DIVERSE CHEMOSELECTIVITY DURING ACYLATION OF NUCLEOSIDES

Michal SOBKOWSKI

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznań, Poland; e-mail: msob@ibch.poznan.pl

The acylation of nucleosides with acid chlorides under very mild conditions (diluted solution in neutral solvents containing a few equivalents of an amine) was investigated. The rate and the sites of the reaction were found to depend strongly on the kind of amine used. In the presence of DMAP acylation occurred chemoselectively on the hydroxy groups of the ribose moiety, while a mixture of triethylamine and pyridine promoted acylation of the thymine residue.

INTRODUCTION

During preparation of nucleoside derivatives, the imido system in thymine and uracil residues is often left unprotected due to its relatively low reactivity. However, for a number of reactions aimed to the sugar moiety, e.g. alkylation, Mitsunobu reactions, or some condensations, such protection is highly desirable or obligatory to prevent base modifications. This demand is usually realized by introduction of a benzoyl group into the N3 position of the nucleobase[1]. The known methods for this purpose often require lengthy procedures of acylation and an employment of transient protection-deprotection strategy. In this paper some new findings regarding rapid and regioselective acylation of thymidine are presented.

RESULTS AND DISCUSSION

During ribonucleoside 3′-*H*-phosphonate condensations a decrease in the yield of *H*-phosphonate diester formation was noted when the reaction was carried out in the presence of trialkyl amines or strong nucleophilic catalysts (e.g. DMAP)[2]. It appeared that the reason for this deterioration of the yield was partial consumption of pivaloyl chloride (PvCl) used as a condensing agent in unexpected rapid pivaloylation of a nucleoside moiety. This was rather surprising observation, as under very mild conditions used for the condensation (diluted solution of reagents in DCM, 3 equiv. of an amine, 1.2 equiv. of PvCl) no competing acylation was anticipated.

In follow-up experiments, the reactivity of PvCl toward protected thymidine derivatives (3′,N3-diprotected for 5′-OH, and 3′,5′-diprotected

for N3 acylation) in the presence of DMAP, TEA, pyridine, or TEA/Py mixture, was investigated (Table I)[3]. In the presence of DMAP the pivaloylation of the 5′-OH group was over within a few seconds, while N3 acylation required several minutes for completion (chemoselectivity ratio >30:1). For pyridine, both reactions were much slower, and the advantage of *O*- over *N*-acylation was significantly reduced (ratio 2:1). In contrast, in the presence of triethylamine (TEA) acylation occurred in the thymine residue with an excellent chemoselectivity of >240:1 and with high rate ($t_{1/2}$ ~ 1 min)[5]. When a mixture of pyridine and TEA was used, *N*3-Pv derivative was formed over 600 times faster than the 5′-*O*-Pv one, and required only a few seconds to complete. This high acceleration of the acylation rate might be explained in terms of base catalysis operating on the pyrimidine residue and a nucleophilic catalysis of pyridine operating on acyl chloride. The observed diversification of reactivity of acyl chlorides toward thymidine due to these two types of catalysis is drafted in Fig. 1.

The above findings were exploited for rapid and efficient preparation of 5′-*O*-pivaloyl-, 3′,5′-*O*,*O*-dipivaloyl-, and *N*3-pivaloyl-thymidine[4] by a reaction of unprotected thymidine with pivaloyl chloride (1, 3, and 2 equiv., respectively) in acetonitrile in the presence of appropriate amines, DMAP or TEA/Py. After simple work-up (solvent extractions) the desired compounds were obtained as white solids in good yields.

TABLE I
Estimated kinetics of acylation of N3H and 5′-OH positions of thymidine derivatives protected in the all remaining sites (i.e. 3′,5′- or 3′,N3-deprotected). Reaction conditions: 0.1 mmol of nucleoside, 5 equiv. of amine(s), 3 equiv. of PvCl in 1 ml of acetonitrile. The progress of reactions was analyzed by TLC

	pivaloylation ($t_{½}$)		benzoylation ($t_{½}$)	
Amine Unprotected site for acylation:	N3H	5'-OH	N3H	5'-OH
DMAP (5 equiv.)	~ 30 s	< 1 s	~ 30 min	~ 10 s
Py (5 equiv.)	~ 2 h	~ 1 h	~ 24 h	~ 60 s
TEA (5 equiv.)	~ 60 s	> 4 h	< 1 s	~ 60 s
TEA (2.5 equiv.) + Py (2.5 equiv.)	< 1 s	-[a]	-[a]	~ 60 s

[a] Not complete due to side reactions.

Benzoylation in the thymine residue (using 3′,5′-diprotected thymidine) under the same conditions (5 equiv. of a base in ACN solution) differed from the pivaloylation in terms of kinetics and product distribution. In the presence of pyridine alone, the reaction was sluggish ($t_{1/2}$ ~ 24 h), while the

use of both pyridine and TEA caused an immediate (<1 s) benzoylation of ca. 50% of the starting material. However, then the reaction stopped and did not progress further even after addition of more BzCl. In contrast, in the presence of TEA alone, the still very rapid reaction (completion <1 s) was almost quantitative.

Benzoylation of the 5′-OH group of thymidine in the presence of TEA and/or pyridine was much faster ($t_{1/2}$ ~ 1 min, Table I) than the respective pivaloylation (several hours). In consequence, although the reaction of unprotected thymidine with BzCl in ACN containing TEA yielded a heterobase-benzoylated derivative as a main compound, it was accompanied by a variety of other products, presumably due to competitive acylation in the ribose moiety. Changing the solvent to DMF improved partly the selectivity of benzoylation (base vs sugar)[6] and allowed the preparation of *N*3-Bz-thymidine[1b].

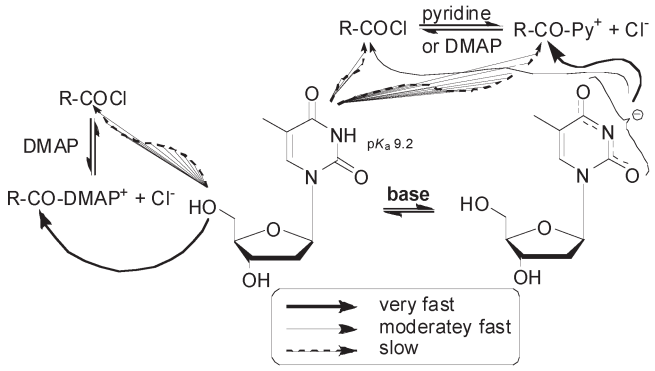

FIG. 1
The influence of base and nucleophilic catalysis on the rates of acylation

The rapid benzoylation of the 5′-OH group in the presence of pyridine along with a particularly slow benzoylation in the thymine residue under these conditions (Table I), explains the known selectivity of benzoylation in sugar moiety of nucleosides in pyridine[7]. In contrast, pivaloylation in the presence of pyridine showed poor selectivity.

Interestingly, during benzoylation of thymidine and its derivatives in the presence of TEA, besides the expected *N*3-Bz product (3′-*O*-DMTr, 5′-*O*-Pv: R_f 0.90; DCM–MeOH 97:3; "fast"), another compound was formed (R_f 0.75; "slow") as a main product (ratio of ca. 2:1 for Py/TEA used as amines, and 9:1 for TEA alone; TLC analysis). After several hours, the compound desig-

nated as "slow" was converted quantitatively into the "fast" one. A similar phenomenon was observed by Sekine during benzoylation of uridine derivatives, for which the analogous "slow" intermediate product was identified as an O^4-benzoylated nucleoside, while the final product ("fast"), as an $N3$-benzoylated one[1d]. By analogy, the "slow" and "fast" compounds described in this paper were tentatively assigned also as O^4- and $N3$-Bz derivatives, respectively. Since the assumed O → N acyl migration was found to proceed similarly in anhydrous and aqueous conditions, the participation of acyl chloride in this process was excluded, leaving an intramolecular nucleophilic substitution as a most probably mechanism of this isomerization.

The O^4-Bz thymidine derivatives were relatively stable in the presence of water, alcohols, and TEA (10% v/v), while upon addition of pyridine, a rapid debenzoylation of these compounds occurred with development of intensive reddish colorization of the reaction mixture. This reaction could arise from an attack of pyridine molecule on the O^4-Bz carbonyl carbon atom with the formation of *N*-benzoyl-pyridinium cation that might further undergo a substitution in the pyridine moiety, e.g. due to Zincke-type reactions, yielding red-brown colored products[8]. Similar deacylation of O^4-Bz kinetic products by pyridine could be also responsible for the incomplete benzoylation of nucleobase residue of thymidine derivatives observed in the presence of TEA/Py (vide supra).

Noteworthy, while the ^{1}H NMR signals of $N3$-Pv-thymidine in DMSO-d_6 were sharp and well resolved, in $CDCl_3$ all the signals, but these derived from the methyl groups, were broad. Additionally, the resonances for H6 and H1′ protons were split into two pairs of signals. At low temperature (from –30 to –50 °C) the H1′ signals appeared as two overlapping triplets, while those of H6, as two sharp singlets separated by 0.21 ppm. At elevated temperature (+50 °C) these pairs merged into one singlet for H6, and a poorly resolved triplet for H1′. These suggested that in $CDCl_3$ the thymine-pivaloylated nucleoside exists in two isomeric forms that may interconvert easily, presumably due to a fast O^4-Pv/$N3$-Pv equilibrium. If it would be the case, the protonation of the pivaloylated thymine residue should slow down the acyl migration rate, and the protonation site might be expected to be preferentially the N3 nitrogen atom of the O^4-Pv isomer. Indeed, in $CDCl_3$ acidified with TFA, the aforementioned splittings of signals were as strong as those at low temperatures, and the ^{1}H NMR resonances of almost all but two protons were well resolved. Broadening of the two signals, tentatively assigned as H6 and H1′ protons of $N3$-protonated O^4-Pv derivative, might arise from fast $N3H^+$ proton exchange.

These experiments seem to confirm the putative rapid O^4-Pv/N3-Pv equilibration in $CDCl_3$. In the case of N3-Bz thymidine, no such phenomenon was observed in ^1H NMR, pointing to significant kinetic and thermodynamic differences of pivaloylation and benzoylation of thymidine and its derivatives.

The author would like to thank Prof. Adam Kraszewski and Prof. Jacek Stawinski for helpful discussions. The financial support from Polish Ministry of Science and Higher Education is gratefully acknowledged.

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