Kinetics and Mechanism of the Acid-catalysed Hydrolysis of Regioisomeric Benzotriazole and 8-Azaadenine Nucleosides

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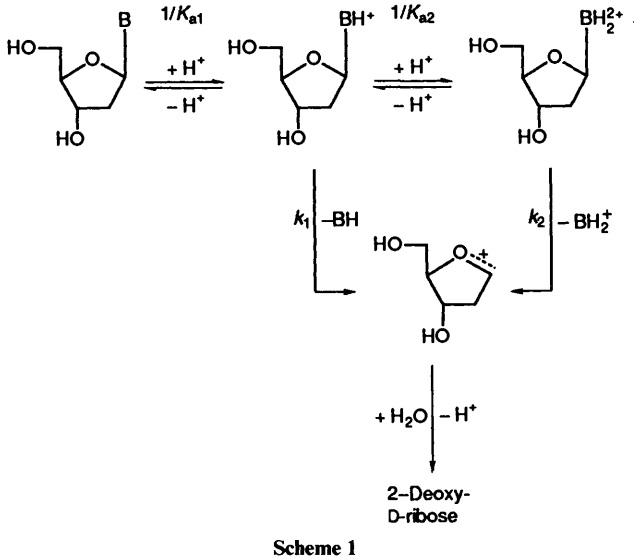
First-order rate constants for the acid-catalysed hydrolysis of ribo- and 2′-deoxyribonucleosides derived from 1*H*-, 2*H*- and 3*H*-benzotriazoles and 7*H*-, 8*H*- and 9*H*-8-azaadenines have been determined over a wide pH range. Partial rate constants for reactions *via* mono- and di-protonated substrates have been calculated with the aid of spectrophotometrically determined pK_a values. The mechanisms of hydrolysis reactions have been elucidated by the effects that polar substituents on the base moiety exert on the protonation and heterolysis steps. Reactivities of various regioisomers have been compared, and reasons for the exceptional hydrolytic instability of *N*3-alkylated purine nucleosides briefly discussed.

It is widely accepted[1,2] that purine nucleosides are hydrolysed under acidic conditions by a mechanism involving a rapid initial protonation of the base moiety and a rate-limiting unimolecular cleavage of the *N*-glycosidic bond to give the free base and a cyclic glycosyl oxocarbenium ion (Scheme 1). Among the base-



Scheme 1

modified analogues of purine nucleosides, benzimidazole[3] and indazole nucleosides[4] have been shown to react by the same mechanism. In contrast, 7-deazaadenosine in acidic solutions undergoes anomerization concurrent with the hydrolysis, suggesting that depurination is preceded by preequilibrium formation of an acyclic cationic Schiff base.[5] It is also known that those purine nucleosides which do not bear a substituent at C6 are depurinated only under very acidic conditions. At low hydronium ion concentrations these nucleosides undergo imidazole ring-opening with subsequent formation of 4-amino-5-formamidopyrimidine.[6–9]

The present paper describes the results of kinetic studies on the acid-catalysed hydrolysis of two series of base-modified analogues of purine nucleosides, *viz.* benzotriazole (1,2,3-triazolo[4,5-*d*]pyrimidine) and 8-azaadenine nucleosides, both of which are of considerable biological interest.[10,11] The mechanisms of the hydrolysis reactions have been elucidated from structural effects and pH–rate profiles. Reactivities of various regioisomers have been compared, and the reasons for the well known hydrolytic instability of *N*3-substituted purine nucleosides discussed in light of kinetic results obtained with *N*1- and *N*3-glycosylated 4-methylbenzotriazoles.

Table 1 NOE data for the benzotriazole derivatives prepared[a]

Compound	Irradiated proton	Observed NOE (%)
2b	1′	H2′ 7.0, H4′ 2.1, H7 6.9
4b	H1′	H2′ 3.5, H4′ 2.0
1c	H1′	H_a2′ 7.3, H4′ 2.0, H7 6.5
3c	H1′	H_a2′ [b], H4′ 1.8
5c	H1′	H_a2′ 8.2, H4′ 1.4, CH_3 9.2

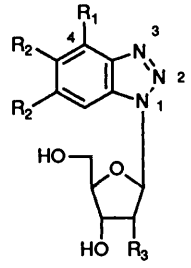
[a] In [$^2H_6$]DMSO at 296 K. [b] Overlapping with DMSO.

Results and Discussion

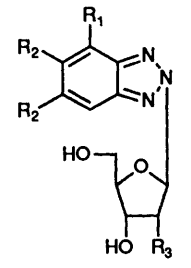
Syntheses.—The preparation of most of the benzotriazole 2′-deoxyribonucleosides[12] (**1a**, **b**, **d–f**; **3a**, **b**, **d–f**; **5b**, **d**) and ribonucleosides[13,14] (**2a**, **e**, **f**; **4a**) used in kinetic measurements, as well as that of the regioisomeric 8-azaadenine 2′-deoxyribonucleosides[15] (**9–13**), has been previously described. The other compounds were synthesized as follows.

The synthesis of 4-nitrobenzotriazole *N*1- and *N*2-β-D-ribofuranosides (**2d**; **4d**) was accomplished by fusing the free base with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose. The reaction provides two main products, which were separated as deblocked materials by column chromatography. The isomeric 4-nitrobenzotriazole ribonucleosides were further hydrogenated to the corresponding 4-aminobenzotriazole nucleosides (**2b**; **4b**).

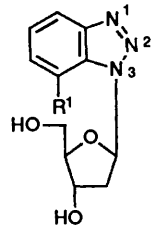
The structures of **2b**, **d** and **4b**, **d** were verified by UV and NMR NOE-difference spectroscopy. The UV absorption spectra of the regioisomeric 4-nitro- and 4-amino-benzotriazole ribonucleosides were observed to be similar to those of the corresponding 2′-deoxyribonucleosides synthesized earlier.[12] NOE data (Table 1) established a β-configuration in all cases.[16] Moreover, an NOE on H7 of **2b** confirmed *N*1-ribosylation with this compound, and hence with **2d**. Compound **4b** exhibited no NOEs on the nucleobase upon irradiation of H1′, consistent with *N*2-ribosylation.



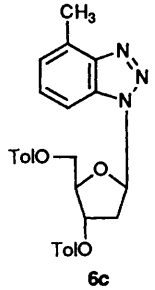
1a: $R^1 = R^2 = R^3 = H$
b: $R^1 = NH_2$; $R^2 = R^3 = H$
c: $R^1 = CH_3$; $R^2 = R^3 = H$
d: $R^1 = NO_2$; $R^2 = R^3 = H$
e: $R^1 = R^3 = H$; $R^2 = CH_3$
f: $R^1 = R^3 = H$; $R^2 = Cl$
2a: $R^1 = R^2 = H$; $R^3 = OH$
b: $R^1 = NH_2$; $R^2 = H$; $R^3 = OH$
d: $R^1 = NO_2$; $R^2 = H$; $R^3 = OH$
e: $R^1 = H$; $R^2 = CH_3$; $R^3 = OH$
f: $R^1 = H$; $R^2 = Cl$; $R^3 = OH$



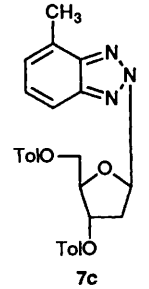
3a: $R^1 = R^2 = R^3 = H$
b: $R^1 = NH_2$; $R^2 = R^3 = H$
c: $R^1 = CH_3$; $R^2 = R^3 = H$
d: $R^1 = NO_2$; $R^2 = R^3 = H$
e: $R^1 = R^3 = H$; $R^2 = CH_3$
f: $R^1 = R^3 = H$; $R^2 = Cl$
4a: $R^1 = R^2 = H$; $R^3 = OH$
b: $R^1 = NH_2$; $R^2 = H$; $R^3 = OH$
d: $R^1 = NO_2$; $R^2 = H$; $R^3 = OH$



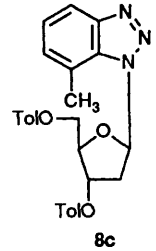
5b: $R^1 = NH_2$
c: $R^1 = CH_3$
d: $R^1 = NO_2$



6c



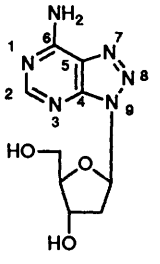
7c



8c

Glycosylation of the anion of 4-methylbenzotriazole with 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-*erythro*-pentofuranosyl chloride[17] provided three products (**6c**; **7c**; **8c**), which were separated by column chromatography and deprotected with methanolic sodium methoxide to give **1c**, **3c** and **5c**. The structures of the three regioisomers obtained were elucidated by ^1H NMR NOE-difference spectroscopy. β-Configuration was established by the observed NOEs of $H_\alpha 2'$ and H4′ upon irradiation of H1′.[16] Additional NOEs on H7 of **1c** and on the methyl group of **5c** suggested these compounds to be the *N*1- and *N*3-glycosides, respectively. The third isomer, **3c**, exhibited no NOE on the base moiety, as expected for an *N*2-glycosylated derivative. The UV absorption spectra of **1c**, **3c** and **5c** were almost identical with those of the corresponding non-methylated nucleosides, **1a** and **3a**.[12]

Hydrolysis of Benzotriazole Nucleosides.—Table 2 records the pK_{a1} values obtained spectrophotometrically for the conjugate acids of benzotriazole 2′-deoxyribonucleosides, and second-order rate constants, $k = k_{obs}/[H^+]$, determined by HPLC, for their hydrolysis at pH ≫ pK_{a1}, *i.e.* under conditions where



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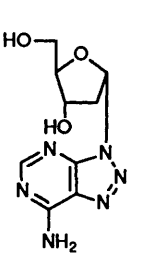

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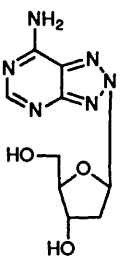

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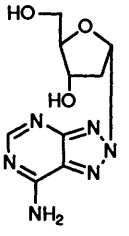

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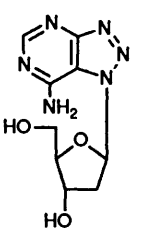

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Table 2 Second-order rate constants, k, for the acid-catalysed hydrolysis of benzotriazole 2′-deoxyribonucleosides at 333.2 K, pK_{a1} values of their monocations at 298.2 K, and rate constants, k_1, for heterolysis of the monocations at 333.2 K[a]

Compound	$k^b/10^{-3}$ dm^3 mol^{-1} s^{-1}	pK_{a1}[c]	log k_1[d]
*N*1-Deoxyribofuranosides			
4-H (**1a**)	81	−0.5	−0.4
4-NH_2 (**1b**)	9.0	1.9	−3.7
4-CH_3 (**1c**)	57	−0.3	−0.7
4-NO_2 (**1d**)	990	−2.7[e]	2.9
5,6-di-CH_3 (**1e**)	61	0.2	−1.2
5,6-di-Cl (**1f**)	88	−2.0[e]	1.1
*N*2-Deoxyribofuranosides			
4-H (**3a**)	780	−0.4[e]	0.5
4-NH_2 (**3b**)	530	2.4	−2.5
4-CH_3 (**3c**)	690	0.0	0.0
4-NO_2 (**3d**)	890	−2.5[f]	2.6
5,6-di-CH_3 (**3e**)	610	0.5	−0.5
5,6-di-Cl (**3f**)	240	−1.7[f]	1.3
*N*3-Deoxyribofuranosides			
4-NH_2 (**5b**)	2 600	1.8	−1.2
4-CH_3 (**5c**)	640	−0.5	0.5
4-NO_2 (**5d**)	11 000	−3[f]	4.2

[a] Ionic strength adjusted to 0.1 mol dm^{-3} with sodium chloride. [b] $k = k_{obs}/[H^+]$. The first-order rate constants, k_{obs}, were measured at 333.2 K under conditions where the reaction *via* substrate monocation predominates (pH ≫ pK_{a1}). [c] At 298.2 K. [d] Calculated by eqn. (1) at 333.2 K; pK_{a1} (333 K) = pK_{a1} (298 K) − 0.2.[1] [e] pK_{a1} of the corresponding riboside +0.3. [f] An approximate value.

hydrolysis *via* the monoprotonated substrate is the only reaction that may be expected to contribute significantly to the observed first-order rate constant, k_{obs}. It is clear that substituents at C4, C5 or C6 markedly affect the basicity of *N*1-(**1a–f**; **2a–f**) and *N*2-glycosides (**3a–f**; **4a**, **b**, **d**), while the influence on the hydrolysis rate is much smaller. The electronegative 4-nitro group, for example, reduces the basicity by two orders of magnitude, but enhances the hydrolysis of **1a** and **3a** by factors of only 12 and 1.1, respectively. The observed insensitivity of the hydrolysis rate to the basicity of the base moiety is consistent with rate-limiting formation of a cyclic glycosyl oxocarbenium ion (Scheme 1). Electron-withdrawal by polar substituents, for example, diminishes the electron density

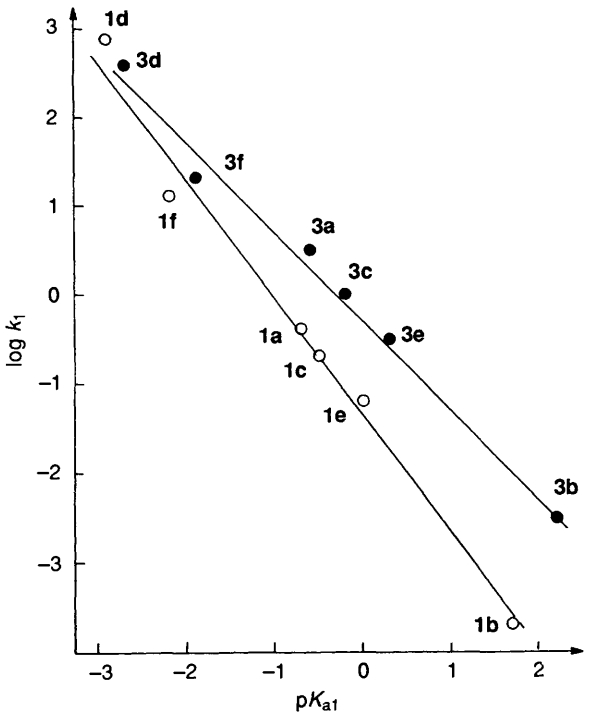

Fig. 1 Logarithmic rate constants for the heterolysis of monoprotonated benzotriazole 2′-deoxyribonucleosides plotted against their pK_{a1} values at 333.2 K. Notation: *N*1-glycosides, open circles and *N*2-glycosides, filled circles

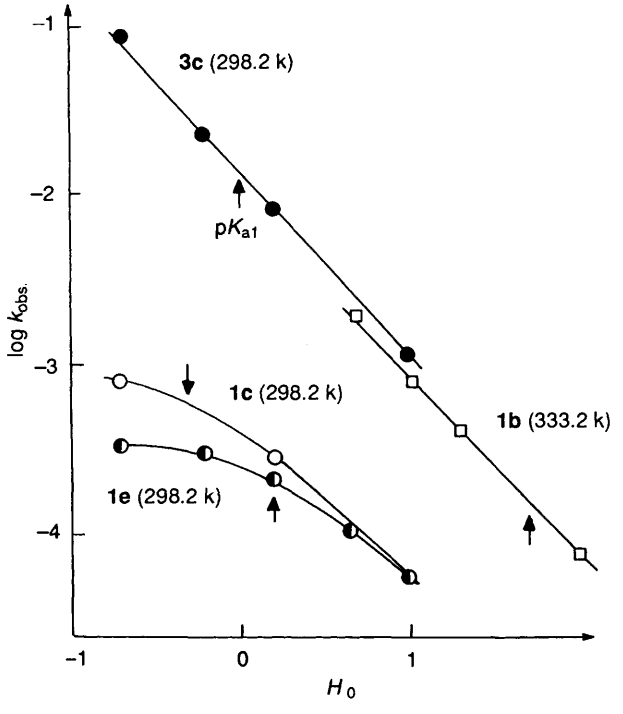

Fig. 2 Rate profiles for the hydrolysis of some benzotriazole 2′-deoxyribonucleosides in aqueous hydrogen chloride

at the triazole ring, and hence the initial protonation is markedly retarded. Simultaneously the *N*-glycosidic bond becomes more strongly polarized, resulting in acceleration of the heterolysis step. These two opposing effects partially cancel each other, and hence the influence on the observed rate constant remains small. As seen from Fig. 1, the logarithmic rate constants calculated by eqn. (1) for the heterolysis of substrate

$$\log k_1 = \log k - pK_{a1} \quad (1)$$

monocations exhibit an inverse dependence on the pK_{a1} values, the slopes of the plots $\log k_1$ *vs.* pK_{a1} being -1.3 ± 0.1 with *N*1-glycosides and -1.0 ± 0.1 with *N*2-glycosides. Similar reaction constants have previously been reported for purine,[2,6] benzimidazole[3] and indazole[4] nucleosides, which all react *via* cyclic oxocarbenium ions. If a route *via* an acyclic Schiff base intermediate were operating, as with 7-deazaadenosine,[5] the effect on initial protonation would be of minor importance, owing to the large distance between the base moiety substituents and the site of protonation. Accordingly, electron-withdrawing groups that destabilize the developing Schiff base by reducing the electron density at the triazole ring would be strongly rate-retarding, which is clearly not the case. It is also worth noting that no sign of anomerization was detected when the hydrolysis of **2a** and **4a** was followed by ^{1}H NMR spectroscopy in deuterium oxide (pD 2).

Comparison of the hydrolysis rates of 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-4-methyl-1*H*-benzotriazole (**1c**) and 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-7-methyl-1*H*-benzotriazole (**5c**) helps to elucidate the susceptibility of the hydrolysis of purine nucleosides to steric factors. The monocation of **5c** is heterolysed 15 times as fast as that of **1c**. This reactivity difference may result partly from a different site of protonation (N2 or N3 with **1c**; N1 or N2 with **5c**) and partly from steric acceleration. The 4-methyl group increases non-bonded repulsive interactions between the base and sugar moieties when the deoxyribosyl group is attached at N3 (**5c**), but not at N1 (**1c**). Since these interactions are weakened on going from the initial state to the transition state, owing to lengthening of the *N*-glycosidic bond, hydrolysis of the *N*3-isomer is sterically accelerated. It should be noted, however, that the observed acceleration is a rather modest one; introduction of a methyl group at a position equivalent to N3 of purine nucleosides enhances the heterolysis only by a factor of 15. Accordingly, the exceptionally rapid hydrolysis of 3-methyl purine nucleosides and wyosine (1,N^2-isopropeno-3-methylguanosine) can hardly be accounted for by steric acceleration, as suggested previously.[18] Most probably steric factors are responsible for only a minor part of the total acceleration, which has been estimated to be of the order of 10^6.[18] The studies of Czarnik[19] on hydrolysis of cyclonucleosides have led to a similar conclusion.

The reactivity ratio of the monocations of 4-nitrobenzotriazole nucleosides (**5d**; **1d**) is almost equal to that of the 4-methyl derivatives. In contrast, with 4-amino substituted *N*3- and *N*1-deoxyribosides (**5b**; **1b**) the corresponding reactivity ratio is considerably higher, *viz.* 270. One should bear in mind, however, that it has been proposed that 4-aminobenzotriazole nucleosides undergo protonation of the amino group rather than one of the triazole N-atoms.[12]

Fig. 2 shows some illustrative examples of pH–rate profiles for the hydrolysis of *N*1- and *N*2-glycosylated benzotriazole nucleosides. The rate profile of *N*2-glycosylated 4-methylbenzotriazole (**3c**) remains linear on passing the pK_{a1} value of the substrate monocation, indicating that hydrolysis *via* the dicationic species becomes predominant at pH < pK_{a1}. In contrast, the reaction from the dication appears not to occur with the *N*1-glycosylated nucleosides, as shown by the fact that the hydrolysis rates of **1c** and **1e** become pH-independent in highly acidic solutions. This difference in behaviour most likely results from the fact that with *N*1-glycosides the consecutive protonations take place at adjacent nitrogen atoms, N2 and N3, which is obviously an extremely unfavourable process. With *N*2-glycosides the potential sites of protonation, N1 and N3, are separated by an additional nitrogen atom, and the attachment of the second proton is thus easier than with *N*1-glycosides. In striking contrast to the other *N*1-glycosides, 4-amino-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-1*H*-benzotriazole (**1b**) exhibits a linear pH–rate profile. As mentioned

Table 3 First-order rate constants for the hydrolysis of benzotriazole ribonucleosides in aqueous hydrogen chloride (0.1 mol dm^{-3}) at 363.2 K, and pK_{a1} values of their monocations at 298.2 K

Compound	$k_{obs}/10^{-3}$ s^{-1}	pK_{a1}
*N*1-Ribofuranosides		
4-H (**2a**)	0.375 ± 0.005	−0.8
4-NH_2 (**2b**)	0.0583 ± 0.0007	
4-NO_2 (**2d**)	4.12 ± 0.05	−3.0
5,6-di-CH_3 (**2e**)	0.300 ± 0.005	−0.2
5,6-di-Cl (**2f**)	0.409 ± 0.018	−2.3
*N*2-Ribofuranosides		
4-H (**4a**)	2.80 ± 0.20	−0.7
4-NH_2 (**4b**)	0.972 ± 0.014	
4-NO_2 (**4d**)	3.60 ± 0.04	−2.8[a]

[a] An approximate value.

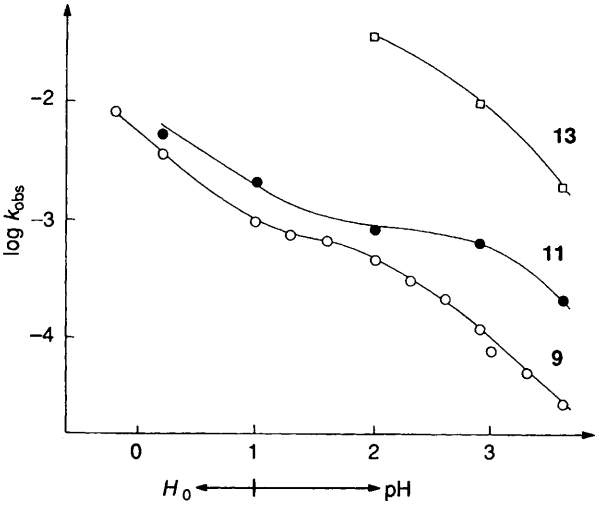

Fig. 3 Rate profiles for the hydrolysis of regioisomeric 8-azaadenine 2′-deoxyribofuranosides at 333.2 K (ionic strength adjusted to 0.1 mol dm^{-3} with sodium chloride)

above, the first protonation of this nucleoside occurs at the exocyclic nitrogen atom.[12] The dication may thus be formed by attachment of the second proton at N2 or N3 of the unprotonated triazole ring, which is obviously a facile process compared to formation of an *N*2,*N*3-diprotonated species.

As seen from Table 2, the monocations of *N*2-glycosylated benzotriazoles are heterolysed considerably faster than their *N*-glycosylated counterparts. A tentative explanation for this reactivity difference is that the quinoid electron structure of *N*2-glycosides increases the energy level of the initial state of their hydrolysis compared to that of *N*1-glycosides. Since the releasing base does not exhibit a similar quinoid electron structure, the Gibbs energy of activation may be expected to be smaller with *N*2- than with *N*1-glycosides.

2′-Deoxyribonucleosides of benzotriazoles are hydrolysed almost 500 times as fast as the corresponding ribonucleosides (Table 3). The reactivity difference, which results from destabilization of the oxocarbenium ion intermediate by the electronegative 2′-hydroxyl group, is thus of the same magnitude as that observed with purine nucleosides.

Hydrolysis of 8-*Azaadenine Nucleosides.*[20]—Fig. 3 shows the pH–rate profile for the hydrolysis of 8-aza-2′-deoxyadenosine (**9**). The hydrolysis rate increases continuously with increasing hydronium ion concentration, suggesting that the reaction proceeds through a cyclic oxocarbenium ion (Scheme 1) rather than through an acyclic cationic Schiff base. The latter reaction would be expected to exhibit an inverse dependence of rate on acidity under very acidic conditions. It has been shown[21] that Schiff bases are hydrolysed *via* carbinolamine intermediates. Under very acidic conditions the decomposition of carbinolamines, which is a base-catalysed reaction, becomes rate limiting, and hence a deceleration is observed.

The observed first-order rate constant, k_{obs}, depends on hydronium ion concentration according to eqn. (2),[6] where the

$$k_{obs} = \frac{\frac{k_1}{K_{a1}}[H^+] + \frac{k_2}{K_{a1}K_{a2}}[H^+]^2}{1 + \frac{[H^+]}{K_{a1}} + \frac{[H^+]^2}{K_{a1}K_{a2}}} \text{ at pH} \gg pK_{a2} = \frac{[H^+]}{K_{a1} + [H^+]}\left(k_1 + \frac{k_2}{K_{a2}}[H^+]\right) \quad (2)$$

partial rate and equilibrium constants are those defined in Scheme 1. With 2′-deoxyadenosine the rate profile is linear, indicating that k_1/K_{a1} and k_2/K_{a2} are almost equal (0.027 and 0.025 dm^3 mol^{-1} s^{-1} at 323.2 K).[2] The rate profile of **9**, in turn, exhibits an inflection point at pH < pK_{a1}. The values obtained by least-squares fitting for this compound at 333.2 K are: k_1/K_{a1} = 0.11 dm^3 mol^{-1} s^{-1} and k_2/K_{a2} = 0.0047 dm^3 mol^{-1} s^{-1}. In other words, the reaction *via* the monocation is approximately as rapid with **9** as with 2′-deoxyadenosine (taking the difference in temperature into account), while the reaction *via* the dicationic species proceeds at least 10 times as fast with 2′-deoxyadenosine as with its 8-aza analogue. The monocation of 2′-deoxyadenosine bears a proton at N1,[22–24] and the same site of protonation has been suggested for 8-azaadenosine on the basis of the X-ray structure of its hydrochloride.[25] The second protonation takes place with adenine nucleosides at N7, the difference between pK_{a1} and pK_{a2} values being 5 log units.[26,27] With 8-aza derivatives this difference may be larger, since the adjacent N8 atom reduces the basicity of the N7 site. Obviously this decrease in basicity (increase in K_{a2}) is not completely compensated for by the concomitant increase in the heterolysis rate (k_2), and hence hydrolysis *via* the diprotonated species (k_2/K_{a2}) is slower than with 2′-deoxyadenosine.

8-Aza-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)adenine (**13**) is hydrolysed 75 times as fast as its *N*9-isomer (**9**), but the shape of the pH–rate profile appears to be similar to that of **9**. The corresponding reactivity ratio of 7-(β-D-ribofuranosyl)adenine and adenosine has been reported to be 34.[28] The observed rate enhancement may be partly of steric and partly of electronic origin. The relatively modest reactivity difference between *N*1- and *N*3-2′-deoxyribofuranosides of 4-methylbenzotriazole (**1c**; **5c**) suggests that the electronic effects play a more important role. For comparison, 2-amino-7-(2-hydroxyethoxymethyl)-purine is depurinated 50 times as fast as its *N*9-isomer,[9] and this reactivity difference cannot be accounted for by steric influences.

The pH–rate profile for the hydrolysis of 8-aza-8-(2-deoxy-β-D-*erythro*-pentofuranosyl)adenine (**11**) also resembles that of the corresponding *N*9-isomer, but goes through an inflection point at a higher pH. The values obtained for the partial rate constants are: k_1/K_{a1} = 1.2 dm^3 mol^{-3} s^{-1} and k_2/K_{a2} = 0.0076 dm^3 mol^{-1} s^{-1} (pK_{a1} = 3.10). Comparison with the data presented above reveals that the monocations of the *N*8- and *N*9-isomers are heterolysed at approximately the same rate, but the *N*8-isomer is more basic, and hence is protonated to a greater extent at low concentrations of hydronium ion. In highly acidic solutions, where the reaction *via* the dication predominates, the reactivity difference is much smaller.

8-Aza-9-(2-deoxy-α-D-*erythro*-pentofuranosyl)adenine (**10**) is hydrolysed slightly faster than 8-aza-2′-deoxyadenosine, the

Table 4 ^{13}C NMR chemical shifts of the benzotriazole nucleosides prepared[a]

Compound	Chemical shift											
	C4	C5	C6	C7	C3a	C7a	C1′	C2′	C3′	C4′	C5′	CH_3
1c[d]	129.7	124.0	127.7	108.5	145.6	132.5	86.4	*b*	70.8	88.2	61.9	16.4
3c[d]	128.4[c]	125.7[c]	127.6[c]	115.4	144.0[c]	143.6[c]	93.1	*b*	70.7	87.3	62.1	16.7
5c[d]	121.8	128.8	124.3	116.8	132.2	146.0	86.4	37.9	70.9	88.3	62.2	17.8
2d[e]	135.3	121.8	127.5	119.2	137.8[c]	138.0[c]	91.3	73.1	70.4	86.3	61.4	
4d[e]	145.7	126.9	126.3	125.6	137.4[c]	136.4[c]	98.0	75.3	70.6	86.7	61.9	
2b[e]	140.6	104.4	129.2	96.4	136.2	133.8	90.3	72.9	70.5	85.6	61.8	
4b[e]	145.0	104.3	128.8	103.5	139.4	136.6	96.8	74.8	70.8	86.1	62.3	

[a] In [$^2H_6$]DMSO; ppm from internal TMS. [b] Overlapping with solvent. [c] Tentative. [d] Assignment of the signals according to **1a**[12] using increments of methyl substituent. [e] Aglycon signals according to the 2′-deoxyribofuranoside.[12]

ratio of the rate constants being *ca.* 1.5. With the corresponding *N*8-glycosides (**12**; **11**) the reactivity difference is slightly smaller [$k_{obs}(\alpha)/k_{obs}(\beta)$ = 1.2]. For comparison, 9-(2-deoxy-α-D-*erythro*-pentofuranosyl)adenine has been reported to be hydrolysed 1.47 times as fast as 2′-deoxyadenosine.[29]

In summary, the acid-catalysed hydrolysis of benzotriazole and 8-azaadenine nucleosides, is mechanistically similar to that of purine nucleosides. However, the reaction *via* substrate dication is usually exceptionally slow, owing to impeded formation of this species. Only with 4-aminobenzotriazole nucleosides, which undergo *N*4-protonation, and with *N*2-glycosides of benzotriazoles is no such anomalous behaviour observed.

Experimental

Materials.—General. Acetonitrile was distilled from calcium hydride. Glass plates coated with a 0.25 mm layer of silica gel Sil G-25 with fluorescent indicator UV_{254} (Merck) were employed for TLC analyses. Column flash chromatographic separations were carried out at 0.8 bar* on silica gel 60H (Merck). NMR spectra were recorded on a Bruker-AC-250 spectrometer using tetramethylsilane as an internal standard. Coupling constants are in Hz. ^{13}C NMR data for selected nucleosides are given in Table 4. Elemental analyses were performed by Mikroanalytisches Laboratorium Beller, Göttingen.

1-[2-*Deoxy*-3,5-*di*-O-(p-*toluoyl*)-β-D-erythro-*pentofuranosyl*]-4-*methyl*-1H-*benzotriazole* (**6c**), 2-[2-*deoxy*-3,5-*di*-O-(p-*toluoyl*)-β-D-erythro-*pentofuranosyl*]-4-*methyl*-2H-*benzotriazole* (**7c**) *and* 1-[2-*deoxy*-3,5-*di*-O-(p-*toluoyl*)-β-D-erythro-*pentofuranosyl*]-7-*methyl*-1H-*benzotriazole* (**8c**). The solution of 4-methylbenzotriazole[30] (5 mmol, 660 mg) in acetonitrile (30 cm^3) was treated with sodium hydride (80% in oil; 6 mmol; 180 mg) and stirred for 10 min. 2-Deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-*erythro*-pentofuranosyl chloride[31] (5 mmol, 1.95 g) was added and the stirring was continued for 15 min. The reaction mixture, filtered through Celite, was evaporated to an oil, which was applied to a silica gel 60H column (4 × 21 cm). Elution with 9:1 and 8:2 light petroleum–ethyl acetate afforded three zones. The fast migrating one, consisting of **6c**, gave colourless needles (870 mg, 36%) when crystallized from methanol. M.p. 111–112 °C; TLC (silica gel, light petroleum–ethyl acetate, 8:2) R_f 0.65; δ_H([$^2H_6$]DMSO) 2.34, 2.39 and 2.54 (3 s, 3 CH_3), 2.96 (m, $H_\alpha2'$), 3.41 (m, $H_\beta2'$), 4.55 (m, H5′ and H5″), 4.70 (m, H4′), 5.97 (q, H3′), 6.88 (dd, *J* 4.9 and 1.6, H1′), and 7.2–8.0 (m, arom. H) (Found: C, 68.9; H, 5,5; N, 8.7. $C_{28}H_{27}N_3O_5$ requires: C, 69.26; H, 5.61; N, 8.65%).

The second zone, consisting of **7c**, crystallized as colourless needles (520 mg, 22%) from methanol. M.p. 109–110 °C. TLC (silica gel, light petroleum–ethyl acetate, 8:2) R_f 0.52; δ_H([$^2H_6$]DMSO) 2.36, 2.40 and 2.68 (3 s, 3 CH_3), 2.93 (m, $H_\alpha2'$), 3.57 (m, $H_\beta2'$), 4.44 (m, H5′ and H5″), 4.67 (q, H4′), 5.90 (m, H3′), 6.99 (t, *J* 6.1, H1′) and 7.2–8.0 (m, arom. H) (Found: C, 68.75; H, 5.5; N, 8.8. $C_{28}H_{27}N_3O_5$ requires: C, 69.26; H, 5.61; N, 6.65%).

The slow migrating zone, consisting of **8c**, afforded a solid foam on evaporation (540 mg, 22%). TLC (silica gel, light petroleum–ethyl acetate, 8:2) R_f 0.47. δ_H([$^2H_6$]DMSO) 2.35, 2.40 and 2.77 (3 s, 3 CH_3), 2.95 (m, $H_\alpha2'$), 3.85 (m, $H_\beta2'$), 4.28 (m, H5′ and H5″), 4.86 (q, H3′), 5.92 (m, H4′), 7.06 (dd, *J* 4.5 and 2.1, H1′) and 7.2–8.0 (m, arom. H) (Found: C, 69.4; H, 5.6; N, 8.6. $C_{28}H_{27}N_3O_5$ requires: C, 69.26; H, 5.61; N, 8.65%).

1-(2-*Deoxy*-β-D-erythro-*pentofuranosyl*)-4-*methyl*-1H-*benzotriazole* (**1c**). A suspension of **6c** (1 mmol, 490 mg) in methanol (15 cm^3) was treated with methanolic sodium methoxide (2 cm^3, 1 mol dm^{-3}), stirred overnight at room temperature and finally refluxed for 10 min. The product mixture was adsorbed on silica gel, loaded on the top of a silica gel 60 column (2 × 15 cm) and eluted with a mixture of chloroform and methanol (19:1). The fractions containing **1c** were evaporated, and the residue was crystallized from a mixture of ethyl acetate and ethanol to give colourless needles (180 mg, 72%). M.p. 133–134 °C. λ_{max}/nm (pH 7) 260 (ε/dm^3 mol^{-1} cm^{-1} 7200) and 287 (4600); TLC (silica gel, chloroform–methanol, 9:1) R_f 0.40; δ_H([$^2H_6$]DMSO) 2.41 (m, $H_\alpha2'$), 2.69 (s, CH_3), 3.04 (m, $H_\beta2'$), 3.45 (m, H5′ and H5″), 3.93 (q, H4′), 4.53 (m, H3′), 4.81 (t, HO5′), 5.42 (d, HO3′), 6.73 (t, *J* 6.3, H1′), 7.20 (d, *J* 7.0, H5), 7.44 (t, *J* 7.1, H6) and 7.76 (d, *J* 8.3, H7) (Found: C, 57.7; H, 6.0; N, 16.8. $C_{12}H_{15}N_3O_3$ requires: C, 57.82; H, 6.07; N, 16.86%).

2-(2-*Deoxy*-β-D-erythro-*pentofuranosyl*)-4-*methyl*-2H-*benzotriazole* (**3c**). **7c** was deprotected as described for **6c**. The colourless oily residue obtained crystallized upon storing (170 mg, 68%). M.p. 52–54 °C. λ_{max}/nm (pH 7) 279 (8900); TLC (silica gel, chloroform–methanol, 9:1) R_f 0.60; δ_H([$^2H_6$]DMSO) 2.50 (m, $H_\alpha2'$), 2.60 (s, CH_3), 2.94 (m, $H_\beta2'$), 3.52 (m, H5′ and H5″), 3.97 (q, H4′), 4.62 (m, H3′), 4.79 (t, HO5′), 5.41 (d, HO3′), 6.64 (dd, *J* 4.5 and 2.2, H1′), 7.23 (d, *J* 6.3, H5), 7.40 (t, *J* 6.8, H6) and 7.78 (d, *J* 8.6, H7) (Found: C, 57.8; H, 6.2; N, 16.9. $C_{12}H_{15}N_3O_3$ requires: C, 57.82; H, 6.07; N, 16.86%).

1-(2-*Deoxy*-β-D-erythro-*pentofuranosyl*)-7-*methyl*-1H-*benzotriazole* (**5c**). **8c** was deprotected as described for **6c**. Crystallization from ethyl acetate gave small colourless needles (190 mg, 76%). M.p. 83–85 °C; TLC (silica gel, chloroform–methanol, 9:1) R_f 0.40; λ_{max}/nm (pH 7) 261 (6000) and 284 (3700). δ_H([$^2H_6$]DMSO) 2.50 (m, $H_\alpha2'$), 2.77 (s, CH_3), 3.40 (m, $H_\beta2'$), 3.97 (q, H4′), 4.58 (m, H3′), 4.70 (t, HO5′), 5.44 (d, HO3′), 6.83 (dd, *J* 4.7 and 1.8, H1′) and 7.35 and 7.85 (m, H5, H6 and H7) (Found: C, 57.8; H, 6.2; N, 16.9. $C_{12}H_{15}N_3O_3$ requires: C, 57.82; H, 6.07; N, 16.86%).

4-*Nitro*-1-(β-D-*ribofuranosyl*)-1H-*benzotriazole* (**2d**) *and*

* 1 bar = 10^5 Pa.

4-nitro-2-(β-D-ribofuranosyl)-2H-benzotriazole (**4d**). A well-ground mixture of 4-nitrobenzotriazole[32] (5 mmol, 820 mg) with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (5.15 mmol, 1.64 g) was heated for 10 min at 135–145 °C under reduced pressure (aspirator). The orange product mixture obtained was dissolved in methanol (50 cm^3), basified with methanolic sodium methoxide (10 cm^3, 1 mol dm^{-3}), and the mixture was stirred for 2 h at room temperature and finally refluxed for 10 min. The product was adsorbed on silica gel and loaded on the top of a silica gel 60H column (5 × 25 cm). Elution with a mixture of chloroform and methanol (9:1) gave two zones. The fast migrating product (**4d**) crystallized as yellow plates from water (514 mg, 35%). M.p. 147–148 °C; TLC (silica gel, chloroform–methanol, 9:1) R_f 0.43; λ_{max}/nm (pH 7) 314 (9700); δ_H([$^2H_6$]DMSO) 3.67 (m, H5′ and H5″), 4.14 (q, H4′), 4.42 (br. s, H3′), 4.65 (br. s, H2′), 4.84 (t, HO5′), 5.38 (d, HO3′), 5.81 (d, HO2′), 6.34 (d, *J* 3.1, H1′), 7.74 (t, *J* 7.7, H6) and 8.53 (m, H5 and H7) (Found: C, 44.8; H, 4.3; N, 19.0. $C_{11}H_{12}N_4O_6$ requires: C, 44.60; H, 4.08; N, 18.91%).

The slow migrating zone (**2d**) gave yellow needles (500 mg, 34%) when crystallized from water and then from a mixture of ethyl acetate and ethanol. M.p. 167–168 °C (lit.,[33] 159–160 °C); TLC (silica gel, chloroform–methanol, 9:1) R_f 0.23; λ_{max}/nm (pH 7) 306 (9800); δ_H([$^2H_6$]DMSO) 3.60 (m, H5′ and H5″), 4.09 (q, H4′), 4.29 (q, H3′), 4.77 (q, H2′), 4.98 (t, HO5′), 5.36 (d, HO3′), 5.66 (d, HO2′), 6.43 (d, *J* 5.0, H1′), 7.83 (t, *J* 8.0, H6), 8.35 (d, *J* 7.6, H5) and 8.62 (d, *J* 8.3, H7) (Found: C, 44.4; H, 4.1; N, 18.9. $C_{11}H_{12}N_4O_6$ requires: C, 44.60; H, 4.08; N, 18.91%.

4-Amino-1-(β-D-ribofuranosyl)-1H-benzotriazole (**2b**). The solution of **2d** (1.4 mmol, 400 mg) in ethanol (40 cm^3) was hydrogenated for 6 h on palladium (10% on C, 80 mg). The filtered solution was evaporated and the residue crystallized as small needles (255 mg, 71%) from ethanol. M.p. 159–161 °C; TLC (silica gel, chloroform–methanol, 9:1) R_f 0.23; λ_{max}/nm (pH 7) 224 (22 000), 265 (2800) and 318 (4600); δ_H([$^2H_6$]DMSO) 3.60 (m, H5′ and H5″), 4.01 (q, H4′), 4.26 (q, H3′), 4.73 (q, H2′), 4.91 (t, HO5′), 5.28 (d, HO3′), 5.53 (d, HO2′), 6.07 (br. s, NH_2), 6.16 (d, *J* 5.3, H1′), 6.45 (d, *J* 7.4, H5), 6.97 (d, *J* 7.9, H7) and 7.20 (t, *J* 7.9, H6) (Found: C, 50.0; H, 5.2; N, 21.0. $C_{11}H_{14}N_4O_4$ requires: C, 49.62; H, 5.30; N, 21.04%).

4-Amino-2-(β-D-ribofuranosyl)-2H-benzotriazole (**4b**). The solution of **4d** (1.0 mmol, 300 mg) in ethanol (30 cm^3) was hydrogenated for 8 h on palladium (10% on C, 60 mg). The product was adsorbed on silica gel and chromatographed on a silica gel 60H column (2 × 10 cm). Elution with a mixture of chloroform and methanol (9:1) yielded a yellow oil (190 mg, 70%). TLC (silica gel, chloroform–methanol 9:1) R_f 0.35; λ_{max}/nm (pH 7) 226 (22 000), 278 (3600), 287 (3500) and 328 (3200); δ_H([$^2H_6$]DMSO) 3.60 (m, H5′ and H5″), 4.06 (q, H4′), 4.37 (q, H3′), 4.62 (q, H2′), 4.81 (t, HO5′), 5.26 (d, HO3′), 5.65 (d, HO2′), 5.81 (br. s, NH_2), 6.12 (d, *J* 3.6, H1′), 6.40 (d, *J* 7.3, H5), 6.98 (d, *J* 8.4, H7) and 7.14 (t, *J* 8.5, H6) (Found: C, 49.8; H, 5.45; N, 21.0. $C_{11}H_{14}N_4O_4$ requires: C, 49.62; H, 5.30; N, 21.04%).

Kinetic and Equilibrium Measurements.—The first-order rate constants were obtained by the HPLC technique described previously.[2] In some illustrative cases the hydrolysis reactions were also followed by ^1H NMR spectroscopy (JEOL GX-400) in deuterium oxide containing deuteriated perchloric acid. The pK_a values of substrate monocations were determined spectrophotometrically (Cary 17D) by recording the UV spectra at increasing acid concentrations. Calculation of the pK_{a1} values were based on the H_0 scale.[34]

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