favored in chloroethane but the σ/n type being favored in 1-chloropropane. These results reflect the decreased electronegativity of chlorine relative to fluorine (yielding a more competitive n state) and the alkyl stabilization order discussed previously.

The alcohols are perhaps the most interesting class of those studied. The MNDO calculations find both n and σ/n (long bond) minima for ethanol, the latter being slightly favored (Table IV), but only a σ/n minimum for 1-propanol. Fully optimized ab initio calculations on the ethanol cation radical employing an STO-3G basis set locate only an n-type minimum, but with a 4-31G basis set both minima are found, the n type still being favored. The same results are obtained with a 6-31G** basis set, but the energy difference is reduced to only 2 kcal. The ab initio results for the cation radical of 1-propanol are entirely analogous (Table IV) but the σ/n (long bond) cation radical emerges as the ground state in the 6-31G* calculation, as expected from the alkyl stabilization effect. With use of the CC bond as the reaction coordinate, ab initio calculations were also used to probe for the existence of a σ/n minimum in acetaldehyde, but again none was found. The excellent correspondence between the MNDO and extended basis set ab initio calculations for alcohols and carbonyl compounds justifies considerable confidence in these results and further enhances the credibility of MNDO, especially in its cation radical applications. Apparently, long-bond minima do indeed exist generally in alcohol cation radicals and, in most cases, probably represent the ground-state structure, at least in the gas phase. Although they have not yet been explicitly investigated, the cation radicals of ethers should presumably be subject to the same generalization. Esters appear (Table IV) to have n-type minima corresponding to carbonyl ionization accompanied by considerably less stable σ/n-type minima corresponding to stretching the appropriate CC bond in the ether fragment of the ester.

Acknowledgment. We thank the Welch Foundation (F-149) for research support.

Registry No. Ethane radical cation, 34488-65-8; propane radical cation, 34479-70-4; butane radical cation, 34479-72-6; isobutane radical cation, 34479-71-5; cyclobutane radical cation, 34474-99-2.

Synthesis of Oligodeoxyribonucleotides Using *N*-Benzyloxycarbonyl-Blocked Nucleosides

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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 has been successfully deblocked by using transfer hydrogenation. The deblocking conditions result in no reduction of the pyrimidine bases.

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 reevaluated.

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 reexamined[2] with emphasis upon developing groups that 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 that 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 that 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 olignonucleotide 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 that 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 oligonucleotide

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

diester (mmol)	nucleoside (mmol)	coupling agent (mol %)	3'→5' dimer, % yield (t_R)[a]	3'→3' dimer, % yield (t_R)	other products, % yield
16a (1.0)	12b (1.2)	MS-NT (650)	23, 53 (9.4, 12.6)[b]	25, <2 (7.6, 8.0)[b]	27, 11; 12b, 44
16a (1.0)	12b (1.2)	TPS-NT (650)	23, 58	25, <2	12b, 53
16a (1.0)	12b (1.2)	TPS-NT (150)	23, 63	25, 0	12b, 35
16c (0.88)	12d (1.1)	TPS-NT (200)	24, 73 (16.8)	26, 7 (12.0)[c]	12d, 15

[a] HPLC retention times, t_R, in minutes. [b] Column B, solvent system 1c. [c] Column B, solvent system 1a.

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] that minimize reaction at the 6-oxygen of guanosine; however, recent reports indicate that phosphate coupling reagents do react with 2-*N*-acylguanosines 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.

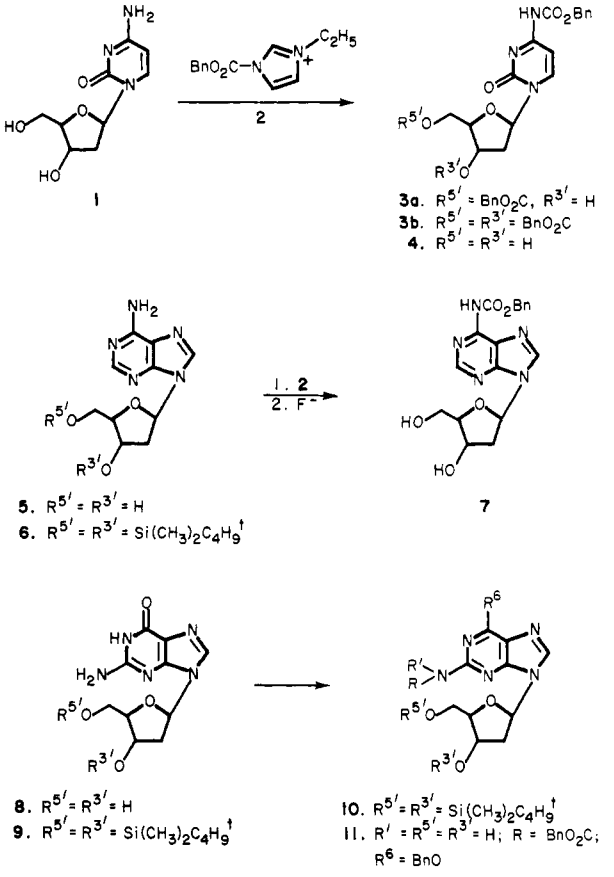
What is clearly needed is a blocking group for the guanosine 6-O position. Acyl groups are far too labile,[2] so we propose an ether-type blocking group, specifically a benzyl ether, for this role. Benzyl ethers have been used to protect the 6-O position of guanosine previously,[7] although not in oligonucleotide 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 since the use of benzyl chloroformate under all the usual conditions was unsatisfactory for the preparation of *N*-benzyloxycarbonyl nucelosides.[8] 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 nucleoside carbamates along with more details and extensions of the current methodology is being presented elsewhere.[8]

2'-Deoxycytidine (**1**) reacts with 1-((benzyloxy)carbonyl)-3-ethylimidazolium tetrafluoroborate (**2**) to give a mixture of polyacylated nucleosides. Treatment of this mixture with dilute sodium hydroxide gives 4-*N*-Cbz-2'-deoxycytidine (**4**) in 98% 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 ether cytidine or adenosine derivatives that 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.

Scheme I. Preparation of Base-Blocked Deoxyribonucleosides



Preparation of Blocked Oligonucleotides. The base-blocked deoxynucleosides **12b–d** were then incorporated into an oligonucleotide synthetic scheme by using the popular phosphotriester approach.[1] In this strategy nucleoside 3'-phosphodiesters are coupled with the 5'-hydroxyl of another oligomer. 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 was undertaken. 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[9] or Lewis[10,11] 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–d**) were prepared according to literature methods in 80–90% yield.

The nucleoside-3'-phosphodiesters **16a,c** were readily prepared as reported.[12] Thus **12a** and **12c** were phosphorylated with the bis(triazolide) **14**, and the intermediate triazolide **15** hydrolyzed

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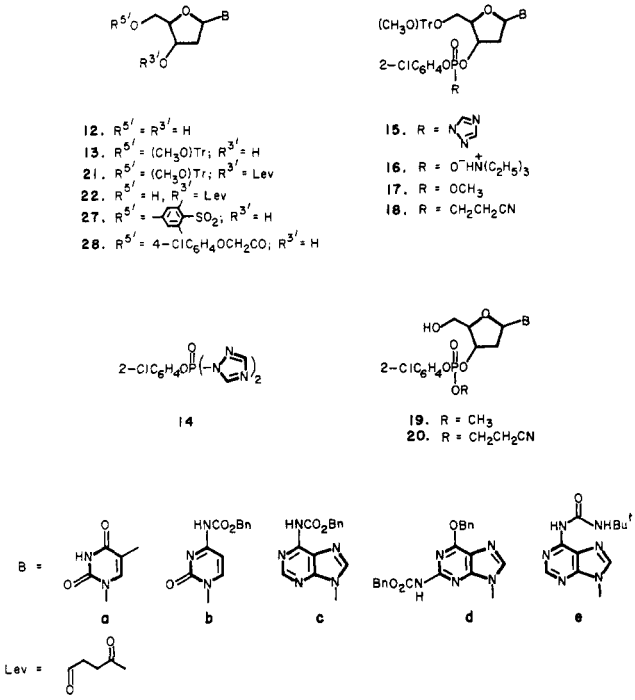
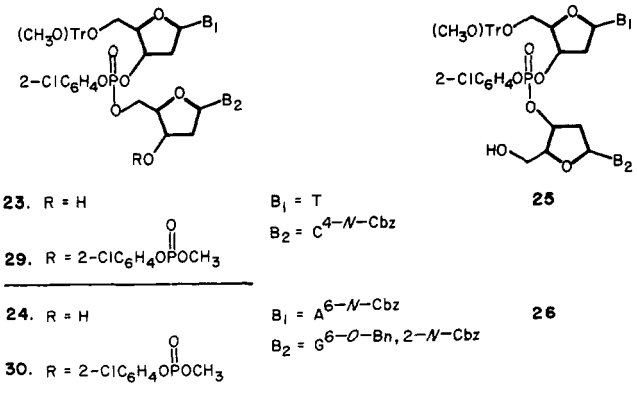
with water and triethylamine to give the phosphodiesters **16** in >95% yield with no detectible 3′ → 3′ coupled products. The 2-chlorophenyl group was chosen as the internucleotide phosphate blocker because it is reported to be removed via an oximate anion[13] with a minimum of internucleotide bond 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. Recent reports[12,14] describe the synthesis of nucleotide dimers via the coupling of a nucleotide phosphodiester with an N-blocked 2′-deoxyribonucleoside. We first examined the coupling between the phosphodiesters **16a** and **16d** and the N-blocked nucleosides **12c** and **12d**. These results are summarized in Table I, and we

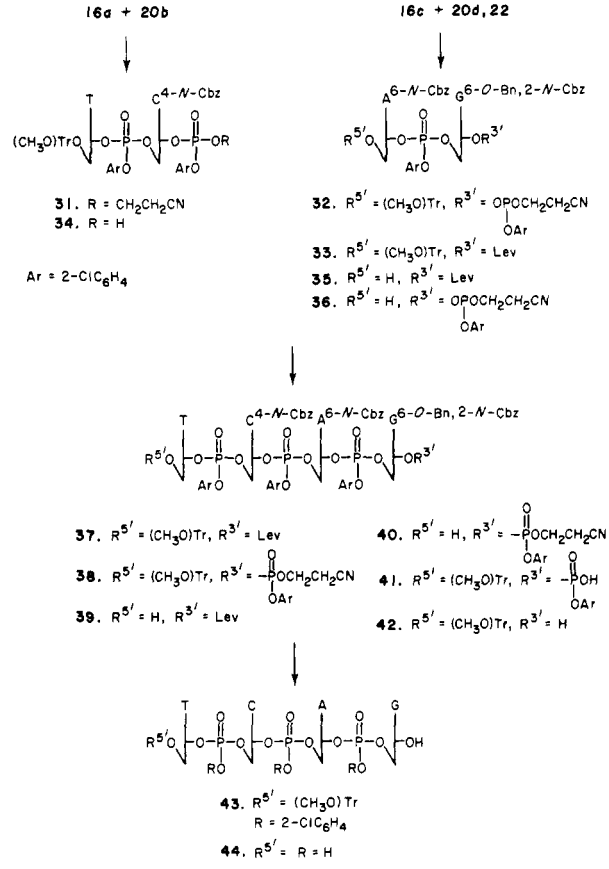


concluded that this methodology for producing nucleotide dimers was not satisfactory for the following reasons: (a) when MS-NT is used as the coupling agent, loss of starting material by 5′-O-sulfonation was too large; and (b) not each set of 3′→5′ and 3′→3′ nucleoside dimers were conveniently separable. 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**.

Scheme II. Preparation of Oligodeoxyribonucleotides



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 β-cyanoethyl group has been shown[9] to be effective for blocking a 3′ terminal phosphate, and this methodology was adopted for the synthesis of tetramers **37** and **38** (Scheme II). Phosphotriesters **18b,d** were prepared in 80% and 78% yields, via the intermediate triazolides **15b,d**, respectively. These were detritylated with 2% benzenesulfonic acid to give the 3′-blocked nucleotides **20b** and **20d**, which were coupled with the phosphodiesters **16a** and **16c**, respectively, in the presence of TPS-NT[15] 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).[15]

The use of the methyl group[16,17] was also investigated, and the monomers **17a-d** and **19b,d** and the dimers **29** and **30** were prepared analogously. The methyl group was found to be unstable to the phosphorylation conditions used or to storage at −78 °C and gave side products (e.g., **16e**) upon its removal. For these reasons, we concluded that the use of methyl as a phosphate blocking group in oligonucleotide syntheses is inadvisable, although there are mixed observations from others.[11b,16-18]

Removal of the methoxytrityl from the purine dimers **31** and **32** was investigated in some detail in an effort to prevent depurination that occurs during acid treatment of oligonucleotides.[9,10] Recent reports describe improved removal of trityl groups with

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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** (0.19)	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 parentheses. 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

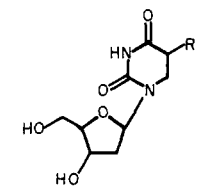
substrate	catalyst		
	10% Pd/C	$Pd(OH)_2/C$	Pd black
$dG^{6\text{-}O\text{-}Bn,2\text{-}N\text{-}Cbz}$ (**12d**)	2 h	1.5	<30 min
5′-$(CH_3O)TrdG^{6\text{-}O\text{-}Bn,2\text{-}N\text{-}Cbz}$-3′-OH (**13d**)	6 h	8 h	30 min
5′-$(CH_3O)TrA^{6\text{-}N\text{-}Cbz}pG^{6\text{-}O\text{-}Bn,2\text{-}N\text{-}Cbz}$-3′-p$OCH_2CH_2CN$ (**32**)	~3 days	~3 days	12 h
5′-$(CH_3O)TrTpC^{4\text{-}N\text{-}Cbz}pA^{6\text{-}N\text{-}Cbz}pG^{6\text{-}O\text{-}Bn,2\text{-}N\text{-}Cbz}$-3′-OH (**42**)	very slow	>4 days	24 h

$ZnBr_2$ in methylene chloride[11] or nitromethane.[10] When comparing these methods in our systems to the older 2% benzenesulfonic acid method,[9] 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[19] 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 that 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–d** can be readily deblocked by 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[20] 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 overreduction of the pyrimidine bases, we turned to transfer hydrogenolysis.[21] Using cyclohexadiene as the hydrogen source and 10% palladium on carbon as the catalyst, we removed both *O*-benzyl and *N*-Cbz groups cleanly from **12b–d** in 30–90 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 h to give **43**. Again, no reduction of the pyrimidine bases was observed. The latter was demonstrated by showing that



45. R = CH_3
46. R = H

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[22] if 2′-deoxy-5,6-dihydrocytidine had been formed.

Complete Deblocking of Oligonucleotides. The phosphate blocking groups were removed as described[13] by 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 80% 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,[15] showing that only the 3′→5′-phosphate linkage was present and that the tetramer has the expected composition.

Conclusions

The *N*-Cbz-blocked deoxynucleosides **12b** and **12c** have been synthesized by the use of the 1-(benzyloxycarbonyl)-3-ethylimidazolium ion. 2′-Deoxyguanosine has been blocked as its 6-*O*-benzyl ether, 2-*N*-Cbz carbamate. The functionalization of the guanosine 6 position was accomplished via a 6-thioether, which

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was prepared from 2′-deoxyguanosine and phenyl chlorothioformate. These base-blocked nucleosides (**12b–d**) then have been incorporated into an efficient oligonucleotide synthesis. They were found to be stable to phosphorylation and to conditions used to remove other blocking groups. The benzyl ether and *N*-Cbz groups may be removed quantitatively when desired from oligonucleotides by transfer hydrogenolysis without any detectable side reactions.

Experimental Section

Melting points were obtained with Buchi (capillary) and Kofler (microscope slide) apparatus 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 δ downfield from Me_4Si. Coupling constants are given in hertz. 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 obtained 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 280 nm unless otherwise stated. Preparative medium-pressure liquid chromatography (MPLC) was done by using an Altex 110A pump equipped with a preparative liquid head and an Altex 151 UV detector set at 254 or 280 nm. The following Altex stainless steel columns were used: (A) 4.6 × 250 mm, 5-μm Ultrasphere ODS; (B) 3.2 × 250 mm, 5-μm LiChrosorb C-18; (C) 10 × 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 gradient, 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 × 130 mm or 40 × 240 mm, 40–63-μm silica Gel 60 (EM Reagents)) were used for MPLC. Column chromatography (CC) was performed with 63–200-μm Silica Gel 60 (EM Reagents). Analytical thin-layer chromatography (TLC) was done with aluminum-backed silica plates (Merck). Preparative TLC was carried out on 2000-μm thick Silica Gel GF (Analtech).

Unless otherwise noted, reactions were conducted under a nitrogen atomosphere with magnetic stirring at room temperature (20–26 °C). 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 3-Å 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 concentrated aqueous HF and rendered anhydrous by repeated addition and evaporation of pyridine. 2-Chlorophenyl phosphodichloridate,[23] ((2,4,6-triisopropylphenyl)sulfonyl)nitrotriazole,[15] ((2,4,6-trimethylphenyl)sulfonyl)nitrotriazole,[13] triethyloxonium tetrafluoroborate,[24] 5′-*O*-(monomethoxytrityl)thymidine,[25] 5,6-dihydrothymidine,[26] 2′-deoxydihydrouridine,[27] and phenyl chlorothioformate[28] were prepared as described.

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

***N*-(Benzyloxycarbonyl)imidazole.** 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 crystallized from petroleum ether to yield 230 g, 78%, of Cbz-imidazole: mp 37.0–38.5 °C (lit.[29] oil); ^1H NMR δ 5.3 (s, 2 H), 7.0 (m, 1 H), 7.3 (m, 6 H), 8.0 (m, 1 H). 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 and then stirred for 2 h. This solution was used to acylate the deoxyribonucleosides. Evaporation of a portion left **2**: ^1H NMR δ 1.47 (t, 3 H, J = 7), 4.20 (q, 2 H, J = 7), 5.42 (s, 2 H), 7.3 (m, 6 H), 7.65 (m, 1 H), 8.98 (m, 1 H).

5′-*O*,4-*N*-Bis(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 monoacylated cytidines. The bis(carbobenzoxy) material **3a** was isolated by chromatography on silica gel (5/95 ethanol/$CHCl_3$). Recrystallization from methanol gave **3a** as needles: mp 89–90 °C; IR 1740 cm^{-1}; ^1H NMR δ 5.05 (s, 2 H), 5.15 (s, 2 H), 6.15 (t, 1 H, J = 6), 7.85 (d, 1 H, J = 7); UV λ_{max} (nm (ϵ)) 242 (14 000), 294 (7000). 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 acylcytidines was treated with sodium hydroxide as described[25] for O-deacylations, the Dowex 10N resin was washed with $CHCl_3$, and the filtrate was extracted with 4 × 100 mL of $CHCl_3$/isopropyl alcohol (75/25). Combining the organic extracts and evaporating left a residue that 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, 2 H), 6.05 (t, 1 H, J = 6), 8.25 (d, 1 H, J = 7); UV λ_{max} (nm (ϵ)) 242 (15 400), 294 (7700). 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), *tert*-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 × 300 mL), and the $CHCl_3$ was evaporated to give a 1/1 mixture of **6** and a trissilylated adenosine. The residue was taken up in 200 mL of 80% aqueous acetic acid in order to convert the tris(silylated) material to **6**. 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 that was column chromatographed (Et_2O) to yield bis(silyl) ether **6** (18.9 g, 92%): mp 128–130 °C (lit.[30] mp 132.5–133 °C); ^1H NMR δ 0.10 (s, 6 H), 0.13 (s, 6 H), 0.97 (s, 18 H), 6.37 (t, 1 H, J = 6), 8.05 (s, 1 H), 8.25 (s, 1 H).

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[30] in THF and evaporating the THF, followed by column chromatography (7/93 EtOH/$CHCl_3$), 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, 2 H), 6.30 (t, 1 H, J = 7), 7.3 (s, 5 H), 8.13 (s, 1 H), 8.47 (s, 1 H); 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*-(Benzyloxycarbonyl)adenine. 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, 2 H), 7.2 (s, 5 H), 8.15 (s, 1 H), 8.40 (s, 1 H); 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.

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6-(Phenylthio)-2-*N*-(phenylthiocarbonyl)-3′-5′-*O*-bis(*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 1 week at room temperature and 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 was 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, 6 H), 0.12 (s, 6 H), 0.93 (s, 18 H), 6.33 (t, 1 H, J = 6), 7.3 (s, 15 h), 8.30 (s, 1 H); 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; N, 8.1; S, 11.2. Found: C, 58.3; H, 6.2; N, 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 °C) left a residue that was treated with tetrabutylammonium fluoride in THF and column chromatographed (7/93 EtOH/$CHCl_3$) to give **11** in 95% yield: IR 1750 cm^{-1}; ^1H NMR δ 5.18 (s, 2 H), 5.53 (s, 2 H), 6.30 (t, 1 H, J = 6), 7.3 (s, 10 H), 8.00 (s, 1 H); 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*-(Methoxytrityl)nucleosides (13b–d). The general procedure[25] for tritylation of acylated nucleosides was employed to prepare these nucleosides that 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, 3 H), 5.1 (s, 2 H), 6.15 (t, 1 H, J = 6), 8.05 (d, 1 H, J = 6); UV λ_{max} (nm (ϵ)) 235 (24 900), 295 (7600). 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, 3 H), 5.2 (s, 2 H), 6.4 (t, 1 H, J = 6), 8.1 (s, 1 H), 8.7 (s, 1 H); 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; N, 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, 2 H), 5.48 (s, 2 H), 6.43 (t, 1 H, J = 7), 7.83 (s, 1 H); 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; H, 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 **16c**. 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*-(Methoxytrityl)thymidine 3′-*O*-(2-Chlorophenyl phosphate) Triethylammonium Salt (16a): ^1H NMR δ 1.2–1.4 (m, 12 H), 3.1–2.9 (m, 6 H), 3.8 (s, 3 H), 6.5 (m, 1 H), 7.4–6.8 (m, 18 H).

5-*O*-(Methoxytrityl)-6-*N*-(benzyloxycarbonyl)-2′-deoxyadenosine 3′-*O*-(2-Chlorophenyl phosphate) Triethylammonium Salt (16c): ^1H NMR δ 1.26 (t, 9 H, J = 7), 2.98 (q, 6 H, J = 7), 3.75 (s, 3 H), 5.28 (s, 2 H), 6.51 (t, 1 H, J = 6.5), 8.02 (s, 1 H), 8.65 (s, 1 H).

Preparation of Fully Blocked Nucleotides (17a,c,d; 18b,d). Intermediates **17** and **18a–d** were generated from 2-(chlorophenyl)phosphodichloridate (250 mol %), triazole (500 mol %), and triethylamine (500 mol %) in THF according to a reported procedure[31] from **13a–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, 3 H), 3.7 (s, 3 H), 3.8 (2d, 3 H, J = 12), 6.3 (m, 1 H); 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, 3 H), 3.75 (d, 3 H, J = 12), 5.2 (s, 2 H), 6.3 (m, 1 H), 7.9 (s, 1 H), 8.5 (s, 1 H); 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, 3 H), 3.85 (d, 3 H, J = 11), 5.18 (s, 2 H), 5.57 (s, 2 H), 6.33 (t, 1 H, J = 7), 7.83 (s, 1 H); 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, 2 H), 3.80 (s, 3 H), 4.3–4.5 (m, 3 H), 5.25 (s, 2 H), 6.35 (m, 1 H), 8.10 (d, 1 H, J = 7.5); UV λ_{max} (nm (ϵ)) 236 (22 000), 294 (7000). Anal. Calcd for $C_{46}H_{42}ClN_4O_{10}PH_2O$: C, 61.7; H, 5.0; N, 6.3. Found: C, 61.7; H, 4.9; N, 6.5.

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, 2 H), 3.74 (s, 3 H), 4.4 (s, 2 H), 5.20 (s, 2 H), 5.60 (s, 2 H), 6.4 (m, 1 H), 8.8 (s, 1 H); 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.[9,19] 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 anhydrous $ZnBr_2$ 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; ^1H NMR δ 2.8 (t, 2 H, J = 6), 5.20 (s, 2 H), 6.20 (m, 1 H), 8.13 (d, 1 H, J = 7); UV λ_{max} (nm (ϵ)) 242 (16 100), 294 (7500). 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; ^1H NMR δ 3.92 (d, 3 H, J = 11), 5.18 (s, 2 H), 5.51 (s, 2 H), 6.23 (t, ^1H, J = 7), 7.90 (s, 1 H); 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; ^1H NMR δ 2.8 (t, 2 H, J = 7), 5.24 (s, 2 H), 2.61 (s, 2 H), 6.3 (m, 1 H), 7.82 (s, 0.5 H), 7.85 (s, 0.5 H); 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[19] 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,[32] and the mixture was stirred at room temperature for 12 h. The reaction was quenched with 5% $NaHCO_3$, the solution was extracted with $CHCl_3$, the solvent was evaporated, the residue was treated with 2% benzenesulfonic acid,[19] and the crude product column chromatographed on silica gel (95/5 $CHCl_3$/EtOH) to give **22** in 79% yield as a glass: ^1H NMR δ 2.15 (s, 3 H), 2.6 (m, 4 H), 5.17 (s, 2H), 5.50 (s, 2 H), 6.2 (dd, 1 H), 7.85 (s, 1 H); UV λ_{max} (nm (ϵ)) 257 (13 100), 268 (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 of CH_3CN and 5 mL of pyridine at room temperature was added over 2 h via syringe (4-(chlorophenoxyacetyl) chloride[33] (250

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mg, 1.2 mmol) in 5 mL of CH_3CN, and the reaction mixture was stirred for 2 h at room temperature and 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 (97/3 $CHCl_3$/EtOH) to give 0.56 g, 84% yield, of **28**: ^1H NMR δ 4.50 (s, 2 H), 5.13 (s, 2 H), 5.47 (s, 2 H), 6.38 (t, 1 H, $J = 7$), 7.87 (s, 1 H); 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.[13,15] The products were column chromatographed by 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[32] without isolating the fully protected dimer.

5'-(CH_3O)TrTpC$^{4N\text{-}Cbz}$pA$^{6\text{-}N\text{-}Cbz}$pG$^{6\text{-}O\text{-}Bn,2\text{-}NCbz}$-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 of 3/2 pyridine/HOAc. The mixture was stirred at room temperature for 5 min, cooled to 0 °C, 2,4-pentanedione (0.5 mL) added, and the solution stirred for 15 min more. The mixture was added to 50 mL of rapidly stirring ether and 65 mg of precipitated product collected. This product was contaminated with low-molecular-weight byproducts and was purified by HPLC (4/96 EtOH/$CHCl_3$) to give pure **42**, 49 mg, 0.20 mmol, 74% yield, t_R (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 × 3 cm test tube was added a solution (1/1 EtOAc/EtOH) 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 atomosphere 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) and then washed twice with water and twice with ethanol. For the hydrogenolysis of **42**, 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.[13b] 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 0.01 M 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 0.1 M NH_4OAc (pH 6.5), and one unit of spleen phosphodiesterase was added. The mixture was incubated at 37 °C for 18 h and 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 Hydrogenation Conditions. When thymidine (**12a**) was subjected to the transfer hydrogenation conditions described above for 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 **12a**. When 4-*N*-Cbz-dC was subjected to these reaction conditions for 24 h, 2'-deoxycytidine (**1a**) was isolated as the sole nucleosidic product, and no 2'-deoxydihydrouridine (**46**) was found to be present (HPLC system CA, 4a). When 2'-deoxycytidine 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.

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Registry No. 1, 951-77-9; **2**, 82892-54-4; **3a**, 82892-55-5; **3b**, 82892-74-8; **4**, 82892-56-6; **5**, 958-09-8; **6**, 51549-32-7; **7**, 82892-57-7; **8**, 961-07-9; **9**, 51549-35-0; **10**, 82892-58-8; **11**, 82892-59-9; **12a**, 50-89-5; **13b**, 82902-24-7; **13c**, 82892-60-2; **13d**, 82892-61-3; **15a**, 82892-62-4; **15c**, 82902-25-8; **16a**, 82892-63-5; **16c**, 82902-27-0; **17a**, 76512-73-7; **17c**, 82892-64-6; **17d**, 82902-28-1; **18b**, 82892-65-7; **18d**, 82902-29-2; **19d**, 82892-66-8; **20b**, 82892-67-8; **20d**, 82892-68-0; **21**, 82892-76-0; **22**, 82892-69-1; **23**, 82902-38-3; **24**, 82892-81-7; **25**, 82892-72-6; **26**, 82892-73-7; **27**, 82892-82-8; **28**, 82892-70-4; **29**, 82902-36-1; **30**, 82892-77-1; **31**, 82892-75-9; **32**, 82902-34-9; **33**, 82902-35-0; **34**, 82892-78-2; **35**, 82892-79-3; **36**, 82892-80-6; **37**, 82902-31-6; **38**, 82902-33-8; **39**, 82902-39-4; **40**, 82892-83-9; **41**, 82902-37-2; **42**, 82902-30-5; **43**, 82902-32-7; **44**, 82892-71-5; *N*-benzyloxy carbonyl imidazole, 22129-07-3; imidazole, 288-32-4; benzyl chloroformate, 501-53-1; triethyloxonium tetrafluoroborate, 368-39-8; 6-*N*-benzyloxycarbonyladenine, 82919-04-8.

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Applications of the Intramolecular Diels-Alder Reaction to the Formation of Strained Molecules. Synthesis of Bridgehead Alkenes[1]

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Abstract: The synthesis and thermolysis of a number of 2-alkenyl-1,3-butadienes are reported. In all cases studied, the dominant reaction manifold is intramolecular Diels-Alder cycloaddition, which results in formation of bicyclo[*n*.3.1] bridgehead alkenes. Electron-withdrawing substituents accelerate the reaction, thereby permitting synthesis of a variety of functionalized bridgehead olefins. These cycloadditions are found to be highly regio- and stereospecific. Kinetic studies permit evaluation of the effectiveness of various electron-withdrawing groups to accelerate the reaction, in addition to establishing the reaction's exo selectivity.

There are several ways that a diene and dienophile can be joined in the intramolecular Diels-Alder reaction. In the example illustrated in eq 1, the dienophile is joined at the 1-position of the diene (we refer to this as type 1 IDA). This variant has assumed

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