REACTIONS OF N-ACYLIMIDAZOLE WITH NUCLEOSIDES AND NUCLEOTIDES#

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Abstract — By controlling the pH of the reaction system and the addition of a base of an appropriate basicity, the selective acylation of ribonucleosides and ribonucleotides can be achieved by using N-acylimidazole. Ribonucleoside 2',3'-cyclophosphates can be easily prepared by the reaction of N-acylimidazole with the corresponding 3'-nucleotides. The mechanisms and kinetics of the reactions of N-acylimidazoles with nucleosides and nucleotides were studied by means of ^{31}P and ^{1}H nmr.

INTRODUCTION

N-Acylimidazole as acylating reagent was first introduced into organic synthesis by Staab[1] near thirty years ago. Since then, it has been occasionally used for the protection of the hydroxyl and amino groups of sugars, nucleosides and nucleotides. Cramer et al.[2,3] reported the selective benzoylation of 6-amino groups of cytidine and its 2',3'-cyclophosphate with benzoylimidazole. Corey and Hodgson[4] claimed selective acylation of 2-hydroxyl group of glucose by using acylimidazole. In the author's laboratory, acylimidazole has been successfully employed in the preparation of intermediate oligoribonucleotides for the total synthesis of yeast alanine tRNA.[5-7]

Because ribonucleoside and ribonucleotide are multifunctional compounds, which may react with acylimidazole in complicated ways. Well understanding the reactivities of the different functional groups of the nucleoside and nucleotide toward the acylimidazole and carefully controlling the conditions of the reaction system are necessary for the successfulness in the application of this reagent in the nucleic acid synthesis. In the present communication, the results of our studies on the reactions of acylimidazoles with nucleosides and nucleotides are presented.

SELECTIVE ACYLATION OF NUCLEOSIDES

The selective acylation of nucleosides is always interesting to synthetic nucleic acid chemists. Recently, Wagner[8] reported that ribonucleosides could be

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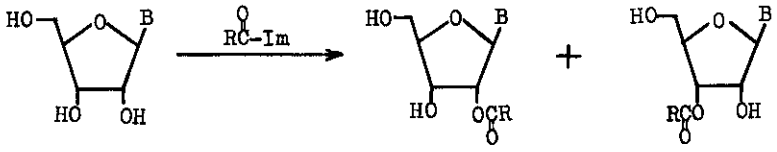
Dedicated to Professor Sir Derek Barton, F.R.S., on the occasion of his seventieth birthday.

acylated with organic tin reagent-acyl chlorides to form 3'-O-acyl derivatives. Kempe[9] claimed that 5'-O-DMT-ribonucleoside was acylated by acyl chloride at low temperature to give 2'-O-benzoylribonucleoside as the main product. Very recently, Matsuda[10] reported the selective acylation of the hydroxyl groups of the sugar moiety of guanosine to provide the fully O-acylated guanosine bearing free 2-amino group with acid anhydride-DMAP-MeCN-Et_3N system.

Wang et al. using the acylimidazole as acylating agent, observed that the acylation of the nucleoside was very slow in the absence of a base, but achieved quick selective acylations of the different hydroxyl groups of the ribose moiety of the nucleosides in the presence of different, appropriate bases.[5]

In the presence of morpholino-N,N'-dicyclohexylcarboxamidine (MDCAI, 0.2 mmol), acylimidazole (1.1 mmol) reacted with ribonucleosides (1 mmol) in DMF at room temperature for about 30 minutes to form a mixture of 2'- and 3'-O-acylribonucleosides with a ratio around 3:7 in a total yield of 76-85% (Scheme 1). The nucleosides used were uridine (B=uracil), pseudouridine (B=pseudouracil) and adenosine (B=adenine), and the N-acylimidazoles (RCOIm) used for uridine were anisoyl-, benzoyl-, pivaloyl- and acetylimidazoles, and only anisoylimidazole was employed for pseudouridine and adenosine. No acylation of the 5'-OH and the 6-amino group of the heterocyclic base was detected (Scheme 1).



Scheme 1

The 2'- and 3'-O-acylated products are usually in an equilibrium in the solution. The 3'-O-acyl derivatives are in general less soluble and more thermostable. The 2'-O-acyl derivatives could be readily converted into the 3'-O-isomer during the continuous concentration of the solution of the mixture of 2'- and 3'-O-acyl isomers in a polar solvent. Therefore, it is understandable why Kempe et al. obtained 2'-O-acyl-5'-O-DMT-uridine by acylating 5'-O-DMT-uridine with acyl chloride at -78°C.

By using tetraethylammonium hydroxide (TEAH) as the base, instead of MDCAI, and uridine and anisoylimidazole as starting materials, 5'-O-anisoyluridine was produced as the major product (yield ca. 50%) together with about 10% yield of a mixture of 2'- and 3'-O-monoanisoyluridines. Arabinofuranosyluridine and 2'-deoxyuridine were also used instead of uridine to react with N-acylimidazoles under the same condition (in the presence of MDCAI), only 5'-O-derivatives were

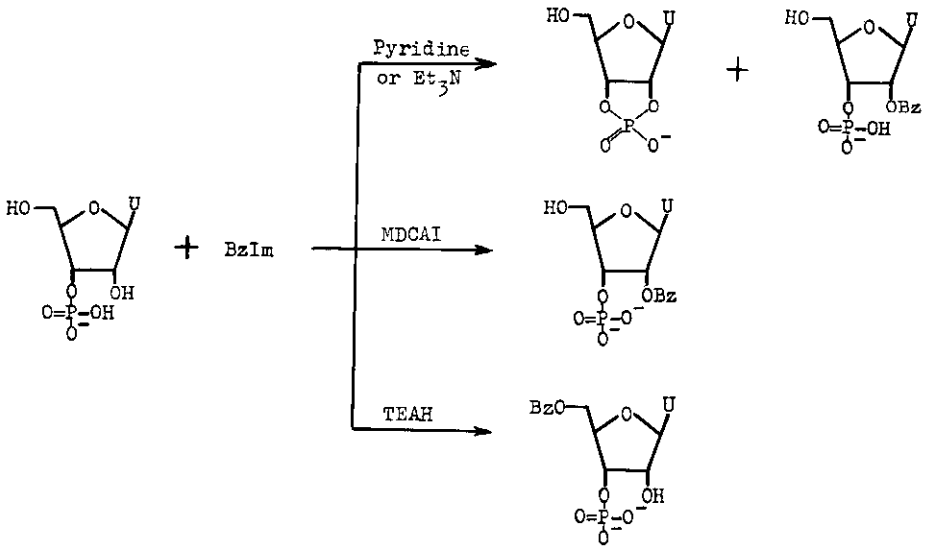
formed but much slowly than those in the case of uridine, so that the cis-diol has anchimeric assistance to the acylation.[5]

SELECTIVE ACYLATION OF NUCLEOTIDES

In the early work of Wang and his coworkers on oligoribonucleotide syntheses[6,7] by the classical phosphodiester method, N-acylimidazoles were used as acylating agents for the preparation of fully or partially acylated nucleotides from the corresponding ribonucleotides or their 5'-O-MMT-derivatives. In comparison with other acylating agents, e.g., pyridine-carboxylic acid anhydrides and -acyl chlorides, N-acylimidazoles have the advantages of mild and quick reactions (room temperature and 10 minutes to a few hours) and high yields (70-90%) of clean products.[6]

In the acylation of 3'-ribonucleotides, ribonucleoside 2',3'-cyclophosphate is ususlly formed as an undesirable by-product. In order to depress the formation of the cyclophosphate, Khorana et al.[11] had developed a procedure with the use of the tetraethylammonium salt of the same carboxylic acid as employed for the acylation. This procedure has been widely used, but it has some drawbacks, such as long reaction time, dark-colored products and the incomplete depression in the formation of the 2',3'-cyclophosphate.

It has been found[6] that as the pyridinium salt of the 3-ribonucleotide (Uppy) is allowed to react with the N-acylimidazole,the course of the reaction depends on the kind of the base added to the reaction system. In the absence or in the presence of an added weak base like pyridine or triethylamine, a mixture of the

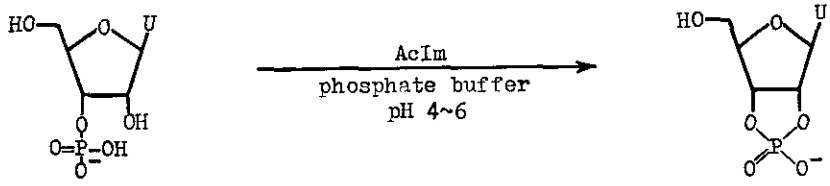


Scheme 2

2',3'-cyclophosphate and the 2'-O-acyl derivatives are formed. In the presence of a strong base like MDCAI or TEAH, the production of the 2',3'-cyclophosphate can be completely inhibited and O-acyl derivatives are exclusively formed in a short time. With MDCAI, the 2'-O-acyl derivative is isolated as the main product, while with TEAH, the 5'-O-acyl derivative is the main product. Scheme 2 shows a typical case. In an excess of the acylimidazole and longer time reaction, the monoacyl products may undergo further acylation on the 2'- or 5'-hydroxyl group.

N-ACYLIMIDAZOLE AS CYCLIZING REAGENT FOR 3'-RIBONUCLEOTIDES

In general, the formation of 2',3'-cyclophosphate during the acylation of 3'-ribonucleotide is an undesirable side reaction, but 2',3'-cycloribonucleotides are sometimes valuable starting materials in the synthetic nucleic acid chemistry. Khorana et al.[12] used DCCI to cyclize 3'-ribonucleotide into the ribonucleoside 2',3'-cyclophosphate, but it usually takes long time to heat the reaction mixture. With N-acetyl- or other N-acyl-imidazole in DMF or even in aqueous solution,[13] the 3'-ribonucleotide or its pyridinium salt is easily cyclized in 10-30 minutes at room temperature without significant formation of 2'-acylated product, provided that the pH of the solution is being kept at about 4 to 6 (Scheme 3).

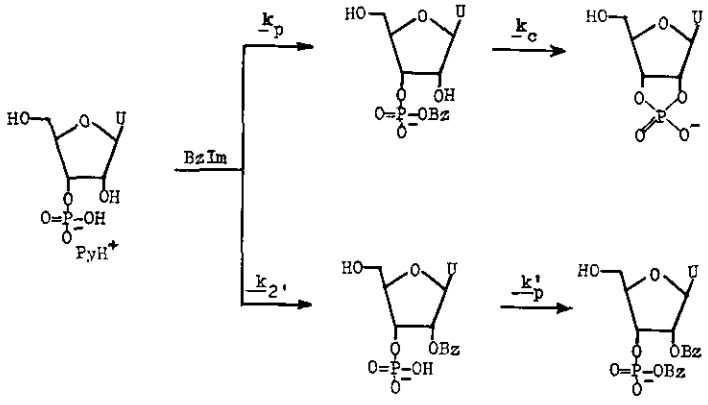


Scheme 3

MECHANISMS AND KINETICS OF THE ACYLATION OF NUCLEOSIDES AND NUCLEOTIDES WITH N-ACYLIMIDAZOLE

In general, the acylation of both the ribonucleoside and the ribonucleotide depends on the base added to the reaction medium and the pH of the system. For the ribonucleoside, in the presence of MDCAI, the N-acylimidazole only attacks either 2'- or 3'-hydroxyl group and leaves the 5'-OH and the 6-amino groups free. In the presence of an excess of TEAH, uridine reacts with anisoylimidazole to yield only 5'-O-acyl derivative. The discrepancy between the result of Cramer et al.[3] and that of Wang et al. can be explained in terms of the order of the reactivities of the -OH and $-NH_2$ groups of the ribonucleosides or the ribonucleotides, the difference of the pK_a's of the bases utilized and the pH's of the reaction systems as well as the pK_a's of the 2'- and 5'-OH, the heterocyclic base and $-PO_3H_2$ groups of the ribonucleotides in the two cases. Cramer et al.[6] used the tetraethylammonium salt of the same carboxylic acid employed for the acylation of cytidine 2',3'-cyclophosphate while Wang et al. used triethylamine (TEA), MDCAI or TEAH as the base catalyst. The pH of the latter reaction system was significantly higher than that of the former. The

pK_a's of cytidine are 4.22 and 12.2,[14] and those of adenosine 3.63 and 12.51.[15] Here 4.22 and 3.63 are most likely the pK_a's of the heterocyclic bases of cytidine and adenosine, and 12.2 and 12.5, the pK_a's of the 2'-OH groups, respectively. The lower pH of the experimental condition of Cramer et al. might be favorable to the acylation of the amino group. In addition, the 2'- and 3'-OH groups of cytidine had been protected, only the 5'-OH group was free in their case. The acylation of 5'-OH group of the ribose moiety requires much higher pH. Therefore Cramer and his coworkers obtained N-acylated product. Similarly, Matsuda's finding could be rationlized. He carried out the acylation of guanosine with acid anhydride in a basic system.



Scheme 4

Due to the induction effect of the hemiacetal C-1 atom of the aldose, the order of the activities of the hydroxyl groups of glucose is 2-OH>3-OH>6-OH.[16] It is resonable to consider the order of reactivities of the hydroxyl groups of the ribose moiety of the ribonucleoside to be 2'-OH>3'-OH>5'-OH. Because of the higher reactivity of 2'-OH than that of the 3'-OH, the 2'-O-acylation should be favored, but the 3'-O-derivative is more thermostable and less soluble than the 2'-O-derivative, so that the formation of the 3'-O-derivative is thermodynamically more favored in the equilibrium of the reaction mixture. The pure, crystallized 3'-O-acyl derivative once dissolved in a polar solvent is also quickly isomerized into and equilibrated with its 2'-O-isomer in the solution.

In the case of the nucleotide, the reaction course of the acylation of the pyridinium salt of 3'-UMP (Uppy) with benzoylimidazole (BzIm) in DMF as a model has been followed by ^{31}P- and ^{1}H-nmr methods and by the determinations of the intermediates and the final products formed in the reaction and the sequence of their emergences. The reaction courses under different conditions have been established.[17] Scheme 4 shows the reaction courses both in the absence of an

added base and in the presence of the weak base Et_3N in DMF.
The intermediate mixed anhydride of 3'-uridylic acid and benzoic acid (Upbz) was formed first in the reaction at about 1 minute,reached its maximum at about 20 minutes, and then decreased gradually, which could be detected and confirmed by its ^{31}P-nmr chemical shift(δ-6.7 ppm). The compound with 18.0 ppm,identified as uridine 2',3'-cyclophosphate (U>p), was formed a little later,and became the major product with the simultaneous exhaustion of Upbz. The 2'-O-benzoylated product (Ubzp, 1.3 ppm) emerged at about 20 minutes and finally was converted into the mixed anhydride of 2'-O-benzoyl uridylic and benzoic acid (Ubzpbz, 7.9 ppm). When a strong base like MDCAI or TEAH was added into the reaction system, the reaction course was extensively and even completely changed. The formation of Upbz and consequently that of U>p were extensively or completely inhibited while the 2'-O-benzoylated compound (Ubzp, in the case of MDCAI) or the 5'-O-benzoylated one (BzUp, 2.2 ppm, in the case of TEAH) was formed as the major product.

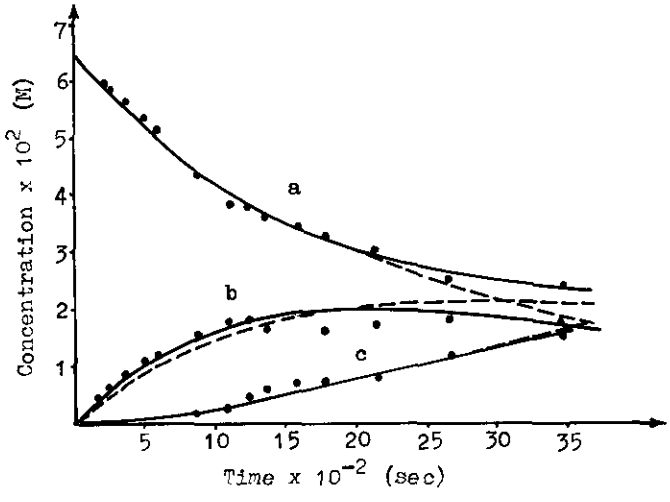


Fig. 1. The time course of the reaction of Uppy with BzIm in DMF
Experimental: ———— ; computor-simulated: --------.
a. Uppy; b. Upbz; c. U>p.

In the study on the kinetics of the reaction of Uppy (0.065 mmol) with BzIm (0.26 mmol) in DMF (2.5 ml) in the absence of an added base at 20°, the rate constants for different steps in Scheme 4 were calculated out by solving a series of differential equations and the kinetic curves were simulated with the assistance of a computor (Fig. 1).The simulated curves quite agreed with those of the experimental ones though with some deviations, which indicated that, as the time of reaction was going on, the actual rates of the consumptions of the starting material 3'-UMP and of the formation of the mixed anhydride (Upbz) were

Table 1. Rate constants of the reactions of Uppy with BzIm in DMF under the influence of an added base at 20°C

Base added	Concentrations of reactants(mM)			Rate constants of reactions ($\underline{k}$ x 10^4)					
	[Uppy]	[BzIm]	[Base]	$\underline{k}_p$(a)	$\underline{k}_c$(b)	$\underline{k}_2$,(a)	$\underline{k}_5$,(a)	$\underline{k}_p$,(a)	$\underline{k}_{p2}$,(a)
None	26.0	156	0	13	2.8	2.7	0	6.2	0.76
Et_3N	26.0	182	91.0	0.83	0.83	5.0	0	0.5	0
MDCAI	30.8	216	92.4	0.025	0	1	0.5	0	0

Units of $\underline{k}$'s: (a) $mol^{-1}sec^{-1}$; (b) sec^{-1}.

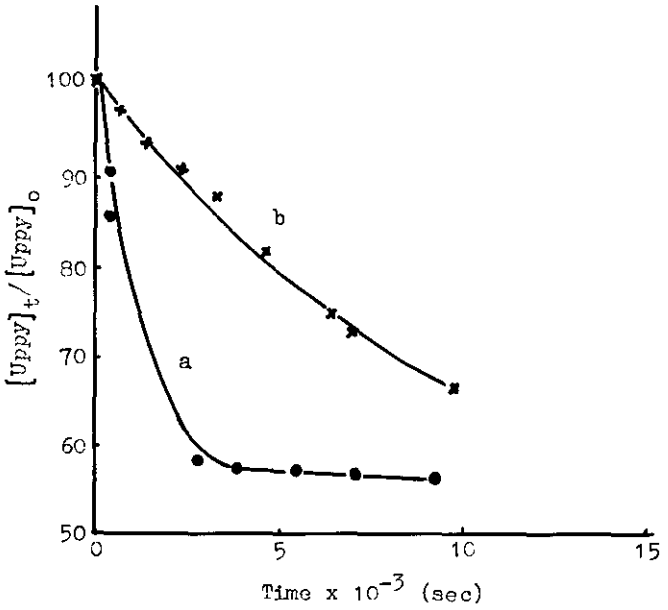


Fig. 2. Effects of extra added imidazole on the consumption rate of Uppy in the reaction with AcIm in DMF at 20°C
a. [Im]/[Uppy]=0; b. [Im]/[Uppy]=0.2.

a little slower than the computor-simulated ones.The deviation of the simulated curves from the experimental ones could be rationalized by that the imidazole, which was continuously liberated during the reaction, could increase the pH of the reaction system and affect the reverse reaction with the result of slowing down the reaction of BzIm with the phosphate group, i.e., the consumption of 3'-UMP and the formation of Upbz correspondingly. In fact, an extra addition of

free imidazole(0.025 mmole) to the above reaction system of the pyridinium salt of uridine 3'-phosphate (Uppy, 0.125 mmole) and AcIm (0.625 mmloe) in DMF (2.5 ml) clearly exhibited an inhibiting effect on the rate of the consumption of Up (Fig. 2).[18] Hence it is important to control the pH of the reaction system during the acylation with acylimidazole.

Table 1 listed the calculated rate constants of the consumption of UMP and the formations of the intermediates,and also showed that the addition of bases like Et_3N and MDCAI did slow down the reaction of Uppy with BzIm ($\underline{k}_p + \underline{k}_2' + \underline{k}_5'$) obviously due to the depression of the formation of Upbz. From the experimental results, the rate contants of the reaction of the hydroxyl and phosphate groups of Uppy with BzIm may be ordered as follows: (1) without added base, $\underline{k}_p \gg \underline{k}_2' \gg \underline{k}_5'$; (2) with added Et_3N, $\underline{k}_2' > \underline{k}_p \gg \underline{k}_5'$;(3) with MDCAI, $\underline{k}_2' > \underline{k}_5' \gg \underline{k}_p$; and (4) with Et_4NOH, $\underline{k}_5' \gg \underline{k}_2' \gg \underline{k}_p$.

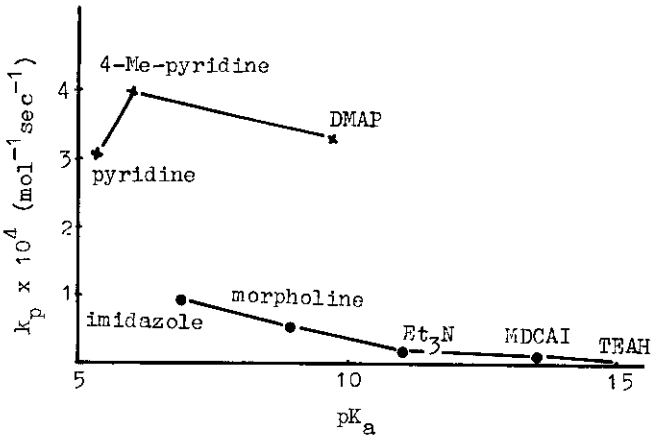


Fig. 3. The effects of different bases (0.125 mmol) on the $\underline{k}_p$ of the reaction of Uppy (0.125 mmol) with AcIm (0.625 mmol) in DMF (2.5 ml) at 20°C.

As mentioned above, the acylations of nucleosides and nucleotides by acylimidazoles are base-catalyzed reactions. The bases like imidazole, morpholine, Et_3N, MDCAI and TEAH all showed general base-catalytic effects, while preliminary experiments[19] showed that on the addition of equal moles of pyridine, 4-methylpyridine or 4-dimethylaminopyridine (DMAP) to the reaction system containing 3'-UMP and AcIm, the $\underline{k}_p$ was significantly enhanced (Fig. 3). Here, the latter three bases seem to have nucleophile catalytic effects.[20]

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