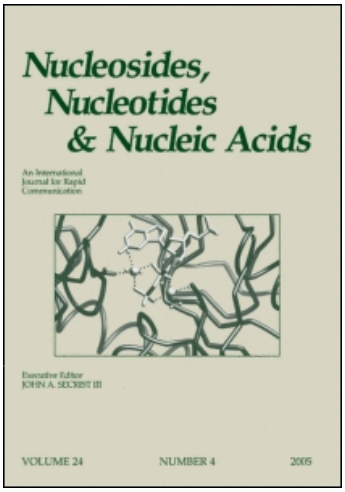

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MECHANISM FOR ACID-CATALYZED HYDROLYSIS OF NUCLEOSIDE AND ACYCLONUCLEOSIDE ANALOGUES OF BENZIMIDAZOLE

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Abstract: A study has been made of a broad series of nucleosides and acyclonucleosides of a variety of benzimidazole analogues. Quantitative kinetic data are presented, and the mechanism of the reactions described, taking into account steric, electronic and conformational effects. The overall results are also compared with those of purine nucleosides.

We have previously reported upon, and described, the mechanism of the acid-catalyzed hydrolysis of a series of 2-substituted 1-(1-alkoxyethyl)benzimidazoles.[1,2] The subsequent accessibility of a variety of benzimidazole nucleoside analogues, some of which exhibit significant biological activities, prompted us to extend our investigation to this class of compounds.

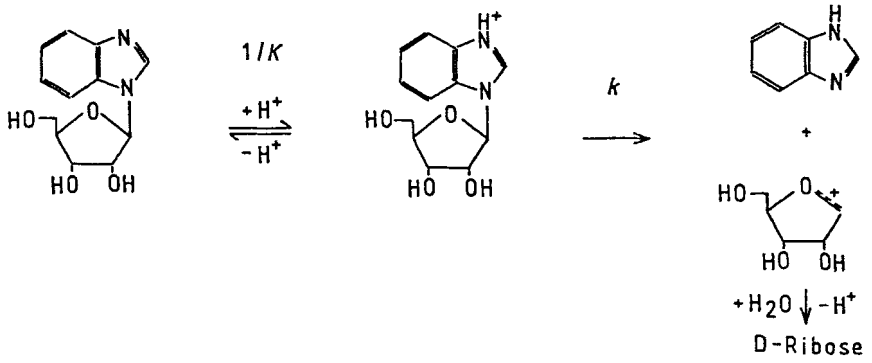
The chemical and physico-cnemical properties of benzimidazole and its nucleosides, of obvious intrinsic interest in relation to the properties of purine nucleosides, are equally relevant to their multitudinous biological properties, some of which have been reviewed by Sehgal and Tamm.[3] It is worth noting that the α-anomer of 5,6-dimethyl-1-(D-ribofuranosyl)benzimidazole, as well as some of its variants, are key constituents of vitamin B_{12}.[4] Benzimidazole nucleosides and nucleotides were long ago shown to be substrates of higher plant enzymes and to play some role in protein biosynthesis, and a benzimidazole nuc-

leoside has been found to be a component of an enzyme isolated from wheat embryos.[5] Various benzimidazole nucleoside analogues exhibit significant in vitro antiviral activities.[3] The well-known 5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole, commonly denoted as DRB, is a superinductor of interferon in human fibroblasts.[3] The same analogue is better known, and widely applied as a specific reversible inhibitor of initiation of transcription in eukaryotic systems.[6] More recently it has been demostrated that this inhibitory effect is mediated via inhibition of casein kinase II, which is apparently involved in some phosphorylation step essential to initiation of transcription.[7] Benzimidazole analogues have also been implicated as acting the functional FAD cofactor[8] and as antimicrobial agents with potential utility in control of periodontal diseases.[9]

Apart from the foregoing, studies of benzimidazole nucleosides might be expected to furnish useful data for comparison with corresponding data already available for purine nucleosides, since with benzimidazole derivatives there is only one potential site of protonation, the ring N(3), irrespective of other substitution in the benzimidazole ring.

RESULTS AND DISCUSSION

We had previously shown that 1-(1-alkoxyethyl)benzimidazoles,[1,2] and the corresponding 1-(3-chlorotetrahydro-2-furyl) analogues,[10] are hydrolyzed in aqueous acid by a rate-limiting cleavage of the N(3)-protonated substrate to the free benzimidazole base and a resonance-stabilized carbonium ion. The following facts strongly suggest that a similar mechanism, depicted in Scheme 1, is applicable to the hydrolysis of benzimidazole ribonucleosides. (i) The observed first-order rate constants, k, are proportional to the oxonium ion concentration at low acidities, and then level off to constant values, $k(SH^+)$, under conditions where the substrate is completely protonated. Illustrative examples are the rate profiles for 5,6-disubstituted benzimidazole ribonucleosides (1a-1e, Fig. 1). (ii) The logarithmic values of the rate constants, $k(SH^+)$, are linearly dependent on the logarithmic values of the acidity constants, $K(SH^+)$, of the substrate monocations (Fig. 2), the reaction constant (1.05, r=0.9998, n=5) being almost identical to that obtained earlier with 2-substituted 1-(1-ethoxyethyl)benzimidazo-



Scheme 1

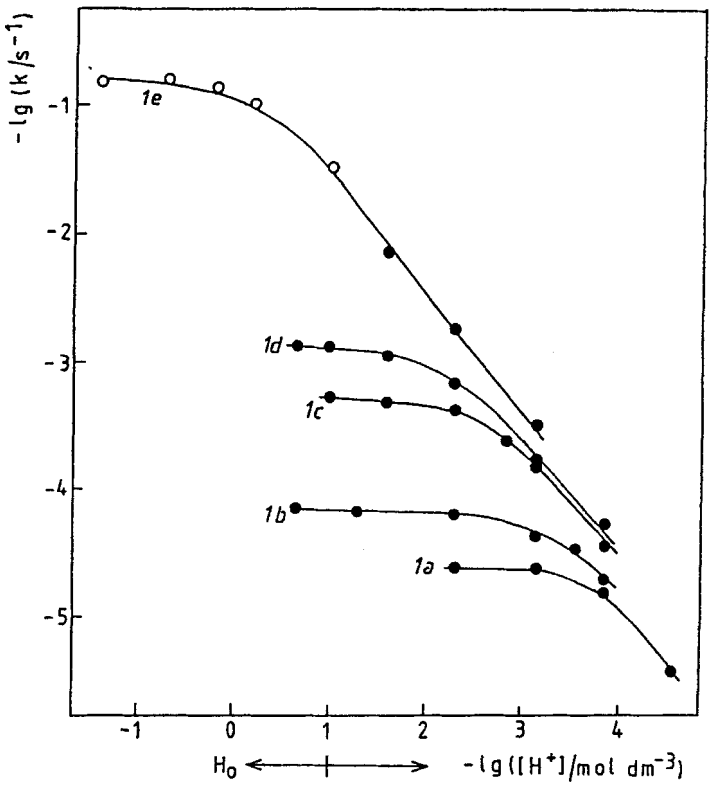
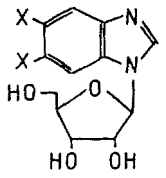
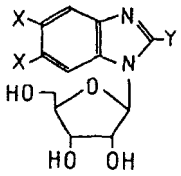


Fig. 1: Rate-profiles for the hydrolysis of 5,6-disubstituted benzimidazole ribonucleosides at 393.2 K. The ionic strength was adjusted to 0.20 mol dm^{-3} at $[H^+]$ < 0.20 mol dm^{-3}.

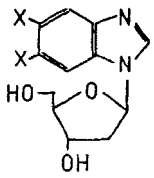
les.[2] (iii) The possible anomerization during hydrolysis, as has been observed for some deaza analogues of purine nucleosides,[11,12] can be eliminated in the present instance, since no sign of anomerization could be detected when the hydrolysis of 1b was followed by ^{1}H NMR spectro-



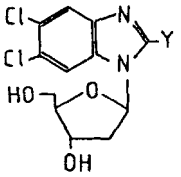
1a: X = CH_3
1b: X = H
1c: X = F
1d: X = Cl
1e: X = NO_2



3a: X = H , Y = CH_3
3b: X = H , Y = CF_3
3c: X = F , Y = CH_3
3d: X = F , Y = $CH(CH_3)_2$
3e: X = Cl , Y = CH_3



2a: X = H
2b: X = F
2c: X = Cl



4a: Y = CH_3
4b: Y = $CH(CH_3)_2$

scopy. And, finally (iv) the entropy of activation for the hydrolysis of the monocation of 1e is slightly positive, viz. (28 + 3)J K^{-1} mol^{-1} at 333.2 K, consistent with the suggested unimolecular nature of the rate-limiting step.

Although our data for hydrolysis of benzimidazole 2'-deoxyribonucleosides (2a-2c) are more limited, the similarity of the structural effects in the hydrolysis of ribo- and 2'-deoxyribo-nucleosides may be regarded as compelling evidence for similar mechansims (Table 1). The observed reactivity ratio for the ribo- and 2'-deoxyribo-nucleosides, viz. 4 10^{-3} at 363.2 K, is that to be anticipated from the known[13] difference in stabilities between ribo- and 2'-deoxyribo-furanosyl oxocarbenium ions.

As mentioned above, the plot of $\lg[k(SH^+)/s^{-1}]$ vs. $-\lg[K(SH^+)/mol\ dm^{-3}]$ is strictly linear for the 5,6-disubstituted benzimidazole ribonucleosides (1a-1e). By contrast, the points for the 2-substituted nucleosides (3a-3e) are displaced by 0.3 to 1.2 units above this correlation line. The situation is analogous for the 2-substituted benzimidazole 2'-deoxyribonucleosides (4a,4b). The magnitude of the deviation appears to correlate with the size of the 2-substituent, being 0.3 ~ 0.6

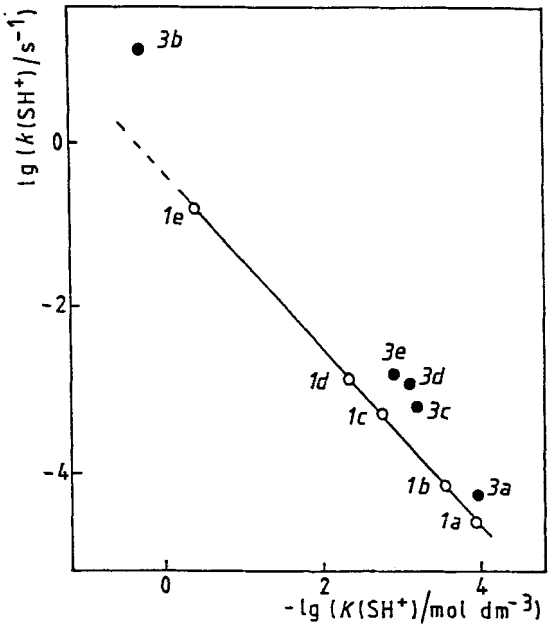


Fig. 2: Logarithmic rate constants for the heterolysis of monoprotonated benzimidazole ribonucleosides plotted against the logarithmic acidity constants of the same species at 393.2 K. Notation: 1a-1e are 5,6-disubstituted and 3a-3e 2-substituted compounds.

units for a methyl, 0.7 - 0.8 for an isopropyl, and 1.2 for the trifluoromethyl. For comparison, the steric parameters of these groups are 0.0, 0.48 and 0.78, respectively.[14] This acceleration of rate, which is not of electronic origin, may result from two different factors. On one hand, a bulky substituent at C(2), adjacent to the N-glycosidic bond, undoubtedly enhances steric compression in the initial state. On the other hand, substitution at C(2) shifts the syn-anti equilibrium towards the syn conformation,[15-17] where non-bonded repulsive interactions between the sugar and base moieties are more pronounced than in the anti conformation. Since the steric strain due to both these factors is partially relieved in the activated complex, the reaction is accelerated.

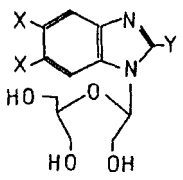
One possible approach to distinguish between the contributions of the syn-anti equilibrium and normal steric acceleration to the observed

Table 1: Acidity constants, $K(SH^+)$, for the monocations of nucleoside and acyclonucleoside analogues of benzimidazole, and first-order rate constants, $k(SH^+)$, for their hydrolysis in aqueous solution.[a]

Benzimidazole analogue	T/K	$-\lg[K(SH^+)/\text{mol dm}^{-3}]$	$k(SH^+)/10^{-3}\ s^{-1}$
Ribonucleosides			
5,6-Dimethyl- (1a)	393.2	3.97 ± 0.12	0.025 ± 0.001
Unsubstituted (1b)	393.2	3.58 ± 0.06	0.069 ± 0.002
5,6-Difluoro (1c)	393.2	2.76 ± 0.05	0.54 ± 0.02
5,6-Dichloro (1d)	393.2	2.34 ± 0.10	1.35 ± 0.06
5,6-Dinitro (1e)	393.2	0.38 ± 0.10	154 ± 6[b]
2-Methyl (3a)	393.2	3.98 ± 0.11	0.045 ± 0.002
2-Trifluoromethyl (3b)	393.2	-0.34 ± 0.15[c]	12300 ± 1300[b]
5,6-Difluoro-2-methyl (3c)	393.2	3.20 ± 0.11	0.61 ± 0.04
5,6-Difluoro-2-isopr. (3d)	393.2	3.10 ± 0.02	1.17 ± 0.05
5,6-Dichloro-2-methyl (3e)	393.2	2.91 ± 0.06	1.45 ± 0.07
2'-Deoxyribonucleosides			
Unsubstituted (2a)	363.2	3.89 ± 0.03	0.65 ± 0.01
5,6-Difluoro (2b)	363.2	3.06 ± 0.03	5.76 ± 0.09
5,6-Dichloro (2c)	363.2	2.75 ± 0.04	11.6 ± 0.3
5,6-Dichloro-2-methyl (4a)	363.2	3.28 ± 0.02	13.1 ± 0.3
5,6-Dichloro-2-isopr. (4b)	363.2	3.05 ± 0.09	27.0 ± 0.6
2',3'-seco-nucleosides			
Unsubstituted (5a)	393.2	3.80 ± 0.08	0.27 ± 0.02
2-Methyl (5b)	393.2	4.19 ± 0.11	0.116 ± 0.005
5,6-Difluoro (5c)	393.2	2.90 ± 0.01	2.42 ± 0.08
Arabinonucleosides			
Unsubstituted (6)	393.2		0.047 ± 0.009
α-Ribonucleosides			
5,6-Dimethyl (7)	393.2		0.026 ± 0.008

[a]Ionic strength was adjusted to 0.20 mol dm^{-3} with sodium chloride. [b]Extrapolated by the Arrhenius equation from the rate constants obtained at lower temperatures. [c]Extrapolated by the van't Hoff equation.

rate-accelerations is to compare the influence of a given C(2)-substituent on the rates of hydrolysis of a ribonucleoside and its 2',3'-seco counterpart (5a-5c) under conditions where both are fully protonated.



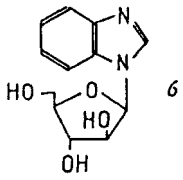
5a: X = H, Y = H
5b: X = H, Y = CH_3
5c: X = F, Y = H

Bearing in mind that the acyclic chain of a seco nucleoside is much more flexible, the syn-anti equilibrium would be expected to affect the reactivity to a lesser extent than for the parent ribonucleoside. A clear indication of the flexibility of the acyclic moiety of seco nucleosides is furnished by their $k(SH^+)$ values, which are 4-fold larger than those for the corresponding parent ribonucleosides. Opening of the sugar ring stabilizes the developing oxocarbenium ion, since formation of a partial double-bond between the C(1) and $O^{4'}$ is not hindered by conformational changes required in the case of a ribofuranosyl ring to obtain a planar structure in this portion of the molecule.

From Table 1 it will be noted that a C(2)-methyl substituent increases the basicity of 1-substituted benzimidazole derivatives by 0.5 log units. Since the reaction constant is close to unity, the electronic influence on the rate constant, $k(SH^+)$, should be almost the same. In other words, a C(2)-methyl group should decrease the value of $k(SH^+)$ to one-third if steric effects can be ignored. This appears to be the case with 2',3'-seco-nucleosides. The ratio of the $k(SH^+)$ values for the compounds 5b and 5a is 0.43, i. e. only slightly larger than the predicted value of 0.33, and comparable to our previous[2] corresponding value of 0.48 for 1-(1-ethoxyethyl)benzimidazoles. Hence steric factors appear to play only a minor role in the hydrolysis of acyclonucleoside analogues of benzimidazole. With the parent nucleosides (3a,3c,3e,4a) the 2-methyl substituent is only slightly rate-retarding, or even rate-accelerating, the $k(SH^+,CH_3)/k(SH^+,H)$ ratios being 0.65 (3a), 1.1 (3c), 1.1 (3e) and 1.1 (4a). Hydrolysis of ribonucleosides is thus more susceptible to the steric nature of the 2-substituent than that of seco nucleosides. One possible explanation for this is that the syn conformer of ribonucleosides is somewhat more labile than the anti conformer. A C(2)-methyl shifts the syn-anti equilibrium appreciably towards the syn form ($x_{syn}>0.9$),[15] with concomitant acceleration of hydrolysis. However, the role of the syn-anti equilibrium should not be overestimated; e. g. 2-isopropyl (3d,4b) and 2-trifluoromethyl (3b) congeners exhibit an even stronger preference for the syn conformation,[16] but their $k(SH^+)$ values deviate from the reactivity-acidity correlation line only slightly more than those of the 2-methyl analogues. Furthermore, part of the observed rate-enhancement may undoubtedly be attributed to normal steric

acceleration. The real difference in reactivity between the syn and anti conformers is most probably less than one order of magnitude.

The configuration of the carbohydrate moiety does not appear to play a significant role in the hydrolysis of benzimidazole nucleosides, as may be seen from the behaviour of 1-(β-D-arabinofuranosyl)benzimidazole (6, Table 1), which is hydrolyzed about as readily as the corresponding riboside, and the similar reactivities of anomeric 5,6-dimethyl-1-(D-ribofuranosyl)benzimidazoles (1a and 7). It is worth noting, in this connection, that York[18] observed only an 8 percent difference in rates of acidic hydrolysis of the α- and β-anomers of adenosine.



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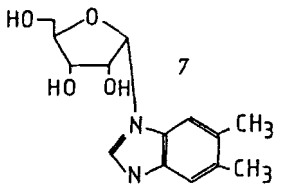

7

We had previously[19] demonstrated that the rate constants for the the breakdown of the monocations of 6-substituted purine ribonucleosides are linearly dependent on the acidity constants of the monocations. As may be seen from Fig. 3, the points referring to the hydrolysis of benzimidazole ribonucleosides are almost on this correlation line, indicating that there is no real difference between the reactions of the monoprotonated species of benzimidazole and purine nucleosides. Benzimidazole is as good leaving group as purine, as long as reactions via monoprotonated substrates are concerned. The higher reactivity of purine nucleosides in very acidic solutions results from the fact that under such conditions purine nucleosides react via diprotonated species, which is not possible in the case of benzimidazole derivatives. In slightly acidic solutions the hydrolytic stabilities do not differ markedly.

Our findings on the influence of C(2)-substituents on the stability of the N-glycosidic bond appear to be consistent with the data of Townsend et al.,[20,21] who showed that C(6)-substituents enhance the reactivity of 7-(β-D-ribofuranosyl)purine by a factor of 2-3, whereas analogous substitution retards the hydrolysis of 9-(β-D-ribofuranosyl)-purine by a factor of 5. The increase in rate for the N(7)-glycoside

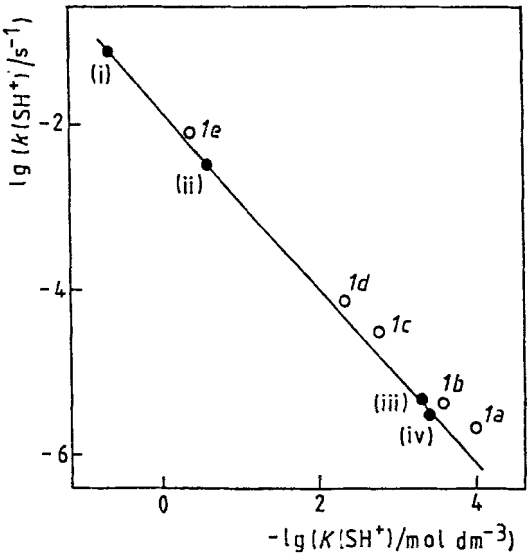

Fig. 3: Logarithmic rate constants for the heterolysis of monoprotonated purine nucleosides plotted against the logarithmic acidity constants of the same species at 363.2 K. Notation: (i) 6-chloro, (ii) 6-methoxy, (iii) 6-amino and (iv) 6-dimethylamino derivatives of 9-(β-D-ribofuranosyl)purine. Points 1a-1e refer to the monocations of benzimidazole nucleosides at the same temperature.

series was attributed to the added effect of steric acceleration. The 5 to 30-fold higher reactivity of the N(7)-glycosides relative to their N(9)-glycoside counterparts may be assumed to reflect the reactivity difference between syn and anti conformers, though normal steric acceleration undoubtedly contributes. Moreover, recent ^{15}N NMR spectroscopic studies have demonstrated that N(9) of the N(7) substituted isomers has an enhanced basicity compared to N(7) of the N(9) substituted isomers, and this may also increase the reactivity of the N(7)-glycosides.[22]

Also relevant in this context is the report of Jordan and Niv,[23] according to which replacement of an 8-amino or 8-methylamino by 8-dimethylamino enhanced the rate of hydrolysis 110-fold in the case of adenosine, and as much as 800-fold in the case of guanosine. This was interpreted by the authors as due to the dimethylamino nucleosides being "locked" in the syn conformation about the N-glycosidic bond. While it is true that 8-dimethylamino-adenosine and -guanosine are predominantly in the conformation syn, the dimethylamino substituent is not suffi-

ciently bulky to liquidate the syn-anti equilibrium. In fact, the situation in this case is probably not significantly different from that for the riboside of 2-trifluoromethylbenzimidazole. But comparisons of the relative reactivities of 8-substituted guanosines and adenosines are additionally complicated by the fact that an 8-substituent may influence not only the syn-anti equilibrium, but also the relative basicities of the nitrogen atoms of the purine ring. This latter factor is of paramount importance in that the site of protonation has a significant effect on the leaving-group properties of the purine base. For comparison, we have recently demonstrated that N(7)H-adenine is a far better leaving-group than its N(1)H tautomer.[24]

EXPERIMENTAL

Materials. Compounds 1a-1d, 2a-2c, 3b-3e, 4a, 4b and 6 were synthesized as previously described.[15-17,25,26] The 2',3'-seco nucleosides (5a-5c) were prepared in yields of 80 %, 76 % and 71 % respectively as elsewhere reported.[27] The α-anomer of 5,6-dimethyl-1-(D-ribofuranosyl)benzimidazole (7) was a gift of Prof Z. Ruzic-Toros of Zagreb. 2-Methyl-1-(β-D-ribofuranosyl)benzimidazole was prepared according to the general procedure of Vorbruggen, using $SnCl_4$ as a catalyst.[28] The crude product was chromatographed on silica gel (CH_2Cl_2 containing CH_3OH from 0 to 10 % v/v) and crystallized from water (m.p. 222-224 °C). Elemental analysis: calculated for $C_{13}H_{16}N_2O_4$ C 59.08 %, H 6.10 %, N 10.60 %; found C 59.20 %, H 6.05 %, N 10.48 %. ^1H NMR (DMSO-d_6-D_2O, ppm from TMS) 2.10 (3H, s, CH_3) 4.11 (2H, m, 5'H), 4.33 (3H, m, 2',3',4'H), 5.79 (1H, d, J = 7.2 Hz, 1'H), 7.20 (2H, m, 5H and 6H), 7.57 (2H, m, 4H and 7H). 5,6-Dinitro-1-(β-D-ribofuranosyl)benzimidazole (1e) was synthesized via 5,6-dinitrobenzimidazole and obtained in crystalline form, with satisfactory elementary analyses and ^1H NMR spectrum in agreement with the structure. The synthesis will be described in detail elsewhere.

Kinetic measurements. The first-order rate constants were obtained by a sealed tube technique described previously.[29] The compositions of the samples were analyzed by HPLC on a reversed phase column.[30] The oxonium ion concentrations of the acetic and formic acid buffers, employed in the kinetic measurements, were calculated on the bases of the data

reported in literature.[31-33] The values of $\underline{k}(SH^+)$ and $\underline{K}(SH^+)$ were calculated *via* eqn. (1) by the the method of least squares.

$$\lg[\underline{K}(SH^+)/\text{mol dm}^{-3}] = \lg[\underline{k}(SH^+)/\underline{k} - 1] + \lg[H^+] \qquad (1)$$

^{1}H NMR measurements. The progress of the hydrolysis of 1b was followed by recording the ^{1}H NMR spectra (Jeol GX-400) of the samples of 1b allowed to hydrolyze in acidic deuterium oxide from 0.2 to 2 half-lives. The only signal detected in the anomeric proton region was the 1'H doublet of the starting material.

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