Kinetics of the Multistage Reactions of 6-Substituted Purine Nucleosides with Aqueous Alkalies*

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Reactions of some 6-substituted 9-(β-D-ribofuranosyl)purines with aqueous sodium hydroxide have been studied by liquid chromatography. The main reaction pathway for the decomposition of 6-chloro, 6-methyl and 6-methylthio derivatives has been shown to consist of three consecutive reactions: an attack of hydroxide ion on the C8 atom with rapid subsequent opening of the imidazole ring and anomerization of the glycone moiety, deformylation of the resulting 5-formamido-4-ribosylaminopyrimidines, and cleavage of the N-glycosidic bond. With the 6-chloro derivative, the first step is irreversible, while with the 6-methyl and 6-methylthio derivatives, recyclization to purine ribosides competes with the deformylation of 5-formamido-4-ribosylaminopyrimidines. 6-Chloro- and 6-methylthio-9-(β-D-ribofuranosyl)purines also yield some inosine, but this reaction is of minor importance. In contrast, 6-methoxy-9-(β-D-ribofuranosyl)purine is converted quantitatively to inosine. The rate constants for the different partial reactions have been determined at several concentrations of hydroxide ion. The kinetic data, and those reported earlier for adenosine and 9-(β-D-ribofuranosyl)purine, have been used to evaluate the susceptibility of the consecutive steps to the polar nature of the 6-substituent.

The alkaline hydrolysis of unsubstituted 9-(β-D-ribofuranosyl)purine has been shown[1,2] to involve three kinetically distinguishable steps. Firstly, a nucleophilic attack of hydroxide ion on the C8 atom of the purine moiety leads to opening of the imidazole ring and anomerization of the ribosyl group. Secondly, the resulting anomeric mixture of 5-formamido-4-ribosylaminopyrimidines is deformylated to a mixture of 5-amino-4-ribosylaminopyrimidines, and finally the N-glycosidic bond is cleaved. The alkaline cleavage of adenosine is also initiated by an attack of hydroxide ion on the C8 atom.[3] However, intramolecular cyclizations to anomeric adenine nucleosides and N6-ribosyladenines compete efficiently with the deformylation of the 5-formamidopyrimidine intermediate.[3] N6-Ribosyladenines are finally hydrolyzed to D-ribose and adenine. Adenosine is also partly deaminated to inosine,[3,4] but this reaction contributes only a few percent to the total disappearance of adenosine. The initial attack of hydroxide ion on the C8 site of the adenine ring takes place less readily than all the subsequent reactions. For this reason the mole fractions of all the intermediates remain less than 0.05 during a kinetic run. The consecutive steps are thus kinetically less clearly distinguishable than with unsubstituted purine riboside. Comparative kinetic studies with 2'-deoxy-, 2',3'-O-isopropylidene- and 5'-O-methyladenosine, and 9-(β-D-arabinofuranosyl)adenine have indicated that the glycone moiety hydroxyl groups do not play any important role in the alkaline cleavage of adenine nucleosides.[3]

The present study was aimed at elucidating the influences that polar groups on the C6 atom of the purine ring have on the mechanism of the alkaline cleavage of purine ribosides and on the rates of the parallel and consecutive steps involved. For this purpose, the reactions of

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6-chloro-, 6-methyl-, 6-methylthio- and 6-methoxy-9-(β-D-ribofuranosyl)purines with aqueous sodium hydroxide were studied by liquid chromatography. The kinetic results obtained are compared to those reported previously[2,3] for adenine nucleosides and unsubstituted 9-(β-D-ribofuranosyl)purine. The susceptibilities of different partial reactions to the polar nature of the 6-substituent are discussed.

Results and discussion

Reaction pathways. LC analyses of aliquots withdrawn at different intervals from alkaline solutions of 6-methyl-9-(β-D-ribofuranosyl)purine strongly suggest that the mechanism described[2] for the alkaline cleavage of unsubstituted 9-(β-D-ribofuranosyl)purine may also apply to the hydrolysis of its 6-methyl derivative. As seen from Fig. 1, the starting material (*1a*) is converted quantitatively to 4,5-diamino-6-methylpyrimidine (*4a*) via consecutive accumulation of two intermediates, both of which are equilibrium mixtures of two major and two minor components. The first intermediate was observed to be spectroscopically identical (UV) with 4-amino-5-formamido-6-methylpyrimidine,[5] and most probably consists of four isomeric 5-formamido-4-methyl-6-ribosylaminopyrimidines (*2aαp, 2aβp, 2aαf* and *2aβf*). When the isomeric mixture, *2a*, was separated preparatively by LC and treated with aqueous alkali, it was converted to a mixture of four isomeric compounds (*3a*) that spectroscopically (UV) closely resembles *4a*.[6] Besides *3a*, a compound (*5a*) that was spectroscopically identical (UV) with *1a*, but exhibited a shorter retention time on an RP column, was obtained. The same compound also appeared during the alkaline cleavage of *1a*. When separated by LC and treated with aqueous alkali, it gave quantitatively *4a* via intermediary formations of *2a* and *3a*. These findings, and the fact that adenosine is partly isomerized to 9-(β-D-ribopyranosyl)adenine concurrent with its cleavage in alkali,[3] suggest that *5a* is 6-methyl-9-(β-D-ribopyranosyl)purine. The alkaline hydrolysis of *3a,* separated by LC, yielded only *4a*. Accordingly, the reactions of *1a* with aqueous sodium hydroxide can be summarized by Scheme 1.

The reasoning presented above for 6-methyl-9-(β-D-ribofuranosyl)purine may also be applied to the corresponding 6-methylthio derivative (*1b*).
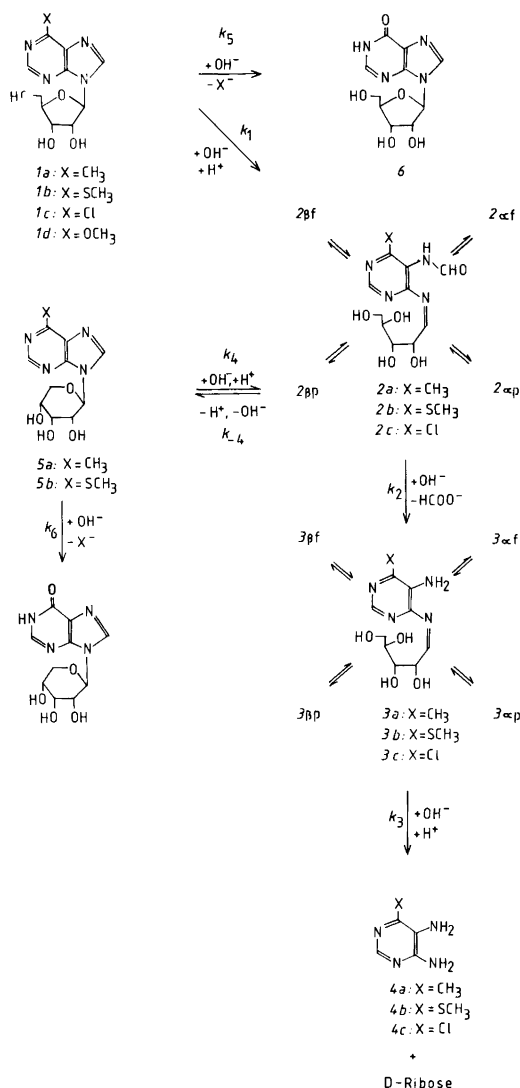
Scheme 1.

However, *1b* was additionally converted to inosine (*6*), although this reaction is not quantitatively important. Fig. 2 shows the time-dependence of the mole fractions of the intermediates and products detected.

LC analyses also indicated that 6-chloro-9-(β-D-ribofuranosyl)purine (*1c*) is partly converted to *6* and partly to *4c* via *2c* and *3c*. However, with this compound, no anomerization of the starting material was observed during the hydrolytic decomposition. The situation is thus
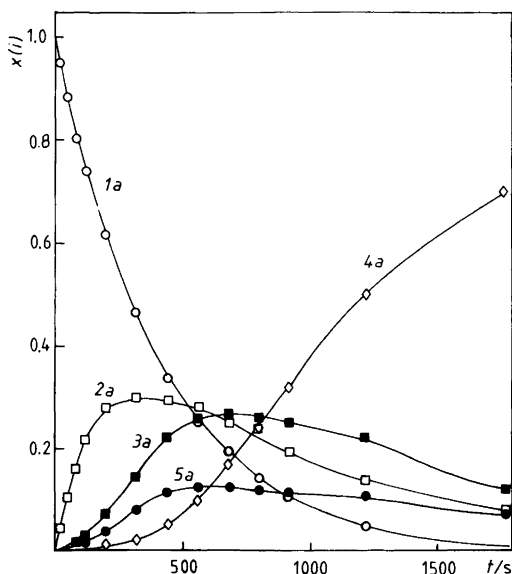
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Fig. 1. Time-dependent product distribution for the reaction of 6-methyl-9-(β-D-ribofuranosyl)purine with aqueous sodium hydroxide (0.20 mol dm^{-3}, ionic strength 0.50 mol dm^{-3} with NaCl) at 363.2 K. The enumeration refers to Scheme 1.

analogous to the hydrolysis of unsubstituted 9-(β-D-ribofuranosyl)purine.[2] These findings are consistent with the results[5] according to which 4-amino-5-formamidopyrimidine and its 6-chloro derivative are cyclized to purines less efficiently than 6-methyl- and 6-amino-substituted compounds. The time-dependent product distribution for the hydrolysis of *1c* is presented in Fig. 3. It should be noted that the data refer to low substrate concentrations ($<10^{-3}$ mol dm^{-3}). At higher concentrations, *1c* polymerizes to insoluble products.[7] The suggested reaction pathway receives further support from the studies of Montgomery and Thomas.[7] When treated with a mixture of dioxane and aqueous sodium hydroxide (50 % v/v), the 2',3'-*O*-isopropylidene derivative of *1c* gave a 47 % yield of 4-chloro-5-formamido-6-[(2',3'-*O*-D-isopropylidene-β-ribofuranosyl)amino]pyrimidine and a 7.6 % yield of 2',3'-*O*-isopropylideneinosine.

6-Methoxy-9-(β-D-ribofuranosyl)purine was observed to be converted quantitatively to inosine.

Table 1. Pseudo first-order rate constants for the partial reactions involved in the alkaline cleavage of some 6-substituted 9-(β-D-ribofuranosyl)purines.[a]

Substituent at C6	T/K	[OH$^-$]/mol dm^{-3}	$k_1/10^{-3}$ s^{-1}	$k_2/10^{-3}$ s^{-1}	$k_3/10^{-3}$ s^{-1}	$k_4/10^{-3}$ s^{-1}	$k_{-4}/10^{-3}$ s^{-1}	$k_5/10^{-3}$ s^{-1}
CH$_3$ (*1a*)	363.2	0.50	4.21(8)	4.0(3)	3.6(1)	6.6(3)	2.0(1)	–
		0.40	3.59(6)	2.8(2)	3.0(1)	5.6(3)	2.0(1)	–
		0.30	2.89(6)	2.1(2)	2.4(1)	4.4(2)	2.0(1)	–
		0.20	2.43(4)	1.6(1)	1.6(1)	3.8(2)	2.0(1)	–
		0.10	1.31(4)	0.81(9)	1.0(1)	2.5(1)	1.9(1)	–
SCH$_3$ (*1b*)	363.2	0.50	6.72(6)	5.2(3)	7.3(3)	7.9(2)	2.0(1)	0.14(2)
		0.40	5.41(7)	4.2(2)	6.2(3)	6.9(2)	2.0(1)	0.13(2)
		0.30	4.28(4)	3.3(2)	5.2(2)	5.6(2)	2.0(1)	0.10(1)
		0.20	3.43(3)	2.2(1)	4.0(2)	3.9(2)	2.0(1)	0.08(1)
		0.10	1.95(3)	1.5(1)	2.3(1)	2.9(2)	2.0(1)	0.05(1)
Cl (*1c*)	323.2	0.50	5.88(8)	0.52	0.091	–	–	1.21(2)
		0.40	5.14(5)	0.49	0.078	–	–	0.98(1)
		0.30	4.07(6)	0.45	0.065	–	–	0.72(1)
		0.20	2.85(4)	0.48	0.043	–	–	0.50(1)
		0.10	1.80(3)	0.48	0.023	–	–	0.27(1)
H[b]	323.2	0.20	0.397(17)	0.384	0.0571	–	–	–
	363.2	0.20	11.0(2)	4.60	1.14	–	–	–
NH$_2$[c]	363.2	0.20	0.049(1)	0.24	–	0.060	0.26	0.002

[a]The rate constants indicated in Scheme 1. The ionic strength was adjusted to 0.50 mol dm^{-3} with NaCl.
[b]From Ref. 2. [c]From Ref. 3.

Kinetics of the partial reactions. Table 1 lists the pseudo first-order rate constants for the partial reactions involved in the alkaline cleavage of compounds *1a–1c*. With all these compounds, the rate constant, k_1, for the attack of hydroxide ion on the C8 atom depends curvilinearly on [OH$^-$]. At low concentrations of hydroxide ion the plots of k_1 vs. [OH$^-$] are convex; at high alkalinities they become linear. This kind of behavior has been explained as resulting from the ionization of the 2'-hydroxyl group.[2,3] Hydroxide ion may be expected to attack more readily the neutral substrate than its 2'-oxyanion. Accordingly, the plot of k_1 vs. [OH$^-$] exhibits a downward curvature in the alkalinity region where the 2'-hydroxyl group becomes ionized (Fig. 4). With purine nucleosides, this happens between pH 12 and 13.[8] Under more basic conditions, the substrate is present entirely as 2'-oxyanion, and k_1 is hence linearly related to [OH$^-$].

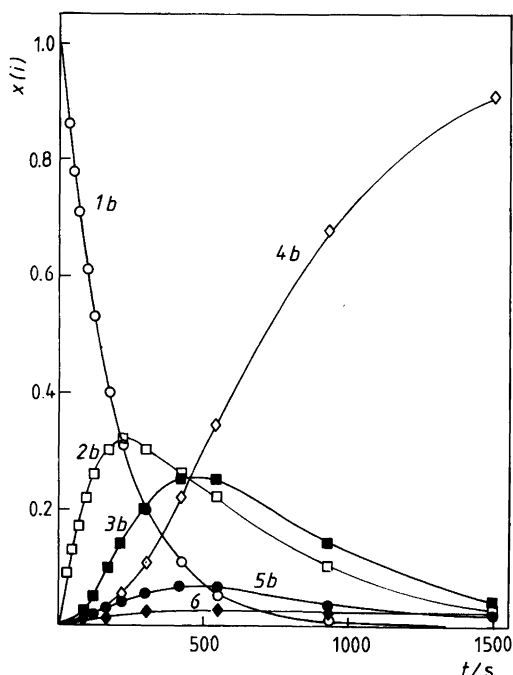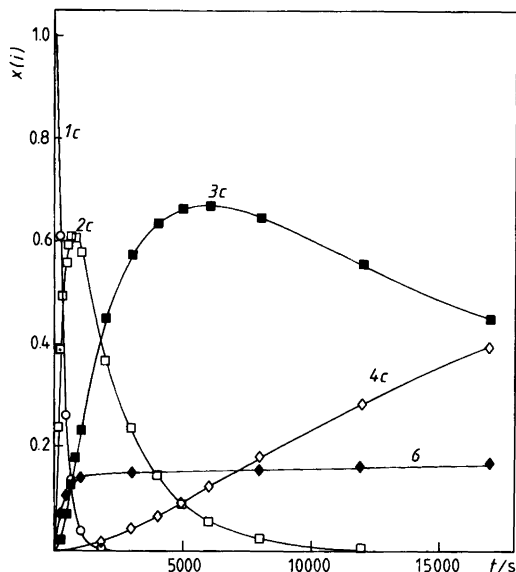The data in Table 1 indicate that k_1 increases markedly with increasing electronegativity of the

Fig. 3. Time-dependent product distribution for the reaction of 6-chloro-9-(β-D-ribofuranosyl)purine with aqueous sodium hydroxide (0.20 mol dm^{-3}, ionic strength 0.50 mol dm^{-3} with NaCl) at 323.2 K. The enumeration refers to Scheme 1.

Fig. 2. Time-dependent product distribution for the reaction of 6-methylthio-9-(β-D-ribofuranosyl)purine with aqueous sodium hydroxide (0.20 mol dm^{-3}, ionic strength 0.50 mol dm^{-3} with NaCl) at 363.2 K. The enumeration refers to Scheme 1.

6-substituent. This is expected, since electron-withdrawing groups, for example, diminish the electron density at the reaction center and thus facilitate the attack of hydroxide ion. As seen from Fig. 5, an approximatively linear correlation exists between the logarithmic rate constants and the pK_a values for the corresponding free purines, used as a measure of the electron density at C8. However, the unsubstituted purine riboside is exceptionally susceptible to attack by hydroxide ion. The correlation between the reactivity and the base moiety structure is thus very similar to that reported for the corresponding reaction of 6-substituted 9-(1-ethoxyethyl)purines, 9-(β-D-ribofuranosyl)purines reacting uniformly from 2 to 4 times more rapidly than their 1-ethoxyethyl counterparts.[6] Clearly, the glycone moiety does not play any decisive role in the reaction. The somewhat higher reactivities of purine nucleosides may well be accounted for by the stronger electron-withdrawing ability of the ribofuranosyl group. For comparison, a similar reactivity difference exists between the 1-(2-chloroethoxy)ethyl and 1-ethoxyethyl derivatives of purine.[10] 9-(β-D-Ribopyranosyl)purines are cleaved slightly more
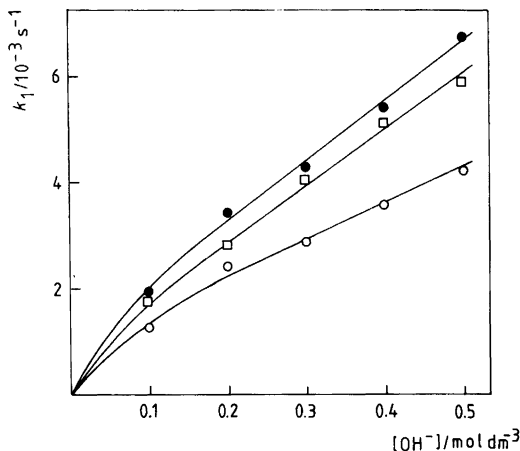
Fig. 4. Pseudo first-order rate constants for the attack of hydroxide ion on the C8 atom of 6-methyl- (O, 363.2 K), 6-methylthio- (●, 363.2 K) and 6-chloro-9-(β-D-ribofuranosyl)purine (□, 323.2 K). The ionic strength was adjusted to 0.50 mol dm⁻³ with sodium chloride.

rapidly than the furanoid nucleosides, which partly explains why ribopyranosyl derivatives do not efficiently accumulate during the decomposition of ribofuranosylpurines.

As mentioned above, displacement of the 6-substituent by hydroxide ion competes with the opening of the imidazole ring. The proportion of this side reaction is about 3 % for *1b* and 15 % for *1c*. Accordingly, it is considerably less important than with the correspondingly substituted 9-(1 -ethoxyethyl)purines. Evidently, the ribosyl group at N9 reduces the electron density at the adjacent C8 atom, while the inductive influence on the C6 site is negligible. The 6-methoxy derivative (*1d*) reacts solely by displacement of the methoxy group, the second-order rate constant being 6.2×10^{-3} dm³ mol⁻¹ s⁻¹ at 363.2 K.

The rate of deformylation of 5-formamido intermediates, *2a–2c*, responds in two different manners to changes in the concentration of hydroxide ion. The rate constants, k_2, obtained for *2a* and *2b* are proportional to [OH⁻], whereas those obtained for *2c* are completely independent of [OH⁻]. A similar difference has been observed in the behavior of 6-substituted 4-amino-5-formamidopyrimidines, and it has been accounted for by a change of the rate-limiting step.[5] In fact, introduction of a ribosyl group at the N⁴ atom retards the deformylation by less than 20 %. The

glycone moiety most probably does not participate in the deformylation, and the mechanisms suggested[5] for 4-amino-5-formamidopyrimidines can also be applied to the deformylation of *2a–2c*. Accordingly, hydroxide ion performs a rapid initial attack on the carbonyl carbon of the formamido group, and the tetrahedral intermediate formed undergoes base-catalyzed decomposition.[5] The reaction in alkaline solution is approximately first-order with respect to hydroxide ion, since the prevailing ionic form of the substrate is an unreactive N^5-monoanion. The strongly electronegative chlorine atom makes the tetrahedral intermediate so acidic that its decomposition does not require base catalysis. The initial attack of hydroxide ion thus becomes rate-limiting, and, at the same time, the reaction order with respect to hydroxide ion changes from one to zero.[5]

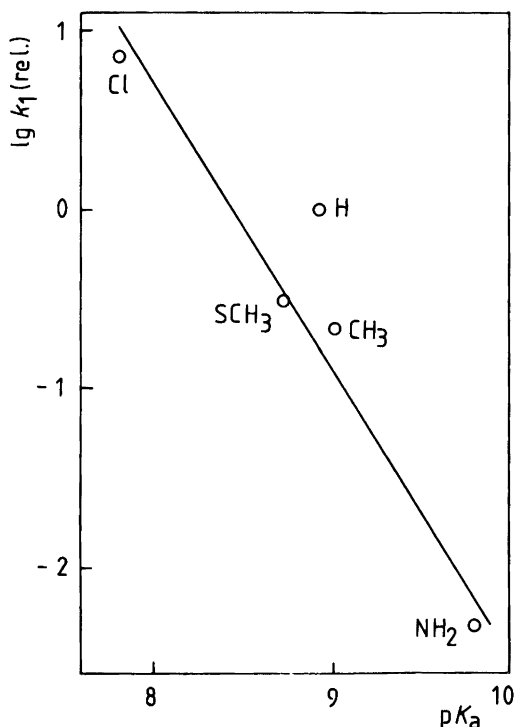As seen from Table 1, the susceptibility to the

Fig. 5. The influence of the 6-substituent on the reaction of 9-(β-D-ribofuranosyl)purines with aqueous sodium hydroxide. The logarithms of the relative rate constants plotted against the pK_a values of the correspondingly substituted purines.[9] The rate constant, k_1, is defined in Scheme 1.

polar nature of the 6-substituent is lower in the deformylation reaction than in the initial attack of hydroxide ion on the C8 atom. Although electron withdrawal by a polar group may be expected to facilitate the attack of hydroxide ion on the carbonyl carbon of the formamido group as efficiently as the attack on the C8 atom of the purine ring, part of this rate enhancement is cancelled out by the concomitant increase in the concentration of the unreactive N^5-monoanion. The rate constants, k_3, for the final step of the alkaline cleavage of purine nucleosides are rather insensitive to the 6-substituent. This partial reaction may be assumed to involve rate-limiting attack of hydroxide ion on the anomeric carbon of the acyclic Schiff base intermediate and rapid subsequent breakdown of the carbinolamine formed.[2] The reaction thus proceeds via a species the proportion of which is extremely small in the equilibrium mixture of 5-amino-4-ribosylaminopyrimidines. Since the dependence of the equilibrium composition on the nature of the 6-substituent is unknown, the effect of the 6-substituent on the rate of decomposition is difficult to rationalize.

In summary, the multistage pathway presented[1,2] for the alkaline cleavage of unsubstituted 9-(β-D-ribofuranosyl)purine is generally followed in the decomposition of its 6-substituted derivatives, although the 6-substituent may also be displaced by hydroxide ion. The consecutive partial reactions are kinetically fairly well separated when the 6-substituent is strongly electronegative, but overlap severely when this group becomes electropositive.

Experimental

Materials. 6-Methyl-9-(β-D-ribofuranosyl)purine (*1a*) was prepared from commercial 6-methylpurine and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (Sigma) by the method of Vorbruggen,[11] using the trimethylsilyl ester of trifluoromethanesulfonic acid as catalyst. The product crystallized from methanol was homogeneous on a reversed-phase column and exhibited ^{13}C NMR signals (D$_2$O; DSS as internal standard) at 21.0 (C6–CH$_3$), 64.1 (C5'), 73.1 (C2'), 76.5 (C3'), 88.3 (C4'), 91.1 (C1'), 135.2 (C5), 147.0 (C8), 152.0 (C4), 154.1 (C2) and 162.3 (C6) ppm. 6-Methylthio- (*1b*), 6-chloro- (*1c*) and 6-methoxy-9-(β-D-ribofuranosyl)purine (*1d*) and inosine

(*6*) were commercial products (Sigma) that were employed as received after checking their purity on a reversed-phase column. The preparation of the 6-substituted 4,5-diamino- and 4-amino-5-formamidopyrimidines used as reference materials has been described previously.[5]

Preparative separations. The compounds (*2a, 2b, 3a, 3b, 5a and 5b*) accumulated during the alkaline cleavage of *1a* and *1b* were separated preparatively on a Spherisorb RP-18 column (250×8 mm, 5 μm) using aqueous acetonitrile as eluant. The fractions obtained were evaporated to dryness under reduced pressure.

Kinetic measurements. The LC method described previously[2] was used to follow the reactions of *1a–1d, 2a, 2b, 3a, 3b, 5a* and *5b* with aqueous sodium hydroxide. The separations were carried out on a LiChrosorb RP-18 column (250×4 mm, 10 μm) using mixtures of acetonitrile and acetic acid buffer (pH 4.3) as eluant. The acetonitrile content was 4, 11, 4 and 10 % (v/v) for *1a, 1b, 1c* and *1d*, respectively. However, when the formation of 4,5-diaminopyrimidines (*4a–4c*) was followed, the proportion of acetonitrile ranged from 20 to 30 %. The peak heights were transformed to concentrations with the aid of calibration solutions of known concentration.

Calculation of the rate constants. The rate constants, $k_d(1)$, for the disappearance of *1a–1d* were calculated from the integrated first-order rate law. These were transformed to the rate constants for the parallel partial reactions giving *6* and *2a–2c* with the aid of eqns. (1) and (2), where $x(6)$ stands for the mole fraction of *6* in the

$$k_5 = x(6)k_d(1) \tag{1}$$
$$k_1 = k_d(1) - k_5 \tag{2}$$

final product mixture. The rate constants, k_4, for the disappearance of *5a* and *5b*, separated preparatively, were also calculated from the first-order rate law, but the reaction was followed only from 0 to 30 % to minimize overlap with the recyclization reaction. The rate constants, $k_d(2)$, for the disappearance of *2a* and *2b*, separated preparatively, were obtained in a similar manner, and were further transformed to the rate constants for the parallel cyclization and deformylation reactions by eqns. (3) and (4). Here, $x(5)$ denotes the mole fraction of *5a* or *5b* in the

$$k_{-4} = x(5)k_d(2) \tag{3}$$
$$k_2 = k_d(2) - k_{-4} \tag{4}$$

product mixture during the early stages of the disappearance of *2a* or *2b*. The rate constants, k_3, for the disappearance of *3a* and *3b*, separated preparatively, were determined by following their decomposition from 0 to 80 % and applying the first-order rate law.

Intermediates *2c* and *3c* were not separated preparatively, but the rate constants k_2 and k_3 were determined by applying the kinetics of consecutive first-order reactions. Accordingly, k_2 was obtained by least-squares fitting from eqn. (5) and k_3 from eqn. (6). In these eqns., $[2c]_t$ and $[4c]_t$ are the concentrations of *2c* and *4c* at time *t*, $[2c]_T$ is the maximum concentration of *2c* reached at time $t = T$, and $[1c]_0$ is the initial concentration of *1c*. The derivation of eqns. (5) and (6) is presented in Ref. 12.

$$\frac{[2c]_t}{[2c]_T} = \frac{e^{-k_d(I)t} - e^{-k_2 t}}{e^{-k_d(I)T} - e^{-k_2 T}} \tag{5}$$

$$\frac{[4c]_t}{[1c]_0} = \frac{k_1}{k_d(I)}$$
$$- \frac{k_1 k_2 k_3}{k_d(I)[k_2 - k_d(I)][k_3 - k_d(I)]} e^{-k_d(I)t}$$
$$- \frac{k_1 k_3}{[k_d(I) - k_2][k_3 - k_2]} e^{-k_2 t}$$
$$- \frac{k_1 k_2}{[k_d(I) - k_3][k_2 - k_3]} e^{-k_3 t} \tag{6}$$

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