Preparation of Phosphonate 10a from Phosphite 19. $FeCl_3$ (341 mg, 2.1 mmol) was added to a solution of the phosphite **19** (500 mg, 2.1 mmol) in anhydrous CH_2Cl_2 (15 mL) at -20 °C. The reaction mixture was allowed to warm to room temperature overnight, washed with brine (2 × 5 mL), and then dried ($MgSO_4$). GCMS analysis indicated two products (ratio (70:30) identified on the basis of their spectral data as the H-phosphonate diester **10a** (major) and the phosphate **21**: (^{31}P NMR -5.6; EIMS, m/z (relative intensity) 255 ($M^+ + 1$, 0.2), 239 (1), 209 (6), 195 (100), 191 (9), 167 (15), 155 (54), 127 (75), 99 (85), 81 (23), 59 (18)).

Acknowledgment. We thank Professor Darrell P. Eyman for his helpful discussions, the University of Iowa Faculty Scholars Program for the research leave (to D. F.W.) during which this work was conducted (1985–1988), and the Alfred P. Sloan Foundation for their financial support.

Rapid and Efficient Synthesis of Nucleoside 5′-*O*-(1-Thiotriphosphates), 5′-Triphosphates and 2′,3′-Cyclophosphorothioates Using 2-Chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one

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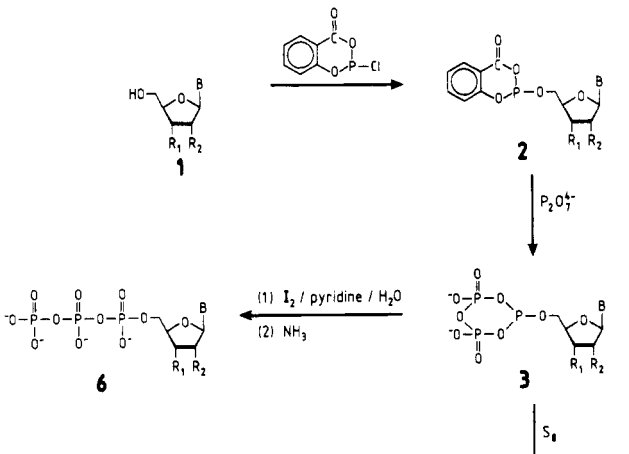
Received May 28, 1988

2-Chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one phosphitylates the 5′-hydroxy group of a nucleoside to form an intermediate (**2**), which on subsequent reaction with pyrophosphate produces, in a double displacement process, a P^2,P^3-dioxo-P^1-5′-nucleosidylcyclotriphophite (**3**). Oxidation with sulfur gives a nucleoside 5′-(1-thiocyclotriphosphate) (**4**), which is hydrolyzed to the diastereomeric mixture of a nucleoside 5′-*O*-(1-thiotriphosphate) (**5**). Alternatively, **3** can be oxidized with iodine/water to furnish nucleoside 5′-triphosphates **6**. This reagent can also be applied to the synthesis of nucleoside 2′,3′-cyclic phosphorothioates. Protection of nucleobase functional groups is not required.

Ribonucleoside and 2′-deoxyribonucleoside 5′-*O*-(1-thiotriphosphates) (NTPαS and dNTPαS) are finding widespread application in biochemistry and molecular biology[1] as substrates for DNA and RNA polymerases for sequencing,[2] mutagenesis,[3] and the labeling of hybridization probes.[4] The development of new methods of synthesis for these compounds is therefore warranted.[5] 2-Chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one[6] (salicyl phosphorochloridite) has been employed earlier for the preparation of nucleoside H-phosphonates as synthons in oligonucleotide synthesis.[7] However, this reagent is capable of undergoing three nucleophilic displacement reactions. We have exploited this multifunctionality in developing a facile synthesis of the 5′-*O*-(1-thiotriphosphates) and 5′-triphosphates of the eight common deoxyribonucleosides and ribonucleosides. This compound can also be readily employed for the synthesis of nucleoside

Scheme I



	1 - 4			5 - 6		
	B	R_1	R_2	B	R_1	R_2
a	A^{Bz}	OMeOAc	H	A	OH	H
b	G^{ib}	OMeOAc	H	G	OH	H
c	T	OAc	H	T	OH	H
d	C^{Bz}	OMeOAc	H	C	OH	H
e	A	OAc	OAc	A	OH	OH
f	G	OAc	OAc	G	OH	OH
g	U	O,OC(H,OCH_3)		U	OH	OH
h	C	O,OC(H,OCH_3)		C	OH	OH

Bz, benzoyl; ib, isobutyryl.

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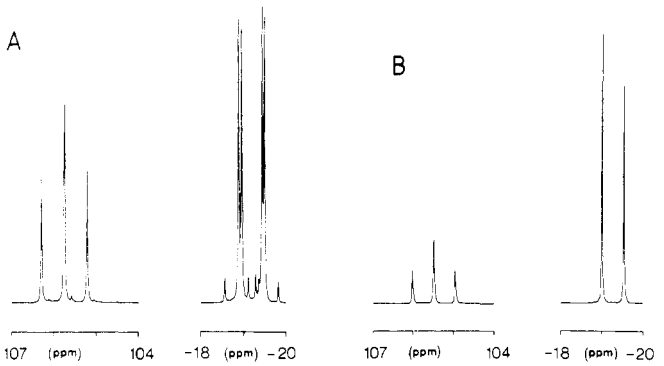


Figure 1. (A) Selected regions of the ^{31}P NMR spectrum of **3c**. Calculated ABX parameters: δ_X = 105.74 ppm; δ_A = −19.08 ppm; δ_B = −19.29 ppm; J_{AB} = 26.20 Hz; J_{BX} = 42.45 Hz; J_{AX} = 45.06 Hz. (B) Selected regions of the ^{31}P NMR spectrum of P^1-ethyl P^2,P^3-dioxo-cyclotriphophite (**7**). δ_A = 105.5 ppm (t); δ_B = −19.26 ppm; J_{AB} = 42.90 Hz. Spectra were recorded of reaction mixtures prepared as described in Methods. Parameters were as follows: Offset, 6150 Hz; sweep width 15151 Hz; pulse width, 4.0 ms; 32 K transients; acquisition time, 1.08 s; line broadening, 0.9 Hz; number of transients, 114 for spectrum 1B and 1820 for spectrum 1A.

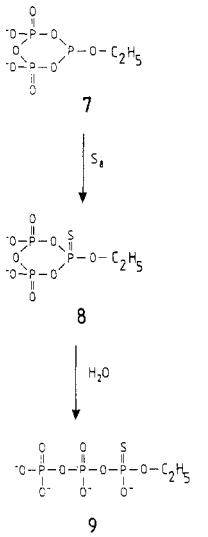
2′,3′-cyclophosphorothioates.

Results and Discussion

The sequence of reactions for the synthesis of the nucleoside 5′-*O*-(1-thiotriphosphates) and 5′-triphosphates is indicated in Scheme I. The reaction of the salicyl phosphorochloridite with a nucleoside yields the two diastereomers of **2**. They are identified by the appearance of two signals at 127.6 ppm and 126.3 ppm observed in the ^{31}P NMR spectrum of the reaction solution. Similar chemical shifts have been reported for the corresponding 3′-derivatives of nucleosides.[7] When **2** is reacted with pyrophosphate ^{31}P NMR spectroscopy reveals that a new compound has been formed. Two quartets appear at around −19 ppm along with a triplet centered at 105.5 ppm (Figure 1A), suggesting the formation of **3**. In the ^1H-coupled spectrum (not shown) each line of the triplet is split into a triplet with J_{POCH} = 5.5 Hz. This indicates that the trivalent phosphorus is attached to the 5′-oxygen of the nucleoside. No splitting of the quartets is observed in the ^1H-coupled spectrum. This spectrum is of the ABX type[8] where the AB part corresponds to the two phosphorus atoms of the pyrophosphate group with close but not identical shifts and the X part corresponds to the trivalent phosphorus atom. The nonequivalence of the two phosphorus atoms is due to the presence of the chiral nucleoside moiety. This can be ascertained by inspection of the spectrum of the corresponding ethyl derivative **7** (Scheme II) (Figure 1B). In this spectrum the two phosphorus of the pyrophosphate are identical and appear as a simple doublet. The existence of intermediate **3** shows that the nucleoside phosphite **2** is bifunctional in that it can undergo two subsequent nucleophilic substitution reactions. We assume that the leaving group in the first reaction with pyrophosphate is the carboxyl group and that the phenolic group leaves in the intramolecular second reaction. The nucleoside cyclic triphosphate resulting from reaction of **3** with iodine cannot be detected as it hydrolyzes during the oxidation reaction in the aqueous medium.

The intermediate **3** may be oxidized with sulfur to give the nucleoside 5′-thiotriphosphates or with iodine/water to yield the normal triphosphates. Upon addition of sulfur

Scheme II



to the reaction solution containing **3** the triplet of the original spectrum (Figure 1A) at δ(X) = 105.74 ppm shifts to 45.06 ppm whereas the two quartets are shifted only slightly upfield to δ(A) = −22.58 ppm, d (1 P); δ(B) = −22.75 ppm, d (1 P); J_{AB} = 26.43 Hz; J_{BX} = 35.72 Hz; J_{AX} = 35.77 Hz. This spectrum is consistent with the formation of the intermediate nucleoside 5′-(1-thiocyclotriphosphate) **4**. This cyclic intermediate reacts with water to form the nucleoside 5′-thiotriphosphate **5**. To demonstrate that nucleophilic attack by water occurs exclusively at the phosphate and not at the phosphorothioate, we carried out the hydrolysis reaction in $H_2{}^{18}O$. The resulting NTPαS showed the expected ^{18}O-induced upfield chemical shift only for Pγ. We have not detected any branched triphosphate[9] derived from opening of the pyrophosphate bond. Based on the relative pK_a values of the possible leaving groups, this is to be expected. The α-phosphorothioate, pK_a approximately 1.7, is a better leaving group than either of the phosphates, pK_a approximately 6.6.

^{31}P NMR spectroscopy of the reaction mixture showed, in addition to the signals of the NTPαS and of some nucleotide byproducts described below, a triplet at −21.82 ppm and a doublet at −7.94 ppm (J = 20.81 Hz). We attribute these to contamination by a small amount of tripolyphosphate, probably derived from reaction of pyrophosphate with activated phosphate contained in the pyrophosphate as a contaminant. Another triplet at 34.18 ppm and a doublet at −21.83 ppm (J = 29.8 Hz) is attributed to the presence of 1-thiocyclotriphosphate, the equivalent of **4** without the nucleoside moiety. This is derived from reaction between the phosphitylating agent and pyrophosphate and oxidation with sulfur. Both these contaminants copurify with the pyrimidine but not the purine deoxyribonucleoside 5′-thiotriphosphates on DEAE-Sephadex chromatography. Reduction of the amount of phosphitylating agent to 1.0 equiv and a change in the gradient of the elution buffer resulted in separation of these impurities from the pyrimidine deoxyribonucleoside 5′-thiotriphosphates.

The products **5** were isolated by chromatography on DEAE-Sephadex in 60–75% yield and identified by ^{31}P NMR spectroscopy and reverse-phase HPLC by comparison with authentic material.[1] Obviously this reaction sequence yields a mixture of diastereomers of NTPαS and

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dNTPαS, but these can easily be separated preparatively with the same reverse-phase HPLC system used analytically as described in the Materials and Methods section. In addition to the desired product and small amounts of unreacted nucleoside, two byproducts, chromatographically well separated from the nucleoside thiotriphosphates, were isolated. One was identified as the nucleoside 5′-H-phosphonate resulting from hydrolysis of **2**, the other as the dinucleoside 5′,5′-phosphorothioate with a chemical shift of 56.6 ppm for the dithymidine derivative, presumably formed by reaction of unreacted **1** with **2**. Similar reactivity as of **2** has been reported for nucleoside 5′-diacylphosphites.[10] Replacement of pyrophosphate by phosphate in the reaction with **2** yields the nucleoside 5′-O-(1-thiodiphosphate) although at lower yield and contaminated by the nucleoside 5′-O-(1-thiotriphosphate). Attempts to increase the ratio of thiodi- to thiotriphosphate by employing a smaller excess of phosphate in the reaction were unsuccessful. The elucidation of the mechanism leading to the formation of the thiotriphosphate requires further study.

The successful synthesis of the two diastereomers of 2′,3′-cAMPS and 2′,3′-cCMPS reported here shows that reaction of ribonucleosides with salicyl phosphorochloridite represents an improvement over existing methods for the synthesis of such cyclic phosphorothioates. The yields obtained are moderate, yet better than those previously reported for the synthesis of the uridine[11] and guanosine[12] derivatives, but no effort has been made to optimize the reaction conditions. The assignment of the absolute configuration of the two diastereomers of the two examples given rests on the X-ray structural analysis of the endo isomer of uridine 2′,3′-cyclic phosphorothioate[13] and the virtual identity of the ^{31}P NMR chemical shifts of these isomers with those of uridine 2′,3′-cyclic phosphorothioate.

It has been reported by Imai and Torrence[14] that bis-(2,2,2-trichloroethyl) phosphorochloridite selectively reacts at the sugar hydroxyls of nucleosides as long as the reaction is carried out at −78 °C. We, therefore, initially assumed that the reaction with salicyl phosphorochloridite would require the protection of the base NH_2 functions if the reaction was to be performed at room temperature. However, when working with the ribonucleoside derivatives as **1e,f,h**, where only the vicinal hydroxyl groups and not the bases had been protected, no product indicating phosphitylation at any of the base functional groups could be detected either by ^{31}P NMR spectroscopy or HPLC. Thus, this method does not require protection of the functional groups of the nucleobases. We have not investigated whether the selectivity for the hydroxyl group in this reaction is kinetically or thermodynamically controlled. Barone et al.[15] had observed in a related system that a phosphoramidite group can be rearranged in a slow process from the base to the sugar. As our phosphitylation reaction is finished in less than 10 min, we assume that the occurrence of such a rearrangement is not the determining factor in the selectivity we observe.

In an attempt to determine whether the phosphitylating agent might selectively react with the nucleoside 5′-hydroxyl group, we carried out the phosphitylation reaction with unprotected deoxyadenosine in a mixture of DMF and pyridine at −78 °C. However, after hydrolysis the ^{31}P NMR spectrum indicated only a small preference for the nucleoside 5′-H-phosphonate.

Table I. Chromatographic Data

	HPLC retention times					TLC (R_f values)	
	S_p			R_p			
	min	%		min	%	system I	system II
dATPαS (**5a**)	9.02	50.7		9.96	49.2	0.60	0.17
dGTPαS (**5b**)	8.41	49.8		8.98	50.2	0.14	0.16
TTPαS (**5c**)	9.87	47.6		10.77	52.3	0.45	0.16
TDPαS	9.13	48.8		9.81	51.2	0.81	0.33
dCTPαS (**5d**)	5.10	49.8		5.92	50.2	0.25	0.16
ATPαS (**5e**)	8.56	49.8		9.86	50.1	0.48	0.18
GTPαS (**5f**)	6.93	51.6		8.12	48.3	0.13	0.11
UTPαS (**5g**)	7.61	51.2		8.70	48.7	0.17	0.11
CTPαS (**5h**)	6.41	49.9		7.41	50.1	0.21	0.13
cAMPS (exo)	10.94	42.6	(endo)	13.93	53.8		
cCMPS (exo)	4.24	41.1	(endo)	5.62	41.3		

The method described here represents a very efficient synthesis of deoxyribonucleoside and nucleoside 5′-O-(1-thiotriphosphates) that has certain advantages over existing methods. It is a rapid one-pot reaction that does not require protection of the nucleobases. No solubility problems are encountered and the very simple introduction of sulfur makes it well suited for the synthesis of the ^{35}S-labeled compounds. The oxidation with sulfur can probably also be performed with the sulfur dissolved in a mixture of CS_2/pyridine as described for the synthesis of oligonucleotides.[16,17]

The examples reported here show the wide applicability of salicyl phosphorochloridite in synthetic nucleotide chemistry, which can probably be extended to other alcohols as well as to pyrophosphate analogues such as the imino and methylene analogues. In fact, in addition to the synthesis of TTP we have also prepared the triphosphates of 3′-azido-2′,3′-dideoxy- and 3′-fluoro-2′,3′-dideoxythymidine[18] by this method in approximately 65% yield.

Experimental Section

Materials and Methods. The phosphitylating agent 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one was either purchased from Aldrich or prepared according to Cade and Gerrard[6] and was stored under argon. Sulfur (sublimed) was from Merck Darmstadt and dried before use in a desiccator over P_2O_5. Dry dioxane and pyridine (containing less than 0.01% water) were also from Merck Darmstadt and were used as supplied. DMF and tri-*n*-butylamine were obtained from Fluka. All solvents were stored over 4-Å molecular sieves.

2′,3′-Diacetyladenosine and 2′,3′-diacetylguanosine were purchased from Sigma. N^6-Benzoyl-2′-deoxyadenosine, N^4-benzoyl-2′-deoxycytidine, and N^2-isobutyryl-2′-deoxyguanosine were synthesized by the transient protection method.[19] These compounds were reacted with dimethoxytrityl chloride by standard procedures and the products isolated by chromatography on silica gel. The protected 2′-deoxynucleosides were prepared by reaction with methoxyacetic anhydride and subsequent removal of the dimethoxytrityl group as reported for the deoxyadenosine derivative.[20] 3′-Acetylthymidine was synthesized according to Verheyden and Moffatt.[21] 2′,3′-Methoxymethylidene uridine and

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Table II. ^{31}P NMR Spectral Data

		chemical shifts, ppm			coupling constants, Hz	
		P_α	P_β	P_γ	$J_{\alpha\beta}$	$J_{\beta\gamma}$
dATPαS (**5a**)	S_p	44.40 (d)	−23.06 (m)	−9.04 (d)	27.50	19.74
	R_p	43.75 (d)	−23.18 (m)	−9.04 (d)	28.20	19.74
dGTPαS (**5b**)	S_p	43.86 (d)	−23.06 (q)	−8.31 (d)	27.89	20.21
	R_p	43.59 (d)	−23.08 (q)	−8.31 (d)	28.41	20.21
TTPαS (**5c**)	S_p	43.82 (d)	−23.21 (q)	−9.18 (d)	28.06	19.92
	R_p	43.39 (d)	−23.25 (q)	−9.18 (d)	29.04	19.92
TDPαS	S_p	41.99 (d)	−7.10 (d)	-	30.20	-
	R_p	41.45 (d)	−7.10 (d)	-	31.85	-
dCTPαS (**5d**)	S_p	43.59 (d)	−22.70 (q)	−6.18 (d)	27.49	20.51
	R_p	43.35 (d)	−22.74 (q)	−6.81 (d)	28.41	20.51
ATPαS (**5e**)	S_p	44.01 (d)	−23.39 (q)	−10.03 (d)	27.75	19.73
	R_p	43.83 (d)	−23.43 (q)	−10.03 (d)	28.67	19.73
GTPαS (**5f**)	S_p	43.94 (d)	−23.27 (q)	−9.82 (d)	27.93	19.83
	R_p	43.74 (d)	−23.55 (q)	−9.82 (d)	28.58	19.83
UTPαS (**5g**)	S_p	43.76 (d)	−22.46 (m)	−7.79 (d)	23.08	19.29
	R_p	43.54 (d)	−22.88 (m)	−7.79 (d)	23.34	19.29
CTPαS (**5h**)	S_p	43.93 (d)	−23.30 (q)	−10.08 (d)	27.64	19.64
	R_p	43.79 (d)	−23.58 (q)	−10.08 (d)	28.34	19.67
ethyl TPαS (**9**)		43.49 (d)	−23.49 (dd)	−10.01 (d)	29.59	19.59

cytidine were prepared as described.[22] 5′-Acetyladenosine[23] and O^5,N^4-diacetylcytidine[24] were prepared from the corresponding 2′,3′-methoxymethylidene nucleosides[22] by acetylation with acetic anhydride in pyridine/DMF for 3 h at room temperature and subsequent hydrolysis of the product mixture with 60% formic acid.[22] The desired compounds were isolated in approximately 50% yield after chromatography on SiO_2.

^{31}P NMR spectra were recorded on a Bruker WP200SY spectrometer operating at 81.01 Hz with broad band decoupling. Spectra of intermediates were recorded by addition of DMF-d_7 or dioxane-d_8 to the reaction solution. For recording the spectra of the chromatographed products, 10 mM aqueous solutions of the triethylammonium salts were prepared containing 20% D_2O and approximately 10 mM EDTA pH 8. Samples were transferred to 5-mm tubes with a concentric capillary containing 80% phosphoric acid as external standard. Chemical shifts are given in ppm and are positive when downfield from the reference.

TLC was performed on either HPTLC Fertigplatten Si 5000F 254S (Merck Darmstadt) developed with 2-propanol/NH_3/H_2O (7:1:2, v/v) (system I) or on Kieselgel 60 Platten (Merck Darmstadt) eluted with 1-propanol/NH_4OH/H_2O (11:7:2, v/v) (system II). Reverse-phase HPLC was performed with a Waters dual-pump 6000A system in combination with a Waters 680 gradient controller and a Model 440 UV detector operating at 254 nm. Columns were packed with ODS Hypersil (5 μm, from Shandon Southern, Runcon, UK) and were eluted with 100 mM triethylammonium bicarbonate (TEAB), pH 7.5, containing a linear gradient of acetonitrile from 0% to 15% in 20 min.

Chromatography on DEAE-Sephadex was carried out at 4 °C. Fractions containing product were combined and evaporated to dryness on a rotary evaporator and the residue was coevaporated with methanol to remove traces of buffer.

Bis(tri-*n*-butylammonium) pyrophosphate was prepared according to Moffatt[25] as follows: Tetrasodium diphosphate decahydrate (2.23 g, 5 mmol) was dissolved in water (50 mL), the solution was applied to a column of Dowex 50WX8 in the H^+ form, and the column was washed with water. The eluate was directly dropped into a cooled (ice water) and stirred solution of tri-*n*-butylamine (2.38 mL, 10 mmol) in ethanol (20 mL). The column was washed until the pH of the eluate increased to 5.0 (approximately 70 mL of water). The ethanol/water solution was evaporated to dryness and reevaporated twice with ethanol and finally with anhydrous DMF (30 mL) on an oil pump. The residue was dissolved in DMF and diluted to 10 mL. This slightly yellow solution was stored over 4-Å molecular sieves.

Deoxyribonucleoside and Ribonucleoside 5′-*O*-(1-Thiotriphosphates) 5a–h. One of the protected nucleosides [N^6-benzoyl-3′-(methoxyacetyl)-2′-deoxyadenosine (**1a**), N^2-isobutyryl-3′-(methoxyacetyl)-2′-deoxyguanosine (**1b**), 2′,3′-diacetyladenosine (**1e**), 2′,3′-diacetylguanosine (**1f**), 3′-acetylthymidine (**1c**), N^4-benzoyl-3′-(methoxyacetyl)-2′-deoxycytidine (**1d**), (2′,3′-(methoxymethylidene)uridine (**1g**), 2′,3′-(methoxymethylidene)cytidine (**1h**)] (100 μmol) was dissolved in anhydrous pyridine (2 mL) (or pyridine/DMF, 1/4, v/v for **1f**) and evaporated to dryness in vacuo. The residue was dried further over P_2O_5 under vacuum for 1 h at room temperature. The reaction flask was filled with argon by introducing the gas into the desiccator and closed with a rubber septum. During all the following manipulations a small positive pressure of argon was maintained in the reaction vessel by connecting it with an argon-filled balloon. Anhydrous pyridine (100 μL) was injected through the septum followed by anhydrous dioxane (300 μL) (the volume of each solvent was doubled for reaction with **1d**; 200 μL of pyridine and 800 μL of DMF were used for reactions with **1f** and **1h**). A freshly prepared 1 M solution of 2-chloro-4*H*-1,2,3-dioxaphosphorin-4-one in anhydrous dioxane (110 μL, 110 μmol; but only 100 μL for reactions with **1c** and **1d**) was then injected into the well-stirred solution of the nucleoside. A white precipitate was formed when DMF was omitted from the solution. After 10 min a well-vortexed mixture of a 0.5 M solution of bis(tri-*n*-butylammonium) pyrophosphate in anhydrous DMF (300 μL) and tri-*n*-butylamine (100 μL) was quickly injected. The white precipitate immediately dissolved and 10 min later a suspension of sulfur (6.4 mg, 200 μmol) in DMF (200 μL) followed by an additional 100 μL of DMF to ensure complete transfer of sulfur was injected. After stirring for 10 min, the septum was removed and water (5 mL) added. After 30 min the reaction mixture was evaporated to dryness and the residue dissolved in concentrated ammonia (20 mL) for **4a**, **4b**, **4c**, **4d**, **4e**, and **4f**. Ammonolysis was carried out for 5 h at 60 °C for **4a**, **4b**, and **4d** and for 2 h at room temperature for **4c**, **4e**, and **4f**. For removal of the methoxymethylidene groups of **4g** and **4h** the residue was dissolved in water (25 mL) and glacial acetic acid (5 mL) to give a solution of pH 2.2. After reaction for 1 h at room temperature, the solution was evaporated to dryness, the residue dissolved in water (10 mL), and the pH of the solution adjusted to approximately 9 with ammonia and left at room temperature for 30 min.

These reaction solutions were then evaporated to dryness, the residue dissolved in water (5 mL), and the solution applied to a DEAE-Sephadex A-25 column (2 × 30 cm). Chromatography was performed with a linear gradient of 1 L each of 0.05 M and 1 M TEAB for the purine derivatives **5a**, **5b**, **5e**, and **5f** and of 0.4 and 0.8 M for the pyrimidine derivatives **5c**, **5d**, **5g**, and **5h**. Fractions of 17 mL were collected. The purine derivatives were eluted between 0.7 and 0.8 M buffer and the pyrimidine derivatives between 0.55 and 0.65 M. Fractions containing product were combined and evaporated to dryness on a rotary evaporator and the residue coevaporated with methanol to remove traces of buffer.

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Yields were between 60% and 75%. HPLC, TLC, and NMR characteristics are summarized in Tables I and II.

Ethyl 1-Thiotriphosphate (9). P^1-Ethyl P^2,P^3-Dioxocyclotriphophite (7). Dry ethyl alcohol (100 μmol) was reacted with 100 μmol of salicyl phosphorochloridite and pyrophosphate as described for the reaction with nucleosides. After reaction for 10 min with pyrophosphate, 200 μL of DMF-d_7 was added to the reaction solution, the solution transferred to a NMR tube that previously had been flushed with argon, and the ^{31}P NMR spectrum recorded (see Figure 1B).

Ethyl 1-Thiocyclotriphosphate (8). Addition of sulfur (6.4 mg, 200 μmol) to the above reaction solution resulted in the appearance of new peaks in the ^{31}P NMR spectrum as follows: δ(A) = 44.21 ppm, t (1 P); δ(B) = −23.01 ppm, d (2 P); J_{AB} = 34.44 Hz.

Ethyl 1-Thiotriphosphate (9). Water was added to the above reaction solution, which was evaporated to dryness after 1 h. The residue was dissolved in D_2O and the ^{31}P NMR spectrum recorded (see Table II).

Thymidine 5′-Triphosphate (6c). 3′-Acetylthymidine (1c) (100 μmol) was phosphitylated as described above. Pyrophosphate and tri-*n*-butylamine were added and the reaction mixture was stirred for 10 min. A solution of 1% iodine in pyridine/water (98/2, v/v) (2 mL, 157 μmol) was then added. After 15 min excess iodine was destroyed by adding a few drops of a 5% aqueous solution of $NaHSO_3$ and the reaction solution evaporated to dryness. The residue was dissolved in water (10 mL). After standing at room temperature for 30 min, concentrated ammonia (20 mL) was added. After 1 h the solution was evaporated to dryness, the residue dissolved in water, and the solution applied to a DEAE Sephadex column, which was eluted with a linear gradient of 800 mL each of 0.05 M and 1 M TEAB. The product was eluted between 0.50 and 0.55 M buffer, yield 72%. The product was compared by HPLC and ^{31}P NMR with a commercial sample.

Thymidine 5′-*O*-(1-Thiodiphosphate) (TDPαS). 3′-Acetylthymidine (1c) (100 μmol) was phosphitylated as described above for the synthesis of TTPαS. Instead of pyrophosphate a mixture of 0.5 M tri-*n*-butylammonium orthophosphate in anhydrous DMF (500 μL) and tri-*n*-butylamine (100 μL) was added. After addition of sulfur and removal of the protecting group, the reaction mixture was chromatographed on a DEAE-Sephadex column using a linear gradient of 800 mL each of 0.05 M and 0.8 M TEAB. TDPαS (yield 23%) was eluted between 0.52 and 0.55 M buffer and TTPαS (yield 22%) between 0.56 and 0.62 M buffer. HPLC, TLC, and NMR data are summarized in Tables I and II.

Adenosine and Cytidine 2′,3′-Cyclophosphorothioates. 5′-Acetyladenosine or 5′-*O*-,N^4-diacetylcytidine (100 μmol) was reacted with salicyl phosphorochloridite as described for the synthesis of the nucleoside thiotriphosphates. After being stirred for 5 min, a mixture of 0.3 mL of DMF and 0.1 mL of tri-*n*-butylamine was injected. After a further 10 min, a suspension of 10 mg of sulfur in 0.2 mL of DMF was added. After stirring for 30 min, 2 mL of a mixture of H_2O/dioxane (1:3, v/v) was added and the solution evaporated. The residue was dissolved in 10 mL of 25% aqueous ammonia. After reaction for 1 h at room tempeature for the reaction with 5′-acetyladenosine and reaction for 1.5 h for that with 5′-*O*,N^4-diacetylcytidine, the solution was evaporated to dryness, the residue dissolved in 5 mL of H_2O, any sulfur removed by filtration and the solution chromatographed on a DEAE-Sephadex column (2 × 30 cm) with a linear gradient of 1 L each of 0.05 and 0.4 M TEAB. The diastereomers of adenosine 2′,3′-cyclophosphorothioate eluted between 0.23 and 0.26 M buffer and those of cytidine 2′,3′-cyclophosphorothioate between 0.16 and 0.20 M buffer. Yield of 2′,3′ cAMPS 32%, of 2′,3′ cCMPS 45%. ^{31}P NMR (H_2O): δ 77.26 (exo isomer, S_p configuration) and 75.69 ppm (endo isomer, R_p configuration) for 2′,3′-cAMPS and 77.03 (exo isomer, S_p configuration) and 75.72 ppm (endo isomer, R_p configuration) for 2′,3′-cCMPS. HPLC data are in Table I.

Acknowledgment. We thank G. Gish, D. B. Olsen, and J. Sayers for help in the preparation of the manuscript.

Registry No. **1a**, 25152-96-9; **1b**, 118275-88-0; **1c**, 21090-30-2; **1d**, 96666-99-8; **1e**, 29886-19-9; **1f**, 42167-65-7; **1g**, 16628-81-2; **1h**, 16628-82-3; **4a**, 118275-89-1; **4b**, 118275-90-4; **4c**, 118275-91-5; **4d**, 118275-92-6; **4e**, 118275-93-7; **4f**, 118275-94-8; **4g**, 118275-95-9; **4h**, 118275-96-0; (R_p)-**5a**, 87358-15-4; (S_p)-**5a**, 80875-87-2; (R_p)-**5b**, 80902-29-0; (S_p)-**5b**, 80902-28-9; (R_p)-**5c**, 83199-35-3; (S_p)-**5c**, 83199-32-0; (R_p)-**5d**, 80951-76-4; (S_p)-5d, 80951-75-3; (R_p)-**5e**, 58976-49-1; (S_p)-**5e**, 58976-48-0; (R_p)-**5f**, 81570-50-5; (S_p)-**5f**, 81570-51-6; (R_p)-**5g**, 71214-30-7; (S_p)-**5g**, 71214-29-4; (R_p)-**5r**, 118374-59-7; (S_p)-**5h**, 118353-34-7; **6c**, 365-08-2; **7**, 118297-34-0; **8**, 118275-97-1; **9**, 118275-98-2; (R_p)-2′,3′-cAMPS, 118275-99-3; (S_p)-2′,3′-cAMPS, 118276-00-9; (R_p)-2′,3′-cCMPS, 118276-01-0; (S_p)-2′,3′-cCMPS, 118276-02-1; TDPαs, 81811-21-4; 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one, 5381-99-7; bis(tri-*n*-butylammonium)pyrophosphate, 5975-18-8; 5′-acetyladenosine, 2140-25-2; 5′-*O*,N^+-diacetylcytidine, 6554-13-8.

Products from Reactions of Methyl (*E*)-2-Cyano-3-(*p*-substituted-phenyl)acrylates with 1-Phenyldiazoethane and Cycloreversion of Secondary Pyrazolines

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Received August 27, 1987

In dichloromethane, the reaction of methyl (*E*)-2-cyano-3-(*p*-substituted-phenyl)acrylates (**2**) with 1-phenyldiazoethane (**1**), prepared from 2 molar equiv of acetophenone hydrazone, produced methyl 2-cyano-4-phenyl-4-(*p*-substituted-phenyl)-2-pentenoates (**3**), 1-cyano-1-(methoxycarbonyl)-2-methyl-2-phenyl-3-(*p*-substituted-phenyl)cyclopropanes (**4**), and 4-cyano-4-(methoxycarbonyl)-3-methyl-3-phenyl-5-(1-phenyl-1-(*p*-substituted-phenyl)ethyl)-1-pyrazolines (**5**). Compounds **5** were found to be derived from compounds **3** and **1**. Compounds **5** were different from the primary pyrazolines derived from compounds **1** and **2**, both in mode of formation and properties. Compounds **5**, when decomposed thermally or photochemically, produced the initial olefins **3**. This decomposition is an example of "true" cycloreversion.

The 1,3-dipolar cycloadditions of diazomethane derivatives to olefins activated by electron-attracting groups have been extensively studied, especially in connection with the preparation of cyclopropane derivatives.[1] In a previous paper,[2] we reported that the reaction of 1,1-disubstituted olefins containing electron-attracting substituents with 1-phenyldiazoethane produced both cyclopropane and olefin derivatives. In the reactions with 2-

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