ANALOGS OF PYRIMIDINE NUCLEOSIDES.

16.* RACEMIC 2',3'-DIDEOXYNUCLEOSIDES AND THEIR DERIVATIVES

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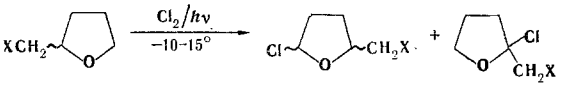
The chlorination of 2-halomethyltetrahydrofurans and acyl derivatives of tetrahydrofuryl alcohol was studied; mixtures of 2,5- and 2,2-disubstituted tetrahydrofurans are formed as a result of the reaction. 2,4-Bis(trimethylsilyl) derivatives of uracil, 5-substituted uracils, and cytosine are alkylated by the resulting mixtures of α-chloro ethers without separation, and mixtures of cis and trans isomers of 1-(5-substituted-2-tetrahydrofuryl) and 1-(2-substituted-2-tetrahydrofuryl) derivatives of uracil, 5-substituted uracils, and cytosine are obtained. The reaction products were identified on the basis of their PMR spectra.

5'-Triphosphates of 2',3'-dideoxynucleosides inhibit the growth of DNA chains [2, 3] and, owing to this, are used as instruments for the determination of the primary structure of DNA [4]. 2',3'-Dideoxynucleosides of pyrimidine and purine bases, particularly their 5'-deoxy-5'-halo derivatives, have antitumorigenic and antivirus properties [5, 6] and also act as coronary dilators [7]. The development of new methods for the synthesis of 2',3'-dideoxynucleosides and their derivatives is therefore an urgent problem.

For its solution we investigated the chlorination of 2-substituted tetrahydrofurans, and we used the resulting α-chloroethers as alkylating agents in reactions with 2,4-bis(trimethylsilyl)uracil, 5-substituted uracils, and cytosine.

Gross [8] and Kratokhvil [9] have shown that the photochemical chlorination of tetrahydrofuran (THF) at −30 to −20°C leads to 2-chlorotetrahydrofuran in 40% yield. We have previously established that the chlorination of 2-substituted tetrahydrofurans with electronegative substituents in the 2 position proceeds with the formation of primarily 2,5-disubstituted tetrahydrofurans [10, 11]. Esters of 5-chlorotetrahydrofuran-2-carboxylic acid [10], 2-chloro-5-trifluoromethyltetrahydrofuran, and 2-chloro-5-perfluoro-tert-butyltetrahydrofuran [11] were obtained, and it was shown that they are more stable than 2-chlorotetrahydrofuran.

In the present research we studied the chlorination of 2-halomethyltetrahydrofurans and acyl derivatives of tetrahydrofuryl alcohol (1a–d).



1 a-d 2 a-d 3 a-d

1-3 a X = Cl; b X = Br; c X = $OCOC_6H_5$; d X = $OSO_2C_6H_4$—CH_3-*p*

The reactions were carried out in a quartz flask with illumination with a PRK-4 lamp in carbon tetrachloride at −15 to −10°C. The reaction mixtures were analyzed by means of PMR spectroscopy; we were unable to use gas–liquid chromatography (GLC) because of the instability of the reaction products under the conditions of chromatographic separation. Decompositon of the reaction products is also observed in the case of fractionation *in vacuo* (10^{-2} mm, mercury column). An analysis of the PMR spectra showed that the principal reaction products are 2,5-disubstituted tetrahydrofurans 2a-d (50-80%), 2,2-disubstituted tetrahydrofurans 3a-d (9-13%) are formed as side products, and the remaining constituent is unchanged starting compound. The ratios of the reaction products in the mixtures were determined from the integral intensi-

*See [1] for Communication 15.

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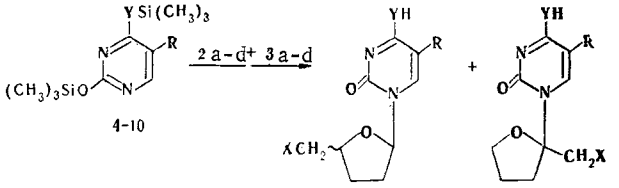
ties of the 2-H protons (6.2 ppm) and the intensities of the signals of the 3-H and 4-H protons at 1.8-2.2 ppm. The greatest amount (13%) of 2,2-disubstituted tetrahydrofuran 3c was observed in the chlorination of 2-benzoxytetrahydrofuran 1c. It should be noted that 2-chloro-5-halomethyltetrahydrofurans 2a,b are less stable than esters of 5-chlorotetrahydrofuran-2-carboxylic acid or 2-chloro-5-trifluoromethyltetrahydrofuran, evidently as a consequence of the less pronounced electron-acceptor properties of the substituent. At the same time, 2,2-disubstituted tetrahydrofurans 3a,b are more stable than the corresponding halo derivatives of tetrahydrofurancarboxylic acid [10]. The chlorination of acyl derivatives of tetrahydrofuryl alcohol proceeds similarly. In the case of benzoyl protection of the hydroxy group of tetrahydrofuryl alcohol 1c the yield in chlorination is higher than in the case of an acetyl protective group. 2-Chloro-5-(p-tolylsulfonylmethyl)tetrahydrofuran is formed in good yield in the chlorination of the tosyl derivative. In view of their instability, the products of the chlorination of 2 and 3 were used without separation for the alkylation of 2,4-bis(trimethylsilyl) derivatives of uracil, 5-substituted uracils, and cytosine (4-10). The reaction takes place at 20-25°C, whereas 2-chlorotetrahydrofuran reacts at a lower temperature (-15°C) [12].



4 Y=O, R=H; 5 Y=O, R=CH_3; 6 Y=O, R=F; 7 Y=O, R=Cl; 8 Y=O, R=Br; 9 Y=O, R=I; 10 Y=NH, R=H; a — cis isomer; б — trans isomer; 11, 25 Y=O, R=H, X=Cl; 12, 26 Y=O, R=CH_3, X=Cl; 13, 27 Y=O, R=F, X=Cl; 14, 28 Y=O, R=X=Cl; 15, 29 Y=O, R=Br, X=Cl; 16, 30 Y=O, R=I, X=Cl; 17, 31 Y=NH, R=H, X=Cl; 18, 32 Y=O, R=H, X=Br; 19, 33 Y=O, R=CH_3, X=Br; 20, 34 Y=O, R=F, X=Br; 21, 35 Y=O, R=Cl, X=Br; 22, 36 Y=O, R=X=Br; 23, 37 Y=O, R=I, X=Br; 24, 38 Y=NH, R=H, X=Br; 39, 45, 51 Y=O, R=H, 39—50 X=$OCOC_6H_5$; 40, 46, 52 Y=O, R=CH_3; 41, 47, 53 Y=O, R=F; 42, 48, 54 Y=O, R=Cl; 43, 49, 55 Y=O, R=Br; 44, 50, 56 Y=NH, R=H; 51—56 X=OH; 57 Y=O, R=F, X=$OSO_2C_6H_4CH_3$-*p*

As a result of the reactions we obtained mixtures of cis and trans isomers of 2,5-disubstituted tetrahydrofurans (11a,b-24a,b and 39a,b-44a,b) and 2,2-disubstituted compounds (25-38 and 45-50). The yields of IIa,b-24a,b and 39a,b-44a,b reached 60% (based on the sums of the isomers), while the yields of 25-38 and 45-50 did not exceed 8%. The mixtures of isomers of the 2,5-disubstituted tetrahydrofurans can be separated from the 2,2-disubstituted tetrahydrofurans by fractional crystallization from ethanol and column chromatography on silica gel. In connection with the fact that 2,2-disubstituted tetrahydrofurans are formed in small amounts, they were identified from the PMR spectra, and an individual compound was isolated in only one case, viz., 1-(2-hydroxymethyl-2-tetrahydrofuryl)-5-methyluracil (see the experimental section). After removal of the benzoyl protective group from 39a,b-44a,b with ammonia in methanol, we obtained mixtures of the cis and trans isomers of 1-(D,L-5-hydroxymethyltetrahydro-2-furyl)uracil, 5-substituted uracils, and cytosine (51a,b-56a,b). The individual racemic 2',3'-dideoxynucleosides (51a-53a) and their trans isomers (51b-53b) (Tables 3 and 4) were obtained by chromatography on plates with silica gel.

Compounds 57a,b are formed in 36% yield in the alkylation of 2,4-bis(trimethylsilyl)-5-fluorouracil with 2-chloro-5-(p-tolylsulfonylmethyl)tetrahydrofuran (Tables 1 and 2); however, these compounds were not subsequently used in view of the difficulty involved in removal of the protective group.

The assignment of the individual isomers of the 2',3'-dideoxynucleosides and their derivatives was made on the basis of a study of the Overhauser nuclear effect (ONE) of the 5'-H protons of the tetrahydrofuran ring during irradiation of the 6-H protons of the pyrimidine ring. The ONE was studied beforehand for ftorafur as a model compound. In the PMR spectrum of this compound the 5'-H protons of the tetrahydrofuran form two multiplets that differ considerably with respect to their chemical shifts (4.19 and 3.77 ppm). When the 6-H proton of the pyrimidine ring was irradiated, a 5% increase in the intensity of only the weak-field mul-

TABLE 1. PMR Spectra of Mixtures of the cis and trans Isomers of 1-(D,L-5-Substituted-2-tetrahydrofuryl)uracils, 5-Substituted Uracils (11a,b-16a,b, 18a,b-23a,b, 39a,b-43a,b, 51a,b-55a,b, and 57a,b), and Cytosine (17a,b, 24a,b, 44a,b, and 56a,b) in d_6-DMSO

Compound	δ, ppm (relative to hexamethyldisiloxane)											trans:cis ratio
	NH(NH₂)	6-H (Ph—H)		5'-H (5-CH₃)		2'-H	3'-H—4'-H	5'-H		6'-H		
		trans	cis	trans	cis			trans	cis	trans	cis	
11a,b	10,6	7,33[a]	7,58[a]	5,65[a]		6,06	2,65—1,82	4,56	4,27	3,56	3,92	1,2 : 1
12a,b	11,3	7,08	7,44	(1,72)		6,08	2,65—1,60	4,59	4,33	3,54	3,83	1 : 1,2
13a,b	11,8		7,87	—		5,97[b]	2,20—1,70		4,16		3,89	0 : 1
14a,b	11,8	7,86	7,95	—		5,94	2,34—1,60	4,61	4,16	3,63	3,92	1 : 1,6
15a,b	11,8	7,93	8,06	—		5,73	2,28—1,67	4,63	4,19	3,63	3,87	1 : 2
16a,b	11,8	7,98	8,05	—		5,94	2,34—1,60	4,61	4,20	3,67	3,90	1 : 2,2
17a,b	(7,29)	7,56	7,60	5,75		5,95	2,30—1,40	4,50	4,07	3,48	3,75	1 : 1,5
18a,b	11,0	7,59[a]	7,63[a]	5,57[a]	5,54[a]	5,96	2,47—1,62	4,51	4,14	3,51	3,67	1 : 1,5
19a,b	11,2	7,47	7,56	(1,71)		5,99	2,39—1,63	4,51	4,24	3,52	3,67	1 : 1
20a,b	11,7	7,87[c]	7,97[c]	—		5,92[b]	2,65—1,67	4,57	4,10	3,53	3,71	1 : 1,2
21a,b	11,9	7,95		—		5,96	2,47—1,59	4,58	4,16	3,49	3,74	1 : 1,1
22a,b	11,8	7,94	8,03	—		5,92	2,38—1,68	4,59	4,03	3,57	3,67	1 : 1,1
23a,b	11,6	7,99	8,06	—		5,91	2,36—1,67	4,60	4,16	3,53	3,73	1 : 1,4
24a,b	(8,38)	7,99[a]		6,10[a]	6,18[a]	5,94	2,38—1,62	4,67	4,28	3,56	3,74	1 : 1,1
39a,b	11,2	(8,04—7,37)		5,54[a]	5,41[a]	6,01	2,42—1,75	4,67		4,25	4,45	1 : 1
40a,b	11,2	(8,07—7,31)		(1,71)		6,02	2,40—1,60	4,67		4,24	4,43	1 : 1,1
41a,b	11,7	(7,94—7,30)		—		5,96	2,30—1,80	4,72		4,28	4,51	1 : 1,4
42a,b	11,4	(8,07—7,40)		—		5,92	2,60—1,84	4,75		4,30	4,47	1 : 1,4
43a,b	11,4	(8,05—7,41)		—		5,96	2,60—1,80	4,76		4,27	4,49	1 : 1,1
44a,b	(9,63) (8,56)	(8,05—7,30)		6,10[a]	5,97[a]	5,97	2,35—1,61	4,76		4,29	4,49	1 : 1,1
51a,b	11,1	7,56[a]	7,90[a]	5,52[a]		5,92	2,30—1,64	4,32	3,96	3,32	3,51	1 : 1,1
52a,b	11,2	7,37	7,72	(1,72)		5,91	2,34—1,60	4,30	3,90	3,29	3,52	1 : 1,7
53a,b	11,7	7,87[c]	8,30[c]	—		5,87	2,71—1,62	4,36	3,95	3,34	3,63	1 : 1,1
54a,b	11,7	7,90	8,50	—		5,85	2,29—1,64	4,36	4,00	3,31	3,56	1 : 15
55a,b	11,7	7,91	8,53	—		5,85	2,20—1,60	4,38	4,03	3,38	3,54	1 : 7
56a,b	(8,35) (7,86)	7,72[a]	8,10[a]	5,87[a]		5,87	2,29—1,62	4,01	3,72	3,38 4,28	3,56 4,51	1 : 3,3 1 : 1,4
57a,b	11,7	(7,99—7,28)		—		5,97	2,30—1,80	4,72				

[a]In this case $^3J_{6\text{-H}-5\text{-H}}$ = 8.0 Hz. [b]In this case $^5J_{2'\text{-H}-\text{F}}$ = 1.8 Hz. [c]In this case $^3J_{6\text{-H}-\text{F}}$ = 7.1 Hz.

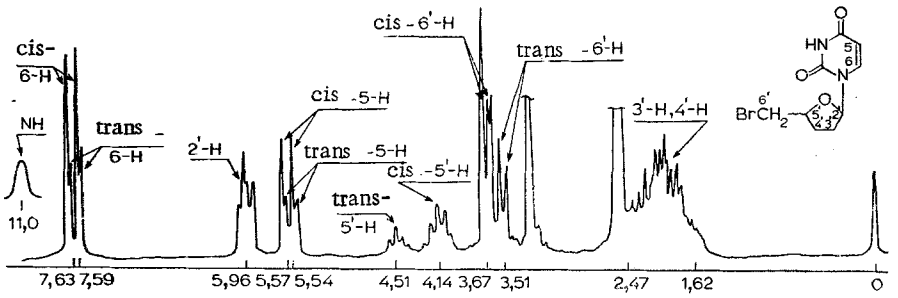


Fig. 1. PMR spectrum of a mixture of the cis and trans isomers of 1-(D,L-5-bromomethyl-2-tetrahydrofuryl)uracil (18a,b) (d_6-DMSO, hexamethyldisiloxane as the standard).

tiplet of the 5'-H protons was observed in the PMR spectrum, whereas the intensity of the strong-field multiplet remained unchanged. Consequently, the absorption at 4.19 ppm corresponds to a cis 5'-H proton, and the absorption at 3.77 ppm corresponds to a trans 5'-H proton relative to the ring of the pyrimidine base. This result is in agreement with the literature data, for which the assignment of the 5'-H protons of ftorafur was accomplished on the basis of an analysis of the spin-spin coupling constants (SSCC) in the PMR spectra recorded at 360 MHz [13]. Similarly, when the 6-H proton of the pyrimidine ring of the cis isomer of 3'-deoxythymidine (52a) was irradiated, no change in the intensity of the resonance of the 5'-H proton of the tetrahydrofuran ring was observed, whereas, as in the case of trans isomer 52b, we noted an 8% increase in the intensity of the absorption of the 5'-H proton. Consequently, the difference in the chemical shifts of the 5'-H proton can be used as a criterion in the assignment of the 2',3'-dideoxynucleosides and their derivatives to the β or α series. In the spectra of

TABLE 2. Physicochemical Properties of 1-(D,L-5-Substituted-2-tetrahydrofuryl)uracils, 5-Substituted Uracils (11a,b-16a,b, 18a,b-23a,b, 39a,b-43a,b, 51a,b-55a,b, and 57a,b), and Cytosines (17a,b, 24a,b, 44a,b, and 56a,b)

Compound	UV spectra, λ_{max}, nm ($\varepsilon \cdot 10^{-3}$)			IR spectra νOH, cm^{-1}	R_f[a]	Found, %			Empirical formula	Calc., %			Yield, %
	pH 2	pH 7	pH 12			C	H	N		C	H	N	
11a,b	264 (9,2)	262 (8,9)	264 (7,0)	—	0,79	47,1	4,6	12,4	$C_9H_{11}ClN_2O_3$	46,9	4,8	12,1	40,0
12a,b	268 (10,0)	267 (9,8)	270 (6,5)	—	0,81	48,9	4,9	11,1	$C_{10}H_{13}ClN_2O_3$	49,1	5,4	11,4	39,0
13a,b	270 (9,8)	269 (8,0)	270 (7,0)	—	0,80	44,0	4,1	11,6	$C_9H_{10}ClFN_2O_3$	43,5	4,1	11,3	25,0
14a,b	276 (9,3)	278 (7,2)	279 (6,2)	—	0,77	40,6	3,9	10,5	$C_9H_{10}Cl_2N_2O_3$	40,9	3,8	10,6	18,5
15a,b	280 (9,0)	280 (7,8)	280 (5,8)	—	0,72	34,6	3,1	9,5	$C_9H_{10}BrClN_2O_3$	34,9	3,3	9,0	6,5
16a,b	288 (6,7)	288 (6,3)	283 (5,0)	—	0,80	30,4	2,7	8,3	$C_9H_{10}ClJN_2O_3$	30,3	2,8	7,9	5,0
17a,b	282 (12,9)	272 (11,6)	273 (8,7)	—	0,48[b]	40,1	4,9	15,6	$C_9H_{12}ClN_3O_2 \cdot HCl$	40,8	4,9	15,8	12,0
18a,b	264 (9,9)	264 (9,8)	264 (7,8)	—	0,88	39,9	4,1	9,8	$C_9H_{11}BrN_2O_3$	39,3	4,0	10,2	39,0
19a,b	269 (9,3)	269 (9,1)	269 (6,9)	—	0,92	41,2	4,6	9,4	$C_{10}H_{13}BrN_2O_3$	41,5	4,5	9,7	47,0
20a,b	268 (9,0)	268 (8,5)	268 (7,8)	—	0,91	36,5	3,5	9,8	$C_9H_{10}BrFN_2O_3$	36,9	3,4	9,5	28,0
21a,b	279 (9,6)	279 (9,0)	279 (6,6)	—	0,89	34,9	3,2	9,3	$C_9H_{10}BrClN_2O_3$	34,9	3,3	9,0	22,0
22a,b	280 (8,2)	280 (8,0)	280 (5,7)	—	0,92	30,0	2,8	7,3	$C_9H_{10}Br_2N_2O_3$	30,5	2,9	7,9	18,0
23a,b	290 (6,2)	290 (6,0)	290 (4,6)	—	0,94	27,3	2,6	7,0	$C_9H_{10}BrJN_2O_3$	27,0	2,5	7,0	11,0
24a,b	280 (18,4)	274 (10,0)	274 (9,4)	—	0,44[b]	35,1	4,7	13,6	$C_9H_{12}BrN_3O_2 \cdot HCl$	34,8	4,2	13,5	6,5
39a,b	265 (9,5)	265 (10,1)	260 (7,7)	—	0,57	59,3	5,1	9,2	$C_{16}H_{16}N_2O_5$	60,8	5,1	8,9	41,0
40a,b	272 (8,8)	271 (9,2)	269 (7,7)	—	0,84	60,2	5,4	8,8	$C_{17}H_{18}N_2O_5$	61,8	5,5	8,5	40,0
41a,b	272 (8,9)	271 (9,0)	269 (7,3)	—	0,68	57,2	4,3	8,7	$C_{16}H_{15}FN_2O_5$	57,5	4,5	8,4	35,5
42a,b	278 (8,2)	279 (8,3)	275 (6,2)	—	0,82	54,5	4,4	8,4	$C_{16}H_{15}ClN_2O_5$	54,8	4,3	8,0	21,0
43a,b	280 (7,9)	281 (8,3)	277 (6,8)	—	0,78	49,5	4,2	7,4	$C_{16}H_{15}BrN_2O_5$	48,6	3,8	7,1	10,0
44a,b	282 (12,1)	275 (9,8)	271 (8,9)	—	0,56[b]	55,6	5,5	12,3	$C_{16}H_{17}N_3O_4 \cdot HCl$	54,8	4,9	12,0	26,5[c]
51a,b	263 (10,7)	261 (10,9)	263 (8,4)	3320	0,21; 0,16	51,1	6,0	12,2	$C_9H_{12}N_2O_4$	50,9	5,7	13,2	23,0[c]
52a,b	266 (10,2)	265 (9,8)	268 (8,2)	3380	0,26; 0,20	53,8	6,7	12,5	$C_{10}H_{14}N_2O_4$	53,1	6,2	12,4	21,7[c]
53a,b	272 (8,5)	273 (8,8)	271 (7,4)	3470	0,23; 0,28	46,6	4,7	11,8	$C_9H_{11}FN_2O_4$	47,0	4,8	12,2	21,0[c]
54a,b	280 (8,5)	280 (8,4)	278 (7,1)	3390	0,20	43,2	4,3	10,9	$C_9H_{11}ClN_2O_4$	43,8	4,5	11,3	10,0[c]
55a,b	283 (8,5)	282 (8,7)	280 (6,4)	3380	0,23	36,6	3,5	9,8	$C_9H_{11}BrN_2O_4$	37,1	3,8	9,6	6,6[c]
56a,b	282 (13,1)	272 (8,8)	272 (8,9)	3310	0,15[b]	44,2	5,0	17,3	$C_9H_{13}N_3O_3 \cdot HCl$	43,6	5,7	17,0	10,0[c]
57a,b	271 (7,9)	270 (8,1)	279 (7,3)		0,71	48,8	3,8	7,7	$C_{16}H_{17}FN_2O_6$	50,0	4,4	7,3	5,1[c]

[a]Chloroform–ethanol (9:1). [b]Ethyl acetate–acetone–ammonium hydroxide–ethanol (20:10:1.5:7). [c]The overall yield of two steps based on the starting compound (4-10).

TABLE 3. PMR Spectra of the cis and trans Isomers of 1-(D,L-5-Hydroxymethyl-2-tetrahydrofuryl)uracil and 5-Substituted Uracils (51a-53a, 51b-53b) in D_2O (δ, ppm, relative to DSS)

Compound	Chemical shift						
	NH	6-H	5-H (5-CH_3)	2'-H	3'-H—4'-H	5'-H	6'-H
51a	11,1	7,89[a]	5,85[a]	6,08	2,60—1,65	4,22	3,75
51b	11,0	7,74[a]	5,85[a]	6,10	2,60—1,75	4,66	3,65
52a	11,2	7,76	(1,90)	6,13	2,56—1,80	4,25	3,83
52b	11,1	7,51	(1,90)	6,15	2,63—1,75	4,60	3,62
53a	11,7	8,10[b]	—	6,06	2,65—1,60	4,21	3,82
53b	11,7	7,90[b]	—	6,08	2,67—1,70	4,54	3,62

[a]In this case $^3J_{6-H-5-H}$ = 8.0 Hz. [b]In this case $^3J_{F-6-H}$ = 7.1 Hz.

the trans isomers the signal of the 5'-H proton is shifted 0.3-0.5 ppm to weak field as compared with the cis isomers. The resonances of the 6'-H protons of the substituents in the tetrahydrofuran ring for the cis isomers are found at weaker field as compared with the signals of these protons in the spectra of the trans isomers, probably because of the anisotropic effect of the pyrimidine ring. The signal of the 6-H protons in the cis isomers of the compounds obtained is found at weaker field as compared with the trans isomers, as in the case of other nucleosides [14] (Figs. 1 and 2).

It should be noted that the certain increase in the ONE for the 2'-H proton in the case of irradiation of the 6-H proton on passing from 2',3'-dideoxyuridine and 3'-deoxythymidine (4%) to ftorafur (8%) probably indicates an increase in the syn conformation relative to the glycoside bond in the latter. The fact that the resonance of the NH proton is found at weak field (∿11 ppm) constitutes evidence that the tetrahydrofuran ring added to the N_1 atom.

The PMR spectrum of the cis isomer of 1-(D,L-5-hydroxymethyl-2-tetrahydrofuryl)-5-methyluracil (59a) coincides completely with the PMR spectrum of 3'-deoxythymidine obtained from thymidine.

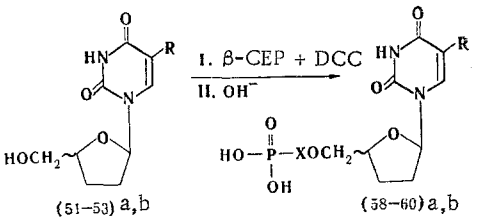
A broad AB quartet corresponding to the signals of the protons of the substituent in the tetrahydrofuran ring is characteristic for the PMR spectra of 2,2-disubstituted tetrahydrofurans (Fig. 3 and the experimental section). In the case of 1-(2-hydroxymethyl-2-tetrahydrofuryl)-5-methyluracil the signals of the 5'-H protons and the protons of the substituent in the tetrahydrofuran ring merge to form a broad multiplet at 3.4-4.0 ppm (Fig. 3b).

The UV spectra of the compounds obtained are characteristic for 1-substituted uracils; the IR spectra of 51a,b-56a,b contain a ν_{OH} band at 3450-3500 cm^{-1}, which is absent in the spectra of all of the 5'-deoxy-5'-substituted 2',3'-dideoxynucleosides.

Thus we have found a convenient method for the synthesis of racemic 2',3'-dideoxynucleosides and their 5'-deoxy-5'-halo derivatives, as well as the corresponding α anomers.

Isomeric 1-(D,L-5-hydroxy-2-tetrahydrofuryl)uracil 5'-monophosphates were synthesized by phosphorylation of a mixture of the cis and trans isomers.

β-Cyanoethyl phosphate (CEP) in the presence of dicyclohexylcarbodiimide (DCC) [15] and a mixture of phosphorus oxychloride and trimethyl phosphate [16, 17] were used as the phosphorylating agents. In the first case the alcohol—β-CEP—DCC ratio was 1:2:5. The preparative isolation of the reaction products was realized by means of ion-exchange chromatography on



58 R=H; 59 R=CH_3; 60 R=F; 58—60; a — cis isomer ; b — trans isomer

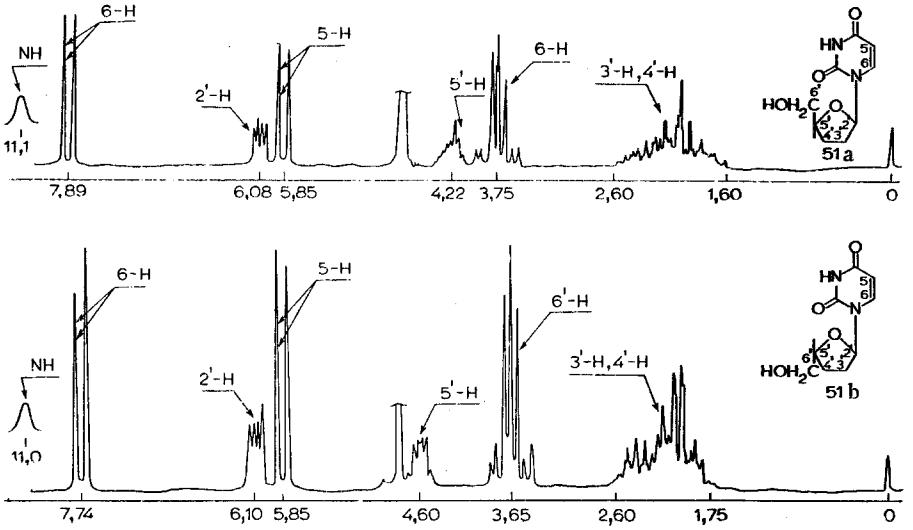


Fig. 2. PMR spectra of the cis and trans isomers (51a,b) of 1-(D,L-5-hydroxymethyl-2-tetrahydrofuryl)uracil (D_2O, DSS as the standard).

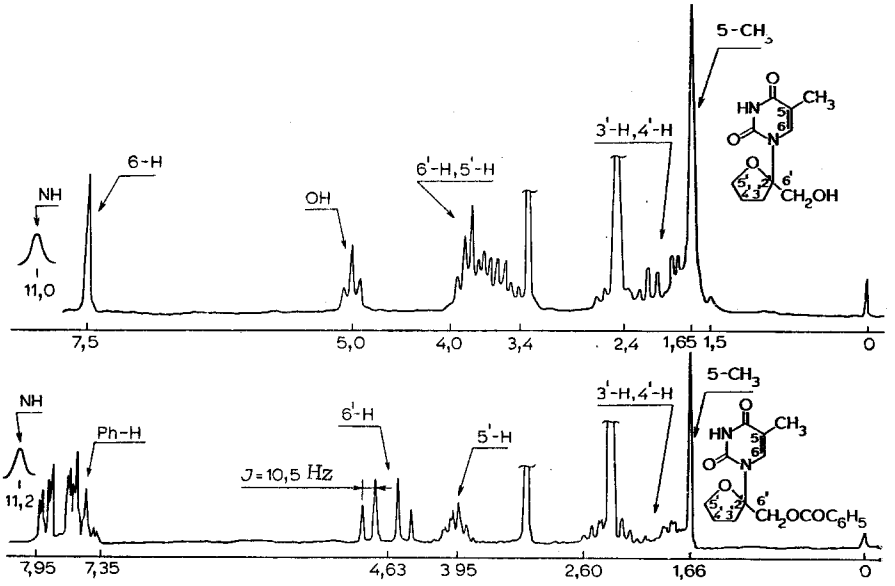


Fig. 3. PMR spectra of 1-(2-benzoxymethyl-2-tetrahydrofuryl)-5-methyluracil (a) and 1-(2-hydroxymethyl-2-tetrahydrofuryl)-5-methyluracil (b) (d_6-DMSO).

QAE-Sephadex A-25 by elution with an $(NH_4)_2CO_3$ stepwise gradient buffer. The unchanged starting compound was eluted with a 0.1 N buffer solution, while the phosphates were eluted with 0.3-0.5 N $(NH_4)_2CO_3$ solutions. Phosphates 58a,b-60a,b were obtained in ∿50% yields and were isolated in the form of ammonium salts. Although it did not increase the yields, the use of a mixture of phosphorus oxychloride and trimethyl phosphate as the phosphorylating agent simplified the reaction in a preparative respect.

To determine the antitumorigenic activity we used mixtures of the cis and trans isomers of 1-(5-halomethyl-2-tetrahydrofuryl)-5-fluorouracil in a ratio close to 1:1 (13a,b, 20a,b). The compounds were injected intraperitoneally over a wide range of doses into mice with lympholeucosis L 1210 and adenocarcinoma AC 755, the dose-effect curve was constructed, and the antitumorigenic activity at the optimum dose was determined. Since 5-fluorouracil derivatives (13a,b, 20a,b) did not significnatly increase the life span of the animals and in view of the difficulty involved in preparative separation, the individual isomers were not investigated.

The authors thank T. N. Shubina for providing us with a sample of 3'-deoxythymidine.

EXPERIMENTAL

The purity of the substances was monitored by thin-layer chromatography (TLC) on Silufol UV-254 and by elemental analysis. Preparative separation of the isomers was accomplished by means of thin-layer chromatography (TLC) on Merck PSC-Fertidplatten F-254 plates. Monitoring of the separation during column chromatography was accomplished by means of a Uvikord II flow absorptiometer connected to a recorder and an Ultrarak fraction collector (LKB, Sweden). The buffer was fed into the column with a Varioperpex pump. The PMR spectra were recorded with a Bruker WH/DS 90 spectrometer (West Germany) with d_6-DMSO as the solvent and hexamethyldisiloxane (HMDS) as the internal standard, with D_2O as the solvent and DSS as the internal standard, and with $CDCl_3$ as the solvent and HMDS as the internal standard. The IR spectra were obtained with a UR-20 spectrometer (East Germany). The UV spectra were recorded with a Unicam SP 1800 spectrophotometer (England). The melting points were determined with a Boëtius microblock (East Germany). Electrophoresis was carried out on FN-12 paper in a phosphate buffer (pH 7.5) at 900 eV for 2 h. The electrophoretic mobilities (E_f) were determined relative to the mobility of 2'-uridine monophosphate. 5-Halomethyltetrahydrofurans Ia,b were obtained by the methods described in [18, 19].

General Method for the Chlorination of **1a-d.** A solution of the starting compound in 100 ml of dry CCl_4 was placed in a quartz flask, the flask was cooled to −15°C, and a mixture of dry nitrogen and 0.12 mole of chlorine was introduced into the mixture with stirring and irradiation with a PRK-4 mercury lamp (400 W at a distance of 30-40 cm from the flask) while maintaining the temperature at −15 to −10°C. Irradiation was continued for another 1 h with bubbling of nitrogen into the reaction mixture to remove the hydrogen chloride, after which NaA molecular sieves were added, and the mixture was stirred for 30 min and filtered. The reaction mixture was concentrated *in vacuo* to half its original volume, and the concentrate was used for the subsequent reaction. The yields of chlorination products were determined on the basis of the PMR spectra of the reaction mixtures.

1-(D,L-5-Bromomethyl-2-tetrahydrofuryl)uracil (18a,b). A mixture of 6.75 g (0.055 mole) of uracil, 40 ml of hexamethyldisilazane, and 2.0 ml of trimethylchlorosilane was heated at 150-170°C until the uracil dissolved completely, after which it was heated for another 2 h. The excess hexamethyldisilazane was removed by vacuum distillation, 10 ml of dry acetonitrile was added to the residue, and a 9.1-g (0.055 mole) sample of the mixture of ethers 2b and 3b (8.5% ether 3b was present in the mixture) was added slowly dropwise. The mixture was stirred at 20°C for 4 h, and 10 ml of ethanol was added at such a rate that the temperature did not exceed 40°C. Stirring was continued at 20°C for another hour, and the resulting precipitate was separated and extracted with chloroform. The chloroform-insoluble part of the precipitate (1.2 g) was unchanged uracil. The chloroform solution was evaporated, and the residue was combined with the mother liquor and purified with a column filled with silica gel [elution with ethanol—chloroform (1:9)]. The separation was monitored by means of a flow absorptiometer. The product was recrystallized from ethanol to give 5.3 g (31%) of 18a,b (a mixture of the cis and trans isomers).

Synthesis of 11-17 and 19-24. These compounds were synthesized in the form of mixtures of cis and trans isomers, which were not separated. The PMR spectra are given in Table 1.

1-(D-L-5-Hydroxymethyl-2-tetrahydrofuryl)-5-methyluracil (52a,b) and 1-(2-Hydroxymethyl-2-tetrahydrofuryl)-5-methyluracil. A 15-ml sample of dry acetonitrile was added, as described above, to 2,4-bis(trimethylsilyl)-5-methyluracil, obtained from 6.27 g (0.055 mole) of 5-methyluracil, 30 ml of hexamethyldisilazane, and 2.0 ml of trimethylchlorosilane, and 13.3 g (0.055 mole) of a mixture of ethers 2c and 3c (containing 13% ether 3c) was added slowly dropwise with stirring. The mixture was stirred at room temperature for 4 h, after which 15 ml of ethanol was added, and stirring was continued for another hour. The resulting precipitate was separated and extracted with chloroform. The chloroform-insoluble part of the precipitate was unchanged 5-methyluracil (1.85 g). The chloroform solution was evaporated, and the residue was combined with the mother liquor and purified with a column filled with silica gel [elution with chloroform—ethanol (9:1)]. The separation was monitored by means of a flow absorptiometer. Recrystallization of the product from ethanol gave 0.7 g (4%) of 46 and 4.9 g (29%) of 40a,b.

A 4.9-g sample of 40a,b was suspended in 100 ml of dry methanol, and the suspension was saturated with dry ammonia at 0°C and allowed to stand in a refrigerator for 3 days. The meth-

TABLE 4. 1-(D-L-5-Hydroxymethyl-2-tetrahydrofuryl)uracil and 5-Substituted Uracils (51a-53a, 51b-53b)

Compound	R	mp, °C	R_f		UV spectra (in H_2O), λ_{max}, nm	Yield, %
			A[a]	B[b]		
51a	H	173—174	0,21	0,25	265 (6,5)	13,6
51b	H	120—121	0,16	0,20	262 (10,3)	9,1
52a	CH_3	187—189	0,26	0,44	264 (9,2)	13,5
52b	CH_3	133—135	0,20	0,30	266 (11,3)	8,2
53a	F	144—146	0,28	0,35	273 (6,6)	11,0
53b	F	128—130	0,23	0,28	271 (9,0)	10,0

[a]System A: acetone—hexane (1:1). [b]System B: chloroform—ethanol (9:1).

anol was evaporated to dryness, 60 ml of a mixture of chloroform and water (1:1) was added to the residue, the mixture was shaken until the solid material dissolved completely, and the layers were separated. The aqueous layer was evaporated, 20 ml of absolute ethanol was added to the oily residue, and the mixture was again evaporated to dryness. The residue was recrystallized from 20 ml of absolute ethanol. Cooling of the solution precipitated 2.05 g of 52a,b. Evaporation of the filtrate gave an additional 0.3 g of substance (according to the PMR data, the precipitate was a mixture of the cis and trans isomers in a ratio close to 1:1). The overall yield of the isomers was 20.6% (based on 5-methyluracil).

The pure cis and trans isomers were obtained by means of preparative TLC on silica gel (Tables 3 and 4).

A 0.68-g sample of 46 was suspended in 30 ml of dry methanol, and the suspension was saturated with dry ammonia at 0°C and then worked up as described above to give 0.32 g (2.8% based on 5-methyluracil) of 1-(2-hydroxymethyl-2-tetrahydrofuryl)-5-methyluracil with mp 177-179°C and R_f 0.44 (system B). UV spectrum, λ_{max}: (pH 2) 266 (9500); (pH 7) 269 (8500); (pH 12) 271 nm (7800). PMR spectrum (in d_6-DMSO): 1.55-2.4 (m, 4, 3-H, 4-H), 3.4-4.0 (m, 4, 5-H, 6-H), 5.0 (t, 1, OH), 7.5 (s, 1, 6-H), 11.0 (s, 1, NH) (Fig. 3b).

Synthesis of 51a,b and 53a,b-56a,b. These compounds were synthesized in the form of mixtures of the isomers, and their separation was carried out similarly. The characteristics of the compounds are given in Tables 2-4.

1-(D,L-5-Hydroxymethyl-2-tetrahydrofuryl)-5-methyluracil 5'-Phosphates (59a,b). A 0.54-g sample of the barium salt of β-cyanoethyl phosphate was dissolved in 50 ml of water in the presence of 2 ml of Dowex-50 (H^+), and the mixture was passed through a column filled with Dowex-50 (H^+) with collection of the acidic solution, which was treated with 20 ml of pyridine and evaporated *in vacuo*. The residue was dried with absolute pyridine (two 15-ml portions), and the oily residue was treated with 0.29 g (1.3 mmole, 13,000 optical units) of 52a,b (a mixture of the cis and trans isomers in a ratio of 1:1). The solution was dried with absolute pyridine (two 15-ml portions), 10 ml of absolute pyridine and a solution of 1.34 g (6.5 mmole) of dicyclohexylcarbodiimide in 10 ml of absolute pyridine were added to the residue. The reaction mixture was shaken vigorously for 30 min, after which it was maintained at 20°C for 24 h. Water (10 ml) was added, and the mixture was allowed to stand at 20°C for 24 h. The precipitated dicyclohexylurea was separated and washed with pyridine. The filtrate was treated with 36 ml of 9 N ammonium hydroxide, and the mixture was heated at 100°C for 3 h. Evaporation of the solution *in vacuo* gave a yellowish oil and a small amount of dicyclohexylurea. Water (15 ml) was added, and the mixture was filtered and evaporated with water and ammonia to remove traces of pyridine. The residue was dissolved in water, and the aqueous solution was applied to a column filled with AGN activated charcoal. The phosphoric acid was eluted with water until the eluate was neutral, and elution was continued with 50% aqueous ethanol containing 5% ammonium hydroxide. A total of 200 ml of eluate (10,000 optical units) was collected and evaporated to dryness at 30°C. The residue was dissolved in water, and the volume of the solution was increased to the point at which the concentration of phosphates did not exceed 0.01 N (18 ml). The solution was applied to a column containing 50 ml of QAE-Sephadex A-25 and eluted with an ammonium carbonate stepwise gradient (from 0.01 to 1 N); the separation was monitored by means of a flow absorptiometer. The unchanged alcohol 52a,b was eluted with 0.01-0.1 N buffer, and phosphates 59a,b were eluted with a 0.3-0.5 N buffer (6300 optical units). The R_f value of 52a,b is 0.72 and the R_f value of phosphates 59a,b is

0.45 on silica gel plates in system C [isobutyric acid–aqueous ammonia–water (66:1.5:33)]. The yield of phosphates 59a,b was 50%. The eluate containing the phosphates was evaporated to dryness, water was added to the dry residue, and the mixture was again evaporated (five 20-ml portions) to dryness for complete removal of the ammonium carbonate. Evaporation of the water left a white crystalline substance (0.22 g), which was identified as a mixture of isomeric phosphates 59a,b in the form of ammonium salts with E_f 0.93. The yield was 50%.

1-(D,L-5-Hydroxymethyl-2-tetrahydrofuryl)uracil 5'-Phosphates (58a,b). A 0.38-g (1.8 mmole) sample of alcohol 51a,b (containing 90% of the cis isomer) was added to a cooled mixture of 0.66 g [0.45 ml (3.6 mmole)] of phosphorus oxychloride and 2.5 g [2.1 ml (18 mmole)] of trimethyl phosphate, and the mixture was allowed to stand at 0°C for 24 h. Ice water (2 ml) was added to the reaction mixture, and the mixture was allowed to stand at 4°C for 2 h. It was then treated with 8 ml of water, and the solution was applied to a column filled with AGN activated charcoal. Workup as described above gave 0.28 g (49%) of a mixture of isomeric phosphates 58a,b in the form of ammonium salts with R_f 0.37 (system C) and E_f 1.04. The R_f value for 51a,b was 0.78.

1-(D,L-5-Hydroxymethyl-2-tetrahydrofuryl)-5-fluorouracil 5'-Phosphates (60a,b). A 0.85-g (3.7 mmole) sample of alcohol 53a,b (a mixture of the cis and trans isomers in a ratio of 1:1.1) was added to a cooled mixture of 1.13 g [0.78 ml (7.4 mmole)] of phosphorus oxychloride and 5.2 g [4.35 ml (37 mmole)] of trimethyl phosphate, and the mixture was then worked up by the method described above to give a mixture of isomeric phosphates 60a,b in the form of ammonium salts [0.57 g (45%)] with R_f 0.41 (system C) and E_f 0.87. The R_f value of 53a,b was 0.68.

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