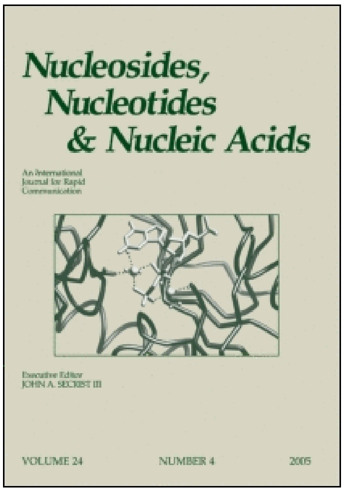

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Leonidas Kiriasis[a]; Silke Farkas[a]; Wolfgang Pfleiderer[a]
[a] Fakultät für Chemie, Universität, Konstanz, West Germany

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NUCLEOSIDES, XLIII[1)]

SYNTHESIS AND PROPERTIES OF O^4-ALKYLTHYMIDINES

Leonidas Kiriasis, Silke Farkas, and Wolfgang Pfleiderer
Fakultät für Chemie, Universität Konstanz
Postfach 5560, D-7750 Konstanz / West Germany

Abstract. Various O^4-alkylthymidines 14-20 have been synthesized by two different methods. O^4-Alkylation takes place with 3',5'-di-O-acetyl- (2) and 3',5'-di-O-benzoylthymidine (3) respectively in a silver ion catalysed reaction with alkyl halides, whereas the azolide approach makes use of a nucleophilic displacement of the appropriate intermediate by alkoxides and subsequent deacylation to the free nucleosides. Structural proofs are based on elemental analyses, UV- and ^{1}H-NMR-spectra.

Introduction. - Action of alkylating agents on DNA causes modifications at various nucleophilic sites which may affect the replication as well as the transcription of the genetic material[2,3]. Especially N-alkyl-N-nitrosoureas are regarded as potent carcinogens, whereas N-alkyl-N-nitrosoguanidines are in addition also highly mutagenic[4]. Since N-nitroso alkylating agents show a pronounced affinity for O-al-

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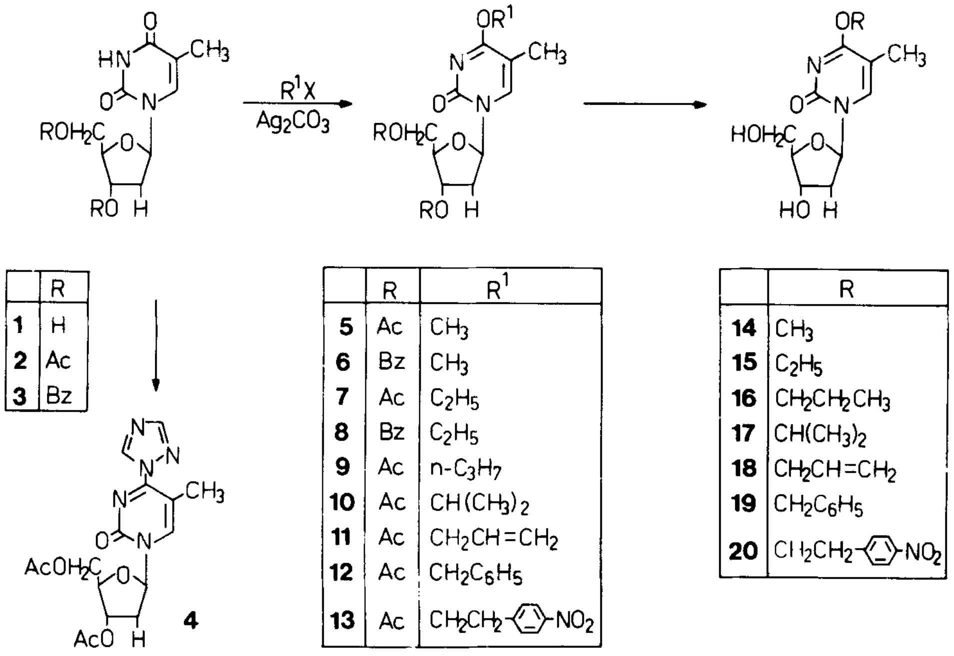
kylations due to the S_N1-type mechanism of such reactions[5], electrophilic attack at O^2 and O^4 of thymine, O^2 of cytosine as well as at O^6 of guanine are seen as the main targets[6-9]. The modified pyrimidine and purine bases will then directly influence the base-pairing behavior giving rise to unexpected transitions by mispairing.

In order to provide a series of O^4-alkyl-thymidine derivatives for studies on repair mechanisms and other biological effects their chemical syntheses have now been achieved by various procedures in reasonable to good yields.

Action of diazoalkanes on thymidine dates back to 1934[10] but leads to low yields and mixtures of O- and N-alkyl derivatives, which are often difficult to separate[6,11,12]. Other methods include silver-catalyzed alkylations of the amide function[13] as well as its chemical modification to intermediary triazoles[14-19] and imidazoles[20] followed by nucleophilic displacement by the corresponding alcohols and alkoxides respectively.

Syntheses. - A first approach to O^4-alkylations of thymidine (1) was achieved by reaction of 3',5'-di-O-acetyl- (2) and 3',5'-di-O-benzoylthymidine (3) respectively with the appropriate alkyl halide in toluene and in the presence of silver carbonate at slightly elevated temperatures. Heating of the starting material with silver carbonate in toluene under reflux for 1 h with azeotropic removal of moisture prior to the addition of the alkylating agent is recommended. The reaction produces in general two products, the expected O^4-alkyl-3',5'-di-O-acylthymidine (5-13) and a faster running component which turned out to be the corresponding 2,4-di-O-alkylthymine, as shown by the isolation and characterization of 2,4-di-O-ethylthymine. The formation of the 2,4-dialkoxy-5-methylpyrimidines can be explained by additional O^2-alkylation followed by nucleophilic attack of the halide ion at the anomeric C-1' atom of the sugar moiety and cleavage of the glycosidic linkage. The main reaction products are separated by silica gel chromatography and are subsequently deacylated by alkoxides or ammonia to give the O^4-

alkylthymidines 14-19 as colourless crystals in yields of 37-74 %.



	R
1	H
2	Ac
3	Bz

	R	R^1
5	Ac	CH_3
6	Bz	CH_3
7	Ac	C_2H_5
8	Bz	C_2H_5
9	Ac	n-C_3H_7
10	Ac	$CH(CH_3)_2$
11	Ac	$CH_2CH=CH_2$
12	Ac	$CH_2C_6H_5$
13	Ac	CH_2CH_2-⌬-NO_2

	R
14	CH_3
15	C_2H_5
16	$CH_2CH_2CH_3$
17	$CH(CH_3)_2$
18	$CH_2CH=CH_2$
19	$CH_2C_6H_5$
20	CH_2CH_2-⌬-NO_2

O^4-Methylthymidine (14) has been synthesized before from 2,4-dimethoxy-5-methylpyrimidine and 3,5-di-O-p-toluoyl-2-deoxyribofuranosyl chloride in a Hilbert-Johnson reaction followed by deacylation[21]. The O^4-ethylthymidine (15) has recently been characterized by X-ray analysis[22], whereas in the earlier literature only its 3',5'-di-O-acetyl derivative was mentioned[23].

The triazole approach was achieved by conversion of 3',5'-di-O-acetylthymidine (2) with phosphoryl chloride and 1,2,4-triazole into 1-(3,5-di-O-acetyl-2-deoxyribofuranosyl)-5-methyl-4-(1,2,4-triazol-1-yl)-2(1H)pyrimidinone (4) and subsequent nucleophilic displacements at C-4. Methanol and ethanol respectively reacted in the presence of triethylamine on boiling under reflux whereas isopropanol gave a low yield under these conditions even after 3 days. Introduction of the 4-p-nitrophenylethoxy group required refluxing for 2 days in acetonitrile/triethylamine to give after ammonia treatment of the crude intermediate 13 a 39 % yield of 20[24]. The use of 4-dimethylaminopyridine instead of triethylamine catalyzes the reaction to some extent, but the yield could not be improved over 38 % even on boiling for 24 h. Alkoxides react as expected much faster, give better yields, and deacetylate simultaneously to give the free O^4-alkylthymidines 14, 15 and 17.

Activation of the amide function in 2 by $POCl_3$/N-methylimidazole[20] seems to play even a much stronger role since displacement of the azolide group proceeds with methanol/triethylamine even at room temp. in 40 % yield in 6 h. This reaction has not yet been applied to other types of alcohols.

Characterization of the O^4-alkylthymidine derivatives 14-19 is based on elemental analyses, UV- and ^{1}H-NMR-spectra (Tab. 1).

EXPERIMENTAL

UV Spectra were recorded on a Cary Recording Spectrometer, Model 118, from Appl. Physics Corp. - NMR Spectra were measured with a Bruker WM 250 high resolution spectrometer with tetramethylsilane as an internal standard and on a δ-scale in ppm. - Thin layer chromatography was performed on silica gel sheets F 1550 LS 254 of Schleicher & Schüll, preparative thick layer chromatography on glass plates 40 x 20 cm coated with a 0.2 cm layer of silica gel PF_{254} of Merck/Darmstadt and column chromatography on Merck silica gel 60 (particle size 0.063-0.2 mm). - Drying of the substances was achieved in a vacuum desiccator or in a Büchi-TO 50 drying oven under vacuum at room temp. and slightly elevated temp. respectively. Melting points were determined in a Tottoli apparatus and are uncorrected.

Tab. 1 - Physical Data of O^4-Alkylthymidines

-thymidine	UV-Spectra in MeOH				^{1}H-NMR-Spectra in D_6-DMSO (δ-values in ppm)				Sugar Protons
	λ_{max}(nm)		lg ε		6-H	1'-H	5-CH_3	O^4-Substituent	
O^4-Methyl- (<u>14</u>)	205	282	4.26	3.79	8.00s	6.13pt	1.87s	3.83s (3H, OCH_3)	5.23d (3'-OH)
O^4-Ethyl- (<u>15</u>)	205	282	4.27	3.80	8.00s	6.13pt	1.86s	4.27q (2H, OCH_2), 1.28t (3H, CH_3)	5.05t (5'-OH)
O^4-n-Propyl- (<u>16</u>)	205	282	4.26	3.79	8.00s	6.12pt	1.87s	4.22t (2H, OCH_2), 1.69m (2H, CH_2), 0.93t (3H, CH_3)	4.21m (3'-H)
O^4-i-Propyl- (<u>17</u>)	205	282	4.28	3.80	7.98s	6.12pt	1.84s	5.23sep (1H, OCH) 1.27d (6H, CH_3)	3.79m (4'-H)
O^4-Allyl- (<u>18</u>)	205	283	4.31	3.82	8.03s	6.12pt	1.89s	6.00m (1H,-CH=), 5.28m (2H, =CH_2), 4.77d (2H, OCH_2-)	3.60m (5'-H)
O^4-Benzyl- (<u>19</u>)	205	283	4.45	3.86	8.05s	6.14pt	1.90s	7.40m (5H, Arom.), 5.34s (2H, CH_2)	2.17m (2'-H)
O^4-p-Nitro-phenylethyl- (<u>20</u>)	205	277	4.46	4.20	8.00s	6.11pt	1.79s	8.17d (ortho-H), 7.57d (meta-H), 3.16t (2H, CH_2)	1.98m (2'-H)

s = Singlet; d = doublet; t = triplet; pt = pseudotriplet; sep = septet; m = multiplet.

A. General Procedure for O^4-Alkylation of Thymidine (1). 3',5'-Di-O-acetylthymidine (2)[25] (3.26 g, 10 mmol) of 3',5'-di-O-benzoylthymidine (3)[26] (4.5 g, 10 mmol) is refluxed with silver carbonate (4.14 g, 15 mmol) in toluene (120 ml) for 1 h and then 20 ml of the solvent distilled off to remove any moisture. On cooling to 50°C the alkyl halide (20 mmol) is added and the reaction mixture is stirred at this temp. for 15 to 30 h. The insoluble silver salts are filtered off, washed with ethyl acetate and then the filtrate evaporated to dryness. The residue is dissolved in a small amount of ethyl acetate, put on a silica gel column 20x4 cm (100 g silica gel) and chromatographed using the same solvent. The main fraction containing the fully protected material 5-12 is evaporated yielding a colourless oil, which on treatment with either sodium ethoxide in methanol (0.1 N solution) for 3 h or with conc. aqueous ammonia (50 ml) in dioxane (50 ml) and methanol (50 ml) for 12-18 h at room temp. causes deacylation. The reaction solution is evaporated to dryness and the residue is purified by column chromatography (10x4 cm) with a mixture of chloroform/methanol (20/1). The main fraction is evaporated to dryness again and the residue triturated with ether or recrystallized from ethanol.

B. General Procedure for the Synthesis of O^4-Alkylthymidines via the Triazolide Method. To a solution of 1,2,4-triazole (6.9 g, 0.1 mol) and phosphoryl chloride (3.0 g, 20 mmol) in acetonitrile (20 ml) are added at 0°C with stirring triethylamine (10 g, 0.1 mol) dropwise within 15 min. After stirring for 15 min a solution of 3',5'-di-O-acetylthymidine (2) (3.26 g, 10 mmol) in acetonitrile (50 ml) is added slowly and then the reaction mixture kept for 18 h at room temp. Triethylamine (13 ml) and water (3ml) is added and the solution stirred for 15 min and finally carefully evaporated in vacuum. The residue is dissolved in chloroform (100 ml), washed twice with water (10 ml) and a saturated solution of sodium bicarbonate (10 ml). The chloroform layer is dried over $MgSO_4$ and then evaporated to give crude 1-(3,5-di-O-acetyl-2-deoxyribofuranosyl)-5-methyl-4-(1,2,4-triazol-1-yl)-2(1H)pyrimidinone (4), which is pure enough for the displacement reactions.

Sodium (0.25 g, 11 mmol) is dissolved in the corresponding alcohol (60 ml) and then the triazole derivative 4 is added and the solution stirred at room temp. for 3-18 h depending on the alkoxide residue. The reaction solution is neutralized with acetic acid, evaporated to dryness and the residue is recrystallized from acetone or ethanol.

O^4-Methylthymidine (14). Alkylation of 2 according to method A for 64 h at 40°C, with addition of extra methyl iodide (2 ml) after each 24 h, afforded colourless crystals from acetone (1.07 g, 42 %) of m.p. 172-174°C. Lit.[21] 171.5-173.5°C.

Application of the triazolide method yielded after 3 h colourless crystals (1.61 g, 63 %) of m.p. 172-174°C.

Anal. Calc. for $C_{11}H_{16}N_2O_5$ (256.3): C, 51.57; H, 6.29; N, 10.93. Found: C, 51.57; H, 6.22; N, 10.90.

O^4-Ethylthymidine (15). The alkylation of 2 with ethyl iodide was performed 33 h at 50°C followed by 15 h stirring at room temp. Deacylation and work-up led to colourless crystals from a small quantity of ethanol (1.21 g, 45 %) of m.p. 185°C.

Under analogous conditions 3',5'-di-O-benzoylthymidine (3) gave 2.0 g (74 %) 15 of m.p. 186°C.

The triazolide method gave after 3 h treatment colourless needles (1.7 g, 63 %) of m.p. 185°C.

Anal. Calc. for $C_{12}H_{18}N_2O_5$ (270.3): C, 53.30; H, 6.70; N, 10.45. Found: C, 53.24; H, 6.75; N, 10.45.

O^4-n-Propylthymidine (16). Treatment of 2 with n-propyl iodide at 50°C over night yielded colourless crystals from ether (1.16 g, 41 %) of m.p. 105-106°C.

Anal. Calc. for $C_{13}H_{20}N_2O_5$ (284.3): C, 54.92; H, 7.09; N, 9.85. Found: C, 54.98; H, 7.22; N, 10.09.

O^4-Isopropylthymidine (17). Alkylation of 2 was carried out with isopropyl iodide at 50°C for 30 h. After deacylation, a colourless powder (1.53 g, 54 %) of m.p. 160°C was obtained by trituration with diethylether.

The triazolide method afforded, after displacement with isopropoxide for 18 h and treatment with ammonia for removal of the acetyl groups, a colourless powder (1.59 g, 56 %) of m.p. 160°C.

Anal. Calc. for $C_{13}H_{20}N_2O_5$ (284.3): C, 54.92; H, 7.09; N, 9.85. Found: C, 54.82; H, 7.05; N, 9.69.

O^4-Allylthymidine (18). Alkylation of 2 with allylbromide at 50°C for 16 h gave colourless crystals (1.24 g, 41 %) of m.p. 106-107°C.

Anal. Calc. for $C_{13}H_{18}N_2O_5$ (282.3): C, 55.31; H, 6.42; N, 9.92. Found: C, 55.78; H, 6.48; N, 10.00.

O^4-Benzylthymidine (19). The alkylation of 2 was carried out with benzylbromide at 50°C for 16 h. Deacetylation by sodium ethoxide for 1 h at room temp. and acidification of the concentrated aqueous solution gave colourless crystals (1.36 g, 41 %) of m.p. 179-180°C.

Anal. Calc. for $C_{17}H_{20}N_2O_5$ (332.4): C, 61.44; H, 6.06; N, 8.42. Found: C, 61.66; H, 6.17; N, 8.51.

O^4-[2-(4-nitrophenyl)ethyl]-thymidine (20). The crude triazolide 4 (0.75 g, 2 mmol) was dissolved in acetonitrile (10 ml) and 2-(4-nitrophenyl)-ethanol (0.86 g, 5 mmol) and N,N-diisopropylethylamine (0.9 ml, 5 mmol) were added and the mixture refluxed for 66 h. The reaction mixture was evaporated to dryness and purified by column chromatography (20x3 cm, 50 g silica gel) in ethyl acetate. The main fraction was evaporated to dryness and the protected intermediate 13 deacylated by conc. aqueous ammonia (3 ml) in methanol (10 ml) by stirring for 18 h at room temp. Evaporation and coevaporation with methanol gave on recrystallization from ethyl acetate colourless needles (0.52 g, 66 %) of m.p. 143-146°C.

Anal. Calc. for $C_{18}H_{21}N_3O_7$ (391.4): C, 55.24; H, 5.41; N, 10.74. Found: C, 55.26; H, 5.21; N, 10.71.

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