2-(Phenylethynyl)-1,3-cyclohexadiene (34) was the sole product from reactions of the bromo carbonate **15** and the bromo phosphate **21** with phenylacetylene. This compound was as unstable as **33**. It therefore did not have enough stability during storage to allow for consistent analysis: ^{1}H NMR (CCl_4) δ 7.25 (m, 5 H), 6.25–5.75 (m, 3 H), 2.15 (br s, 4 H); MS, *m/e* (relative intensity) 180 (M^+, 100), 178 (95), 165 (70), 126 (10).

2-(3-Hydroxy-3-methyl-1-butynyl)-2-cyclohexen-1-ol, 1-Acetate (29). A solution of 2.36 g (10.72 mmol) of **3**, 1.10 g (13.08 mmol) of 3-methyl-1-butyn-3-ol, 0.15 g (0.22 mmol) of $(PPh_3)_2PdCl_2$, and 0.02 g (0.15 mmol) of CuI in 10 mL of *N*-methylpyrrolidine was heated at 80 °C for 4 h. The solvent was removed on a rotary evaporator, and the residual viscous liquid was extracted with hexane. After concentration, the sequence of extraction with hexane and concentration was repeated until a homogeneous solution was obtained when the residual liquid was taken up in hexane. The product, which was a mixture of **3** and **29**, weighed 1.79 g. This mixture was subjected to column chromatography, first with a 1:1 mixture of hexane and CH_2Cl_{25} to recover 0.64 g (27%) of **3**. Further elution with a 1:3 mixture of hexane and ether gave 0.61 g (26%) of **29**. This substance progressively turned into material insoluble in hexane: ^{1}H NMR (CCl_4) δ 6.10 (br t, J = 3 Hz, 1 H), 5.30 (br s, 1 H), 3.25 (br s, 1 H), 2.30–1.10 (m, 6 H), 2.10 (s, 3 H), 1.50 (s, 6 H); IR (neat, cm^{-1}) 3440, 3000, 2220, 1720, 1250; MS, *m/e* (relative intensity) 222 (M^+, 2), 162 (40), 147 (80), 129 (20), 91 (35), 73 (30), 43 (100); high resolution mass spectrum calcd for $C_{13}H_{18}O_3$ 222.12560, found 222.12753.

2-(Phenylethynyl)-2-cyclohexenol (37). To 4.99 g (22.68 mmol) of the bromo acetate **3** in 20 mL of pyrrolidine were added 0.21 g (0.92 mmol) of palladium acetate and 0.47 g (1.81 mmol) of triphenylphosphine. The stirred mixture was heated to 80 °C, and 4.65 g (45.65 mmol) of phenylacetylene was added. The reaction mixture was kept at 80 °C for 6 h.

The solvent was removed on a rotary evaporator, and the dense reddish liquid residue was extracted with 3 × 100 mL of hexane. The extract was washed with water and brine and dried ($MgSO_4$). After concentration, the product was chromatographed with a 1:1 mixture of hexane and CH_2Cl_2. The first fraction eluted was a mixture of unknown substances. Continued elution with the same solvent mixture, followed by 100% CH_2Cl_2, gave 3.02 g (67%) of **37** as a viscous oil: ^{1}H NMR (CCl_4) δ 7.30 (m, 5 H), 6.20 (t, J = 4 Hz, 1 H), 4.20 (br, 1 H), 2.80 (br s, 1 H), 2.30–1.50 (m, 6 H); IR (neat, cm^{-1}) 3400, 3060, 2940, 2200, 1600, 1050; MS, *m/e* (relative intensity) 198 (M^+, 80), 180 (75), 170 (100), 141 (80), 115 (70); high resolution mass spectrum calcd for $C_{14}H_{14}O$ 198.10447, found 198.10664.

This compound was also prepared by the same procedure from the bromo alcohol **8**.

Reaction of Bromo Acetate 11 with Phenylmagnesium Bromide. To a solution of 1.56 g (4.71 mmol) of **11** and 0.11 g (0.10 mmol) of $(PPh_3)_4Pd$ in 10 mL of *N*-methylpyrrolidine at 80 °C was added by syringe phenylmagnesium bromide prepared from 2.07 g (13.13 mmol) of phenyl bromide and excess magnesium in THF. Heating under reflux was continued for 5 h. After concentrating the reaction mixture, the residue was extracted with ether, and the ether extract was washed consecutively with water and brine and dried ($MgSO_4$). The residue, after removal of ether was chromatographed, first eluting with hexane. Subsequent use of a 3:1 mixture of hexane and ethyl acetate gave 1.48 g (82%) of 2-bromo-3,3-diphenyl-2-propenol (**12**) with physical characteristics identical with **12** obtained by other methods.

Reaction of the Bromo Acetate 3 with PhMgBr. To a solution of 2.15 g (9.78 mmol) of **3** and 0.22 g (0.20 mmol) of $(PPh_3)_4Pd$ in 10 mL of *N*-methylpyrrolidine at 80 °C was added PhMgBr prepared from 1.70 g (10.76 mmol) of PhBr and excess Mg in THF. After the addition (15 min), the mixture was heated for 1 h and then diluted with 50 mL of hexane and filtered. The filtrate was evaporated carefully, and the pale yellow oil was chromatographed with hexane to obtain 1.00 g (65%) of 2-bromo-1,3-cyclohexadiene (**6**) identical with samples obtained by other methods.

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Notes

Solid-Phase Synthesis, Separation, and Stereochemical Aspects of P-Chiral Methane- and 4,4′-Dimethoxytriphenylmethanephosphonate Analogues of Oligodeoxyribonucleotides

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Oligonucleotides that are modified at the internucleotide phosphate moiety(ies) have attracted considerable attention. In addition to phosphorothioate analogues of oligodeoxyribonucleotides, which have a stereogenic phosphorus center and are therefore valuable probes for stereochemical investigations of the mechanism of action of phosphorolytic enzymes,[2] other analogues of interest include *O*-alkyl phosphotriester **1** and alkanephosphonate **2** congeners of oligodeoxyribonucleotides. Miller and Ts'o, and their associates,[3] have demonstrated that oligonucleotide phosphotriesters and methanephosphonates form base-paired complexes with complementary nucleic acids and that these complexes have greater stability toward dissociation than those containing the corresponding diesters, presumably due to less electrostatic repulsion between

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(3) (a) Miller, P. S.; Agris, C. H.; Blake, K. R.; Murakami, A.; Spritz, S. A.; Reddy, P. M.; Ts'o, P. O. P. In "Nucleic Acids: The Vectors of Life"; Pullman, B., Jortner, J., Eds.; D. Reidel Publishing Co.: Boston 1983; pp 521–535, and references cited therein. (b) Miller, P. S.; Fang, K. N.; Kondo, N. S.; Ts'o, P. O. P. *J. Am. Chem. Soc.* **1971**, *93*, 6657.

Table I. Numbering of 4,4′-Dimethoxytriphenylmethanephosphonate Analogues of Di- and Octadeoxyribonucleotides and Analytical Data

compd no.	yield, rel %	formula[a]	elution time, min[b]	^{31}P NMR, ppm[c]	FAB-MS, m/z $(M + H)^+$
3a-fast	56	$T_{DMT}T$	18.80	27.55	833
3a-slow	44	$T_{DMT}T$	19.95	27.75	833
3b-fast	58	$T_{DMT}C$	16.07	27.77	
3b-slow	42	$T_{DMT}C$	16.62	27.56	
3c-fast	53	$C_{DMT}C$	14.40	27.12	803
3c-slow	47	$C_{DMT}C$	14.95	27.00	803
3d-fast	57	$A_{DMT}A$	17.10	27.47[d]	851
3d-slow	43	$A_{DMT}A$	18.03	27.47[d]	851
3e-fast	52	$GG_{DMT}AATTCC$	15.16	27.40[e]	
3e-slow	48	$GG_{DMT}AATTCC$	15.81	27.52[e]	
3f-fast	42	$GGAA_{DMT}TTCC$	15.06	27.92[f]	
3f-slow	58	$GGAA_{DMT}TTCC$	16.54	27.42[f]	

[a] DMT = 4,4′-dimethoxytriphenylmethanephosphonate moiety. [b] Refers to C_{18} HPLC, see Experimental Section for details. [c] Refers to chemical shift of the phosphonate group measured in 1:1 v/v MeOH:0.1 M Tris-HCl buffer pH 7.6 at 20 °C in the presence of 5 mM EDTA, except for **3e** and **3f** where DMF was used in place of MeOH; the ppm values are downfield, relative to 25% H_3PO_4 in D_2O as an external reference. [d] Measured as separated and admixed diastereomers. [e] Measured as the diastereomer mixture; phosphate signals at 0.90 to 1.26 ppm; methanephosphonate:phosphodiester = 1:6. [f] Phosphate signals at 1.21 to 1.54 ppm; methanephosphonate:phosphodiester = 1:6.

phosphate groups.[3b] These observations have kindled hopes that **1** and **2** might block transcription[4a] or translation and could thus specifically inhibit the growth of tumor cells or replication of viruses in infected cells.[4b,c] In addition, analogues of DNA having modified internucleotide linkages can be used to study protein–nucleic acid interactions.[5]

As part of our investigations of the synthesis of modified oligodeoxyribonucleotides,[6] we have examined the possibility of obtaining **1** and **2** by means of adapting the solid-phase phosphoramidite-coupling chemistry that was developed by Caruthers and co-workers[7] and is now widely used either manually or with an automated synthesizer. The present report concerns out exploratory findings for this route to alkanephosphonates by using a commerically available automated DNA synthesizer.

The formation of a support-bound internucleotide *O*-methyl phosphite intermediate (Scheme I, A) during each cycle of synthesis by the phosphoramidite method,[7] and the well-known nucleophilic reactivity of phosphites toward alkyl and acid halides (R′X) could, in principle, offer a convenient, direct route to a phosphorus–carbon bond at any internucleotide linkage (B). Subsequent *O*-demethylation with PhSH–Et_3N followed by NH_4OH-mediated cleavage from the support and base-deprotection would give the desired oligonucleotide analogue C.

Nemer and Ogilvie[8] were the first to report a solution-phase Arbusov-type reaction involving a dinucleoside *O*-methyl phosphite and methyl iodide, while Caruthers and co-workers[5b] have noted more recently that reactions of support-bound internucleotide phosphites A with alkyl iodides at 50 °C gave very low yields of alkanephosphonates of undefined sterochemistry. During our initial work in this area, we also found that reactions of support-bound oligonucleotide *O*-methyl phosphites with relatively simple alkyl halides [CH_3I, *p*-$BrC_6H_4CH_2Br$, and $Br(CH_2)_6CO_2Bu$-*t*] in CH_3CN at 60 °C gave alkanephosphonate products in rather low yield. For example, when 5′-(4,4′-dimethoxytriphenylmethyl)dithymidyl (3′→5′) *O*-methyl phosphite (DMT = 4,4′-dimethoxytriphenylmethyl) bound to "long chain alkylamine"-type controlled-pore glass[7e] was reacted with 5 M [^{13}C]CH_3I in CH_3CN for 10 h at 60 °C, we obtained a 9% isolated yield of dithymidyl (3′→5′) [^{13}C]methanephosphonate ($T_{Me*}T$)[9a] as a mixture of two isomers, after cleavage from the support with concentrated NH_4OH and detritylation with 3% aqueous HOAc. These P-chiral ^{13}C-labeled diastereomers of $T_{Me*}T$ were fractionated by means of reverse-phase C_{18} HPLC to give fast- and slow-eluting compounds that were characterized by comparison of their NMR spectroscopic parameters with those reported for the diastereomers of 5′-DMT-3′-Ac-$T_{Me}T$:[5b,9b] "fast" $T_{Me*}T$ (14.13 min), 45% $\delta(^{31}P)$ 35.19 ppm, $^1J(^{31}P-^{13}C)$ = 141.76 Hz; "slow" $T_{Me*}T$ (14.83 min), 55% $\delta(^{31}P)$ 35.23 ppm, $^1J(^{31}P-^{13}C)$ = 141.76 Hz. Similar results were also obtained for $A_{Me}T$, the stereochemistry of which is discussed below. In connection with the fact that the diastereomers of $T_{Me*}T$ and $A_{Me}T$ were obtained in near equimolar amounts, it should be noted that this reflects the diastereomeric composition of the internucleotide *O*-methyl phosphite, which we have shown[10] to be completely epimerized at phosphorus as the result of relatively rapid, tetrazole-mediated epimerization of the putative tetrazoylamidite intermediate prior to the coupling reaction per se.

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(9) (a) Throughout this report, 5′→3′ oligodeoxyribonucleotides d-(N′N″···N‴) are designated simply as N′N″···N‴, and modified internucleotide linkages are indicated by a predefined subscript. (b) The diastereomer of 5′-DMT-3′-Ac-$T_{Me}T$ designated[5b] as isomer "I" was reported[5b] to have $^1J(^{31}P-^{13}C)$ = 14.49 Hz, which is substantially different from our value of 142 Hz for $T_{Me*}T$, presumably due to a misplaced decimal point, i.e., 14.49 should be taken to mean 144.9.

(10) Stec, W. J.; Zon, G. *Tetrahedron Lett.* **1984**, *25*, 5279.

Reaction of support-bound dithymidyl (3′→5′) *O*-methyl phosphite with benzoyl chloride was expected to ultimately afford a diastereomeric pair of dithymidyl (3′→5′) benzoylphosphonates or their base-catalyzed hydrolysis products, namely, (R_P)- and (S_P)-dithymidyl (3′→5′) phosphonate, $T_{P(O)H}T$. The latter compounds were especially interesting in that they could be used for the first *chemical* assignment of absolute configuration at the alkanephosphonate center in $T_{Me}T$, given that dialkyl phosphonates can be alkylated and sulfurated with retention of configuration, thus allowing $T_{P(O)H}T$ to serve as a "link" between $T_{Me}T$ and dithymidyl (3′→5′) phosphorothioates, $T_{PS}T$, whose absolute configurations are firmly established.[11] Surprisingly, the material obtained from the attempted Arbusov reaction with benzoyl chloride (0.8 M in CH_3CN, 60 °C, 20 h) did *not* give rise to a ^{31}P NMR spectrum characteristic of compounds containing either P–H or P–C(O)Ph moieties. $T_{P(O)H}T$ was expected to show a pair of signals in the range of 5–8 ppm[8] having a spin–spin coupling constant $^1J_{PH} \simeq 700$ Hz, while the P–C(O)Ph case should have given signals at 8 ppm;[12] the observed chemical shift values were 28.96 and 28.85 ppm. Fractionation of the reaction product by HPLC afforded samples of fast-eluting (14.91 min) and slow-eluting (15.73 min) substances for analysis by positive fast-atom bombardment mass spectrometry (FAB-MS) using a matrix of either glycerol or 3-mercapto-1,2-propanediol. Ions corresponding to a protonated molecular ion, $(M + H)^+$, and a kaliated molecular ion, $(M + K)^+$, were observed at m/z 833 and 871, respectively, for both samples, thus establishing their isomeric nature. On the basis of this data it was deduced that the two products (18% yield) were the diastereomers of dithymidyl (3′→5′) 4,4′-dimethoxytriphenylmethanephosphonate, $T_{DMT}T$.[13]

The lability of the 5′-DMT group in the presence of acids and the known[14] reaction of a triarylmethyl chloride with a trialkyl phosphite to give an *O,O*-dialkyl triarylmethanephosphonate suggested that a trace amount of HCl in the benzoyl chloride could have catalytically led to the formation of DMTCl, which then reacted with the internucleotide *O*-methyl phosphite to give support-bound $T_{DMT}T$ and/or 5′-benzoyl-$T_{DMT}T$. That this Arbusov-type reaction involving DMTCl was possible was confirmed by treatment of support-bound 5′-DMT-dithymidyl (3′→5′) *O*-methyl phosphite with a saturated solution of DMTCl in CH_3CN for 8 h at 60 °C, which afforded a pair of diastereomers that was identical (HPLC, FAB-MS, ^{31}P NMR) to the $T_{DMT}T$ products originally derived from the aforementioned reaction with benzoyl chloride. Other control experiments revealed that treatment of the support-bound phosphite with a solution of benzoyl chloride (0.8 M) in CH_3CN containing either Et_3N (1.6 M) or 2,6-lutidine (1.6 M) *did not* lead to detectable amounts of $T_{DMT}T$ (<0.5%, HPLC) after 7–20 h at 60 °C, while heating the support-bound phosphite in CH_3CN at 60 °C for 10 h in the *absence* of benzoyl chloride *did* afford a small amount of $T_{DMT}T$ (3–4% isolated yield). The results obtained by using either Et_3N or 2,6-lutidine as a scavenger for acid further supported the possibility of HCl-catalyzed, DMTCl-mediated formation of the support-bound precursor(s) of $T_{DMT}T$ during the originally attempted P-benzoylation reaction that was conducted in the absence of an added base. The control experiment employing heat but no added benzoyl chloride indicated that detritylation leading to formation of a P–DMT linkage can be a potential problem during attempts to chemically modify the support-bound *O*-methyl phosphite using relatively slow-reacting electrophiles.[15a] The ^{31}P NMR spectrum of a buffered (pH 7) aqueous solution of the crude product derived from the reaction of the support-bound phosphite with benzoyl chloride–Et_3N (or 2,6-lutidine) consisted of two signals of approximately equal intensity at 5 and 7 ppm, which were in the range of chemical shifts (5–8 ppm) expected for either P–H[8,12] or P–C(O)Ph moieites;[12] however, preparative reverse-phase HPLC of this material using a strongly eluting gradient of CH_3CN led to the collection of only thymidine (22% yield). The known[12] occurrence of base-catalyzed debenzoylation of dithymidyl (3′→5′) benzoylphosphonate and base-catalyzed dealkylation of diethyl phosphonate, plus the release of products from the controlled-pore glass support using ethylenediamine–EtOH (1:3, 20 °C, 7 h), which does not normally cleave internucleotide phosphodiester linkages, suggested that the thymidine may have resulted from decomposition of a phosphonate. The reaction of dithymidyl (3′→5′) *O*-methyl phosphite with benzoyl chloride in the presence of an HCl scavenger was therefore studied by first synthesizing the phosphite and then removing it from the support by using ethylenediamine–EtOH (1:1, 70 °C, 30 min) to allow real-time monitoring by means of ^{31}P NMR spectroscopy. The approximately equal intensity ^{31}P NMR signals for the diastereomers of dithymidyl (3′→5′) phosphite (2.8 mM) in CH_2Cl_2 containing 10% v/v C_6D_6 were seen at 135 and 136 ppm. The addition of a 200-fold molar excess of Et_3N followed by addition of a 100-fold molar excess of benzoyl chloride led to slow (7 days, 20 °C), partial conversion (50%) of the starting phosphites into two major phosphorus-containing products (127 and 128 ppm, ca. 1:1 ratio) and two minor hydrolysis products (ca. 0 ppm). While the chemical shifts of the major products suggested that they might be diastereomers having a P–OC(O)Ph moiety, neither positive-ion nor negative-ion FAB-MS analyses of the crude EtOH-quenched reaction mixture led to identifiable molecular ions. No further experiments were conducted reagarding the desired P-benzoylation reaction.

With the hope of ultimately synthesizing other alkanephosphonate analogues of oligonucleotides bearing either

(11) Lesnikowski, Z. J.; Niewiarowski, W.; Zielinski, W. S.; Stec, W. J. *Tetrahedron* **1984**, *40*, 15 and references cited therein.

(12) Dithymidyl (3′→5′) benzoylphosphonate with protected 5′- and 3′-hydroxyl groups gives rise to a ^{31}P NMR absorption signal ca. 8 ppm downfield from H_3PO_4 [Krime, A.; Fujii, M.; Sekine, M.; Hata, T. *J. Org. Chem.* **1984**, *49*, 2139].

(13) The positive FAB mass spectra of $T_{DMT}T$ and other $N_{DMT}N'$ compounds (vide infra) were complex, particularly at lower m/z values. However, there were four discernible features common to the mass spectra data. First, protonated molecular ions, $(M + H)^+$, were observed for all $N_{DMT}N'$ samples. In addition, "sequence" ions corresponding to the loss of one nucleoside were present, though generally in low abundance. Fission of the C–P bond could allow formation of a 4,4′-dimethoxytriphenylmethyl cation, thus accounting for the ions observed at m/z 303. Finally, ions corresponding to either a purine or pyrimidine base having two hydrogen atoms (base + 2 H)$^+$, were seen, as has been observed in chemical ionization mass spectrometry (CI–MS) of simple nucleosides [Wilson, M. S., McCloskey, J. A. *J. Am. Chem. Soc.* **1975**, *97*, 3436].

(14) Drew, M. G. B.; Rodgers, J.; White, D. W.; Verkade, J. G. *J. Chem. Soc., Chem. Commun.* **1971**, 227.

(15) (a) Adventitious formation of a low level of P–DMT linkages during the normal coupling cycle with phosphoramidites represents, to our knowledge, a heretofore unreported possible side reaaction in the synthesis of oligonucleotides. However, a control study of the 1-μmol-scale synthesis of TT using standard coupling and capping conditions (see Experimental Section) demonstrated that the crude product was free of detectable $T_{DMT}T$ (<0.5%, HPLC). (b) The model compound used for comparison of ^{31}P NMR chemical shifts was *O,O*-dimethyl 4,4′-dimethoxytriphenylmethanephosphonate, which was obtained by the reaction of $(MeO)_3P$ with DMTCl: mp 140–141 °C (crystallized from MeOH), $\delta(^{31}P)$ 30.2 ppm (DMF as solvent), EI–MS at 70 eV (direct probe inlet) showed the presence of the molecular ion, m/z 412 (1.4%); base-peak ion, m/z 303.

lipophilic or fluorescent groups attached to phosphorus through a relatively stable type of phosphorus–carbon bond, additional support-bound di- and oligonucleotide *O*-methyl phosphites were treated with DMTCl as preliminary model reactants. It was found that 8 h of reaction at 60 °C using a saturated solution of DMTCl in CH_3CN led to moderate isolated yields (ca. 15%) of the expected di- and oligonucleotide 4,4′-dimethoxytriphenylmethanephosphonates (**3**), which are listed in Table I. ^{31}P NMR spectra of products **3a–f** showed in each case an absorption signal ca. 27 ppm downfield from H_3PO_4, which was consistent with the presence of an *O,O*-dialkyl alkanephosphonate moiety.[15b] The octamers **3e** and **3f** also exhibited a 1:6 ratio of integrated absorption intensities for the phosphonate:phosphodiester signals. The nucleotide composition of the HPLC-separated diastereomers of **3e** and **3f** was confirmed by our modified version of a degradation procedure[16] using formic acid, which cleanly liberated the purine and pyrimidine bases for separation by HPLC [Cyt (4.87 min), Gua (8.43 min), Thy (10.11 min), and Ade (15.56 min)] and quantification by integration of peak areas, using GGAATTCC as a structurally defined[17] reference compound that was degraded with formic acid in parallel with samples of **3e** and **3f**.

In contrast to some oligonucleotide methanephosphonates, which undergo 5–10% hydrolysis after 24 h in ethylenediamine–EtOH at room temperature,[18] $T_{DMT}T$ was largely unreacted after 20 h in concentrated NH_4OH–MeOH (1:1 v/v) at 70 °C. This stability, which presumably results from steric hindrance at phosphorus due to the DMT group, was further evidenced by the fact that $T_{DMT}T$ survived heating at 70 °C for 16 days in 0.1 M Tris (pH 7.4)–MeOH (1:1 v/v).

It was further established that compounds **3a–d** do not undergo detectable internucleotide bond cleavage in the presence of nuclease P1 in 0.025 M Tris, pH 7, during 24 h of incubation at 37 °C. This finding was similar to results which have shown that the hydrolytic activity of nucleases can be markedly influenced by the nature of the phosphorus group at potential reaction sites in di- and oligonucleotide analogues having either phosphorothioate[6a,b] or phosphotriester[3b,6c] linkages. Nevertheless, the inhibitory influence of the DMT group toward the action of nuclease P1 was localized to the phosponate moiety: the diastereomers of the octamers **3e** and **3f** were hydrolyzed by nuclease P1 to give dG, dA, dT, and dC together with the diastereomers of $G_{DMT}A$ and $A_{DMT}T$, respectively, following treating with alkaline phosphatase to remove the 5′-phosphate groups. The identity of the $G_{DMT}A$ and $A_{DMT}T$ products, which could not be synthesized directly (vide infra), was based on their slow HPLC elution, relative to authentic GA and AT, and their subsequent degradation with formic acid to give 1:1 molar ratios of Gua:Ade and Ade:Thy, respectively.

Although the syntheses of **3a–d** led to only minor amounts of detectable (HPLC, ^{31}P NMR) side-reaction products, repeated attempts to similarly synthesize $G_{DMT}G$, $G_{DMT}A$, and $A_{DMT}T$ were unsuccessful. A combination of ^{31}P NMR, FAB-MS, and nucleoside-composition data indicated that the major isolated products were mixtures of the corresponding nucleoside 3′- and/or 5′-(4,4′-dimethoxytriphenyl)methanephosphonic acids. Alkanephosphonic acids were also present as major contaminants (20–30%) in the crude samples of **3e** and **3f**, as judged from HPLC characteristics and, more reliably, ^{31}P NMR absorption signals at 22–23 ppm. The hydrolytic lability of a 4,4′-dimethoxytriphenylmethanephosphonate moiety could, by analogy to oligonucleotide methanephosphonates,[18] be strongly influenced by intramolecular structural factors; however, the elucidation of such effects required further studies that were beyond the scope of the presently reported work.

The absolute configurations of the alkanephosphonate phosphorus centers in **3a–f** are unknown at this time, and each diastereomer in Table I has been temporarily referred to as either a "fast" or "slow" isomer based upon its relative elution time from a reverse-phase C_{18} HPLC column. To date, the only unambiguous assignment of absolute configuration at phosphorus within the family of dinucleoside alkanephosphonates has been achieved through X-ray crystallography by Miller and co-workers[19a] for "isomer 2"[19b] for adenosyl (3′→5′) thymidyl methanephosphonate ($A_{Me}T$), which had the S_P configuration. A sample of $A_{Me}T$ containing "isomers 1 + 2" and a sample of $A_{Me}T$ containing the crystalline (S_P)-"isomer 2" were kindly provided by Dr. Miller for comparison by HPLC with samples of the "fast" and "slow" diastereomers of $A_{Me}T$ that we prepared according to the procedure described above for $T_{Me*}T$. It was found that the elution time for our "fast" $A_{Me}T$ (15.05 min) compound was identical with the elution time for Miller's (R_P)-$A_{Me}T$ ("isomer 1"), and that the "slow" $A_{Me}T$ (16.16 min) compound was identical with Miller's samples of (S_P)-"isomer 2". By analogy to the empirical relationship between phosphorothioate chirality in $N_{PS}N'$ and HPLC elution times,[6a,b] it was tentatively concluded that the "fast" isomers of $A_{Me}T$, $T_{Me}T$, and other $N_{Me}N'$ have the R_P configuration, while the corresponding "slow" isomers have the S_P configuration.[20] The physicochemical interactions between a 4,4′-dimethoxytriphenylmethanephosphonate moiety and the C_{18} stationary phase of the HPLC column could be significantly different from the interactions of a methanephosphate group with the C_{18} stationary phase, which would thus preclude correlations between $N_{Me}N'$ and $N_{DMT}N'$ compounds. If an $N_{DMT}N'$ compound is obtained in the future as a sterochemical "anchor", then it may be possible to correlate absolute configuration at phosphorus in $N_{DMT}N'$ with either HPLC mobilities or ^{31}P NMR chemical shifts;[6a] however, attempts to establish the latter type of correlation may be complicated by the fact that samples of the HPLC-separated diastereomers of $A_{DMT}A$ and their admixture were found, somewhat surprisingly, to have isochronous ^{31}P NMR signals even at a relatively high field strength.

While the presently reported studies have demonstrated that alkanephosphonate analogues of oligonucleotides are obtainable by altering the chemistry cycle used with automated DNA synthesizers that employ phosphoramidite-coupling, further work is needed to clarify the scope and limitations of this synthetic approach. The contrasting results obtained for the reaction of various dinucleoside *O*-methyl phosphites with DMTCl are especially intriguing, and it will therefore be informative to

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(18) Miller, P. S.; Agris, C. H.; Murakami, A.; Reddy, P. M.; Spritz, S. A.; Ts'o, P. O. P. *Nucleic Acids Res.* **1983**, *11*, 6225 and references cited therein.
(19) (a) Chacko, K. K.; Linder, K.; Saenger, W.; Miller, P. S. *Nucleic Acids Res.* **1983**, *11*, 2801. (b) Miller, P. S., private communication.
(20) Previous attempts to deduce the absolute configuration of the diastereomers of $T_{Me}T$ have failed.[5b] If our tentative configurational assignments for $T_{Me}T$ are correct, then it is now possible to consider, in further detail, the interactions of $T_{Me}T$-containing *lac* operators with *lac* repressor.[5b]

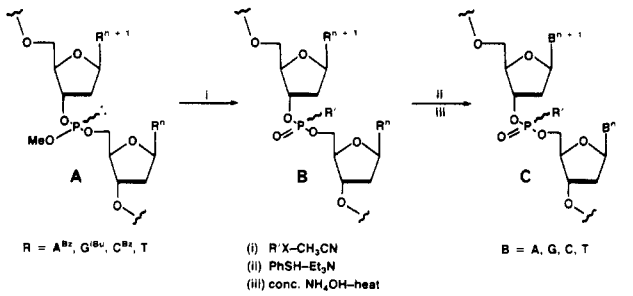
study the detailed solution chemistry for reactions of electrophilic R′X reagents with the intermediary dinucleoside *O*-methyl phosphites, which are readily accessible via solid-phase synthetic routes. Finally, it should be noted that the presently obtainable low yields of the alkanephosphonate analogues of oligodeoxyribonucleotides are offset, to some extent, by their largely automated synthesis and are nevertheless more than adequate for biological scale (i.e., nmol–pmol) experiments with these novel compounds.[21]

Experimental Section

All NMR spectra were recorded in the pulsed Fourier transform mode. ^{31}P NMR spectra recorded at 121.47 MHz were obtained by using 10-mm sample tubes. The general acquisition conditions consisted of 60–90° flip angle pulses, 10 000 Hz spectral width, 8192 data points, complete ^1H decoupling gated on during the acquisition time, ambient temperature (23 °C), and a repetition time for signal averaging of 1.2–2 s. ^1H NMR spectra recorded at 400 MHz were obtained by using 5-mm samples tubes. The general acquisition conditions consisted of low-level gated irradiation for water–solvent suppression, 90° flip angle pulses, 5000 Hz spectral width, 16 384 data points, ambient temperature (22 °C), and a repetition time of 8 s. Mass spectra were recorded with a VG 7070E/HF mass spectrometer equipped with a VG 11/250 data system. Positive and negative fast-atom bombardment (FAB) mass spectra were scanned from samples introduced directly into the ion source as colloidal suspensions in either a glycerol or 3-mercapto-1,2-propanediol sample matrix, which was placed on an unheated stainless steel sample probe tip; bombardment was with a 16-μA beam of 9-keV xenon fast atoms generated in a Saddle–Field neutral-beam gun.

DNA Synthesis. General Methods. 5′-Dimethoxytrityl 3′-methoxy(*N*,*N*-diisopropylamino)phosphine deoxynucleosides having either *N*-benzoyl (for dA and dC) or *N*-isobutyryl (for dG) blocking groups were purchased from Applied Biosystems (Foster City, CA) as powders that were >95% pure by ^{31}P NMR analysis. 1*H*-Tetrazole ("99+%", Aldrich, Milwaukee, WI) was resublimed before dissolution in HPLC-grade CH_3CN (Burdick & Jackson, Muskegon, MI), which had been dried over type 4A molecular sieves before use. This grade of dried CH_3CN was also used as the solvent for the phosphoramidite reagents (0.1 M). All other reagents and solvents were purchased (Aldrich, "Gold Label" or highest purity available) and used as received. Commercially available (either Applied Biosystems or American BioNuclear, Emeryville, CA) "long chain alkylamine"-type controlled pore glass[7e] having 20–40 μmol/g of 3′-linked 5′-DMT deoxynucleoside was used as the solid support for automated synthesis with an Applied Biosystems Model 380A DNA synthesizer, which was equipped with three column ports for independently programmable parallel syntheses on scales of either 1 μmol or 10 μmol of support-bound nucleoside. The essential chemical steps[7d] and step times for the 1-μmol scale syntheses were as follows: detritylation (100 s) with Cl_3CCO_2H–CH_2Cl_2 (3% w/v), 1*H*-tetrazole-catalyzed coupling (180 s) of the phosphoramidite (catalyst:amidite:5′-HO acceptor ≃ 50:10:1), capping (120 s) of residual 5′-HO groups with Ac_2O–2,6-lutidine–THF (1:1:8 v/v/v) and 4-(dimethylamino)pyridine in THF (6% w/v), and oxidation (30 s) of the resultant phosphite with a solution prepared from I_2 (2.5 g) and H_2O (2 g) in THF (80 mL) containing 2,6-lutidine (20 mL). The total time for these steps and washing with CH_3CN (HPLC grade, <0.01 ppm H_2O) between steps was 20 min. Apparent coupling yields were generally >97% based on a colorimetric assay for the DMT cation in 0.1 M *p*-toluenesulfonic acid in CH_3CN at 530 nm. The steps and step times for the 10 μmol operating scale were as follows: detritylation (150 s), neutralization with 2,6-lutidine (120 s), coupling (360 s, catalyst:amidite:5′-HO acceptor ≃ 25:5:1), capping (240 s), and oxidation (120 s). The total time for these steps and washing with CH_3CN between steps was

Scheme I



80 min. The apparent coupling yields were generally >94%. Reaction of an intermediary phosphite with relatively nonvolatile R′X was performed by automatically interrupting the synthetic cycle prior to the oxidation step for removal of the fritted column from the synthesizer, introduction (by syringe) of a solution of R′X in CH_3CN, and then placement of the stoppered column in a heating block at 60 °C. The R′X–CH_3CN was subsequently removed by syringe, and the solid support was thoroughly washed with CH_3CN prior to reconnection of the column to the synthesizer, and continuation of the synthetic cycle. Reaction of the intermediary phosphite with a relatively volatile R′X (e.g., CH_3I) was performed as described above, except that the solid support, which was removed from the column was heated with R′X–CH_3CN in a tightly sealed vial. The solid support was subsequently repacked in the column for continuation of the synthetic cycle. The 1-μmol and 10-μmol scale syntheses both utilized essentially the same ending methods: either the inclusion or exclusion of a final detritylation step to respectively provide either 5′-HO or 5′-DMT oligonucleotide termini and then *O*-demethylation (10 min, 3 times) with PhSH–Et_3N in *p*-dioxane (1:2:3 v/v/v) followed by cleavage of the product from the support with concentrated NH_4OH solution, which was delivered to the column in 8 portions over a 1-h period (total volume ≃ 2 mL or 10 mL for 1 μmol or 10 μmol, respectively). The volume of the ammoniacal solution was increased by 30–50% by the addition of more concentrated NH_4OH solution before heating at 60 °C for 10 h to deblock the bases. An alternative procedure, which was used to minimize hydrolysis of labile internucleotide linkages, involved cleavage of the product from the support with a solution of ethylenediamine–absolute EtOH (1:1 v/v)[18] that was delivered to the column as described above for NH_4OH. The resultant solution was then kept at room temperature for 7 h. In those cases where the expected products might be poorly soluble in either the concentrated NH_4OH or ethylenediamine–absolute EtOH solutions (e.g., $N_{DMT}N'$), the support was washed with either formamide, ethanol, or CH_3CN after the basic cleavage procedure, and the wash solvent was then removed under reduced pressure. The ammoniacal or ethanolic solutions of the crude product were first concentrated under a stream of N_2 and were then taken to dryness by using a vacuum centrifuge after the addition of ca. 3% v/v Et_3N to prevent detritylation of those products having a 5′-DMT group. The resultant residue containing the crude product was either first analyzed by ^{31}P NMR spectroscopy or it was dissolved in a suitable solvent containing ca. 3% v/v Et_3N for analysis and product collection by HPLC, as described in the next section.

HPLC Methods. The high-performance liquid chromatography system, which included two pumps, an automatic injector, a variable wavelength UV detector (set at 254 nm), a signal recorder–integrator, and a system controller, was used with a μBondapak C_{18} reverse-phase column (7.8 mm × 30 cm) and linear gradients of CH_3CN vs. aqueous triethylammonium acetate ("TEAA", 0.1 M, pH 7) with a flow rate of 4 mL/min for analysis and collection of synthetic products, as specified below. The same HPLC conditions were employed for the analysis of enzymatic digests; HPLC conditions used to analyze formic acid-catalyzed degradation mixtures are described in a following section. Products collected by HPLC were obtained by removal of CH_3CN under a stream of N_2 and then removal of water and buffer using a vacuum centrifuge.

(21) Our brief study of the effect of temperature and solvent on the yield of $T_{Me}T$ derived from the presently reported solid-phase synthetic procedure has revealed that a ca. 70% isolated yield of $T_{Me}T$ can be obtained by using CH_3I–CH_3CN at 100 °C for 3 h.

compd	init condns, CH_3CN: TEAA	CH_3CN gradient
$T_{Me*}T$	5:95	1% min^{-1} for 30 min
$A_{Me}T$	5:95	1% min^{-1} for 30 min
3a	30:70	1% min^{-1} for 10 min, then isocratic
3b	30:70	1% min^{-1} for 10 min, then isocratic
3c	30:70	1% min^{-1} for 10 min, then isocratic
3d	30:70	1% min^{-1} for 10 min, then isocratic
3e	5:95	2% min^{-1} for 10 min, then 1% min^{-1}
3f	5:95	2% min^{-1} for 10 min, then 1% min^{-1}

Nuclease P1 Catalyzed Hydrolysis of 3e and 3f. A dry sample (ca. 0.2 OD_{260} unit) of each diastereomer of the oligonucleotide analogue was dissolved in 100 μL of 0.025 M Tris-HCl buffer (pH 7.0), and a buffered solution (3 μL) of nuclease P1 from Penillium citrinum (Sigma Chem., Co., St. Louis, MO; 370 units of protein dissolved in 2 mL of buffer) was added at 37 °C. After 24 h of incubation at 37 °C, $MgCl_2$ was added (concentration = ca. 10 mM) followed by alkaline phosphatase (Sigma; 5 μL of a solution of 44 units of alkaline phosphatase, Type III-R from *Escherichia coli*, in 2 mL of 0.01 M Tris-acetate buffer, pH 8.8). Incubation was continued at 37 °C for an additional 2 h. Aliquots were heated for 3 min at 100 °C (protein denaturation) prior to HPLC analysis as described above. Digests of **3e** "fast" and **3e** "slow" gave products with elution times of 22.98 and 23.87 min, respectively, which were collected, concentrated in vacuo, and hydrolyzed with formic acid as described above. The 1:1 ratio of Gua:Ade found for both products were taken as evidence for $G_{DMT}A$ "fast" and "slow", respectively. The digests of **3f** "fast" and **3f** "slow" gave products with elution times 21.01 and 21.83 min, respectively, which were collected and identified as $A_{DMT}T$ "fast" and $A_{DMT}T$ "slow", respectively, based on the 1:1 ratio of Ade:Thy given by formic acid hydrolysis.

Removal of 5′-DMT Group. HPLC-collected products having a 5′-DMT group were detritylated with 3% v/v HOAc–H_2O (1 mL, pH 2.5–2.7) at room temperature for 5–10 min, which was followed by extraction of DMT-OH with EtOAc and then concentration to dryness using a vacuum centrifuge.

Formic Acid Degradation. One OD_{260} unit of GGAATTCC[17] standard was dissolved in formic acid (90%, 1 mL) and the resultant solution was transferred to a vial (4 mL) for heating at 120 °C in a heat block for 12 h. The cooled solution was evaporated to dryness under reduced pressure and the resultant residue was dissolved in 0.1 M TEAA buffer, pH 7 (200 μL) for analysis by HPLC (μBondapak reverse-phase C_{18} column, 7.8 mm × 30 cm; eluent: 0.1 M TEAA buffer, pH 7 containing 2% (v/v) of CH_3CN, flow rate = 4 mL/min, isocratic). The average ratio of absorptions measured at 280 nm for quadruplicate injections of the resultant equimolar amounts of Cyt (4.76 min), Gua (8.21 min), Thy (10.18 min), and Ade (15.26 min) were used to calculate[16] the base composition of all of the presently reported di- and oligonucleotide phosphonates and their side products, which were treated with formic acid and analyzed as described above for GGAATTCC.

Acknowledgment. We thank Dr. Paul S. Miller (The Johns Hopkins University) for providing authentic samples of $A_{Me}T$ for comparison with our products. James Cone (Laboratory of Experimental Carcinogenesis, National Cancer Institute) provided assistance in obtaining the FAB-MS data, and Dr. Michael F. Summers was helpful in recording NMR spectra. The comments of a referee were useful in prompting some of the control experiments regarding the attempted benzoylation reaction described herein.

Registry No. **3a** (isomer 1), 97352-74-4; **3a** (isomer 2), 97414-05-6; **3b** (isomer 1), 97352-75-5; **3b** (isomer 2), 97414-06-7; **3c** (isomer 1), 97352-76-6; **3c** (isomer 2), 97414-94-3; **3d** (isomer 1), 97352-77-7; **3d** (isomer 2), 97414-07-8; **3e** (isomer 1), 97352-78-8; **3e** (isomer 2), 97414-08-9; **3f** (isomer 1), 97352-79-9; **3f** (isomer 2), 97414-09-0; $T_{Me*}T$ (isomer 1), 97352-80-2; $T_{Me*}T$ (isomer 2), 97414-10-3; "Fast" $A_{Me}T$ (*R*p), 71830-18-7; "Slow" $A_{Me}T$ (*S*p), 71790-90-4; $[^{13}C]CH_3I$, 4227-95-6; benzoyl chloride, 98-88-4.

Photochemistry of the Anthracene Chromophore: The Dimerization of *trans*-1-(9-Anthryl)-2-phenylethylene

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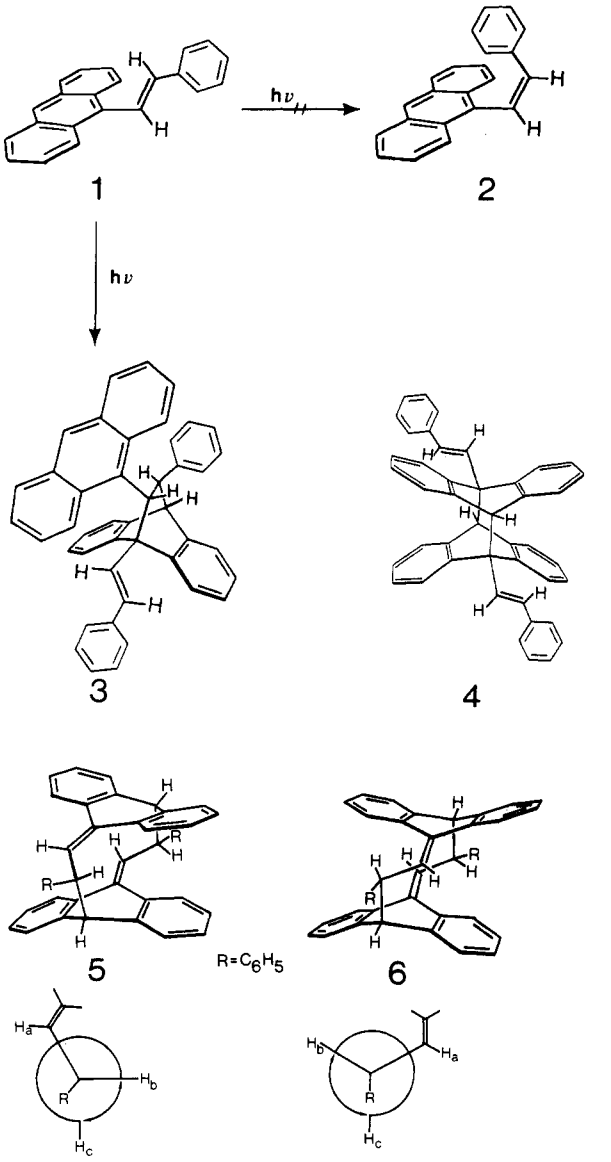
Most *trans*-1,2-diaryl substituted ethylenes upon photoexcitation undergo geometrical isomerization,[1] but all our attempts to prepare *cis*-1-(9-anthryl)-2-phenylethylene (**2**) by irradiation of its trans isomer (**1**) have been unsuccessful.[2,3] When a 10^{-4} M solution of **1** in degassed



benzene is irradiated, a seemingly clean[4] unimolecular reaction proceeds with a quantum efficiency of 0.0014, but neither is the cis isomer **2** detectable by UV spectroscopy nor has it been possible to isolate or characterize any other

(1) Mazzucato, U. *Pure Appl. Chem.* **1982**, *54*, 1705 and references cited therein.
(2) Becker, H.-D.; Andersson, K. *J. Org. Chem.* **1983**, *48*, 4549.
(3) For other examples of one-way photoisomerizations, see: Arai, T.; Karatsu, T.; Sakuragi, H.; Tokumaru, K. *Tetrahedron Lett.* **1983**, *24*, 2873. Cf. also: Lewis, F. D.; Petisce, J. R.; Oxman, J. D.; Nepras, M. J. *J. Am. Chem. Soc.* **1985**, *107*, 203.
(4) The absorption spectral changes during the disappearance of **1** give rise to an isosbestic point at 328 nm.

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