Bis(2,2,2-trichloroethyl) Phosphorochloridite as a Reagent for the Phosphorylation of Oligonucleotides: Preparation of 5'-Phosphorylated 2',5'-Oligoadenylates

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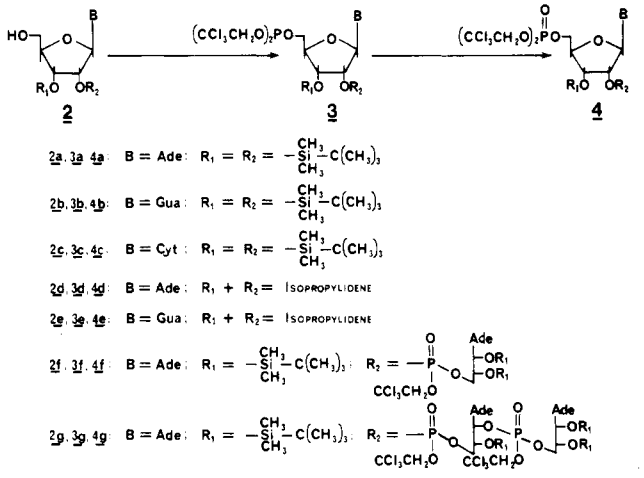
Bis(2,2,2-trichloroethyl) phosphorochloridite was found to be a useful reagent for the phosphorylation of protected nucleosides and oligonucleotides especially when the phosphate blocking group was 2,2,2-trichloroethyl and the hydroxyl protecting group was *tert*-butyldimethylsilyl. Thus compounds **2a-c,f,g** could be phosphorylated to the corresponding 5'-phosphotriesters **4a-c,f,g** in yields of 75-99%. Removal of the protecting groups (to give the 5'-monophosphates **6a-c,f,g**) was achieved by zinc-copper couple/2,4-pentanedione/DMF treatment to remove the 2,2,2-trichloroethyl group and tetrabutylammonium fluoride/THF treatment to remove the *tert*-butyldimethylsilyl groups. A method was found that permits preparation of reproducibly active zinc-copper couple. As a hydroxyl protecting group, the isopropylidene moiety was somewhat less useful in conjunction with the use of bis(2,2,2-trichloroethyl) phosphorochloridite. Thus, upon phosphorylation of **2d** and **2e**, the phosphotriesters **4d** and **4e** were obtained in yields of 83% and 61%, respectively. Deblocking of the isopropylidene groups was accomplished with formic acid at room temperature to give **6a** and **6b** in yields of 74% and 70%, respectively. The 5'-phosphorylated 2'-5'-linked oligonucleotides **6f** and **6g** were converted to the corresponding 5'-triphosphates to give compounds **1a** and **1b** which are found in extracts of interferon-treated cells upon incubation with double-stranded RNA.

In the past several years, the popularity of the phosphotriester[1-3] method of oligonucleotide synthesis has increased dramatically.[4] One approach to this phosphotriester method has been introduced and developed by Letsinger and Lunsford[5] and Ogilvie and associates.[6] This method relies upon the *tert*-butyldimethylsilyl moiety as a hydroxyl protecting group,[7] 2,2,2-trichloroethyl phosphorodichloridite[5,8] to effect internucleotide bond formation, and, as a consequence, the 2,2,2-trichloroethyl group for protection of the internucleotidic phosphite (or phosphate) moieties.[5,6,9] A significant advantage of this procedure is that no base amino protecting group is required.

Coincident with increased use of the phosphotriester methodology has been th development of a large number of phosphorylating reagents with a variety of phosphate protecting groups.[4,10] Many of these reagents were specifically designed for the introduction of an internucleotide linkage, whereas relatively few methods have been reported as suitable for the direct introduction of a phosphate moiety at the 5'-terminus of an oligoribonucleotide. Such 5'-phosphorylated oligonucleotides are of considerable interest for at least the following reasons: 5'-phosphorylated oligonucleotides may be required for ligase reactions or physicochemical studies; native tRNA is a 5'-phosphorylated molecule; the unique 2',5'-oligoadenylates associated with the interferon-induced antiviral

Scheme I



state are also 5'-phosphorylated.[11] A number of procedures have been described[12] as suitable for 5'-phosphorylation; however, many of them require protection of the base amino group (of cytidine, adenosine, or guanosine) to avoid phosphoramide formation.

We have sought a reagent that may be suitable for the 5'-phosphorylation of protected oligonucleotides which have been prepared via the phosphite method and which bear the 2,2,2-trichloroethyl protecting group but possess no heterocyclic base protecting groups. Such a reagent is bis(2,2,2-trichloroethyl) phosphorochloridite [bis(2,2,2-trichloroethyl) chlorophosphite, BTEP, $(Cl_3CCH_2O)_2PCl$]. We describe here its application to the phosphorylation

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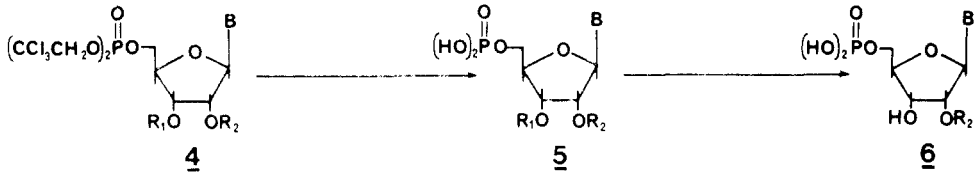
Scheme II



4 5 6

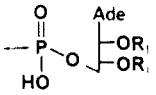
5a: B = Ade; $R_1 = R_2 = -\overset{CH_3}{\underset{CH_3}{Si}}-C(CH_3)_3$ 6a: B = Ade; R_2 = H

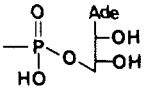
5b: B = Gua; $R_1 = R_2 = -\overset{CH_3}{\underset{CH_3}{Si}}-C(CH_3)_3$ 6b: B = Gua; R_2 = H

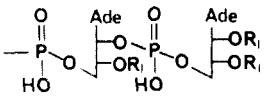
5c: B = Cyt; $R_1 = R_2 = -\overset{CH_3}{\underset{CH_3}{Si}}-C(CH_3)_3$ 6c: B = Cyt; R_2 = H

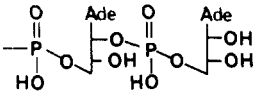
5d: B = Ade; $R_1 + R_2$ = Isopropylidene

5e: B = Gua; $R_1 + R_2$ = Isopropylidene

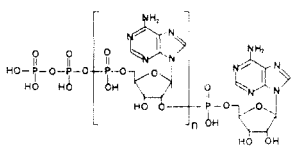
5f: B = Ade; $R_1 = -\overset{CH_3}{\underset{CH_3}{Si}}-C(CH_3)_3$, R_2 = 

6f: B = Ade; R_2 = 

5g: B = Ade; $R_1 = -\overset{CH_3}{\underset{CH_3}{Si}}-C(CH_3)_3$, R_2 = 

6g: B = Ade; R_2 = 

of various nucleosides as well as 2′,5′-linked oligoribonucleotides which serve as precursors to the unusual 5′-*O*-triphosphoadenylyl-(2′→5′)-adenylyl-(2′→5′)-adenosine (2-5A, **1b**).[11]



1a: n = 1
1b: n = 2

Results and Discussion

Phosphorylation of Protected Nucleosides and Oligoribonucleotides with Bis(2,2,2-trichloroethyl) Phosphorochloridite (BTEP; Scheme I, Table I). BTEP was examined as a reagent for the phosphorylation of seven different protected nucleosides and oligoribonucleotides in dry THF with pulverized molecular sieves as an acid scavenger at a temperature of −78 °C. When the *tert*-butyldimethylsilyl group was used to protect the 2′- and 3′-hydroxyl functions of either adenosine (**2a**), cytidine (**2b**), or guanosine (**2c**), excellent yields of the corresponding 5′-phosphorylated products (**4a–c**) were obtained after iodine oxidation of the intermediate phosphite triesters (**3a–c**). The quantitative yield obtained for the case of the guanosine derivative (**2c** → **4c**) is especially noteworthy since of all the common nucleosides, guanosine often gives rise to the greatest difficulties.[13] When the 2′,3′-isopropylidene derivatives of adenosine (**2d**) or guanosine (**2e**) were phosphorylated with BTEP and then oxidized to the phosphotriesters with iodine, the yield of products (**4d,e**) was only moderate to good (Table I). The relative lack of success of the isopropylidene protecting group in this context may be related to its instability to the potential acidity that may be generated during the phosphorylation reaction, even though this acidity is presumably minimized by the added pulverized molecular sieves. Phosphorylation of the appropriately protected oligoribonucleotides, **2f** or **2g**, also led to high yields of 5′-phosphotriesters (**4f,g**).

One advantage of the use of BTEP is that no protecting group need be used for the amino group of the heterocyclic base (adenine, cytosine, or guanine). It is, however, critical that the phosphorylation reaction should be performed at −78 °C since when the reaction is carried out at ambient temperature, considerable N-phosphorylation can occur. For example, the reaction of 2′,3′-*O*-bis(*tert*-butyldimethylsilyl)adenosine (**2a**) with BTEP at room temperature gave a 1:1 mixture of two products as determined by TLC. The product with the higher R_f value (0.9, system B) was quite labile to mild (0.01 N HCl) acid treatment. Its proton NMR spectrum showed two doublets (each four protons) at approximately 4.5 ppm which could be assigned as the methylene protons of the 2,2,2-trichloroethyl groups; in addition, this compound gave a broad singlet (~7.1 ppm) which integrated to one proton and corresponded to the adenine 6-amino proton. The product with the lower R_f value (0.4, system B) was stable to mild acid treatment, and its NMR showed methylene signals at about 4.5 ppm with half the intensity (R_f ~ 0.9) of the former compound. Both of these products gave the same product (**4a**) upon iodine oxidation; however, the N-phosphorylated material also gave rise to an additional unidentified product. When the same phosphorylation reaction with BTEP was carried out with **2a** at −78 °C, the product of higher R_f was no longer generated, and the product of low R_f (**3a**) was isolated in 98% yield. Thus, the use of low temperature for the phosphorylation reaction leads to higher reaction yields and also simplifies

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Table I. Phosphorylation of Protected Nucleotides and Oligonucleotides with Bis(2,2,2-trichloroethyl) Phosphorochloridite

substrate	product[a]	yield, %	mp, °C	R_f[b]	^{31}P NMR[c]	^{1}H NMR,[c] δ									
						sugar			base			protecting group			
						H1′	H2′	H3′	H2	H8	NH_2	*t*-Bu	CH_3[e]	CH_3[f]	Cl_3CH_2[g]
2a	**4a**	92	174–176	0.56	−4.22	5 92 (d, J = 4.5)	5.03 (t, J = 4.5)		8.01 (s)	8.36 (s)	6.18 (br s)	0.85 (s, 9 H), 0.98 (s, 9 H)	−0.18 (s, 3 H), 0.02 (s, 3 H), 0.18 (s, 6 H)		4.66 (d, J = 7), 4.93 (d, J = 7)
2b	**4b**	99	191–191.5	0.32	−4.28	5.72 (d, J = 7)	4.90 (dd, J = 5, 7)	4.52 (t, J = 5)		7.65 (s)		0.80 (s, 9 H), 0.93 (s, 9 H)	−0.26 (s, 3 H), −0.04 (s, 3 H), −0.13 (s, 6 H)		4.59 (d, J = 7), 4.63 (d, J = 7)
2c	**4c**	94	98–100	0.34	−4.74	5 59 (br s)			5.83 (d, J = 7,[d] H5)	7.65 (d, J = 7,[d] H6)		0.91 (s, 18 H)	0.09 (s, 6 H), 0.11 (s, 3 H), 0.19 (s, 3 H)		4.66 (d, J = 7, 4 H)
2d	**4d**	83	150–152	0.39	−4.42	6.15 (d, J = 1.5)	5.46 (dd, J = 6, 1.5)	5.18 (dd, J = 3, 6)	7.94 (s)	8.33 (s)	6.41 (br s)			1.40 (s), 1.62 (s),	
2e	**4e**	61	225–231	0.22	−4.46	5.98 (br s)	5.35 (dd, J = 7, 3)	5.28 (d, J = 7)		7.69 (s)	6.82 (br s)			1.32 (s), 1.53 (s)	4.60 (d, J = 7), 4.68 (d, J = 7)
2f	**4f**	91		0.31	−4.37, −2.75	5.86 (d, J = 4.5), 6.16 (d, J = 4)	4.95 (m), 5.76 (m)		8.01 (s, 2 H)	8.25 (s), 8.27 (s)	6.52 (br s, 4 H)	0.76 (s, 9 H), 0.91 (s, 18 H)	−0.21 (s, 3 H), −0.02 (s, 3 H), 0.07 (s, 3 H), 0.09 (s, 6 H), 0.17 (s, 3 H)		4.52 (d, J = 6.5), 4.70 (d, J = 6.5)
2g	**4g**	75		0.17	−4.76, −3.30, −3.00	5.85 (d, J = 3.7	4.93 (m), 5.58 (m, 2 H)		7.96 (s), 8.01 (s), 8.02 (s)	8.24 (s, 2 H), 8.28 (s)	6.12 (br m, 6 H)	0.79 (s, 9 H), 0.88 (s, 27 H)			

[a] Elemental analysis. **4a**: calcd for $C_{26}H_{44}O_7N_5Si_3Cl_6P$: C, 37.24; H, 5.29; N, 8.35; Cl, 25.37; P, 3.69. Found: C, 37.10; H, −0.39; N, 8.05; Cl, 25.51; P, 3.64. **4b**: Calcd for $C_{26}H_{44}O_8N_5Si_2Cl_6P$: C, 36.54; H, 5.19; N, 8.20; Cl, 24.90; P, 3.51. Found: C, 36.85; H, 4.93; N, 8.01; Cl, 24.8; P, 3.43. **4c**: Calcd for $C_{25}H_{44}O_8N_3Si_2Cl_6P$: C, 36.86; H, 5.45; N, 5.16; Cl, 26.12; P, 3.80. Found: C, 37.20; H, 5.42; N, 4.74; Cl, 26.06; P, 3.91. **4d**: Calcd for $C_{17}H_{20}O_7N_5Cl_6P$: C, 31.41; H, 3.10; N, 10.77; Cl, 32.72; P, 4.77. Found: C, 31.43; H, 3.22; N, 9.93; Cl, 32.64; P, 5.18. **4e**: Calcd for $C_{17}H_{20}O_8N_5Cl_6P$: C, 30.65; H, 3.03; N, 10.52; Cl, 31.94; P, 4.65. Found: C, 31.25; H, 2.71; N, 10.07; Cl, 31.31; P, 4.48. [b] R_f values were determined on silica gel TLC with solvent system A [c] In parts per million. J values are given in hertz. For ^{1}H NMR data, only the well-resolved and characteristic signals are reported. [d] For compound **4c** the base portions are H5 and H6 of the cytidine ring. [e] CH_3's of the *tert*-butyldimethylsilyl group. [f] CH_3's of the isopropylidene group. [g] Long-range phosphorus-hydrogen coupling.

Table II. Deblocking of Protected Nucleotides and Oligonucleotides

protected nucleotide or oligonucleotide		step 1, removal of Cl_3Et group					step 2, removal of silyl groups		product				
							0.7 M					R_f[a]	
compd	amt, μmol	Zn-Cu, mmol	acac, mmol	DMF, mL	time, h	temp, °C	TBAF, mL	time, h	compd	amt, μmol	% yield	B	C
4a	50.0	1.0	0.5	1.0	1	55	3.75	2	6a	46.3	93	0.47	0.62
4b	25.0	0.5	0.25	0.5	2	55	1.9	2	6b	23.5	94	0.31	0.58
4c	3.3	0.66	0.33	0.66	1	55	2.5	2	6c	29.0	88	0.59	0.81
4f	5.2	1.56	0.78	0.10	1.5	60	1.0	16	6f	4.3	83	0.32	0.65
4g	5.0	2.0	1.0	0.15	4.5	60	1.2	16	6g	3.75	75	0.17	0.65
4d	28.0	0.56	0.28	0.56	4	60	0.2[b,c]	18[b]	6a	20.6	74		
4e	28.0	0.56	0.28	0.56	4	60	0.2[b,c]	18[b]	6b	19.5	70		

[a] As determined on PEI plates at 20 °C. 4a-g all had R_f's identical with those of authentic samples. [b] Temperature of 25 °C. Values for the removal of the isopropylidene group at 25 °C. [c] For 88% HCOOH.

purification of intermediates by preventing phosphoramidate formation.

Removal of the 2,2,2-Trichloroethyl Group (Table II, Scheme II). Since its introduction by Eckstein,[14] the 2,2,2-trichloroethyl group has been widely employed as a phosphate protecting group; however, one occasional problem associated with its use is difficulty in its quantitative removal. In addition to the orthodox methods to remove this group by reductive elimination with zinc or zinc–copper couples,[15] some interesting attempts at its removal have been reported with reagents such as radical anions, fluoride anion, cobalt/phthalocyanine, zinc/methanol/NH_4Cl, or catalytic hydrogenation over palladium catalyst.[16] We were reluctant to use catalytic hydrogenation methods because such deblocking conditions would not be applicable to reduction-sensitive nucleotides derived from uracil or cytosine. While nucleophilic agents such as fluoride anion seemed to be ideal for removal of the trichloroethyl group since this reagent also deblocks the *tert*-butyldimethylsilyl protecting group, serious cleavage or isomerization of the internucleotidic linkage can occur when fluoride anion is used to deblock a protected nucleoside such as **4g**.[17]

When the more classical method of reductive elimination with zinc or zinc–copper couple was applied to the trichloroethyl blocked intermediates, we observed only variable and partial deblocking. The reaction could not be forced to completion no matter how much excess zinc or zinc–copper couple was added to the reaction mixture. Typically, the reaction would stop just after a white precipitate was deposited from the solution. This precipitate was presumed to be the zinc salt of partially deblocked nucleotide phosphodi- or -monoesters. This precipitate may effectively cover the surface of the metal, thus poisoning it for further reaction. In order to overcome this problem, we added acetylacetone[15b] (2,4-pentanedione) to the reaction to chelate the zinc cation and maintain the surface of the metal in a cleaner state. Under such conditions, the reaction proceeded faster, and no precipitate formed, but the overall yield of the reaction was still rather low (**5a**, ~45%, **5b**, ~34%). The combination of zinc and triisopropylbenzenesulfonic acid in pyridine[15c] also gave rise to a rather low yield of deblocked product (**5a**, 42%; **5b**, 30%).

Conventional zinc–copper couple, prepared by the method of LeGoff,[18] contains approximately 2% copper. We considered the possibility that a new highly active couple might be obtained by increasing the ratio of copper in the couple. Therefore, a series of zinc–copper couples with different nominal copper contents of 3.2%, 6.4%, 9.6%, 16%, and 32% were prepared by treating zinc dust with cupric acetate in hot acetic acid. These were then examined for their ability to remove the trichloroethyl group from the protected nucleotide **4a**. Quantitative deblocking was obtained, with the couples containing either nominally 16% or 32% copper. These couples were gradually dissolved during the reaction with protected nucleotide in a solvent of DMF and acetylacetone to give a blue-green solution. The nearly homogeneous solution then was diluted with water–methanol and freed of divalent copper and zinc cations with Chelex resin. By this method, nearly quantitative yields of deblocked products could be obtained.

Removal of the *tert*-Butyldimethylsilyl Protecting Group (Scheme II, Table II). Facile removal of the *tert*-butyldimethylsilyl protecting group from the ribose hydroxyls could be achieved by the same method used by Ogilvie and co-workers,[6] that is, tetrabutylammonium fluoride in THF. As alluded to above, however, it was critical that the fluoride treatment be subsequent to removal of the trichloroethyl protecting group for the case of the oligonucleotides. Otherwise, considerable phosphodiester bond isomerization took place.

Synthesis of 5′-*O*-Triphosphoadenylyl-(2′→5′)-adenylyl-(2′→5′)-adenosine (2-5A, 1b) and 5′-*O*-Triphosphoadenylyl-(2′→5′)-adenosine (1a). The unusual oligoribonucleotides 5-*O*-triphosphoadenylyl-(2′→5′)-adenosine (**1a**) and 5-*O*-triphosphoadenylyl-(2′→5′)-adenylyl-(2′→5′)-adenosine (2-5A, **1b**) are among a series of 2′,5′-linked oligonucleotides that are synthesized by the enzyme 2′,5′-oligoadenylate synthetase which is induced in several cell lines upon interferon treatment.[11] The trinucleotide triphosphate **1b** is a potent inhibitor of protein synthesis, whereas the dimer triphosphate **1a**, as isolated from biosynthetic sources, has been reported to be devoid of inhibitory activity.

Quite a few of synthetic studies on these 2′,5′-linked oligoribonucleotides have been published recently.[19] A

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number[19f-l] of these have dealt with only the so-called "core" trinucleotide which bears no 5'-terminal phosphate function and which is relatively inactive as an inhibitor of protein synthesis in cell-free systems. We chose **1a** and **1b** as synthetic targets to test the utility of the BTEP approach to introduction of a terminal phosphate group.

The starting materials for the synthesis of **1a** and **1b** were the previously prepared[19h,l] blocked oligonucleotides **2f** and **2g**, respectively. As delineated in Table I, **2f** and **2g** could be converted to the 5'-phosphotriesters **4f** and **4g** in yields of 91% and 75%, respectively. These intermediates, **4f** and **4g**, were deblocked (Table II) to the monophosphates **6f** and **6g**. The 5'-monophosphates were converted, via reaction of the corresponding imidazolidate[20] with pyrophosphate anion, to the 5'-triphosphates **1a** and **1b**. This represents a yield of 34% of **1a** based on protected dimer **2f** and a yield of 20.2% of **1b** based on protected trimer **2g**. When calculated on the basis of the starting blocked monomers used to prepare the appropriately blocker dimer and trimer, this represents an overall yield of 31% for the dimer triphosphate **1a** and an overall yield of 14.2% for the trimer triphosphate **1b**. Clearly, the major limitation of this sequence is the conversion of the monophosphate to the triphosphate, a reaction which proceeds in poor to moderate yield.

The 5'-triphosphate **1b** obtained in this manner was equipotent with an authentic sample of **1b** as an inhibitor of protein synthesis in a system derived from mouse L cells. The 5'-triphosphate of this dimer, **1a**, was without significant biological activity, in accord with findings based on material isolated and fractionated from biosynthetic sources.

Conclusions

As a phosphorylating agent for properly protected oligoribonucleotides, bis(2,2,2-trichloroethyl) phosphorochloridite (BTEP) has the following specific merits: (a) it is readily accessible from trichloroethanol and PCl_3 in the same reaction that gives 2,2,2-trichloroethyl phosphorodichloridite, a reagent used for introduction of internucleotide bonds by the phosphite triester method; (b) no protection of base (cytosine, guanine, or adenine) amino group is required; (c) the resultant triester products are relatively nonpolar and may be easily purified on silica gel; (d) it is perfectly compatible with the other protecting groups, condensation conditions, and deblocking procedures employed in the phosphite triester method of synthesis developed by Letsinger[5] and Ogilvie[6]; (e) the 2,2,2-trichloroethyl protecting group may be readily removed by zinc–copper couple/acetylacetone treatment in DMF solution. Ogilvie et al.,[21] in a comparison of various protecting groups used in the dichloridite procedure, found the 2,2,2-trichloroethyl group of greatest advantage in regard to chemical stability. It was noted, however, that efficient removal of the trichloroethyl group was sometimes difficult, depending on the quality of the zinc–copper couple. The observations reported herein may obviate that difficulty. The utility and efficiency of this reagent for 5'-phosphorylation of oligonucleotides has been demonstrated by its application to the synthesis of **6f** and **6g** which were converted to **1a** and **1b**, unusual oligonucleotides formed in extracts of interferon-treated cells.

Experimental Section

Materials and Methods. Except where otherwise indicated, chemicals were obtained from standard sources and used without further purification. Analytical TLC was done on Analtech silica gel GF, Merck PEI-cellulose-F, or Merck cellulose-F plates by using the following solvent systems: A, $CHCl_3$–MeOH, 20:1; B, 0.1 M NH_4HCO_3; C, 1 M LiCl; D, *n*-BuOH–EtOH–H_2O–concentrated NH_4OH, 60:20:20:1; E, isobutyric acid–1 M NH_4OH–0.2 M EDTA, 100:60:0.8; F, 2-propanol–NH_4OH–H_2O, 55:10:35. Melting points (uncorrected) were determined on a Thomas-Hoover apparatus, UV spectra on a Cary 15, and ^1H and ^{31}P NMR spectra on a Varian instrument at 220 and 109 MHz, respectively. ^{31}P chemical shifts (parts per million) are reported relative to external 0.85% H_3PO_4, and proton chemical shifts are with reference to Me_4Si (internal). Reactions with BTEP were done in small vials or flasks sealed with a rubber septum to exclude moisture. Reagents were introduced via the septum with a hypodermic syringe.

Preparation of Bis(2,2,2-trichloroethyl) Phosphorochloridite (BTEP) and 2,2,2-Trichloroethyl Phosphorodichloridite. The literature[8] preparation of these trichloroethanol derivatives was modified to provide maximum yield of the disubstituted phosphorochloridite. In a well-ventilated fume hood, trichloroethanol (44.8 g, 29 mL, 0.30 mol) was added dropwise to phosphorus trichloride (41.2 g, 26.2 mL, 0.30 mol) over a period of 15 min while the reaction mixture was maintained at −50 °C under a dry argon atmosphere. The mixture was then warmed to 0 °C and stirred under a dry atmosphere for 2 h. The reaction mixture was heated to 55 °C in vacuo (~15 mmHg) for 1 h and subsequently distilled to yield 2,2,2-trichloroethyl phosphorodichloridite [18.7 g, 0.074 mol, 24.7%; bp 52 °C (0.65 mm Hg)] and bis(2,2,2-trichloroethyl) phosphorochloridite (BTEP): 16.7 g (0.046 mol, 15.3%); bp 98 °C (0.15 mmHg); d^{25}_4 1.75 g/mL. Anal. Calcd for $C_4H_4O_2Cl_7P$: Cl, 68.32; P, 8.52. Found: Cl, 68.81, P, 8.15. These reagents have been kept under argon in glass vials fitted with rubber septums, and these in turn have been kept in a desiccator at room temperature. Reagent was removed as needed with the aid of a hypodermic syringe. Under these conditions, the reagents have remained stable and satisfactory for phosphorylations for at least 6 months.

Preparation of Active Zinc–Copper Couple. Zinc dust (2.1 g, 32 mmol) was added with vigorous stirring to a hot (100 °C) solution of cupric acetate monohydrate (1.2 g, 6 mmol) in glacial acetic acid (30 mL). The reaction proceeded with violent evolution of hydrogen gas, and the blue color of the cupric cation disappeared in 3 min. The resulting chocolate brown couple was allowed to settle for 1 min, and then the acetic acid was decanted. The metallic couple was washed with acetic acid (30 mL) and ether (2 × 30 mL) and stored under ether.

Preparation of Tetrabutylammonium Fluoride (TBAF) Solution (0.7 M) in THF. Commercial (Eastman) 10% tetrabutylammonium hydroxide (25.7 mL) was neutralized with 52% aqueous hydrogen fluoride solution (7.7 mL). Colorless crystals separated when the solution was cooled to 5 °C. The supernate was decanted, and the remaining crystals were washed with cold H_2O (20 mL) and drained free of H_2O. The crystals were then dissolved in ethanol (30 mL), and the ethanolic solution was evaporated. The resulting residue was coevaporated with benzene–acetonitrile several times. After being dried over P_2O_5 at 35 °C (0.5 torr) for 10 h, the residue was transferred to a 10-mL volumetric flask, and THF (dry) was added to the mark. This solution (0.7 M in TBAF) was stored over molecular sieves (5 Å) in a sealed bottle kept at −20 °C.

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Phosphorylation of Nucleosides or Oligonucleotides with BTEP. 5′-O-[Bis(2,2,2-trichloroethyloxy)phosphoryl]-2′,3′-di-O-silyladenosine (4a). 2′,3′-O-Bis(*tert*-butyldimethylsilyl)adenosine[22] (**2a**; 49.5 mg, 0.1 mmol) dried in vacuo at 60 °C for 4 h was dissolved in dry[23] THF (2 mL). Finely pulverized molecular sieves[24] (4 Å, 750 mg) were added, and then BTEP (43 μL, 0.21 mmol) was added while the reaction mixture was maintained at a temperature of −78 °C. After the reaction mixture was stirred for 30 min at −78 °C, it was warmed to room temperature, the pulverized molecular sieves were removed by filtration, and the filtrate was diluted with ether (10 mL). To this solution was added a solution of $NaHCO_3$ (5 mL of 0.5 M solution) followed by an ether solution of iodine (38.0 mg, 0.30 mmol in 5 mL of ether) which was added dropwise to the vigorously stirred biphase mixture. When the oxidation was judged complete[25] (~15 min), the organic layer was separated, shaken with aqueous Na_2SO_3 to destroy excess iodine, washed with saturated NaCl solution, dried over anhydrous Na_2SO_4, and then evaporated in vacuo. Chromatography on a short column of silica gel (3 g) with chloroform–methanol (50:1) afforded the phosphate triester **4a**: 77.1 mg (0.092 mmol, 92%); mp 174–176 °C. Compound **4a** had an R_f of 0.56 in $CHCl_3$/MeOH (20:1). The ^{31}P NMR showed a singlet with δ −4.234.

Quite similar procedures were used for the phosphorylation of protected adenosine, guanosine, and cytidene nucleosides as well as protected oligonucleotides. Relevant conditions, yields, and product characterization are presented in Table I.[26]

Deblocking Procedure: Removal of Trichloroethyl, Isopropylidene, and *tert*-Butyldimethylsilyl Protecting Groups. Adenosine 5′-Monophosphate (6a). Compound **4a** (41.9 mg, 795 OD_{260} units, 0.05 mmol) was dissolved in dry[27] DMF (1 mL), and activated zinc–copper couple (65 mg, 1.0 mmol) and acetylacetone (50 μL, 0.5 mmol) were added. The mixture was placed in an oil bath at 55 °C and stirred for 1 h. At the end of this period no metal remained, and the solution had become green. This solution was diluted with methanol (30 mL) and water (15 mL), and Chelex resin[28] (15-mL settled volume) was added. The mixture was stirred until the solution became colorless (~1 h). The Chelex was removed by filtration, and the filtrate was evaporated to dryness in vacuo (<40 °C). The residue (**5a**) was treated for 2 h with 0.7 M TBAF in THF. After evaporation of the solvent, the residue was washed with ether to remove nonpolar substances and then dissolved in triethylammonium bicarbonate buffer (TEAB, 0.05 M, pH 7.2). This solution was applied to a DEAE Sephadex (HCO_3^-) A-25 column (0.9 × 12 cm), and the column was eluted with a linear gradient (200 mL/200 mL) of 0.05 M TEAB–0.20 M TEAB (pH 7.2). Pure 5′-AMP (**6a**) was obtained as the triethylammonium salt (41.3 mg, 735 OD_{260} units, 0.0463 mmol, 92.5%) after removal of the solvent and repeated evaporation with water to remove the volatile TEAB.

Deblocking of 4d to 5′-AMP (6a). Activated zinc–copper couple (54.6 mg, 840 μmol) was added to a mixture of **4d** (27.3 mg, 42.0 μmol, 648 A_{258} units), acetylacetone (430 μL, 420 μmol), and DMF (840 μL). The reaction mixture was heated at 60 °C for 4 h with stirring. During this time, the progress of the reaction was monitored by TLC (silica gel, solvent system D). After the reaction was judged complete, the mixture was cooled to room temperature and diluted with CH_3OH (10 mL) and H_2O (5 mL). This mixture was stirred with Chelex resin (4.8-mL settled volume) for 1 h at room temperature. During this time the blue-green color of the original solution disappeared. The Chelex was removed by filtration, and the filtrate was slowly evaporated in vacuo, taking care to ensure that the temperature did not exceed 20 °C. The resultant residue was then treated with formic acid (88%, 300 μL). The formic acid solution was maintained at room temperature for 24 h and then evaporated to dryness in vacuo. The residue was dissolved in H_2O and the water removed in vacuo. This process was repeated several times, and then the residue was taken up in triethylammonium bicarbonate (0.05 M, 500 μL). The pH of this solution was adjusted to 7.5 with 0.1 M NH_4OH, and it was applied to a DEAE Sephadex (HCO_3) A-25) column (0.9 × 12 cm) which was eluted with a linear gradient of 0.05 M TEAB–0.15 M TEAB (200 mL/200 mL) buffer (pH 7.5). Appropriate fractions were pooled and evaporated. Residual triethylammonium bicarbonate was removed by repeated addition and evaporation of H_2O. 5′-AMP (**6a**) was obtained in 74% yield (480 A_{258} units).

Nearly identical procedures were employed to prepare 5′-CMP and 5′-GMP as well as 5-phosphorylated oligonucleotides. Specifics are outlined in Table II.

Preparation of 5′-O-Triphosphoadenylyl-(2′→5′)-adenylyl-(2′→5′)-adenosine (2-5A, 1b) and 5′-O-Triphosphoadenylyl-(2′→5′)-adenosine (1a). Trimer monophosphate **6g** (200 A_{258} units, 5.41 μmol) was dissolved in dry pyridine (1 mL), and the pyridine was evaporated in vacuo to remove traces of moisture. This procedure was repeated once with dry pyridine and then twice with dry benzene. The resulting residue was dissolved in dry DMF (135 μL), and triethylamine (5.4 μL), tri-*n*-octylamine (2.7 μL), and *N,N′*-carbonyldiimidazole (4.4 mg, 27 μmol) were added to the DMF solution. The reaction mixture was stirred at room temperature for 1 h and then treated with CH_3OH (2 μL) to destroy excess carbonyldiimidazole. To this mixture was added a solution (108 μL, 5.4 μmol) of tri-*n*-butylammonium pyrophosphate in DMF (0.5 M), and the reaction was kept under a dry argon atmosphere at room temperature for 24 h. DMF (800 μL) was added to the reaction mixture, and the white precipitate which formed was removed by centrifugation. The supernatant was evaporated in vacuo, and the residue was diluted with triethylammonium bicarbonate solution (0.225 M, 500 μL). The pH of this solution was adjusted to 7.5, and it was applied to a column of DEAE-Sephadex (HCO_3^-) A-25 (0.9 × 18 cm). The column was eluted with a linear gradient (250 mL/250 mL) of 0.225 M TEAB–0.60 M TEAB buffer (pH 7.5). Fractions 43–55 contained the triphosphate **1b** and were pooled and evaporated repeatedly with water to remove the volatile triethylammonium bicarbonate. The triphosphate of trimer **1b** was obtained in 36% yield (72 A_{258} units, 1.95 μmol) as the triethylammonium salt. On cellulose F TLC, **1b** had an R_f of 0.30 in solvent system E and an R_f of 0.24 in solvent system F. This product had identical chromatographic properties with those of authentic 2-5A. ^{31}P NMR (D_2O) δ −22.5 (t, $J \approx 19$ Hz), −11.3 (d, $J \approx 19$ Hz), −9.9 (d, $J \approx 19$ Hz), −1.3 (s), −0.9 (s).

Compound **1a**, the 5′-triphosphate of adenylyl-(2′→5′)-adenosine, was prepared from dimer monophosphate **6f** in a similar manner. Thus, **6f** (159 A_{258} units, 6.07 μmol) was reacted with carbonyldiimidazole (4.9 mg, 30.3 μmol) in DMF (135 μL) in the presence of triethylamine (6 μL) and tri-*n*-octylamine (13 μL). Excess carbonyldiimidazole was destroyed with CH_3OH (2 μL), and pyrophosphate solution (134 μL of 0.5 *M* tri-*n*-butylammonium pyrophosphate in DMF) was added. After 24 h, the reaction mixture was worked up as above, and the products were separated on a DEAE Sephadex (HCO_3^-) A-25 (0.9 × 12 cm) column eluted with a linear gradient (200 mL/200 mL) of 0.20 M TEAB–0.5 M TEAB buffer (pH 7.5). The yield of **1a** was 45% (71.3 A_{258} units) based on monophosphate: ^{31}P NMR (D_2O) δ −22.1 (5, $J \approx 19$ Hz), −11.1 (d, $J \approx 19$ Hz), −9.6 (d, $J \approx 19$ Hz), −1.1 (s).

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(23) THF used herein was distilled in the presence of $LiAlH_4$ (2.5 g/500 mL) and stored over metallic sodium.

(24) Molecular sieves (4 Å) were dried at 110 °C for 4 h under reduced pressure and then powdered with the aid of a mortar and pestle.

(25) Completeness of oxidation was judged by the persistence of color of the added iodine or by TLC ($CHCl_3$/MeOH, 10/1). Excess iodine was destroyed by treatment of the reaction mixture with aqueous sodium sulfite.

(26) BTEP is a compound of unexplored pharmacology; we would emphasize the need for appropriate caution in its manipulation.

(27) DMF was distilled from calcium hydride (1 g/250 mL) and stored over molecular sieves (4 Å).

(28) The Bio-Rad product was washed with 0.1 M NH_4HCO_3 and H_2O before use.

(29) Note Added in Proof: The yield of **1b** has been considerably improved from 36% to 47% by two simple alterations in the synthetic procedure: first, Me_2SO was used as a solvent for the preparation of the imidazolide of **6g**; second, the intermediate imidazolide was isolated as the sodium salt (via sodium iodide treatment) prior to reaction with pyrophosphate. This change results in an overall yield of **1b** of 18% starting from blocked monomers. We have also found that the trimer monophosphate (**6g**) is an antagonist of the action of 2,5A (**1b**) and double-stranded RNA (Torrence, P. F.; Imai, J.; Johnston, M. *Proc. Natl. Acad. Sci. U.S.A.*, in press).

Toshiro Inubushi and Herman Yeh of this Institute for determination of ^{31}P NMR spectra.

Registry No. **1a**, 65954-94-1; **1b** triethylammonium salt, 78549-90-3; **2a**, 69504-15-0; **2b**, 72409-42-8; **2c**, 72409-47-3; **2d**, 362-75-4; **2e**, 362-76-5; **2f**, 78549-91-4; **2g**, 78549-92-5; **4a**, 78571-57-0; **4b**, 78571-58-1; **4c**, 78571-59-2; **4d**, 78549-93-6; **4e**, 78549-94-7; **4f**, 78549-95-8; **4g**, 78571-60-5; **5a**, 78571-61-6; **5b**, 78571-62-7; **6a**, 61-19-8; **6a** triethylammonium salt, 65411-70-3; **6b**, 85-32-5; **6c**, 63-37-6; **6f**, 78549-96-9; **6g**, 78549-97-0; bis(2,2,2-trichloroethyl) phosphorochloridite, 41662-41-3; 2,2,2-trichloroethyl phosphorochloridite, 78549-98-1; tetrabutylammonium fluoride, 429-41-4.

Synthesis of and Absolute Configurational Assignment to Enantiomerically Pure Unsaturated [4.4.2]Propellanes

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The chiral unsaturated propellanes **6a**, **6b**, and **19** have been prepared by sequences which hinge upon Ramberg–Bäcklund rearrangement as a pivotal step. These hydrocarbons have been obtained in enantiomerically pure form and with established absolute configuration. The procedure involves Diels–Alder addition of (−)-*endo*-bornyltriazolinedione, followed by efficient separation of the two diastereomeric urazoles involving crystallization or high-pressure liquid chromatography techniques. Diastereomeric purity could be ascertained by ^1H NMR spectroscopy at 300 MHz, and absolute configuration was determined by X-ray crystal structure analysis. Subsequent hydrolysis or hydride reduction and oxidation of the diastereomerically pure urazoles efficiently delivered the optically pure propellanes. The relationship of the various substitution patterns to the magnitude of the observed specific rotations is briefly discussed.

Interest in propellane compounds has become rather widespread in recent years for several reasons.[3] Small-ring propellanes, for example, have tested various extreme limits of carbon hybridization and provided additional insight into the stability of highly strained systems.[4] Unsaturated propellanes have proven to be a rich source of fascination because of their often observed involvement in multifarious electrocyclic reactions, including fluxional isomerism, which stem in large part from the structurally enforced proximity of the reacting centers.[5] The existence of many naturally occurring propellanes[3] has likewise engendered interest in the control of stereochemistry within such carbocyclic frameworks.[6] While literally hundreds of propellane derivatives are now known, very few have been prepared by design in optically active form,[7] and no attention has, to our knowledge, been paid to the establishment of absolute configuration.

Our past interest in the chemistry of unsaturated [4.4.2]propellanes[8] and in the utilization of optically active triazolinediones for asymmetric transfer[9–11] has led presently to the preparation of several enantiomerically pure

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