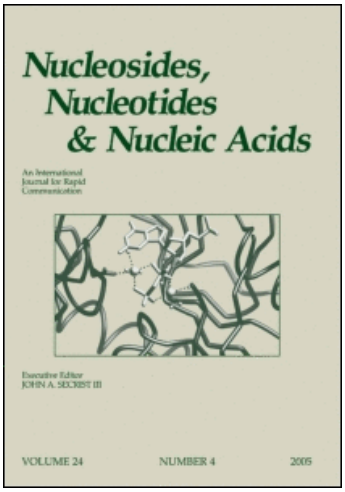

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Evaluation of the Kinetics of Hydrolysis of Diamino Analogues of 2'- Or 3'-Deoxyadenosine and of 9-(2-Deoxy-β-D-*threo*-Pentofuranosyl)adenine or 9-(3-Dexoy-β-D-*threo*-Pentofuranosyl)adenine By Liquid Chromatography

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EVALUATION OF THE KINETICS OF HYDROLYSIS OF DIAMINO ANALOGUES OF 2′- OR 3′-DEOXYADENOSINE AND OF 9-(2-DEOXY-β-D-*THREO*-PENTOFURANOSYL)ADENINE OR 9-(3-DEOXY-β-D-*THREO*-PENTOFURANOSYL)ADENINE BY LIQUID CHROMATOGRAPHY

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ABSTRACT

The degradation of diamino analogues of 2′- or 3′-deoxyadenosine and of 9-(2-deoxy-β-D-*threo*-pentofuranosyl)adenine or 9-(3-deoxy-β-D-*threo*-pentofuranosyl)adenine in buffers of acid, neutral and alkaline pH and constant ionic strength was followed by liquid chromatography. The rate of hydrolysis at acid pH was found to be related to the position and configuration of the amino group on the sugar moiety. The compounds under study were found to be more stable than corresponding monoaminated nucleosides, which have been reported to be more stable than the hydroxyl nucleosides. Liquid chromatographic analyses indicate that acid hydrolysis involves cleavage of the N-glycosyl bond as the major degradative process, together with another minor process. pH-rate profiles, activation parameters and deuterium isotope solvent effects are discussed.

1. Introduction

Antisense oligonucleotides are oligomers aimed at hybridizing with RNA within cells and block gene expression. These oligonucleotides must be enzymatically stable before they can be used as potential therapeutics for viral diseases and cancers. For this purpose,

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the oligonucleotides have to be chemically modified. Moreover, strong hybridization with its target mRNA might be advantageous for generating biological effects with oligonucleotides. Conformational rigidity and hydration are recognised as being important factors determining the stability of oligonucleotide duplexes[1]. Oligonucleotides built up from 3'-amino-2',3'-dideoxy nucleosides (N3'→P5' phosphoramidates) hybridize with RNA with increased duplex stability[2]. This is attributed to the more rigid backbone structure of 3'-NHP(O)(O^-)O-5' linked oligomers and to the formation of interstrand hydrogen bonding. This enhancement in duplex stability is not observed when the oligomer is built up from 5'-amino-2',5'-dideoxy nucleosides (N5'→P3' phosphoramidates)[3]. However, 3',5'-diaminonucleosides have not been used yet for the construction of oligomers mainly due to the difficult synthetic accessibility of such oligonucleotides. Before starting this research, we became interested in studying first the chemical stability of diaminonucleosides.

The structures of the compounds under study (**1-4**) are shown in Fig. 1. Although there are many reports on the stability of normal deoxynucleosides and dideoxynucleosides[4-18], little is known about the stability of aminated nucleosides[19]. Stability data on **1-4** might likewise be important when carrying out derivatisation reactions with these nucleosides. Moreover, there is intense research going on for development of antigene and antisense oligonucleotides because they are potential therapeutics for viral diseases and cancers. Compounds **1-4** are potential candidates for incorporation into oligonucleotides, serving as enzymatically stable substitutes for natural deoxynucleosides. We therefore found it interesting to carry out stability studies on **1-4**.

2. Experimental

2.1. Samples and reagents

The synthesis of compounds **1-4** has been described elsewhere[20]. Adenine, sodium octanesulphonate, deuterium oxide (99.8 %) and deuterium chloride (99 %) were purchased from Acros Organics (Geel, Belgium). Glycine was from Tessenderlo Chemie (Tessenderlo, Belgium). HPLC-grade tetrahydrofuran from Rathburn (Walkerburn, Scotland) was used. Water was distilled twice in glass apparatus. All other reagents were of pro-analysi quality (Acros Organics).

2.2. Kinetic studies

Samples for kinetic studies were prepared by diluting 0.1 ml of a 5 x 10^{-3} M stock solution with 4.9 ml of 0.1 M buffer of appropriate pH and ionic strength 0.4 (adjusted with

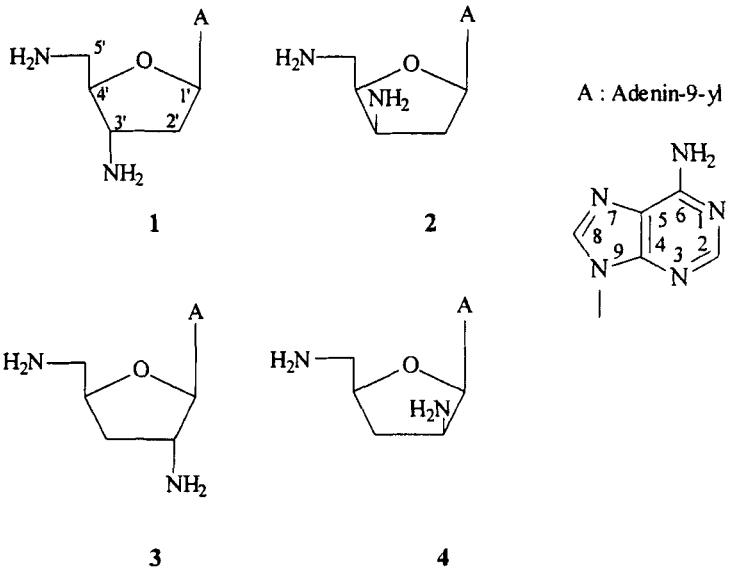

FIGURE 1. Structures of compounds **1-4**
1: 9-(3,5-diamino-2,3,5-trideoxy-β-D-*erythro*-pentofuranosyl)adenine
2: 9-(3,5-diamino-2,3,5-trideoxy-β-D-*threo*-pentofuranosyl)adenine
3: 9-(2,5-diamino-2,3,5-trideoxy-β-D-*erythro*-pentofuranosyl)adenine
4: 9-(2,5-diamino-2,3,5-trideoxy-β-D-*threo*-pentofuranosyl)adenine

KCl before measuring the final pH). Aliquots (0.5 ml) of this solution were put in vials, capped and degraded at 100 °C in a Memmert (Schwabach, Germany) oven which was fitted with a calibrated thermometer. The vials were removed at appropriate intervals and quenched with an equal volume of corresponding neutralizing KOH or HCl solution, mixed thoroughly and stored at -20 °C until they could be analysed as a series by liquid chromatography. Temperature studies were carried out at pH 1.15 and 11.93. Solvent isotope effects were determined by comparing rates of hydrolysis in 0.1 N DCl (made by diluting deuterium chloride with deuterium oxide) and in 0.1 N HCl at 100 °C.

2.3. Liquid chromatography

Analysis of samples for kinetic studies was carried out on liquid chromatographic (LC) apparatus consisting of an SP 8700 XR solvent delivery system (Thermo Separation Products, Fremont, CA, U.S.A.), used at a flow rate of 1 ml/min, a Marathon autosampler (Spark Holland, Emmen, The Netherlands) equipped with a 20 µl loop, a Waters Model 440

detector (Milford, MA, U.S.A.) at 254 nm and a Hewlett-Packard Model 3396 integrator (Avondale, PA, U.S.A.). A PLRP-S 1000 Å 8 μm (Polymer Laboratories, Church Stretton, Shropshire, U.K.) (250 mm x 4.6 mm I.D.) column, equilibrated at 60 °C by means of a water bath was used with a mobile phase that consisted of tetrahydrofuran - 0.2 M sodium octanesulphonate - 0.2 M potassium phosphate buffer pH 2.0 - water (15:25:30:30, v/v). A Waters Model 990 Photodiode Array (PDA) detector (Milford, MA, U.S.A.) was used to record online UV spectra. pH was measured at room temperature with a Consort P 514 pH meter (Turnhout, Belgium) using a Schott pH electrode (Mainz, Germany). Development of the LC method is described elsewhere[21]. Isolation of the unknown minor degradation product of compound **2** (**2MDP**) was done using the same mobile phase and a PLRP-S 1000 Å 8 μm semi-preparative column (250 mm x 12.5 mm I.D.). Four methods were used in an attempt to remove the salts (sodium octanesulphonate, potassium phosphate and potassium chloride) from **2MDP**, without any success however.

3. Results and discussion

The degradation of **1-4** exhibits pseudo-first order kinetics and the observed rate constants of degradation, k_{obs}, are given in Table 1. For **1** and **3** at pH 1.15-8.85 the only UV-absorbing degradation product that was characterized by its retention time and UV spectrum, was adenine. Liquid chromatograms of the partially degraded samples of **1** and **3** at pH 11.93 and of **2** and **4** at pH 1.15-11.93 indicated that besides adenine, the main degradation product, there were other minor degradation products (Fig. 2). The concentration of the minor products for **2** and **4** was low at pH 1.15 (2.5 and 5 % of the total chromatographic area, respectively) and higher at pH 2.88-11.93 (up to 25 and 9 %, respectively). For **1** and **3** at pH 11.93 the total concentration of the minor degradation products did not exceed 3 %. The UV-spectra of these products could not be determined, because of their low concentrations, except for that of **2MDP** (λ_{max} = 207 and 258 nm) which was very similar to that of **2** (λ_{max} = 208 and 258 nm). It seems therefore that **2MDP** is a carbohydrate modified adenine analogue.

Attempts to isolate **2MDP** were futile, partly because of desalting problems, and partly because the sample was degrading to adenine during purification. Compound **2MDP** may be an intermediate in the degradation of **2** to adenine because as the reaction proceeded, the peak corresponding to **2MDP** decreased and finally disappeared while the one of adenine increased. It was not possible to identify **2MDP** by LC-MS because it could not be resolved

TABLE 1. Pseudo-first order rate constants (± standard error) observed for degradation of **1-4** at 100°C and ionic strength 0.4 as a function of pH

Compound	pH	$k_{obs}(h^{-1}) \times 10^2$	$t_{1/2}(h)$
1	1.15	82.14 ± 2.76 (n=27, x=8, y=4)	0.84 ± 0.03
	2.88	2.58 ± 0.09 (n=21, x=6, y=4)	26.86 ± 0.94
	4.77	1.12 ± 0.05 (n=20, x=7, y=2)	61.88 ± 2.76
	6.87	0.85 ± 0.03 (n=29, x=8, y=2)	81.53 ± 2.88
	8.85	0.74 ± 0.03 (n=28, x=9, y=2)	92.40 ± 3.70
	11.93	5.64 ± 0.09 (n=32, x=8, y=2)	12.29 ± 0.20
2	1.15	52.02 ± 1.63 (n=32, x=8, y=2)	1.33 ± 0.04
	2.88	4.09 ± 0.31 (n=21, x=6, y=1)	16.94 ± 1.28
	4.77	4.61 ± 0.23 (n=14, x=4, y=2)	15.03 ± 0.75
	6.87	2.73 ± 0.21 (n=16, x=5, y=2)	25.38 ± 1.95
	8.85	1.77 ± 0.17 (n=12, x=5, y=1)	39.15 ± 3.76
	11.93	7.08 ± 0.64 (n=18, x=5, y=1)	9.79 ± 0.88
3	1.15	2.46 ± 0.21 (n=36, x=9, y=1)	28.17 ± 2.40
	2.88	1.54 ± 0.09 (n=21, x=7, y=2)	45.00 ± 2.63
	4.77	1.12 ± 0.04 (n=31, x=8, y=2)	61.88 ± 2.21
	6.87	0.39 ± 0.02 (n=35, x=9, y=2)	177.69 ± 9.11
	8.85	0.38 ± 0.01 (n=28, x=9, y=2)	182.37 ± 4.80
	11.93	5.24 ± 0.08 (n=40, x=10,y=2)	13.23 ± 0.20
4	1.15	26.42 ± 0.55 (n=38, x=10,y=2)	2.62 ± 0.05
	2.88	12.12 ± 0.29 (n=24, x=6, y=1)	5.72 ± 0.14
	4.77	7.90 ± 0.14 (n=16, x=4, y=3)	8.77 ± 0.16
	6.87	2.56 ± 0.13 (n=26, x=7, y=3)	27.07 ± 1.37
	8.85	3.11 ± 0.12 (n=32, x=8, y=2)	22.28 ± 0.86
	11.93	3.22 ± 0.13 (n=28, x=7, y=4)	21.52 ± 0.87

Glycine hydrochloride buffer was used at pH 1.15 and potassium phosphate buffer at all other pH values. n: total number of chromatographic observations. x: points on the time axis. y: amount of half-lives tested. All experiments were done in duplicate.

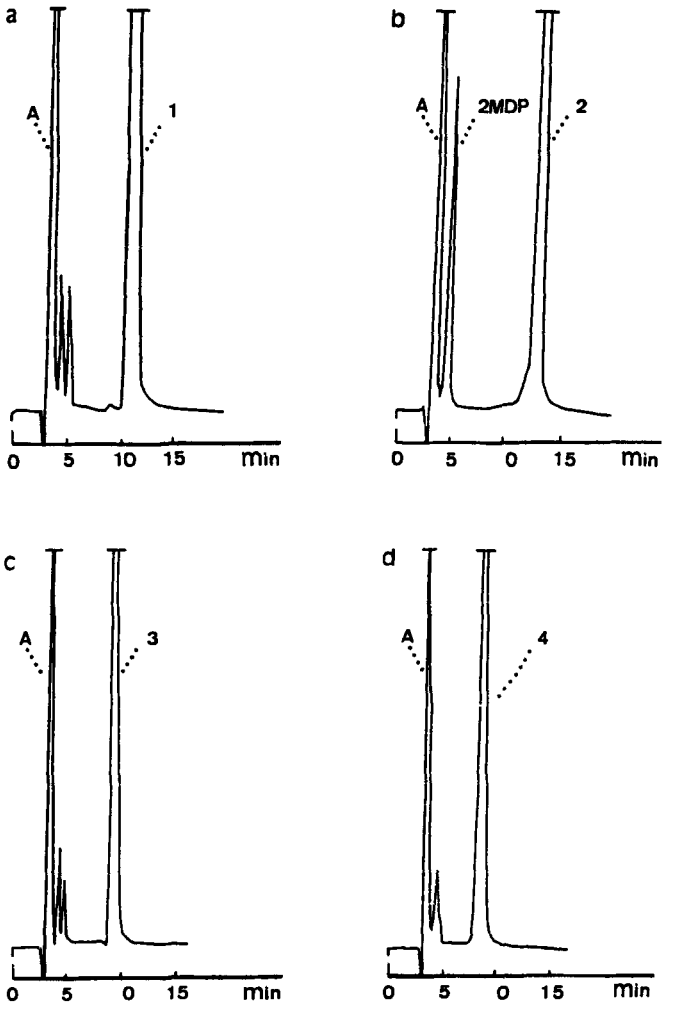

FIGURE 2. Liquid chromatograms of compounds **1** and **3** partially degraded at pH 11.93 and of **2** and **4** partially degraded at pH 4.77. Mobile phase: tetrahydrofuran - 0.2 M sodium octanesulphonate - 0.2 M potassium phosphate buffer pH 2.0 - water (15:25:30:30, v/v). Flow: 1 ml/min. Column: PLRP-S 1000 Å, 8 µm. Temperature: 60 °C. Detection: UV at 254 nm. A: adenine. **2MDP**: minor degradation product of **2**.

from **2** and adenine using a mobile phase containing only an organic modifier (methanol, acetonitrile) and a volatile buffer (ammonium acetate) and without an ion-pairing agent.

It has been established that acid-catalyzed hydrolysis of many purine nucleosides follows a unimolecular mechanism, A_1, in which the N-protonated nucleoside dissociates in the rate determining step to a free purine base and a glycosyl oxocarbenium ion[5,7-11]. However, exceptions to this behaviour are known. Purine ribonucleosides bearing no substituents at C_6 are hydrolyzed at low hydronium ion concentrations by opening of the imidazole ring. For example, 9-(β-D-ribofuranosyl)purine[22,23], 2-amino-9-(β-D-ribofuranosyl)purine and 2-methyl-9-(β-D-ribofuranosyl)purine[24] undergo imidazole ring opening under mildly acidic solutions and depurination at high hydronium ion concentration. 7-Deaza-2'-deoxyadenosine and 2-methyl-7-deaza-2'-deoxyadenosine undergo anomerization and isomerization to furanoid and pyranoid derivatives, which suggests their hydrolysis proceeds *via* a cationic acyclic Schiff base[25].

Cleavage of the N-glycosyl bond is the only mechanism of hydrolysis for **1** and **3** at pH 1.15-8.85 and it is the predominant mechanism for **2** and **4** at pH 1.15. At higher pH values (2.88-6.87) a second mechanism plays a greater role for **2** and **4**. The pK_a values of **1-4** are not known, but it is likely that they are in a similar range to those of their monoaminated analogues (pK_a 3-4 for N_1 and 6-9 for sugar $-NH_2$)[19]. At pH 2.88-6.87, N_1 of compounds **1-4** is probably not fully protonated and so other mechanisms, besides hydrolysis of a protonated nucleoside, may play a larger part in hydrolysis.

At pH 1.15, the position of the amino group on the sugar has an influence on the rates of hydrolysis (Table 1). The compounds with an amino group on $C_{2'}$ (**3** and **4**) are more stable than the ones having the same group on $C_{3'}$ (**1** and **2,** respectively). This is in agreement with a previous report on monoaminated nucleosides[19]. Compounds with a hydroxyl group on $C_{2'}$ are also known to be more stable than the ones having the same group on $C_{3'}$ [5,13]. Like a hydroxyl group[16], a protonated amino group retards nucleoside acid hydrolysis by a negative inductive effect which reduces the electron density at the $C_{1'}$-$O_{4'}$ region and hence destabilises the intermediate cyclic oxocarbonium ion which is formed in the rate determining step. Due to proximity, the $C_{2'}$ amino group has a greater stabilizing effect against acid hydrolysis than the $C_{3'}$ one.

Configuration of the amino groups on the sugar also affects the rates of hydrolysis at pH 1.15. Compound **3**, which has the amino group at $C_{2'}$ in the 'down' position, is more stable than compound **4**, which has the same group in the 'up' position. In compound **4** the amino

group is in *cis* configuration to the N -glycosidic bond and this causes steric strain which increases the hydrolysis rate. Previous studies have shown that compounds with a hydroxyl[7,13] or an amino group[19] at $C_{2'}$ in the 'up' position are less stable than those with the same group in the 'down' position. In contrast, the epimer with a $C_{3'}$-NH_2 in the 'down' position (**1**) is less stable at pH 1.15 than the one with a $C_{3'}$-NH_2 in the 'up' position (**2**). Similar results were obtained for monoaminated nucleosides[19] and for hydroxyl nucleosides[13]. It seems that other conformational factors, such as intramolecular hydrogen bonding, play a role here.

At pH 11.93 compounds **1-3** have similar reactivity, but **4** is about twice as stable. In alkaline media, decomposition of purine nucleosides is initiated by a nucleophilic attack of the hydroxide ion on the C_8 in the base moiety[6,26]. There is no significant effect of sugar amino groups on the electron density of C_8, therefore there is little or no influence on alkaline hydrolysis. However, the $C_{2'}$ amino group of **4**, which is in the 'up' position, offers steric hindrance to hydroxide ions and so makes the compound more stable.

The rate constants of compounds **1-4** were compared with those of their monoamino analogues (i.e. monoamino analogues of 2'- or 3'-deoxyadenosine and of 9-(2-deoxy-β-D-*threo*-pentofuranosyl)adenine or 9-(3-deoxy-β-D-*threo*-pentofuranosyl)adenine with an amino group in position 2' up or down, 3' up or down, or 5') and some hydroxyl analogues[19]. At pH 1.15, compounds **1-4** were 5-48 times more stable than their corresponding monoaminated analogues which in turn are more stable than corresponding hydroxyl analogues. Protonation of sugar amino groups takes place before that of the heterocyclic base. A protonated amino group has a stronger negative inductive effect and therefore reduces the electron density at $C_{1'}$-$O_{4'}$ more than a corresponding hydroxyl group and this retards hydrolysis more. It is also likely that the protonated sugar amino groups retard hydrolysis by repulsion of approaching protons due to their charge. Compounds **1-4** have two sugar amino groups and therefore show greater stability than the monoamino analogues. At alkaline pH, there is little or no difference in stability beween the three series of nucleosides. At pH 11.93 the sugar amino groups are not protonated and the -NH_2 or -OH groups at $C_{3'}$ and $C_{5'}$ are too far to have an effect on the electron density at C_8 and so the rate of hydrolysis is about the same. However, the compounds with an -OH group at $C_{2'}$ are less stable than those with an -NH_2 group at $C_{2'}$. A $C_{2'}$-OH has a greater negative inductive effect than a $C_{2'}$-NH_2, and this makes C_8 more electropositive and therefore more susceptible to nucleophilic attack. In addition, all the compounds with a $C_{2'}$ functional

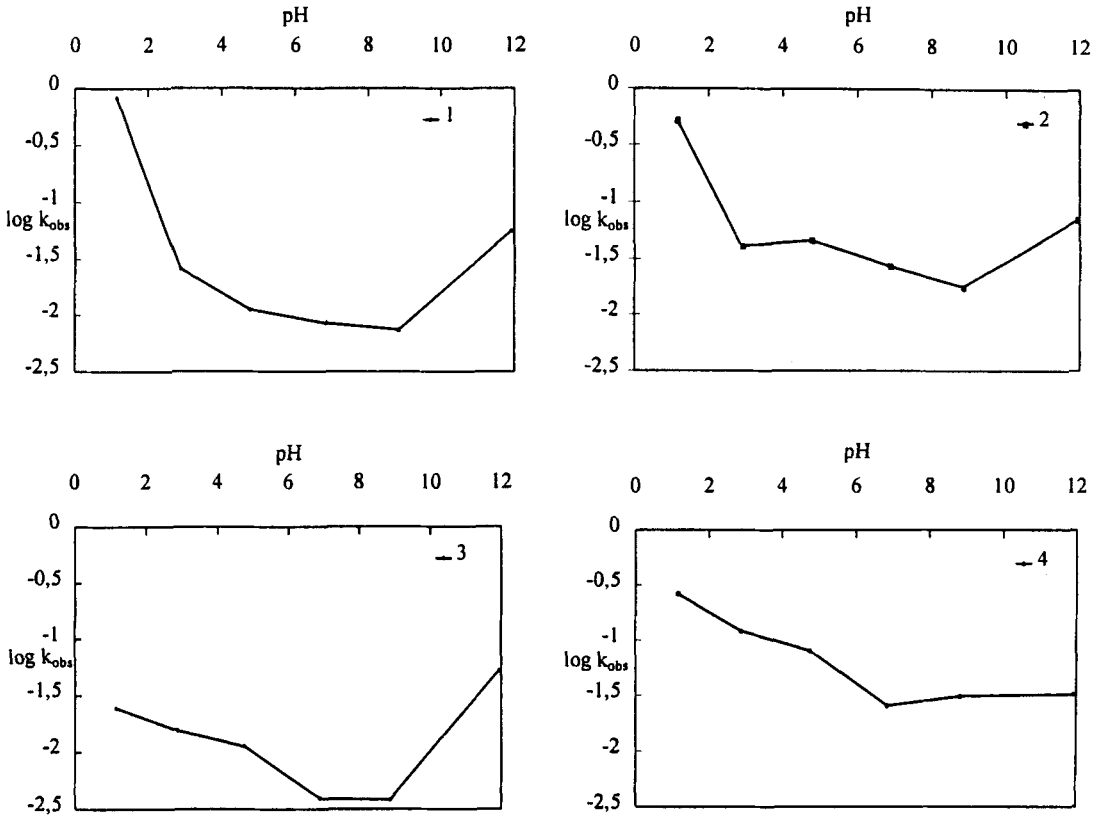

FIGURE 3. Logarithm of observed pseudo-first order rate constant, log k_{obs}, for the decay of compounds **1-4** as a function of pH.

group in the 'up' position are more stable than their epimers with the group in the 'down' position due to the steric hindrance they offer to a hydroxide group attacking the molecule.

Throughout the study the ionic strength, μ , was maintained at 0.4 because of its possible influence on the reaction rate[27,28]. There was no general catalysis by either glycine hydrochloride or potassium phosphate buffers at pH 2.88, μ = 0.4 and 100°C (concentration range 0.05 M to 0.2 M) for compound **4**, therefore the results obtained with the two buffers were pooled to establish the relationship between hydrolysis rates and pH (Fig. 3). In the acid pH region all the compounds exhibit negative slopes which are non-unity. Factors that are known to cause non-unity slopes are general acid-base catalysis, variable ionic strength, different processes being in operation and presence of different protonated forms which are each degraded at different rate constants[27-29]. The non-unity slopes of **1-4** may be caused by

acid-catalyzed hydrolysis of a molecule with a monoprotonated base being superimposed on uncatalyzed hydrolysis of the molecule with an unprotonated base. Besides, LC analyses indicated that acid hydrolysis of **2** and **4** involves two processes which may have different rate constants. For **1-3**, hydrolysis is lowest between pH 6.87 and 8.85 and then increases again towards pH 11.93 due to hydroxide ion catalysis. For **4**, the curve tends to flatten out in the region of pH 6.87-11.93 because of steric hindrance of hydroxide ion attack by the $C_{2'}$ amino group in the 'up' position.

The rate constants for degradation of **1-4** at pH 1.15 and 11.93 and at different temperatures are given in Table 2. Regression analysis was performed on these data in accordance with the logarithmic form of the Arrhenius equation,

$$\log k_{obs} = \log A - \frac{E_a}{2.303RT} \qquad \text{eq 1}$$

where k_{obs} is the observed pseudo first-order reaction rate constant, A is a pre-exponential factor and is a constant, E_a is the activation energy, T is the absolute temperature and R is the universal gas constant. The enthalpy and entropy of activation, $\Delta H^{\neq}$ and $\Delta S^{\neq}$, were calculated for a second-order reaction from the equations

$$\Delta H^{\neq} = E_a - RT \qquad \text{eq 2}$$

$$\Delta S^{\neq} = \frac{\Delta H^{\neq}}{T} - R\ln\frac{T}{k} - R\ln\frac{\tilde{k}}{h} \qquad \text{eq 3}$$

where $\tilde{k}$ is the Boltzmann constant (the gas constant per molecule) and h is Planck's constant.

In order to derive the values of the acid and base catalyzed second-order rate constants k_{obs} was fitted over the entire pH-range to eq 4:

$$k_{obs} = k_S + k_A[H^+] + k_B[OH^-] \qquad \text{eq 4}$$

where k_S stands for the rate constant of the solvent catalyzed reaction, k_A and k_B for the acid and base catalyzed reaction respectively. The simulation was carried out with the statistical package SAS[30], using a weighted non-linear regression method to calculate the rate constants k_S, k_A and k_B. The simulation allowed to conclude that at pH = 1.15 the reactions were dominated by the acid catalysis (k_A $[H^+]$) while at pH = 11.93 the base catalyzed reactions (k_B $[OH^-]$) were prevailing, except for component **4**. The activation parameters $\Delta H^{\neq}$ and $\Delta S^{\neq}$derived from these second-order rate constants k_A and k_B at a pH of 1.15 and 11.93 respectively, are shown in Table 3.

TABLE 2. Pseudo-first order rate constants (± standard error) observed for degradation of compounds **1-4** in glycine hydrochloride buffer at pH 1.15 and potassium phosphate buffer at pH 11.93 and ionic strength 0.4 as a function of temperature

Compound	pH	Temperature (°C)	$k_{obs}(h^{-1}) \times 10^2$	$t_{1/2}(h)$
1	1.15	90	29.49 ± 0.71 (n=36, x=9, y=3, z= 2)	2.35 ± 0.06
		100	82.14 ± 2.76 (n=27, x=8, y=4, z=2)	0.84 ± 0.03
		111	233.15 ± 17.88 (n=36, x=9, y=2, z=2)	0.30 ± 0.02
	11.93	90	1.73 ± 0.03 (n=38, x=9, y=2, z=2)	40.06 ± 0.69
		100	5.64 ± 0.09 (n=32, x=8, y=2, z=2)	12.29 ± 0.20
		114	16.98 ± 1.02 (n=28, x=10, y=1, z=2)	4.08 ± 0.25
		117	23.21 ± 1.56 (n=12, x=7, y=1, z=1)	2.99 ± 0.20
		124	46.98 ± 1.77 (n=11, x=7, y=2, z=1)	1.48 ± 0.06
2	1.15	90	18.91 ± 0.52 (n=38, x=10, y=2, z=2)	3.66 ± 0.10
		100	52.02 ± 1.63 (n=32, x=8, y=2, z=2)	1.33 ± 0.04
		111	105.11 ± 6.68 (n=23, x=10, y=1, z=2)	0.66 ± 0.04
		118	169.69 ± 3.10 (n=6, x=5, y=1, z=1)	0.41 ± 0.01
	11.93	90	2.09 ± 0.07 (n=26, x=8, y=1, z=2)	33.16 ± 1.11
		100	7.08 ± 0.64 (n=18, x=5, y=1, z=2)	9.79 ± 0.88
		114	21.69 ± 1.42 (n=36, x=10, y=1, z=2)	3.20 ± 0.21
		123	32.96 ± 0.63 (n=28, x=8, y=2, z=2)	2.10 ± 0.04
3	1.15	90	0.65 ± 0.02 (n=38, x=10, y=1, z=2)	106.62 ± 3.28
		100	2.46 ± 0.21 (n=36, x=9, y=1, z=2)	28.17 ± 2.40
		114	12.59 ± 0.65 (n=30, x=9, y=1, z=2)	5.50 ± 0.28
		123	30.68 ± 0.74 (n=22, x=8, y=2, z=2)	2.26 ± 0.05
	11.93	90	1.63 ± 0.05 (n=28, x=7, y=1, z=2)	42.52 ± 1.30
		100	5.24 ± 0.08 (n=40, x=10, y=2, z=2)	13.23 ± 0.20
		114	18.28 ± 1.46 (n=38, x=10, y=1, z=2)	3.79 ± 0.30
		123	25.91 ± 0.47 (n=30, x=9, y=1, z=2)	2.67 ± 0.05
4	1.15	90	8.22 ± 0.13 (n=38, x=10, y=1, z=2)	8.43 ± 0.13
		100	26.42 ± 0.55 (n=38, x=10, y=3, z=2)	2.62 ± 0.05
		114	105.69 ± 5.50 (n=32, x=9, y=2, z=2)	0.66 ± 0.03
		118	154.35 ± 10.10 (n=16, x=9, y=1, z=2)	0.45 ± 0.03
	11.93	90	0.89 ± 0.02 (n=36, x=10, y=2, z=2)	77.87 ± 1.75
		100	3.21 ± 0.13 (n=28, x=7, y=4, z=2)	21.59 ± 0.87
		114	11.12 ± 0.18 (n=38, x=10, y=4, z=2)	6.23 ± 0.10
		117	17.41 ± 0.98 (n=14, x=7, y=2, z=1)	3.98 ± 0.22
		123	43.80 ± 0.94 (n=9, x=5, y=2, z=1)	1.58 ± 0.03

n: total number of chromatographic observations. x: points on the time axis. y: amount of half-lives tested. z: number of experiments done

TABLE 3. Arrhenius relationships and activation parameters of compounds **1-4**

Compound	Arrhenius relationship	E_a (kcal mol^{-1})	$\Delta H^{\neq}$ (kcal mol^{-1})	$\Delta S^{\neq}$ (e.u.)
1[a]	$\log k_{obs} = 15.89-5960(1/T)$ r=0.9999	27.3	26.5	-4.6
2[a]	$\log k_{obs} = 12.39-4749(1/T)$ r=0.9956	21.7	21.0	-20.4
3[a]	$\log k_{obs} = 17.93-7295(1/T)$ r=0.9995	33.4	32.6	+4.5[c]
4[a]	$\log k_{obs} = 16.63-6426(1/T)$ r=0.9997	29.4	28.7	-1.3[c]
1[b]	$\log k_{obs} = 14.44-5873(1/T)$ r=0.9977	26.9	26.1	-11.3
2[b]	$\log k_{obs} = 12.77-5223(1/T)$ r=0.9892	23.9	23.2	-19.5[c]
3[b]	$\log k_{obs} = 12.94-5325(1/T)$ r=0.9891	24.4	23.6	-18.0
4[b]	$\log k_{obs} = 15.96-6530(1/T)$ r=0.9972	29.9	29.1	d

a: Glycine hydrochloride buffer was used at pH 1.15 and 0.4 ionic strength, b: potassium phosphate buffer at pH 11.93 and 0.4 ionic strength, c: result to be interpreted with caution because of large error on simulated rate value, d: simulated rate value could not be obtained and entropy not calculated, e.u. = cal mol^{-1} K^{-1}.

It has been suggested[31] that A_1 reactions should be expected to have small entropy of activation ($\Delta S^{\neq}$) values of either positive or negative sign and all A_2 reactions largely negative $\Delta S^{\neq}$ values. Other workers[5,7,10,11,13,16,18] found slightly negative or positive $\Delta S^{\neq}$ values (-11.3 to +13.7 e.u.) for purine nucleosides and in conjunction with other criteria, concluded that these nucleosides hydrolyse by A_1 mechanism. The $\Delta S^{\neq}$ value of **1**, **3** and **4** at pH 1.15 indicates the rate determining step of hydrolysis is of unimolecular nature, but that of **2** at pH 1.15 and of **2** and **3** at pH 11.93 points to a bimolecular rate determinig step.

The observed solvent isotope effects at pH 1.22 are shown in Table 4. The data indicates that water is involved in the rate determining step. These results do not correlate with the

TABLE 4. Observed pseudo-first order rate constants (± standard error) observed for degradation of **1-4** in 0.1 N deuterium chloride and 0.1 N hydrochloric acid

Compound	Solvent	$k_{obs}(h^{-1}) \times 10^2$	k_H/k_D
1	DCl	30.95 ± 1.75 (n=10, x=5 , y=1)	1.3
	HCl	41.59 ± 1.75 (n=12, x=6 , y=1)	
2	DCl	20.45 ± 0.72 (n=14, x=7 , y=1)	2.3
	HCl	46.43 ± 0.98 (n=17, x=9 , y=2)	
3	DCl	14.79 ± 0.08 (n=13, x=7 , y=1)	2.1
	HCl	31.74 ± 0.14 (n=14, x=7 , y=1)	
4	DCl	16.54 ± 0.65 (n=11, x=6 , y=2)	1.4
	HCl	23.80 ± 0.77 (n=11, x=6 , y=2)	

n: total number of chromatographic observations. x: points on the time axis. y: amount of half-lives tested.

$\Delta S^{\neq}$ values for **1**, **3** and **4**, which indicate that the rate determining step is of unimolecular nature. The results are also in contrast to previous observations on purine nucleosides[5,10,13] where reverse isotope effects were obtained and it was concluded that a unimolecular mechanism was involved. York[13] stated that in A_1 mechanisms the rate determining step is the cleavage of a protonated nucleoside and since deuteroacids are weaker acids than protoacids, there is a higher concentration of the deuterated nucleoside in D_2O, therefore giving a greater rate of hydrolysis. In this study, the solvent isotope effect could be caused by exchange of hydroxyl and amino protons with deuterium, and for **2** and **4** the isotope effects observed are due to several reactions. The solvent isotope effects therefore do not give conclusive information on the mechanism(s) taking place.

4. Conclusion

As a conclusion, at pH 1.15, compounds **1-4** were more stable than their corresponding monoaminated analogues, which in turn are more stable than corresponding hydroxyl analogues. At alkaline pH, there is little or no difference in stability beween the three series of nucleosides.

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