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Fluoride ion catalyzed alkylation of purines, pyrimidines, nucleosides and nucleotides using alkyl halides

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ABSTRACT

Alkyl halides react rapidly with purines and pyrimidines in the presence of fluoride ion. Alkylation of thymidine leads to novel dimeric nucleoside derivatives bridged through N^3. Alkylation of thymidine mono and dinucleotides leads to alkylation at the base (N^3) as well as diester and triester formation at the phosphate.

INTRODUCTION

The alkylation of purines, pyrimidines, nucleosides and nucleotides has been vigorously investigated during the past decade. Such investigations have been motivated by factors including the natural occurrence of alkylated nucleosides, the mutagenic properties of alkylating agents and the need for modified nucleosides in the study of nucleic acids. Much of this work has recently been reviewed.[1]

Among the most actively investigated alkylating agents are alkyl halides, trialkyl phosphates, alkyl sulfates and alkyl sulfonates. Yields in the synthetic experiments have usually been low (10-60%) even when vigorous conditions are used.[1-3] Rarely have products of alkylation at the phosphate of nucleotides been observed in yields above 10%.

We wish to describe in this manuscript the results of reactions between alkyl halides and nucleic acid components in the presence of fluoride ion as catalyst. The conditions described lead to near quantitative alkylation at purines and pyrimidines as well as high yields of diesters and triesters of nucleotides. The procedures allow the synthesis of novel "bridged" nucleosides. A preliminary report of some of these results has appeared.[4]

DISCUSSION

Alkyl halides have been used mainly to alkylate purine and pyrimidine bases[2,5,6], particularly adenine. However Singer[7] and Shapiro[8] have found high yields in reactions of alkyl iodides with cytidine while several products have been obtained with guanosine.[9] Jones and Robins[10] reported good yields of methylated guanosine and inosine derivatives using methyl iodide.

We have found that alkyl halides react rapidly with purines and pyrimidines at room temperature in the presence of fluoride ion to produce high yields (Table I) of the alkylated bases. Guanine was insoluble in the reaction media under the conditions used. The table shows that by using an excess of the alkyl halide very high yields of 1,3-dialkylpyrimidines are obtained from cytosine and uracil and that adenine yields the 9-alkyl derivative.

When methyl bromide was used as the limiting reagent (condition B), 1-methyluracil was the major product (60%) of the reaction with uracil while 34% of the 1,3-dimethyluracil was also obtained. However with cytosine and adenine only the 1-methyl and 9-methyl derivatives were obtained respectively.

Table 1.

Products of Alkylation of Purines and Pyrimidines.

Base	Alkylating Agent	Conditions	Products (%)
uracil	CH_3Br	A	1,3-dimethyluracil (99)
uracil	CH_3Br	B	1,3-dimethyluracil (34) 1-methyluracil (60) 3-methyluracil (6)
uracil	ϕCH_2Cl	A	1,3-dibenzyluracil (96)
uracil	$CH_3CH_2CH_2CH_2Br$	A	1,3-di-n-butyluracil (52) 1-n-butyluracil (24)
uracil	$CH_3CH_2CH_2CH_2Cl$	A	1-n-butyluracil (10)
uracil	$CH_3CH_2CH_2CH_2Cl$	C	1,3-di-n-butyluracil (95)
uracil	$CH_3CH_2CH(CH_3)Br$	A	1,3-di-sec-butyluracil(7) 1-sec-butyluracil (18)
cytosine	CH_3Br	A	1,3-dimethylcytosine (85)
cytosine	CH_3Br	B	1-methylcytosine (99)
adenine	CH_3Br	A	9-methyladenine (95)
adenine	CH_3Br	B	9-methyladenine (77)
adenine	ϕCH_2Cl	A	9-benzyladenine (93)
xanthine	CH_3Br	A	caffeine (85)

In all of these cases no change in product distribution was observed for reactions that were allowed to proceed for 16-20 hrs when methyl bromide or benzyl chloride were used.

To compare the effects of halogen and steric factors, the butyl halides were reacted with uracil. As expected, the alkyl chloride gave lower yields than the bromide and sec-butyl bromide gave lower yields than the n-butyl bromide. It should be noted that even in these cases when the alkyl halide was present in large excess, very high yields of the 1,3-dialkyluracils were obtained (conditions C).

Our major interest in this study was the possibility of obtaining novel nucleoside and nucleotide derivatives. To this end thymidine and deoxyuridine were studied as model compounds.

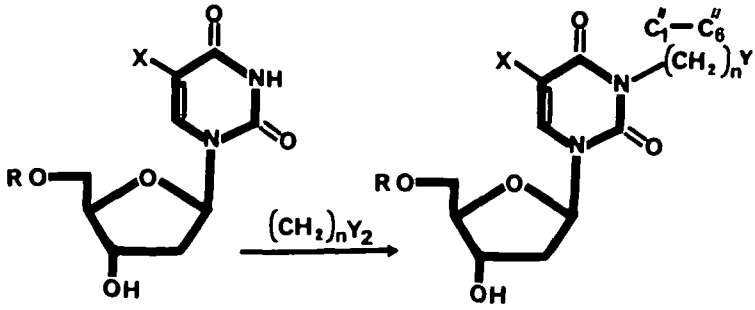
Because of the observation that a large excess of alkyl halide would produce virtually quantitative yields (followed by TLC) of the 3-alkylthymidines, such a procedure was used with methyl bromide, n-butyl chloride and isopropyl chloride. While TLC's showed that thymidine had been quantitatively converted to a single product in each case, isolation of the products by an extraction procedure led to isolated yields of 63, 55 and 45% respectively of the 3-alkylthymidines. However when the reaction product was passed through an exchange column before work-up, yields improved to the 85-90% range.

Recently attention has been drawn to studies of nucleosides "bridged" through their bases[11-17] with most reports involving purines. We have found that the fluoride ion catalyzed reaction of dihalides with thymidine and deoxyuridine presents an easy route to the dimers 3.

The treatment of 5'-O-monomethoxytritylthymidine (MMT-T, 1a) with tetra-(n-butyl)ammonium fluoride (TBAF) in methylene chloride led to a 91% yield of the dimer 3a and an 8% yield of 2a. These products were detritylated with acetic acid and the dimer di(thymidin-N^3-yl)methane 3b and the monomer N^3-fluoromethylthymidine (2b) were fully characterized by high resolution mass spectrometry (Table 2) and by CMR (Table 3). If TBAF is eliminated and replaced by triethylamine, absolutely no such products are obtained.

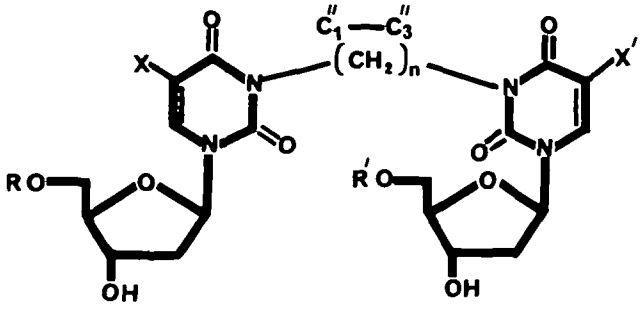
The dimer 3c was obtained directly from deoxyuridine (1c) in 84% yield. The mixed dimer 3d was prepared in 27% yield by mixing equal amounts of 1a and 1c in methylene chloride with TBAF. In this manner the products 3a and 3d were easily separated by extraction from 3c which is soluble in water. Products 3a and 3d separated cleanly on TLC. The product 3d was fully characterized after detritylation to 3e.

When methylene chloride was replaced by 1,2-dichloroethane, 1,6-dichloro-



1 a R=MMT, X=CH_3
b R=H, X=CH_3
c R=X=H

2 X=CH_3 a R=MMT, Y=F, n=1
b R=H, Y=F, n=1
c R=MMT, Y=Cl, n=2
d R=H, Y=Cl, n=2
e R=MMT, Y=Cl, n=6
f R=H, Y=Br, n=6



3 a R=R'=MMT; X=X'=CH_3; n=1
b R=R'=H; X=X'=CH_3; n=1
c R=R'=X=X'=H; n=1
d R=MMT, R'=H; X=CH_3, X'=H; n=1
e R=R'=H; X=CH_3, X'=H; n=1
f R=MMT; R'=H, X=X'=CH_3; n=2
g R=R'=H; X=X'=CH_3; n=2
h R=MMT, R'=H; X=X'=CH_3; n=6
i R=R'=H; X=X'=CH_3; n=6

hexane or 1,6-dibromohexane dimers were not obtained directly but rather the monomers 2c-f were obtained in good yields. The best yields of dimers 3f-i were obtained by condensing the tritylated chloroalkylthymidines 2c and 2e with thymidine. In this way the dimers 3f and 3h were obtained in 27% and 34% respectively. The methoxytrityl group aids in the separation of the products from TBAF. Thus when 2d was condensed with thymidine only a 21% yield of 3g

Table 2.

High Resolution Measurements on

Alkylated Purines, Pyrimidines and Thymidines.

Compound	Formula	Calculated Mass	Observed Mass
1-methyluracil	$C_5H_6N_2O_2$	126.0429	126.0426
3-methyluracil	$C_5H_6N_2O_2$	126.0429	126.0427
1,3-dimethyluracil	$C_6H_8N_2O_2$	140.0586	140.0582
1-methylcytosine	$C_5H_7N_3O$	125.0589	125.0589
1,3-dimethylcytosine	$C_6H_9N_3O$	139.0746	139.0743
9-methyladenine	$C_6H_7N_5$	149.0700	149.0701
9-benzyladenine	$C_{12}H_{11}N_5$	225.1014	225.1008
N^3-methylthymidine	$C_{11}H_{16}N_2O_5$	256.1059	256.1058
N^3-isopropylthymidine	$C_{13}H_{20}N_2O_5$	284.137	284.137
2b (TCH_2F)X2TMS	$C_{19}H_{34}N_2O_5FSi_2$	445.1991	445.1990
3b (TCH_2T)X4TMS	$C_{33}H_{60}N_4P_{10}Si_4$	784.3387	784.3390
3c ($dUCH_2dU$)X4TMS	$C_{31}H_{56}N_4O_{10}Si_4$	756.307	756.308
2d (TCH_2CH_2Cl)X2TMS	$C_{18}H_{33}N_2O_5Si_2Cl$	448.162	448.161
2e ($T(CH_2)_6Br$)		492	492*
3e (TCH_2dU)X4TMS	$C_{32}H_{58}N_4O_{10}Si_4$	770.323	770.322
3g ($T(CH_2)_2T$)X4TMS	$C_{34}H_{62}N_4O_{10}Si_4$	798.354	798.357
3i ($T(CH_2)_6T$)X4TMS	$C_{38}H_{70}N_4O_{10}Si_4$	854.417	854.418

*low resolution measurement.

was isolated.

The alkylation of mono and dinucleotides was investigated using the above procedures. The yields and product distribution depend heavily on the exact condition used. For example using n-butyl chloride in large excess led to the triester 5a in 20% yield and the diester 6a in 67% yield. Increasing the temperature of the reaction to 85°C produced 5a in 50% yield. n-Butyl bromide gave the triester in 40% yield at 22°C and in 50% yield at 60°C.

The alkylation of dinuclotides of thymidine was also investigated. Compound 7 was converted into the fully alkylated triester 8a in 30% yield using n-butyl bromide at 60°C. A 63% yield of the alkylated diester 9a was also obtained. When this reaction was performed at 22°C only 15% of the triester was formed along with 70% of the diester 9a. The alkylation of 7 was repeated at 22°C using methyl bromide as alkylating agent. In this

Table 3

CMR Chemical Shift* Assignments for N^3-Haloalkyl and Bridged Nucleosides

Carbon	1b	2b	2d	2f	3b	3g	3i	1c	3c	3e
2	150.5†	151.7	152.5	152.3	151.6	152.5	152.2	151.2	151.7	151.8
4	166.1	164.2	165.3	165.4	164.6	165.6	165.3	163.8	164.2	164.7
										164.3
5	111.6	110.7	110.6	110.7	110.4	110.3	110.6	102.2	101.9	110.6
										101.9
6	138.1	137.8	136.9	136.2	136.7	136.5	136.6	141.6	140.9	140.9
		137.4	136.6		136.4					136.7
1'	86.3	87.1	87.1	87.1	86.8	87.4	87.0	88.3	88.9	88.9
										87.0
2'	41.2	41.0	41.3	41.3	41.1	41.3	41.2	74.0	41.3	41.4
										41.2
3'	72.2	71.9	72.0	72.0	71.8	71.8	72.0	70.3	71.9	71.9
4'	88.8	88.7	88.8	88.8	88.5	88.8	88.7	85.2	87.3	88.8
										87.4
5'	62.8	62.6	62.7	62.8	62.6	62.6	62.7	61.3	62.6	62.7
5-CH_3	12.4	12.9	13.1	13.2	13.0	13.1	13.2		46.3≠	46.8≠
1"		84.5	43.3	42.1	40.4	40.0	42.1			
		75.8								
2"			40.7	27.0			27.5			
3"				28.8			28.3			
4"				28.3						
5"				33.8						
6"				34.3						

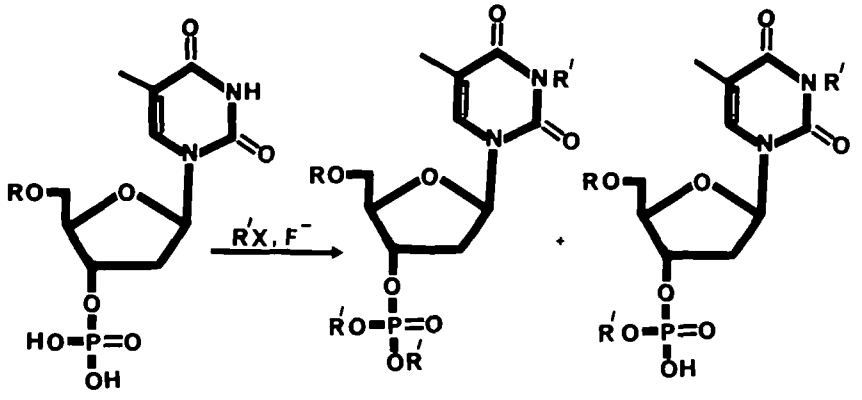
* δ in ppm relative TMS in CD_3OD

† from DMSO d-6

≠ from the fully silylated derivative

experiment the triester 8b was obtained in 55% yield. The diester 9c was obtained in 42% yield.

The structures of the diesters were easily verified by detritylation to 9b and 9d followed by enzymatic degradations. The production of the N^3-



4 a) R=Tr b) R=H

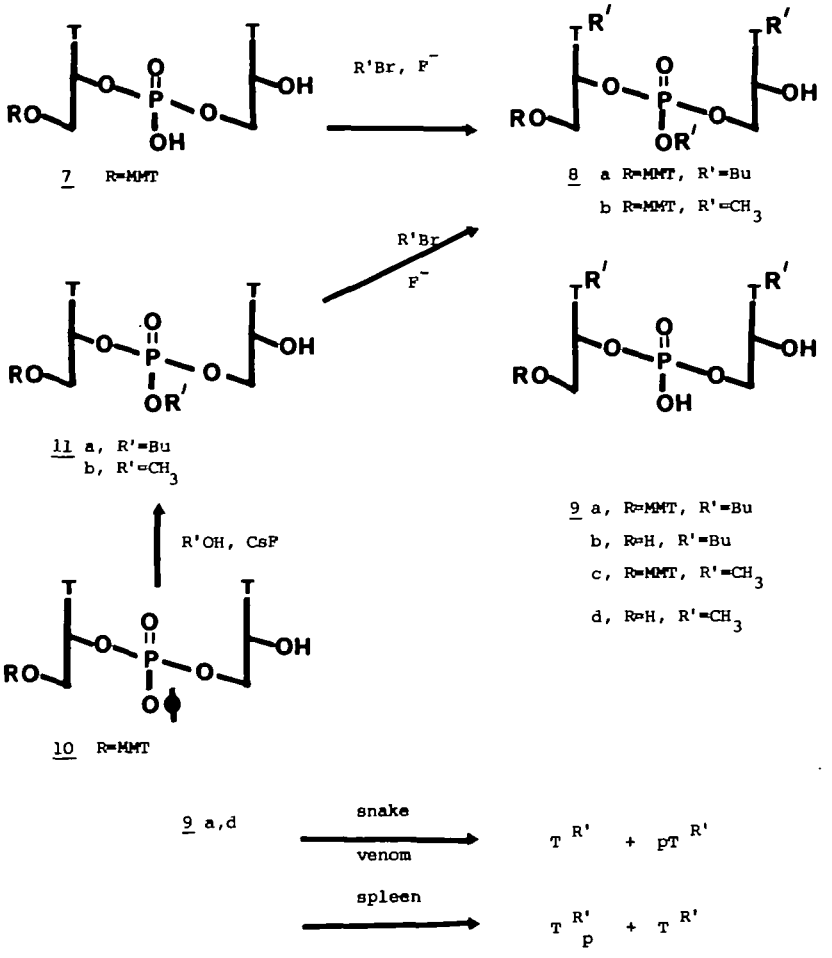
5 R' = nBu

6 R' = nBu

alkylthymidines as the only nucleosidic material from both snake venom and spleen phosphodiesterases completely confirmed the structures. The structures of the triesters 8a and 8b were confirmed by using our previously reported phosphate exchange procedure.[19,20] The phenyl triester 10 is easily converted into the butyl ester 11a or the methyl ester 11b in butanol or methanol with cesium fluoride. Alkylation of 11 with the appropriate alkyl halide produced 8a and 8b identical to those described above.

MATERIALS AND METHODS

Descending paper chromatography was carried out using Whatman 3MM paper. The solvent systems employed were: solvent A, isopropyl alcohol - concentrated ammonium hydroxide - water (7:1:2); solvent B', n-butanol - acetic acid - water (4:1:5, organic phase). The solvents were prepared on a volume basis. Thin-layer chromatography was carried out employing the ascending technique in closed jars which were not coated with absorbent paper. All thin layer chromatography was run on Eastman Chromagram Sheets 13181 on strips 10 cm x 2 cm. Thick-layer chromatography was carried out on glass plates (20 cm x 20 cm) coated with a 1 mm thick layer of silica gel DSF-5 (Terrochem Laboratories). Paper electrophoresis was performed using Whatman 3MM paper in a Savant Flat Plate electrophoretic chamber with a Savant Model HB power supply operated at 2000 V for 1 h; the solution was a triethylammonium bicarbonate buffer (0.05 M, pH 7.5), prepared by making 15.15 g triethylamine up to 3l volume with water and



then bubbling 20 g carbon dioxide through the solution. Nucleosides and their derivatives were detected on paper chromatograms, thin and thick-layer sheets using a u.v. light source (Mineralite, output ∿254 nm). Compounds containing trityl or p-monomethoxytrityl groups were detected on chromatography by spraying with 10% perchloric acid solution and drying them in a stream of warm air. Spectra. All u.v. spectra reported were obtained on a Cary 17 recording spectrophotometer. Mass spectra were obtained on an AEI, MS-9. CMR spectra were recorded on a Bruker WH-90 FT NMR.

TBAF in THF. This reagent has been prepared in several ways which seem to give similar results. We regularly prepare the reagent as follows: After titration

of a dilute (~10%) aqueous solution of tetra-n-butylammonium hydroxide with a dilute (~5%) aqueous solution of HF, the neutral solution is lyophilized. The colorless and hydroscopic solid is shattered to a powder (simply by shaking the flask) and dried further over P_2O_5 for periods up to 24 hr without heating. The dry powder is then dissolved in freshly distilled THF and stored over molecular sieves (Type 4A) in a tightly stoppered plastic bottle as a 0.68 M solution.

General Procedures for the Reaction of Alkyl Halides with Purines and Pyrimidines and Nucleosides

A. Purines and Pyrimidines.

One mmole of purine or pyrimidine was dissolved in THF (10 ml) containing 5 mmole of TBAF. Alkyl halide (3 mmole) was added and the solution was stirred at room temperature and monitored by TLC (chloroform:ethanol (4:1)). Reactions were complete within 1 hr and no change in product distribution could be detected for reaction times up to 20 hr. Solutions were concentrated and applied to TLC plates which were developed in 4:1 chloroform:ethanol (containing 1% NH_4OH). Occasionally rechromatography was necessary to completely eliminate tetrabutylammonium salts.

Products were identified by comparison of their physical properties (mp, uv, NMR) to those reported in the literature[21]. The exact mass of each product was determined by high resolution mass spectrometry and are reported in Table 2.

B. Alkylating Agent as Limiting Reagent.

To 3 mmole of purine or pyrimidine in THF (10 ml) containing 5 mmole of TBAF was added 1 mmole of alkyl halide. The procedure was continued as in A above. Yields are based on the amount of alkylating agent used.

C. Alkyl Halide in Large Excess.

To 1 mmole of purine or pyrimidine in THF (10 ml) containing 5 mmole of TBAF was added 1-5 ml of alkyl halide and the procedure was continued as in A above.

D. Alkyl Halides with Thymidine.

To thymidine (1 mmole) in THF (15 ml) containing 10 mmole of TBAF was added 5 ml of alkyl halide and the resulting solution was stirred for 1-24 hr. Solvents were removed at reduced pressure and the residue was dissolved in chloroform. The chloroform solution was washed with water (3X), concentrated and applied to TLC plates developed in chloroform:methanol (4:1).

The isolated yields of N^3-alkylthymidines were 63%, 55% and 45% when methyl bromide, n-butyl chloride and isopropyl chloride were used respectively.

TLC's had indicated quantitative conversion of thymidine to a single product in each case. The large losses were experienced during the extraction procedures.

All of the products showed λ_{max} (95% EtOH) at 267 nm and the exact masses of parent ions are shown in Table 2.

E. Procedure D was repeated except that the residue from the reaction was dissolved in water (instead of $CHCl_3$) and was passed through a column of Dowex 50W-X8 (Na^+-form). The eluate was concentrated and applied to TLC plates as above. Yields obtained from this procedure were in the 85-90% range.

N^3-Fluoromethylthymidine (2b) and Di(thymidin-N^3-yl)methane (3b)

Compound 1a (0.42 mmole), TBAF (2.1 mmole) in THF (3.1 ml) and methylene chloride (10 ml) were stirred together for 4 hr and worked-up as in procedure D. Compounds 2a (8%, λ_{max} (EtOH) 271 nm, $R_f^{Et_2O}$ 0.57) and 3a (91%, λ_{max} (EtOH) 268 nm, $R_f^{Et_2O}$ 0.10) were obtained. Each product was detritylated with 80% acetic acid (85°C, 30 min) and compounds 2b (λ_{max} (EtOH) 271 nm, R_f^{THF} 0.73, $R_f^{B'}$ 0.76) and 3b (λ_{max} (EtOH) 268 nm, R_f^{THF} 0.39, $R_f^{B'}$ 0.53) were obtained. The structures were assigned on the basis of their exact mass (Table 2) and CMR spectra (Table 3).

Di-(2'-deoxyuridin-N^3-yl)methane (3c)

Deoxyuridine (2.19 mmole) and TBAF (17 mmole) were mixed in a solution of THF (25 ml) and methylene chloride (5 ml) for 24 hr and after evaporation of the solvents the residue was dissolved in pyridine (6 ml) containing acetic anhydride (6 ml). After 3 hr, solvents were removed and the residue was dissolved in chloroform. After the normal extraction procedure and evaporation of chloroform the residue was treated with a solution of concentrated ammonium hydroxide in pyridine (1:1, 25 ml) for 15 hr at 22°C. The products were isolated from TLC plates developed first in EtOAc-THF (1:1) and then twice in $CHCl_3$-CH_3OH (7:3). Compound 3c (429 mg, 84%, λ_{max} (EtOH) 263 nm, R_f^{THF} 0.15, $R_f^{B'}$ 0.32) was obtained and characterized as shown in Tables 2 and 3.

2'-Deoxyuridin-N^3-yl-thymidin-N^3-ylmethane (3e)

Compound 1a (1.1 mmole) and 2'-deoxyuridine (1.1 mmole) were added with TBAF (17 mmole) in THF (25 ml) to methylene chloride (5 ml). The reaction was worked-up as in D. TLC plates were developed first in ether and then in ethyl acetate. Compound 3a (25%) was eluted at this point and the plates were re-developed (twice) in EtOAc-THF (1:1). Compound 3d (27%, λ_{max} (EtOH) 266 nm, R_f^{THF} 0.56) was obtained and after detritylation compound 3e (λ_{max} (EtOH) 265 nm, R_f^{THF} 0.24, $R_f^{B'}$ 0.43) was obtained and characterized further in Tables 2 and 3.

General Preparation of N^3-Haloalkylthymidines 2c-f

The general procedure used was procedure D for the alkylation of thymidines. The yields and general properties are listed below.

Product	Yield	λ_{max}^{EtOH} (nm)	$R_f^{Et_2O}$	R_f^{THF}	$R_f^{B'}$
2c	97	269	0.58		
2d	66	268		0.71	0.80
2e	99	268	0.60		
2f	64	268		0.65	0.88

Further properties of 2d and 2f are recorded in Tables 2 and 3.

Preparation of Dimers 3f and 3g

Compound 2c (143 mg, 0.25 mmole) and thymidine (1.24 mmole) were added to 3.7 ml of TBAF solution (2.48 mmole). The mixture was evaporated to dryness and the residue was dissolved in DMF (1 ml). The resulting solution was stirred at 22°C for 62 hr. After the usual extraction procedure the products were separated on TLC plates developed first in ether, then ethyl acetate and finally ethyl acetate-THF (1:1). The product 3f (λ_{max} (EtOH) 267 nm, R_f^{THF} 0.74) was obtained in 27% yield. On detritylation the dimer 3g (λ_{max} (EtOH) 267 nm, R_f^{THF} 0.40, $R_f^{B'}$ 0.53) was obtained and spectral properties are recorded in Tables 2 and 3.

When this experiment was repeated by condensing 2d and thymidine, a 21% yield of 3g was obtained directly.

Preparation of Dimers 3h and 3i

Compound 2e (274 mg, 0.42 mmole) and thymidine (508 mg, 2.1 mmole) were condensed in 6.2 ml of the TBAF solution (4.2 mmole) for 15 hr. The product was worked-up in the usual manner and compound 3h (119 mg, 34%, λ_{max} (EtOH) 267 nm, R_f^{THF} 0.76) was obtained and yielded compound 3i (λ_{max} (EtOH) 267nm, R_f^{THF} 0.44, $R_f^{B'}$ 0.75) on detritylation (see Tables 2 and 3).

Procedures for the Alkylation of 5'-O-Tritylthymidine 3'-Phosphate (4a) with Butyl Halides

(1) Compound 4a (126 mg, NH_4^+ salt, 0.21 mmole) was suspended in 1-chlorobutane (5 ml). TBAF (2.1 mmole) solution was added and the solution was stirred at 85°C for 24 hr. The solvents were removed at reduced pressure and the residue was dissolved in chloroform (10 ml). After extraction with water, the chloroform layer was concentrated and applied to TLC plates which were developed with ether-hexane (3:1). The triester 5a moved up the plates while the diester 6a remained near the origin. Both products were eluted

and as a result 77 mg of 5a (50%, λ_{max} (EtOH) 267 nm, $R_f^{Et_2O\text{-hexane}(3:1)}$ 0.37, $R_f^{Et_2O}$ 0.66, $R_f^{Et_2O\text{-EtOAc}(1:1)}$ 0.81) and 82 mg of 6a (Bu_4N^+ salt, 43%, λ_{max} (EtOH) 266 nm were obtained.

The products 5a and 6a were further characterized by detritylation to the triester 5b and diester 6b respectively. Compound 5b ($T_p^{Bu}(Bu)_2$) showed λ_{max} (EtOH) at 267 nm and $R_f^{Et_2O\text{-hexane}(3:1)}$ 0.18 , $R_f^{Et_2O}$ 0.26, $R_f^{Et_2O\text{-EtOAc}(1:1)}$ 0.35 and had no electrophoretic mobility. The parent ion in the mass spectrum occurred at m/e = 490. Compound 6b (T_p^{Bu}-Bu) showed λ_{max} (H_2O) 266 nm, λ_{max} (pH1) 266 nm, λ_{max} (pH13) 266 nm; its chromatographic properties were R_f^A 0.85 and $R_f^{B'}$ 0.67 and it had an electrophoretic mobility (E_m^{Tp}) of 0.45 relative to thymidine 3'-phosphate.

(2) The above reaction was repeated at room temperature (22°C) and the yields of 5a and 6a were 20 and 67% respectively.

(3) Reaction (2) above was repeated except that 1-bromobutane was used in place of 1-chlorobutane. Compound 5a was obtained in 40% yield and compound 6a in 36% yield. Interestingly a new compound was obtained from this reaction which was identified as the dibutyl ester of 5'-O-trityl-thymidine 3'-phosphate (Tr-Tp$(Bu)_2$). The yield of this compound was 15%. It was further identified by detritylation to the dibutyl ester of thymidine 3'-phosphate (Tp$(Bu)_2$) for which the parent ion in the mass spectrum occurred at m/e = 434.

This compound was also prepared by our phosphate exchange procedure[19] from the bisphenyl ester of thymidine 3'-phosphate and n-butanol. The product was identical in all respects to the compound Tp$(Bu)_2$. When this compound was subjected to alkylation with 1-chlorobutane and TBAF it was converted in high yield into compound 5b ($T_p^{Bu}(Bu)_2$). This serves as an independent proof of structure of 5b.

(4) Reaction (3) was repeated at 60°C and the yields were 5a (50%), 6a (25%) and 8% of TrTp$(Bu)_2$.

(5) Reaction (1) was repeated except that thymidine 3'-phosphate (4b) was used as starting material. Compound 5b was obtained in 28% yield and compound 6b in 44% yield.

The Alkylation of Thymidine Dinucleotides

(1) The ammonium salt of 5'-O-monomethoxytritylthymidylyl-(3'-5')-thymidine (7, 0.105 mmole) was treated with TBAF solution (1.05 mmole) in 1-bromobutane (2.5 ml) at 60°C for 24 hr. After the normal work-up the triester 8a (λ_{max} (EtOH) 265 nm, R_f^{EtOAc} 0.45, $R_f^{EtOAc:THF}$ 0.69) was obtained in 30%

yield. The diester 9a (λ_{max}(EtOH) 266 nm) was obtained in 63% yield as the Bu_4N^+ salt. Compound 9a was detritylated to the diester 9b (λ_{max} (H_2O) 267 nm, (pH1) 267 nm, (pH13) 268 nm; R_f^A 0.87, $R_f^{B'}$ 0.57, E_m^{Tp} 0.33). This compound (9b) was further characterized by complete enzymatic degradation. Snake venom produced N^3-butylthymidine and N^3-butylthymidine 5'-phosphate while spleen phosphodiesterase produced N^3-butylthymidine 3'-phosphate and N^3-butylthymidine in the correct ratios.

(2) The above reaction was repeated at 22°C yielding 8a (15%) and 9a (70%).

(3) Reaction (2) in this series was repeated except that methyl bromide replaced 1-bromobutane. The triester 8b (λ_{max} (EtOH) 265 nm, R_f^{EtOAc} 0.09, $R_f^{EtOAc:THF(1:1)}$ 0.55) was obtained in 55% yield. The diester 9c was obtained in 42% yield. The structure of the diester was confirmed by treatment with acetic acid to produce 9d (λ_{max}(H_2O) 265 nm, (pH1) 266, (pH13) 266 nm; R_f^A 0.72, $R_f^{B'}$ 0.23, E_m^{Tp} 0.39). On treatment with snake venom phosphodiesterase, 9d was completely degraded to N^3-methylthymidine and N^3-methylthymidine 5'-phosphate. Spleen phosphodiesterase completely degraded 9d to N^3-methylthymidine 3'-phosphate and N^3-methylthymidine.

Proof of Structure of 8a and 8b

The phenyl ester of 5'-O-monomethoxytritylthymidyl-(3'-5')-3'-acetylthymidine was dissolved in dry 1-butanol containing cesium fluoride. The product was isolated and alkylated with 1-chlorobutane and TBAF at 22° for 17 hr. The product was deacetylated to yield a compound indistinguishable from 8a.

The procedure was repeated using dry methanol in place of 1-butanol and a compound identical to 8b was produced in the same manner.

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