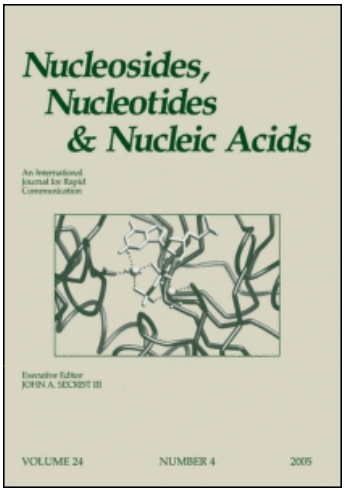

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Efficient Preparation of Cyclic 3',5'-Phosphoramidites and -Amidates of Antiviral and Antitumor 5-X-2'-Deoxyuridines (X = H, CH_3, I, F, CF_3, trans-CH=CHBr)

Wesley G. Bentrude[a]; Mamunur R. Khan[a]; M. R. Saadein[a]; Alan E. Sopchik[a]
[a] Department of Chemistry, University of Utah, Salt Lake City, Utah

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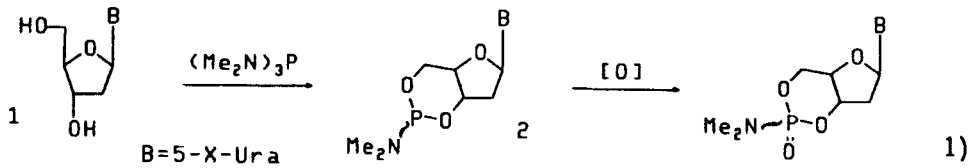
EFFICIENT PREPARATION OF CYCLIC 3',5'-PHOSPHORAMIDITES AND -AMIDATES OF ANTIVIRAL AND ANTITUMOR 5-X-2'-DEOXYURIDINES (X = H, CH_3, I, F, CF_3, trans-CH=CHBr).

Wesley G. Bentrude*, Mamunur R. Khan, M.R. Saadein,[1] and Alan E. Sopchik
Department of Chemistry, University of Utah
Salt Lake City, Utah 84112

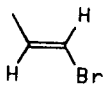
Abstract: Direct cyclization of the title nucleosides with $(Me_2N)_3P$ followed by oxidation with N_2O_4 or t-BuOOH affords the individual cyclic 3',5'-phosphoramidate diastereomers shown to be isolable in 45-77% yields.

Neutral derivatives of nucleoside cyclic 3',5'-monophosphates have proven to be valuable in studies of enzyme active site mapping,[2] as mimics or antagonists of cAMP or cGMP,[3] as possible antitumor or antiviral prodrugs,[4] and in investigations of the energetics of the chair-twist equilibrium available to the six-membered phosphate ring.[5] Nucleoside cyclic phosphoramidates ($P(O)NR_2$) have been of considerable interest in this regard and in addition, as the anilidates (P(O)NHPh), are very useful precursors of phosphorothioates and cyclic phosphate diesters which are chiral by virtue of isotopic oxygen substitution on phosphorus.[6] A number of preparations of nucleoside cyclic 3',5'-phosphoramidates have been reported.[6,7]

The potentially most straightforward access to the cyclic phosphoramidates of 2'-deoxyribonucleosides is by the way of reaction 1:



1)

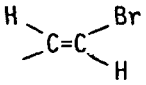
1a X=H 1b X=Me 1c X=I 1d X=F 1e $X=CF_3$ 1f X= 

The phosphoramidite, **2**, thus formed is an extremely valuable intermediate as has been demonstrated for thymidine (**Scheme**). Thus, phosphoramidite **2b** has been shown previously to be converted nearly quantitatively to the corresponding phenyl[5a] or methyl phosphite (**4**)[8] from which cyclic phosphate triesters (**5**[8] and **10**[5a]), methylphosphonates (**6**),[9] phosphorothioates (**7**),[10] and ^{17}O- and ^{18}O-labeled cyclic diesters (**8**)[11] can be readily prepared in high yields. Furthermore, **4** is obtained as a mixture of diastereomers which results in the formation of useful amounts of both diastereomers of products **5-9**.

Unfortunately, up to now the yields of phosphoramidite **2b** have never been reliably above 10-20%[5a,8,12] which has precluded the application of these methods to more costly 2'-deoxyribonucleosides. We report here the preparation of the cyclic phosphoramidites of a series of 5-substituted 2'-deoxyribonucleosides (**1a-1f**) in good yields from 0.5-1.0 g amounts of nucleoside as evidenced by the ^{31}P NMR spectra of the crude phosphoramidites (**2**). After oxidation, HPLC separation applied to a portion of the crude product gave the corresponding phosphoramidates (**3**) in 45-77% yields based on starting nucleoside. Useful amounts of the cis (relationship of Me_2N and base) as well as the predominant trans diastereomer were formed (See **Table 1**). It should be emphasized, however, that the trans phosphoramidite can be converted to the methyl phosphite (e.g. **4**) with either the cis or trans isomer in excess and thus can yield cis phosphoramidate predominantly (**4**→**9**).[7h,i] Thus, formation of cis-**2** is only important when the cis phosphoramidate is required to be prepared directly (**1** → **2** → **3**). Nucleosides **1c-1f** are all in clinical use or in various stages of testing as antivirals or antitumor agents.[13] Reaction 1 and those of the **Scheme** provide an excellent approach, therefore, to potential prodrug forms of **1c-1f**.[14]

The crucial aspects of the experimental procedure that ensure high yields are the use of carefully purified acetonitrile as solvent, thorough deoxygenation of the reaction, slow increase of the temperature of the reaction, and its control below 65 °C. The cyclization with 2'-deoxyuridine is sluggish unless tetrazole is added to catalyze the process. A greater percentage of cis phosphoramidate for direct oxidation to cis-**3** can usually be obtained, at a small sacrifice in overall yield, by reduction of the reaction time. This approach gives minor amounts of what appear by ^{31}P NMR to be uncyclized 3'- and 5'-

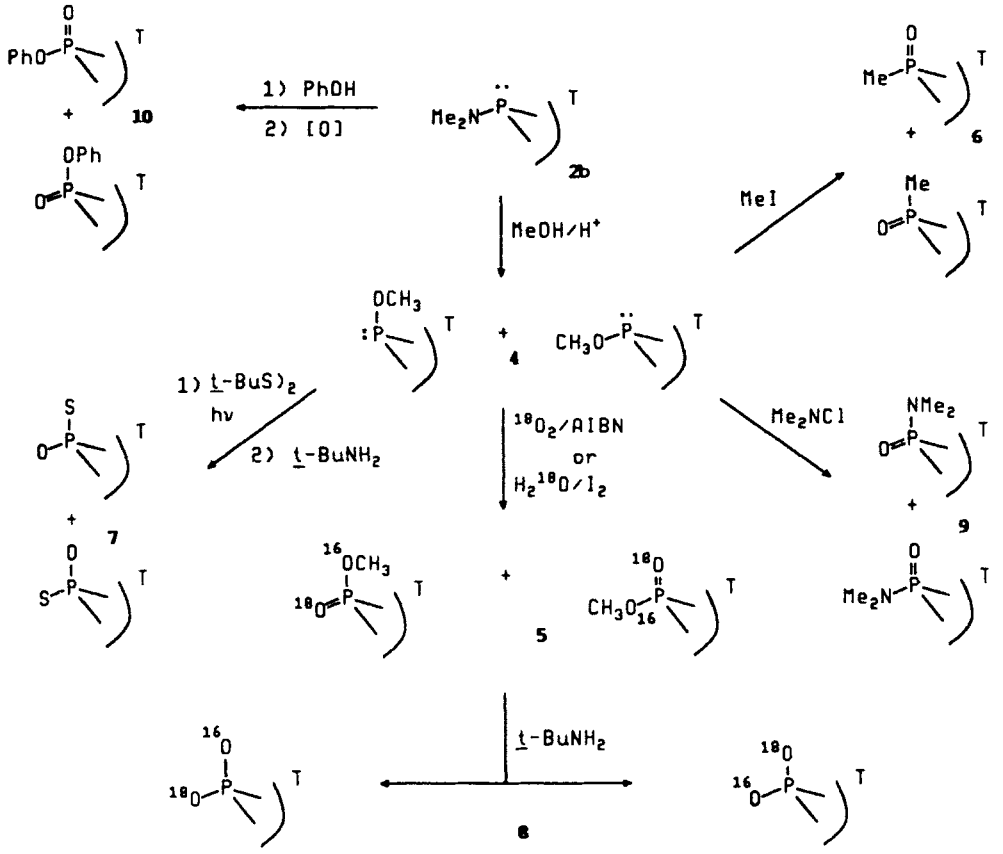
TABLE 1. Product Data for 5-X-2'-Deoxyuridine Cyclic 3',5'-Phosphoramidates.

Compd.	X	Total Isolated[a] Yield (%) of **3**	$\delta^{31}P$[b] cis	trans	Ratio of[c] trans/cis
3a	H	45	7.69	9.43	83/17
3b	CH_3	77	7.92	9.15	77/23
3c	I	64	7.80	9.26	78/22
3d	F	70	7.68	9.23	84/16
3e	CF_3	55	7.73	9.27	86/14
3f		66	7.87	9.35	81/19

[a]By HPLC, 50-100 mg scale, Dynamax Macro SiO_2 column, single pass. Solvent system CH_2Cl_2/MeOH = 97/3 for compounds **3a-3c** and CH_2Cl_2/MeOH = 98/2 for compounds **3d-3f**. [b]Acetone-d_6 121.3 MHz. Chemical shifts (ppm) are reported relative to external 85% H_3PO_4. [c]By ^{31}P quantitative NMR and/or HPLC.

phosphorodiamidites. These assumed precursors of **3** do not interfere, however, with workup of the reaction.

The structures of the individual diastereomers of **3a** and **3c-3f** were verified by their ^1H, ^{31}P, and ^{13}C NMR parameters on comparison to those of the known phosphoramidate, **3b**.[7f,g,15] The structures of both the cis and trans diastereomers of **3b** have been proven by X-ray crystallography.[7i,16] Cis and trans geometries were assigned to the individual diastereomers of **3a** and **3c-3f** on the basis of their relative ^{31}P chemical shifts, as is well precedented.[7f] Quantitative elemental analyses are given in Table 2. ^1H chemical shifts appear in Table 3. J_{HH} and J_{HP} values for the phosphate rings of **3a** and **3c-3f** are similar to the published values[7h,5d] for **3b** and will appear elsewhere in connection with conformational analysis of the phosphoramidate rings. Trends in



SCHEME

TABLE 2. Analytical Data for **3a** and **3c-3f**.[a]

Compound	Formula		%C	%H	%P
3a	$C_{11}H_{16}N_3O_6P$	Calcd.	41.64	5.04	9.78
		Found	41.96	5.01	9.97
3c	$C_{11}H_{15}N_3O_6PI$	Calcd.	29.79	3.39	6.99
		Found	29.69	3.47	7.17
3d	$C_{11}H_{15}N_3O_6PF$	Calcd.	39.40	4.48	9.25
		Found	39.26	4.56	8.93
3e	$C_{12}H_{15}N_3O_6PF_3$	Calcd.	37.40	3.89	8.05
		Found	37.30	4.06	7.89
3f	$C_{13}H_{17}N_3O_6PBr$	Calcd.	36.97	4.03	7.35
		Found	36.57	4.07	7.55

[a]Carried out on 40/60 to 60/40 cis/trans mixtures.

TABLE 3. ^{1}H Chemical Shifts for the Diastereomers of 3a-3f at 500 MHz in Acetone-d_6 at 26°.[a]

Compound	H1'	H2'[b]	H3'	H4'	H5'a	H5'b	H5	H6	NH	NMe_2	Other
cis-3a	6.33	2.50-2.59	4.85	4.16	4.32	4.58	6.03	7.83	9.10	2.65	
trans-3a	6.35	2.52-2.64	4.86	3.93	4.46	4.50	5.63	7.74	7.99	2.67	
cis-3b[d]	6.40	2.64-2.67	4.94	4.20	4.31	4.61		7.51	c	2.68	1.84[e]
trans-3b[d]	6.42	2.53-2.60	4.92	3.93	4.48	4.49		7.62	c	2.70	1.84[e]
cis-3c	6.35	2.56-2.70	4.94	4.17	4.34	4.58		8.02	c	2.56	
trans-3c	6.33	2.58-2.75	4.94	3.94	4.47	4.52		8.12	c	2.67	
cis-3d	6.39	2.59-2.68	4.91	4.17	4.39	4.58		7.83	c	2.56	
trans-3d	6.35	2.53-2.65	4.98	3.94	4.48	4.54		7.97	10.47	2.67	
cis-3e	6.32	2.60-2.72	4.92	4.22	4.35	4.60		8.11	c	2.64	
trans-3e	6.30	2.58-2.74	4.92	3.99	4.50	4.54		8.16	c	2.66	
cis-3f	6.41	2.58-2.68	4.91	4.21	4.38	4.59		7.79	10.34	2.65	7.39, 6.81[f]
trans-3f	6.42	2.56-2.65	4.89	3.96	4.47	4.53		7.81	c	2.67	7.41, 6.92[f]

[a]In ppm downfield from internal TMS. [b]2H multiplet. [c]Peak not observed. [d]At 300 MHz. [e]5-Me. [f]5-CH=CHBr.

TABLE 4. ^{13}C NMR Parameters for **3a-3f** in Acetone-d_6 at 75.5 MHz, 26°.

	Chemical Shift (J_{CP}, Hz[a])										
Compound	C1'	C2'[b]	C3'	C4'	C5'	C2	C4	C5	C6	Me_2N	5-X
cis-**3a**[e]	87.4	36.7 (7.5)	78.4 (5.2)	74.6 (10.6)	69.4 (8.3)	151.0	164.1	102.8	143.4	36.2 (4.7)	
trans-**3a**[b]	87.3	36.1 (8.5)	77.8 (4.2)	75.4 (4.7)	70.2 (7.7)	151.7	166.0	103.4	143.2	36.4 (5.0)	
cis-**3b**	85.7	35.1 (7.6)	77.9 (5.2)	73.7 (11.3)	68.6 (8.4)	150.5	163.7	110.9	137.0	35.8 (3.7)	11.7[f]
trans-**3b**	85.0	35.7 (8.4)	76.9 (4.0)	74.9 (4.5)	69.0 (7.6)	151.1	164.2	111.8	137.2	36.2 (4.6)	12.0[f]
cis-**3c**	86.7	35.9 (7.1)	78.1 (5.2)	74.5 (11.1)	69.0 (8.4)	150.3	160.3	69.6	146.1	36.3 (3.6)	
trans-**3c**	86.2	36.0 (8.4)	76.7 (3.7)	75.2 (4.1)	69.1 (7.6)	150.8	160.7	69.6	146.3	36.4 (4.7)	
cis-**3d**[e]	86.1	35.9 (7.1)	78.0 (5.1)	74.5 (10.9)	69.0 (8.4)	149.3	157.3	141.3 (232.2^c)	125.6 (34.2^d)	36.3 (3.5)	
trans-**3d**[b]	87.2	36.0 (8.5)	77.6 (4.2)	75.4 (4.6)	70.1 (7.8)	150.4	159.2	142.0 (232.4^c)	126.8 (34.4^d)	36.4 (5.1)	
cis-**3e**[e]	88.1	36.6 (7.3)	78.0 (5.2)	74.8 (11.2)	69.0 (8.4)	149.7	158.9	143.4 (32.6^c)	104.8 (5.8^d)	36.3 (3.4)	123.4[g] (268.4)
trans-**3e**	87.5	36.0 (8.5)	76.5 (4.0)	75.4 (4.3)	68.9 (7.4)	150.1	159.2	143.4 (32.6^c)	105.0 (5.9^d)	36.2 (4.8)	123.2[g] (268.8)
cis-**3f**[e]	86.3	36.1 (7.1)	78.1 (5.2)	74.6 (11.3)	68.9 (8.2)	149.4	161.9	108.3	140.3	36.3 (3.7)	111.5[h] 130.3
trans-**3f**	85.4	36.0 (8.2)	76.6 (3.9)	75.1 (4.3)	69.0 (7.5)	149.9	162.3	108.6	140.3	36.3 (4.5)	112.0[h] 130.5

[a] Acquisition times 2.1 s. Errors in J values ± 0.5 Hz. [b] In CD_3OD. [c] J_{C5F}. [d] J_{C6F}. [e] At 125.8 MHz. [f] 5-Me. [g] 5-CF_3. [h] 5-CH=CHBr.

relative ^{1}H chemical shifts potentially useful in assigning cis and trans structures are evident in Table 2. ^{13}C parameters are recorded in Table 4. The relative ^{13}C chemical shifts for C3' and C4' correlate well with the cis or trans identity of the particular diastereomer as do J_{PC} values for C2'-C5'. These correlations were pointed out previously.[7f]

EXPERIMENTAL

^{1}H NMR spectra were taken on a Varian VXR-500 spectrometer at 500 MHz. ^{13}C NMR spectra were obtained on a Varian XL-300 MHz spectrometer at 75.5 MHz and on a VXR-500 MHz at 125.8 MHz. ^{31}P NMR spectra were recorded on a Varian XL-300 instrument at 121.3 MHz.

Anhydrous solvents were obtained as follows: acetonitrile by successive distillations from calcium hydride, phosphorus pentoxide and then calcium hydride; dichloromethane by distillation first from phosphorus pentoxide and then from calcium hydride. Hexamethylphosphorus triamide was obtained from Aldrich Chemical Co. and distilled before use. Tetrazole from Aldrich Chemical Co. was sublimed prior to use. HPLC grade solvents were Omnisolve materials from EM Industries. A toluene solution of t-BuOOH from Fluka Chemie AG was used as received.

Preparation of 2b-2f and 3b-3f. Reactions were run on 0.5-1.0 g of nucleoside. The procedure for thymidine is typical. Thymidine (1.0 g, 0.41 mmol) was mixed with 100 mL of carefully purified acetonitrile in a flask equipped with a rubber septum. Argon was bubbled through the mixture for 15 min, and freshly distilled $(Me_2N)_3P$ (0.67 g, 0.74 mL, 0.41 mmol), 99.9% pure by GLC, was added. The cloudy mixture was purged with argon for an additional 10 min. The flask was fitted with an argon-flushed condenser topped with a gas bubbler through which a slow flow of argon was maintained. The temperature of the reaction was slowly increased over a 1-2 h period and maintained at 63-65 °C for 18 h, during which time the reaction mixture became clear. Solvent was removed under oil pump vacuum to leave a white, foamy solid (1.25 g, 95% crude yield). ^{31}P NMR ($CDCl_3$) showed peaks at δ 146.1 (trans) and 140.1 (cis), ratio 75/25 (trans/cis), which comprised at least 90% of the total peak area. The crude product was dissolved in 50 mL of purified CH_2Cl_2 and maintained under argon while 1 mL of t-BuOOH (approximately 3 M solution in toluene, diluted with 5 mL of CH_2Cl_2) was added dropwise at 0 °C with stirring. Solvent evaporation left a foamy, white product (1.15 g,

0.34 mmol, 83% crude yield based on thymidine). The crude phosphoramidate (100 mg) was purified by HPLC in one pass on a Dynamax Macro SiO_2 column (25 cm x 21.4 mm; CH_2Cl_2/MeOH, 97/3, flow rate 10 mL/min) to give 71 mg of trans-**3b** and 20 mg of cis-**3b**.[17] (Total yield based on 100 mg of crude product, 77%). As an alternative oxidation, the cyclic phosphoramidite was dissolved in 30 mL of CH_2Cl_2 and oxidized at -70° C with a saturated CH_2Cl_2 solution of N_2O_4 added dropwise until a pale yellow-green color persisted. N_2O_4 was the reagent of choice except for X = Me or I for which t-BuOOH gave equally good yields.

Preparation of 2a and 3a. These products were obtained by a procedure identical to the above except that 20 mg of tetrazole was added to a 500 mg-scale reaction.

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