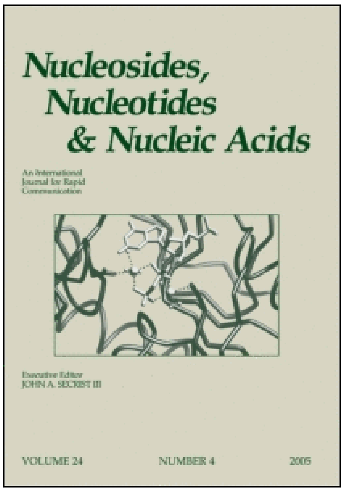


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Improved Synthesis of Adenosine Cyclic 3', 5'-Phosphoramidates via Anhydrides. Preparation of Adenosine Cylic 3', 5'-(R_p) - and ($_p$)-N-Methylphosphoramidate

S. Bottka[a]; J. Tomasz[a]
[a] Institute of Biophysics, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary

To cite this Article Bottka, S. and Tomasz, J.(1988) 'Improved Synthesis of Adenosine Cyclic 3', 5'-Phosphoramidates via Anhydrides. Preparation of Adenosine Cylic 3', 5'-(R_p) - and ($_p$)-N-Methylphosphoramidate', Nucleosides, Nucleotides and Nucleic Acids, 7: 3, 307 — 319

To link to this Article: DOI: 10.1080/07328318808068712

URL: http://dx.doi.org/10.1080/07328318808068712

NUCLEOSIDES & NUCLEOTIDES, 7(3), 307-319 (1988)

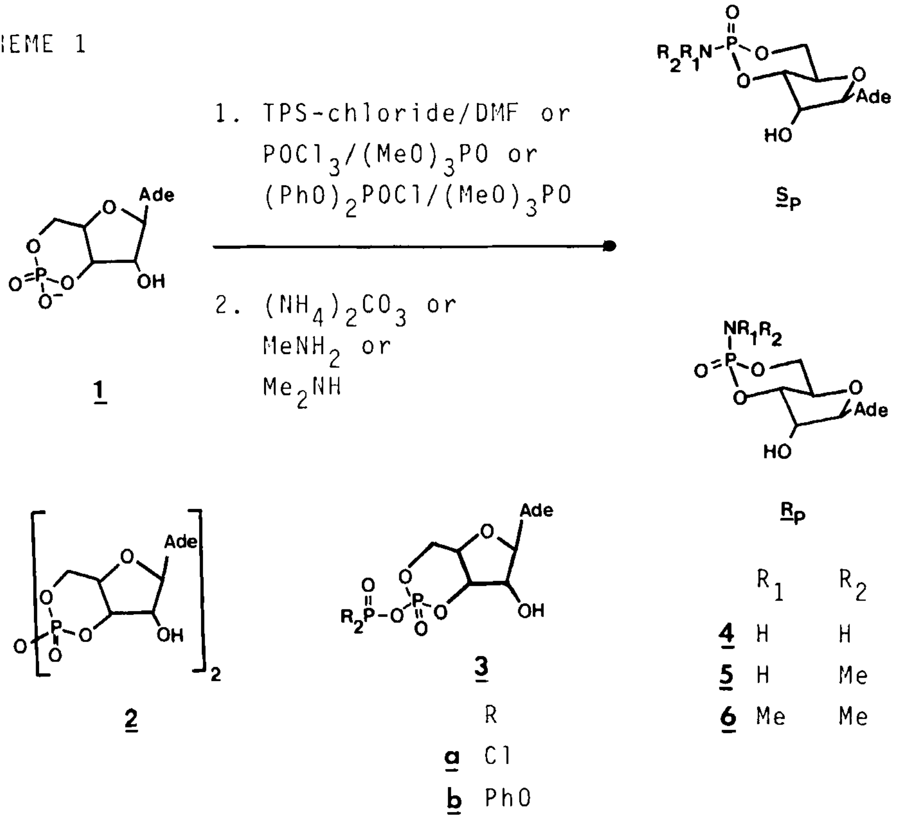
IMPROVED SYNTHESIS OF ADENOSINE CYCLIC 3',5'-PHOSPHORAMIDATES VIA ANHYDRIDES. PREPARATION OF ADENOSINE CYLIC 3',5'-(R_P)- AND (S_P)-N-METHYLPHOSPHORAMIDATE

S. Bottka* and J. Tomasz
Institute of Biophysics, Biological Research Center,
Hungarian Academy of Sciences, H-6701 Szeged, Hungary

Abstract. The two-step method for the preparation of adenosine cyclic 3',5'-phosphoramidate diastereoisomers, which involves the activation of adenosine cyclic 3',5'-monophosphate (**1**) with an acid chloride and *in situ* aminolysis of the anhydride intermediate (Bentrude, W.G.; Tomasz, J. Synthesis 1984, 27; Bottka, S.; Tomasz, J. Tetrahedron Lett. 1985, 24, 2909), has been improved. The best yields were attained when **1** was reacted with 4.4 molar equivalents of phosphorus oxychloride in trimethyl phosphate at 0°C for 3 h, and the solution of phosphorus oxychloride in trimethyl phosphate was pretreated with 0.5 molar equivalent of water at room temperature for 20 min. R_P and S_P diastereoisomers of adenosine cyclic 3',5'-N-methylphosphoramidate and N,N-dimethylphosphoramidate have been synthesized under these experimental conditions.

Phosphorus diastereoisomers of adenosine cyclic 3',5'-N,N-dimethylphosphoramidate (**6**) have proved to be valuable tools for studying the binding sites and activation requirements of the enzymes involved in the metabolism of adenosine cyclic 3',5'-monophosphate (cyclic AMP, **1**), [1-2] a fundamental intracellular mediator and regulatory molecule of living systems. The same diastereoisomers have also been useful probes for the chair $\rightleftharpoons$ twist conformational equilibrium of the 1,3,2-dioxaphosphorinane ring,[3]. It is expected that diastereoisomeric pairs of other cyclic AMP amides can also be successfully used in the above mentioned biochemical and conformational investigations. Synthetic work was, therefore, undertaken in our laboratory in the early eighties to get new compounds according to a more

SCHEME 1



	R
a	Cl
b	PhO

	R_1	R_2
4	H	H
5	H	Me
6	Me	Me

convenient route than the multi-step ways to *6*.[4] This work led to the elaboration of a simple, two-step method for the preparation of the individual diastereoisomers of *6*[5] and of adenosine cyclic 3',5'-phosphoramidate (*4*).[6] The method involved the activation of unblocked *1* with either 2,4,6-triisopropylbenzenesulfonyl chloride (TPS-chloride), phosphorus oxychloride or diphenyl phosphorochloridate in anhydrous N,N-dimethylformamide or trimethyl phosphate, followed by in situ treatment of the reactive anhydride intermediate with ammonium carbonate under anhydrous conditions or with aqueous dimethylamine (SCHEME 1). Phosphoramidate *6* was prepared both by TPS-chloride in N,N-dimethylformamide[5] and by diphenyl phosphorochloridate in trimethyl phosphate.[7] For the synthesis of **4**, phosphorus oxychloride in trimethyl phosphate was used.[6] The structure of the intermediate depended on the nature of the acid chloride used for activation. ^{31}P NMR studies showed that it was cyclic AMP symmetrical anhydride (**2**) for TPS-chloride[8] and cyclic AMP diphenyl phosphoric mixed anhydride (**3b**) for diphenyl phosphorochloridate.[7] The method preferentially gave S_P-amide diastereoisomers. The isolated yields were: 31.0% (S_P) and 1.6% (R_P) for **6**[5], 38.6% (S_P) and 9.2% (R_P) for **4**.[6]

To increase the yield and change the diastereoisomeric ratio in favor of (R_P) diastereoisomer, we have compared the acid chlorides and solvents used earlier as well as investigated the effects of the reaction time and the water content of the reaction mixture on the yield and the distribution of diastereoisomers. The synthesis of **6** has been selected for these studies because of the greater stability of **6** compared to **4**. On the basis of the results obtained, the method for the preparation of **6** could significantly be improved. In addition, the first synthesis of the diastereoisomeric pair of a new cyclic AMP amide derivative, adenosine cyclic 3',5'-N-methylphosphoramidate (**5**) has also been performed under the experimental conditions found optimal for the preparation of **6**. Results are presented in this paper.

RESULTS AND DISCUSSION

Studies on the synthesis of adenosine cyclic 3',5'-N,N-dimethylphosphoramidate (**6**)

The synthesis of **6** was performed according to SCHEME 1. Tri-n-butylammonium salt of **1** was reacted with 2.2 molar equivalents of phosphorus oxychloride, diphenyl phosphorochloridate or TPS-chloride in anhydrous N,N-dimethylformamide or trimethyl phosphate, at 0°C. Aliquots of the mixtures were removed at intervals, mixed with excess 40% aqueous dimethylamine solution that had been precooled to -20°C and analyzed by HPLC. Yields and relative percentages of S_P-**6** are summarized in TABLE 1.

TABLE 1. Changes in the yield and the relative percentage of $\underline{S}_P$ diastereoisomer[1] with the activating acid chloride, solvent and reaction time in the synthesis of adenosine cyclic 3',5'-N,N-dimethylphosphoramidate (6).[2]

Activating acid chloride		$POCl_3$		$(PhO)_2POCl$		TPS-Cl	
Solvent	Reaction time (min)[3]	% of 6	rel. % of $\underline{S}_P$-6	% of 6	rel. % of $\underline{S}_P$-6	% of 6	rel. % of $\underline{S}_P$-6
DMF	5(10)	25.5[4]	85.1	72.8	84.2	49.7	78.3
	30(60)	22.0[4]	83.6	68.3	86.5	-	-
$(MeO)_3PO$	10	70.6	92.4	74.8	90.5	43.6	82.1
	60	74.3	93.6	72.6	91.5	48.4	81.3

As shown in TABLE 1, of the three acid chlorides tested, phosphorus oxychloride and diphenyl phosphorochloridate gave similar results in trimethyl phosphate. The yields were between 70-75%. Earlier it was shown,[7] that the maximal yield of the reaction with diphenyl phosphorochloridate is ∿75%. This is a consequence of the fact that nucleophilic attack of dimethylamine at the two phosphorus atoms of anhydride intermediate **3b** occurs in a ratio of P^1/P^2 ∿3:1.[7] The maximal yield (74.3%) obtained for the reaction with phosphorus oxychloride may indicate cyclic AMP dichloro phosphoric mixed anhydride (**3a**) as reactive intermediate, but it is also possible that a mixture of **3a** and **2** is generated. The use of N,N-dimethylformamide instead of trimethyl phosphate, did not cause noticeable change for the reaction with diphenyl phosphorochloridate. The yield significantly decreased, however, and many by-products were formed in the reaction with phosphorus oxychloride. This finding may be interpreted on the basis of the known interaction between phosphorus oxychloride and N,N-dimethylformamide.[9] TPS-chloride was less effective than phosphorus oxychloride or diphenyl phosphorochloridate. The yield never exceeded 50% in either solvents, as expected on the basis of the structure of reactive intermediate **2**.[8]

In all cases S_P-**6** was the dominate diastereoisomer formed. Of the two solvents, the quantity of S_P-**6** was larger in trimethyl phosphate than in N,N-dimethylformamide. A maximal yield for S_P-**6** (69.5%) was attained when phosphorus oxychloride in trimethyl phosphate was used for 60 min. For the preparation of R_P-**6** a 10 min reaction with diphenyl phosphorochloridate in N,N-dimethylformamide gave the best yield (11.5%).

Earlier it was found[6] that the relative percentage of R_P-**4** could be increased from 19.2% to 62.9% by changing the solvent used in the ammonium carbonate treatment.[6] However, a similar effect was not observed in the analogous synthesis of **6**. The relative percentage of R_P-**6** remained practically unaltered (6.8±1.0%), irrespective of whether the intermediate was treated with dimethylamine in an aqueous solution or in four different anhydrous organic solvent systems.

The relative proportion of $\underline{R}_P$-**6** could, however, be increased, if the solution of phosphorus oxychloride in trimethyl phosphate was pretreated with 0.5 or 1.0 molar equivalent of water. Since the pretreatment reduced the rate of the reaction, the quantity of phosphorus oxychloride was doubled. Reproducible results were obtained only, when the pretreatment was carried out at room temperature for at least 20 min. The effect of added water on the yield and the relative percentage of $\underline{R}_P$-**6** is summarized in TABLE 2.

Data in TABLE 2 show, that practically the same yields could be obtained in the reactions with added water during 3-6 h as found for the analogous reactions run without added water for 10 or 60 min (see TABLE 1). The quantity of $\underline{R}_P$-**6** in the reactions with added water was about three times as large as in the blank reaction run without water. An unidentified by-product ($\leq$ 5%) was formed in the reaction, when 1.0 molar equivalent of water was employed. For this reason, the use of 0.5 molar equivalent is recommended. On a preparative scale by using 0.5 molar equivalent of water and a reaction time of 3 h, $\underline{R}_P$-**6** and $\underline{S}_P$-**6** were obtained by reversed phase MPLC in isolated yields of 13.0% and 54.0%, respectively. These values are significantly higher than those obtained for the preparation of **6** with TPS-chloride (1.6% $\underline{R}_P$ and 31.0% $\underline{S}_P$[5]) and represent the best yields reported so far for the synthesis of $\underline{R}_P$-**6** and $\underline{S}_P$-**6**.[4]

Synthesis of adenosine cyclic 3',5'-N-methylphosphoramidate (**5**)

Compound **5** was synthesized under the conditions found optimal for the preparation of **6**, *i.e.* by reacting **1** at 0°C for 3 h with a solution of phosphorus oxychloride in trimethyl phosphate that had been pretreated with water at room temperature for 20 min. The components were employed in a molar ratio of **1**/phosphorus oxychloride/water = 1.0:4.4:0.5.

TABLE 2. Effect of water on the yield and the relative percentage of $\underline{R}_P$ diastereoisomer[1] in the synthesis of adenosine cyclic 3',5'-N,N-dimethylphosphoramidate ($\underline{6}$) with phosphorus oxychloride in trimethyl phosphate. Molar equivalents of components were: cyclic AMP/$POCl_3$/H_2O = 1.0 : 4.4 : X.[2]

Reaction time (min)	Molar equivalents of water, X = 0.0 % of $\underline{6}$	0.0 rel. % of $\underline{R}_P$-$\underline{6}$	0.5 % of $\underline{6}$	0.5 rel % of $\underline{R}_P$-$\underline{6}$	1.0 % of $\underline{6}$	1.0 rel. % of $\underline{R}_P$-$\underline{6}$
30	65.9	4.1	56.8	15.7	57.4	18.5
60	69.8	5.7	60.8	19.1	60.0[5]	20.7
180	72.6	6.9	71.0	20.6	67.4[5]	22.1
360	72.6	7.6	73.0	21.0	74.4[5]	21.8

[1] Determined by UV spectrophotometry. [2] For details see EXPERIMENTAL. [3] Values in parentheses are times for the reaction with $(PhO)_2POCl$. [4] Many by-products were formed. [5] Formation of a by-product (≤5%) was found.

Treatment with methylamine was done in anhydrous N,N-dimethylformamide at -20°C, because compound 5 is unstable in aqueous alkaline solution. The diastereoisomers were formed in a ratio of R_P/S_P = 27:73 as detected by ^{31}P NMR. On a preparative scale R_P-5 and S_P-5 were isolated by reversed phase MPLC in yields of 23.3% and 45.9%, respectively. Configurations at phosphorus were assigned by ^{31}P NMR spectroscopy. On the basis of literature data,[5,10-13] R_P-5 having axial methylamino group, is expected to absorb at higher field.

Attempted synthesis of adenosine cyclic 3',5'-N,N-diisopropylphosphoramidate

Attempts to prepare adenosine cyclic 3',5'-N,N-diisopropylphosphoramidate according to this anhydride route, have failed. Both phosphorus oxychloride and TPS-chloride was used for the activation and the aminolysis was performed with anhydrous diisopropylamine. Adenosine cyclic 3',5'-N,N-diisopropylamidate could not be detected by HPLC in either case. Similar unreactivity of diisopropylamine has been found in the Appel-reaction and attributed to steric hindrance by the two bulky isopropyl groups.[14]

EXPERIMENTAL

Materials and Methods

Cyclic AMP tri-n-butylammonium salt was prepared according to Ref. 5. Phosphorus oxychloride and diphenyl phosphorochloridate (b.p. 140-142°C/52 Pa) were distilled prior to use. TPS-chloride was recrystallized from n-hexane. Solvents were distilled (N,N-dimethylformamide over phosphorus pentoxide, trimethyl phosphate at reduced pressure) and stored over 0.4 nm molecular sieves.

HPLC analyses were performed by using a Waters liquid chromatograph with a UV absorbance monitor working at 254 nm (Waters Model 440) and an integrator (Labor MIM MP-85). The reversed phase column was a Hibar LiChrosorb RP-18 (250x4.0 mm I.D., 5 µm, Merck). An isocratic elution mode was used with a mixture of 100 mM aqueous potassium phosphate solution (pH 6.6)/methanol = 63:37 (v/v). The flow rate was 1.0 mL/min.

Preparative reversed phase MPLC was conducted on a Li Chroprep RP-18 (25-40 µm, Merck) column (1.6x96.0 cm) in water/methanol mixtures with 1.2 MPa overpressure. The elution rate was 20.0 mL/2.4 min/fraction. Triply distilled water was used for all analyses.

TLC was carried out on HPTLC Kieselgel 60 F_{254} (Merck) chromatoplates in the following solvent systems: S1; chloroform/methanol = 6:1 (v/v) (two successive developments), S2; chloroform/ethanol = 1:1 and S3; n-buthanol/ethanol/water = 16:2:5. Spots were visualized under a short-wave (254 nm) UV lamp (Desaga-Uvis).

^{31}P NMR spectra were recorded on a Bruker WM-250 FT spectrometer operating at 101.2 MHz. Positive chemical shifts are downfield from external 85% H_3PO_4.

Studies on the synthesis of adenosine cyclic 3',5'-N,N-dimethylphosphoramidate (**6**)

Effect of activating acid chloride, solvent and reaction time

Cyclic AMP tri-n-butylammonium salt (0.1 mmol) was dissolved in 0.5 mL of N,N-dimethylformamide (A) or trimethyl phosphate (B). To the solution 0.22 mmol of the acid chloride {20.0 µL phosphorus oxychloride (a) or 45.6 µL diphenyl phosphorochloridate (b) or solution of 66.4 mg TPS-chloride (c) in 6.0 mL of N,N-dimethylformamide or trimethyl phosphate} was added at 0°C. The six mixtures thus obtained, were stirred with the exclusion of atmospheric moisture at 0°C. Aliquots (100 µL) were removed from mixtures Aa and Ac after 5 and 30 min, from mixtures Ab, Ba, Bb and Bc after 10 and 60 min and were pipetted into 40% aqueous dimethylamine solution (2 mL) that had been cooled to -20°C. After evaporation and dissolution of the residues in 100 mM aqueous potassium phosphate (pH 6.6) /methanol = 63:37 (v/v) mixture (2.0 mL), the solutions were analysed by HPLC. Retention times (min) were: 5.4 (for **1**), 6.3 (for $\underline{R}_P$-**6**) and 11.3 (for $\underline{S}_P$-**6**). Yields and relative percentages of $\underline{S}_P$-**6** were tabulated (TABLE 1).

Effect of solvent used for the aminolysis

Mixture Ba was used in these studies. After 60 min stirring at 0°C, 50 μL aliquots were pipetted into 6 mL of the following solutions that contained 350 μL of anhydrous dimethylamine and were cooled to -20°C. I. trimethyl phosphate, II. N,N-dimethylformamide, III. pyridine and IV. N,N-dimethylformamide/pyridine = 9:1 (v/v). HPLC analysis of the samples, % of **6** (rel. % of $\underline{R}_P$-**6**): I. 75.9 (7.8), II. 72.5 (5.9), III. 72.5 (6.5) and IV. 73.8 (7.1).

Effect of water

Phosphorus oxychloride (40.0 μL, 0.44 mmol) was dissolved in trimethyl phosphate (0.2 mL). After stirring 20 min at room temperature, the solution was added to a solution of cyclic AMP tri-n-butylammonium salt (0.1 mmol) in trimethylphosphate (0.3 mL) that had been cooled to 0°C. The reaction mixture was stirred with the exclusion of atmospheric moisture at 0°C. After 30, 60, 180 and 360 min, 100 μL aliquots were removed, reacted with aqueous dimethylamine, and analyzed by HPLC as described. The reaction was repeated with solutions of phosphorus oxychloride in trimethylphosphate that contained deionized water (0.9 μl, 0.05 mmol or 1.8 μL, 0.1 mmol). Yields and relative percentages of $\underline{R}_P$-**6** were summarized in TABLE 2.

Preparation of $\underline{R}_P$-**6** and $\underline{S}_P$-**6**

Experiment underlined in TABLE 2, was repeated on a fivefold scale. The reaction mixture was dropped into 40% aqueous dimethylamine solution (50 mL) that had been cooled to -20°C, under vigorous stirring. After warming up to room temperature, the solution was evaporated. The residue from evaporation was dissolved in a mixture of water/methanol = 75:25 (v/v) (1.0 mL) and applied onto the MPLC column. A mixture of water/methanol of the same composition was used for elution. Diastereoisomers were isolated by evaporation of the respective fractions in yields of 24.3 mg (13.0%, $\underline{R}_P$-**6**, from fractions 22-30) and 101.1 mg (54.0%, $\underline{S}_P$-**6**, from fractions 35-48), respectively. The products were identified by ^{31}P NMR and TLC comparisons with authentic samples.[5]

$\underline{R}_P$- and $\underline{S}_P$-Adenosine cyclic 3',5'-N-methylphosphoramidates (5)

Phosphorus oxychloride (200.0 µL, 2.2 mmol) and deionized water (4.5 µL, 0.25 mmol) were added to trimethyl phosphate (1.5 mL) (A). The solution was stirred with the exclusion of atmospheric moisture at room temperature for 20 min. Cyclic AMP tri-n-butylammonium salt (0.5 mmol) was dissolved in trimethyl phosphate (1.0 mL) (B), and the solution was cooled to 0°C. Solution A was poured into solution B, then the reaction mixture was stirred with the exclusion of atmospheric moisture at 0°C for 3 h. The reaction mixture was poured into an anhydrous solution of methylamine (5 mL) in N,N-dimethylformamide (20 mL) that had been cooled to -20°C. Excess methylamine was immediately removed under reduced pressure. The solid deposited was filtered off, and the solution was evaporated. The evaporational residue was dissolved in a mixture of water/methanol = 85:15 (v/v, 5 mL). A precipitate started to deposit by slightly cooling the solution. After a couple of hours standing, the precipitate was filtered by suction, and the mother liquor was applied onto the MPLC column. The precipitate after recrystallization from hot deionized water (5 mL) gave 42.0 mg of pure $\underline{S}_P$-**5**. The column was washed with mixtures of water/methanol = 85:15 (v/v, 1200 mL) and 82:18 (800 mL). After evaporating the appropriate fractions, 39.8 mg (23.3%) $\underline{R}_P$-**5** (from fractions 45-58) and 36.5 mg $\underline{S}_P$-**5** (from fractions 84-97) were obtained. The yield for $\underline{S}_P$-**5** was 45.9%. For recrystallization hot deionized water (for $\underline{S}_P$-**5**, ∿ 70 fold) and methanol (for $\underline{R}_P$-**5**, ∿20 fold) were used. The compounds can be stored without decomposition at -5°C for 6 months. Anal. ($\underline{S}_P$-**5**) Calcd for $C_{11}H_{15}N_6O_5P$ (342.25): C 38.60, H 4.42, N 24.56,

P 9.05; found: 38.89, H 4.47, N 24.53, P 9.16.
^{31}P NMR (DMSO-d_6): δ +7.75 (R_P-5), +9.37 (S_P-5); relative signal intensities: S_P/R_P = 11:4.
M.S. m/e rel. int. of S_P-5 and R_P-5 (%) , $M(TMS)_3^+$ 558(6.4 and 2.2), 543 (23 and 23), 264(24 and 24), 236(100 and 97) and 147(23 and 100).
TLC, R_f values of R_P-5 and S_P-5: 0.21 and 0.25 (S1), 0.28 and 0.34 (S2), 0.36 and 0.42 (S3).

REFERENCES AND NOTES

1. de Wit, R.J.W.; Hoppe, J.; Stec, W.J.; Baraniak, J.; Jastorff, B. Eur.J.Biochem. 1982, 122, 95.
2. de Wit, R.J.W.; Hekstra, D.; Jastorff, B.; Stec, W.J.; Baraniak, J.; van Driel, R.; van Haastert, P.J.M. Eur.J. Biochem. 1984, 142, 255.
3. Bentrude, W.G.; Sopchik, A. E. Tetrahedron Lett. 1981, 307; Bentrude, W.G.; Béres, J.; Chandrasekaran, S.; Nelson, K.; Szakály-Quin, G.; Setzer, W.N.; Sopchik, A.E.; Tomasz, J. Phosphorus and Sulfur 1983, 18, 389; Nelson, K.A.; Bentrude, W.G.; Setzer, W.N.; Hutchinson, J.P. J. Am. Chem. Soc. 1987, 109, 4058.
4. For a critical review of the earlier synthesis of **6**, see ref. 5.
5. Bentrude, W.G.; Tomasz, J. Synthesis 1984, 27.
6. Bottka, S., Tomasz, J. Tetrahedron Lett. 1985, 24, 2909.
7. Bottka, S.; Pelczer, I.; Tomasz, J. Nucleosides and Nucleotides 1988, 7, 2nd issue.
8. Tomasz, J.; Bottka, S.; Pelczer, I. Nucleosides and Nucleotides 1987, 6, 785.
9. Alunni, S; Linda, P.; Marino, G.; Santini, S.; Savelli, G. J.Chem.Soc.Perkin Trans. II. 1972, 2070 and references cited.
10. Mosbo, J.A.; Verkade, J.G. J.Am.Chem.Soc. 1973, 95, 4653.
11. Bentrude, W.G.; Tan, H.-W. J.Am.Chem.Soc. 1973, 95, 4666.
12. Stec, W.J.; Mikolajczyk, M. Tetrahedron 1973, 29, 539.

13. Stec, W.J.; Zielinsky, W.S. Tetrahedron Lett. 1980, 21, 1361.

14. Béres, J.; Bentrude, W.G.; Párkányi, L.; Kálmán, A.; Sopchik, A.E. J.Org.Chem. 1985, 50, 1271.

Received July 24, 1987