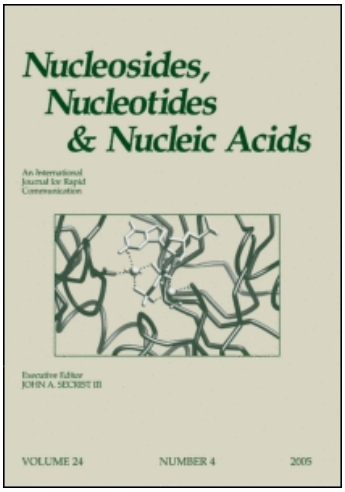

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HYDROLYSIS OF ADENOSINE CYCLIC 3',5'-(R_P)- AND (S_P)-PHOSPHORAMIDATES. STEREOELECTRONIC EFFECTS IN THE ACID HYDROLYSIS.

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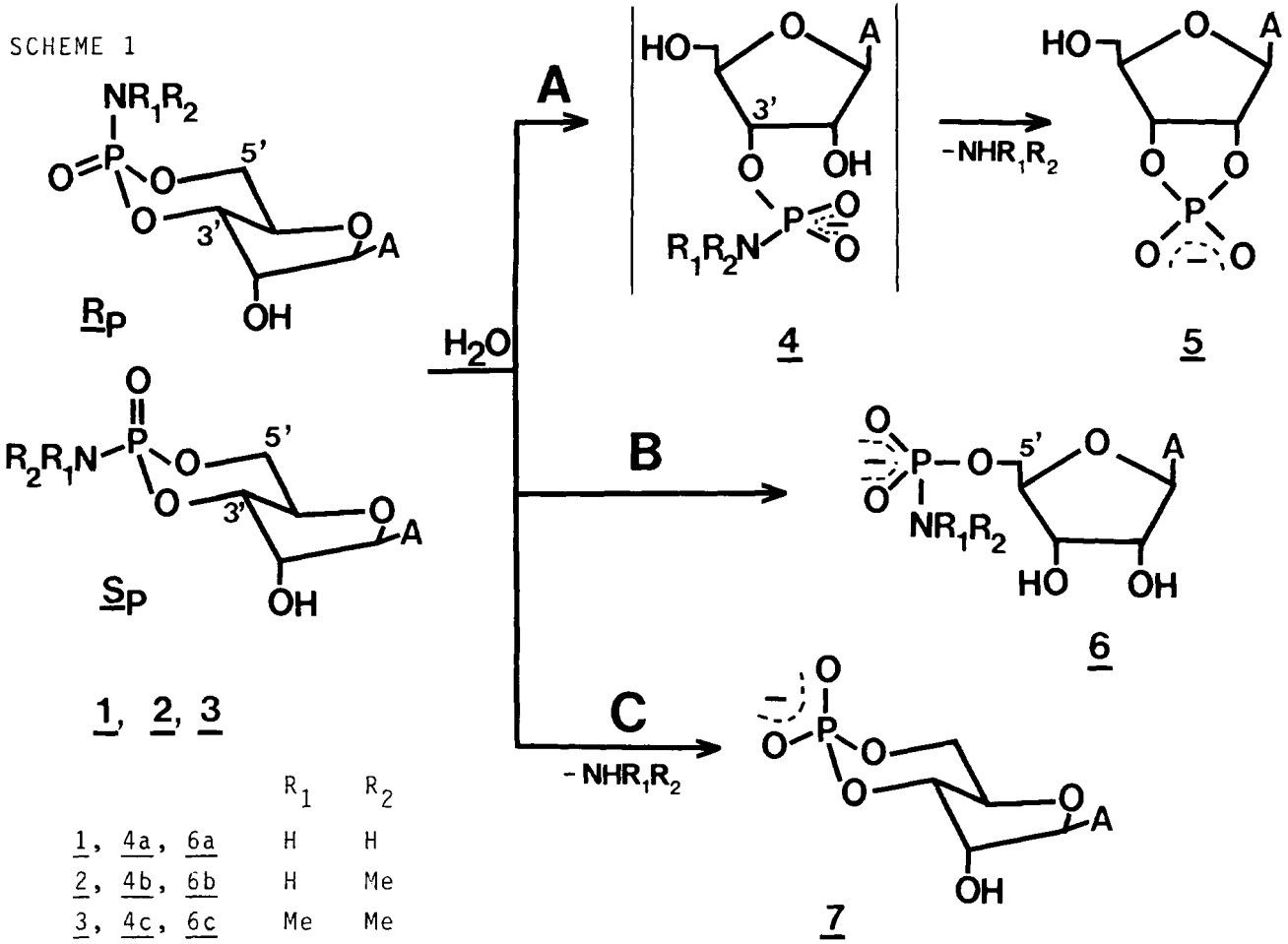
Abstract. Hydrolyses of R_P and S_P diastereoisomers of adenosine cyclic 3',5'-phosphoramidate (**1**), -N-methylphosphoramidate (**2**) and -N,N-dimethylphosphoramidate (**3**) in 0.1 N sodium hydroxide and 0.1 N hydrochloric acid, respectively, have been studied. In 0.1 N sodium hydroxide, the ester bonds of the compounds are broken. In 0.1 N hydrochloric acid, despite predictions to the contrary, **1** hydrolyzes with predominant ester bond breakings, while **3** decomposes with exclusive amide bond fission. In the acid hydrolysis of **2**, cleavages of amide and ester bonds are of comparable degree. Significant differences exist between R_P and S_P diastereoisomers. The peculiar behavior of **1** and **2** in acid hydrolysis is interpreted on the basis of ground state stereoelectronic effects.

Hydrolyses of adenosine cyclic 3',5'-(R_P)- and (S_P)-phosphoramidates (R_P-**1** and S_P-**1**) and adenosine cyclic 3',5'-(S_P)-N,N-dimethylphosphoramidate (S_P-**3**) in 0.1 N sodium hydroxide and 0.1 N hydrochloric acid, respectively, have recently been studied by product analysis in our laboratory.[1,2] It was found that in 0.1 N sodium hydroxide the compounds hydrolyzed as expected,[3] i.e. with ester bond breakings to give a mixture of adenosine cyclic 2',3'-monophosphate (**5**) and adenosine 5'-phosphoramidate (**6a**) or adenosine 5'-N,N-dimethylphosphoramidate (**6c**) (routes **A** and **B** in SCHEME **1**) in molar percentages of **5**/**6a**(**6c**) ~70:30 (for R_P-**1**), ~60:40 (for S_P-**1**) or ~90:10 (for S_P-**3**). Cyclic phosphate **5** can be derived from 3'-phosphoramidate **4a** (or **4c**), the unstable product of P-O-C5' bond breaking. It was demonstrated that in aqueous solution **4a** rapidly converts

SCHEME 1



	R_1	R_2
1, 4a, 6a	H	H
2, 4b, 6b	H	Me
3, 4c, 6c	Me	Me

into **5** by the action of the 2'-hydroxyl group.[4] Remarkable differences were observed in the acid hydrolysis of phosphoramidates **1** and S_P-**3**. In 0.1 N hydrochloric acid, S_P-**3** hydrolyzed with practically exclusive P-N bond breaking (>99%) to adenosine cyclic 3',5'-monophosphate (**7**) (route **C** in SCHEME 1), as anticipated on the basis of the known sensitivity of P-N bond towards acid.[5,6] In the acid hydrolysis of R_P-**1** and S_P-**1**, however, P-N bond fission was insignificant (<10%) and cleavage of the ester bonds prevailed **5**/**6a**/**7** ~83:8:9 (for R_P-**1**) and ~87:10:3 (for S_P-**1**). This indicates an unexpected stability of the P-N bond in **1** towards acid. A semi-quantitative comparison showed significant differences in the rates of hydrolysis of S_P-**1** and S_P-**3** both in 0.1 N sodium hydroxide and in 0.1 N hydrochloric acid. The difference in rates was much larger in alkali than in acid.

Hydrolysis studies on R_P-**1**, S_P-**1** and S_P-**3** have now been repeated by using HPLC for product analysis, instead of preparative TLC and DEAE-cellulose column chromatography employed earlier. The investigations have also been extended to adenosine cyclic 3',5'-(R_P)- and (S_P)-N-methylphosphoramidates (R_P-**2** and S_P-**2**) and to R_P-**3**. The behavior of the diastereoisomeric pairs of three adenosine cyclic 3',5'-phosphoramidates differing in one N-methyl group under hydrolysis conditions in 0.1 N sodium hydroxide and 0.1 N hydrochloric acid, could thus be compared. Results of these comparative hydrolysis studies are described in the present paper. A possible interpretation based on ground state stereoelectronic effects for the enhanced stability of the P-N bond of **1** towards acid, will also be discussed.

RESULTS AND DISCUSSION

The diastereoisomeric pairs, R_P and S_P of three adenosine cyclic 3',5'-phosphoramidates differing in one N-methyl group, **1**, **2** and **3**, were hydrolyzed at 37°C in 0.1 N sodium

hydroxide and 0.1 N hydrochloric acid. Percentage bond breakings, half-lives and pseudo-first-order rate constants together with the standard deviations, are summarized in TABLE 1.

Alkaline hydrolysis

Alkaline hydrolysis results almost exclusively in the cleavage of the ester bonds. There is a preferential fission of the P-O-C5' linkage that contains the primary carbon atom. The P-O-C5' bond breaking exceeds the P-O-C3' bond fission by a factor of approximately 3 for **1** and **2** and takes place about 10 times as rapidly as the P-O-C3' bond fission occurs for **3**. The P-N bond breaking is insignificant (<1%), except in $\underline{S}_P$-**2**, where it approaches 3%.

Striking differences can be found in the rate of hydrolysis of phosphoramidates **1**, **2** and **3**. The rate of hydrolysis of **1** exceeds that of **2** by a factor of approximately 26 and is greater by a factor of about 8,000 ($\underline{R}_P$) or 11,000 ($\underline{S}_P$) than that of **3**. The hydrolysis of **2**, thus, occurs about 300-400 times as fast as does that of **3**. The differences in the rate of hydrolysis may be interpreted in a similar way as Westheimer suggested[7] for the alkaline hydrolysis of phosphoramidic fluorides that proceeds much more rapidly with amides of ammonia and of primary amines than with those of secondary amines.[8] Hence, the alkaline hydrolysis of phosphoramidates **1** and **2** having hydrogen atom(s) attached to nitrogen, would proceed according to an elimination-addition type mechanism through a nitrogen analogue of monomeric metaphosphate (**8**) (SCHEME 2), while that of phosphoramidate **3** containing no hydrogen atom attached to nitrogen, would be a concerted process.

$\underline{R}_P$ Diastereoisomers of the individual phosphoramidates hydrolyze about twice as rapidly as do the $\underline{S}_P$ diastereoisomers. In contrast, the phosphorus diastereoisomers of adenosine cyclic 3',5'-monophosphate benzyl triesters having the benzyloxy group at the equatorial position hydrolyze approximately four times faster than those with the benzyloxy group at the axial position.[9]

TABLE 1
Reaction rates, half-life values and percentage of bond breakings of the hydrolysis of R_P and S_P diastereoisomers of adenosine cyclic 3',5'-phosphoramidate (**1**), -N-methylphosphoramidate (**2**) and N,N-dimethylphosphoramidate (**3**) in 0.1 N sodium hydroxide (OH^-) and 0.1 N hydrochloric acid (H^+) at 37°C.[a]

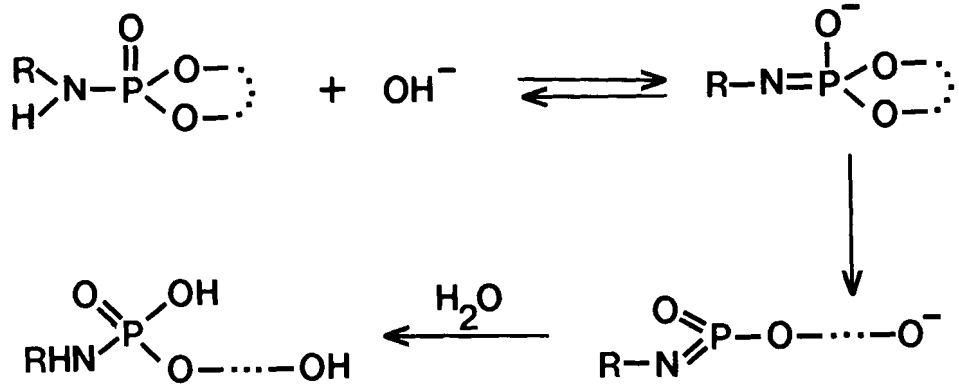
Compound		Bond breaking (%) P-N	P-O-C5'	P-O-C3'	$t_{1/2}$ sec.	$k \times 10^5$ sec^{-1}	$\sigma^b \times 10^5$
R_P-**1**	OH^-	<0.2[c]	74.5	25.5	1.75	39600	4800
	H^+	8.6	80.8	10.6	1620	42.8	1.001
S_P-**1**	OH^-	<0.2[c]	64.6	35.4	2.80	24800	4300
	H^+	3.2	86.1	10.7	1920	36.1	0.951
R_P-**2**	OH^-	0.4	73.7	25.9	47	1470	31.6
	H^+	35.1	54.1	10.8	59040	1.17	0.0062
S_P-**2**	OH^-	2.9	73.6	23.5	74	937	7.77
	H^+	76.1	21.6	2.3	28080	2.46	0.0107
R_P-**3**	OH^-	0.7	90.1	9.2	13680	5.06	0.163
	H^+	99.5	0.4	0.1	69840	0.99	0.0073
S_P-**3**	OH^-	0.4	91.6	8.1	32040	2.16	0.035
	H^+	99.6	0.4	<0.1[c]	8640	8.02	0.155

[a] For details see EXPERIMENTAL [c] Neglected for calculation

[b] Standard deviation

$$\sigma = \sqrt{\frac{\Sigma x_i^2 - n(\bar{x})^2}{n - 1}}$$

SCHEME 2



8

Acid hydrolysis

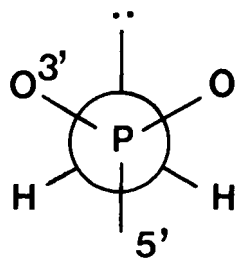
In acid hydrolysis, phosphoramidates 1, 2 and 3 behave differently. In the case of 1 - contrary to expectations[5,6] - the cleavage of the ester bonds predominates, and the degree of P-N bond breaking is less than 10%. The P-N bond breaking for R_P-1 is about three times faster than that for S_P-1. In the hydrolysis of 2 the P-N bond fission is remarkably increased, and the cleavage of either both ester bonds (for S_P-2) or only of the P-O-C5' ester linkage (for R_P-2), is decreased accordingly. The amount of P-N bond breaking is about two times greater for S_P-2 compared to R_P-2. Only compound 3 hydrolyzes as expected,[5,6] i.e. with practically exclusive P-N bond breaking (>99%). Of the two ester bonds, the P-O-C5' linkage that contains the primary carbon atom, is the more unstable bond even in acid. The fission of the P-O-C5' bond is approximately 8 times faster than that of the P-O-C3' linkage for R_P-1 and S_P-1. This factor is remarkably different for the two diastereoisomers of 2, being equal to about 5 for R_P-2 and approximately 10 for S_P-2.

With the exception of S_P-3, the acid hydrolysis of the phosphoramidates studied proceeds slower than does the alka-

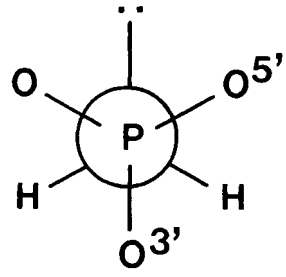
line hydrolysis. Similarly as with alkaline hydrolysis, the rate of decomposition of $\underline{R_P}$-**1** in acid exceeds that of $\underline{S_P}$-**1**. On the other hand, $\underline{S_P}$-**2** and $\underline{S_P}$-**3** are less stable than $\underline{R_P}$-**2** and $\underline{R_P}$-**3** by factors of approximately 2 and 8, respectively.

Results of acid hydrolysis may be interpreted on the basis of ground state stereoelectronic effects. The nitrogen atom of a phosphoramidate may be either of planar or of pyramidal geometry.[10] For example, in crystalline phenyl phosphorodiamidate one nitrogen atom is quasi-planar and the second one is pyramidal.[11] Assuming that the amide nitrogen atom in **1** is of pyramidal geometry, one may consider a stereoelectronic effect due to the overlap of the lone pair of the nitrogen atom and the σ^* antibonding orbital of one of the two P-O ester bonds. This $n_N <--> \sigma^*_{P-O}$ orbital overlap is expected to cause the relatively highest energy gain on the basis of the electronegativity differences between oxygen and nitrogen and requires the antiperiplanar orientation of the lone pair on nitrogen to one of the two P-O ester bonds.[12] As shown in SCHEME 3, two of the three possible staggered conformations about the P-N bond, **9a** and **9b** fulfil

SCHEME 3



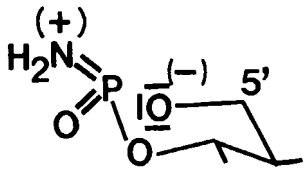
9a



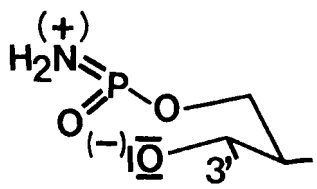
9b

the latter requirement i.e. these are the stereoelectronically favored conformations. In terms of a simple resonance picture, this stereoelectronic effect may be described by the no-bond-double-bond resonance structures **10a** and **10b**,[13] which clearly show the weakening of the ester bonds and the

strengthening of the P-N linkage. The stereoelectronic effect may thus be responsible for the predominant ester bond breaking and the unimportant P-N bond breaking in the acid hydrolysis of 1.



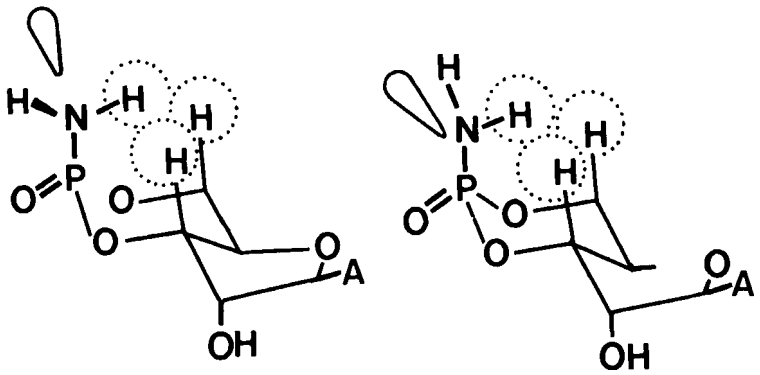
10a



10b

The approximately threefold P-N bond breaking found for R_P-1 compared to S_P-1, can be understood by considering the 1,3-synaxial steric repulsion between the amino group and the 3'or 5' hydrogen as schematically shown in SCHEME 4.

SCHEME 4



This synaxial steric repulsion destabilizes the two stereoelectronically favored conformations of R_P-1, 9a and 9b, but has no effect on S_P-1.

Why does the above-mentioned stereoelectronic effect not operate in the acid hydrolysis of 3? On the basis of the analogous thymidine compound of known geometry,[14] it seems reasonable to suppose that S_P-3 has a planar dimethylamino group that lies in the same plane as the phosphoryl group

does. This geometrical arrangement is not favorable for the above-mentioned orbital overlap. On the other hand, the attraction of the nitrogen lone pair by phosphorus may be compensated by the electron transfer from phosphorus towards nitrogen along the σ bond.[10,15,16] Consequently, in this case no factor that would strengthen the P-N linkage, is available. If in $\underline{R_P}$-**3** the dimethylamino group is not planar but of pyramidal geometry as is e.g. in cis-2-dimethylamino-2-thio-5-t-butyl-1,3,2- oxazaphosphorinane (axial dimethylamino group),[17] it is expected that the 1,3- syn-axial steric repulsion destabilizes the two stereoelectronically favored conformations **9a** and **9b** to a much greater extent than it does for $\underline{R_P}$-**1**.

Monomethylamide **2** occupies an intermediate position between unsubstituted amide **1** and dimethylamide **3**. On the basis of bond breakings, we may say in a very rough approximation, that $\underline{R_P}$-**2** resembles **1**, while $\underline{S_P}$-**2** is similar to **3**. Accordingly, a pyramidal or quasi-pyramidal nitrogen atom may be suggested for $\underline{R_P}$-**2**, and a planar or quasi-planar methylamino group may be proposed for $\underline{S_P}$-**2**.

Experiments to check the validity of the outlined proposal for the stereoelectronic control of the acid hydrolysis of phosphoramidates **1** and **2** and in general of 2-amino-2-oxo-1,3,2-dioxaphosphorinanes of similar structure, are in progress in our laboratory.

EXPERIMENTAL

Materials

Phosphoramidates **1**, **2** and **3** were prepared and separated into $\underline{R_P}$ and $\underline{S_P}$ diastereoisomers as described.[1,18] Adenosine 5'-N-methylphosphoramidate and -N,N-dimethylphosphoramidate used as HPLC controls, were synthesized by the analogy of a literature method.[19] The HPLC homogeneous phosphoramidates were characterized by acid hydrolysis: both compounds gave adenosine 5'-monophosphate. All other control nucleotides were purchased from Sigma.

HPLC analyses were carried out by using a Waters liquid chromatograph on a Hibar LiChrosorb RP-18 (250x4.0 mm I.D., 5 μm, Merck) reverse phase column in the following mixtures of 100 mM aqueous potassium phosphate buffer (pH 6.6)/methanol (v/v) = 90:10 (I), 83:17 (II), 82:18 (III), 72:28 (IV) and 63:37 (V). The flow rate was 1.0 mL/min. Retention times (min) were: adenosine 5'-monophosphate, 2.8 (II) and 3.7 (I); adenosine 3'-monophosphate, 3.3 (II) and 5.5 (I); adenosine 2'-monophosphate, 4.6 (II) and 9.2 (I); 5, 5.7 (II); 7, 8.1 (II) and 19.3 (I); **6b**, 10.3 (II); **6c**, 11.3 (II); R_P-1, 9.1 (III); S_P-1, 10.4 (III); R_P-2, 6.1 (IV); S_P-2, 8.9 (IV); R_P-3, 6.3 (V) and S_P-3, 11.3 (V). Separations were followed by UV at 254 nm.

Hydrolysis studies

5×10^{-3} M solutions of the phosphoramidates (1.0 mL of each) in 0.1 N sodium hydroxide or 0.1 N hydrochloric acid were kept at 37°C for 10 (for 1 in NaOH) 120 (for 1 in HCl and for 2 in NaOH), 600 (for 2 in HCl and for 3 in NaOH) or 1440 min (for 3 in HCl). Aliquots (40 μL) were removed and, with the exception of the hydrolysates of 1 in NaOH, were neutralized with 0.2 N HCl or 0.2 N NaOH (20 μL) then mixed with 100 mM aqueous potassium phosphate buffer, pH 6.6 (40 μL). To the hydrolysates of 1 in NaOH, 0.2 N HCl (80 μL) was added. The solution was kept at 37°C for 2 h then neutralized and diluted with the potassium phosphate buffer as above. Aliquots of the solutions thus obtained were analyzed by HPLC. For HPLC analyses system I (for 2 in NaOH) or II (for all other cases) was used. In systems I and II the unreacted phosphoramidates were not eluted from the column. Compound 5, one of the products of hydrolysis, was unstable both in NaOH and in HCl and converted to a mixture of adenosine 2'(3')-monophosphates. Adenosine 5'-phosphoramidates **6b** and **6c** were labile in HCl and transformed into adenosine 5'-monophosphate. Phosphoramidate **6a** and cyclic phosphate 5 in the hydrolysates of 1 in NaOH, were converted by acid treatment to a mixture of adenosine phosphates. Percentage bond breakings were, thus, obtained from the distribution of

the following products: % P-N = %**7**, % P-O-C5' = % adenosine 2'(3')-monophosphate; % P-O-C3' = % adenosine 5'-monophosphate (for **1** in NaOH and HCl, for **2** and **3** in HCl) or **6b** (for **2** in NaOH) or **6c** (for **3** in NaOH). Pseudo-first-order rate constants were obtained by measuring the decrease of the concentration of **1**, **2** or **3** at four different points of time. Each measurement is the mean value of three HPLC analyses. In HPLC systems **III**-**V** used for kinetic measurements, decomposition products were eluted before and separated cleanly from **1**, **2** and **3**. Percentage bond breakings, half-lives, pseudo-first-order rate constants are summarized in TABLE 1.

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