3 x 20 ml). Solvent was removed under vaccum and coevaporated with toluene to dryness. The residue was purified on a silica gel column, yield 53 mg (62%). $[\alpha]_D^{20}$ -24.6 (c 0.13, MeOH); UV (MeOH): λ_{max} = 325 nm (ϵ = 10.200), 290 nm (ϵ = 6.800), 250 nm (ϵ = 11.500), 227 nm (ϵ = 24.200) [pH 7]; 324 nm (ϵ = 3.400), 288 nm (ϵ = 9.300), 225 nm)ϵ = 28.400) [pH 13]; 309 nm (ϵ = 6.800), 282 nm (ϵ = 8.200), 229 nm (ϵ = 20.500) [pH 2]. ^{1}H-NMR ($CDCl_3$): 11.1 (s, 1H)-CHO; 7.79 (s, 1H) H-2; 6.18-5.82 (m, 3H) H-1', H-2', H-3'; 4.40 (m, 2H) H-5', H-5''; 4.16 (m, 1H) H-4'; 3.76 (s, 3H) N-CH_3; 2.73 (s, 3H) 6-CH_3; 2.14, 2.13, 2.07 (3s, 3 x 3H) 3 x OAc. ^{13}C-NMR ($CDCl_3$): 183.3 (d, J_{CH} = 194 Hz) CHO; 153.6 (C-9); 147.8 (C-4a); 144.4 (C-3a); 139.3 (d, J_{CH} = 215 Hz) C-2; 137.6 (C-6); 121.3 (C-7); 118.6 (C-9a); 87.2 (d, J_{CH} = 167 Hz) C-1'; 79.2 (d, J_{CH} = 153 Hz) C-4'; 72.6 (d, J_{CH} = 149 Hz) C-2'; 70.2 (d, J_{CH} = 156 Hz) C-3'; 63.1 (t, J_{CH} = 146 Hz) C-5', 28.9 (N-CH_3); 10.6 (6-CH_3). MS (FAB^+): calc. for $(M+H)^+$ 490.1574, found 490.1555.

Preparation of 4,9-dihydro-5,6-dimethyl-7-iodo-9-oxo-3-(2',3',5'-O-triacetyl-β-D-ribofuranosyl)imidazo[1,2-a]purine (20)

To a suspension of N^5-methyl isomer of wyosine-triacetate (184 mg, 0.4 mmol), silver trifluoroacetate (88 mg, 0.4 mmol) in dichloromethane (5 ml), a solution of iodine (102 mg, 0.4 mmol) in dichloromethane (2 ml) was added dropwise. After 3 h, the mixture was poured into a cold solution of sodium bicarbonate (25 ml) which was filtered and extracted with dichloromethane (2 x 20 ml). The organic phase was washed with saturated $Na_2S_2O_3$ solution (20 ml), water (20 ml) and then dried on $MgSO_4$. After evaporation, the residue was purified on a sillica gel column, yield 120 mg (51%). $[\alpha]_D^{20}$ -19.8 (c 0.096, MeOH); UV (MeOH): λ_{max} = 290 nm (ϵ = 9.400),

Table 2: ^{15}N chemical shifts[a] of tricyclic desmethyl wyosine derivatives in neutral and acidic DMSO (T = 30 °C) and/or CH_2Cl_2 (T = 25 °C) solutions.

Compound	TFA (equiv.)	N^1	N^3	N^4	N^5	N^8
5a[b]	0	-134.8	-213.5	-221.8	-243.2	-195.2
	1	-175.8	-209.7	-221.7	-240.7	-194.2
5b[c]	0	-139.8	-219.1	-221.7	-244.4	-195.8
	0.1	-147.3	-218.3	-221.6	-244.0	-195.8
5d[c]	0	-138.9	-219.8	-224.4	-248.9	-196.7
	0.1	-147.0	-219.0	-	-	-
	1	-188.6	-215.0	-224.4	-246.6	-195.6
5e[d]	0	-133.4	-215.7	-221.5	-241.8	-194.9
	1	-146.7	-213.1	-222.3	-242.7	-194.9
5f[c]	0	-134.3	-215.6	-211.3	-206.8	-198.9
	1	-162.4	-213.0	-211.8	-205.7	-198.2
18b[c]	0	-135.7	-218.3	-223.8	-254.0	-197.5
	1	-172.3	-214.3	-222.6	-251.2	-197.5
18b[b]	0	-133.7	-216.4	-223.4	-251.7	-196.7
	1	-145.5	-215.6	-223.3	-251.1	-196.6
18c[b]	0	-134.1	-214.1	-225.2	-256.8	-197.9
	1	-158.3	-212.0	-225.0	-255.1	-197.3
18d[c]	0	-135.8	-218.9	-226.2	-257.9	-198.1
	1	-170.0	-216.1	-225.5	-254.6	-197.2
18d[d]	0	-133.0	-217.0	-225.8	-255.8	-197.4
	1	-145.5	-216.1	-225.5	-255.0	-197.2

[a] in ppm with respect to $CH_3{}^{15}NO_2$ in a capillary.
[b] ~0.25 M in DMSO. [c] ~0.5 M in CH_2Cl_2. [d] ~0.2 M in DMSO.

233 nm (ε = 20.500) [pH 7]; 290 nm (ε = 8.900), 233 nm (ε = 23.000) [pH 13]; 286 nm (ε = 7.400) 234 nm (ε = 16.900) [pH 2]. ^{1}H-NMR ($CDCl_3$): 7.67 (s, 1H) H-2; 6.20-5.81 (m, 3H) H-1', H-2', H-3'; 4.37 (m, 2H) H-5', H-5''; 4.16 (m, 1H) H-4'; 3.72 (s, 3H) N-CH_3; 2.32 (s, 3H) 6-CH_3; 2.13, 2.12, 2.06 (3s, 3 x 3H) 3 x OAc. ^{13}C-NMR ($CDCl_3$): 152.9 (C-9); 147.5 (C-4a); 146.0 (C-3a); 137.0 (d, J_{CH} = 210 Hz) C-2; 131.8 (C-6); 117.1 (C-7); 102.0 (C-9a) 87.0 (d, J_{CH} = 166 Hz) C-1'; 79.1 (d, J_{CH} = 153 Hz) C-4'; 72.6 (d, J_{CH} = 148 Hz) C-2'; 70.1 (d, J_{CH} = 157 Hz) C-3'; 63.1 (t, J_{CH} = 149 Hz) C-5'; 30.0 (N-CH_3); 11.2 (6-CH_3). MS: $(FAB)^+$: calc. for $(M+H)^+$ 588.0591, found 588.0624.

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Table 3: ^{15}N chemical shifts[a] of some 6-oxo purine nucleosides in DMSO.

Compound	TFA (equiv.)	N^1	N^3	N^7	N^9	NH_2
21[b]	0	-233.9	-215.1	-133.8	-211.2	-307.8
	1	-233.0	-217.0	-179.6	-207.2	-303.9
22[c]	0	-233.5	-216.1	-131.5	-215.7	-307.0
	1	-233.1	-217.0	-157.8	-213.8	-304.8
23[b]	0	-206.6	-167.1	-131.9	-206.6	-
	1	-206.2	-167.3	-141.2	-205.6	-
24[d]	0	-205.8	-168.1	-130.7	-211.2	-
	1	-205.8	-168.1	-134.6	-210.9	-

[a]in ppm, with respect to $CH_3{}^{15}NO_2$.
[b]from ref. 16, [c]present work ~0.45 M in DMSO, T = 35 °C, [d]present work ~0.3 M in DMSO, T = 30 °C.

Table 4: Measurements of acidity constants and the second order rate constant for the acid-catalyzed hydrolysis of N-glycosidic bonds of wyosine and derivatives.

Compounds	[Acidity constants][a] $-lg\ (K_a/mol\ dm^{-3})$	[Acid-catalyzed hydrolysis: Rate constant] $k/10^{-4}\ dm^3\ mol^{-1}\ s^{-1}$
1	2.66 ± 0.03	798 ± 15[b] (1590 ± 30)[c]
4	2.36 ± 0.04	7.34 ± 0.09[a]
6*	1.10	-
7	-0.3 ± 0.1	6.95 ± 0.07[b]
11	1.10 ± 0.05	12.6 ± 0.02[d]
15	3.10 ± 0.03	1.01 ± 0.02[b]
16	3.20 ± 0.03	776 ± 12[b]
17	2.85 ± 0.03	8.79 ± 0.12[b]
18a	1.69 ± 0.04	-
18b	1.63 ± 0.03	-
18c	1.97 ± 0.05	-
18d	2.01 ± 0.05	-

[a]pK_a values are logarithmic acidity constants measured at 298.2 K at an ionic concentration of 0.1 mol dm^{-3} adjusted with sodium chloride [*measured in DMSO-water, 8:2, v/v]. [b]first order rate constants measured in 0.1 mol dm^{-3} aqueous hydrogen chloride; [c]ionic strength adjusted to 1.0 mol dm^{-3} with sodium perchlorate; [d]the first-order rate constant measured in 0.5 mol dm^{-3} aqueous hydrogen chloride.

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