Transition Metal Labeling of Oligodeoxyribonucleotides: Synthesis and Characterization of (Pentacarbonyl)tungsten(0) Nucleoside Phosphites

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(Pentacarbonyl)tungsten(0) nucleoside phosphite complexes were prepared by reaction of nucleoside methyl phosphites generated *in situ* with pentacarbonyl(η^2-*cis*-cyclooctene)tungsten(0) (**1**). The mononucleoside metal complexes 5′-*O*-(4,4′-dimethoxytrityl)thymidine 3′-*O*-[dimethyl (pentacarbonyl)tungsten(0) phosphite] (**5a**) and *N*-benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyadenosine 3′-*O*-[dimethyl(pentacarbonyl)tungsten(0) phosphite] (**5b**) and the dinucleoside analogs (*Rp*,*Sp*)-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxythymidylyl-(3′→5′)-3′-*O*-acetylthymidine *O*-[methyl(pentacarbonyl)-tungsten(0) phosphite] (**8a**) and (*Rp*,*Sp*)-*N*-benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyadenylyl-(3′→5′)-3′-*O*-levulinylthymidine-*O*-[methyl(pentacarbonyl)tungsten(0) phosphite] (**8b**) were prepared and characterized by ^{1}H NMR, ^{31}P NMR, IR, and mass spectrometry. Extension of the methodology to longer oligomers using solid-phase methods was unsuccessful. HPLC, IR, and enzymatic digestion analysis of the products of the oligomer solid-support reactions indicated that reaction with **1** did not give the tungsten-derived oligonucleotide.

Introduction

Nonradioactively labeled polynucleotides are extensively employed in molecular biology and diagnostics.[1] Many studies have been directed toward the development of novel and relatively simple procedures for obtaining such derivatives. An important group of potential labels are the transition metals. Transition metal complexes have become important instruments for probing structural and functional details of genetic systems.[2]

Transition metal labeled polynucleotides have been employed in electron microscopic and X-ray crystallographic studies to examine polynucleotide microstructure.[3-4] Specifically, electron-dense heavy metal atoms are often employed to solve the phase problem during the determination of biopolymer structure.[3] Scanning transmission electron microscopy (STEM) has been used in conjunction with heavy metal labeling to discern the structural features of biomolecules.[5-7] The molecular conformation and structure of osmium-labeled poly-[d(AT)] was easily visualized using STEM.[7]

There are diagnostic implications for the role of transition metal labeled oligodeoxyribonucleotides as well. Group VI transition metal carbonyl complexes exhibit extremely intense absorptions in their IR spectra in the 2150–1900 cm^{-1} region due to terminal metal carbonyl linkages. Additionally, these signals fall into a region of absorption which excludes most other organic molecules, including those of proteins and DNA. Metalloimmunoassays involving chromium tricarbonyl and manganese tricarbonyl labeled biomolecules have successfully demonstrated the detection of such metal carbonyl complexes in biological samples using FT-IR in the picomole range.[8,9] With these results, we have anticipated that incorporation of such zero-valent metal carbonyl complexes into oligodeoxyribonucleotides could potentially result in their application in DNA probe based diagnostics.

Electron microscopic sequencing of polynucleotides labeled with heavy metals positioned at specific nucleotides was first discussed by Beer in the early 1960's.[10,11] In order for such a method to become feasible, it is necessary to develop procedures to quantitatively and specifically introduce heavy metal labels into polynucleotides. Previous labeling attempts have involved selective metal ion attachment either directly to purine or pyrimidine moieties[12-15] or to chemically modified pyrimidine or purine derivatives.[16,17] The latter methods thus required a number of synthetic trials.

We have undertaken routes to introduce transition metal complexes into oligodeoxyribonucleotides using a far simpler approach. This involves the introduction of the metal labels during the course of solid-phase DNA synthesis.[18-20] Our approach involves the substitution of a metal carbonyl coupling procedure for the oxidation

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(1) Landegren, U.; Kaiser, R.; Caskey, C. T.; Hood, L. *Science* **1988**, *242*, 229.
(2) Tullius, T. D., Ed. *Metal-DNA Chemistry*; American Chemical Society Symposium Series; American Chemical Society: Washington DC, 1989.
(3) Barton, J. K.; Lippard, S. J. Heavy metal Interactions with Nucleic Acids. In *Metal Ions Biol.*; Spiro, T. J., Ed.; John Wiley & Sons: New York, 1980, pp 31-113.
(4) Kennard, O.; Hunter, W. N. *Quart. Rev. Biophys.* **1989**, *22*, 327.
(5) Beer, M. *Ultramicroscopy* **1979**, *4*, 481.
(6) Wall, J. S.; Hainfield, J. F.; Bittner, J. W. *Ultramicroscopy* **1978**, *3*, 81.
(7) Cole, M. D.; Wiggins, W.; Beer, M. *J. Mol. Biol.* **1977**, *117*, 387.
(8) Butler, I. S.; Vessierères, A.; Jaouen, G. *Comments Inorg. Chem.* **1989**, *8*, 269.
(9) Ismail, A. A.; Jaouen, G.; Cheret, P.; Brossier, P. *Clin. Biochem.* **1989**, *22*, 297.
(10) Beer, M.; Zobel, C. R. *J. Mol. Biol.* **1961**, *3*, 717.
(11) Beer, M.; Moudrianakis, E. N. *Proc. Nat. Acad. Sci., U.S.A.* **1962**, *48*, 409.
(12) Whiting, R. F.; Ottensmeyer, F. P. *Biochim. Biophys. Acta* **1977**, *474*, 334.
(13) Dale, R. M. K.; Ward, D. C. *Biochemistry* **1975**, *14*, 2458.
(14) Dale, R. M. K.; Martin, E.; Livingston, D. C.; Ward, D. C. *Biochemistry* **1975**, *14*, 2447.
(15) Dale, R. M. K.; Livingston, D. C.; Ward, D. C. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 2238.
(16) Cai, S. X.; Wu, Y.; Keanna, J. F. W. *New J. Chem.* **1993**, *17*, 325.
(17) Lindsay, M.; Philipp, M. *GATA* **1991**, *5*, 311.
(18) Bergstrom, D. E.; Schmaltz, T. *J. Org Chem.* **1992**, *57*, 873.
(19) Bergstrom, D. E.; Schmaltz, T. *Nucleosides Nucleotides* **1989**, *8*, 1057.

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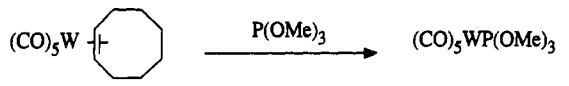
Scheme 1



1 2

procedure during standard oligonucleotide synthesis using phosphoramidite methodology. Specifically, reaction of oligonucleoside methyl phosphites generated *in situ* with metal carbonyl complexes affords metal carbonyl nucleoside methyl phosphite triesters.

This approach is generally more attractive since (1) the labels are positioned at the polymer backbone, (2) there is flexibility in this method such that several different metal labels may be introduced at various stages of DNA synthesis, and (3) the product metal carbonyl–phosphite complexes are extremely stable. This report describes the results of attempts to introduce a (pentacarbonyl)-tungsten(0) moiety into polynucleotides using standard DNA chemistry.

Results and Discussion

We first examined the specificity of reaction of metal complex 1[21] with phosphites. The model compound pentacarbonyl(trimethyl phosphite)tungsten(0) (**2**) was prepared by reaction of pentacarbonyl(η^2-*cis*-cyclooctene)-tungsten(0) (**1**)[21] with trimethyl phosphite. This compound was examined for its stability toward reagents and conditions of automated DNA synthesis.[22] This included conditions for detritylation, capping, oxidation and deprotection. The compound was found stable to all conditions as noted by an absence of change in the properties of the complex by TLC or IR.

Of initial concern with these reactions was the possibility for reaction of metal carbonyl complexes with purine and pyrimidine rings. With many types of metal complexes, there exists the potential for reaction at N-3 or O-4 of thymine, N-7 or N-3 of deoxyadenosine, N-7, N-1, or O-6 of deoxyguanosine, and N-3 of deoxycytidine.[23,24] Thus, in order to examine the reactivity of pentacarbonyl(η^2-*cis*-cyclooctene)tungsten(0) (**1**) toward other potential electron donors, 1 was combined with 5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-levulinylthymidine (**6c**) or with *N*-benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-acetyl-2′-deoxyadenosine (**6d**). Infrared and ^{1}H-NMR analysis confirmed the absence of nonspecific interactions of the protected nucleosides with metal complex **1**.

We have investigated the reaction of pentacarbonyl-(η^2-*cis*-cyclooctene)tungsten(0) (**1**) with mononucleoside phosphite esters, dinucleoside phosphite esters, and an oligodeoxyribonucleotide phosphite ester positioned at the 5′-terminus of an oligodeoxyribonucleotide during automated solid-phase DNA synthesis. The mononucleosides 5′-*O*-(4,4′-dimethoxytrityl)thymidine-3′-*O*-[dimethyl (pentacarbonyl)tungsten(0) phosphite] (**5a**) and *N*-benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyadenosine 3′-*O*-[dimethyl (pentacarbonyl)tungsten(0) phosphite] (**5b**) were prepared by reaction of phosphoramidites **3a** or **3b** with methanol and tetrazole to generate *in situ* intermediates **4a** or **4b**. Reaction of **4a** or **4b** with **1** afforded (pentacarbonyl)tungsten(0) nucleoside dimethyl phosphite derivatives **5a** or **5b**.

Phosphorus signals for **5a** or **5b** exhibit (natural abundance of ^{183}W, 14.3%) $J_{^{31}P-^{183}W}$ coupling constants equal to 390.2 Hz for **5a** and 393.4 Hz for **5b**. The ^{1}H-NMR spectra exhibited a pair of doublets for the two methyl phosphites [$J_{^{31}P-^1H}$ coupling constants equal to 10.0 Hz for **5a** and 12.0 Hz for **5b**]. FT-IR spectra displayed three carbonyl absorption bands for both **5a** and **5b**; such spectra are typical for (pentacarbonyl)tungsten(0) trialkyl phosphite complexes.[25,26]

Stereoisomeric dinucleoside analogs **8a** and **8b** were synthesized in a similar fashion by condensation of 3′-protected nucleosides **6a** or **6b** with phosphoramidites **3a** or **3b** in the presence of tetrazole to generate phosphites **7a** or **7b**. Metal coupling with **1** afforded complexes **8a** or **8b**. Using reversed-phase HPLC, stereoisomers (*R*p,*S*p) were separated. Hydrolysis of the 3′-acetyl group of **8a** with concentrated NH_4OH at 55 °C for 8 h afforded complex **8d**.

The ^{1}H-NMR spectra of the diastereomers of **8a** exhibited no apparent differences in chemical shifts. Infrared absorbances were similar to those obtained for model compound **2** and for mononucleosides **5a** and **5b**. The absolute configuration of the epimeric products was not determined since efforts to obtain suitable crystals for X-ray analysis were unsuccessful. Purification of the tungsten–phosphite complexes generally proved difficult. None could be crystallized, and estimated purity by NMR was generally less than 95%.

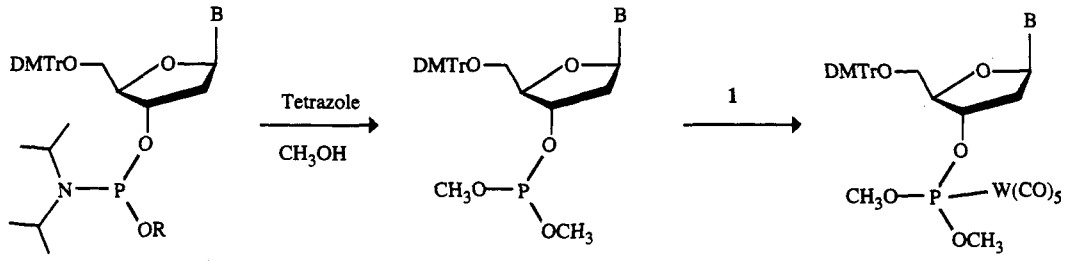
Substitution of β-cyanoethyl phosphoramidite **3c** for the methyl phosphoramidite **3b** resulted in the production of the tungstate(−1) complex **8c** as a triethylammonium salt upon basic workup. Confirmation of the structure of **8c** was based upon mass spectral analysis and upon comparison of spectroscopic data for model compound **9**. These types of compounds were examined for their stability to reagents and conditions of automated DNA synthesis and appear stable. However, since previous studies have suggested that such compounds would be easily destroyed by nucleophiles,[27] they were not pursued further.

The first step in the extension of $W(CO)_5$ derivatization to oligonucleotides was to determine if compound **1** would react with a phosphite triester which was covalently attached to a CPG support. Dinucleoside **8d** (dT-dT) was prepared in low yield (~25%) on a CPG solid support using an automated synthesizer and was characterized by ^{31}P NMR. The 3′-deprotected derivative of dinucleoside **8b** (dA-dT) was also prepared on a solid support and was characterized by mass spectrometry. Yields were low as indicated by HPLC. Modification of the reaction conditions, including solvent and reaction times, did not improve the efficiency of the reaction.

Despite the relatively low yields for synthesis of the tungsten-modified dinucleosides on the CPG support, extension of the described methodology to the synthesis of oligonucleotides containing a single $W(CO)_5$-derived phosphite triester linkage was attempted on an automated

(20) Dalla Riva Toma, J. M.; Toma, P. H.; Fanwick, P. E.; Bergstrom, D. E.; Byrn, S. R. *J. Crystallogr. Spectrosc. Res.* **1993**, *23*, 41.
(21) Atkinson, T.; Smith, M., In *Oligonucleotide Synthesis: A Practical Approach*; Gait, M. J., Ed.; IRL Press: Oxford, 1984, pp 35–81. Brown, T.; Brown, D. J. S. In *Oligonucleotides and Analogues: A Practical Approach*; Eckstein, F., Ed.; IRL Press: Oxford, 1991, pp 1–24.
(22) Franke, F.; Jenks, I. D. *Aust. J. Chem.* **1978**, *31*, 595.
(23) Beck, W.; Kottmair, N. *Chem. Ber.* **1976**, *109*, 970.
(24) Mathieu, R.; Lenzi, M.; Poilblanc, R. *Inorg. Chem.* **1970**, *9*, 2030. Poilblanc, R.; Bigorgne, M. *Bull. Soc. Chim. Fr.* **1962**, 1301.
(25) Andrews, G. T.; Colquhoun, J.; McFarlane, W.; Grim, S. O. *J. Chem. Soc., Dalton Trans.* **1982**, 2353.
(26) Brill, T. B.; Landon, S. J. *Chem. Rev.* **1984**, *84*, 577.

Scheme 2

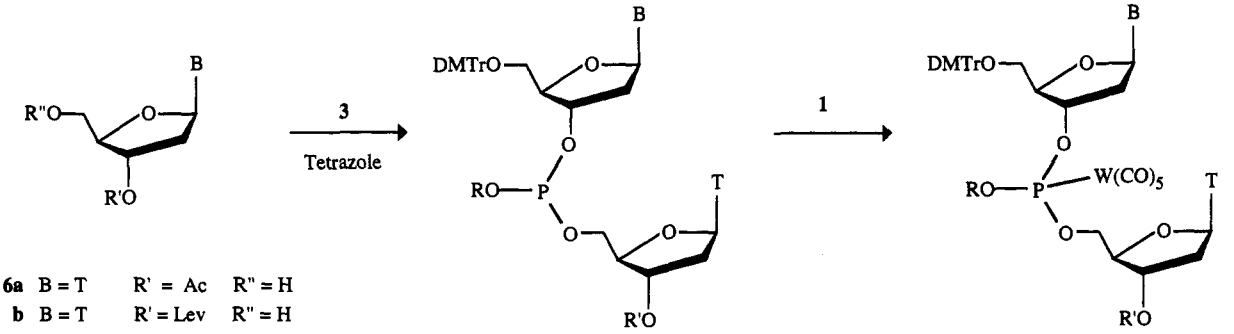


3a B = T R = CH_3
b B = A^{Bz} R = CH_3
c B = A^{Bz} R = CH_2CH_2CN

4a B = T
b B = A^{Bz}

5a B = T
b B = A^{Bz}

Scheme 3

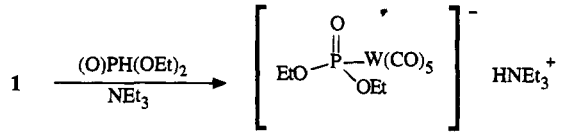


6a B = T R' = Ac R" = H
b B = T R' = Lev R" = H
c B = T R' = Lev R" = DMTr
d B = A^{Bz} R' = Ac R" = DMTr

7a B = T R = CH_3 R' = Ac
b B = A^{Bz} R = CH_3 R' = Lev
c B = A^{Bz} R = CH_2CH_2CN R' = Ac

8a B = T R = CH_3 R' = Ac
b B = A^{Bz} R = CH_3 R' = Lev
c B = A^{Bz} R = $HNEt_3^+$ R' = Ac
d B = T R = CH_3 R' = H

Scheme 4



9

DNA synthesizer. The attempted preparation of 5′-T*TpTpTpTpTpTpTpTpTp-TpTpTpTpT-3′ and 5′-A*TpTpTpGpCpCpApApApApT-3′ (where * indicates a tungsten carbonyl methyl phosphite triester linkage and *p* a phosphate triester linkage) was undertaken by introducing **3a** or **3b** in the final coupling step. The oxidation procedure was then replaced by a metal complexation procedure with **1**. Following deprotection, the crude products were analyzed by HPLC, IR, and enzymatic degradation. HPLC profiles observed for both oligomer reactions exhibited peaks with retention times corresponding to oligomer fragments. Analysis of all peaks using an IR spectrometer equipped with an IR plane microscope and an MCT detector confirmed the absence of metal carbonyl absorption bands; such spectra were identical to unmodified oligonucleotides with the same sequences. Enzymatic digestion analyses of all HPLC peaks suggested that metal complexation had not occurred; truncated sequences corresponding to cleavage at the intended site of metal complexation were obtained instead. Ligand exchange reactions are highly susceptible to steric effects which may be significantly greater for a phosphite triester that is part of a polymer bound oligonucleotide.

Experimental Section

Materials. 3′-*O*-Acetylthymidine was purchased from Aldrich Chemical Co., Inc., and used as obtained. Phosphoramidites and tetrazole were purchased from either Glenn Research, Inc., or from Applied Biosystems and used without further purification. Reagents for DNA synthesis were purchased from MilliGen/Biosearch, Inc. Solvents for chromatography were spectroscopic or HPLC grade. Reaction solvents were obtained from Aldrich Chemical Co., Inc., in Sure/Seal packages. Thin-layer chromatography (TLC) was performed on plastic-backed plates of Merck 60 F_{254} neutral (type E; 200 μm) alumina or Whatman UV_{254} (250 μm) silica gel. All TLC plates were preeluted prior to use. Visualization was accomplished under UV radiation (254 nm) or by iodine complexation. All 5′-(O-4,4′-dimethoxytrityl)-containing species were identified as orange spots on TLC plates by spraying with a fine mist of <0.01 M sulfuric or hydrochloric acid. Silica gel used for column chromatography was Kieselgel 60 (70–230 mesh; 0.063–0.200 mm). Preparative TLC was performed on Merck 60 F_{254} neutral (type E; 20 × 20 cm; 1.5 mm) alumina or Whatman PK5F (20 × 20 cm; 1 mm or 2 mm) silica gel.

Equipment and Procedures. All reactions were performed in Schlenkware under a nitrogen environment. Solvents were removed *in vacuo* at ambient temperature. Melting points were recorded uncorrected. Infrared spectra (IR) were recorded with liquid cells in suitable solvents between NaCl plates at 4 cm^{-1} resolution. For diastereomers **8a** and oligomers, IR spectra were recorded using solid samples on an instrument equipped with an IR plan microscope and a mercury cadmium telluride photodetector.

Chemical shifts (δ) for ^1H spectra are reported as parts per million downfield from the internal standard tetramethylsilane; chemical shifts (δ) for ^{31}P spectra are reported as parts per million downfield from the external standard 85% H_3PO_4.

Analytical HPLC columns were Beckman C18 reversed phase (5 μm; 4 mm × 26 cm); semipreparative columns were Econosil C18 (10 μm; 10 × 250 mm). Acetonitrile/water gradients were employed for both analytical and semipreparative work of dinucleoside derivatives. Flow rates for analytical analyses were 1.0 mL/min; flow rates for semipreparative analyses were 5.0 mL/min.

Preparation of pentacarbonyl(η^2-*cis*-cyclooctene)tungsten(0) (1) was discussed previously.[21] 3′-*O*-Levulinylthymidine (**6b**) was prepared according to the procedure of Kumar and Poonian.[28] Protected nucleosides **6c** and **6d** were prepared by standard procedures.[22] Compound **9** was prepared by a modification of the procedure of McFarlane *et al.*[29]

Mass spectrometric (MS) analyses were performed by the Mass Spectrometry facilities at the Purdue University Department of Medicinal Chemistry & Pharmacognosy. Compounds were analyzed by fast atom bombardment in either positive- or negative-ion mode using appropriate matrixes (nitrobenzyl alcohol, glycerol, or dithiothreitol:dithioerythritol (1:1)).

Pentacarbonyl(trimethyl phosphite)tungsten(0) (2). A solution of 0.24 g of complex **1**[21] (0.55 mmol) and 64.9 μL of freshly distilled $P(OMe)_3$ (0.55 mmol) in 30 mL of pentane was stirred overnight under N_2. Solvent was removed *in vacuo*, and **2** was purified by preparative TLC (alumina; hexane–acetone (95:5 v/v); R_f = 0.50) to a white oil: ^{1}H-NMR ($CDCl_3$) δ 3.68 (d; $J_{^{31}P,^1H}$ = 11.8 Hz); ^{31}P-NMR (acetone-d_6) δ 138.84 (s, $J_{^{31}P,^{183}W}$ = 386.1 Hz); IR (hexane) 2078.6 s, 1989.3 vs.

5′-*O*-(4,4′-Dimethoxytrityl)thymidine 3′-*O*-[dimethyl (pentacarbonyl) tungsten(0) phosphite] (5a). A mixture of 150 mg of **3a** (0.22 mmol), 30 mg of 1*H*-tetrazole (0.43 mmol), 65 μL of anhydrous CH_3OH (1.6 mmol), and 10 mL of anhydrous CH_3CN was stirred for 30 min under N_2. A solution of 0.30 g of **1** (0.69 mmol) in 5 mL of hexane was added, and stirring was continued for an additional 3 h. Solvent was removed under reduced pressure, the residue was washed with several volumes of hexane, and 39 mg of **5a** (0.04 mmol, 19%) was purified by preparative TLC (silica; CH_2Cl_2–MeOH (90:10 v/v); R_f = 0.65): mp 134 °C dec; ^{1}H-NMR (acetone-d_6) δ 7.35 (9H, m), 6.80 (5H, d), 6.42 (1H, dd), 5.28 (1H, m), 4.35 (1H, m), 3.80 (6H, s), 3.75 (3H, d; $J_{^{31}P,^1H}$ = 10.0 Hz), 3.70 (d, 3H; $J_{^{31}P,^1H}$ = 10.0 Hz), 3.46 (1H, m), 3.30 (1H, m), 2.70 (2H, m), 1.52 (3H, s); ^{31}P-NMR (acetone-d_6) δ 138.63 (s; $J_{^{31}P,^{183}W}$ = 390.2 Hz); IR (CH_2Cl_2) 2079.0 m, 1989.2 m, 1940.1 vs; MS calcd (MH^+) 961.1571, found (MH^+) 961.1502.

N-Benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyadenosine-3′-*O*-[dimethyl (pentacarbonyl)tungsten(0) phosphite] (5b). A mixture of 100 mg of **3b** (0.12 mmol), 17 mg of 1*H*-tetrazole (0.24 mmol), 54 μL of distilled anhydrous CH_3OH (1.33 mmol), and 10 mL of CH_3CN was stirred under N_2 for 30 min. A solution of 0.17 g of **1** (0.39 mmol) in 5 mL of hexane was added, and the mixture was stirred for an additional 5 h. Solvent was removed *in vacuo*, the residue was washed with several volumes of hexane, and 30 mg of **5b** (0.03 mmol, 25%) was purified using preparative TLC (silica; CH_2Cl_2–MeOH (95:5 v/v), 1% NEt_3; R_f = 0.68): mp 142 °C dec; ^{1}H-NMR ($CDCl_3$) δ 10.60 (s), 8.95 (1H, s), 8.71 (1H, s), 8.04 (5H, m), 7.30 (8H, m), 6.81 (5H, d), 6.48 (1H, dd), 5.24 (1H, m), 4.40 (1H, m), 4.05 (1H, m), 3.77 (6H, s), 3.66 (3H, d; $J_{^{31}P,^1H}$ = 12.0 Hz), 3.62 (3H, d; $J_{^{31}P,^1H}$ = 12.0 Hz), 3.41 (1H, m), 3.11 (1H, m), 2.75 (1H, m); ^{31}P-NMR ($CDCl_3$) δ 140.90 (s; $J_{^{31}P,^{183}W}$ = 391.4 Hz); IR (CH_2Cl_2) 2079.6 m, 1984.6 m, 1942.4 vs; MS calcd (MH^+) 1074.1949, found (MH^+) 1074.2016.

(*Rp*,*Sp*) 5′-*O*-(4,4′-Dimethoxytrityl)-2′-deoxythymidylyl-(3′→5′)-3′-*O*-acetylthymidine *O*-[Methyl (pentacarbonyl)-tungsten(0) phosphite] (8a). A mixture of 400 mg of **3a** (0.58 mmol), 200 mg of **6a** (0.70 mmol), and 120 mg of 1*H*-tetrazole (1.71 mmol) in 20 mL of CH_3CN was stirred under N_2 for 5 min and then combined with a solution of 450 mg of **1** (1.04 mmol) in 5 mL of hexane and 8 mL of distilled acetone. The reaction was stirred vigorously for 7 h and then poured into 100 mL of deionized H_2O which had been adjusted to pH 9 with NEt_3. The mixture was extracted four times with 100 mL of CH_2Cl_2 (with four drops of NEt_3 added). The CH_2Cl_2 extracts were dried over Na_2SO_4, concentrated in volume, and purified on 65 g of silica using CH_2Cl_2–MeOH–NEt_3 (96:3:1 v/v) as the eluent. Fractions with R_f = 0.40 were combined and dried under vacuum to give 156 mg of **8a** (0.13 mmol, 22%). Diastereomers were separated using reversed-phase HPLC and a gradient system consisting of 50%–100% B in 60 min (A = H_2O; B = CH_3CN). Peaks eluting at 32.6 and 33.6 min corresponded to (pentacarbonyl)tungsten-(0) nucleoside phosphite isomers **8a**. Fractions of the two diastereomeric tungsten–phosphite complexes were concentrated to pale yellow solids. For the diastereomer eluting at 32.6 min: mp 117 °C; ^{1}H-NMR (acetone-d_6) δ 10.04 (s, br), 7.65–7.34 (10H, m), 6.94 (5H, d), 6.44 (1H, m), 6.19 (1H, m), 5.31 (2H, m), 4.31 (2H, m), 4.18 (2H, m), 3.80 (s, 6H), 3.76 (3H, d; $J_{^{31}P,^1H}$ = 12.4 Hz), 3.46 (2H, m), 2.85 (3H, s), 2.65 (2H, m), 2.40 (2H, m), 1.80 (3H, s), 1.47 (3H, s); ^{31}P-NMR (acetone-d_6) δ 138.17. IR 2079.5 m, 1989.9 m, 1941.7 vs; MS calcd (MH^+) 1213.2, found (MH^+) 1213.3. For the diastereomer eluting at 33.6 min: mp 120 °C; ^{1}H-NMR (acetone-d_6) δ 10.04 (s, br), 7.65–7.35 (10H, m), 6.95 (5H, d), 6.43 (1H, m), 6.23 (1H, m), 5.30 (2H, m), 4.34 (2H, m), 4.18 (2H, m), 3.80 (6H, s), 3.73 (3H, d; $J_{^{31}P,^1H}$ = 12.2 Hz), 3.46 (2H, m), 2.85 (3H, s), 2.64 (2H, m), 2.41 (2H, m), 1.78 (3H, s), 1.47 (3H, s); ^{31}P-NMR (acetone-d_6) δ 137.42; IR 2079.2 m, 1989.9 m, 1941.4 vs; MS calcd (MH^+) 1213.2, found (MH^+) 1213.3.

(*Rp*,*Sp*)-*N*-Benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxy-adenylyl-(3′→5′)-3′-*O*-levulinylthymidine *O*-[Methyl (pentacarbonyl)tungsten(0) phosphite] (8b). A mixture of 200 mg of **3b** (0.35 mmol), 30 mg of 1*H*-tetrazole (0.43 mmol), and 10 mL of anhydrous CH_3CN was stirred under N_2 for 10 min. A solution of 135 mg of **6b**[28] (0.40 mmol) in 5 mL of CH_3CN was added, and the reaction mixture was stirred for an additional 15 min, whereby 217 mg of **1** (0.5 mmol) in 3 mL of hexane was introduced. After 5 h of vigorous stirring, the solvent was removed *in vacuo*, and 53 mg of compound **8b** (0.04 mmol, 16%) was purified by a combination of column chromatography (65 g silica; 90:10 $CHCl_3$–MeOH (v/v)) followed by preparative TLC (silica; 90:10 $CHCl_3$–MeOH (v/v); R_f = 0.60). Diastereomers were separated using reversed-phase HPLC and a gradient system consisting of 80–100% B (A = H_2O; B = CH_3CN) in 30 min; the two diastereomers eluted at 6.9 and 7.5 min: ^{1}H-NMR (acetone-d_6) δ 10.10 (s, br), 8.44 (1H, s), 8.15 (1H, s), 7.69–7.44 (6H, m), 7.34–7.21 (8H, m), 6.86–6.81 (5H, m), 6.64 (1H, m), 6.21 (1H, m), 5.48 (1H, m), 5.36 (1H, m), 4.42 (1H, m), 4.39 (1H, m), 3.77 (2H, m), 3.47 (2H, m), 2.85 (m), 2.82 (m), 2.57–2.51 (4H, m), 2.09 (s), 1.79 (s); ^{31}P-NMR (acetone-d_6) δ 138.14, 137.30; IR (CH_2Cl_2) 2080.7 m, 1991.2 w, br, 1945.1 vs; MS calcd (M^+) 1382.9, (MNa^+) 1405.9, found (M^+) 1382.5, (MNa^+) 1405.5.

***N*,*N*,*N*-Triethylammonium *N*-Benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxy-3′-*O*-phosphoadenylyl-*P*-[(pentacarbonyl-)tungstate(−1)]-(3′→5′)-3′-*O*-acetylthymidine (8c).** Compound **3c** (100 mg, 0.12 mmol), 68 mg of **6a** (0.24 mmol), 42 mg of 1*H*-tetrazole (0.60 mmol), and 10 mL of anhydrous CH_3CN were combined and stirred for 30 min under N_2. A solution of 0.17 g of **1** (0.36 mmol) in 5 mL of pentane was added, and the reaction mixture was stirred overnight. Solvent was removed *in vacuo*, and the residue was washed with several volumes of hexane followed by several volumes of deionized H_2O. The residue was dissolved in 10 mL of CH_2Cl_2, combined with 3–4 drops NEt_3, and extracted with several volumes of deionized H_2O until the aqueous effluent was clear. The CH_2Cl_2 layer was dried over Na_2SO_4 and concentrated to a gold residue. The material was loaded onto 90 g of silica and eluted with CH_2Cl_2–CH_3OH–NEt_3 (90:5:5 v/v). Fractions with R_f = 0.68 were concentrated to a gold granular solid, washed with Et_2O, and dried under vacuum: ^{1}H-NMR (acetone-d_6) δ 10.1 (s, br), 8.54 (2H, m), 8.15 (3H, m), 7.70–7.15 (18H, m), 6.87 (5H, m), 6.59 (1H, m), 6.32 (1H, m), 5.40 (2H, m), 4.20 (2H, m), 3.78 & 3.77 (12H, 2s), 3.38 (m), 3.20 (6H, q; J = 7.0 Hz), 2.35 (2H, m), 1.89 (3H, m), 1.33 (9H, t; J = 7.0 Hz); ^{31}P-NMR (acetone-d_6) δ 104.78, 103.19; IR (CH_2Cl_2) 2065.0 m, 1967.9 sh, 1922.3 vs; MS calcd (MH^+) 1310.24, (MNa^+) 1333.2, found (MH^+) 1309.8, (MNa^+) 1332.75.

(*Rp*,*Sp*) 5′-*O*-(4,4′-Dimethoxytrityl)-2′-deoxythymidylyl-(3′→5′)-thymidine *O*-[Methyl (pentacarbonyl)tungsten(0) phosphite] (8d). A milligram portion of **8a** was warmed at 55 °C in 5 mL of concentrated methanolic NH_3 for 8 h. HPLC analysis of the resulting mixture and a gradient system consisting of 50%–100% B in 60 minutes (A = H_2O; B = CH_3CN) exhibited

(28) Kumar, G.; Poonian, M. S. *J. Org. Chem.* **1984**, *49*, 4905.
(29) McFarlane, H. C.; McFarlane, W.; Rycroft, D. S. *J. Chem. Soc., Dalton Trans.* **1975**, 1616.

a set of doublet peaks at 26.1 and 27.2 minutes: ^{31}P-NMR (acetone-d_6) δ 138.17, 137.41.

***N,N,N*-Triethylammonium Pentacarbonyl(diethylphosphonato)tungstate(-1) (9).**[29] Complex 1 (0.50 g, 1.2 mmol), 148 μL of diethyl phosphite (1.2 mmol), and 160 μL of NEt_3 (1.2 mmol) in 20 mL of pentane were stirred under N_2 for 5 h and then concentrated to a yellow residue. Compound **9** was purified by preparative TLC (silica; hexane–acetone (50:50 v/v); R_f = 0.75) and recrystallized from hexane–Et_2O to give a white granular solid: ^{1}H-NMR (acetone-d_6) δ 3.94 (4H, m), 3.41 (6H, q; J = 7.5 Hz), 1.36 (6H, t; J = 6.9 Hz), 1.26 (9H, t; J = 7.5 Hz); ^{31}P-NMR (acetone-d_6) δ 112.85 (d; $J_{^{31}P,^{183}W}$ = 359.6 Hz); IR (CH_2Cl_2) 2064 m, 1984 sh, 1931.3 vs; negative ion MS calcd 460.96, found 461.0.

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Supplementary Material Available: ^{1}H NMR spectra of compounds **2**, **5a,b**, and **8a-c**, ^{31}P NMR spectra of **2**, **5a,b**, **8a-c**, and **9**, IR spectra of **5a,b** and **8a,b**, and a HPLC profile of **8a** (19 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.