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THE INFLUENCE OF THE PURINE 2-AMINO GROUP ON DNA CONFORMATION AND STABILITY—II

SYNTHESIS AND PHYSICAL CHARACTERIZATION OF d[CGT(2-NH_2)ACG], d[CGU(2-NH_2)ACG], AND d[CGT(2-NH_2)AT(2-NH_2)ACG]

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Abstract—Synthesis of an *N*-protected derivative of 2-amino-2′-deoxyadenosine (dA′) from 2′-deoxyguanosine is reported. The syntheses of several oligodeoxynucleotides containing this modified nucleoside are described, together with physical characterization via melting studies and CD conformational analysis. As expected, the 2-amino group is seen to add to the duplex stability. Although the sequence $d(TA')_3$ was found to undergo a salt-induced conformational transition, mixed sequences such as d(CGTA′CG) did not display this behavior. All guanine residues present in these sequences were O^6 protected, either with the cyanoethyl group or the 4-nitrophenylethyl group, to eliminate guanine degradation during phosphorylation and condensation reactions. Procedures for the introduction and removal of these O^6 protecting groups are described.

Polynucleotides containing the 2-aminoadenine residue, usually homopolymers or alternating polymers, have been investigated in detail, especially in the ribonucleotide series.[1-9] However, largely due to the lack of adequate synthetic methods, little work has been done on deoxyoligonucleotides of defined sequence containing this modified base. Interest in such molecules has been stimulated by the recent finding that the DNA of cyanophage S-2L contains exclusively 2-aminoadenine in place of adenine,[10] and also by speculation about the role of the purine 2-amino group in the transition from B to Z DNA.[11]

Circular dichroism gave the first clue to the left-handed form of poly d(CG) induced by 4 M NaCl,[12] while ^1H and ^{31}P NMR,[13] and finally a single-crystal X-ray structure,[14,15] have clearly established the parameters of the Z conformation. Moreover, structural features that facilitate Z formation have been identified. For example, poly d(CG) containing 5-methylcytosine,[16] 7-methylguanine,[17] or 8-bromoguanine[18] has been shown to more readily adopt the Z form than does poly d(CG) itself. Z DNA formation also has been demonstrated using magnesium perchlorate,[19] tetramethylammonium chloride,[20] cobalthexammine,[21] and polyarginine[22] in addition to 4 M sodium chloride. Yet the minimal structural requirements of Z DNA have not been delineated. It has been suggested that a strictly alternating pyrimidine/purine sequence is one key requirement.[11] The CG-rich sequence d(CGCGAATTCGCG), for example, does not adopt a Z form,[23,24] nor does $d(AT)_3(CG)_3$,[25] but $d(TA)_3(CG)_3$ does form concatamers in which the $d(CG)_3$ portion appears to be Z.[26] A second requirement may be the purine 2-amino group, since poly d(CG), but not poly d(TA) nor poly d(CI), will undergo the B → Z transition.[11] The $d(CG)_3$ X-ray structures, moreover, show a water bridged N^2–H-phosphate H-bond which may stabilize the Z structure,[15] and the 2-amino group appears to disrupt a spine of hydration that may be important in stabilizing the B form.[27]

We have sought to introduce specific modifications into short oligonucleotides to provide insight into DNA conformational requirements through analysis of the properties of these specifically modified sequences. Thus we have shown that the alternating hexanucleoside pentaphosphate of thymidine and 2-amino-2′-deoxyadenosine [$d(TA')_3$] will undergo a conformational transition in either sodium chloride or tetramethylammonium chloride.[28] The resultant CD spectrum is similar although not identical to that seen for the Z form of $d(CG)_n$ and for poly d(AC) · poly d(GT) at high concentrations of cesium chloride, a putative Z structure.[29,30] In addition, we have shown that the alternating pyrimidine/purine molecule d(CGTACG) does not undergo a salt-induced conformational change.[31] The logical next step is to examine mixed sequences such as d(CGTA′CG), in which each purine has the 2-amino group. The synthesis and physical characterization of this and some related molecules are reported below.

The synthesis of guanine-rich oligonucleotides has long been known to proceed in somewhat reduced yield and with a higher proportion of side products than is the case with guanine-poor sequences.[32] Despite the major improvements in oligonucleotide synthesis that have taken place recently, problems with guanine residues continue to be cited.[33-37] The demonstration by Reese and by Hata that the guanine O^6 atom reacts readily with a variety of reagents, including commonly used condensing agents[38,39] and activated nucleotides,[40] has led to a realization of the necessity of O^6 protection. Thus Hata has introduced O^6-silyl, sulfonyl, phosphoryl, and phosphino thioyl derivatives[41] while Reese has prepared O^6-(2-nitrophenyl) substituted guanine nucleosides.[42] We have developed a general route, based on the work of both Reese and Hata, for 6-substitution of guanine.[43,44] Our route may be used for synthesis of 2-amino-2′-deoxyadenosine (see below), simple O^6-alkyl-2′-deoxyguanosines,[43] or for preparation of several derivatives suitable for use as O^6 protected

nucleosides.[44] Pfleiderer has recently reported application of the Mitsunobu reaction to the synthesis of one of these, the O^6-(4-nitrophenylethyl) compound.[45] We have been examining the use of the most promising of these protected derivatives in oligodeoxynucleotide synthesis: the O^6-phenylthioethyl,[31] 4-nitrophenylethyl[46] and cyanoethyl derivatives. Each of these protecting groups is a substituted ethyl group susceptible to cleavage by β-elimination at the end of the synthesis. The phenylthioethyl moiety, however, requires a prior oxidation to the corresponding sulfoxide. Alternatively, both the 4-nitrophenylethyl and the cyanoethyl groups are removed without additional deprotection steps, making them inherently more attractive. At this time we wish to report the first example of oligonucleotide synthesis using the cyanoethyl group for guanine protection as well as additional examples of use of the 4-nitrophenylethyl group, to complete our preliminary evaluation of these three O^6 protecting groups.

Derivatives of 2-amino-2′-deoxyadenosine were obtained by the synthetic routes shown in Figs. 1 and 2. Triisobutyryl deoxyguanosine (1) is sulfonylated in methylene chloride using triisopropylbenzenesulfonyl chloride and triethylamine along with a catalytic amount of 4-dimethylaminopyridine. The sulfonylated derivative **2** is obtained in high yield within 1 hr, and can be isolated if desired. It is generally more convenient to instead immediately react **2** with excess trimethylamine, at 0°, to give the trimethylammonium salt **3**. Not more than 10 min are required for the complete conversion of **2**–**3**. We have not been able to isolate **3** due to its ready demethylation. However, if used immediately, and kept cold, little degradation to the dimethylamino derivative is observed. Thus excess liquid ammonia is added to the reaction mixture immediately after the addition of trimethylamine, and the mixture is allowed to reflux until reaction is complete (TLC); this is usually about 5–8 hr. The reaction of **2** itself with liquid ammonia is much slower than is the reaction of **3**. After evaporation to remove ammonia, **4** is treated with benzoyl chloride in pyridine to give **5** which is reacted with 1 N NaOH to cleave the 3′ and 5′ isobutyryl groups leaving the 6-*N*-benzoyl-2-*N*-isobutyryl derivative **6**. Thus the overall transformation of triisobutyryl deoxyguanosine to **6** is performed without an intermediate purification step, in good overall yield.

Although **6** is immediately usable, without 3′ protection, for synthesis of a dinucleoside phosphate,[34,35,47,48] we decided instead to use the 3′-*O*-levulinyl compound **7c** for dimer preparation. Conversion of **6**–**7c** was readily effected by selective protection of the 5′ hydroxyl group via reaction with *tert*-butyldiphenylsilyl chloride, in pyridine, to give **7a**, reaction of **7a** with levulinic anhydride to give **7b**,

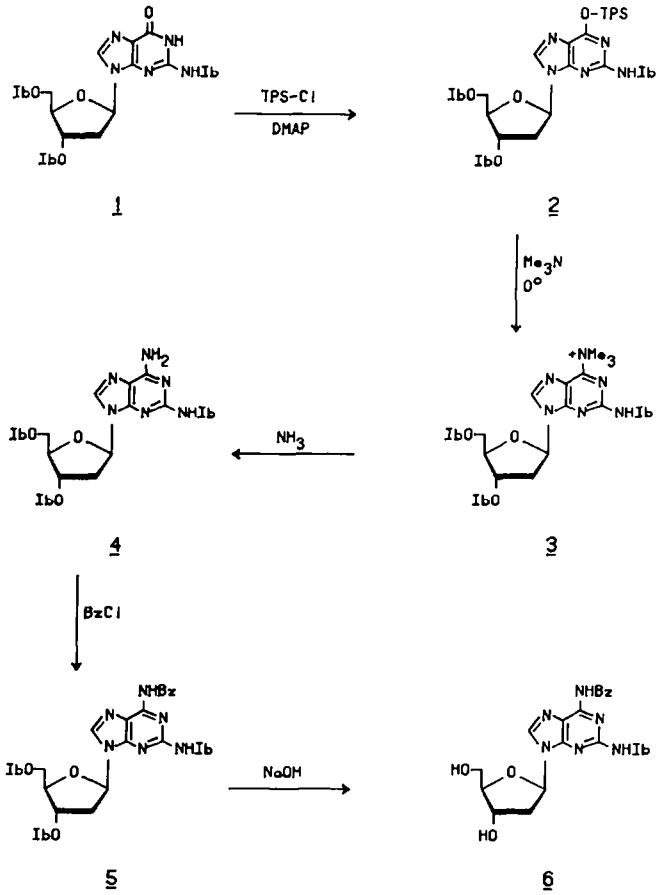
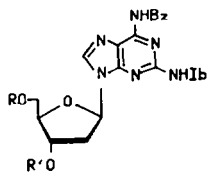
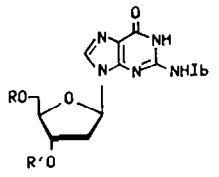


Fig. 1. Ib = isobutyryl; TPS-Cl = 2,4,6-triisopropylbenzenesulfonyl chloride; DMAP = 4-dimethylaminopyridine; BzCl = benzoyl chloride.



7

a R = BDPS; R′ = H
b R = BDPS; R′ = $COCH_2CH_2COMe$
c R = H; R′ = $COCH_2CH_2COMe$



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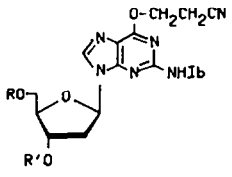
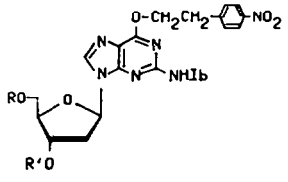
a R = R′ = BDMS
b R = DMT; R′ = $COCH_2CH_2COMe$



9

a R = R′ = BDMS
b R = R′ = H
c R = DMT; R′ = H
d R = DMT; R′ = $COCH_2CH_2CO_2H$
e R = DMT; R′ = $COCH_2CH_2COMe$
f R = H; R′ = $COCH_2CH_2COMe$



10

a R = DMT; R′ = $COCH_2CH_2COMe$
b R = DMT; R′ = H
c R = DMT; R′ = $COCH_2CH_2CO_2H$
d R = H; R′ = $COCH_2CH_2COMe$

Fig. 2. BDPS = *tert*-butyldiphenylsilyl; BDMS = *tert*-butyldimethylsilyl; DMT = 4,4′-dimethoxytrityl.

and cleavage of the silyl group with tetra-*n*-butylammonium fluoride to give **7c**. In this way we were able to ensure that our preparation of dimer would contain only correctly joined material. The silyl compound **7a** was used, rather than a dimethoxytrityl derivative, due to the extreme acid lability of the glycosidic linkage of these di-*N*-acyl derivatives of 2-amino-2′-deoxyadenosine. Use of protic acids for detritylation is entirely precluded; even with use of zinc bromide depurination is detectable after about 1 hr. During the oligonucleotide synthesis itself we of course could not use the silyl group for 5′ protection since fluoride ion treatment would cleave the triester group. But we were able to absolutely minimize the number of times that the diacylated 2-aminoadenine residues were exposed to zinc bromide to one treatment for the two hexanucleoside pentaphosphates d(CGTA′CG) and d(CGUA′CG) and to two for d(CGTA′TA′CG). Moreover, we found that the efficiency of detritylation could be greatly increased, and the time thereby reduced, by swelling the resin in plain methylene chloride between the zinc bromide treatments. In this way we were able to effect nearly complete detritylation with three 20 min reactions, despite the fact that in two cases we were using a highly functionalized Merrifield resin (1.3 meq NH_2/g) which has since been shown to retard zinc bromide catalyzed detritylation.[49] For the synthesis of d(CGUA′CG) we switched to a less functionalized resin (0.3 meq NH_2/g). It is noteworthy that the actual amount of the succinylated nucleoside initially bound is about the same, 0.1 to 0.2 meq/g, for both the 1.3 meq NH_2/g resin and the 0.3 meq NH_2/g resin. Moreover, in addition to having an improved rate of detritylation, the less functionalized resin did not become darkly colored during synthesis, as did the more highly functionalized resin.

The O^6-cyanoethyl-2′-deoxyguanosine derivatives **9a,b** were synthesized from the 3′,5′-*O*-bis(*tert*-butyldimethylsilyl) compound **8a** as we reported elsewhere.[44] The 5′-dimethoxytrityl derivatives **9c** and **9d** were prepared from **9b** by standard procedures. Alternatively, we have applied our *O*-alkylation procedure to the 5′-*O*-dimethoxytrityl-3′-*O*-levulinyl compound **8b**, to effect a one-flask synthesis of either **9e** or the corresponding O^6-(4-nitrophenylethyl) derivative **10a**.[46] Both **9e** and **10a** are appropriately protected for elongation in either the 5′ or 3′ directions after a single deprotection step. Since **8b** is readily available in high yield by standard procedures this is a very direct route to fully protected deoxyguanosine derivatives. This works particularly well for **10a**, giving a yield of *ca* 75%, although for **9e** only a 30–40% yield is generally obtained. Moreover, we have found that **10b**, obtained by delevulinylation of **10a**, may be readily crystallized from ether, making this derivative conveniently available in high purity. We began the syntheses of both the hexamer d(CGTA′CG) and the octamer d(CGTA′TA′CG) using the O^6-cyanoethyl protected deoxyguanosine derivative, but we completed the octamer using the more readily available O^6-nitrophenylethyl protected compound. The four dinucleoside phosphates required for all three sequences were prepared as shown in Table 1. Although we have found 2,4,6-triisopropylbenzenesulfonyl chloride (TPS) plus *N*-

Table 1. Formation of dimers in solution[a]

Phosphate component (mmol)	Hydroxy component (mmol)	TPS/catalyst (mmol)	Product after delevulinylation (yield)
DMT-Cp (2.5)	HO-G**-lev (1.7)	7.5/23Tet	DMT-CpG** (75)
DMT-Cp (1.8)	HO-G*-lev (1.4)	5.4/16Tet	DMT-CpG* (67)
DMT-Tp (3.4)	HO-A'-lev (2.8)	8.4/25MI	DMT-TpA' (71)
DMT-dUp (1.8)	HO-A'-lev (1.5)	5.4/16Tet	DMT-dUpA' (43)

[a]Abbreviations are: DMT=4,4'-dimethoxytrityl; lev=levulinyl; C=4-N-benzoyl-2'-deoxycytidine; T=thymidine; dU=2'-deoxyuridine; A'=6-N-benzoyl-2-N-isobutyryl-2-amino-2'-deoxyadenosine; G*=6-O-(4-nitrophenylethyl)-2-N-isobutyryl-2'-deoxyguanosine; G**=6-O-(cyanoethyl)-2-N-isobutyryl-2'-deoxyguanosine; Tet=tetrazole; MI=N-methyl imidazole. These abbreviations are based on the simplified scheme suggested by Reese.[34]

methylimidazole to be an excellent combination for condensations,[50] the methylimidazole is sometimes a problem during subsequent chromatography. Thus for solution phase condensations we often used tetrazole, rather than methylimidazole, solely for this reason. The anomalously low yield for preparation of the dimer d(DMT–Up*A'*) may be due to side reactions of the uracil base in the presence of TPS/tetrazole.[39] Both of the guanine containing dimers, however, were obtained without formation of the polar, fluorescent impurities and dark colors formerly so characteristic of condensations involving guanine nucleosides.[51]

The oligonucleotide syntheses were carried out as indicated in Table 2. We have found it most efficient to begin by attaching a succinylated monomer to the resin, rather than a dimer, since there is no alternative use for the excess succinylated material recovered after the loading step. A single preparation of **9d** bound to the resin was elongated first to the dimer d(DMT–*C*p*G***–R) and then to the tetramer d(DMT–Tp*A'*p*C*p*G***–R). At this point the resin was divided, one portion was used to prepare the hexamer d(DMT–*C*p*G***pTp*A'*p*C*p*G***–R) while the remainder was used for the octamer. The general sequence followed for elongation was: (1) Detritylation with either 2% benzenesulfonic acid or with zinc bromide. (2) Condensation with the next monomer or dimer 3'-*O*-(2-chlorophenyl)phosphate. (3) Capping with acetic anhydride. The hexamer d(DMT–*C*p*G**pUp*A'*p*C*p*G**–R) was similarly prepared from **10c** attached to a portion of resin.

The cleavage of the guanine O^6-cyanoethyl group was effected during the now standard deprotection of the triesters with 2-nitrobenzaldoxime and tetramethylguanidine.[34] For sequences with the guanine O^6-nitrophenylethyl group we used 2-nitrobenzaldoxime and DBU, under anhydrous conditions, as the first step. Water was then added to allow cleavage of the product from the resin. Thus in each case the guanine O^6 protecting groups were removed without difficulty in the first deprotection step. We have noted that, with the more highly functionalized resin (1.3 meq NH_2/g), some 10–20% of the trityl functionality is usually not cleaved in this step, while with the lower functionalized resin

Table 2. Condensation reactions on resin[a]

Phosphate component (mmol)	Hydroxy component (mmol)	TPS/MI (mmol)	Product (yield)
DMT-Cp (5.0)	HO-G**-R_1 (0.91) BSA[b]	15/45	DMT-CpG**-R_1 (96)
DMT-TpA'p (2.8)	HO-CpG**-R_1 (0.87) $ZnBr_2$[b]	8.5/26	DMT-TpA'pCpG**-R_1 (85)
DMT-CpG**p (1.3)	HO-TpA'pCpG**-R_1 (0.56) $ZnBr_2$[b]	3.8/11	DMT-CpG**pTpA'pCpG**-R_1 (64)
DMT-TpA'p (0.73)	HO-TpA'pCpG**-R_1 (0.18) $ZnBr_2$[b]	2.2/6.6	DMT-TpA'pTpA'pCpG**-R_1 (69)
DMT-CpG*p (1.2)	HO-TpA'pTpA'pCpG**-R_1 (0.12) $ZnBr_2$[b]	3.3/9.8	DMT-CpG*pTpA'pTpA'pCpG**-R_1 (83)
DMT-Cp (2.3)	HO-G*-R_2 (0.84) BSA[b]	7.0/21	DMT-CpG*-R_2 (80)
DMT-dUpA'p (0.63)	HO-CpG*-R_2 (0.32) BSA[b]	1.9/5.7	DMT-dUpA'pCpG*-R_2 (63)
DMT-CpG*p (0.60)	HO-dUpA'pCpG*-R_2 (0.20) $ZnBr_2$[b]	1.8/5.4	DMT-CpG*pdUpA'pCpG*-R_2 (75)

[a]Abbreviations are: R_1=resin with functionalization of 1.3 meq/g; R_2=resin with functionalization of 0.3 meq/g; others are as shown in Table 1.

[b]The hydroxy component shown was obtained by detritylation with either benzene sulfonic acid (BSA) or $ZnBr_2$.

(0.3 meq NH_2/g), less than 5%, usually only 1–2%, is not cleaved. Further base treatment removes some, but not all, of this residual trityl containing functionality. The amino protecting groups were then removed by heating with aqueous ammonia. This step was carried out at 65° for 5–7 days due to the slow hydrolysis of the aminoadenine N^2-isobutyryl group. The mixtures were then applied to a Sephadex G-10 column to remove small molecules, and the pure 5′-*O*-dimethoxytrityl oligonucleotides were obtained by HPLC on a semi-preparative C_{18} column. Final purification on the same column followed detritylation with 80% acetic acid. HPLC of each purified sequence, and of the mixture obtained from each by enzymatic degradation, are shown in Figs. 3–5.

The stabilities of the sequences described above, together with certain related sequences, are compared in Fig. 6 in a plot of Tm^{-1} *vs* ln C. The difference between d(CGTACG)[31] and d(CGTA′CG) is perhaps the clearest demonstration of the additional stabilization afforded by the 2-amino group. Although the T:A′ pair is definitely more stable than a T:A pair, it is clearly less stable than a C:G pair. This is seen by the comparison of d(CGTA′CG) to $d(CG)_3$ and to $d(TA')_3$. Moreover, even the octamer d(CGTA′TA′CG) is no more stable than is the $d(CG)_3$ hexamer. It is also interesting that we do not detect a significant difference in stability between the d(CGTA′CG) and d(CGUA′CG) sequences. We had thought that these molecules might be small enough to display, in their respective Tm values, the de-

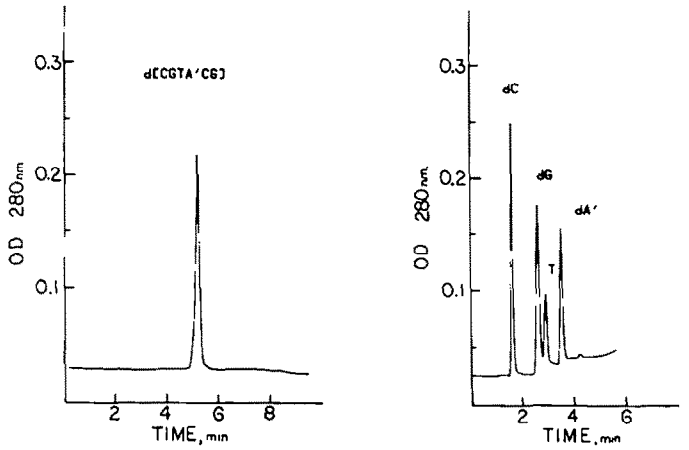


Fig. 3. d[CGTA′CG] after purification (left), using a gradient of 5–20% CH_3CN:0.1 M TEAA in 5 min at 4 mL/min, and after degradation with venom phosphodiesterase and alkaline phosphatase (right), using a gradient of 6–20% CH_3CN:0.1 M TEAA in 5 min at 4 mL/min, where dC, dG, T, and dA′, respectively were obtained in a ratio of 2.1:2.1:1.0:1.1.

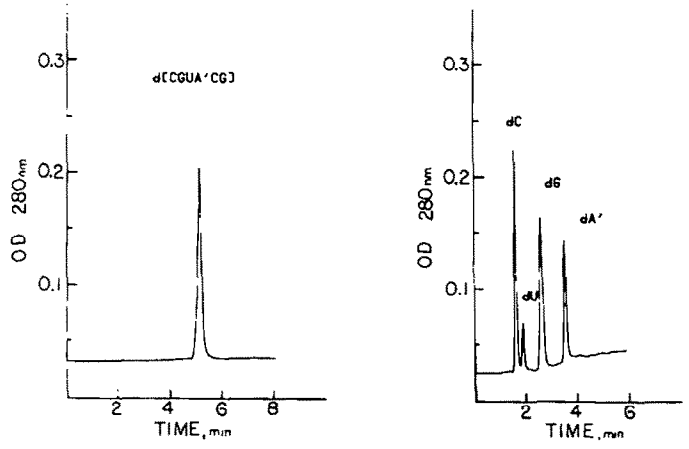


Fig. 4. d[CGUA′CG] after purification (left), using a gradient of 5–20% CH_3CN:0.1 M TEAA in 5 min at 4 mL/min, and after degradation with venom phosphodiesterase and alkaline phosphatase (right), using a gradient of 6–20% CH_3CN:0.1 M TEAA in 5 min at 4 mL/min, where dC, dU, dG and dA′,respectively, were obtained in a ratio of 1.9:1.0:1.9:1.0.

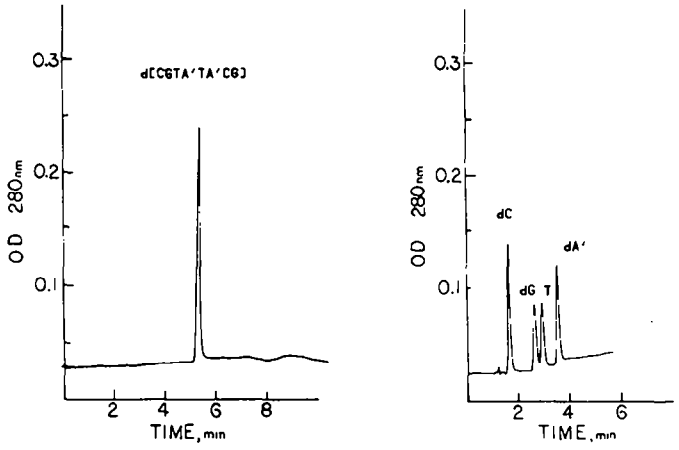


Fig. 5. d[CGTA'TA'CG] after purification (left), using a gradient of 6–20% CH_3CN:0.1 M TEAA in 5 min at 4 mL/min, and after degradation with venom phosphodiesterase and alkaline phosphatase (right), using the same gradient, where dC, dG, T, and dA', respectively, were obtained in a ratio of 1.2:0.9:1.0:0.9.

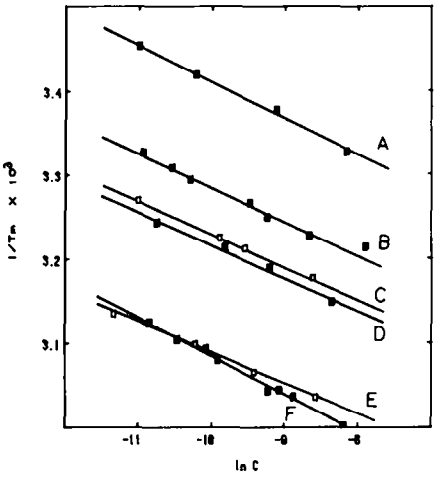


Fig. 6. Plot of inverse of melting temperature vs ln of concentration for: A, d[TA']$_3$; B, d[CGTACG]; C, d[CGUA'CG]; D, d[CGTA'CG]; E, d[CGTA'TA'CG], F, d[CG]$_3$.

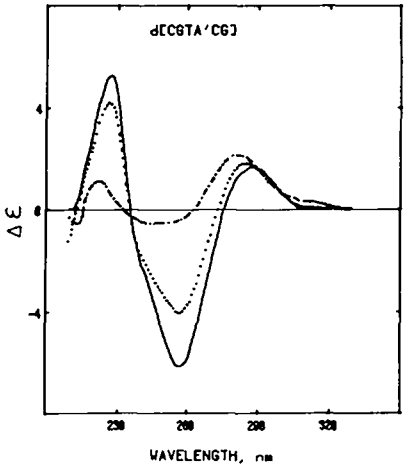


Fig. 7. Circular dichroism spectra of d[CGTA'CG] at 1° in 1 M NaCl (———); 5M NaCl (• • • •); and at 80° (– • –•).

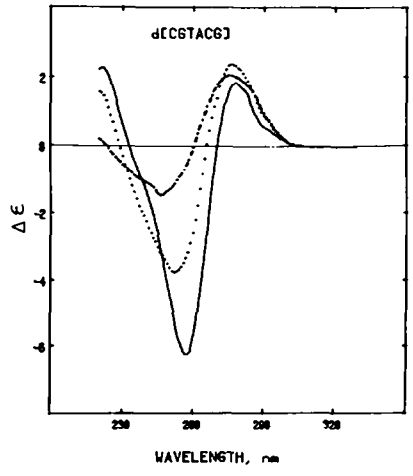


Fig. 8. Circular dichroism spectra of d[CGTACG] at 3° in 1 M NaCl (———); 5 M NaCl (• • • •); and at 80° (– • –•).

stabilization of B DNA postulated for the pyrimidine 5-Me group.[52]

Figure 7 shows CD spectra of d(CGTA'CG) in 1 M and 5 M NaCl. Notably, there is no evidence of a salt-induced conformational transition, as there is for d(TA')$_3$. In fact, the spectra are quite similar to those of d(CGTACG)[31] under the same conditions, as shown in Fig. 8. Moreover, the CD spectra of d(CGUA'CG) and d(CGTA'TA'CG), Figs. 9 and 10, indicate that for these molecules as well the B form is maintained in 5 M NaCl solution. Thus none of the mixed sequences undergo a salt-induced conformational transition, while the homologous sequences d(TA')$_3$ and d(CG)$_3$ both do. But it is not at all clear that d(TA')$_3$ and d(CG)$_3$ undergo the same transition. Comparison of the CD spectra of d(TA')$_3$ in Fig. 11 and d(CG)$_3$ in Fig. 12 reveals only that some change occurs on going to high salt. In fact,

both the low-salt spectra and the high-salt spectra differ appreciably. These CD spectra simply do not provide sufficiently specific information to resolve this question. Indeed, the fact that no transition occurs with the mixed sequences may indicate that the high-salt forms of $d(TA')_3$ and $d(CG)_3$ are not the same, and even may be mutually exclusive. It is interesting that what appears to be a partial $B \rightarrow Z$ transition does take place with the hexamer d[CGC(O^6Me)GCG][46] (Fig. 13). Yet it is also possible that these molecules are simply too small to provide

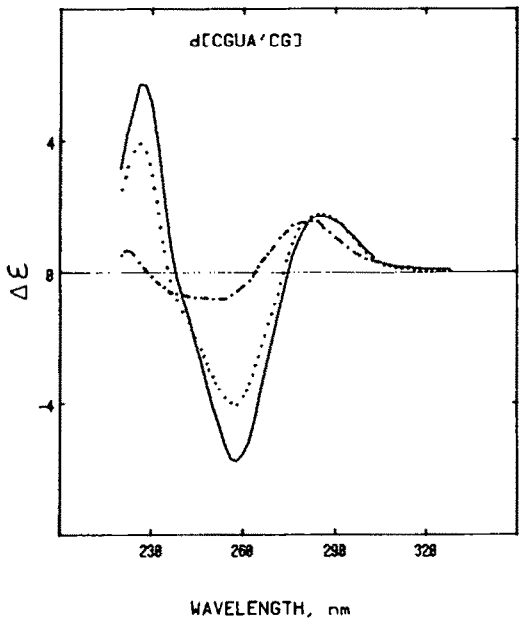


Fig. 9. Circular dichroism spectra of d[CGUA'CG] at 3° in 1 M NaCl (———); 5 M NaCl (• • • •); and at 70° (-•-•).

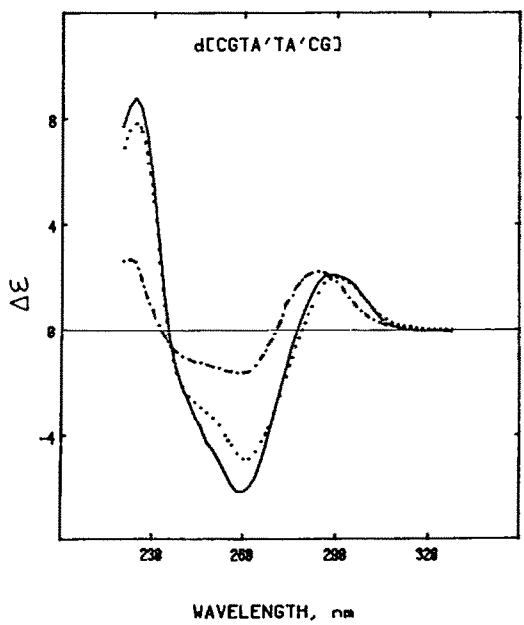


Fig. 10. Circular dichroism spectra of d[CGTA'TA'CG] at 3° in 1 M NaCl (———); 5 M NaCl (• • • •); and at 70°(-•-•).

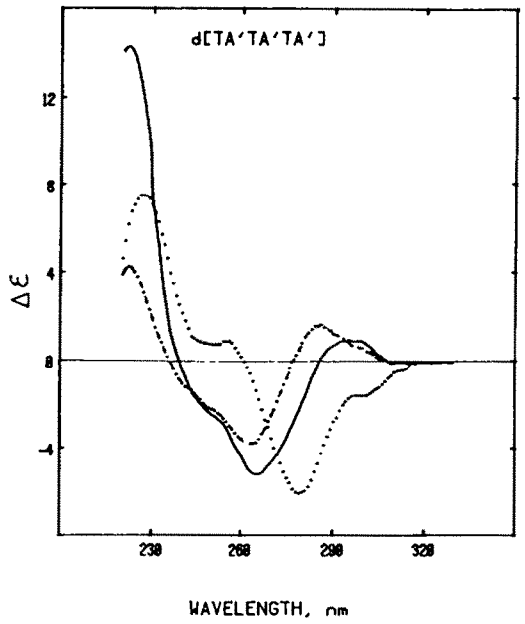


Fig. 11. Circular dichroism spectra of $d[TA']_3$ at 0° in 1 M NaCl (———); 5 M NaCl (• • • •); and at 70° (-•-•).

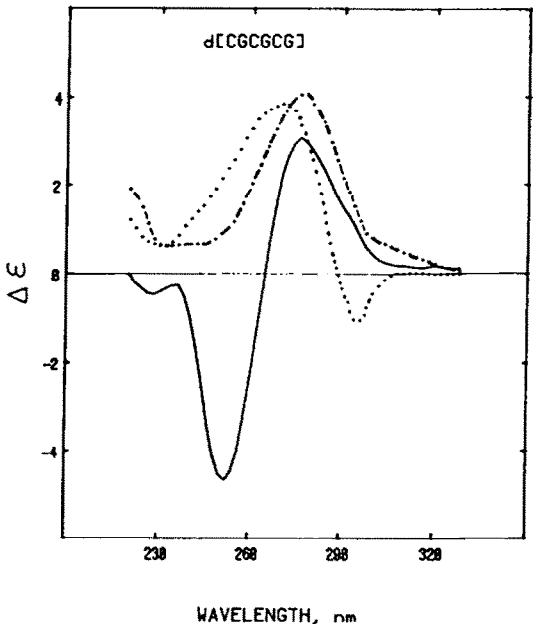


Fig. 12. Circular dichroism spectra of $d[CG]_3$ at 15° in 1 M NaCl (———); 5 M NaCl (• • • •); and at 80° (-•-•).

a complete answer. For example, the hexamer $d(GC)_3$, unlike poly d(GC), does not adopt a Z-form in high-salt solution.[12,20,53] Thus the failure of the mixed sequences reported above to undergo a salt-induced conformational change may be misleading. We are currently pursuing enzymatic polymerization of these duplex sequences to extend our studies to include large molecules.

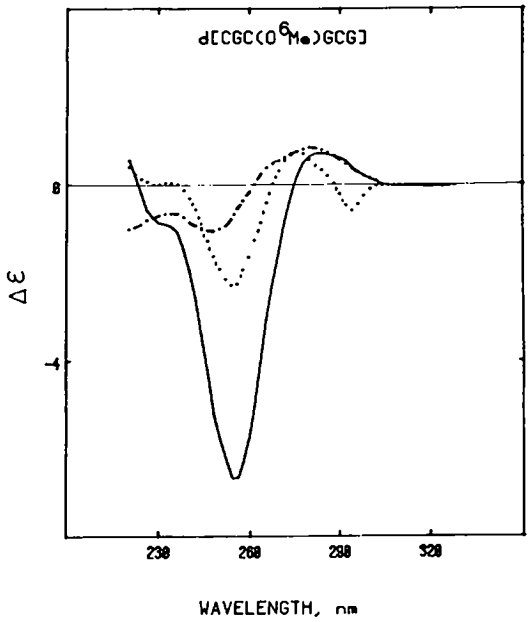


Fig. 13. Circular dichroism spectra of d[CGC(O⁶Me)GCG] at 0° in 1 M NaCl (———); 5 M TMA (• • • •); at 60° (– • – •).

EXPERIMENTAL

Polystyrene and chloromethylpolystyrene were purchased from Bio Rad Laboratories and derivatized according to the literature.[49] The 2-amino-2′-deoxyadenosine derivatives used were synthesized as reported below. Other deoxynucleosides were purchased from the U.S. Biochemical Co. and protected as reported elsewhere[44,46,54] or below. Reagents were obtained from Aldrich Chemical Co. Microanalyses were performed by Galbraith Laboratories. Pyridine, triethylamine, tetrahydrofuran and dioxane were dried by distillation from calcium hydride. Venom phosphodiesterase and alkaline phosphatase were obtained from Sigma. Purification of oligonucleotides was effected on a 7.8 mm × 30 cm μ Bondapak C_{18} column manufactured by Waters Associates. Analytical HPLC was performed using a Radial-Pak Bondapak C_{18} cartridge in a Waters Z Module. Two Waters M6000A pumps, a model 660 Solvent Programmer and a model 440 UV detector were employed.

The absorption spectra and melting curves reported were obtained on a Perkin-Elmer 575 spectrophotometer interfaced to a Tetronix 4051 for data acquisition and analysis. The temperature was controlled electrically using a peltier heated and cooled cell holder. For melting studies the temperature was increased continuously from 0 to 70° at a rate of 0.5°/min.

CD spectra were recorded on a Cary 60 spectropolarimeter equipped with a Cary 6001 CD accessory and a thermostattable cell holder. A 1 cm or 0.1 cm pathlength cylindrical cell was employed.

All physical measurements were carried out in buffer solutions containing 10 mM sodium phosphate or tetramethylammonium phosphate, 1mM sodium EDTA and the indicated salt concentration, adjusted to pH 7.0.

2-N,3′-O,5′-O-*Triisobutyryl-2′-deoxyguanosine* (**1**)

To 2.7 g (10 mmol) 2′-deoxyguanosine and 100 mL dry pyridine cooled in an ice bath was added 10.5 mL (100 mmol) isobutyryl chloride. After 1 hr the mixture was poured into 200 mL water containing 13 g (150 mmol) $NaHCO_3$. The soln was concentrated to about 100 mL to induce crystallization. The product (**1**) was collected by filtration to give 4.4 g (92%), m.p. 104–112°, softening from 75°. ^{1}H NMR ($CDCl_3$) δ 1.22 (m, 18, 3[CH_3]$_2$C), 1.78–3.35 (m, 5, 3Me$_2$C-H, $H_{2'}$, $_{2''}$), 4.12 5.02 (m, 3, $H_{4'}$, $H_{5',5''}$), 5.45 (m, 1, $H_{3'}$), 6.27 ("t", 1, J_{app} = 7Hz, $H_{1'}$), 7.83 (s, 1, H_8), 9.67 (brs, 1, N^2H), 12.15 (brs, 1, N^1H). UV_{max} (MeOH) 259 nm (ε 18, 600); UV_{sh} 280 nm (ε 13, 700); UV_{min} 227 nm (ε 4000). (Found: C, 53.70; H, 6.68; N, 14.25. Calc for $C_{22}H_{31}N_5O_7 \cdot H_2O$: C, 53.33; H, 6.71; N, 14.13%).

6-N-*Benzoyl*-2-N-*isobutyryl*-2-*amino*-2′-*deoxyadenosine* {*dA′*} (**6**)

To 2.4 g (5.0 mmol) of **1** dissolved in 50 mL CH_2Cl_2 was added 2.8 mL (20 mmol) Et_3N, 3.0 g (10 mmol) 2,4,6-triisopropylbenzenesulfonyl chloride and 30 mg (0.25 mmol) 4-dimethylaminopyridine. The mixture was stirred at room temp for 2 hrs, was cooled to 0°, and 10 mL Me_3N was added. Ammonia gas was then allowed to condense into the soln by means of a dry ice cold finger until about 200 mL was added. The soln was refluxed for 5 hr and then the ammonia was allowed to evaporate. The residue was dried by evaporation of pyridine, suspended in 50 mL dry pyridine and cooled in an ice bath. This mixture was then treated with 2.3 mL (20 mmol) benzoyl chloride for 1 hr, poured into a 100 mL portion of 5%$NaHCO_3$, and extracted with two 100 mL portions of EtOAc. The combined organic layers were concentrated and treated with 25 mL of 1 N NaOH in pyridine/MeOH/ water (65/30/5) at 0° for 10 min. The mixture was neutralized by the addition of a 100 mL portion of 5% NH_4Cl, concentrated to remove ammonia, and extracted with three 100 mL portions of EtOAc. The combined organic layers were concentrated to a gum, dissolved in CH_2Cl_2 and purified by flash chromatography on silica gel to give 1.4 g (65%) of **6**. The compound is fluorescent on TLC. ^{1}H NMR ($CDCl_3$/Me_2SO-d_6)δ 1.22 (*d*, J = 6 Hz, 6, [CH_3]$_2$C), 2.15–3.23 (m, 3, Me_2C–H, $H_{2',2''}$), 3.82 (m, 2, $H_{5',5''}$), 4.07 (m, 1, $H_{4'}$), 4.67 (m, 2, $H_{3'}$, OH), 5.37 (brs, 1, OH), 6.50 ("t", 1, J_{app} = 6 Hz, $H_{1'}$), 7.58 (m, 3, Ar), 8.13 (m, 2, Ar), 8.50 (s, 1, H_8), 10.28 (brs, 1, N–H). UV_{max} (EtOH) 297, 245 nm (ε 15,300, 35,800); UV_{min} 276 nm (ε 10,000). (Found, C, 56.48; H, 5.75; N, 18.09. Calc for $C_{21}H_{24}N_6O_5 \cdot \frac{1}{2}$ CH_3OH: C, 56.57; H, 5.74; N, 18.41).

3′-O-*Levulinyl*-6-N-*benzoyl*-2-N-*isobutyryl*-2-*amino*-2′-*deoxyadenosine*{*dA′-lev*}(**7c**)

To 1.8 g (4.0 mmol) of **6** dissolved in 40 mL dry pyridine was added 2.0 mL (7.5 mmol) *tert*-butyldiphenylsilyl chloride. After 3 hr the mixture was poured into 100 mL 5% $NaHCO_3$ and extracted with two 100 mL portions of ether. The combined organic layers were concentrated to a gum, dissolved in CH_2Cl_2, and purified by flash chromatography. The product fractions were concentrated to a gum, dried by evaporation of pyridine and dissolved in 40 mL dry pyridine. To this soln was added, with filtration, an ethereal soln of levulinic anhydride (10 mmol) prepared by reaction of 1.0 mL (10 mmol) levulinic acid with 1.0 g (5.0 mmol) dicyclohexylcarbodiimide in 30 mL ether. To this soln was added 31 mg (0.25 mmol) 4-dimethylaminopyridine and 1.7 mL (12 mmol) Et_3N. The mixture was concentrated to remove the ether and stirred at room temp overnight. It was then poured into 100 mL 5% $NaHCO_3$ and extracted with two 100 mL portions of ether. The combined organic layers were concentrated to a gum and dried by evaporation of pyridine. To the resulting oil was added 5 mL of 1 M tetra-*n*-butylammonium flouride in THF. After 30 min the mixture was concentrated and applied to an 80 mL column of Dowex 50W-X2 (pyridinium form). The product fractions were concentrated to a gum, dissolved in CH_2Cl_2 and purified by flash chromatography on silica gel to give 1.6 g (74%) of **7c**. This compound appears fluorescent on TLC, ^{1}H NMR ($CDCl_3$) δ 1.25 (d, J = 6 Hz, 6, [CH_3]$_2$C), 2.22 (s, 3, CH_3CO), 2.28–3.38 (m, 7, Me_2C–H, –CH_2–CH_2–CO, $H_{2',2''}$), 3.97 (m, 2, $H_{5',5''}$), 4.23 (m, 1, $H_{4'}$), 5.08 (brs, 1, 5′-OH), 5.58 (m, 1, $H_{3'}$), 6.42 ("t", 1, J_{app} = 7 Hz, $H_{1'}$), 7.63(m, 3, Ar), 8.15 (m, 3, Ar, H_8), 8.83 (brs, 1, N–H) 9.70

(brs, 1N–H). UV_{max} (EtOH) 297, 245 nm (ε 14,600, 33,500); UV_{min} 276 nm (ε 9,600). Found C, 57.78; H, 5.81; N, 15.12. Calc for $C_{26}H_{30}N_6O_7 \cdot \frac{1}{4} H_2O$: C, 57.50; H, 5.66; N, 15.48).

2-N-*Isobutyryl*-3′,5′-O-*bis*-(*tert*-*butyldimethylsilyl*)-2′-*deoxyguanosine* (**8a**)

To 2.8 g (8.3 mmol) 2-*N*-isobutyryl-2′-deoxyguanosine and 100 mL dry pyridine was added 2.5 g (36 mmol) imidazole and 2.8 g (18 mmol) *tert*-butyldimethylsilyl chloride. The mixture was stirred for 15 hr, poured into 200 mL 5% $NaHCO_3$, and stirred for another hr. The crystalline product was collected by filtration to give 3.3 g (72%) of **8a**, m.p. 105–112°, softening at 86°. ^1H NMR ($CDCl_3$, Me_2SO-d_6) δ 0.10 (s,12, 4 CH_3–Si), 0.90 (s, 18, 2 $[CH_3]_3C$), 1.25 (d, J = 6 Hz, 6, $[CH_3]_2C$) 2.40 (m, 3, Me_2C–H, $H_{2',2''}$), 3.82 (m, 3, $H_{4'}$, $H_{5',5''}$), 4.55 (m, 1, $H_{3'}$), 6.25 ("t", 1, J_{app} = 7 Hz, $H_{1'}$), 8.00 (s, 1, H_8). UV_{max} (MeOH) 259 nm (ε 18,000); UV_{sh} 280 nm (ε 13,200); UV_{min} 227 nm (ε 3,800). (Found: C, 52.75; H, 8.28; N, 11.85; Si, 9.15. Calc for $C_{26}H_{47}N_5O_5Si_2 \cdot 1\frac{1}{2} H_2O$: C, 52.67; H, 8.50; N, 11.81; Si, 9.47).

2-N-*Isobutyryl*-6-O-(*cyanoethyl*)-2′-*deoxyguanosine* {*dG***} (**9b**)

To 3.4 g (6.0 mmol) of **8a** and 25 ml CH_2Cl_2 was added 3.3 mL (24 mmol) Et_3N, 37 mg (0.3 mmol) 4-dimethylaminopyridine and 3.6 g (12 mmol) 2,4,6-triisopropylbenzenesulfonyl chloride. The mixture was stirred for 2 hr and concentrated to 10 mL, whereupon 2.0 mL (29 mmol) 3-hydroxypropionitrile was added. The mixture was cooled to 0° and 6 mL Me_3N was added. After 10 min 3.1 mL (21 mmol) 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) was added and the mixture was stirred at 0° for 30 min. The mixture was then partitioned between 10 mL EtOAc and 100 mL 10% NH_4Cl. The aqueous layer was extracted again with EtOAc and the combined organic layers were concentrated, dried by evaporation of pyridine and treated with 10 mL of a pyridine soln that was 1 M in tetra-*n*-butylammonium fluoride and 2 M in HF. After 10 hr 50 mL of Dowex 50W-X2 (pyridinium form) was added. The mixture was filtered, concentrated and partitioned between 50 mL each of water and EtOAc. The aqueous layer was washed 5 times with 100 mL portions EtOAc. The product crystallized upon concentration of the aqueous layer to give 0.75 g (32%) of **9b**, m.p. 168–169°. ^1H NMR ($CDCl_3$, Me_2SO-d_6) δ 1.20 (d, J = 7 Hz, 6, $[CH_3]_2C$), 2.57 (m, 3, Me_2C–H, $H_{2',2''}$), 3.12 ("t", 2, J_{app} = 6 Hz, $-CH_2$–CN), 3.73 (m, 2, $H_{5',5''}$), 4.00 (m, 1, $H_{4'}$), 4.30–5.33 (m, 5, 3′OH, 5′OH, $H_{3'}$, –O–CH_2–), 6.47 ("t", 1, J_{app} = 7 Hz, $H_{1'}$), 8.40 (s, 1, H_8), 9.97 (brs, 1, N^2H). UV_{max} (MeOH) 268, 220 nm (ε 17,400, 32,300); UVmin 233 nm (ε 5,300). (Found: C, 52.46; H, 5.81; N, 21.38. Calc for $C_{17}H_{22}N_6O_5$: C, 52.30; H, 5.68; N, 21.53).

2-N-*Isobutyryl*-6-O-(*cyanoethyl*)-5′-O-*dimethoxytrityl*-2′-*deoxyguanosine* {*d*[*DMT*-*G***]} (**9c**)

To 0.74 g (1.9 mmol) of **9b** dissolved in 20 mL dry pyridine was added 0.62 mL (4.5 mmol) Et_3N, 11 mg (0.09 mmol) 4-dimethylaminopyridine, and 1.3 g (3.7 mmol) 4,4′-dimethoxytritylchloride. After 1 hr the mixture was poured into 100 mL 5% $NaHCO_3$ and extracted twice with 100 mL portions EtOAc. The combined organic layers were concentrated to a gum, dissolved in CH_2Cl_2 and purified by flash chromatography on silica gel to give 1.2 g (91%) of **9c**.

2-N-*Isobutyryl*-6-O-(*cyanoethyl*)-5′-O-*dimethoxytrityl*-3′-O-*levulinyl*-2′-*deoxyguanosine* {*d*[*DMT*-*G***-*lev*]} (**9e**)

To 3.0 g (4.1 mmol) of **8b**[46] and 25 mL CH_2Cl_2 was added 2.2 mL (16 mmol) Et_3N, 25 mg (0.2 mmol) 4-dimethylaminopyridine, and 2.5 g (8.2 mmol) 2,4,6-triisopropylbenzenesulfonyl chloride. The mixture was stirred for 2 hr and concentrated to 10 mL whereupon 1.4 mL (21 mmol) 3-hydroxypropionitrile was added. The mixture was cooled to −20° and 7 mL Me_3N was added. After 25 min 2.1 mL (14 mmol) DBU was added and the mixture was stirred at −20° for 1 hr whereupon 10 mL of a mixture of Ac_2O:pyridine (1:9) was added. After 10 min the mixture was poured into 200 mL 5% $NaHCO_3$ and extracted with three 100 mL portions ether. The organic layers were concentrated to a gum which was dissolved in ether and purified by careful flash chromatography on silica gel to give 1.1 g (34%) of **9e**. ^1H NMR ($CDCl_3$) δ 1.18 (m, 6, $[CH_3]_2C$), 2.18 (s, 3, CH_3CO), 2.70 (m, 7, Me_2C–H, $-CH_2-CH_2-CO$, $H_{2',2''}$), 3.00 ("t", 2, J_{app} = 7 Hz, $-CH_2$–CN), 3.40 (m, 2, $H_{5',5''}$), 3.78 (s, 6, 2 CH_3O–), 4.27 (m, 1, $H_{4'}$), 4.80 ("t", 2, J_{app} = 7 Hz, –O–CH_2–), 5.55 (m, 1, $H_{3'}$), 6.40 ("t", 1, J_{app} = 7 Hz, $H_{1'}$), 6.60–7.57 (m, 13, Ar), 7.77 (brs, 1, N^2–H), 8.00 (s, 1, H_8). UV_{max} (MeOH) 270, 235 nm (ε 20,900, 26,600); UV_{min} 251 nm (ε 17,800). (Found: C, 65.12; H, 5.97; N, 10.42. Calc for $C_{43}H_{46}N_6O_9$: C, 65.30; H, 5.86; N, 10.63).

2-N-*Isobutyryl*-6-O-(*cyanoethyl*)-3′O-*levulinyl*-2′-*deoxyguanosine* {*d*[*G***-*lev*]} (**9f**)

To 1.7 g (2.2 mmol) of **9e** dissolved in 90 mL cold 70/30 CH_2Cl_2/MeOH was added 90 mL of cold 4% benzene sulfonic acid in 70/30 CH_2Cl_2/MeOH. The soln was stirred for 7 min, quenched with pyridine, and then poured into 100 mL 5% $NaHCO_3$. The layers were separated and the aqueous layer was extracted once more with a 100-mL portion of 70/30 CH_2Cl_2/MeOH. The combined organic layers were concentrated to a gum, dissolved in CH_2Cl_2, and purified by flash chromatography on silica gel to give 0.88 g (82%) of **9f**.

2-N-*Isobutyryl*-6-O-(4-*nitrophenylethyl*)-5′-O-*dimethoxytrityl*-2′-*deoxyguanosine* {*d*[*DMT*-*G**]} (**10b**)

To 2.8 g (3.2 mmol) of **10a**[46] was added 13 mL 0.5 M hydrazine hydrate in 4/1 pyridine/AcOH. After 30 min the mixture was poured into 10 mL water, extracted with a 100 mL portion of EtOAc, and washed with a 100 mL portion of 5% $NaHCO_3$. The organic layer was concentrated to a gum, dissolved in CH_2Cl_2 and purified by flash chromatography on silica gel to give 2.3 g (91%) of **10b**. A sample was readily crystallized from ether, m.p. 184–186°, with decomposition, softening above 170°. ^1H NMR ($CDCl_3$, Me_2SO-d_6), δ 1.20 (d, J = 7 Hz, 6, $[CH_3]_2C$), 2.60 (m, 3, Me_2C–H, $H_{2',2''}$), 3.33 (m, 4, $ArCH_2$, $H_{5',5''}$), 3.77 (s, 6, 2 CH_3O–), 4.15 (m, 1, $H_{4'}$), 4.57 (m, 1, $H_{3'}$), 4.70 ("t", 2, J_{app} = 6 Hz, –OCH_2–), 5.13 (brs, 1, 3′OH), 6.50 ("t", 1, J_{app} = 6 Hz, $H_{1'}$), 6.65–8.30 (m, 18, Ar, H_8), 9.42 (brs, 1, N^2–H). UV_{max} (MeOH) 270, 235 nm (ε 31,500, 28,200); UV_{min} 248 nm (ε 22,700). (Found: C, 65.27; H, 5.80; N, 10.53. Calc for $C_{43}H_{44}N_6O_9$: C, 65.47; H, 5.62; N, 10.66).

2-N-*Isobutyryl*-6-O-(4-*nitrophenylethyl*)-3′-O-*levulinyl*-2′-*deoxyguanosine* {*d*[*G**-*lev*]} (**10d**)

To 1.4 g (1.6 mmol) of **10a**[46] dissolved in 70 mL cold 70/30 CH_2Cl_2/MeOH was added 70 mL cold 4% benzene sulfonic acid in 70/30 CH_2Cl_2/MeOH. The mixture was stirred for 7 min, quenched with pyridine and poured into 100 mL 5% $NaHCO_3$. The layers were separated and the aqueous layer was extracted one more time with a 100-mL portion of 70/30 CH_2Cl_2/MeOH. The combined organic layers were concentrated to a gum, dissolved in CH_2Cl_2 and purified by flash chromatography on silica gel to give 0.84 g (90%) of **10d**.

5′-O-*Dimethoxytrityl*-2′*deoxyuridine* {*d*[*DMT*-*U*]}

To 1.1 g (5.0 mmol) 2′-deoxyuridine and 50 mL dry pyridine was added 2.0 g (6.0 mmol) 4,4′-dimethoxytrityl chloride, 30 mg (0.25 mmol) 4-dimethylaminopyridine and 1.0 mL (7.0 mmol) Et_3N. The mixture was stirred at room temp for 1 hr, poured into 100 mL 5% $NaHCO_3$, and extracted with two 100 mL portions of ether. The combined organic layers were concentrated to a gum which was dissolved in CH_2Cl_2 and purified by flash chromatography on silica gel to give 2.3 g (87%). ^1H NMR ($CDCl_3$) δ 2.33 (m, 2, $H_{2',2''}$), 2.97 (brs, 1, 3′-OH), 3.43 (m, 2, $H_{5',5''}$),3.77 (s, 6, 2 CH_3O–), 4.03 (m, 1, $H_{4'}$), 4.55 (m, 1, $H_{3'}$), 5.42 (d, 1, J = 8Hz, H_5), 6.33 ("t", 1, J_{app} = 6 Hz, $H_{1'}$), 6.65–7.55 (m, 13, Ar), 7.78 (d, 1, J = 8 Hz, H_6), 9.55 (brs, 1, N^3–H). UV_{max}

(MeOH) 264, 234 nm (ε 10,800, 22,700); UV_{min} 255, 223 nm (ε 10,300, 19,000). (Found: C, 66.26; H, 5.86; N, 5.02. Calc for $C_{30}H_{30}N_2O_7 \cdot \frac{3}{4}H_2O$: C, 66.22; H, 5.83; N, 5.15).

General procedure for synthesis of dimers in solution

For phosphorylation a mixture of 3.2 g (45 mmol) of dry 1,2,4-triazole, 4.2 mL (30 mmol) Et_3N and 2.5 mL (15 mmol) 2-chlorophenylphosphodichloridate in 25 mL anhydrous THF was allowed to stand at room temp for 30 min in a dry box and was then added with rinsing to 5 mmol of a 5′-*O*-dimethoxytritylnucleoside which had been dried 3 times by evaporation of pyridine. The mixture was then concentrated to about 20 mL. After 30 min the mixture was poured into 200 mL 20/80 pyridine/water containing 4.2 mL (30 mmol) Et_3N. The resulting soln was stirred for 15 min and extracted 3 times with 100 mL portions of 70/30 CH_2Cl_2/MeOH. The combined organic layers were washed with water, dried by evaporation of pyridine, concentrated to a gum and used immediately or stored at −80°.

For the condensation, the nucleotide described above, 4.2 mmol of a 3′-*O*-levulinylnucleoside and 3.6 mL (45 mmol) *N*-methylimidazole were combined and dried 3 times by evaporation of pyridine in a dry box. Additional pyridine (about 50 mL) was then added and evaporated to a volume of about 30 mL. To this soln was added 4.5 g (15 mmol) 2,4,6-triisopropylbenzenesulfonyl chloride and the mixture was concentrated further to about 20 mL. After 30 min, the soln was poured into 200 mL 20/80 pyridine/water containing 2.5 g $NaHCO_3$. The mixture was stirred for 5 min and extracted twice with 100 mL portions of 70/30 CH_2Cl_2/MeOH. The combined organic layers were concentrated to a gum which was not further purified.

For delevulinylation, the gum was then treated with 17 mL 0.5 M hydrazine hydrate in pyridine/AcOH (4/1). After 45 min, the mixture was poured into 100 mL water and the soln was extracted twice with 100 mL portions of EtOAc. The combined organic layers were washed with 100 mL 5% $NaHCO_3$, concentrated to a gum, dissolved in CH_2Cl_2, and purified by flash chromatography on silica gel. Yields are shown in Table 1.

General procedure for succinylation and attachment to resin

To 2.0 mmol of 5′-O-dimethoxytritylnucleoside dissolved in 7 mL CH_2Cl_2 was added 0.27 g (2.2 mmol) 4-dimethylaminopyridine and 0.30 g (3.0 mmol) succinic anhydride. After 1 hr 50 mL CH_2Cl_2 was added and the soln was washed with a 50-mL portion 10% NaH_2PO_4. The organic layer was concentrated to a gum and purified by flash chromatography on silica gel using $\frac{1}{2}$% pyridine in the eluant to give about a 90% yield of the product.

The succinylated nucleoside (1.8 mmol) was dissolved in 80 mL CH_2Cl_2 and added to 6.5 g amino functionalized resin. To this mixture was added 0.11 g (0.9 mmol) 4-dimethylaminopyridine and 1.1 g (5.4 mmol) dicyclohexylcarbodiimide. The mixture was shaken for 20 hr, filtered, and washed with three 50 mL portions of CH_2Cl_2, three 50 mL portions of 70/30 CH_2Cl_2/MeOH, three 50 mL portions of MeOH alternating with 50 mL portions of CH_2Cl_2, three 50 mL portions of CH_2Cl_2 and three 50 mL portions of ether. The resin was dried under vacuum and the extent of functionalization was determined (usually *ca.* 0.17 mmol/g) by measuring the absorbance at 500 nm of a weighed sample treated with 2% benzenesulfonic acid in 70/30 CH_2Cl_2/MeOH (ε 9×10^3). Unreacted sites were capped by shaking for 2 hr with 9/1 pyridine/Ac_2O and *N*-methylimidazole. The resin was then washed and dried as before

General procedure for oligonucleotide synthesis

Detritylation of the resin-bound oligonucleotide was accomplished by one of two different methods, as indicated in Table 2. The first procedure employed three 1 min treatments with cold 2% benzene sulfonic acid in 70/30 CH_2Cl_2/MeOH, alternating with washes of solvent. After the third treatment, the mixture was quenched with pyridine. The second method employed three 20 min treatments at room temp with 1 M $ZnBr_2$ in 85/15 CH_2Cl_2/i-PrOH, alternating with washes of CH_2Cl_2. After the third treatment, the mixture was quenched with 0.5 M triethylammonium acetate in dimethylformamide. In each method, volumes were chosen that allowed the resin to be shaken conveniently. After being quenched, the resin was washed as described above and dried under vacuum.

The next monomer or dimer (3–5 equiv) was phosphorylated as described in the previous section and then added to the resin along with 9 equiv (relative to the 3′-phosphate component) of *N*-methylimidazole. This mixture was dried 3 times by evaporation of pyridine. Additional pyridine was added, partially evaporated, and then three equivalents (relative to the 3′-phosphate component) of 2,4,6-triisopropylbenzenesulfonyl chloride were added. After further concentration, the mixture was shaken for 2 hr, filtered, washed and dried as described above. The yield of the reaction was determined by the trityl assay described above.

Unreacted sites were capped by shaking for 20 min with 9/1 pyridine/Ac_2O and *N*-methylimidazole. The resin was then washed and dried.

General procedure for deprotection and purification

A portion of resin was shaken with 2-nitrobenzaldoxime (500 mg/g of resin) and a 10% soln of either DBU or tetramethylguanidine in anhydrous THF (10 mL/g of resin). After 24 hr, 5 mL H_2O was added and the resin was shaken for 2 more days, filtered and washed. The filtrate was concentrated and then heated with aqueous ammonia for 5–7 days at 65°. The soln was again concentrated and applied to a Sephadex G-10 column. The combined product fractions were concentrated and the residue purified on a semipreparative C-18 Bondapak column, using a gradient of 20–50% acetonitrile/0.1 M triethylammonium acetate (TEAA) in 30 min at 2 mL/min. The combined product fractions were concentrated and the residue was detritylated by treatment with 80% AcOH for 20 min. The AcOH was removed by evaporation under reduced pressure and the dimethoxytritanol removed by extraction with ether. The aqueous soln was concentrated and purified on the semipreparative column using a gradient of 5–20% acetonitrile/0.1 M TEAA in 30 min at 2 mL/min. The combined product fractions were concentrated and desalted on the Sephadex G-10 column. A sample of each pure oligonucleotide was treated with venom phosphodiesterase and alkaline phosphatase. The expected ratios of the various monomers were obtained, as determined by analytical hplc (Figs. 3–5).

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REFERENCES

[1]F. B. Howard, J. Frazier and H. T. Miles, *J. Biol. Chem.* **241**; 4293 (1966).
[2]A. Cerami, E. Reich, D. C. Ward and I. H. Goldberg, *Proc. Natl Acad. Sci. U.S.A.* **57**, 1036 (1967).
[3]F. B. Howard, J. Frazier and H. T. Miles, *Biochemistry* **17**, 3783 (1976).
[4]C. Janion and K. H. Scheit, *Biochim. Biophys. Acta* **432**, 192 (1976)
[5]H. R. Rackwitz and K. H. Scheit, *Eur. J. Biochem.* **72**, 191 (1977).

[6]M. Muraska, H. T. Miles and F. B. Howard, *Biochemistry* **19**, 2429 (1980).
[7]K. H. Scheit and H. R. Rackwitz, *Nucleic Acids Res.* **10**, 4059 (1982).
[8]T. R. Webb and L. E. Orgel, *Ibid.* **10**, 4413 (1982).
[9]F. B. Howard and H. T. Miles, *Biopolymers* **22**, 597 (1983).
[10]M. D. Kirnos, I. Y. Khudyakov, N. I. Alexandrushkina and B. F. Vanyushin, *Nature* **270**, 369 (1977).
[11]R. E. Dickerson, H. R. Drew, B. N. Conner, R. M. Wing, A. V. Fratine and M. L. Kopka, *Science* **216**, 475 (1982).
[12]F. M. Pohl and T. M. Jovin, *J. Mol. Biol.* **67**, 375 (1972).
[13]D. J. Patel, L. L. Canuel and F. M. Pohl, *Proc Natl Acad. Sci. U.S.A.* **76**, 2508 (1979).
[14]A. H.-J. Wang, G. J. Quigley, F. J. Kolpak, J. L. Crawford, J. H. van Boom, G. van der Marel and A. Rich, *Nature* **282**, 680 (1979).
[15]A. H.-J. Wang, G. J. Quigley, F. J. Kolpak, G. van der Marel, J. H. Boom and A. Rich *Science* **211**, 171 (1981).
[16]M. Behe, S. Zimmerman and G. Felsenfeld, *Nature* **293**, 233 (1981).
[17]A. Möller, A. Nordheim, S. R. Nichols and A. Rich, *Proc. Natl Acad. Sci. U.S.A.* **78**, 4777 (1981).
[18]E. M. Lafer, A. Möller, A. Nordheim, B. D. Stollar and A. Rich *Ibid.* **78**, 3546 (1981).
[19]E. Hamori and T. M. Jovin, *Biophys. J.* **33**, 67a (1981).
[20]L. A. Marky, unpublished ovservations.
[21]M. Behe and G. Felsenfeld, *Proc. Natl Acad. Sci. U.S.A.* **78**, 1619 (1981).
[22]L. Klevan and V. N. Schumaker, *Nucleic Acids Res.* **10**, 6809 (1982).
[23]H. R. Drew, R. M. Wing T. Takano, C. Broka, S. Tanaka, K. Itakura and R. E. Dickerson, *Proc. Natl Acad Sci. U.S.A.* **78**, 2179 (1981).
[24]R. E. Dickerson and H. R. Drew, *J. Mol. Biol.* **149**, 761 (1981).
[25]F. Quadrifoglio, G. Manzini, M. Vasser, K. Dinkelspiel and R. Crea, *Nucleic Acids Res.* **9**, 2195 (1981).
[26]F. Quadrifoglio, G. Manzini, K. Dinkelspiel and R. Crea, *Ibid.* **10**, 3759 (1982).
[27]H. R. Drew and R. E. Dickerson, *J. Mol. Biol.* **151**, 535 (1981).
[28]B. L. Gaffney, L. A. Marky and R. A. Jones, *Nucleic Acids Res.* **10**, 4351 (1982).
[29]M. Vorlíčková, J. Kypr, S. Štokrová and J. Šponar, *Ibid.* **10**, 1071 (1982).
[30]C. Zimmer, S. Tymen, C. Marck and W. Guschlbauer, *Ibid.* **10**, 1081 (1982).
[31]S. Kuzmich, L. A. Marky and R. A. Jones, *Ibid.* **10**, 6265 (1982).
[32]C. B. Reese, *Tetrahedron* **34**, 3143 (1978).
[33]H. M. Hsiung, R. Brousseau, J. Michniewicz and S. A. Narang, *Nucleic Acids. Res.* **6**, 1371 (1979).
[34]J. B. Chattopadhyaya and C. B. Reese, *Ibid.* **8**, 2039 (1980).
[35]C. Broka, T. Hozumi, R. Arentzen and K. Itakura, *Ibid.* **8**, 5461 (1980).
[36]M. L. Duckworth, M. J. Gait, P. Goelet, G. F. Hong, M. Singh and R. C. Titmas, Ibid. **9**, 1691 (1981).
[37]M. D. Matteucci and M. H. Caruthers, *J. Am. Chem. Soc.* **103**, 3185 (1981).
[38]P. K. Bridson, W. J. Markiewicz and C. B. Reese, *J. Chem. Soc.* Chem Commun. 447 and 791 (1977).
[39]C. B. Reese and A. Ubasawa, *Tetrahedron Letters* 2265 (1980).
[40]H. P. Daskalov, M. Sekine and T. Hata *Ibid.* 3899 (1980).
[41]H. P. Daskalov, M. Sekine and T. Hata, *Bull. Chem. Soc. Jpn* **54**, 3076 (1981).
[42]S. S. Jones, C. B. Reese, S. Sibanda and A. Ubasawa, *Tetrahedron Letters* 4755 (1981).
[43]B. L. Gaffney and R. A. Jones, *Ibid.* 2253 (1982).
[44]B. L. Gaffney and R. A. Jones, *Ibid.* 2257 (1982).
[45]T. Trichtinger, R. Charubala and W. Pfleiderer, *Ibid.* 711 (1983).
[46]S. Kuzmich, L. A. Marky and R. A. Jones, *Nucleic Acids Res.* **11**, 3393 (1983).
[47]K. L. Agarwal and F. Riftina, *Ibid.* **5**, 2809 (1978).
[48]C. B. Reese, R. C. Titmas and L. Yau, *Tetrahedron Letters* 2727 (1978).
[49]H. Ito, Y. Ike S. Ikuta and K. Itakura, *Nucleic Acids Res.* **10**, 1755 (1982).
[50]V. A. Efimov, S. V. Reverdatto and O. G. Chakhmakhcheva, *Tetrahderon Letters* 961 (1982).
[51]A. K. Seth and E. Jay, *Nucleic Acids Res.* **8**, 5445 (1980).
[52]S. Fujii, A. H.-J. Wang, G. van der Marel, J. H. vanVoom and A. Rich, *Ibid.* **10**, 7879 (1982).
[53]T. J. Thamann, R. C. Lord A. H.-J. Wang and A. Rich, *Ibid.* **9**, 5443 (1981).
[54]G. S. Ti, B. L. Gaffney and R. A. Jones, *J. Am. Chem. Soc.* **104**, 1316 (1982).