(46.8), 213 (100). Anal. (exact mass, HR-EIMS) Calcd for $C_{20}H_{23}FO_2$ 314.1682, found 314.1677.

17β-Ethynyl-16α-fluoroestra-1,3,5(10)-triene-3,17α-diol (20) and 17α-Ethynyl-16α-fluoroestra-1,3,5,(10)-triene-3,17β-diol (21). Butyllithium (1.9 M in hexane, 180 μmol) was added to a solution of (trimethylsilyl)acetylene (25 μL, 180 μmol) in pentane at 0 °C. Tetrahydrofuran (100 μL) was added to bring the acetylide into solution. Fluoro ketone **16** (26 mg, 62 μmol) dissolved in THF (0.5 mL) was added, and after 5 min TLC analysis indicated consumption of starting material was complete. The reaction was quenched with dilute aqueous NH_4Cl and extracted once with pentane and once with EtOAc. The organic phase was dried over Na_2SO_4, the solvent removed in vacuo, and the residue dissolved in MeOH (0.5 mL). Three drops of 5 N KOH were added. The solution was heated at 60 °C for 30 min, quenched with aqueous NH_4Cl, and extracted with EtOAc. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure, and the residue was subjected to flash chromatography (15 mm × 6 in. SiO_2, 30% EtOAc in hexane). Two products were formed (R_f 0.34 and 0.17). Each fraction was further purified by preparative HPLC (Whatman M9, 5 mL/min, 90% hexane, 8.7% CH_2Cl_2, 1.3% *i*-PrOH) to yield **20** (5 mg, 26%, t_R = 16 min) and **21** (5 mg, 26%, t_R = 28 min).

20: mp (powder) 183-185 °C; NMR (220 MHz, CD_2Cl_2) δ 0.91 (s, 3 H, 18-CH_3), 1.26-2.37 (m, 11 H), 2.56 (s, 1 H, ≡CH), 2.77-2.80 (m, 3 H), 4.89-4.90 (br s, 1 H, OH), 5.26 (ddd, 1 H, J = 51.5, 8.4, 3.1 Hz, 16β-H), 6.53 (d, 1 H, J = 2.1 Hz, 4-H), 6.60 (dd, 1 H, J = 8.5, 2.6 Hz, 2-H), 7.13 (d, 1 H, J = 8.3 Hz, 1-H); ^{19}F NMR (338 MHz, CD_2Cl_2) φ -194.2 (dddd, J = 58.64, 29.08, 21.16, 7.53 Hz); mass spectrum (70 eV), m/z (relative intensity) 314 M^+ (54.6), 288 (38.5), 213 (100). Anal. (exact mass, HR-EIMS) Calcd for $C_{20}H_{23}FO_2$ 314.1682, found 314.1679.

21: mp (powder) 186-192 °C; NMR (220 MHz, CD_2Cl_2) δ 0.89 (s, 3 H, 18-CH_3), 1.35-2.13 (m, 12 H), 2.77 (s, 1 H, ≡CH), 2.78-2.81 (m, 2 H), 4.83 (br s, 1 H, OH), 5.10 (ddd, 1 H, J = 58.9, 32.3, 20.3 Hz, 16β-H), 6.54 (br s, 1 H, 4-H), 6.59 (dd, 1 H, J = 8.3, 2.8 Hz, 2-H), 7.13 (d, 1 H, J = 8.4 Hz, 1-H); ^{19}F NMR (338 MHz, CD_2Cl_2) φ -185.8 (ddd, J = 53.6, 29.0, 18.1 Hz); mass spectrum (70 eV), m/z (relative intensity) 314 M^+ (46.7), 288 (22.5), 213 (100). Anal. (exact mass, HR-EIMS) Calcd for $C_{20}H_{23}FO_2$ 314.1682, found 314.1679.

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Registry No. **1**, 53-16-7; **2**, 20592-42-1; **3**, 1239-35-6; **4**, 566-76-7; **5**, 92844-09-2; **6**, 92817-03-3; **6** (silylacetylene deriv), 92817-13-5; **7**, 84693-92-5; **11**, 92817-04-4; **12**, 92817-05-5; **13**, 92817-06-6; **14**, 92817-07-7; **15**, 92817-08-8; **16**, 92817-09-9; **17**, 92817-10-2; **18**, 92817-11-3; **19**, 92817-12-4; **20**, 92935-39-2; **21**, 92817-14-6; $(CH_3)_3SiC{\equiv}CH$, 1066-54-2; 16β-chloroestrone triflate, 92817-15-7; 16β-bromoestrone acetate, 65912-80-3; 16α-chloroestrone triflate, 92817-16-8; 16α-bromoestrone acetate, 1239-35-6; 16α-iodoestrone triflate, 92817-17-9.

Supplementary Material Available: NMR studies of 16-fluoroestrogen stereochemistry (10 pages). Ordering information is given on any current masthead page.

Improvements in Oligodeoxyribonucleotide Synthesis: Methyl *N,N*-Dialkylphosphoramidite Dimer Units for Solid Support Phosphite Methodology

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Two procedures for the synthesis of methyl *N,N*-dialkylphosphoramidite dinucleotides (dimer units) compatible with the current solid support phosphite methodology of oligodeoxynucleotide synthesis are described for the first time. In the first procedure a condensation is performed between the 3'-methyl *N,N*-dialkylphosphoramidite moiety of a fully protected nucleotide and the 5'-hydroxyl of a 3'-*O*-levulinyl base protected nucleoside. The phosphite triester internucleotide bond of the resulting dimer is oxidized to the phosphotriester, 3'-*O*-levulinyl is selectively cleaved, and the 3'-hydroxyl of the product is derivatized to methyl *N,N*-dialkylphosphoramidite to give a dimer unit usable in the conventional phosphite methodology of oligonucleotide synthesis. For synthesis of some of the dimer units, especially those containing a 3'-cytidine residue, 3'-*O*-*tert*-butyldimethylsilyl protection in place of the 3'-*O*-levulinyl group was found to be advantageous. Using these dimer phosphite units, the synthesis of several oligonucleotides ranging in size from 16 to 19 units is described. The efficiencies of condensation of the dinucleotide units (98-100%) are quite comparable to the monomer condensations.

Introduction

Chemical synthesis of oligonucleotides has undergone revolutionary developments during the last decade. Although fundamental studies in the synthesis of oligonucleotides were carried out by the classical diester methodology,[1] the development of the triester technique[2,3] in conjunction with the highly efficient and rapid high-performance liquid chromatographic (HPLC) techniques for the purification of oligomeric products added an element of speed to the method. Finally, the use of solid support in the triester method of synthesis[3] accelerated the speed of synthesis dramatically and it became practical

(1) For a comprehensive discussion of the diester method and its applications, see: Khorana H. G. *Bioorg. Chem.* **1978**, *7*, 351-393.

(2) Several laboratories contributed to the development of the triester method. Letsinger, R. L.; Ogilvie, K. K. *J. Am. Chem. Soc.* **1969**, *91*, 3350-3355. Cusack, N. J.; Reese, C. B.; van Boom, J. H. *Tetrahedron Lett* **1973**, 2209-2212. Itakura, K.; Bahl, C. P.; Katagiri, N.; Michinewicz, J.; Wightman, R. H.; Narang, S. A. *Can. J. Chem.* **1973**, *51*, 3649-3651.

(3) Miyoshi, K.; Itakura, K. *Tetrahedron Lett.* **1979**, 3635-3638. Gait, M. J.; Singh, M.; Sheppard, R. C.; Edge, M. D.; Greene, A. R.; Heathcliffe, G. R.; Atkinson, T. C.; Newton, C. R.; Markham, A. F. *Nucleic Acids Res.* **1980**, *8*, 1081-1096. Miyoshi, K.; Arentzen, R.; Huang, T.; Itakura, K. *Nucleic Acids Res.* **1980**, *8*, 5507-5516. Dembek, P.; Miyoshi, K.; Itakura, K. *J. Am. Chem. Soc.* **1981**, *103*, 706-708.

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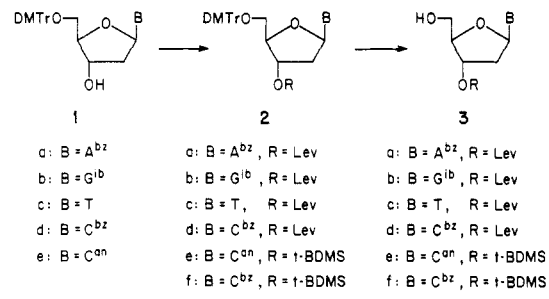
Scheme I



to use synthetic oligonucleotides for the solution of numerous research problems in molecular biology.

The alternate phosphite methodology of oligonucleotide synthesis was introduced by Letsinger and co-workers[4] and subsequently explored in several other laboratories.[5] However, due to the highly reactive nature of nucleoside phosphite intermediates used at that time, the method did not become practical until 1981 when Caruthers[6] discovered new and relatively more stable mononucleoside phosphoramidite condensing units. When these units were used, synthesis of defined oligodeoxynucleotides was achieved on a solid support of silica,[6,7] thus heralding a new and powerful methodology. The inherent high reactivity of the trivalent phosphorus intermediates used in the phosphite approach makes this method exceedingly attractive. For instance, the condensation for the internucleotide bond takes less than 5 min[8] in phosphite methodology compared to about 1 h in the triester method when reactions are performed at room temperature. However, until now, the triester methodology had a distinct advantage over the phosphite technique for the synthesis of longer DNA molecules ($\simeq$30–40 units)[9] because of the availability of appropriate triester dimer and trimer units.[10] The use of dimer and trimer condensing blocks not only enhances the speed of synthesis but makes the purification of the final product easier as well. Recently, Adams and co-workers[11] have reported the synthesis of a long DNA molecule (51 mer) using the monomer phosphoramidite procedure. The yield of the final product was, however, poor due to many condensation and detritylation steps. Part of the loss in the final yield may have resulted from cumulative effects of small amounts of depurination occurring at each detritylation step. The yield losses of this type would be reduced to about half by using appropriate dimer units compatible with the current solid support phosphite method of DNA synthesis. Two recent publications documentating the feasibility of condensing dimers via phosphite methodology to achieve synthesis of natural oligonucleotides[12] or their analogues[13] further support the desirability of having access to efficient synthesis of dimer phosphite units. Considering the advantages of dimer blocks, a project to construct dimer units for the solid support phosphite method of oligodeoxynucleotide synthesis was initiated.

In this report we describe two methods for the synthesis of appropriately protected phosphoramidite dimer units which are compatible with the current solid support phosphite methodology for the synthesis of oligodeoxyribonucleotides. The utility of these dimer units is illustrated by the synthesis of several oligodeoxynucleotide fragments ranging in size from 16 to 29 units.

Results and Discussion

The aim of this work was to develop a practical synthesis of dimer units of the general structure **7** (Scheme II) so that the overall strategy of deoxynucleotide synthesis using these units can be patterned after the scheme currently used in solid support phosphite methodology with stepwise monomer condensations.[6] Thus the protections chosen for various functions of the dimer unit were as follows: dimethoxytrityl for 5′-hydroxyl, methyl for internucleotide phosphate and traditional protections of benzoyl, anisoyl and isobutyryl for the exocyclic amine functions of adenine, cytidine, and guanine, respectively. The 3′-hydroxyl end of the dimers was designed to have a methyl *N,N*-dialkylphosphoramidite linkage. As outlined in Scheme II, the first key step for the synthesis of dimers **7** was the preparation of the corresponding 3′-hydroxyl compounds **6** which in principle can be obtained from suitable 3′-*O*-protected precursors. The choice of 3′-hydroxyl protection of the precursor was, however, critical. The protecting group for 3′-OH should have the following characteristics: (1) The protection reaction should be a high yield step. (2) It should be cleaved selectively without any detrimental effect on the other protecting groups in the molecule such as methyl on internucleotide phosphate, dimethoxytrityl at 5′-hydroxyl, and acyl protections on exocyclic amine. (3) The 3′-OH protecting group should be stable toward various detritylation reactions reported in the literature and the phosphite condensation conditions.

Out of several hydroxyl protecting groups described in the literature, the levulinyl ($-COCH_2CH_2COCH_3$) group was considered appropriate for the 3′-OH protection. It was reported to be selectively cleaved in the presence of other protecting groups by a brief treatment with an ice cold 0.5 M solution of hydrazine monohydrate in pyridine–acetic acid buffer (pH 5.1).[14] However, stability of the methyl protection on internucleotide phosphate during the removal of the levulinyl group needed to be evaluated.

As shown in Scheme II, the two key precursors for the synthesis of a phosphite dimer are a 5′-terminal unit (**4**) and a 3′-terminal unit (**3**). For the synthesis of dimer units (**5**) having a levulinyl as a 3′-hydroxyl protecting group, the 3′-*O*-levulinyl nucleosides (**3**) were used as the 3′-terminal units. These protected nucleosides (**3a–d**) were synthesized (Scheme I) in good yields by the levulinization of 5′-*O*-dimethoxytrityl nucleosides (**1a–d**) with levulinic acid and DCC (dicyclohexylcarbodiimide) to yield completely protected nucleosides (**2a–d**) followed by the removal of the 5′-*O*-dimethoxytrityl group on treatment with 80% aqueous acetic acid. The condensation of 3′-*O*-levulinyl protected nucleosides (**3a–d**) and 5′-*O*-dimethoxytrityl monomer phosphite intermediates **4a–d** in the presence of tetrazole followed by the oxidation of the in-

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(10) Hirose, T.; Crea, R.; Itakura, K. *Tetrahedron Lett.* **1978**, 2449–2452. Wallace, R. B.; Johnson, M. J.; Suggs, S. V.; Miyoshi, K.; Bhatt, R.; Itakura, K. *Gene* **1981**, *16*, 21–26.
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(12) Letsinger, R. L.; Groody, E. P.; Lander, N.; Tanaka, T. *Tetrahedron* **1984**, *40*, 137–143.
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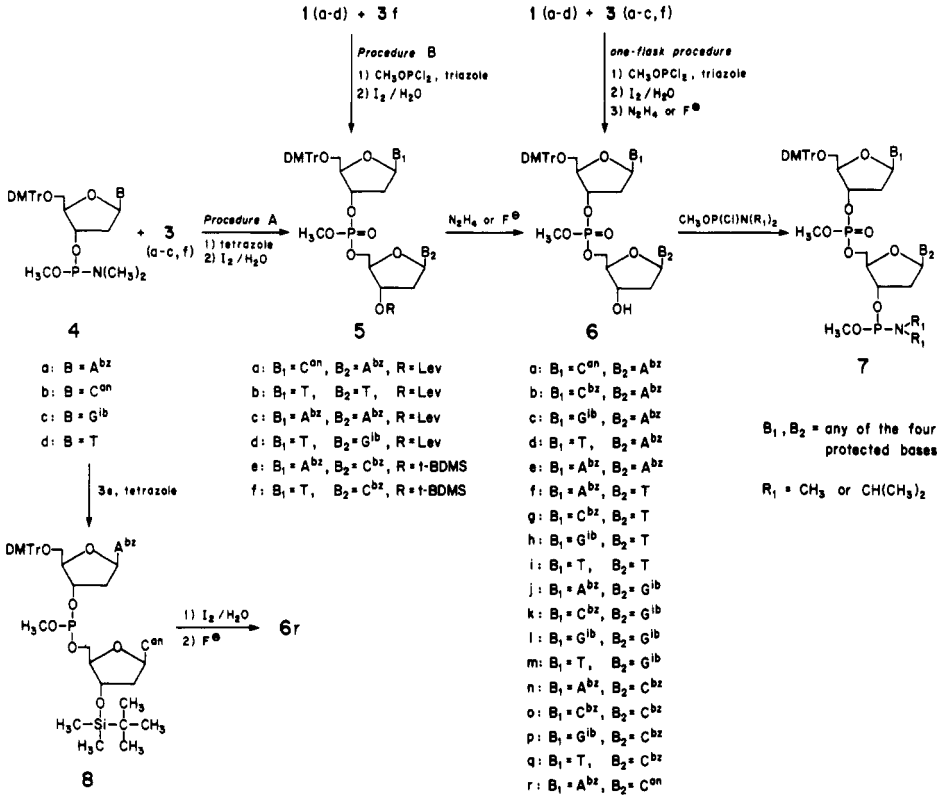
Scheme II



ternucleotide trivalent phosphorous to triester phosphate resulted in the formation of corresponding dimers **5**. The characterization of **5a–f** was based on their proton NMR spectra which show the presence of characteristic signals for the $COCH_3$(3 H) of the levulinyl group at 2.2 ppm, $POCH_3$(3 H) at 3.64–3.8 ppm, and OCH_3(6 H) of the DMTr group at 3.8 ppm. Furthermore, these intermediates on complete deprotection gave the corresponding dinucleoside phosphates (NpN). To obtain the 3′-hyroxyl dimer **6**, the levulinyl protection of **5** was removed by treating it with buffered hydrazine hydrate in pyridine–acetic acid at pH 5.1. The NMR spectra of these 3′-hydroxyl dimers showed the presence of $POCH_3$(3 H) at 3.6–3.8 ppm, OCH_3(6 H) of DMTr at 3.7–3.8 ppm, and appropriate signals for benzoyl, anisoyl, or isobutyryl protecting groups. The presence of these signals and the absence of characteristic $COCH_3$ signals for the levulinic group at 2.2 ppm, clearly indicated the selective removal of levulinic group under these conditions.

Although these reaction sequences provided a convenient route for the synthesis of most of the dimer units (**6**), it was not possible to synthesize the dimer units having cytidine as the 3′-terminal unit due to the poor solubility of the 3′-*O*-levulinyl derivative of cytidine (**3d**) in acetonitrile and THF, the two preferred solvents for condensation. It was, therefore, essential to investigate an alternate 3′-OH protecting group for the synthesis of the NpC type dimer units. *tert*-Butyldimethylsilyl (*t*-BDMSi) group has been widely used in the oligonucleotide synthesis as a protecting group for the hydroxyl function of the ribose moiety.[15] The main advantage of this protecting group is that it can be cleaved by fluoride ions under neutral conditions. The cleavage reaction under these conditions, however, is not selective since it also results in the cleavage of various phosphate protecting groups such as phenyl, chloroethyl, and cyanoethyl. By modifying the above reaction conditions with the addition of acetic acid considerable selectivity was observed in the cleavage of the *t*-BDMSi group in the presence of these phosphate protections and the 5′-*O*-monomethoxytrityl group of a dinucleotide unit.[16] These observations seemed to suggest that development of the appropriate reaction conditions for the selective deprotection of the *t*-BDMSi group in the presence of methyl phosphate protection and the 5′-*O*-dimethoxytrityl substituent is a worthwhile goal.

The 3′-terminal unit with the *tert*-butyldimethylsilyl (*t*-BDMSi) group at the 3′-hydroxyl (**3e,f**) was conveniently synthesized from 5′-*O*-(dimethoxytrityl)-N-protected cytidine (**1d,e**) by its reaction with *tert*-butyldimethylsilyl chloride in the presence of imidazole followed by detritylation of the product with 1 M $ZnBr_2$ in dichloromethane containing 15% 2-propanol.[17] The $ZNBr_2$ treatment of **2** led to a selective detritylation reaction without any significant cleavage of the *tert*-butyldimethylsilyl group. The intermediate **2** and the final products **3e,f** were characterized by their proton NMR spectra. Condensation of **3e** with 5′-*O*-dimethoxytrityl monomer phosphoramidite unit (**4a**) in the presence of tetrazole resulted in the formation of the completely protected phosphite dimer unit (**8**) in 74% yield (Scheme II). The presence of the phosphite linkage in **8** was confirmed by its ^{31}P NMR spectrum showing resonance at −140.97 ppm, whereas its proton NMR spectrum (Experimental Section) showed signals at 0.07, 0.89, 3.53, and 3.78 ppm, indicating the presence of *t*-BDMSi, P–OCH_3, and DMTr groups, respectively. To obtain the 3′-hydroxyl derivative of these dimer units, the removal of the *t*-BDMSi group was first attempted with a 1 M solution of tetrabutylammonium fluoride. Analysis (TLC) of the reaction product after aqueous iodine oxi-

(15) Ogilvie, K. K.; Theriault, N. Y. *Can. J. Chem.* **1980**, *57*, 3140–3144.

(16) Ogilvie, K. K.; Beaucage, S. L. *Tetrahedron Lett.* **1976**, 1255–1256.

(17) Matteucci, M. D.; Caruthers, M. H. *J. Am. Chem. Soc.* **1981**, *103*, 3185–3191.

Table I. Protocol for Synthesis on Solid Support (One Cycle)

operation	reagent	frequency/volume/time
detritylation	0.2 M $Cl_2CHCOOH$ in CH_2Cl_2	2 × 1 mL, 1 min each
wash	0.1% $(C_2H_5)_3N$ in CH_2Cl_2	1 × 2 mL
wash	CH_3CN (anhydrous)	5 × 5 mL
condensation	tetrazole (25 equiv, 70 mg/mL in CH_3CN) and monomer or dimer (10 equiv, 50 mg/mL in CH_3CN)	5 min
wash	CH_3CN	2 × 5 mL
capping	0.15 mL of Ac_2O and 1 mL of pyridine containing 10 mg of (dimethylamino)pyridine	2 min
wash	CH_3CN	2 × 5 mL
oxidation	0.2 M I_2 in THF:lutidine:H_2O (2:1:1)	2 mL, 1 min
wash	CH_3CN	5 × 5 mL
wash	CH_2Cl_2	2 × 5 mL

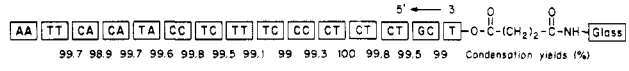
dation indicated the presence of a significant amount of a side product, 5′-*O*-(dimethoxytrityl)-N^6-benzoyladenosine (**1a**) in addition to the desired 3′-hydroxyl dinucleotide **6r**. Apparently some rupture of the internucleotide phosphite bond had taken place during reaction with fluoride ion. Further study of this reaction showed that this internucleotide cleavage becomes observable (TLC) in about 10 min and progresses to completion in an overnight reaction. However, if the dimer phosphite intermediate **8** is oxidized with aqeuous iodine to a phosphotriester dimer first and then treated with tetrabutylammonium floride, the cleavage of the *tert*-butyldimethylsilyl group is achieved selectively to yield **6r** without any side product. The characterization of **6r** was based upon its proton NMR spectrum which indicated the presence of P–OCH_3, DMTr, and other protecting groups and its further deprotection to the corresponding dinucleotide phosphate (ApC).

The procedures described above were used for the synthesis of several dimer phosphite units. However, to build a bank of good amounts of all sixteen dimer phosphite units a simpler synthetic procedure patterned after a literature reference[18] was developed. In this one-flask procedure the 5′-*O*-(dimethoxytrityl)-N-protected nucleoside was treated with a triazole derivative of methyl dichlorophosphite. Without any purification the reaction mixture was reacted with 3′-terminal nucleosides (**3**) to afford the phosphite dimer units. It was then oxidized with aqueous iodine to produce the corresponding triester dimer units (**5**). The crude reaction product thus obtained was then treated with either hydrazine buffer (3′-*O*-levulinic group cleavage) or with fluoride ion (3′-*O*-*t*-BDMSi cleavage). A single silica gel purification at this stage gave the desired 3′-hydroxyl dimer units (**6**) in about 60–65% overall yields. In the case of dimer units with 3′-*O*-(*t*-BDMSi)cytidine at the 3′-end, it was found advantageous to isolate the intermediate 5′-*O*-(dimethoxytrityl)-3′-*O*-(*tert*-butyldimethylsilyl) dimer (**5**) before treatment with fluoride ions for the removal of the *t*-BDMSi group.

Phosphitylation of the 3′-hydroxyl dimer units (**6**) to yield the desired 3′-*O*-methyl *N,N*-dialkylphosphoramidite dimers was achieved by the reaction of **6** with methoxy(*N,N*-dialkylamino)chlorophosphine. The dimer phosphoramidites were characterized by the presence of key proton NMR resonances and ^{31}P NMR signals for the trivalent and pentavalent phosphorous at −147 to 148 and −0.8 ppm, respectively. For structure **7** the integeration of the trivalent ^{31}P signal should be equal to that of the pentavalent ^{31}P signal. Variation from this ratio was used to estimate the percentage of undesired components in the samples. It is pertinent to point out that the workup of a methyl *N,N*-dimethylphosphoramidite reaction mixture with brine[6] invariably resulted in poor yields of the dimer units (≃50%, estimated by ^{31}P NMR) whereas high yields

(18) Fourrey, J. L.; Shire, D. J. *Tetrahedron Lett.* 1981, *22*, 729-732.

Scheme III. Synthesis of 29-mer Using Dimer Units



of methyl *N,N*-diisopropylphosphoramidite dimer units (≃90%) were obtained under similar workup conditions, which supports the previous conclusion regarding lower stability of dimethylphosphoramidite derivatives.[11] However, workup of the phosphitylation reaction mixture with aqueous sodium bicarbonate solution[11] invariably gave higher purity of both dimethyl- and diisopropylphosphoramidite dimers, which were used in the synthesis of oligodeoxyribonucleotides without any further purification. Moreover, both dimethyl and diisopropyl phosphoramidite dimers were stable for months as solids under argon.

Prior to undertaking the synthesis of oligodeoxyribonucleotides with the new dimer phosphite units several model synthetic experiments were carried out. In these studies, the efficiency of the condensation of dimer phosphoramidite units with the nucleoside attached to the solid support was investigated. The conclusion from these experiments was that like monomer condensation, a 10-fold excess of dimer phosphoramidites in acetonitrile was adequate to give quantitative yield of the product. These products, after complete deprotection, were digested with snake venom phosphodiesterase to establish a 3′→5′ internucleotide linkage in the total product. The conditions developed during the synthesis of the model oligomers (Table I, protocol for synthesis) were then used for the synthesis of several oligodeoxyribonucleotides ranging in size 16–29. As shown for the synthesis of a 29-mer (5′-AATTCACATACCTCTTTCCCCTCTCTGCT-3′) in Scheme III, dimers gave consistently high (98.9–100%) condensation yields similar to the monomer condensations. [The yields are based on measurement of the trityl cation released on detritylation.] The overall yield of the 29-mer was 93.4%. The high overall yield of the 29-mer is also evident from the gel electrophoresis pattern of the crude sample (Figure 2). After partial deprotection and cleavage from the resin the desired oligonucleotide was first purified as a trityl component on a C-18 reverse-phase HPLC column. The HPLC profile for the purification of the 29-mer is shown in Figure 1. It should be noted that the tritylated product represents 87–90% of the nucleotide absorbance (260 nm) as measured by peak areas (peak I truncated sequences, peak III the tritylated product). This percentage is in close agreement with the overall yield obtained from trityl cation measurement (93%). The trityl fraction was collected and treated with 80% aqueous acetic acid to cleave the trityl moiety. The detritylated oligomer was purified the second time on a C-18 RP HPLC column. For further analysis, each oligomer was labeled with ^{32}P phosphate at its 5′-terminus using $\gamma[^{32}P]$ ATP and polynucleotide kinase. The labeled product was analyzed for

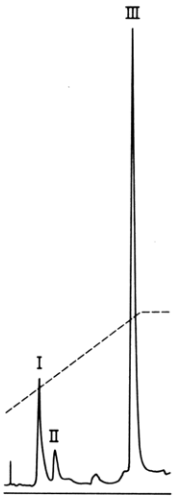


Figure 1. HPLC profile of crude 5′-DMTr-AATTCACATACCTCTTTCCCCTCTCTGCT with reverse-phase C-18 column and a linear gradient of 15–30% of CH_3CN in 0.05 M triethylammonium acetate buffer, pH 7.0. Peak I represents the truncated oligonucleotide sequences, peak II is products resulting from the removal of protecting groups, and peak III represents the 5′-DMTr oligomer(s).

Figure 2. Analysis of ^{32}P-labeled oligonucleotides by polyacrylamide (20%) gel electrophoresis in 7 M urea. Lane 1 represents the crude oligonucleotide mixture containing the 29-mer (5′AATTCACATACCTCTTTCCCCTCTCTGCT). Lane 2 shows the same 29-mer after HPLC purification.

a purity check by electrophoresis on sequencing polyacrylamide gel (Figure 2). The labeled product was also used to confirm the nucleotide sequence by the chemical sequencing method of Maxam and Gilbert[19] and by partial phosphodiesterase digestion (wandering spot)[20] methods. The sequence of oligomer was consistent with the expected structure. The other oligomers synthesized and sequenced by similar procedures are (5′→3′): GCAATTTAACTGTGAT (16-mer), CTTACCGTAAGGTCCTCG (18-mer), CTAACGTTCAGAGGCATG (18-mer), CCTCTTTCCCCTCTTTGCT (19-mer), TTGTAGGAATCCAAGCAAGT (20-mer), AATTCCTCTGTCCTGCTTGCT (21-mer), AATTCATGCCTCTGAACGTTAG (22-mer), GATCCGAGGACCTTGCGATATGA (23-mer), ACGAATTCATATCGCAAGGTCCTCG (25-mer), AATTCATGTATGCCGATGCCATTTTT (26-mer), AATTCATGCCTCTGAACGTTAGCTTCA (27-mer), ATTCGTAAAAATGGCATCGGCATACATG (28-mer), TGTTGGTGAAGCTAACGTTCAGAGGCATG (29-mer).

Experimental Section

General Methods. The proton and ^{31}P NMR were recorded on Varian XL-200 instrument with tetramethylsilane and H_3PO_4 internal standards, respectively. UV spectra were obtained on a Beckman spectrophotometer. Nucleosides were purchased from Vega. The controlled pore glass long chain alkylamine solid support for the synthesis of DNA fragments was purchased from Pierce Chemical Company, Rockford, IL. Acetonitrile (Burdick and Jackson Laboratories, Inc., Muskegon) and tetrahydrofuran (Fisher Scientific Co.) were dried over CaH_2. Pyridine (Burdick Jackson) was dried over 4A molecular sieves. 2,6-Lutidine, *N*,*N*-diisopropylethylamine, and triethylamine were distilled and kept over molecular sieves. Reactions and products were monitored by TLC with 0.25 mm silica gel plates (60F-254) purchased from E.M. Laboratories, Inc. Preparative TLC was performed on silica gel GF 2000, precoated thin-layer chromatography plates. Column chromatography was performed on silica gel prepacked LOBAR columns (E. Merck) by using a linear gradient of methanol in methylene chloride. A pumping pressure of approximately 20 psi was applied to give a flow rate of 2 mL/min, and 10-mL fractions were collected. High-performance liquid chromatography for the purification of DNA fragments was performed on the LDC equipment at 50 °C with a Waters Bondapack C-18 column. A 10-min linear gradient of 15–30% acetonitrile in 0.05 M triethylammonium acetate (TEAA) buffer at pH 7.0 was used for the purification of DMTr-oligomer. The completely deprotected DNA fragments were purified by using the same set up except that a gradient of 5–15% acetonitrile was used.

***N*^{6}-Benzoyl-5′-*O*-(dimethoxytrityl)-3′-*O*-levulinyl-2′-deoxyadenosine (2a).** A sample of N^6-benzoyl-5′-*O*-(dimethoxytrityl)adenosine (13.14 g, 20 mmol) was stirred with anhydrous dioxane (200 mL), levulinic acid (4.4 g, 40 mmol), (*N*,*N*-dimethylamino)pyridine (0.2 g), and dicyclohexylcarbodiimide (10 g, 50 mmol) for 1 h. The reaction mixture was then quenched with ice and filtered. The filtrate was partitioned between dichloromethane and water. The organic layer was dried over sodium sulfate and evaporated to dryness under reduced pressure. The residue so obtained was applied to a silica gel column (4 × 40 cm), and the chromatography was performed by elution with a linear gradient of CH_2Cl_2 and CH_2Cl_2–5% CH_3OH (2 L each). The major fraction was collected and the solvent evaporated in vacuo to yield the desired product (13 g, 17.32 mmol, 86.6%). Anal. Calcd for $C_{43}H_{41}N_5O_8$: C, 68.34; H, 5.43; N, 9.27. Found: C, 67.99; H, 5.58; N, 9.17. The following products were also synthesized by a similar procedure:

2b. Anal. Calcd for $C_{40}H_{43}N_5O_9$: C, 65.10; H, 5.87; N, 9.49. Found: C, 64.62; H, 5.92; N, 9.42.

2c. Anal. Calcd for $C_{36}H_{38}N_2O_9$: C, 67.29; H, 5.92; N, 4.36. Found: C, 67.20; H, 5.82; N, 4.38.

2d. Anal. Calcd for $C_{42}H_{41}N_3O_9 \cdot {}^1/_2H_2O$: C, 68.10; H, 5.67; N, 5.67. Found: C, 68.36; H, 5.61; N, 5.70.

***N*^{6}-Benzoyl-3′-*O*-levulinyl-2′-deoxyadenosine (3a).** The 5′-*O*-dimethoxytrityl derivative of N^6-benzoyl-3′-*O*-levulinyl-2′-deoxyadenosine (7.55 g, 10 mmol) was reacted with 80% acetic acid (150 mL) for $^1/_2$ h. The reaction mixture was then evaporated in vacuo and partitioned between water and methylene chloride. The organic layer was washed with 2% $NaHCO_3$, dried over sodium sulfate, and evaporated to dryness under reduced pressure. The crude product was chromatographed on a silica gel column (4 × 40 cm) by a linear gradient of CH_2Cl_2 and CH_2Cl_2–7% CH_3OH (2 L each) to afford **3a** (4.25 g, 9.3 mmol) in 93% yield. Anal. Calcd for $C_{22}H_{23}N_5O_6$: C, 58.28; H, 5.08; N, 15.45. Found: C, 58.44; H, 5.13; N, 15.44.

Compounds **3b–d** were also prepared by a similar procedure.

3b. Anal. Calcd. for $C_{19}H_{25}N_5O_7$: C, 52.41; H, 5.79; N, 16.09. Found: C, 52.32; H, 5.75; N, 15.82.

3c. A different procedure for its synthesis has also been reported.[21] Anal. Calcd for $C_{15}H_{20}N_2O_7$: C, 52.94; H, 5.88; N, 8.23. Found: C, 52.60; H, 5.90; N, 8.64.

3d. Anal. Calcd for $C_{21}H_{23}N_3O_7$: C, 58.74; H, 5.36; N, 9.79. Found: C, 58.77; H, 5.40; N, 9.76.

***N*^{4}-Anisoyl-5′-*O*-(dimethoxytrityl)-3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxycytidine (2e).** To a stirred solution of N^4-anisoyl-5′-*O*-dimethoxytrityl-2′-deoxycytidine (2 mmol, 1.32 g) and imidazole (7 mmol, 0.5 g) in dry DMF (20 mL) was added

(19) Maxam, A. M.; Gilbert, W. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 560–564.

(20) Tu, C. D.; Jay, E.; Bahl, C. P.; Wu, R. *Anal. Biochem.* **1976**, *74*, 73–93.

(21) Hassner, A.; Strand, G.; Rubinstein, M.; Patchernik, A. *J. Am. Chem. Soc.* **1975**, *97*, 1614–1615.

dropwise a solution of *tert*-butyldimethylsilyl chloride (3 mmol, 0.045 g) in dry DMF (10 mL). The reaction was stirred for 1 h, quenched with ice, extracted with dichloromethane, washed with water, and dried over anhydrous sodium sulfate. The organic layer was concentrated to dryness and purified over silica gel column with dichloromethane as an eluent to yield the desired product (1.33 g, 1.7 mmol) in 85% yield. Anal. Calcd for $C_{44}H_{51}N_3O_8Si\cdot H_2O$: C, 66.33; H, 6.65; N, 5.27. Found: C, 66.26; H, 6.84; N, 5.17.

N^4-Benzoyl-5′-*O*-(dimethoxytrityl)-3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxycytidine (**2f**) was also synthesized by an analogous procedure. Anal. Calcd for $C_{43}H_{49}N_3O_7Si\cdot H_2O$: C, 67.36; H, 6.66; N, 5.48. Found: C, 67.06; H, 6.98; N, 5.08.

N^4-Anisoyl-3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxycytidine (3e). To a solution of 5′-*O*-dimethoxytrityl derivative of 3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxycytidine (1 mmol, 0.77 g) in dichloromethane (5 mL) was added a solution of 1 M $ZnBr_2$ (10 mL) in dichloromethane containing 15% methanol. The reaction mixture was stirred for 30 min, quenched with ice, and extracted with dichloromethane. The organic layer was washed with water, dried over anhydrous sodium sulfate, and concentrated to dryness in vacuo. The product **3e** (0.35 g, 0.74 mmol) was isolated by column chromatography over silica gel (2 × 20 cm) by successive elution with CH_2Cl_2 and CH_2Cl_2–3% MeOH in 74% yield. Anal. Calcd for $C_{23}H_{33}N_3O_6Si$: C, 58.08; H, 6.99; N, 8.84. Found: C, 57.85; H, 7.17; N, 8.73.

N^4-Benzoyl-3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxycytidine (**3f**) was also synthesized by a similar procedure. Anal. Calcd for $C_{22}H_{31}N_3O_5Si$: C, 59.50; H, 7.01; N, 9.43. Found: C, 59.15; H, 6.72; N, 9.44.

N^4-Anisoyl-*P*-methyl-5′-*O*-dimethoxytrityl-2′-deoxycytidylyl-(3′→5′)-N^6-benzoyl-3′-*O*-levulinyl-2′-deoxyadenosine (5a). A mixture of 3′-*O*-[(*N*,*N*-dimethylamino)-methoxyphosphino]-N^4-anisoyl-5′-*O*-(dimethoxytrityl)-2′-deoxycytidine (**4b**, 1.7 g, 2.2 mmol) and **3a** (0.9 g, 2 mmol) was dissolved in anhydrous acetonitrile (25 mL). The reaction mixture was cooled in ice and to it was added dropwise while stirring a solution of tetrazole (4 mmol) in acetonitrile (10 mL). The reaction was allowed to warm up to room temperature which took about 15 min. Iodine (4 mmol) in 20 mL of THF:lutidine:water (2:1:1) was then added and the reaction was further stirred for 15 min. The solvents were removed at reduced pressure and the residue was dissolved in dichloromethane which was extracted with water, 5% sodium bisulfite, 5% sodium bicarbonate, and finally with water. The organic layer was dried over sodium sulfate and reduced to dryness in vacuo. The product **5a** (1.72 g, 1.54 mmol) was isolated by column chromatography on silica gel (4 × 40 cm) with a linear gradient of CH_2Cl_2 and CH_2Cl_2–5% CH_3OH (2 L each) in 77% yield. Anal. Calcd for $C_{61}H_{61}N_8O_{16}$: C, 61.41; H, 5.12; N, 9.39; P, 2.60. Found: C, 61.28; H, 5.08; N, 9.07; P, 2.36.

Compounds **5b–d** were also synthesized by a similar procedure. **5b.** Anal. Calcd for $C_{47}H_{53}N_4O_{16}P$: C, 58.75; H, 5.52; N, 5.83; P, 3.23. Found: C, 58.70; H, 5.75; N, 5.75; P, 3.00.

5c. Anal. Calcd for $C_{61}H_{59}N_{10}O_{14}P$: C, 61.72; H, 4.97; N, 11.80; P, 2.61. Found: C, 61.40; H, 5.23; N, 11.70; P, 2.35.

5d. Anal. Calcd for $C_{51}H_{58}N_7O_{16}P$: C, 58.00; H, 5.49; N, 9.29; P, 2.93. Found: C, 57.85; H, 5.80; N, 9.25; P, 2.70.

N^4-Anisoyl-*P*-methyl-5′-*O*-dimethoxytrityl-2′-deoxycytidylyl-(3′→5′)-N^6-benzoyl-2′-deoxyadenosine (6a). Compound **5a** (0.69 g, 0.62 mmol) was treated with ice cold hydrazine buffer (9.3 mL, 0.5 M $N_2H_4\cdot H_2O$ in pyridine/acetic acid, 3:2 v/v, pH 5.1) for 5 min, quenched with ice, and extracted with methylene chloride/water.[14] The organic layer was dried over sodium sulfate, evaporated, and coevaporated with toluene to dryness in vacuo. (This procedure of eliminating excess hydrazine was preferred over its quenching with acetyl acetone because the latter gives an adduct with hydrazine which interferes with the final purification on silica gel). The solid product thus obtained was chromatographed on silica gel column (3 × 30 cm) by using a linear gradient of CH_2Cl_2 and CH_2Cl_2–7% CH_3OH (2 L each) to afford pure **6a** (0.5 g, 0.49 mmol) in 79% yield.

Anal. Calcd for $C_{56}H_{55}N_8O_{14}P$: C, 61.42; H, 5.06; N, 10.23; P, 2.83. Found: C, 61.38; H, 5.08; N, 9.49; P, 2.49.

Compounds **5b–d** were also similarly treated with hydrazine to afford compounds **6c**, **6d**, and **6e**, respectively. **6c.** Anal. Calcd for $C_{53}H_{55}N_{10}O_{13}P$: C, 59.44; H, 5.14; N, 13.08; P, 2.90. Found: C, 59.64; H, 5.79; N, 13.53; P, 2.90.

6d. Anal. Calcd for $C_{49}H_{50}N_7O_{13}P\cdot H_2O$: C, 59.21; H, 5.24; N, 9.87; P, 3.12. Found: C, 59.22; H, 5.32; N, 9.82; P, 2.77.

6e. Anal. Calcd for $C_{56}H_{53}N_{10}O_{12}P$: C, 61.76; H, 4.87; N, 12.87; P, 2.85. Found: C, 61.36; H, 5.08; N, 12.18; P, 2.85.

One-Flask Synthesis of N^6-Benzoyl-*P*-methyl-5′-*O*-dimethoxytrityl-2′-deoxyadenosyl-(3′→5′)-2′-deoxythymidine (6f) (via Levulinic Route). Methyl dichlorophosphite (0.3 mL, 3 mmol) was added to a cooled solution of triazole (0.84 g, 12 mmol) in 25 mL of THF·pyridine 4:1 at −20 °C and stirred for 20 min. The reaction mixture was then cooled to −70 °C and a solution of N^6-benzoyl-5′-*O*-(dimethoxytrityl)-2′-deoxyadenosine (1.97 g, 3 mmol) in THF (20 mL) was added dropwise and stirred for 10 min. A solution of 3′-*O*-levulinyl-2′-deoxythymidine (1.02 g, 3 mmol) in 20 mL of THF was added, the reaction was allowed to warm to −20 °C, stirred further for 10 min, then treated with iodine (6 mmol) in 30 mL of THF:lutidine:water (2:1:1) for 15 min, reduced to dryness in vacuo, dissolved in dichloromethane (100 mL), and extracted with 5% sodium bisulfite, 5% sodium bicarbonate, and finally with water. The organic layer was dried over anhydrous sodium sulfate, concentrated in vacuo, then treated with ice cold hydrazine buffer (60 mL, 0.5 M $N_2H_4\cdot H_2O$ in pyridine/acetic acid 3:2 v/v, pH 5.1) for 5 min, concentrated again to dryness in vacuo, and extracted with CH_2Cl_2, 5% sodium bicarbonate, and finally with water. The organic layer was dried over sodium sulfate, concentrated, and coevaporated with toluene under reduced pressure several times. The crude reaction product was then purified over silica gel column (4 × 40 cm) by using a linear gradient of CH_2Cl_2–1% MeOH and CH_2Cl_2–10% MeOH (2 L each) to afford the product **6f** (1.8 g, 1.89 mmol) in 63% yield. Anal. Calcd for $C_{49}H_{50}N_7O_{13}P\cdot H_2O$: C, 59.21; H, 5.23; N, 9.87; P, 3.12. Found: C, 59.50; H, 5.09; N, 9.82; P, 3:00.

The following compounds were also synthesized by an analogous precedure.

6b. Anal. Calcd for $C_{55}H_{53}N_8O_{13}P\cdot H_2O$: C, 60.99; H, 5.08; N, 10.35; P, 2.86. Found: C, 60.07; H, 5.20; N, 10.08; P, 2.81.

6g. Anal. Calcd for $C_{48}H_{50}N_5O_{14}P\cdot{}^1/_2\ H_2O$: C, 60.00; H, 5.31; N, 7.29; P, 3.23. Found: C, 59.91; H, 5.57; N, 6.85; P, 3:07.

6h. Anal. Calcd for $C_{46}H_{52}N_7O_{14}P\cdot H_2O$: C, 56.61; H, 5.54; N, 10.05; P, 3.18. Found: C, 56.41; H, 5.66; N, 9.90; P, 2.91.

6i. Anal. Calcd for $C_{42}H_{47}N_4O_{14}P\cdot H_2O$: C, 57.27; H, 5.57; N, 6.36; P, 3.52. Found: C, 57.30; H, 5.51; N, 6.43; P, 3.40.

6j. Anal. Calcd for $C_{53}H_{55}N_{10}O_{13}P$: C, 59.44; H, 5.14; N, 13.08; P, 2.90. Found: C, 59.04; H, 5.50; N, 13.00; P, 2.85.

6k. Anal. Calcd for $C_{52}H_{55}N_8O_{14}P\cdot H_2O$: C, 58.64; H, 5.35; N, 10.52; P, 2.91. Found: C, 58.64; H, 5.66; N, 10.64; P, 3.08.

6l. Anal. Calcd for $C_{50}H_{57}N_{10}O_{14}P$: C, 57.03; H, 5.42; N, 13.31; P, 2.94. Found: C, 56.97; H, 5.48; N, 13.28; P, 2.92.

6m. Anal. Calcd for $C_{46}H_{52}N_7O_{14}P\cdot H_2O$: C, 56.61; H, 5.54; N, 10.05; P, 3.18. Found: C, 56.41; H, 5.38; N, 9.92; P, 3.24.

N^6-Benzoyl-*P*-methyl-5′-*O*-dimethoxytrityl-2′-deoxyadenosyl-(3′→5′)-N^4-benzoyl-3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxycytidine (5e). Procedure A. 3′-*O*-[(*N*,*N*-Dimethylamino)methoxyphosphino]-N^6-benzoyl-5′-*O*-(dimethoxytrityl)-2′-deoxyadenosine (**4a**, 0.42 g, 0.55 mmol) and N^4-benzoyl-3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxycytidine (**3f**, 0.22 g, 0.5 mmol) were dissolved in anhydrous tetrahydrofuran (10 mL) and cooled in ice and a solution of tetrazole (0.09 g, 1.1 mmol) in 5 mL of tetrahydrofuran was added dropwise. The reaction mixture was stirred at room temperature for 15 min, treated with 5 mL of I_2 reagent (0.2 M I_2 in THF:lutidine:water 2:1:1) for 15 min, and then evaporated to dryness under reduced pressure. The crude reaction product was dissolved in dichloromethane (50 mL), washed successively with 5% sodium bisulfite, 5% sodium bicarbonate, and water, and then dried over anhydrous sodium sulfate. The desired product **5e** (0.39 g, 0.33 mmol) was purified (66.6% yield) over preparative TLC plates (silica gel) using CH_2Cl_2:MeOH 9:1 as chromatographic solvent.

Procedure B. Methyl dichlorophosphite 1 mmol, 0.1 mL) was added to a stirred solution of triazole (4 mmol, 0.28 g) and collidine (2 mmol, 0.25 mL) in dry pyridine (2 mL) and dry THF (8 mL) at −20 °C. The reaction was stirred for 20 min and cooled further to −70 °C. A solution of N^6-benzoyl-5′-*O*-(dimethoxytrityl)-2′-deoxyadenosine (1 mmol, 0.54 g) in 5 mL of anhydrous THF was added dropwise. After stirring the reaction for 10 min, a solution of N^4-benzoyl-3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxycytidine (**3f**,

1 mmol, 0.44 g) in 5 mL of dry THF was added. The reaction mixture was allowed to warm to −20 °C, stirred further for 10 min, and then treated with 10 mL, of iodine reagent (0.2 M I_2 in THF:lutidine:H_2O 2:1:1) for 15 min. The crude reaction mixture was concentrated to dryness under reduced pressure, dissolved in dichloromethane (50 mL), and extracted with 5% sodium bisulfite, 5% sodium bicarbonate, and finally with water. The organic layer was dried over anhydrous sodium sulfate and reduced to dryness in vacuo. The product **5e** (0.75 g, 0.64 mmol) was isolated by column chromatography over silica gel (4 × 40 cm) by using a linear gradient of CH_2Cl_2 and CH_2Cl_2–5% CH_3OH (2 L each) in 64.4% yield. Anal. Calcd for $C_{61}H_{67}N_8O_{13}PSi\cdot H_2O$: C, 61.20; H, 5.77; N, 9:37. Found: C, 61.38; H, 6.19; N, 9.14.

5f was also synthesized by a similar procedure. Anal. Calcd for $C_{54}H_{64}N_5O_{14}PSi\cdot H_2O$: C, 58.99; H, 6.29; N, 6.88; P, 3.04. Found: C, 59.02; H, 6.05; N, 6.62; P, 2.84.

***N*⁶-Benzoyl-*P*-methyl-5′-*O*-dimethoxytrityl-2′-deoxyadenosyl-(3′→5′)-*N*⁴-benzoyl-2′-deoxycytidine (6n).** A 1 M solution of tetrabutylammonium fluoride (2 mL, THF) was added to a stirred solution of **5e** (1 g, 0.85 mmol) in tetrahydrofuran (8 mL). The mixture was stirred for 15 min, evaporated to dryness in vacuo, and partitioned between dichloromethane and water. The organic layer was dried over anhydrous magnesium sulfate and then evaporated to dryness. The product **6n** (0.61 g, 0.57 mmol) was purified in 67.7% yield by preparative TLC (silica gel) with CH_2Cl_2:MeOH 9:1 as chromatographic solvents. Anal. Calcd for $C_{55}H_{53}N_8O_{13}P\cdot H_2O$: C, 60.99; H, 5.08; N, 10.35; P, 2.86. Found: C, 60.43; H, 5.00; N, 10.19; P, 2.76.

Compound **6q** was also synthesized by similar procedure. **6q.** Anal. Calcd for $C_{48}H_{50}N_5O_{14}P\cdot H_2O$: C, 59.44; H, 5.26; N, 7.22; P, 3.20. Found: C, 59.77; H, 5.39; N, 7.32; P, 3.21.

The corresponding dinucleotides with a TBDMSi group at the 3′-end (procedure B) were treated, without purification, with fluoride ions as described above to yield **6o** and **6p. 6o.** Anal. Calcd for $C_{54}H_{53}N_6O_{14}P$: C, 62.30; H, 5.10; N, 8.08; P, 2.98. Found: C, 62.09; H, 5.24; N, 8.05; P, 3.14.

6p. Anal. Calcd for $C_{52}H_{55}N_8O_{14}P$: C, 59.65; H, 5.26; N, 10.71; P, 2.96. Found: C, 59.35; H, 5.62; N, 10.27; P, 2.72.

***N*⁶-Benzoyl-5′-*O*-dimethoxytrityl-2′-deoxyadenosyl)-(3′→5′)-*P*-methoxyphosphino-*N*⁴-anisoyl-3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxycytidine (8).** 3′-*O*-[(*N*,*N*-Dimethylamino)methoxyphosphino]-*N*⁶-benzoyl-5′-*O*-(dimethoxytrityl)-2′-deoxyadenosine (**4a**, 0.34 g, 0.45 mmol) and *N*⁴-anisoyl-3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxycytidine (**3e**, 0.14 g, 0.3 mmol) were dissolved in anhydrous acetonitrile (10 mL) and cooled in an ice bath, and a solution of tetrazole (0.063 g, 0.9 mmol) in acetonitrile (5 mL) was added dropwise. After stirring the reaction mixture for 15 min, it was allowed to warm to room temperature. The acetonitrile was removed under reduced pressure and the solid so obtained was partitioned between dichloromethane and water. The organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. The purification of crude reaction product over preparative TLC plates (silica gel) with CH_2Cl_2:CH_3OH 9:1 as the chromatographic solvents afforded pure **8** (0.22 mmol 0.26 gm) in 74% yield: NMR (ppm) 0.07 (s, 6, 2 × $SiCH_3$), 0.89 (s, 9, *tert*-butyl Si), 2.2–3.0 (m, 4, 2 × CH_2), 3.53 (m, 3, $POCH_3$), 3.78 (s, 6, OCH_3 of DMTr), 3.87 (s, 3, OCH_3 of anisoyl), 3.44 (m, 2, 5′-CH_2), 4.4 (m, 4, $POCH_2$, 2 × 4′-CH), 4.7 and 5.1 (2 m, 2, 2 × 3′-CH), 6.25 and 6.54 (2 m, 2, 2 × 1′-CH), 8.28 (m, 2, NCH=N), 6.7–8.1 (m, 24, aromatics). Anal. Calcd for $C_{60}H_{69}N_8O_{13}PSi$: C, 61.59; H, 5.96; N, 9.58; P, 2.65. Found: C, 61.05; H, 5.85; N, 9.04; P, 2.54.

***N*⁴-Anisoyl-*P*-methyl-5′-*O*-dimethoxytrityl-2′-deoxycytidylyl-3′-*O*-[(*N*,*N*-dimethylamino)methoxyphosphino]-(3′→5′)-*N*⁶-benzoyl-2′-deoxyadenosine (7, B_1 = Can; B_2 = Abz; R_1 = CH_3).** The dinucleotide **6a** (0.4 g, 0.4 mmol) and *N*,*N*-diisopropylethylamine (0.2 g, 1.6 mmol) were dissolved in dry acid-free chloroform (5 mL) and to it was added chloro-(*N*,*N*-dimethylamino)methoxyphosphine (0.112 g, 0.8 mmol) dropwise under an atmosphere of dry nitrogen. The reaction was stirred for 15 min, diluted with ethyl acetate, and extracted with an aqueous 2% solution of sodium bicarbonate. The organic phase was dried over magnesium sulfate, concentrated to a small volume, and added dropwise to 20 mL of cold hexane (−78 °C) with vigorous stirring. The precipitated solid was filtered and washed with cold hexane to afford **7** (0.3 g, 0.27 mmol) in 68% yield: NMR 2.6–3.0 (m, 10, $N(CH_3)_2$ and 2 × 2′-CH_2), 3.44 (m, 2, 5′-CH_2), 3.6–3.74 (m, 6, 2 × $POCH_3$), 3.8 (s, 6, 2 × OCH_3), 3.9 (s, 3, OCH_3), 4.32 (m, 4, $POCH_2$ and 2 × 4′-CH), 5.14 and 5.44 (2 m, 2, 2 × 3′-CH), 6.30 and 6.54 (2 m, 2, 2 × 1′-CH), 6.7–8.4 (m, 26, aromatics).

Other dinucleotide phosphoramidites were also prepared by a similar procedure and characterized by proton and ^{31}P NMR. Chloro-(*N*,*N*-diisopropylamino)methoxyphosphine (instead of chloro-(*N*,*N*-dimethylamino)methoxyphosphine) was used for the synthesis of methyl *N*,*N*-diisopropylphosphoramidite dimer units.

Synthesis of a 29-mer 5′-AATTCACATACC-TCTTTCCCCTCTCTGCT-3′ on Solid Support. The nucleoside (5′-*O*-(dimethoxytrityl)-2′-deoxythymidine) was attached via an alkali labile 3′-*O*-succinyl linkage to CPG/long chain alkylamine, controlled pore glass support (pore diameter, 500 Å, particle size 125–177 μm) by using the procedure previously reported for condensing the nucleosides to the silica gel.[17] The loading of nucleoside to the resin was calculated to be approximately 22 μmol of T/g of resin by measuring the acid-released trityl color at 498 nm.

The synthesis of oligonucleotide was carried out in a reaction vessel (5 mL) fitted with a septum on one side and a fritted disk on the other end. A sample of resin-bound 5′-*O*-(dimethoxytrityl)thymidine (0.045 g, 22 μmol of thymidine/g of resin) was taken up in the vessel and successively reacted with the reagents under argon (for protocol, see Table I). The solution was filtered after each operation through the fritted disc. The 5′-*O*-dimethoxytritylated dimer phosphoramidites of the structures **7** (Scheme II), were condensed to the resin in the following sequence: GC, CT, CT, CT, CC, TC, TT, TC, CC, TA, CA, CA, TT, and AA. The yields for respective condensations calculated by measuring (at 498 nm) the amount of trityl group released on acidic cleavage were found to be 99%, 99.5%, 99.8%, 100%, 99.3%, 99%, 99.1%, 99.5%, 99.8%, 99.6%, 99.7%, 98.9%, and 99.7%. The trityl group was not cleaved after the addition of the last dimer as this would be useful in separating the desired 29-mer from the truncated sequences. The sequences of other oligonuleotide synthesized by similar procedure are given under the Results and Discussion Section.

Release from the Resin and Purification of the 29-mer. A sample of oligomer-bound resin (15 mg) was treated with a solution of thiophenol (0.125 mL) and triethylamine (0.125 mL) in dioxane (0.25 mL) for 30 min at room temperature to deblock methyl protection from the phosphates. The resin was centrifuged and thoroughly washed with methanol. To remove the base protections and to release the nucleotidic material from the resin, it was then incubated with concentrated ammonia solution (2 mL) for 15 h at 55 °C. The resin was filtered and the filtrate was evaporated to dryness in vacuo. An aliquot of this crude sample was detritylated with 80% aqueous acetic acid, labeled with ^{32}P phosphate by standard kination procedure, and analyzed by electrophoresis on 20% acrylamide/7 M urea gel (Figure 2). The balance of the material was dissolved in water and then purified over HPLC (RP-18 column) with a linear gradient of CH_3CN (15% to 30%) in 0.05 M triethylammonium acetate buffer, pH 7.0. The peak containing trityl oligomer (III, Figure 1), eluting with 28% CH_3CN, was collected. It was concentrated to dryness, reacted with 80% acetic acid (1 mL) for half an hour at room temperature and reevaporated to dryness. The sample thus obtained was dissolved in 0.5 mL of water and extracted with ether (saturated with water). The aqueous layer was then purified over HPLC (reverse phase C-18 column) with a gradient of 5–15% CH_3CN in 0.05 M triethylammonium acetate buffer, pH 7.0. The fraction eluted at 15% CH_3CN in a single peak was collected and coevaporated with water several times to yield 18.6 O.D.A_{260nm} of pure 29-mer. [The yield of the 29-mer is not absolute since to obtain highly pure material sharp cuts at the middle of the HPLC peaks were pooled.] To finally establish its homogeneity the oligomer was labeled with ^{32}P phosphate using γ[^{32}P] ATP and polynucleotide kinase and analyzed by electrophoresis on 20% polyacrylamide/7 M urea gel (Figure 2).

Characterization of the Oligodeoxynucleotides. Each purified ologonucleotide was labeled with ^{32}P at its 5′-end with T_4 polynucleotide kinase and γ-^{32}P-ATP. The labeled product was sequenced both by partial phosphodiesterase digestion followed by two-dimensional homochromatographic analysis[20] and

by base-specific chemical cleavage method of Maxam and Gilbert.[19] The nucleotide sequence of each oligomer was found to be as expected.

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Supplementary Material Available: Table I (proton NMR of **2a-f** and **3a-f**) and Table II (proton NMR of **5a-d** and **6a-r**) (3 pages). Ordering information is given on any current masthead page.

Stereochemistry of Dithianyllithium Addition to Cyclohexanone[1]

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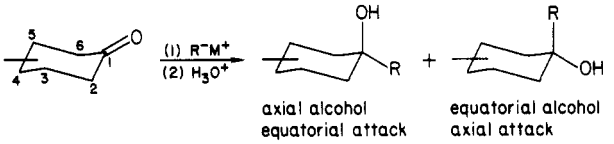
The reaction of 2-phenyl-1,3-dithianyllithium (1-Li) with 4-*tert*-butylcyclohexanone in cyclohexane and tetrahydrofuran may proceed either with kinetic or thermodynamic control. In the latter case carbinol formation involves exclusively equatorial attack. In contrast, only kinetic control is seen in the reaction of the same ketone with 1,3-dithianyllithium (2-Li) or its mono-*S*-oxide (3-Li). No addition but only enolate formation occurs in solvent hexamethylphosphoric triamide; with 1-[2-phenyl-2-(1,3-dithianyl)]-4-*tert*-butylcyclohexanol reversal of the addition reaction is seen in this solvent.

Introduction

In contrast to the significant effort dedicated to the study of the reduction of cyclohexanones by metal hydrides,[3,4] reports concerning the course of addition of organometallic compounds to give isomeric alcohols are limited.[5] This in spite of the fact that the addition of carbanions to ketones has important stereochemical aspects.



It is now well-established that the steric hindrance introduced by the axial hydrogens (or substituents) at C(3,5) tends to direct the nucleophile to approach the carbonyl from the equatorial side.[6] It is also clear that an opposing nonsteric factor may lead to a preference for axial attack.[3,4]

The nature of the second factor is not well understood. It has been suggested that the thermodynamic stability of the products,[7] the torsional strain engendered during equatorial approach,[8] the configuration of the frontier orbitals,[9,10] the hardness vs. softness of the nucleophile,[11] and the importance of two-electron stabilizing interactions[12,13] are responsible for the unexpected ratio of axial addition.

Table I. Products of the Reaction of 1-Li with 4 as a Function of Reaction Time

entry	solvent	time, h	5:6
1	THF	"zero"[a]	79:21
2	THF	1.5[a]	86:14
3	THF	4[b]	100:0
4	C_6H_{12}	"zero"[c]	76:24
5	C_6H_{12}	2[c]	82:18
6	C_6H_{12}	20[c]	95:5
7	C_6H_{12}	48[c]	98.2:1.8
8	HMPTA	16.5[d]	

[a] At -20 °C. [b] 1.5 h at -20 °C plus 2.5 h at 25 °C. [c] At 25 °C. [d] 15 h at 0 °C plus 15 h at 25 °C.

1,3-Dithianyllithium and its 2-substituted derivatives are widely used organometallics in organic synthesis,[14] and their addition to ketones affords the corresponding carbinols in high yields.[15] This paper reports the results of a stereochemical study of the addition of the lithium salt of the parent 1,3-dithiane, as well as its 2-phenyl derivative to the anancomeric[16] 4-*tert*-butylcyclohexanone. Since spectral evidence suggests[17] that 2-phenyl-2-lithio-1,3-dithiane (1-Li) exists as a tight ion pair in tetrahydrofuran (THF) or cyclohexane (C_6H_{12}) but as a solvent-separated, delocalized ion pair in hexamethylphosphoric triamide (HMPTA), it can be argued that the species present in HMPTA should be the softer base. Analysis of the solvent effect (THF or C_6H_{12} vs. HMPTA) thus appeared of in-

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