Title

SYNTHESIS OF OLIGODEOXYRIBONUCLEOTIDES USING N-BENZYLOXYCARBONYL BLOCKED NUCLEOSIDES

Author

Watkins, B.E.

Publication Date

1982

LBL-13981
Preprint

Lawrence Berkeley Laboratory
UNIVERSITY OF CALIFORNIA

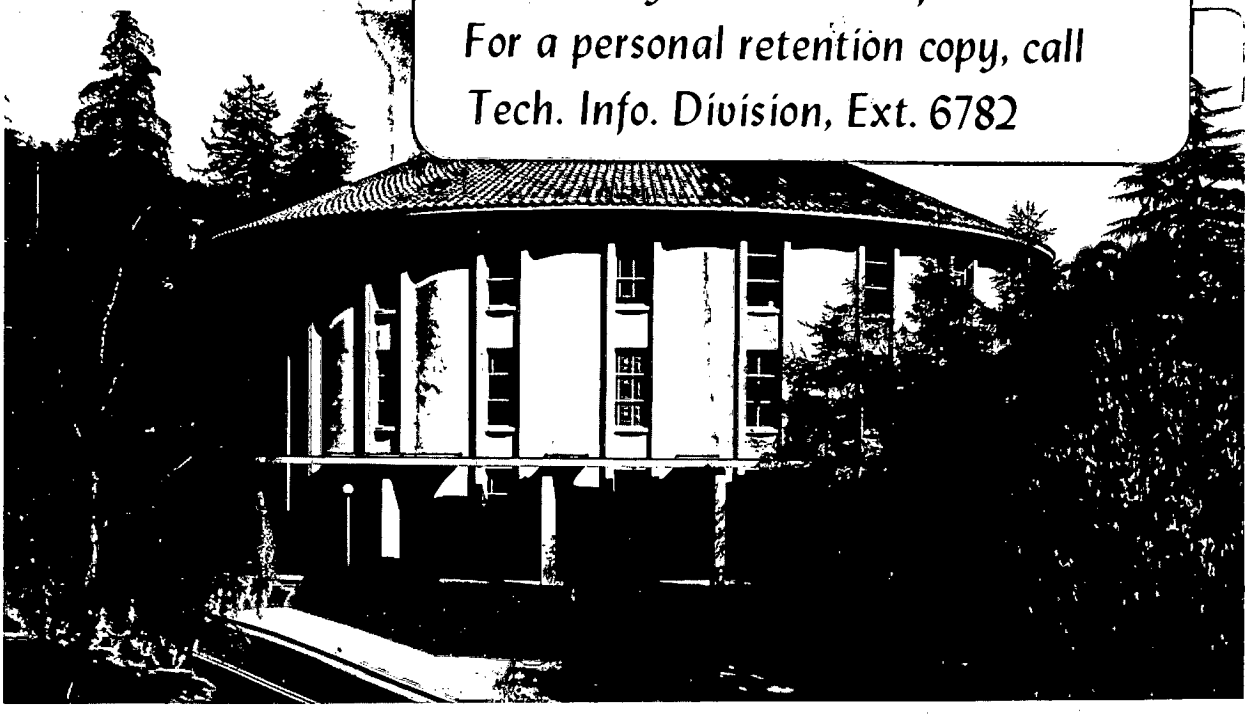
CHEMICAL BIODYNAMICS DIVISION

Submitted to the Journal of the American Chemical Society

SYNTHESIS OF OLIGODEOXYRIBONUCLEOTIDES USING N-BENZYLOXYCARBONYL BLOCKED NUCLEOSIDES

Bruce E. Watkins, John S. Kiely,
and Henry Rapoport

January 1982



Prepared for the U.S. Department of Energy under Contract W-7405-ENG-48

DISCLAIMER

LBL-13981

Synthesis of Oligodeoxyribonucleotides Using N-Benzyloxycarbonyl Blocked Nucleosides.

Bruce E. Watkins, John S. Kiely, and Henry Rapoport*

Contribution from the Department of Chemistry and Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720

January, 1982

Abstract

The exo-amino groups of 2'-deoxyadenosine and 2'-deoxycytidine have been blocked as the benzyl carbamates, and 2'-deoxyguanosine has been blocked as its 2-N-benzyloxycarbonyl carbamate and 6-O-benzyl ether. These blocked nucleosides have been incorporated into an efficient oligodeoxyribonucleotide synthetic scheme and the resulting oligomer successfully deblocked using transfer hydrogenation. The deblocking conditions result in no reduction of the pyrimidine bases.

This work was supported in part by the Director, Office of Energy Research, Office of Basic Energy Sciences, Chemical Sciences Division of the U.S. Department of Energy under Contract No. W-7405-ENG-48.

Introduction

The synthesis of oligodeoxyribonucleotides primarily involves blocking group chemistry. Nucleosides and nucleotides have multiple reactive centers, and selective reagents must be found for the various functional groups or else the other reactive centers must be blocked. Since such high selectivity has not been attained, blocking strategies are required. The blocking groups should be introduced in high yield, be stable to the subsequent reaction conditions, and be selectively removed when necessary. In the most common oligonucleotide synthetic route, the triester method, the choice is to block each reactive center because of the poor specificity of the reagents used, the need for high yield and purity, and the difficult separations often encountered. As new reagents and methods are being developed, the choice of whether or not to block is constantly being re-evaluated.

There has been a great variety in the nature and scope of the blocking groups used in oligonucleotide synthesis with one exception: the exocyclic amine blockers, which are invariably amides.[1] Benzoyl has been most frequently selected for 2'-deoxyadenosine, anisoyl for 2'-deoxycytidine and isobutyryl for 2'-deoxyguanosine, although other acyl groups have been examined.[1,2] These groups have been selected for this purpose, and have survived, because they represent a compromise between stability and ease of removal. Recently, the subject of nucleoside amine blockers has been re-examined[2] with emphasis upon developing groups which are hydrolytically more stable than those currently in vogue. It has not been clearly established at what point hydrolytic stability will require overly harsh conditions for removal such that the resulting oligonucleotide would be seriously degraded. What is needed is a blocking group which possesses a high degree of hydrolytic stability and yet can be easily removed under mild conditions.

An obvious choice is the benzyloxycarbonyl (carbobenzoxy, Cbz) group, which has been used so effectively in polypeptide syntheses. This blocking group is stable to a variety of hydrolytic conditions, imparts a good deal of lipophilicity to otherwise polar molecules, and most notably is removable under neutral, hydrogenolysis conditions.[3] Its stability also can be modified as needed by adding substituents to the phenyl ring and benzylic carbon. Previous reports concerning the use of benzyl groups to block ribose hydroxyls during oligonucleotide synthesis indicate that substantial reduction of the pyrimidine 4,5-double bond occurs upon their reductive removal.[4] However, recent advances in hydrogenation technology hold out the promise that this side reaction could be avoided when removing Cbz groups from oligonucleotides.

There is another aspect of base blocking which has not been satisfactorily addressed. Generally the synthesis of oligomers containing guanosine gives consistently low yields.[1] It had been suspected, and recently confirmed, that another reactive center on guanosine, in addition to the exo-amine, is the 6-oxygen. This oxygen has been blocked in only one[5] previous oligonucleodite synthesis, and blocking of the guanosine 2-amine seems to be done more for solubility than reactivity reasons. Advances have been made in the selectivity of phosphate coupling reagents[1] which minimize reaction at the 6-oxygen of guanosine, however, recent reports indicate that phosphate coupling reagents do react with 2-N-acyl guanosines to produce 6-substituted guanosines.[6] It has been suggested that those side products could revert to guanosine residues in subsequent deblocking steps, but these 6-substituted guanosines are also modified with other nucleophiles and can lead to complex mixtures of oligonucleotides.

What is clearly needed is a blocking group for the guanosine 6-O position. Acyl groups are far too labile,[2] so we propose an ether type blocking group, specifically a benzyl ether, for this role. Benzyl ethers have been used to

protect the 6-O position of guanosine previously,[7] although not in oligonucleotide synthesis, and could probably be removed under the same conditions as Cbz groups. Thymidine could potentially exhibit the same kind of reactivity as guanosine but it apparently does not.[6]

Results and Discussion

Preparation of Base-Blocked Nucleosides. The synthesis of the base-blocked nucleosides proved to be a formidable task. The use of benzyl chloroformate under all the usual conditions was unsatisfactory for the preparation of N-benzyloxycarbonyl nucleosides. Benzyl chloroformate in the presence of hindered amines or inorganic bases was not sufficiently reactive to acylate the weakly nucleophilic exocyclic amino groups of the heterocyclic bases. In the presence of non-hindered amines, such as triethylamine and pyridine, decomposition to benzyl chloride and carbon dioxide took place in preference to acylation of the exocyclic amines. The benzyl chloride then became an unwanted competitor for nucleophilic sites.

Consequently new methods had to be developed. The method of choice involved first the preparation of 1-Cbz-imidazole. With many nucleophiles this reagent is too unreactive to transfer the Cbz group. It becomes an extremely effective acylating agent, however, on quaternization to 1-Cbz-3-ethylimidazolium ion with triethyloxonium tetrafluoroborate. The following discussion (Scheme I) describes the synthesis of suitably blocked deoxyribonucleosides. The general subject of the preparation of nculeoside carbamates along with more details and extensions of the current methodology is being presented elsewhere.[8]

2'-Deoxycytidine (1) reacts with 1-benzyloxycarbonyl-3-ethylimidazolium tetrafluoroborate (2) to give a mixture of polyacylated nucleosides 3. Treatment of this mixture with dilute sodium hydroxide gives 4-N-Cbz-2'-deoxycytidine (4) in 80% yield. Acylation of the bis-tert-butyldimethylsilyl ether of 2'-

deoxyadenosine (6) with 2, followed by removal of the silyl groups gives 6-N-Cbz 2'-deoxyadenosine (7) in 85% overall yield from deoxyadenosine (5). If deoxyadenosine itself is acylated followed by treatment with sodium hydroxide, a 50% yield of 7 is obtained. In no cases did we observe bis-N-acylated products with either cytidine or adenosine derivatives which are often problems with other acylating agents.[2] With 3',5'-bis-tert-butyldimethylsilyl-2'-deoxyguanosine (9), prepared from 2'-deoxyguanosine, 2 did not result in acylation but rather gave an alkylated product.[8] Even if the expected 2-N-Cbz-2'-deoxyguanosine had been formed, it would not have solved the problem of blocking the 6-O-center. Alternatively we found that the reaction of 9 with phenyl chlorothioformate gave the 6-thiophenylpurine 10. This is an intermediate through which the functionality at the 6-O and 2-N positions can be selectively controlled. Thus treatment of 10 with sodium benzyloxide followed by removal of the silyl groups gives 6-O-benzyl-2-N-Cbz-guanosine (11) in 80% overall yield.

Preparation of Blocked Oligonucleotides. The base-blocked deoxynucleosides 12b,c,d were then incorporated into an oligonucleotide synthetic scheme using the popular phosphotriester approach.[1] In this strategy nucleoside-3'-phosphodiesters are coupled with the 5'-hydroxyl of another oligomer. We briefly explored the phosphite approach to this coupling.[9] It produced unacceptably large amounts of symmetrically coupled nucleotides and was consequently abandoned in favor of phosphate methodology. The tetramer d(T-C-A-G) was selected as the first synthetic target to demonstrate the utility of the N-Cbz and O-benzyl blocking groups, and the preparation of the requisite monomers is shown in Scheme II.

The monomethoxytrityl [(MeO)Tr] group was selected to block the 5'-terminal hydroxyl because of its high degree of selectivity for primary centers and stability when compared to other 5'-hydroxyl blockers.[1] This group is reported

to be removable under mild acidic[10] or Lewis[11,12] acid conditions without concomitant depurination. Both conditions have been applied for our purposes and a comparison will be presented below. The four (MeO)Tr nucleosides (13a,b,c,d) were prepared according to literature methods in 80-90% yield.

The nucleoside-3'-phosphodiesters 16a,c were readily prepared as reported[13]. Thus 12a and c were phosphorylated with the bis-triazolide 14, and the intermediate triazolide 15 hydrolyzed with water and triethylamine to give the phosphodiesters 16 in >95% yield with no detectible 3' → 3' coupled products. The 2-chlorophenyl group was chosen as the internucleotide phosphate blocker because it is reported to be removed via an oximate anion[14] with a minimum of internucleotide bond cleavage.

The next step was to make the 3' → 5' phosphate linkage. There is some choice of what functionality should be present at the 3' position of the incoming nucleoside, the options being a blocked phosphate, a blocked hydroxyl or an unblocked hydroxyl. Recently reports[13,15] describe the synthesis of nucleotide dimers via the coupling of a nucleotide phosphodiester with an N-blocked-2'-deoxyribonucleoside in the presence of 600 mol % of 1-mesitylenesulfonyl-3-nitro-1,2,4-triazolide (MS-NT). Although 20% of starting materials were unaccounted for, the reported low yield (<5%) of undesired 3' → 3' nucleotide dimer and good yield (60-80%) of desired 3' → 5' nucleotide dimer made this procedure seem quite attractive.

The coupling of phosphodiester 16a and nucleoside 12b under the described conditions gave the expected 3' → 5' nucleotide dimer 23 in 53% yield. Along with 23, we obtained 2% of unwanted 3' → 3' dimer 25 and 11% of 5'-O-mesitylenyl-4-N-Cbz-2'-deoxycytidine (27). The remainder of the starting material 12b was recovered. The use of triisopropylbenzenesulfonyl-3-nitro-1,2,4-triazolide (TPS-NT)[16] as the coupling agent at 650 or 150 mol % gave cleaner

reaction products and suppressed the sulfonation of 12 but produced a new nucleotide dimer which we have not yet identified. When phosphodiester 16c and nucleoside 12d were coupled with TPS-NT (200 mol %), our best conditions, a good yield of 3' → 5' dimer 24 was obtained but the 3' → 3' dimer 26 could not be separated from 24 by preparative chromatography.

These results are summarized in Table I and we concluded that this methodology for producing nucleotide dimers was not satisfactory for the following reasons: a) when MS-NT is used as the coupling agent loss of starting material by 5'-O-sulfonation was too large; b) with TPS-NT another side reaction, formation of a new dimer, was observed; and c) not each set of 3'→ 5' and 3'→ 3' nucleoside dimers were conveniently separable. As controls, authentic samples of the 3'→ 3' coupled dimers 25 and 26 were prepared from the phosphodiester 16a and 16c and the 5'-blocked nucleosides 3b and 28d; 3b is available as a byproduct during the synthesis of 4.

Since the Cbz-base blocking strategy requires the 3'-hydroxyl of the incoming nucleoside to be blocked, the synthetic scheme would be more convergent if this 3'-hydroxyl were brought in already functionalized as a blocked phosphotriester. The methyl group was initially selected as a blocking group for the terminal phosphate because it is removable under mild conditions by nucleophiles such as thiols[17] or tert-butyl amine.[18]

The four triesters 17a,b,c,d were prepared from the intermediate triazolides 15a,b,c,d. The methyl group can be cleanly removed from these triesters with benzenethiol/triethylamine to produce the phosphodiesters 16a,c. They may also be removed with refluxing tert-butylamine, however, in the case of 17c the t-butyl urea 16e also was formed. Then the monomethoxytrityl group was removed from 16d to give 19d which was coupled with 16c in the presence of TPS-NT to give the A-G dimer 30. Dimer 29 was prepared analogously to 30.

The methyl group was found to be unstable to the phosphorylating conditions. Also, the phosphotriesters 16 were unstable to storage even at -78°C. Probably the nucleophiles encountered during the phosphorylation are reactive enough to demethylate phosphotriesters. Indeed, even the ring nitrogens of the heterocyclic bases are alkylated by the phosphate methyl esters on storage at low temperature. For these reasons we concluded that the use of methyl as a phosphate blocking group in oligonucleotide synthesis is inadvisable, although there are mixed observations from others.[11b,18,19]

The β-cyanoethyl group has been shown[10] to be effective for blocking a 3'-terminal phosphate, and this methodology was adopted for the synthesis of the tetramers 37 and 38 (Scheme III). Phosphotriesters 18a,c were prepared in 80 and 78% yield via the intermediate triazolides 15a,c, respectively. These were detritylated with 2% benzenesulfonic acid to give the 3'-blocked nucleotides 20b and d which were coupled with the phosphodiesters 16a and c, respectively, in the presence of TPS-NT to give the dimers 31 and 32. The dimer 33 was also made from 16c and the 3'-levulinylguanosine 22, the latter being prepared in 78% yield from the monomethoxytritylguanosine 13d and levulinic anhydride followed by detritylation of the intermediate 21. The levulinyl group was chosen as the 3'-terminal hydroxyl blocker because it may be selectively removed under mild conditions (hydrazine, pyridine, acetic acid).[16]

Removal of the methoxytrityl from the purine dimers 31 and 32 was investigated in some detail in an effort to prevent depurination which occurs during acid treatment of oligonucleotides.[10,11] Recent reports describe improved removal of trityl groups with $ZnBr_2$ in methylene chloride[12] or nitromethane.[11] When comparing these methods in our systems to the older 2% benzenesulfonic acid method,[10] we found that $ZnBr_2$ in methylene chloride was inferior in overall yield, and that $ZnBr_2$ in nitromethane and 2% benzenesulfonic acid were comparable.

In our hands, $ZnBr_2$ in methylene chloride and in nitromethane both led to substantial amounts of depurination, producing 35% and 15% of Cbz-adenine, respectively. Other unidentified dimeric products were also found.

The dimer 31 was deblocked at the 3'-terminal phosphate as described[20] with triethylamine to give 34. This was coupled in the presence of TPS-NT with the dimers 35 and 36 to give the tetramers 37 and 38 as summarized in Table II. Each of these may be detritylated, as described above to allow extension at the 5'-terminus, and deblocked at the 3'-terminus to give 41 and 42 with triethylamine and hydrazine, respectively, in high yields. In no case did we observe the loss of Cbz or benzyl groups from any of the above intermediates.

Removal of N-Cbz and O-Benzyl Groups. Nucleosides which are blocked by benzyl and benzyloxycarbonyl groups have thus been incorporated into an efficient oligonucleotide synthetic scheme and it remains to be shown that they can be effectively removed at the end of the synthesis. As was expected, N-Cbz and O-benzyl protected nucleosides 12b,c,d can be readily deblocked using hydrogen over palladium on carbon or palladium on barium sulfate. However, these conditions also lead to significant reduction of the 5,6 double bond of thymidine. Similar observations were made[4] when removing benzyl groups from uridine-containing oligoribonucleotides. Others have made the same observation for cytidine[21] residues. Pyrimidine bases are much more easily reduced than purine bases and reduction of adenosine or guanosine residues was neither observed or expected.

To avoid this over-reduction of the pyrimidine bases, we turned to transfer hydrogenolysis[22]. Using cyclohexadiene as the hydrogen source and 10% palladium on carbon as the catalyst, both O-benzyl and N-Cbz groups were cleanly removed from 12b,c,d in 30 to 90 min. Under these conditions there was no reduction of thymidine even after 18 h, and 6-N-Cbz-2'-deoxycytidine (12b) was quantatively

converted to 2'-deoxycytidine without any other products being formed. With dimer 32 and tetramer 42, the time necessary for the Cbz and benzyl groups to be completely removed increased markedly (Table III). However, transfer hydrogenolysis with cyclohexadiene over a more active catalyst, palladium black,[22b] proceeded more rapidly and tetramer 42 was deblocked in 24 hr to give 43. Again no reduction of the pyrimidine bases was observed. The latter was demonstrated by showing that neither dihydrothymidine (45) nor 2'-deoxydihydrouridine (46) are formed when thymidine or 2'deoxycytidine are subjected to the reaction conditions. 2'-Deoxydihydrouridine would be the expected deaminated product[23] if 2'-deoxy-5,6-dihydrocytidine had been formed.

Complete Deblocking of Oligonucleotides. The phosphate blocking groups were removed as described[14] using p-nitrobenzaldoximate. These conditions cleanly remove the 2-chlorophenyl group with a minimum of internucleotide phosphate bond cleavage. The monomethoxytrityl group was then removed in 90% acetic acid to give the tetramer d(T-C-A-G) (44) in 80% yield from 37. The order of deblocking was to remove the methoxytrityl group last to minimize 3'→ 5' phosphate rearrangements. The hydrogenolysis was performed after removal of the levulinyl group to prevent its reduction and before deprotecting the phosphates since the phosphate-diesters were expected to adsorb more strongly to the catalyst. The resulting tetramer d(T-C-A-G) (44) was completely degraded to 5'-HOdTp, 5'-HOdCp, 5'-HOdAp, and dG with spleen phosphodiesterase[16] showing that only the 3'→ 5' phosphate linkage was present and that the tetramer has the expected composition.

Conclusions

The N-Cbz and O-benzyl blocked deoxynucleosides 12a,b,c,d have been incorporated into an efficient oligonucleotide synthesis. They were found

to be stable to phosphorylation conditions as well as other deblocking conditions, and they can be removed quantatively when desired by transfer hydrolysis without side reactions.

Experimental Section

Melting points were obtained with Buchi (capillary) and Kofler (microscope slide) apparatuses and are uncorrected. IR spectra were determined as KBr pellets unless otherwise noted with Perkin-Elmer 137 or 1944 spectrophotometers using polystyrene film for calibration (1601.4 cm^{-1} absorption). UV spectra were determined on a Cary 219 spectrophotometer in 95% ethanol unless otherwise noted. ^1H NMR spectra were determined on the following spectrometers: Varian T-60 (60 MHz); Varian E-390 (90 MHz); UCB-250 (a homemade FT instrument operating at 250.80 MHz); were recorded in $CDCl_3$ unless otherwise noted; and are expressed in ppm (δ) downfield from Me_4Si. The ^1H NMR spectra reported do not contain the resonances for phenyl protons and 3',4' and 5' ribose protons as they were found of little analytical value. Elemental analyses were performed by the Analytical Laboratory, College of Chemistry, University of California, Berkeley. Field Desorption Mass Spectra were performed by the Bio-Organic, Biomedical Mass Spectrometer Resource supported by Grant No. RR00719 from the Division of Research Resources, NIH.

High pressure liquid chromatography (HPLC) was performed on an Altex analytical system consisting of two 110A pumps, a 155-10 UV-VIS dector, and a 420 microprocessor controller/programmer. Unless otherwise noted, a flow rate of 1.0 mL/min (one column volume equals 1.5 min) was used, with monitoring at 254 or 290 nm unless otherwise stated. Preparative medium pressure liquid chromatography (MPLC) was done using an Altex 110A pump equipped with a preparative liquid head and an Altex 151 UV detector set at 254, 280 nm. The following Altex stainless steel columns were used: (A) 4.6 x 250 mm, 5 μm Ultrasphere ODS; (B) 3.2 x 250 mm, 5 μm LiChrosorb C-18; (C) 10 x 250 mm, 10 μm Spherisorb ODS. The solvent systems used were: (1) CH_3CN/H_2O: a, 80/20; b, 75/25; c, 65/35; d, 60/40; e, 44/56. (2) CH_3CN/0.1 M triethylammonium acetate, pH 7.0: a, 10/90;

b, 10 min gradiant, 8/92 → 10/90. (3) CH_3OH/0.1 M tetrabutylammonium fluoride, 0.1 M KH_2PO_4, pH 2.7, 10 min gradient, 10/90 → 35/65. (4) CH_3OH/H_2O: a, 20/80; b. 30/70. HPLC conditions are specified as (column, solvent).

Ace Michel-Miller glass columns, 25 x 130 mm or 40 x 240 mm, 40-63 μm Silca Gel 60 (EM Reagents) were used for MPLC. Column chromatography (CC) was performed with 63-200 m Silica Gel 60 (EM Reagents). Analytical thin layer chromatography (TLC) was done with aluminum-backed silica plates (E. Merck). Preparative TLC was carried out on 2000 μm thick Silica Gel GF (Analtech).

Unless otherwise noted, reactions were conducted under a nitrogen atmosphere with magnetic stirring at room temperature (20-26°). Organic layers were dried over $MgSO_4$ and evaporated with a Berkeley rotary evaporator using water aspirator or oil pump reduced pressure, followed by static evaporation with an oil pump. All distillations were bulb to bulb (Kugelrohr-type apparatus) unless otherwise noted.

The following solvents were freshly distilled as needed: tetrahydrofuran (THF) and toluene from sodium/benzophenone; methanol from magnesium; pyridine from toluenesulfonyl chloride and then from calcium hydride; acetonitrile and CH_2Cl_2 from P_2O_5. Triethylamine and nitromethane were distilled from calcium hydride and stored over 3Å molecular sieves. Imidazole and 1,2,4-triazole were dried in vacuo over P_2O_5 before use. 2-Cyanoethanol was distilled before use. Tetrabutylammonium fluoride was prepared from an aqueous solution of the hydroxide by neutralization with conc. aq. HF and rendered anhydrous by repeated addition and evaporation of pyridine. 2-Chlorophenyl phosphodichloridate,[24] 2,4,6-triisopropylbenzenesulfonylnitrotrizaole,[16] 2,4,6-trimethylbenzenesulfonylnitrotriazole,[14] triethyloxonium tetrafluoroborate,[25] 5'-O-monomethoxytritylthymidine,[26] 5,6-dihydrothymidine,[27] 2'-deoxydihydrouridine[28], and phenyl chlorothioformate[29] were prepared as described.

Fully protected oligomers were stored as dry powsers at -15°C for short term or at -196°C in a Linde Super -30A liquid nitrogen refrigeration. The fully deprotected tetramer was stored in water at -196°C.

N-Benzyloxycarbonylimidazole. To imidazole (200 g, 2.94 mol) in 2.0 L of toluene at 0°C was added benzyl chloroformate (250 g, 1.47 mol). The mixture was stirred at room temperature overnight and filtered, the filtrate evaporated, and the resulting oil was crystallized from petroleum ether to yield 230 g, 78%, of Cbz-imidazole: mp 37.0-38.5°C (lit.[30] oil); ^{1}H NMR δ 5.3 (s, 2H), 7.0 (m, 1H), 7.3 (m, 6H), 8.0 (m, 1H). Anal. Calcd for $C_{11}H_{10}N_2O_2$: C, 65.3; H, 5.0; N, 13.9. Found: C, 65.1; H, 5.9; N, 13.8.

1-Benzyloxycarbonyl-3-ethylimidazolium Tetrafluoroborate (2). Triethyloxonium tetrafluoroborate (20 mmol, 3.80 g) was added to N-Cbz-imidazole (19 mmol, 3.94 g) in 50 mL of CH_2Cl_2 at 0°C. The reaction mixture was allowed to come to room temperature then stirred for 2 h. This solution was used to acylate the deoxyribonucleosides. Evaporation of a portion left 2: ^{1}H NMR δ 1.47 (t, 3H, J=7), 4.20 (q, 2H, J=7), 5.42 (s, 2H), 7.3 (m, 6H), 7.65 (m, 1H), 8.98 (m, 1H).

Bis-3'-O,4-N-Benzyloxycarbonyl-2'-deoxycytidine (3a) and 4-N-Benzyloxycarbonyl-2'-deoxycytidine (4). The solvent was evaporated from the solution of 2 (80 mmol) and replaced with 200 mL of acetonitrile. To this slurry was added 2'-deoxycytidine (1, 4.54 g, 20.2 mmol), it was stirred at room temperature for 36 h, the reaction was quenched with 3 mL of saturated sodium carbonate, and the acetonitrile was evaporated to give a 60/40/10 mixture of tris, bis and mono acylated cytidines. The bis-carbobenzoxy material 3a was isolated by chromatography on silica gel (ethanol/$CHCl_3$, 5/95). Recrystallization from methanol gave 3a as needles: mp 89-90°C; IR 1740 cm^{-1}; ^{1}H NMR δ 5.05 (s, 2H), 5.15 (s, 2H), 6.15 (t, 1H, J=6), 7.85 (d, 1H, J=7); UV λ_{max} (nm (ε)) 242 (14,000), 294 (7,000). Anal. Calcd for $C_{25}H_{25}N_3O_8$: C, 60.6; H, 5.1; N, 8.5. Found: C, 60.4; H, 5.0; N, 8.4.

The mixture of acyl cytidines was treated with sodium hydroxide as described[26] for O-deacylations, the Dowex 10N resin was washed with $CHCl_3$, and the filtrate was extracted with 4 x 100 ml of $CHCl_3$/isopropyl alcohol (75/25). Combining the organic extracts and evaporating left a residue which was recrystallized from $CHCl_3$/ether to give 7.1 g, 98%, of 4: mp 139-141°C; IR 1740 cm^{-1}; ^1H NMR (CD_3OD) δ 5.05 (s, 2H), 6.05 (t, 1H, J=6), 8.25 (d, 1H, J=7); UV λ_{max} (nm (ϵ)) 242 (15,400), 294 (7,700). Anal. Calcd for $C_{17}H_{19}N_3O_6$ 0.5 H_2O: C, 55.1; H, 5.4; N, 11.3. Found: C, 55.0; H, 5.5; N, 11.4.

6-N-Benzyloxycarbonyl-2'-deoxyadenosine (7). 2'-Deoxyadenosine (5, 10.75 g, 42.8 mmol), imidazole (25 g, 380 mmol), t-butyldimethylsilyl chloride (28.6 g, 189 mmol) and pyridine (250 mL) were stirred for 2 days. The reaction mixture was then poured into ice water (1 L) and extracted with $CHCl_3$ (2 x 300 mL), the $CHCl_3$ was evaporated to give a 1/1 mixture of 6 and a tris-silylated adenosine. In order to convert the tris-silylated material to 6, the residue was taken up in 200 mL of 80% aq. acetic acid. After 30 min at room temperature, the reaction mixture was poured into 1 L of ice water and extracted with $CHCl_3$. Solid $NaHCO_3$ was added to the organic phase which was stirred for 20 min then washed with water. Drying and evaporating left a residue which was column chromatographed (Et_2O) to yield the bis-silyl ether 6 (18.9 g, 92%): mp 128-130°C (lit.[31] mp 132.5-133°C); ^1H NMR δ 0.10 (s, 6H), 0.13 (s, 6H), 0.97 (s, 18H), 6.37 (t, 1H, J=6), 8.05 (s, 1H), 8.25 (s, 1H).

The bis-silyl ether 6 was added in one portion to a CH_2Cl_2 solution of 2 (400 M%), and the solution was stirred for 15 h and quenched with saturated $NaHCO_3$. The CH_2Cl_2 layer was diluted with an equal volume of $CHCl_3$, and the organic phase was separated, washed with water, dried and evaporated. Treating the residue with tetrabutylammonium fluoride[31] in THF and evaporating the THF followed by column chromatography (EtOH/$CHCl_3$, 7/93) gave 6-N-Cbz-2'-deoxyadenosine (7) in

95% yield from 6 and 87% yield from 2'-deoxyadenosine (5): IR 1740 cm^{-1}; ^1H NMR δ 5.20 (s, 2H), 6.30 (t, 1H, J=7), 7.3 (s, 5H), 8.13 (s, 1H), 8.47 (s, 1H); UV λ_{max} (nm (ε)) 267 (20,300). Anal. Calcd for $C_{18}H_{19}O_5N_5$ 0.8 H_2O: C, 54.1; H, 5.2; N, 17.5. Found: C, 54.0; H, 4.9: N, 17.3.

6-N-Benzyloxycarbonyladenine. 6-N-Cbz-2'-deoxyadenosine (7) was dissolved in 80% aqueous acetic acid and stirred at room temperature for 8 h. The solvent was evaporated and the residue recrystallized from methanol giving 6-N-Cbz-adenine: 217°C (dec); ^1H NMR δ 5.1 (s, 2H), 7.2 (s, 5H), 8.15 (s, 1H), 8.40 (s, 1H); UV λ_{max} (nm (ε)) 275 (13,000). Anal. Calcd for $C_{13}H_{11}N_5O_2$: C, 56.0; h, 4.1; N, 26.0. Found: C, 57.6; H, 4.2; N, 25.5.

6-Phenylthio-2-N-phenylthiocarbonyl-bis-3',5'-O-(tert-butyldimethylsilyl)-2'-deoxyguanosine (10). To 2'-deoxyguanosine (8, 1.67 g, 6.25 mmol) in 30 mL of pyridine was added tert-butyldimethylsilyl chloride (2.83 g, 18.8 mmol) and the mixture was stirred for one week at room temperature then cooled to 0°C and phenyl chlorothioformate (21.5 g, 125 mmol) in 15 mL of pyridine was added. The mixture was stirred in the dark at room temperature for 5 h, and then poured into ice water. The resulting mixture was extracted with chloroform, the bulk of the chloroform was evaporated, and the remaining thiophenol removed by bulb to bulb distillation (20 min, 50°C). Column chromatography of the residue on silica gel ($CHCl_3$) and recrystallization from methanol yielded 4.57 g, 85%, of 10: mp 129-130.5°C; IR 1700 (s) cm^{-1}; ^1H NMR δ 0.08 (s, 6H), 0.12 (s, 6H), 0.93 (s, 18H), 6.33 (t, 1H, J=6), 7.3 (s, 15H), 8.30 (s, 1H); UV λ_{max} (nm (ε)) 291 (17,900), 232 (28,700); FDMS (m/e) 859 (M^+), 802 (M^+-$C(CH_3)_3$), 750 (M^+-SC_6H_5). Anal. Calcd for $C_{42}H_{53}N_5O_5S_3Si_2$: C, 58.6; H, 6.2; H, 8.1; S, 11.2. Found: C, 58.3; H, 6.2; H, 8.1; S, 11.4.

6-O-Benzyl-2-N-benzyloxycarbonyl-2'-deoxyguanosine (11). Sodium (2.5 g, 100 mmol) was dissolved in benzyl alcohol (14.6 g, 120 mmol) and 15 mL of THF. The

resulting solution was cooled to 0°C, 10 (8.39 g, 9.76 mmol) was added in 100 mL THF at 0°C. After being stirred at 0°C overnight, the reaction was quenched with acetic acid (7.2 g, 120 mmol), the bulk of the solvent was evaporated at room temperature, and the residue was dissolved in $CHCl_3$ and washed with water. Evaporation at room temperature followed by bulb to bulb distillation (20 μm, 50°) left a residue which was treated with tetrabutylammonium fluoride in THF and column chromatographed (EtOH/$CHCl_3$, 7/93) to give 11 in 95% yield: IR 1750 cm^{-1}; ^1H NMR δ 5.18 (s, 2H), 5.53 (s, 2H), 6.30 (t, 1H, J=6), 7.3 (s, 10H), 8.00 (s, 1H); UV λ_{max} (nm (ε)) 217 (40,400), 258 (14,900), 268 (15,500); FDMS (m/e) 491 (M^+), 384 ($M^+-C_7H_7$), 356 ($M^+-CO_2C_7H_7$). Anal. Calcd for $C_{25}H_{25}N_5O_6$: C, 61.1; H, 5.1; N, 14.2. Found: C, 60.8; H, 5.2; N, 14.0.

Preparation of 5'-O-Methoxytritylnucleosides (13b,c,d). The general procedure[26] for tritylation of acylated nucleosides was employed to prepare these nucleosides which were obtained as glasses in 80-90% yields after silica gel chromatography using the solvent noted.

5'-O-Methoxytrityl-4-N-benzyloxycarbonyl-2'-deoxycytidine (13b): EtOH/$CHCl_3$ (3/97); IR 1750 cm^{-1}; ^1H NMR δ 3.7 (s, 3H), 5.1 (s, 2H), 6.15 (t, 1H, J=6), 8.05 (d, 1H, J-6); UV λ_{max} (nm (ε)) 235 (24,900), 295 (7,600). Anal. Calcd for $C_{37}H_{35}N_3O_7$: C, 70.1; H, 5.6; N, 6.6. Found: C, 69.9; H, 5.6; N, 6.6.

5'-O-Methoxytrityl-6-N-benzyloxycarbonyl-2'-deoxyadenosine (13c): EtOH/$CHCl_3$ (2/98); IR 1750 cm^{-1}; ^1H NMR δ 3.7 (s, 3H), 5.2 (s, 2H), 6.4 (t, 1H, J=6), 8.1 (s, 1H), 8.7 (s, 1H); UV (nm (ε)) 234 (18,200), 268 (20,400). Anal. Calcd for $C_{38}H_{35}N_5O_6$: C, 69.4; H, 5.4; N, 10.7. Found: C, 69.4; H, 5.5; H, 10.5.

5'-O-Methoxytrityl-6-O-benzyl-2-N-benzyloxycarbonyl-2'-deoxyguanosine (13d): EtOH/chloroform (4/96); IR 1750 (s) cm^{-1}; ^1H NMR δ 3.65 (s, 3H), 5.08 (s, 2H), 5.48 (s, 2H), 6.43 (t, 1H, J=7), 7.83 (s, 1H); UV λ_{max} (nm (ε)) 235 (19,500), 268 (16,400). Anal. Calcd. for $C_{45}H_{41}N_5O_7$: C, 70.8; H, 5.4; N, 9.2. Found: C, 70.9; N, 5.5; N, 9.1

Preparation of Blocked Phosphodiesters (16a,c). The general procedure[13] for phosphorylation with the bis-triazolide 14 followed by hydrolysis of the phosphorylated intermediate 15 with pyridine/TEA/H_2O was used to synthesize 16a and c. They were obtained in 99 and 97% yields respectively by precipitation from $CHCl_3$ with isooctane and were pure by HPLC (B, 1b).

5'-O-Methoxytritylthymidine-3'-O-(2-chlorophenyl)phosphate Triethylammonium Salt (16a): ^1H NMR δ 1.2-1.4 (m, 12H), 3.1-2.9 (m, 6H), 3.8 (s, 3H), 6.5 (m, 1H), 7.4-6.8 (m, 18H).

5'-O-Methoxytrityl-6-N-benzyloxycarbonyl-2'-deoxyadenosine-3'-O-(2-chlorophenyl)phosphate Triethylamine Salt (16c): ^1H NMR δ 1.26 (t, 9H, J=7), 2.98 (q, 6H, J=7), 3.75 (s, 3H), 5.28 (s, 2H), 6.51 (t, 1H, J=6.5), 8.02 (s, 1H), 8.65 (s, 1H).

Preparation of Fully Blocked Nucleotides (17a,c,d; 18b,d). Intermediates 17, 18a,b,c,d were generated from 2-chlorophenylphosphodichloridate (250 mol %), triazole (500 mol %) and triethylamine (500 mol %) in THF according to a reported procedure[32] from 13a,b,c,d (100 mol %). They were treated with either methanol or β-cyanoethanol to give the triesters 17a,c,d and 18b,d. All were isolated after silica gel column chromatography with the solvent noted.

5'-O-Methoxytrityl-2'-deoxythymidine-3'-O-methyl-(2-chlorophenyl)phosphate (17a): $CH_3OH/CHCl_3$ (5/95); 40% yield; ^1H NMR δ 1.4 (s, 3H), 3.7 (s, 3H), 3.8 (2d, 3H, J=12), 6.3 (m, 1H); UV λ_{max} (nm (ε)) 231 (16,500), 267 (10,800). Anal. Calcd for $C_{37}H_{36}ClN_2O_2P$: C, 61.8; H, 5.0; N, 3.9. Found: C, 61.7; H, 5.3; N, 4.0.

5'-O-Methoxytrityl-6-N-benzyloxycarbonyl-2'-deoxyadenosine-3'-O-methyl-(2-chlorophenyl)phosphate (17c): $CH_3OH/CHCl_3$ (1/99); 50% yield; ^1H NMR δ 3.65 (s, 3H), 3.75 (d, 3H, J=12), 5.2 (s, 2H), 6.3 (m, 1H), 7.9 (s, 1H), 8.5 (s, 1H); UV λ_{max} (nm (ε)) 237 (17,400), 269 (19,300). Anal. Calcd for $C_{44}H_{41}ClN_5O_9P$:

C, 62.6; H, 4.9; N, 8.2. Found: C, 62.5; H, 5.0; N, 8.1.

5'-O-Methoxytrityl-6-O-benzyl-2-N-benzyloxycarbonyl-2'-deoxyguanosine-3'-O-methyl-(2-chlorophenyl)phosphate (17d): EtOH/$CHCl_3$ (2/98); 60% yield; IR 1750 cm^{-1}; ^1H NMR δ 3.70 (s, 3H), 3.85 (d, 3H, J=11), 5.18 (s, 2H), 5.57 (s, 2H), 6.33 (t, 1H, J=7), 7.83 (s, 1H); UV λ_{max} (nm (ε)) 268 (15,700). Anal. Calcd. for $C_{52}H_{47}ClN_5O_{10}P$: C, 64.5; H, 4.9; N, 7.2. Found: C, 64.4; H, 5.0; N, 7.2.

5'-O-Methoxytrityl-4-N-benzyloxycarbonyl-2'-deoxycytidine-3'-O-(2-chlorophenyl)-(2-cyanoethyl)phosphate (18b): $CH_3OH/CHCl_3$ (5/95); 97% yield; ^1H NMR δ 2.6-2.8 (m, 2H), 3.80 (s, 3H), 4.3-4.5 (m, 3H), 5.25 (s, 2H), 6.35 (m, 1H), 8.10 (d, 1H, J=7.5); UV λ_{max} (nm (ε)) 236 (22,000), 294 (7,000). Anal. Calcd for $C_{46}H_{42}ClN_4O_{10}P \cdot H_2O$: C, 61.7; H, 5.0; N, 6.3. Found: C, 61.7; H, 4.9; N, 6.3

5'-O-Methoxytrityl-6-O-benzyl-2-N-benzyloxycarbonyl-2'-deoxyguanosine-3'-O-(2-cyanoethyl)-(2-chlorophenyl)phosphate (18d): EtOH/$CHCl_3$ (2/98); 78% yield; IR 1750 cm^{-1}; ^1H NMR δ 2.7 (m, 2H), 3.74 (s, 3H), 4.4 (s, 2H), 5.20 (s, 2H), 5.60 (s, 2H), 6.4 (m, 1H), 8.8 (s, 1H); UV λ_{max} (nm (ε)) 257 (15,300), 267 (15,300). Anal. Calcd. for $C_{54}H_{48}ClN_6O_{10}P$: C, 64.4; H, 4.8; N, 8.3. Found: C, 64.5; H, 5.1; N, 8.0.

Detritylation. Procedure A. The monomethoxytrityl group was removed with 2% benzenesulfonic acid as described.[10,20] The crude products were column chromatographed on silica gel in the solvents noted and isolated as glasses in the yields stated (Table I).

Procedure B. The dimer 33 (1.30 g, 0.92 mmol) was dissolved in CH_2Cl_2 (50 mL) and then 1.12 g of anhyd. $ZnBr_2$ and 200 μL of methanol were added. The suspension was stirred at room temperature for 15 min, 100 mL of 1.0 M ammonium acetate was added, the solution was extracted with chloroform, the chloroform was evaporated, and the residue was column chromatographed to give 35 in 60% yield.

Procedure C. To 33 (300 mg, 0.21 mmol) was added 12.5 mL of a saturated solution of $ZnBr_2$ in CH_3NO_2 (0.076 M), the mixture was stirred for 90 min at room temperature, and the isolation was carried out as above to yield 79% of 35.

4-N-Benzyloxycarbonyl-2'-deoxycytidine-3'-O-(2-cyanoethyl)-(2-chlorophenyl)-phosphate (20b): $CHCl_3/CH_3OH$, 97/3; 85% yield; ^{1}H-NMR δ 2.8 (t, 2H, J=6), 5.20 (s, 2H), 6.20 (m, 1H), 8.13 (d, 1H, J=7); UV λ_{max} (nm (ε)) 242 (16,100), 294 (7,500). Anal. Calcd for $C_{26}H_{26}ClN_4O_9P$: C, 51.6; H, 4.3; N, 9.3. Found: C, 51.4; H, 4.5; N, 9.1.

6-O-Benzyl-2-N-benzyloxycarbonyl-2'-deoxyguanosine-3'-O-methyl-(2-chlorophenyl)-phosphate (19d): $CHCl_3$/EtOH, 97/3; 70% yield; ^{1}H NMR δ 3.92 (d, 3H, J=11), 5.18 (s, 2H), 5.51 (s, 2H), 6.23 (t, LH, J=7), 7.90 (s, 1H); UV λ_{max} (nm (ε)) 254 (12,300), 270 (12,300). Anal. Calcd for $C_{32}H_{31}ClN_5O_9P$: C, 55.2; H, 4.5; N, 10.1. Found: C, 55.3; H, 4.4; N, 10.0.

6-O-Benzyl-2-N-benzyloxycarbonyl-2'-deoxyguanosine-3'-O-(2-cyanoethyl)-(2-chlorophenyl)phosphate (20d): $CHCl_3$/EtOH, 99/5; 85% yield; ^{1}H NMR δ 2.8 (t, 2H, J=7), 5.24 (s, 2H), 2.61 (s, 2H), 6.3 (m, 1H), 7.82 (s, 0.5H), 7.85 (s, 0.5H); UV λ_{max} (nm (ε)) 255 (15,400), 267 (15,600). Anal. Calcd for $C_{34}H_{32}ClN_6O_9P$: C, 55.6; H, 4.4; N, 11.4. Found: C, 55.4; H, 4.4; N, 11.3.

Removal of β-Cyanoethyl Groups was accomplished as described[20] to give 34 and 41 in >95% yield, pure by HPLC: 34: R_t 5.2 min (B, 1e). 41: R_t 7.2 min (B, 1d).

3'-O-Levulinyl-6-O-benzyl-2-N-benzyloxycarbonyl-2-deoxyguanosine (22). To trityl ether 13d (1.84 g, 2.41 mmol) in 25 mL of pyridine was added 2.0 mL of levulinic anhydride[33] and the mixture was stirred at room temperature for 12 h. The reaction was quenched with 5% $NaHCO_3$, extracted with $CHCl_3$, the solvent was evaporated, the residue was treated with 2% benzenesulfonic acid,[20] and the crude product column chromatographed on silica gel ($CHCl_3$/EtOH, 95/5) to give

22 in 79% yield as a glass: ^{1}H NMR δ 2.15 (s, 3H), 2.6 (m, 4H), 5.17 (s, 2H), 5.50 (s, 2H), 6.2 (dd, 1H), 7.85 (s, 1H); UV λ_{max} (nm (ε)) 257 (13,100), 269 (13,100). Anal. Calcd for $C_{30}H_{31}N_5O_8$: C, 61.1; H, 5.3; N, 11.9. Found: C, 60.8; H, 5.3; N, 11.6.

5'-O-(4-Chlorophenoxyacetyl)-6-O-benzyl-2-N-benzyloxycarbonyl-2'-deoxyguanosine (28). To blocked guanosine 11 (0.50 g, 1.0 mmol) in 40 mL CH_3CN and 5 mL pyridine at room temperature was added over 2 h via syringe 4-chlorophenoxyacetyl chloride[34] (250 mg, 1.2 mmol) in 5 mL of CH_3CN, and the reaction mixture was stirred for 2 h at room temperature then poured into 5% $NaHCO_3$. The aqueous suspension was extracted with $CHCl_3$, the $CHCl_3$ was dried and evaporated, and the residue was column chromatographed ($CHCl_3$/EtOH, 97/3) to give 0.56 g, 84% yield of 28: ^{1}H-NMR δ 4.50 (s, 2H), 5.13 (s, 2H), 5.47 (s, 2H), 6.38 (t, 1H, J=7), 7.87 (s, 1H); UV λ_{max} (nm (ε)) 257 (14,900), 269 (15,400). Anal. Calcd for $C_{33}H_{30}N_5O_8Cl$: C, 60.0; H, 4.6; N, 10.6. Found: C, 59.6; H, 4.5; N, 10.6.

Phosphate Coupling Reactions. General Procedure. The phosphate coupling reactions were performed as described[14,16]. The products were column chromatographed using 5-10% EtOH or CH_3OH in $CHCl_3$ for elution and the results are summarized in Tables I and II.

The 3'-3' dimers were prepared from phosphodioesters 16a,c and the 5'-blocked monomers from 3b and 28 with TPS-NT as above. The chlorophenoxyacetyl or benzyl carbonate groups were removed as described[33] without isolating the fully protected dimer.

5'-(CH_3O)TrTpC^{4N}-Cbz$_{pA}$^{6}-N-Cbz$_{pG}$^{6}-O-Bn, 2-N-Cbz-3'-OH (42). To the fully protected tetramer 37 (66 mg, 0.027 mmol) in 1.0 mL of pyridine was added 50 mg of hydrazine hydrate in 1 mL 3/2 pyridine/HOAc. The mixture was stirred at room temperature for 5 min, cooled to 0°C, 2,4-pentanedione (0.5 mL) was added, and the solution stirred for 15 min more. The mixture was added to 50 mL of rapidly

stirring ether, and the 65 mg of precipitated product collected. This product was contaminated with low molecular weight byproducts and was purified by HPLC ($EtOH/CHCl_3$, 4/96) to give pure 42, 49 mg, 0.20 mmol, 74% yield, R_t (B, 1a) 9.8, 10.8 min.

Removal of N-Benzyloxycarbonyl and O-Benzyl Groups by Transfer Hydrogenolysis. General procedure. To the catalyst (100 wt % per Cbz and benzyl group) in a 16 x 3 cm test tube was added a solution (EtOAc/EtOH, 1/1) of the substrate (10 mg/mL) and cyclohexadiene (0.5 mL per 30 mg of substrate) and the suspension was mixed with a Vibro Mixer under a nitrogen atmosphere for the time specified in Table I. Pd/C (10%, Engelhard) was used as is. Palladium hydroxide on carbon (Aldrich) was hydrogenated for 1 h (50 psi) in ethanol before use. Palladium black was freshly generated from palladium acetate (Engelhard) in water (50 psi H_2 for 1 h), then washed twice with water and then twice with ethanol. For the hydrogenolysis of 12, 30 mg was treated in 6 ml of solvent with 0.5 mL of cyclohexadiene and palladium black from 120 mg of palladium acetate for 24 h. The resulting diastereomers (30 mg) were readily separated on HPLC (A, 1d).

p(T-C-A-G) (44). Removal of the 2-chlorophenyl groups from 30 mg of 43 was effected with p-nitrobenzaloximate as described.[14b] After 36 h, the solvent was evaporated and the residue, which contained the (MeO)Tr blocked tetramer, was dissolved in 10 mL of 80% HOAc and stirred at room temperature for 5 h to remove the (MeO)Tr group. The solvent was evaporated and the tetramer 44 was freed of organic material by partitioning the residue between 0.01M Et_3N/HOAc buffer (pH 7.0) and ether. The aqueous layer was lyophilized and the residue chromatographed (C, 2b). The tetramer thus obtained was pure by HPLC (A, 2a).

Enzymatic digest of p(T-C-A-G) (44). The tetramer 44 (100 µg) was dissolved in 100 µL of 0.1M NH_4OAc (pH 6.5) and 1 unit of spleen phosphodiesterase was

added. The mixture was incubated at 37° for 18 h, then analyzed by HPLC (A, 3). A mixture of 5'-HOTp, 5'-HOdCp, 5'-HOdAp, and dG was obtained in a ratio of 1.1: 0.9:1.1:0.9.

Stability of Pyrimidine Bases to Transfer Hydronation Conditions. When thymidine (12a) is subjected to the transfer hydrogenation conditions described above after 24 h, the thymidine was recovered unchanged. HPLC analysis (A, 4b) showed that no dihydrothymidine (45) was produced. Under these HPLC conditions, dihydrothymidine (45) is cleanly separated from thymidine (1a). When 4-N-Cbz-dC[12b] was subjected to these reaction conditions for 24 h, 2'-deoxycytidine (1a) is isolated as the sole nucleosidic product, and no 2'-deoxydihydrouridine (46) was found to be present (HPLC system: A, 4a). When 2'-deoxycytidene was treated with hydrogen over 10% Pd/C in EtOAc/95% EtOH in a Parr apparatus, 2'-deoxydihydrouridine was isolated as the major product. The above HPLC separations were monitored at 220 or 230 nm.

Acknowledgement. This work was supported in part by the Division of Biomedical and Environmental Research, DOE. Assistance in the preparation of intermediates was expertly provided by Wendy S. Jacks, undergraduate research participant.

This work was supported in part by the Director, Office of Energy Research, Office of Basic Energy Sciences, Chemical Sciences Division of the U.S. Department of Energy under Contract No. W-7405-ENG-48.

References and Footnotes

(1) (a) Reese, C. B. Tetrahedron 1978, 34, 3143. (b) Amarnath, V.; Broom, A. D. Chem. Rev. 1977, 77, 183. (c) Zhdanov, R. I.; Zhenodarova, S. M. Synthesis 1975, 222.

(2) Koster, H.; Kulikowski, K.; Liese, T.; Heikens, W.; Kohli, V. Tetrahedron 1981, 37, 363.

(3) Boissonnas, R. A. Adv. Org. Chem. 1963, 3, 159.

(4) Reitz, G.; Pfleiderer, W. Chem. Ber. 1975, 108, 2878.

(5) Jones, S. S.; Reese, C. B.; Sibanda, S.; Ubasawa, A. Tetrahedron Lett. 1981, 22, 4755.

(6) Resse, C. B.; Ubasawa, A. Tetrahedron Lett. 1980, 21, 2265.

(7) Robins, M. J.; Robins, R. K. J. Org. Chem. 1969, 34, 2160.

(8) Watkins, B. E.; Rapoport, H. In preparation.

(9) Letsinger, R. L.; Lunsford, W. B. J. Am. Chem. Soc. 1976, 98, 3655.

(10) Stawinski, J.; Hozumi, T.; Narang, S. A.; Bahl, C. P.; Wu, R. Nucleic Acid Res. 1977, 4, 353.

(11) (a) Matteucci, M. D.; Caruthers, M. H. Tetrahedron Lett. 1980, 21, 3243.
(b) Matteucci, M. D.; Caruthers, M. H. J. Am. Chem. Soc. 1981, 103, 3185.

(12) Kohli, V.; Blocker, H.; Koster, H. Tetrahedron Lett. 1980, 21, 2683.

(13) Chattopadhyaya, J. B.; Reese, C. B. Tetrahedron Lett. 1979, 5059.

(14) (a) Reese, C. B.; Titmas, R. C.; Yau, L. Tetrahedron Lett. 1978, 2727.
(b) Chattopadhyaya, J. B.; Reese, C. B. Nucleic Acid Res. 1980, 8, 2039.

(15) Balgobin, N.; Josephson, S.; Chattopadhyaya, J. B. Acta Chem. Scand. B. 1981, 35, 201.

(16) de Rooij, J. F. Ml; Wille-Hazeleger, G.; van Deursen, P. H.; Serdijn, J.; van Boom, J. H. Recl. Trav. Chim. Pays-Bas 1979, 98, 537.

(17) Daub, G. W.; van Tamelen, E. E. J. Am. Chem. Soc. 1977, 99, 3526.

(18) Smith, D. J. H.; Ogilvie, K. K.; Gillen, M. F. Tetrahedron Lett. 1980, 21, 861.

(19) Thuong, N. T.; Chassignol, M. Tetrahedron Lett. 1980, 21, 2063.

(20) Sood, A. K.; Narang, S. A. Nucleic Acids Res. 1977, 4, 2757.

(21) (a) Christensen, L. F.; Broom, A. D. J. Org. Chem. 1972, 37, 3393. (b) Kikugawa, K.; Sato, F.; Tsuruo, T.; Imura, N.; Ukita, T. Chem. Pharm. Bull. 1968, 16, 1110.

(22) (a) Anantharamaiah, G. M.; Sivanandaiah, K. M. J. Chem. Soc. Perkin Trans I 1977, 5, 490. (b) Felix, A. M.; Heimer, E. P.; Lambros, T. J.; Tzougraki, C.; Meienhofer, J. J. Org. Chem. 1978, 43, 4194.

(23) Cohn, W. E.; Doherty, D. G. J. Am. Chem. Soc. 1956, 78, 2863.

(24) Zenftman, H.; McGillivray, R. British Patent 651,656 (to Imp. Chem. Ind.), Apr. 4, 1951.

(25) Meerwein, H. Org. Syn. 1973, Coll. Vol. 5, 1080.

(26) Schaller, H.; Weimann, G.; Lerch, B.; Khorana, H. G. J. Am. Chem. Soc. 1963, 85, 3821.

(27) Kondo, Y.; Witkop, B. J. Am. Chem. Soc. 1968, 90, 764.

(28) Cushley, R. J.; Watanabe, K. A.; Fox, J. J. J. Am. Chem. Soc. 1967, 89, 394.

(29) Tilles, H. U.S. Patent 3,299,114 (to Stauffer Chem. Co.), Oct. 4, 1966.

(30) Babad, E.; Ben-Ishi, D. J. Heterocyclic Chem. 1969, 6, 235.

(31) Ogilvie, K. K. Can. J. Chem. 1973, 51, 3799.

(32) Katagiri, N.; Itakura, K.; Narang, S. A. J. Am. Chem. Soc. 1975, 97, 7332.

(33) Hassner, A.; Strand, G.; Rubinstein, M.; Patchornik, A. J. Am. Chem. Soc. 1975, 97, 1614.

(34) Arentzen, R.; Reese, C. B. J. Chem. Soc. Perkin Trans. 1, 1977, 445.

Table I. Synthesis of Nucleotide Dimers from 3'-Hydroxyl Nucleosides.

diester (mmol)	nucleoside (mmol)	coupling agent (mol %)	3'→5' dimer % yield (R_t)[a]	3'→3' dimer % yield (R_t)	other products, % yield
19 (1.0)	5 (1.2)	MS-NT (650)	53 (9.4,12.6)[b]	<2 (7.6,8.0)[b]	28, 11 5, 44
19 (1.0)	5 (1.2)	TPS-NT (650)	58	<2	28, 3 31, 16
19 (1.0)	5 (1.2)	TPS-NT (150)	63	0	5, 35 31, 6
18 (.88)	8 (1.1)	TPS-NT (200)	73 (16.8)	7 (12.0)[c]	8, 15

[a] HPLC retention times, R_t, in minutes.

[b] Column B, solvent system 1c.

[c] Column B, solvent system 1a.

Table II. Synthesis of Dimers and Tetramers from 3'-Blocked Nucleosides.

Phosphate Coupling Reactions						Detritylation[a]		
3'-phosphodiester (mmol)	5'-hydroxyl component (mmol)	TPS-NT (mmol)	product	% yield	retention time[b]	product	% yield	retention time
16a (3.5)	20b (3.3)	5.4	31	80	8.4 (B,1b)	--	--	--
16c (1.3)	20d (1.1)	2.6	32	82	17.6, 18.8 (A,1a)	36	87	23.0, 26.8 (A,1c)
16c (2.3)	22 (1.9)	3.0	33	78	9.4 (B,1a)	35[c]	84	15.4, 16.2 (A,1c)
						35[d]	60	
						35[e]	79	
34 (0.40)	35 (0.35)	0.70	37	66	12.6, 13.8 (B,1a)	39	50	5.2 (B,1a)
34 (0.25)	36 (.19)	0.65	38	72	13.2, 14.4 (B,1a)	40	50	5.4 (B,1a)

[a] Procedure A, unless otherwise noted. [b] Retention time in minutes. Conditions, column followed by solvent, are in parenthesis. More than one retention time indicates resolution of phosphorus diastereomers. [c] 12% depurination. [d] Using procedure B, 35% purination. [e] Using procedure C, 15% depurination.

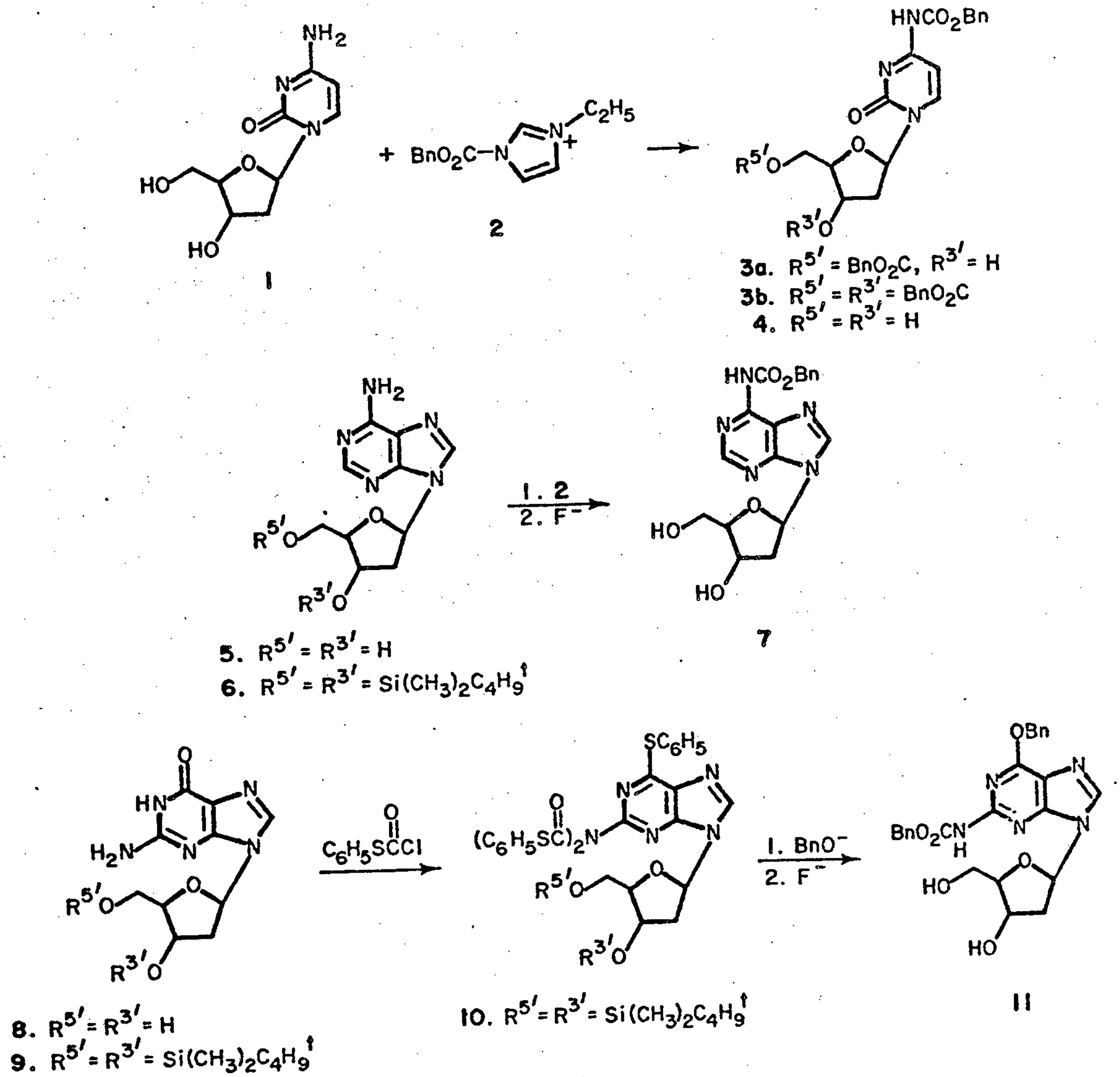
Table III. Time for Completion for Transfer Hydrogenolysis with Various Catalysts.

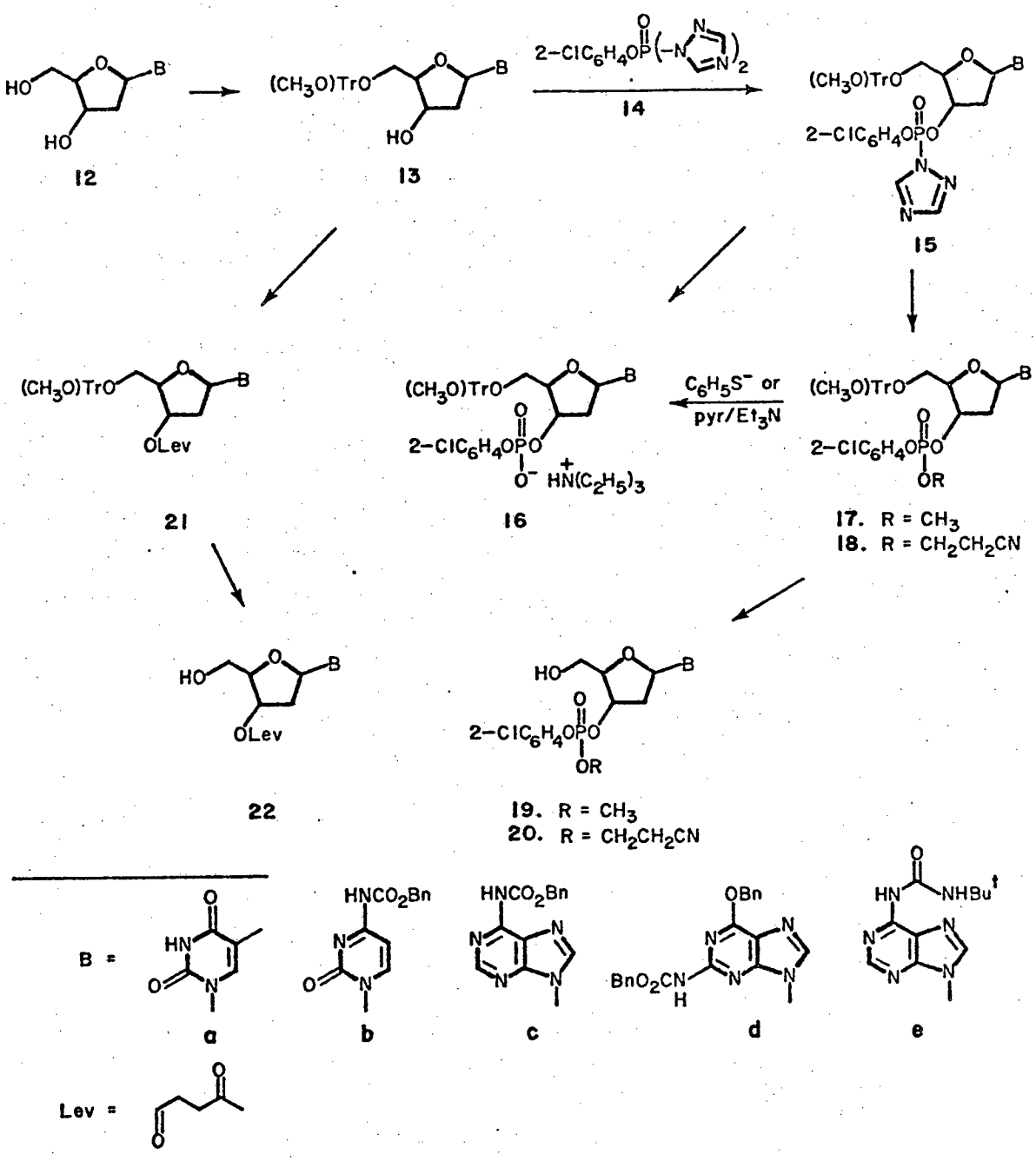
substrate	catalyst		
	10% Pd/C	$Pd(OH)_2/C$	Pd Black
$dG^{6\text{-}O\text{-}Bn,\ 2\text{-}N\text{-}Cbz}$ (12d)	2 hrs	1.5 hrs	<30 min
$5'\text{-}(CH_3O)TrdG^{6\text{-}O\text{-}Bn,\ 2\text{-}N\text{-}Cbz}\text{-}3'\text{-}OH$ (13d)	6 hrs	8 hrs	30 min
$5'\text{-}(CH_3O)TrA^{6\text{-}N\text{-}Cbz}{}_{pG}{}^{6\text{-}O\text{-}Bn,\ 2\text{-}N\text{-}Cbz}\text{-}3'\text{-}pOCH_2CH_2CN$ (32)	~3 days	~3 days	12 hrs
$5'\text{-}(CH_3O)TrTpC^{4\text{-}N\text{-}Cbz}{}_{pA}{}^{6\text{-}N\text{-}Cbz}{}_{pG}{}^{6\text{-}O\text{-}Bn,\ 2\text{-}N\text{-}Cbz}\text{-}3'\text{-}OH$ (42)	very slow	>4 days	24 hrs

Scheme I. Preparation of Base-Blocked Deoxyribonucleosides

Scheme II. Preparation of Blocked Monodeoxyribonucleotides

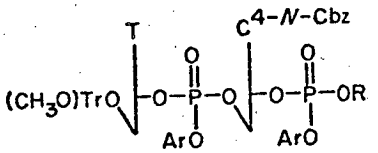
Scheme III. Preparation of Oligodeoxyribonucleotides


NH2
HO
HO
1
+ BnO2C—N
N+
C2H5
2
HNCO2Bn
R5'O
R3'O
3a. R5' = BnO2C, R3' = H
3b. R5' = R3' = BnO2C
4. R5' = R3' = H
NH2
5. R5' = R3' = H
6. R5' = R3' = Si(CH3)2C4H9t
1. 2
2. F−
7
8. R5' = R3' = H
9. R5' = R3' = Si(CH3)2C4H9t
C6H5SCCl
SC6H5
(C6H5SC)2N
10. R5' = R3' = Si(CH3)2C4H9t
1. BnO−
2. F−
OBn
BnO2CN
H
11


12
13
14
15
21
16
17. R = CH3
18. R = CH2CH2CN
22
19. R = CH3
20. R = CH2CH2CN
B =
a
b
c
d
e
Lev =

Wilkinson-Stage I -Tip

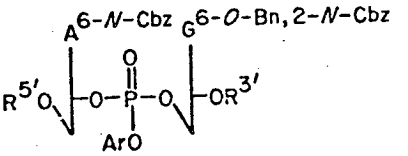
16a + 20b



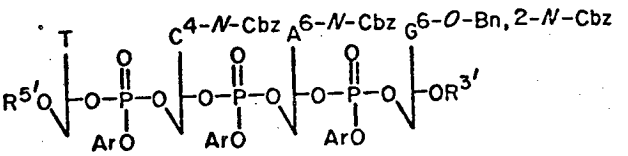
31. R = CH_2CH_2CN
34. R = H

Ar = 2-ClC_6H_4

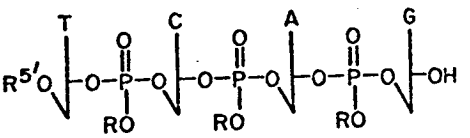
16c + 20d,22



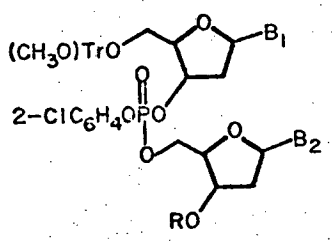
32. $R^{5'}$ = $(CH_3O)Tr$, $R^{3'}$ = $OPOCH_2CH_2CN$ (OAr)
33. $R^{5'}$ = $(CH_3O)Tr$, $R^{3'}$ = Lev
35. $R^{5'}$ = H, $R^{3'}$ = Lev
36. $R^{5'}$ = H, $R^{3'}$ = $OPOCH_2CH_2CN$ (OAr)



37. $R^{5'}$ = $(CH_3O)Tr$, $R^{3'}$ = Lev
38. $R^{5'}$ = $(CH_3O)Tr$, $R^{3'}$ = $-POCH_2CH_2CN$ (OAr)
39. $R^{5'}$ = H, $R^{3'}$ = Lev
40. $R^{5'}$ = H, $R^{3'}$ = $-POCH_2CH_2CN$ (OAr)
41. $R^{5'}$ = $(CH_3O)Tr$, $R^{3'}$ = $-POH$ (OAr)
42. $R^{5'}$ = $(CH_3O)Tr$, $R^{3'}$ = H



43. $R^{5'}$ = $(CH_3O)Tr$
R = 2-ClC_6H_4
44. $R^{5'}$ = R = H

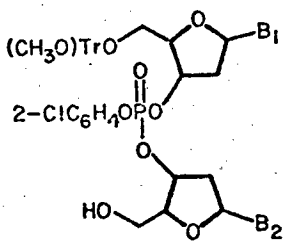


23. R = H

29. R = 2-ClC$_6$H$_4$OP(O)OCH$_3$

B_1 = T

B_2 = C$^{4-N-Cbz}$



25

---

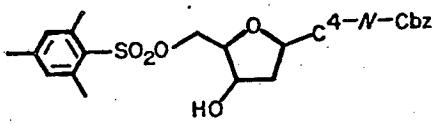
24. R = H

30. R = 2-ClC$_6$H$_4$OP(O)OCH$_3$

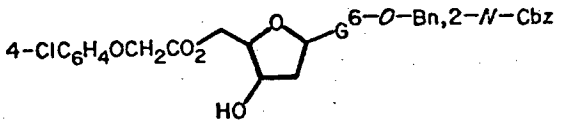
B_1 = A$^{6-N-Cbz}$

B_2 = G$^{6-O-Bn,2-N-Cbz}$

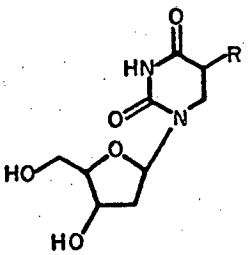
26



27



28d



45. R = CH$_3$

46. R = H

This report was done with support from the Department of Energy. Any conclusions or opinions expressed in this report represent solely those of the author(s) and not necessarily those of The Regents of the University of California, the Lawrence Berkeley Laboratory or the Department of Energy.

Reference to a company or product name does not imply approval or recommendation of the product by the University of California or the U.S. Department of Energy to the exclusion of others that may be suitable.

TECHNICAL INFORMATION DEPARTMENT
LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720