BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 45, 245—247(1972)

Studies on Nucleosides and Nucleotides. II. Selective Acylation of 5′-Hydroxyl Group of Thymidine[1]

Oyo MITSUNOBU, Junji KIMURA, and Yoshiyuki FUJISAWA

Department of Chemistry, College of Science and Engineering, Aoyama Gakuin University, Megurisawa-cho, Setagaya-ku, Tokyo

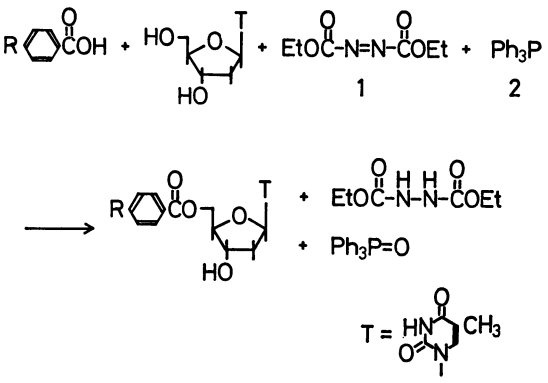
(Received June 15, 1971)

Treatment of thymidine and either acetic acid or benzoic acid with diethyl azodicarboxylate (**1**) and triphenylphosphine (**2**) in dioxane afforded 5′-*O*-acetyl or 5′-*O*-benzoylthymidine along with a trace of 3′,5′-di-*O*-acylated product. When thymidine and benzoic acid or *p*-substituted benzoic acids were allowed to react with **1** and **2** in hexamethylphosphoric triamide(HMPA), the corresponding 5′-*O*-aroylthymidines were selectively obtained. The aroyl groups having electron-withdrawing substituents were much more readily removed than those having electron-releasing groups under mild alkaline conditions.

For the chemical synthesis of nucleotides and oligonucleotides, the hydroxyl groups of sugar moiety should be appropriately protected prior to phosphorylation. Of many blocking groups examined so far, triarylmethyl groups have been widely used because they can be introduced predominantly to 5′-hydroxyl group of nucleosides and removed under acidic conditions. It is known, however, that alkali-labile protecting groups are superior to acid-labile ones for the synthesis of oligonucleotides.[2] Thus acyl groups are used as alkali-labile groups for the protection of the hydroxyl functions as well as amino functions. There are few reports, however, of the selective acylation of 5′-hydroxyl groups of nucleosides.

In a previous paper,[3] we have reported that the 5′-hydroxyl groups of thymidine and uridine were predominantly phosphorylated by means of dibenzyl hydrogen phosphate, diethyl azodicarboxylate (**1**) and triphenylphosphine (**2**). It is of interest to apply this procedure to the selective acylation of 5′-hydroxyl groups of nucleosides.

Acylation of Thymidine. Thymidine was allowed to react with equimolar amounts of acetic acid, **1** and **2** in dioxane at 60°C for 3 hr. The thymidine remaining insoluble disappeared as the reaction proceeded and 5′-*O*-acetylthymidine was isolated in a 55.2% yield by silica gel column chromatography.[4] Differing from the case of phosphorylation of thymidine, a trace of 3′,5′-di-*O*-acetylthymidine was also isolated. Similarly, the reaction of thymidine with benzoic acid, **1** and **2** led to the formation of 5′-*O*-benzoylthymidine in isolated yield of 78.0%. A trace of 3′,5′-di-*O*-benzoylthymidine was also formed.



Since thymidine was found to be sufficiently soluble in hexamethylphosphoric triamide (HMPA), further experiment was carried out using HMPA as the solvent. When a solution of thymidine and **2** in HMPA was

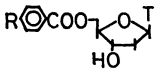
1) Presented at the 24th meeting of the Chemical Society of Japan (April, 1971). Part I: O. Mitsunobu, K. Kato, and J. Kimura, *J. Amer. Chem. Soc.*, **91**, 6510 (1969).

2) a) M. Smith, D. H. Rammler, I. H. Goldberg, and H. G. Khorana, *J. Amer. Chem. Soc.*, **84**, 430 (1962). b) D. H. Rammler and H. G. Khorana, *ibid.*, **84**, 3112 (1962). c) H. Schaller, G. Weimann, B. Lerch, and H. G. Khorana, *ibid.*, **85**, 3821 (1963). d) M. W. Moon, S. Nishimura, and H. G. Khorana, *Biochemistry*, **5**, 937 (1966). e) B. E. Griffin, M. Jarman, and C. B. Reese, *Tetrahedron*, **24**, 639 (1968) and refs. therein.

3) O. Mitsunobu, K. Kato, and J. Kimura, *J. Amer. Chem. Soc.*, **91**, 6510 (1969).

4) No attempt was made to obtain the optimum yield.

Table 1. Preparation of 5′-*O*-aroylthymidines



R	Yield[a] %	Mp °C	R_f[b]	Anal. C% Calcd	Anal. C% Found	Anal. H% Calcd	Anal. H% Found	Recovered thymidine %
NO_2	84.6	182[c]	0.89	52.17	52.59	4.38	4.38	10.8
CN	79.5	168—170	0.82	58.22	58.28	4.61	4.50	20.0
H	74.0	171—172	0.84	58.96	58.72	5.20	5.09	25.0
OCH_3	65.5	176—177	0.85	57.44	57.48	5.35	5.42	33.5
CH_3	65.0	202	0.87	59.99	59.88	5.59	5.13	34.5

a) The yield was calculated by paper chromatography.
b) Solvent system; *n*-butanol-0.1 N HCl=5:1 R_f value of thymidine was 0.57.
c) Lit. mp 180—181°C.[7b]

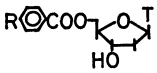
treated with benzoic acid and **1** at room temperature, 5′-*O*-benzoylthymidine was formed in yield of 74.0% and 25.0% of thymidine was recovered as determined by paper chromatography. It is noteworthy that no 3′,5′-di-*O*-benzoylthymidine could be detected by thin layer chromatography (tlc) of the reaction mixture. By a similar way, 5′-*O*-*p*-nitrobenzoyl-, 5′-*O*-*p*-cyanobenzoyl-, 5′-*O*-*p*-methoxybenzoyl- and 5′-*O*-*p*-toluylthymidine were obtained. The results are summarized in Table 1.

As shown in Table 1, benzoic acid derivatives having electron-withdrawing groups gave higher yields of the corresponding 5′-*O*-aroylthymidines than those having electron-releasing groups. 5′-*O*-Aroylthymidine could be isolated by silica gel column chromatography.

Thymidine is soluble in HMPA, while triphenylphosphine oxide formed precipitated nearly quantitatively and could be readily removed by filtration. Although HMPA is known to form complexes with protic acids,[5] no disturbing influence on the present reaction was observed. HMPA is, therefore, a suitable solvent for the present purpose in spite of its high boiling point.

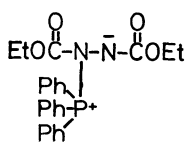
Generally, acid chlorides, acid anhydrides or aryl carboxylates react with 5′-hydroxyl group as well as 3′(2′)-hydroxyl group of nucleosides to give both 5′-*O*-acylated and 3′(2′),5′-poly-*O*-acylated compounds.[6,7] As an example, *p*-nitrobenzoylation of unprotected thymidine (5 mmol) by means of *p*-nitrobenzoyl chloride (5.5 mmol) in pyridine resulted in the formation of 5′-*O*-*p*-nitrobenzoylthymidine (55%) and 3′,5′-di-*O*-*p*-nitrobenzoylthymidine (18%).[8] Thus, the present method might be convenient for the selective formation of 5′-*O*-aroylthymidine.

Table 2. Properties of UV absorbance for 5′-*O*-aroylthymidines

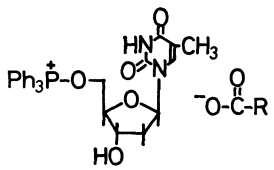


R	$\varepsilon \times 10^{-4}$	λ_{max} (mμ)
NO_2	1.77	265
CN	2.24	242
H	0.884	270
OCH_3	2.46	265
CH_3	2.10	246

Acylation of alcohols by means of carboxylic acids, **1** and **2** may proceed through quaternary phosphonium salts, **3** and **4**, *e.g.*, may involve activation of alcohols rather than carboxylic acids.[9] Acylating and carboxylating reagents having bulky groups predominantly gave corresponding 5′-*O*-protected nucleosides.[10] Thus, it would be reasonable to postulate that the selective formation of 5′-*O*-aroylthymidines by the present method is due to steric hindrance of three phenyl groups around the positively charged phosphorus atom in the intermediate **3**.[3]



3

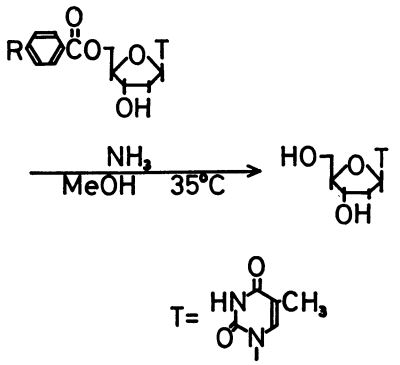


4

Dearoylation of 5′-O-Aroylthymidine. The 5′-*O*-aroylthymidines were treated with methanolic ammonia (saturated at room temperature) at 35°C and the reactions were monitored by paper chromatog-

5) H. Normant, *Angew. Chem.*, **79**, 1029 (1967).
6) P. T. Gilham and H. G. Khorana, *J. Amer. Chem. Soc.*, **80**, 6212 (1958). b) C. Kresze, E. Lodemann, and A. Wacker, *Z. Naturforsch.*, **B22**, 285 (1967); *Chem. Abstr.*, **67**, 117177n (1967).
7) T. Sasaki and Y. Mizuno reported that acylation of cytidine with an equimolar amount of acetic anhydride at refluxing temperature in dry pyridine afforded predominantly N^4-acetylcytidine which on further treatment with acetic anhydride in pyridine predominantly gave N^4, 5′-*O*-diacetyl-cytidine. On the other hand, treatment of cytidine with glacial acetic acid in the absence of pyridine gave 3′(or 2′),5′-*di*-*O*-acetylcytidine and 5′-*O*-acetylcytidine. *Chem. Pharm. Bull.*, **15**, 894 (1967).
8) K. E. Pfitzner and J. G. Moffat, *J. Amer. Chem. Soc.*, **87**, 5661 (1965).
9) O. Mitsunobu and M. Yamada, This Bulletin, **40**, 2380 (1967). b) O. Mitsunobu and M. Eguchi, *ibid.*, in press.
10) a) G. Weimann and H. G. Khorana, *J. Amer. Chem. Soc.*, **84**, 4329 (1962). b) K. Gerzon and D. Kau, *J. Med. Chem.*, **10**, 189 (1967); *Chem. Abstr.*, **66**, 74881x (1967). c) K. K. Ogilvie and R. L. Letsinger, *J. Org. Chem.*, **32**, 2365 (1967).

raphy. As expected,[11] the nature of the *p*-substituents affected the rate of solvolysis; the aroyl groups having electron-withdrawing substituents were much more readily removed than those having electron-releasing groups. The time(hr) required for the complete dearoylation was as follows: *p*-NO_2, 1.0; *p*-CN, 1.5; H, 8.0; *p*-CH_3O, 22; *p*-CH_3, 23.



The results indicate that the acylation of thymidine by means of carboxylic acids, **1** and **2** provides a convenient and selective method available for the preparation of 5′-*O*-acylthymidines.

Experimental

Methods. Paper chromatography was carried out by the ascending technique using Toyoroshi No. 51A paper. The solvent used was *n*-butanol-2NHCl (5: 1). The R_f values of different compounds are listed in Table 1. Estimation of the yields of 5′-*O*-aroylthymidines formed by the reaction in HMPA was carried out spectrophotometrically after elution of spots from paper chromatograms. The spots and appropriate blank areas of the paper were eluted after it was cut into small pieces by soaking in 0.1N hydrochloric acid for 1 day at 37°C in sealed vessels, the contents of the vessels being shaken occasionally. The extracts were measured by a Hitachi EPS-3T recording spectrometer.

Thin layer chromatographic plates were prepared using silica gel PF_{254} (E. Merck) and developed with $CHCl_3$: CH_3CN=1: 1. Silica gel (Wakogel C-300) was used for column chromatography.

Commercial thymidine was dried at 80—90°C over phosphorus pentoxide. Diethyl azodicarboxylate[12] and *p*-toluic acid[13] were prepared by known procedures. Other commercial reagents and solvents were purified and dried by ordinary procedures.

11) On alkaline hydrolysis of 5′-*O*-acyl-2′,3′-isopropylidenuridine, the order of reactivity is CF_3CO>CCl_3CO>CH_2ClCO>CH_3CO. F. Cramer, H. P. Bär, H. J. Phaese, W. Sänger, K. H. Scheit, G. Schneider, and J. Tennigkeit, *Tetrahedron Lett.*, **1963**, 1039.

12) J. C. Kauer, "Organic Syntheses," Coll. Vol. IV, p. 411.

13) W. F. Tuley and C. S. Marvel, "Organic Syntheses," Coll. Vol. III, p. 822.

Preparation of 5′-O-Acetylthymidine. Acetic acid (180 mg, 3×10^{-3} mol) and diethyl azodicarboxylate (522 mg, 3×10^{-3} mol) in dioxane (5 m*l*) were added to a mixture of thymidine (726 mg, 3×10^{-3} mol) and triphenylphosphine (786 mg, 3×10^{-3} mol) in dioxane (5 m*l*) at 60°C under stirring. Stirring was continued for 3 hr and the solution was concentrated. The residue was applied to silica gel column (3.2×70 cm). Elution with ethyl acetate afforded diethyl hydrazodicarboxylate in the first fraction, a trace of 3′,5′-di-*O*-acetylthymidine in the next fraction and 5′-*O*-acetylthymidine in the third fraction. 5′-*O*-Acetylthymidine was obtained by evaporating and crystallizing the residue from ethyl acetate, 55.2% (470 mg). It melted at 153—154°C (lit,[6a] mp 150°C). Found: C, 50.67; H, 5.72; N, 10.34%. Calcd for $C_{12}H_{16}N_2O_4$: C, 50.70; H, 5.63; N, 9.86%.

Similarly, the reaction of benzoic acid (366 mg, 3×10^{-3} mol) with equimolar amounts of thymidine, diethyl azodicarboxylate and triphenylphosphine was carried out in dioxane. Elution with acetonitrile afforded a trace of 3′,5′-di-*O*- benzoylthymidine (mp 154—156°C: Found: C, 63.41; H, 5.02; N, 6.03%. Calcd for $C_{24}H_{22}N_2O_7$: C, 64.00; H, 4.89; N, 6.22%) and a 75.1% (780 mg) yield of 5′-*O*-benzoylthymidine, mp 171—172°C recrystallized from benzene-ethyl acetate. Found: C, 58.72; H, 5.09; N, 8.23%. Calcd for $C_{17}H_{18}N_2O_6$: C, 58.96; H, 5.20; N, 8.09%.

Preparation of 5′-O-Aroylthymidines. General Procedure. A mixture of triphenylphosphine (1×10^{-3} mol) and thymidine (1×10^{-3} mol) in HMPA (1 m*l*) was stirred with exclusion of moisture and a solution of carboxylic acid (1×10^{-3} mol) and diethyl azodicarboxylate (1×10^{-3} mol) in HMPA (1 m*l*) was added over a period of 1 hr at room temperature. Stirring was continued for 5 hr and the mixture was then kept standing overnight at room temperature. After removal of triphenylphosphine oxide (nearly quantitative yield) by filtration, the filtrate was concentrated to a sirup under reduced pressure. The residue was dissolved in dry methanol and the solution was adjusted to exactly 100 m*l* to determine the amounts of the 5′-*O*-aroylthymidine formed and the thymidine unreacted by means of paper chromatography. Methanol was then removed and the residue was chromatographed over silica gel to isolate the product. Elemental analyses and physical properties of 5′-*O*-aroylthymidines thus isolated are summarized in Table 2.

Isolation of 5′-O-p-Methoxybenzoylthymidine. The residue obtained by the removal of methanol was applied to silica gel column (2.2 cm×50 cm), and eluted with a mixture of chloroform-acetonitrile (1: 1) giving a 54.5% yield of 5′-*O*-*p*-methoxybenzoylthymidine. This was purified by recrystallization from methanol.

Solvolysis of 5′-O-Aroylthymidine. 5′-*O*-Aroylthymidine (30 mg) was dissolved in methanolic ammonia (10 m*l*, saturated at room temperature) and stirred at 35°C. The reaction was monitored by paper chromatography and tlc. Time required for the 5′-*O*-aroylthymidines to disappear was as follows; 5′-*O*-*p*-nitrobenzoylthymidine, 1.0; 5′-*O*-*p*-cyanobenzoylthymidine, 1.5; 5′-*O*-benzoylthymidine, 8.0; 5′-*O*-*p*-methoxybenzoylthymidine, 22; 5′-*O*-*p*-toluylthymidine, 23 hr.