with ether. Purification on silica gel gave 916 mg (92%) of ester: IR as above; NMR as above but also displaying minor resonances at 6.2, 5.8, and 5.55 ppm.

Anal. Calcd for $C_{14}H_{24}O_2S_2$: C, 58.31; H, 8.39. Found: C, 58.20; H, 8.43.

(*E*)-Ethyl 4-Cyclohexyl-4-oxo-2-butenoate. A solution of 232 mg (0.81 mmol) of the above thioketal in 3 mL of CH_3CN was added to 430 mg (3.22 mmol) of *N*-chlorosuccinimide and 617 mg (3.62 mmol) of silver nitrate dissolved in 15 mL of CH_3CN–H_2O (8:2) at −15 °C. The resulting mixture was stirred for 15 min at −15 °C and then treated with 3 mL of 10% aqueous sodium sulfite. The mixture was poured into brine, and the product was isolated with ether. Chromatography of the crude product on silica gel afforded 110 mg (69%) of the desired enone ester: IR λ_{max} 3015, 1725, 1700, 1640, 1450, 1370, 1305, 1280, 1180, 1030, 980 cm^{-1}; NMR (CCl_4) δ 6.77 (AB q, J = 15 Hz, $\delta_a - \delta_b$ = 28 Hz, 2 H), 4. 13 (q, J = 7 Hz, 2 H), 2.4 (m, 1 H), 1.28 (t, J = 7 Hz, 3 H).

Anal. Calcd for $C_{12}H_{18}O_3$: C, 68.54; H, 8,63; mol wt 210.1255. Found: C, 68.58; H, 8.67; mol wt (mass spectrum) 210.1267.

(*E*)-Ethyl 4-oxo-2,12-tridecadienoate: IR λ_{max} 3090, 3005, 1730, 1705, 1640, 1590, 1310, 1190, 1040, 990, 915 cm^{-1}; NMR (CCl_4) δ 6.57 (AB q, J = 15 Hz, $\delta_a - \delta_b$ = 24 Hz, 2 H), 5.6 (br m, 1 H), 4.8 (m, 2 H), 4.1 (q, J = 7 Hz, 2 H), 2.5 (br t, 2 H), 1.9 (m, 2 H), 1.3 (t, J = 7 Hz, 3 H), 1.3 (br s, 10 H).

Anal. Calcd for $C_{15}H_{24}O_3$: C, 71.39; H, 9.59; mol wt 252.1725. Found: C, 71.56; H, 9.62; mol wt (mass spectrum) 252.1738.

(*E*)-Ethyl 12-[(*tert*-butyldimethylsilyl)oxy]-4-oxo-2-tridecenoate: IR λ_{max} 1730, 1700, 1640, 1460, 1370, 1300, 1260, 1100, 1040, 840, 780 cm^{-1}; NMR (CCl_4) δ 6.55 (AB q, J = 15 Hz, $\delta_a - \delta_b$ = 22 Hz, 2 H), 4.1 (q, J = 7 Hz, 2 H), 3.65 (m, 1 H), 2.5 (br t, 2 H), 1.3 (t, J = 7 Hz, 3 H), 1.3 (br s, 12 H), 1.08 (d, J = 6 Hz, 3 H), 0.70 (s, 9 H).

Anal. Calcd for $C_{21}H_{40}O_4Si$: C, 65.58; H, 10.48. Found: C, 65.70; H, 10.52.

(*E*)-Ethyl 6-(*p*-methoxyphenyl)-4-oxo-2-hexenoate: IR λ_{max} 3060, 3020, 1720, 1700, 1630, 1610, 1580, 1510, 1300, 1250, 1180, 1030, 980, 820 cm^{-1}; NMR (CCl_4) δ 6.6 (m, 6 H), 4.1 (t, J = 7 Hz, 2 H), 3.65 (s, 3 H), 2.8 (s, m in CD_3COCD_3, 4 H), 1.3 (t, J = 7 Hz, 3 H).

Anal. Calcd for $C_{15}H_{18}O_4$: mol wt 262.1204. Found: mol wt (mass spectrum) 262.1214.

(*E*)-Ethyl 4-[(1α,3β)-3-methoxycyclopentyl]-4-oxo-2-butenoate: IR λ_{max} 3060, 1720, 1700, 1640, 1590, 1460, 1370, 1305, 1280, 1180, 1090, 1030, 980 cm^{-1}; NMR (CCl_4) δ 6.66 (AB q, J = 15 Hz, $\delta_a - \delta_b$ = 25 Hz, 2 H), 4.1 (t, J = 7 Hz, 2 H), 3.7 (m, 1 H), 3.25 (m, 1 H), 3.15 (s, 3 H), 1.3 (t, J = 7 Hz, 3 H).

Anal. Calcd for $C_{12}H_{18}O_4$: mol wt 226.1204. Found: mol wt (mass spectrum) 226.1203.

(*E*)-*tert*-Butyl 4-[(1α,3β)-3-methoxymethoxycyclopentyl]-4-oxo-2-butenoate: IR λ_{max} 1720, 1700, 1635, 1460, 1370, 1310, 1150, 1040, 980, 920, 850 cm^{-1}; NMR (CCl_4) δ 6.6 (AB q, J = 15 Hz, $\delta_a - \delta_b$ = 22 Hz, 2 H), 4.4 (s, 2 H), 4.05 (m, 1 H), 3.3 (m, 1 H), 3.2 (s, 3 H), 1.5 (s, 9 H).

Registry No. I (R = cyclohexyl, R′ = Et), 5452-75-5; I (R = $(CH_2)_7CH{=}CH_2$, R′ = Et), 692-86-4; I (R = $(CH_2)_7CHMe(OSiMe_2Bu\text{-}t)$, R′ = Et), 73434-17-0; I (R = $CH_2CH_2C_6H_4$-*p*-OMe, R′ = Et), 4586-89-4; I (R = *trans*-3-MeO-cyclopentyl, R′ = Bu), 73434-18-1; I (R = *trans*-3-$MeOCH_2O$-cyclopentyl, R′ = Et), 73453-14-2; IIa (R = cyclohexyl, R′ = Et), 73434-19-2; IIa (R = $(CH_2)_7CH{=}CH_2$, R′ = Et), 73434-20-5; IIa (R = $(CH_2)_7CHMe(OSiMe_2Bu\text{-}t)$, R′ = Et), 73434-21-6; IIa (R = $CH_2CH_2C_6H_4$-*p*-OMe, R′ = Et), 73434-22-7; IIa (R = *trans*-3-MeO-cyclopentyl, R′ = Bu), 73434-23-8; IIa (R = *trans*-3-$MeOCH_2O$-cyclopentyl, R′ = Bu), 73434-24-9; IIb (R = cyclohexyl), 73434-25-0; IIb (R = $(CH_2)_7CH{=}CH_2$), 73434-26-1; IIb (R = $(CH_2)_7CHMe(OSiMe_2Bu\text{-}t)$), 73434-27-2; IIb (R = $CH_2CH_2C_6H_4$-*p*-OMe), 73434-28-3; IIb (R = *trans*-3-MeO-cyclopentyl), 73434-29-4; IIb (R = *trans*-3-$MeOCH_2O$-cyclopentyl), 73434-30-7; IIIa (R = cyclohexyl), 73434-31-8; IIIa (R = cyclohexyl), free acid, 73434-32-9; IIIa (R = $(CH_2)_7CH{=}CH_2$), 73434-33-0; IIIa (R = $(CH_2)_7CHMe(OSiMeBu\text{-}t)$), 73434-34-1; IIIa (R = $CH_2CH_2C_6H_4$-*p*-OMe), 73434-35-2; IIIa (R = *trans*-3-MeO-cyclopentyl), 73434-36-3; IIIa (R = *trans*-3-$MeOCH_2O$-cyclopentyl), butyl ester, 73434-37-4; IIIb (R = cyclohexyl), 73434-38-5; IIIb (R = $(CH_2)_7CH{=}CH_2$), 73434-39-6; IIIb (R = $(CH_2)_7CHMe(OSiMe_2Bu\text{-}t)$), 73453-15-3; IIIb (R = $CH_2CH_2C_6H_4$-*p*-OMe), 73434-40-9; IIIb (R = *trans*-3-MeO-cyclopentyl), 73434-41-0; IIIb (R = *trans*-3-$MeOCH_2O$-cyclopentyl), butyl ester, 73434-42-1; $MeSO_2SMe$, 2949-92-0; β,β-bis(methylthio)cyclohexaneethanol, 73434-43-2; triethyl sodiophosphonoacetate, 22822-85-1; ethyl (trimethylsilyl)acetate, 4071-88-9.

Oligonucleotide Analogues with Internucleoside Phosphite Links[1]

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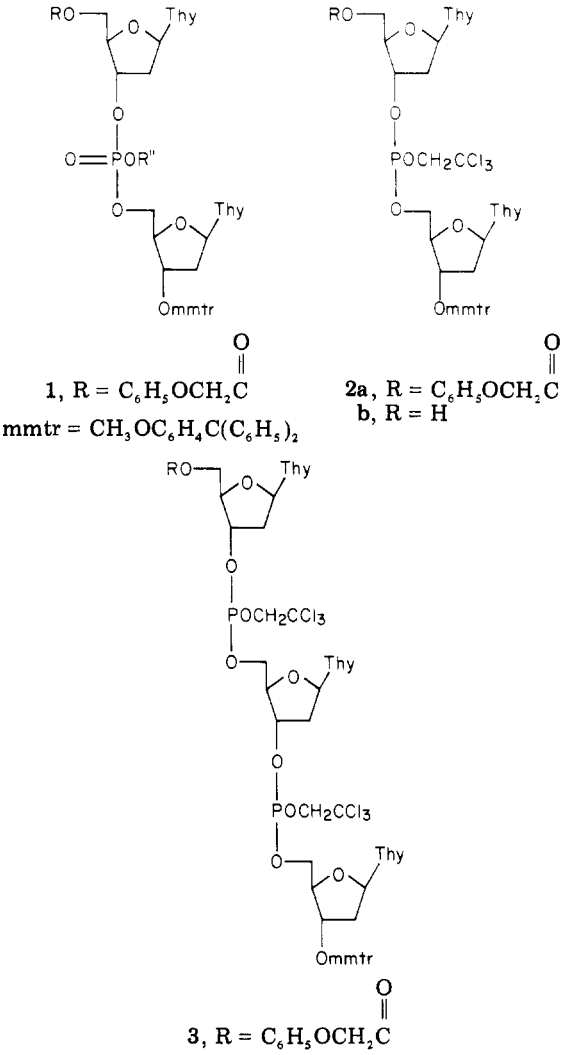
Oligonucleotide derivatives possessing internucleoside phospho triester links (e.g., as in compound 1) have proven



useful in the synthesis of polynucleotides[2] and, in addition, exhibit interesting biochemical properties in their own right.[3] We describe in this note two examples of another class of electrically uncharged oligonucleotide analogues, compounds **2** and **3**, in which nucleosides are joined by phosphite links. Although such compounds are presumed

(1) This work was supported by Grant 10265 from the National Institute of General Medical Sciences of the National Institutes of Health.

(2) For a current review see M. Ikehara, E. Ohtsuka, and A. F. Markham, *Adv. Carbohydr. Chem. Biochem.*, **36**, 135–213 (1979).

(3) J. C. Barrett, P. S. Miller, and P. O. P. Ts'o, *Biochemistry*, **13**, 4897–4906 (1974); R. C. Pless and P. O. P. Ts'o, *ibid.*, **16**, 1239–1250 (1977).

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intermediates in the phosphite triester synthetic sequence,[4] no oligomers of this class have previously been isolated and described. This work was undertaken both to provide further information on parameters in the phosphite triester synthesis and to explore the stability and synthetic potential for compounds of this type.

Dinucleoside phosphite **2a** was synthesized by successive reaction of trichloroethyl phosphorodichloridite with 5′-*O*-(phenoxyacetyl)thymidine and 3′-*O*-(mono-*p*-methoxytrityl)thymidine. It was isolated in 70% yield, based on the (methoxytrityl)thymidine, and was characterized by elemental analysis and the ^{31}P NMR spectrum, which showed a single band at δ 157.2 (relative to triphenyl phosphate) characteristic of trialkyl phosphites. The ultraviolet spectrum, infrared spectrum, and chromatographic properties on silica closely resembled values for the corresponding phosphate (**1**, R″ = Cl_3CCH_2O).

2a consumed a stoichiometric quantity of iodine in water–tetrahydrofuran–2,6-lutidine to give the dinucleoside trichloroethyl phosphate (**1**, ^{31}P NMR δ 15). Thin-layer chromatography of the reaction mixture showed essentially quantitative conversion to this phosphate with less than 1% side products. This experiment demonstrates that this oxidation procedure is highly efficient and that side products observed in the standard phosphite triester sequence (which involves oxidation with iodine–water) must arise primarily in other stages of the synthesis.

2a is stable in pyridine solution (no decomposition was observed on standing in pyridine for 18 h at 25 °C), but it decomposes slowly in a pyridine solution 0.45 M in pyridine hydrochloride (approximately 30% of **2a** was converted to a complex mixture of products within 18 h at 25 °C). Since pyridine hydrochloride is formed in the reaction leading to **2a**, this experiment points up the desirability of short reaction times and low temperatures in work involving the phosphite intermediates.

Especially interesting is the observation that the internucleoside phosphite linkage is stable enough to permit selective cleavage of the phenoxyacetate function. Thus, chromatographic examination of the reaction mixture obtained on treating **2a** with ammonium hydroxide–dioxane (1:1) for 10 min at 57 °C revealed only two products: **2b** and phenoxyacetamide. Alternatively, **2b** was formed on treating **2a** with liquid ammonia for 1.5 h. This finding opened the way to extending the chain. In a preparative run, **2b** was isolated in 79% yield from a reaction with ammonium hydroxide–dioxane and subjected to reaction with the monochloridite intermediate formed from 5′-*O*-(phenoxyacetyl)thymidine and trichloroethyl phosphorodichloridite. Trinucleoside diphosphite **3** was obtained in 40% yield.

Attempts to remove the methoxytrityl group *selectively* from **2a** with aqueous acetic acid, benzenesulfonic acid, or boron trifluoride etherate have thus far been unsatisfactory, although TLC results indicated formation of some of the desired phosphite. It therefore appears that another type of protecting group should be used if regeneration of the 3′-OH group is desired.

Experimental Section

Solvents and nucleosides were dried as previously described.[4] Analytical thin-layer chromatography (TLC) was done on Eastman silica gel sheets (13181). Spots were visualized with UV light and by spraying with 10% perchloric acid in water (after heating, nucleosides appeared gray and methoxytritylated nucleosides, yellow brown). Preparative TLC was done on Analtech precoated silica gel plates (1 mm). ^{31}P NMR spectra were recorded at 36.2 MHz in a pulsed Fourier-transform mode on a JEOL FX90Q spectrometer. Chemical shifts are reported downfield relative to external triphenyl phosphate in hexadeuterioacetone. Condensation reactions were carried out in small flasks equipped with a rubber septum and a $CaCl_2$ drying tube. Reagents were added by means of a hypodermic syringe and the mixtures were stirred throughout the reaction period.

Trichloroethyl 5′-*O*-(Phenoxyacetyl)thymidine-3′ 3′-*O*-(Mono-*p*-methoxytrityl)thymidine-5′ Phosphite (2a). 5′-*O*-(Phenoxyacetyl)thymidine[4] (150 mg, 0.4 mmol) in THF (0.6 mL) was added (3 min) to a solution of $Cl_3CCH_2OPCl_2$ (53 μL, 0.36 mmol) and pyridine (0.2 mL) in THF (0.6 mL) in a flask cooled with dry ice/2-propanol. A white precipitate formed immediately. The tube which had contained the nucleoside was rinsed twice with THF (0.6 mL each) and the rinsings were added to the reaction vessel. After an additional 3 min, 3′-*O*-(mono-*p*-methoxytrityl)thymidine (100 mg, 0.19 mmol) in THF (0.6 mL) was added. Again the tube containing the nucleoside was rinsed twice with THF (0.6 mL) and the rinses were added to the reaction vessel. Stirring was continued at −78 °C for 15 min; then the reaction flask was moved to a bath at −10 °C and after 2 min 2 mL of THF–H_2O (1:1) was added dropwise. When the solution reached room temperature, saturated aqueous NaCl (2 mL) was added and the organic phase was analyzed by TLC with ether–$CHCl_3$–C_2H_5OH (20:20:1): R_f 0.0 (faint trityl positive spot); R_f 0.07 (trityl negative, strong spot under UV, this is the trichloroethyl ester of bis(5′-*O*-(phenoxyacetyl)thymidine-3′ phosphite[5]); R_f 0.25 (very strong trityl positive test, **2a**); and R_f 0.51 (very faint trityl positive test). The organic layer was then concentrated and applied to three silica gel plates (Analtech) for preparative separation of the products. Two developments with $CHCl_3$–C_2H_5OH (24:1), elution of the product band with THF, concentration of the eluate to 2 mL, and precipitation in hexane (40 mL) gave 145 mg (70%) of **2a**: mp 114–117 °C; ^{31}P NMR δ 157.2; UV λ_{max} (C_2H_5OH) 264 nm (ϵ 21 200), λ_{min} 244 (15 000); R_f (24:1 $CHCl_3$–C_2H_5OH) 0.45; R_f (CH_3CN) 0.30.

Anal. Calcd for $C_{50}H_{50}Cl_3N_4O_{14}P$: C, 56.22; H, 4.72; N, 5.24. Found: C, 56.29; H, 4.57; N, 5.36.

Trichloroethyl Thymidine-3′ 3′-(Mono-*p*-methoxytrityl)thymidine-5′ Phosphite (2b). **2a** (128 mg) in THF (5 mL) was stirred with 5 mL of ammonium hydroxide–dioxane (1:1, v/v) at room temperature. Periodic TLC analyses (CH_3CN) showed that the starting material (R_f 0.3) was slowly converted to the desired product (R_f 0.21) and phenoxyacetamide (R_f 0.46). After 100 min the reaction was complete. The solution was concentrated in vacuo to 1 mL and partitioned between CH_2Cl_2 and saturated aqueous NaCl. After separation of the layers and appropriate back extractions, the organic layer was concentrated and chromatographed on silica gel preparative plates with CH_3Cl–C_2H_5OH (50:1) and then CH_3CN as developing solvents. Elution with THF, concentration, and admixture with hexane afforded the title compound: 89 mg (79%); mp 111–113 °C; ^{31}P NMR δ 157.2; R_f (CH_3CN) 0.21.

Anal. Calcd for $C_{42}H_{44}Cl_3N_4O_{12}PH_2O$: C, 52.98; H, 4.87; N, 5.88. Found: C, 52.83; H, 4.86; N, 5.51.

Preparation of Trinucleoside Phosphite 3. This reaction was carried out just like the synthesis leading to **2a**, except that **2a** (50 mg, 0.053 mmol) was substituted for (methoxytrityl)thymidine (100 mg, 0.19 mmol) and the quantities of other reagents were reduced to the smaller scale (i.e., by a factor of 0.053/0.19). Separation of the product mixture by preparative TLC with first $CHCl_3$–C_2H_5OH (50:1) and then $CHCl_3$–C_2H_5OH (25:1) gave 30 mg (40%) of **3**: mp 118–120 °C; ^{31}P NMR δ 157, 157.3; R_f (CH_3CN) 0.14.

Anal. Calcd for $C_{62}H_{64}Cl_3N_6O_{20}P_2$: C, 50.05; H, 4.33; N, 5.65. Found: C, 49.69; H, 4.40; N, 5.34.

Registry No. **1** (R″ = Cl_3CCH_2O), 60010-50-6; **2a**, 73496-62-5; **2b**, 73496-63-6; **3**, 73496-64-7; 5′-*O*-(phenoxyacetyl)thymidine, 38558-16-6; 3′-*O*-(mono-*p*-methoxytrityl)thymidine, 73496-65-8; $Cl_3CCH_2OPCl_2$, 60010-51-7.

(4) R. L. Letsinger and W. B. Lunsford, *J. Am. Chem. Soc.*, **98**, 3655–3661 (1976).

(5) The proportions of the reactants were selected to give some of this 3′-*O*-3′-*O* dinucleoside phosphite derivative (see ref 4). It can be separated readily from **2a** by chromatography.