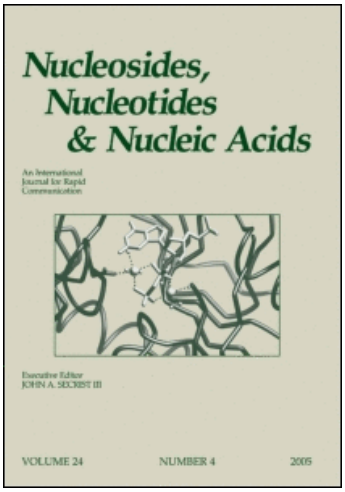


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis of Triple Helix Forming Oligonucleotides with a Stretched Phosphodiester Backbone

T. Sudhakar Rao[a]; Krishna Jayaraman[a]; Ross H. Durland[a]; Ganapathi R. Revankar[a]
[a] Triplex Pharmaceutical Corporation, The Woodlands, TX, U.S.A.

To cite this Article Rao, T. Sudhakar , Jayaraman, Krishna , Durland, Ross H. and Revankar, Ganapathi R.(1994) 'Synthesis of Triple Helix Forming Oligonucleotides with a Stretched Phosphodiester Backbone', Nucleosides, Nucleotides and Nucleic Acids, 13: 1, 255 — 273
To link to this Article: DOI: 10.1080/15257779408013239
URL: http://dx.doi.org/10.1080/15257779408013239

NUCLEOSIDES & NUCLEOTIDES, 13(1-3), 255-273 (1994)

SYNTHESIS OF TRIPLE HELIX FORMING OLIGONUCLEOTIDES WITH A STRETCHED PHOSPHODIESTER BACKBONE[1]

T. Sudhakar Rao,* Krishna Jayaraman, Ross H. Durland, and Ganapathi R. Revankar

Triplex Pharmaceutical Corporation, 9391 Grogans Mill Road, The Woodlands, TX 77380, U.S.A.

ABSTRACT: Total synthesis of novel DMT-phosphoramidites of thymidine (**11** and **15**) and 2'-deoxyguanosine (**8** and **20**) have been accomplished. The utility of these modified building blocks in the preparation of triple helix forming oligodeoxyribonucleotides with a stretched phosphodiester backbone has been evaluated. It was found that the oligonucleotides with extended backbones were unable to enhance the binding to duplex targets containing CG or TA base pairs.

Recently it has been demonstrated that in the presence of divalent cations, certain short guanine rich oligonucleotides can bind to specific sites in duplex DNA to form triple helices[2-4] at physiological pH. It has also been shown that the formation of such sequence-specific triple helices can inhibit DNA replication[5,6] and block transcription initiation, thus resulting in the specific inhibition of the synthesis of disease associated proteins. Therefore, the potential therapeutic significance of these triple helix forming oligonucleotides (TFOs) is obvious.

The major goal of TFO design is to develop molecules which can bind to any duplex DNA sequence, without regard for purine content or other symmetry consideration. *H*-bonding of the Hoogsteen or reverse Hoogsteen type normally occurs with purine bases in the major groove of an underlying duplex. Consequently, a polypurine/polypyrimidine duplex target presents

---

This publication is dedicated to the everlasting memory of Professor Roland K. Robins, 1926-1992.

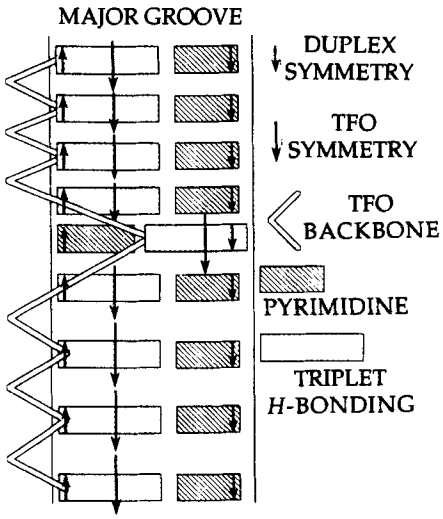
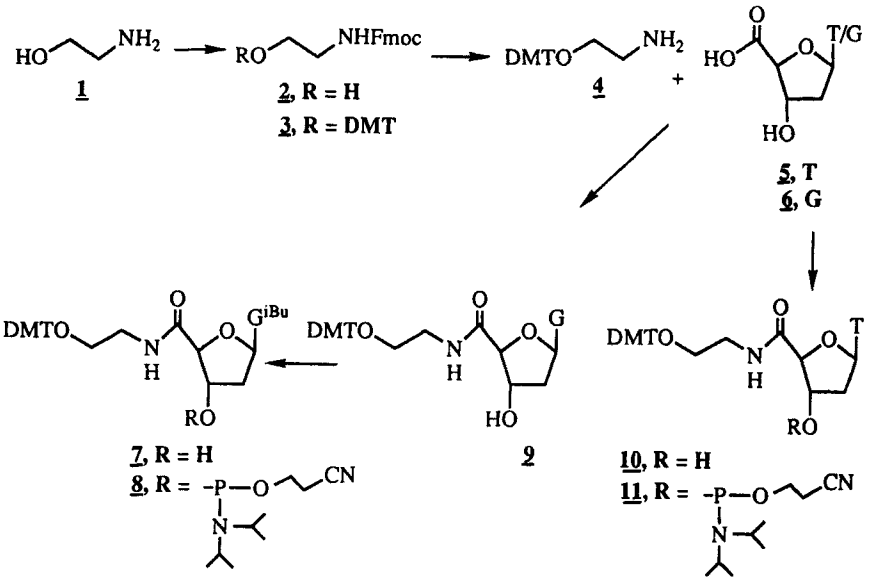


Figure 1
Strand positioning in the major groove of a triple helix

an orderly array of bond formers positioned upon one side of the major helix groove. However, at sites of CG or TA inversion, the corresponding purine target base is placed upon the opposing half of the major groove (*Figure* 1) and can be reached for the purposes of *H*-bonding only (a) upon extension of the TFO backbone, (b) by distension of the duplex, or (c) both.

Indirect data suggests that duplex DNA assumes the **A** form upon triplex formation.[7] In the **A** form, the major groove is deep and narrow, with dimensions well suited to the size of a bound third strand. Therefore, at sites of CG or TA inversion within a polypurine/polypyrimidine domain, at the most 3-5 Å of transverse distortion is required to accommodate *H*-bonding at the "other side" of the major groove. That much lateral distortion can be partially accommodated by the conformational freedom available to the deoxyribose backbone of a duplex. Our preliminary modelling studies suggest that, in order to form a standard TAT or GGC Hoogsteen or reverse Hoogsteen triplet at such sites, distortion of the duplex binding site might also be required.

If triple helix formation could be made stable at sites of CG or TA inversion, TFOs could be designed against any duplex site, rather than just at purine rich targets. One solution to the binding problem at CG inversion sites would be to have an extended backbone. Several reports have appeared in the



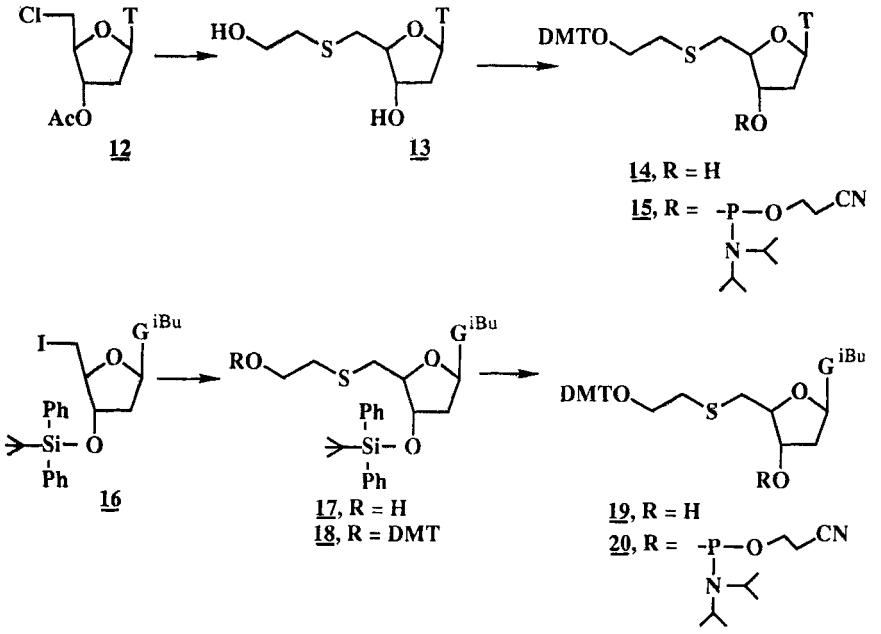
Scheme 1

literature describing the synthesis of oligodeoxyribonucleotides with backbone modification. Some of the analogues reported to date are methylphosphonate,[8] 5'-methylphosphonate,[9] phosphoramidate,[10] phosphorothiolate,[11] phosphorothioate[12] and phosphorodithioate.[13] Other approaches that provide a neutral backbone are methylene,[14] carbonyl,[15] and disubstituted silyl[16] modifications which represent one to one atom replacements. Synthesis of methylsulfonate,[17] methylhydroxylamine[18] and sulfamate[19] as two atom replacements and dimethylenesulfonate,[17] *N*-cyanoguanidine,[20] ethyl sulfide,[21] sulfonamide[22] and all-carbon backbone[23] as three atom replacements of the natural phosphodiester linkage have been reported. Recently recognition of mixed sequence duplex DNA by alternate-strand triple-helix formation has also been reported.[24] We now report our results on the synthesis of triple helix forming oligonucleotides with a novel, extended phosphodiester backbone, employing solid-support, phosphoramidite chemistry. A preliminary account of this work has been published recently.[25]

Chemistry: Synthesis of monomeric units **8** and **11** was accomplished by the condensation of 4,4'-dimethoxytrityl derivative of ethanolamine (**4**) and the

corresponding nucleoside-4'-carboxylic acid (*Scheme* 1). DMT-ethanolamine (**4**) was prepared starting from ethanolamine itself. Thus, ethanolamine on reaction with 1.15 molar equivalents of 9-fluorenylmethyl chloroformate, in the presence of diisopropylethylamine in anhydrous DMF afforded 9-fluorenylmethoxycarbonylethanolamine (**2**). The product **2** was isolated from the reaction mixture in 97% yield. Treatment of **2** with 1.1 molar equivalents of 4,4'-dimethoxytrityl chloride (DMT-Cl) in anhydrous pyridine at ambient temperature for 2 h, gave 9-fluorenylmethoxycarbonyl-*O*-(4,4'-dimethoxytrityl)ethanolamine (**3**) in 86% yield. Removal of fluorenylmethoxycarbonyl group from **3** was accomplished using an excess of piperidine in dichloromethane. The product *O*-(4,4'-dimethoxytrityl)ethanolamine (**4**) was isolated in 64.5% yield as analytically pure material. Compound **4** was allowed to react with 1,2-dideoxy-1-(thymin-1-yl)-β-**D**-ribofuranuronic acid[26] (**5**) in the presence of 1-hydroxybenzotriazole (HOBT), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and triethylamine in dry DMF for 18 h, followed by work up of the reaction mixture and purification of the reaction product by silica gel column chromatography to give a compound which was identified as 4-[1,2-dideoxy-1-(thymin-1-yl)-β-**D**-ribofuranuronamido]-O-(4,4'-dimethoxytrityl)ethanol (**10**). Phosphitylation of **10** was accomplished by using 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite in the presence of *N,N*-diisopropylethylamine in dry dichloromethane. The purified product 3-*O*-(*P*-β-cyanoethoxy-*N,N*-diisopropylaminophosphinyl-4-[1,2-dideoxy-1-(thymin-1-yl)-β-**D**-ribofuranuronamido]-*O*-(4,4'-dimethoxytrityl)ethanol (**11**) was isolated in 85.5% yield.

In a similar manner as described for thymidine, 1,2-dideoxy-1-(guanin-9-yl)-β-**D**-ribofuranuronic acid triethylammonium salt[27] (**6**) was condensed with **4**. A clean reaction was observed and the product 4-[1,2-dideoxy-1-(guanin-9-yl)-β-**D**-ribofuranuronamido]-O-(4,4'-dimethoxytrityl)ethanol (**9**) was isolated in 74% yield. In the case of guanosine monomer (**8**) it is rather necessary to protect the exocyclic amino function prior to phosphitylation in order to avoid any side reactions. Thus, protection of the exocyclic amino function was achieved by transient protection methodology.[28] Compound **9** was treated with chlorotrimethylsilane in anhydrous pyridine, followed by 1.2 molar equivalents of isobutyryl chloride, and after subsequent removal of the silyl protecting groups gave analytically pure (**7**) after silica gel column chromatography in 82% yield. Compound **7** was converted to the corresponding phosphoramidite (**8**) by treatment with 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite. A clean reaction was observed and the product was isolated in 82% yield after silica gel column chromatography.



Scheme 2

Synthesis of the other two building blocks (**15** and **20**) was successfully achieved starting from the corresponding 3'-protected-5'-halonucleosides (*Scheme* 2). Thus, 3'-O-acetyl-5'-chloro-5'-deoxythymidine[29] (**12**) on treatment with the sodium salt of 2-mercaptoethanol[30] in 2 N sodium hydroxide solution gave 5'-deoxy-5'-(2-hydroxyethylthio)thymidine (**13**), which was isolated in 85% yield. Compound **13** was converted to the corresponding DMT derivative (**14**) by treatment with 1.18 molar equivalents of DMT-Cl in dry pyridine. Work up of the reaction mixture and purification of the reaction product by silica gel column chromatography afforded analytically pure **14** in 75% yield. Compound **14** was subsequently converted to the corresponding target building block **15** by a conventional phosphitylation reaction.

In the case of the preparation of 5'-deoxy-5'-(2-hydroxyethylthio)-thymidine (**13**), the reaction was carried out in aqueous 2 N sodium hydroxide solution. However, a similar procedure could not be applied for the preparation of the guanosine analog since the starting material for the synthesis of **17** was 2',5'-dideoxy-5'-iodo-3'-O-(diphenyl-*tert*-butylsilyl)-N^2-isobutyrylguanosine[31]

(16). As expected compound 16 was found to be very unstable under the above reaction conditions , since the isobutyryl group was removed under basic conditions. Thus, the synthesis of 17 was carried out under anhydrous conditions using sodium hydride. The sodium salt of 2-mercaptoethanol was generated *in situ* by the treatment with 1 equivalent of sodium hydride in anhydrous dioxane. A solution of 16 in anhydrous dioxane was added to the sodium salt generated as above . A clean reaction was observed and the analytically pure product 17 was isolated in 86.5% yield after silica gel column chromatography. Compound 17 was reacted with 1.2 molar equivalents of DMT-Cl in anhydrous pyridine to afford the corresponding DMT derivative 18 in 85% yield. Removal of the silyl group from 18 was accomplished by treatment with tetrabutylammonium fluoride in THF. Compound 19 was smoothly converted to the target building block 20 by reaction with 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite and the pure product was isolated in 90% yield.

As we planned to incorporate these monomeric units into natural DNA by standard phosphoramidite methodology, the possibility of hydrolysis of the carboxamide bond during the deprotection step of the oligonucleotide synthesis was of some concern. Thus, a sample of 10 was treated with concentrated ammonium hydroxide for 2 days and no hydrolysis product was observed by thin layer chromatography. Similarly the possibility of oxidation of the sulfur atom in 15 and 20 during the oxidation step of the synthesis cycle was also of some concern. Thus, a sample of 14 and 19 separately dissolved in the oxidizing agent (I_2/pyridine/THF/H_2O), normally used during the oxidation step in the synthesis cycle, was stirred for 20 min. Thin layer chromatography demonstrated that no oxidation occured[21].

Synthesis, Purification and Characterization of Modified TFOs: The TFOs containing a stretched phosphodiester backbone were prepared by the phosphoramidite method using standard solid support methodology on a 0.2 μmole scale on a ABI 380B automated DNA synthesizer. However, the modified building blocks (11, 15 and 20) were used in tenfold excess concentration compared to normal amidites, and with a fourfold increase in coupling time. A series of TFOs was prepared with a stepwise coupling efficiency ranging from 95.6% to 98.5%. All base labile protecting groups on the oligodeoxyribonucleotides were removed by treatment with concentrated NH_4OH at 55 °C for 20 h and the TFO was purified by HPLC using an ion exchange Q-sepharose (Pharmacia) column . The purified product was desalted by passage through C_{18}

sep-pack (Waters) cartridge and analyzed on a 20% denaturing polyacrylamide gel after labeling with ^{32}P-ATP using polynucleotide kinase[32]. Unmodified oligonucleotide was used as the standard for comparison of mobility and purity. The modified TFOs were found to be ≥ 95% pure and with the expected length.

Assay for Triplex Formation: Triplex formation was assessed using the gel shift assay, essentially as described previously.[2,3] In general, trace concentrations (≤ 10^{-11}M) of end labeled duplex were incubated with increasing concentrations of third strand in a buffer consisting of 20mM Tris-HCl, pH 7.6, 10mM $MgCl_2$, and 10% sucrose. Incubations were at 37 °C for 20-24 hours. Samples were then separated on 12% polyacrylamide gels buffered in 89mM Tris, 89mM boric acid, and 10mM $MgCl_2$ (TBM_{10}). Electrophoresis was at 80V for 2-3 hours at room temperature. Gels were dried and autoradiographed.

Triplex formation was detected by the appearance of a discrete band migrating more slowly than the duplex in samples containing added third strand. Apparent dissociation constants for triplex formation were estimated as follows. Triplex formation and dissociation were assumed to be simple bimolecular reactions:

$$\text{triplex} \leftrightarrow \text{duplex} + \text{third strand}$$

It was assumed that each sample was at equilibrium when loaded on the gel, and that gel electrophoresis does not substantially alter the relative amounts of duplex and triplex in the sample. Under these conditions, the apparent dissociation constant, K_d, is expressed by:

$$K_d = SD/T$$

where S, D, and T represent the concentrations of single strand, duplex, and triplex, respectively. Assuming that the amount of duplex is insignificant relative to the amount of added third strand, the K_d is approximately equal to the third strand concentration where 50% of the duplex has been converted to triplex. Although this approximation depends on a number of assumptions, we have found that it is generally useful for comparing the relative affinities of different triplexes, and for estimating the approximate strength of the interaction.

Results: In order to evaluate the relative binding affinities of the modified nucleosides (**x**, **y** and **z** are free nucleosides of **11**, **15** and **20**, respectively), we studied triplex formation using a number of 26-base oligonucleotides (*Table* 1). Binding of different third strand oligonucleotides to appropriate duplex targets was assayed and compared as described above.

Table 1. Oligonucleotides Studied

duplexes:

I	5'-ccccttcccccttcccccttcccc-3' 3'-ggggaaggggggaaggggggaaggggg-5'
II	5'-ccccatccccccatccccccatccccc-3' 3'-ggggtaggggggtaggggggtaggggg-5'
III	5'-cccctttccctccttcctccttctccc-3' 3'-ggggaagggaggaaggaggaagaggg-5'
IV	5'-cccctttcccaccttccaccttcaccc-3' 3'-ggggaagggtggaaggtggaagtggg-5'
V	5'-cccctttcccgccttccgccttcgccc-3' 3'-ggggaagggcggaaggcggaagcggg-5'

third strands:

VI	5'-ggggttgggggggttgggggttggggg-3'
VII	5'-ggggttgggtggttggtggttgtggg-3'
VIII	5'-ggggxtgggggggxtgggggxtggggg-3'
IX	5'-ggggxxgggggggxxgggggxxggggg-3'
X	5'-ggggttgggxggttggxggttgxggg-3'
XI	5'-ggggytgggggggytgggggytggggg-3'
XII	5'-ggggyyggggggyyggggyyggggg-3'
XIII	5'-ggggttgggyggttggyggttgyggg-3'
XIV	5'-ggggttgggzggttggzggttgzggg-3'

Where, **x** is nucleoside of **11**; **y** is nucleoside of **15**; and **z** is nucleoside of **20** for *Tables* 1-4.

Table 2. Comparison of **x**, **y** and T binding to AT base pairs.

duplex	third strand	sequence	apparent K_d
I		5'-ccccttcccccttcccccttcccc-3'	
		3'-ggggaaggggggaaggggggaaggggg-5'	
	VI	5'-ggggttgggggttgggggttggggg-3'	$2x10^{-10}M$
	VIII	5'-ggggxtgggggggxtgggggxtggggg-3'	$1x10^{-8}M$
	IX	5'-ggggxxgggggggxxgggggxxggggg-3'	$8x10^{-8}M$
	XI	5'-ggggytgggggggytgggggytggggg-3'	$1x10^{-8}M$
	XII	5'-ggggyygggggggyygggggyyggggg-3'	$1x10^{-7}M$
III		5'-cccctteeetcettcetcettctccc-3'	
		3'-ggggaagggaggaaggaggaagaggg-5'	
	VII	5'-ggggttgggtggttggtggttgtggg-3'	$5x10^{-10}M$
	X	5'-ggggttgggxggttggxggttgxggg-3'	$2x10^{-8}M$
	XIII	5'-ggggttgggyggttggyggttgyggg-3'	$8x10^{-9}M$

Table 2 evaluates the ability of **x** and **y** to bind to AT base pairs in antiparallel triplexes, using thymidine (T) as a reference. Comparison of the apparent K_ds for I·VI, I·VIII, and I·IX indicate that binding of **x** to AT base pairs is significantly weaker than binding of T. This is confirmed by comparing the K_ds for III·VII and III·X. Similar results are observed with **y** (compare I·VI, I·XI, and I·XII; III·VII and III·XIII). This result is not unexpected, since **x** and **y** have substantially longer backbones than T, and are likely to be suboptimal for binding to AT pairs.

The data in *Table* 3 evaluate whether the longer backbone of **x** or **y** permits binding to TA base pairs as originally proposed. Comparison of I·VI (*Table* 2) and II·VI (*Table* 3) indicates that T clearly binds better to AT base pairs than to TA base pairs, as expected. In this context, the difference in binding affinity is about 3-fold. The data for II·VIII and II·XI (*Table* 3) show that a similar situation holds true for **x** and **y**, despite the longer backbones. In this case, affinity for AT base pairs is about 5-fold higher than for TA base pairs. (Note that relative affinities are expressed for the entire third strand, rather than per modified nucleoside).

Neither the control oligonucleotide VII, nor the modified oligonucleotides X and XIII were able to bind to duplex IV (*Table* 3). Again, there is no evidence that the modified nucleosides **x** or **y** improve binding to the TA pairs, in

Table 3. Comparison of **x**, **y** and T binding to TA base pairs.

duplex	third strand	sequence	apparent K_d
II		5'-ccccatcccccatcccccatcccc-3'	
		3'-ggggtaggggggtaggggggtaggggg-5'	
	VI	5'-ggggttgggggggttgggggttggggg-3'	$5x10^{-10}$M
	VIII	5'-ggggxtgggggggxtgggggxtggggg-3'	$5x10^{-8}$M
	XI	5'-ggggytgggggggytgggggytggggg-3'	$5x10^{-8}$M
IV		5'-ccccttcccaccttccaccttcaccc-3'	
		3'-ggggaagggtggaaggtggaagtggg-5'	
	VII	5'-ggggttgggtggttggtggttgtggg-3'	»$1x10^{-6}$M*
	X	5'-ggggttgggxggttggxggttgxggg-3'	»$1x10^{-6}$M*
	XIII	5'-ggggttgggyggttggyggttgyggg-3'	»$1x10^{-6}$M*

* No triplex was detected at third strand concentrations up to $1x10^{-6}$M.

Table 4. Comparison of **z** and G binding to GC and CG base pairs.

duplex	third strand	sequence	apparent K_d
I		5'-ccccttcccccttcccccttcccc-3'	
		3'-ggggaagggggaagggggaaggggg-5'	
	VI	5'-ggggttgggggttgggggttggggg-3'	$2x10^{-10}$M
	XIV	5'-ggggttgggzggttggzggttgzggg-3'	$5x10^{-9}$M
V		5'-ccccttcccgccttccgccttcgccc-3'	
		3'-ggggaagggcggaaggcggaagcggg-5'	
	VI	5'-ggggttgggggttgggggttggggg-3'	»$1x10^{-6}$M*
	XIV	5'-ggggttgggzggttggzggttgzggg-3'	»$1x10^{-6}$M*

* No triplex was detected at third strand concentrations up to $1x10^{-6}$M.

agreement with the data for duplex II. In the case of duplex IV, the presence of three TA base pairs reduced binding affinity of all third strands by at least 100-1000-fold (relative to duplex I). This is somewhat surprising, since the three TA base pairs in duplex II reduced binding by 5-fold or less. This may be ascribed to sequence context effects, and suggests that a TA base pair flanked by GC on either side is much more restrictive to triplex formation than one flanked on the 3' side by an AT pair.

Table 4 compares the ability of 2'-deoxyguanosine (G) and **z** to bind to GC and CG pairs in antiparallel triplexes. Comparison of the data for I·VI and I·XIV indicates that G binds about 25-fold stronger to GC pairs than does **z**. In order to determine if the longer backbone of **z** improves binding to CG pairs, we compared the relative K_ds for V·VI and V·XIV. Neither third strand was able to bind to duplex V, indicating that the CG pairs are too disruptive to permit triplex formation. Thus, the modified backbones evaluated in this study were unable to increase binding to duplex targets containing TA or CG base pairs.

There are several possible interpretations for these results. Despite the extended backbones, it appears that nucleosides **x**, **y**, and **z** do not interact with the purines at CG and TA pairs to form Hoogsteen-type triplets, as originally intended. However, it is unclear at this time that this is due to an inability of the modified backbone to accommodate such an interaction. It is also possible that the loss of stacking interactions associated with the displacement of the third strand base relative to its neighbors cannot be fully compensated by the formation of the proposed base triplets. Further study is required to resolve these questions.

EXPERIMENTAL

Melting points (uncorrected) were determined in a Thomas-Hoover capillary melting-point apparatus. Elemental analyses were performed by Quantitative Technologies Inc., Whitehouse, NJ. The presence of solvent as indicated by elemental analysis was verified by ^{1}H NMR spectroscopy. Thin layer chromatography (TLC) was performed on plates of silica gel 60F-254 (EM Reagents). Silica gel (Whatman; 230-400 mesh) was used for flash column chromatography. All solvents used were reagent grade. Detection of nucleoside components on tlc was by uv light, and with 10% sulfuric acid in methanol spray followed by heating. Evaporations were conducted under diminished pressure with the bath temperature below 30 °C. Nuclear magnetic resonance (^{1}H NMR)

spectra were recorded at 200 MHz with an Bruker 200 spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as the internal standard. Polyphosphoric acid was used as an external standard for ^{31}P NMR spectra (key: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and br = broad.)

9-Fluorenylmethoxycarbonylethanolamine (2). To a solution of ethanolamine (1.0 g, 16.37 mmoles) and diisopropylethylamine (3.42 mL, 19.65 mmoles) in dry DMF (40 mL) was added 9-fluorenylmethyl chloroformate (4.87 g, 18.8 mmoles). After stirring for 30 min at ambient temperature, the reaction mixture was poured into cold saturated aqueous sodium bicarbonate solution (200 mL). The precipitated product was collected by filtration, washed thoroughly with water (5 x 25 mL) and dried over P_2O_5 under high vacuum to yield 4.5 g (97%) of the title compound; mp 144 - 145 °C. ^1H NMR ($CDCl_3$): δ 2.14 (br s, 1 H, O*H*), 3.33 (m, 2 H, NHCH_2), 3.69 (t, 2 H, CH_2OH), 4.20 (t, 1 H, C*H*), 4.42 (d, 2 H, CHCH_2O), 5.21 (br s, 1 H, CONH) and 7.25 - 7.78 (m, 8 H, *aromatics*). *Anal.* Calcd. for $C_{17}H_{17}NO_3$: C, 72.06; H, 6.05; N, 4.94. Found: C, 71.82; H, 5.99; N, 4.89.

9-Fluorenylmethoxycarbonyl-*O*-(4,4'-dimethoxytrityl)ethanolamine (3). To a solution of **2** (4.0 g, 14.18 mmoles) in anhydrous pyridine (40 mL) was added 4,4'-dimethoxytrityl chloride (5.26 g, 15.53 mmoles). After stirring for 2 h at room temperature, the reaction mixture was partitioned between dichloromethane:water (100 mL each). The aqueous layer was separated and reextracted with dichloromethane (3 x 25 mL). The combined organic phase was dried (Na_2SO_4) and evaporated to dryness. The residual solid was purified by chromatography over a silica gel column using a gradient of hexanes → dichloromethane (20 to 10% of hexanes) as the eluent. The homogeneous fractions were pooled and evaporated to give 7.1 g (85.9%) of the title compound; mp 58 - 60 °C. ^1H NMR ($CDCl_3$): δ 3.21 (t, 2 H, NHCH_2), 3.39 (t, 2 H, CH_2O), 3.76 and 3.78 (2s, 2 x 3 H, 2 OCH_3), 4.12 - 4.44 (m, 3 H, C*H*CH_2), 5.10 (s, 1 H, N*H*), and 6.79 - 7.78 (m, 21 H, *aromatics*). *Anal.* Calcd. for $C_{38}H_{35}NO_5$: C, 77.92; H, 6.02; N, 2.39. Found: C, 77.73; H, 6.02; N, 1.97.

***O*-(4,4'-Dimethoxytrityl)ethanolamine (4).** Compound **3** (5.0 g, 8.5 mmoles) was added to a solution of piperidine (15 mL) in dichloromethane (45 mL). After stirring the reaction mixture for 1 h at room temperature, the solvent was evaporated. The resulting residue was partitioned between dichloromethane

(100 mL) and saturated aqueous sodium bicarbonate solution (50 mL). The organic layer was separated and dried (Na_2SO_4). The solvent was evaporated and the residue was purified by chromatography over a silica gel column packed in 10% triethylamine in dichloromethane. The impurities were eluted using 10% triethylamine in dichloromethane and the desired product was eluted using a solvent system consisting of 10% methanol:10% triethylamine:80% dichloromethane. The homogeneous fractions were pooled and evaporated to give 2.0 g (64.5%) of the title compound as gum. ^1H NMR ($CDCl_3$): δ 1.55 (br s, 2 H, NH_2), 2.87 (t, 2 H, CH_2NH_2) 3.13 (t, 2 H, OCH_2), 3.79 (s, 6 H, 2 OCH_3) and 6.73-7.47 (m, 13 H, *DMT*). *Anal.* Calcd. for $C_{23}H_{25}NO_3 \cdot 1/4\ CH_3OH$; C, 75.17; H, 7.05; N, 3.77. Found: C, 74.99; H, 7.29; N, 3.98.

4-[1,2-Dideoxy-1-(thymin-1-yl)-β-D-ribofuranuronamido]-*O*-(4,4'-dimethoxytrityl)ethanol (10). To an ice-cooled (0-5 °C) solution of 1,2-dideoxy-1-(thymin-1-yl)-β-D-ribofuranuronic acid[24] (**5**, 0.62 g, 2.41 mmoles), 1-hydroxybenzotriazole monohydrate (HOBT, 0.46 g, 3.41 mmoles) and compound **4** (1.1 g, 3.03 mmoles) in anhydrous DMF (25 mL) were added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 0.79 g, 4.13 mmoles) and triethylamine (0.58 mL, 4.16 mmoles). The reaction mixture was allowed to warm to room temperature during 3 h. After stirring for an additional 15 h at ambient temperature, DMF was evaporated under high vacuum. The residue was partitioned between dichloromethane:water (50 mL each). The organic phase was separated and the aqueous layer was extracted with dichloromethane (3 x 50 mL). The combined organic layers was dried (Na_2SO_4) and evaporated under reduced pressure. The resulting residue was dissolved in a minimum amount of dichloromethane and the product was precipitated by the addition of hexanes. The hexane layer was separated by decantation and the remaining solid was purified by chromatography over a silica gel column packed in 10% triethylamine in dichloromethane. A gradient of 0-3% methanol:2% triethylamine in dichloromethane was used as the eluent to yield 1.25 g (86.2%) of analytically pure title compound; mp 164 - 166 °C. ^1H NMR (DMSO-d_6) : δ 1.70 (s, 3 H, CH_3), 2.10 - 2.25 (m, 2 H, $C_{2'}H$ and $C_{2''}H$), 2.98 (t, 2 H, CH_2CH_2O), 3.40 (br s, 2 H, CH_2CH_2O), 3.72 (s, 6 H, 2 OCH_3), 4.30 (br s, 2 H, $C_{3'}H$ and $C_{4'}H$), 5.66 (br s, 1 H, 3'O*H*), 6.34 (dd, 1 H, $C_{1'}H$), 6.85-7.41 (m, 13 H, *DMT*), 8.11 (s, 1 H, C_6H), 8.51 (br s, 1 H, N*H*CO) and 11.30 (br s, 1 H, N_3H). *Anal.* Calcd. for $C_{33}H_{35}N_3O_8 \cdot CH_3OH$: C, 64.44; H, 6.20; N, 6.63. Found: C, 64.23; H, 5.96; N, 6.31.

3-*O*-(*P*-β-Cyanoethoxy-*N,N*-diisopropylaminophosphinyl-4-[1,2-dideoxy-1-(thymin-1-yl)-β-D-ribofuranuronamido]-*O*-(4,4'-dimethoxytrityl)ethanol (11). To a solution of **10** (0.50 g, 0.83 mmole) and *N,N*-diisopropylethylamine (0.58 mL, 3.32 mmoles) in anhydrous dichloromethane (10 mL) was added 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (0.40 mL, 1.7 mmoles) under an argon atmosphere. After stirring for 15 min at room temperature, the reaction mixture was diluted with 10% triethylamine in ethyl acetate solution (100 mL). The solution was washed with aqueous saturated sodium bicarbonate solution (30 mL) and then dried (Na_2SO_4). The solvent was evaporated to dryness. The resulting residue was purified on a silica gel column using ethyl acetate:dichloromethane:triethylamine (45:45:10, v/v) solution as the eluent. The appropriate homogeneous fractions were pooled, the solvent evaporated and the residue was dissolved in a minimum volume of dichloromethane. The product was precipitated by the addition of the dichloromethane solution to pentane at -20 °C to give a colorless amorphous powder, yield 0.57 g (85.5%). ^{31}P NMR ($CDCl_3$): δ 148.61 and 150.76; ^1H NMR (CD_3CN): δ 1.13 (m, 12 H, $N[CH(CH_3)_2]_2$, 1.70 (s, 3 H, C_5CH_3), 3.71 (s, 6 H, 2 OCH_3), 6.25 (m, 1 *H*, $C_{1'}H$), 7.66 and 7.57 (2s, C_6H of the isomers) and other protons. *Anal.* Calcd. for $C_{42}H_{52}N_5O_9P$. pentane: C, 64.58; H, 7.38; N, 8.01; P, 3.54. Found: C, 64.65; H, 7.71; N, 7.71; P, 3.44.

4-[1,2-Dideoxy-1-(guanin-9-yl)-β-D-ribofuranuronamido]-*O*-(4,4'-dimethoxytrityl)ethanol (9). 1,2-Dideoxy-1-(guanin-9-yl)-β-D-ribofuranuronic acid triethylammonium salt[25] (**6**, 0.35 g, 0.9 mmole) and compound **4** (0.38 g, 1.04 mmoles) were dried by coevaporation with anhydrous dioxane (5 x 15 mL). The dry mixture was dissolved in anhydrous DMF (10 mL) to which 1-hydroxybenzotriazole monohydrate (HOBT, 0.17 g, 1.27 mmoles) was added. The mixture was cooled in an ice bath (0-5 °C) before EDC (0.30 g, 1.55 mmoles) and triethylamine (TEA, 216 μL, 1.55 mmoles) were added. The reaction mixture was allowed to warm to room temperature in about 3 h. After stirring for an additional 18 h at ambient temperature, the DMF was evaporated under vacuum at 30 °C. The residue was dissolved in dichloromethane (50 mL) and washed with water (2 x 15 mL). After drying (Na_2SO_4), the organic phase was evaporated and the resulting residue was purified on a silica gel column packed in 10% triethylamine in dichloromethane. The column was eluted with 0-12% methanol:2% triethylamine in dichloromethane to give a homogeneous colorless product, yield 0.41 g (73.6%); mp 189 - 190 °C. ^1H NMR (DMSO-d_6) :δ 2.10-2.30 (m, 1 H, $C_{2'}H$), 2.60-2.80 (m, 1 H, $C_{2''}H$), 2.85-3.00 (m, 2 H, $NHCH_2CH_2O$), 3.42 (br

s, 2 H, $NHCH_2CH_2O$), 3.72 (s, 6 H, 2 OCH_3), 4.35 (s, 1 H, $C_{4'}H$), 4.45 (br s, 1 H, $C_{3'}H$), 5.75 (d, 1 H, $C_{3'}OH$), 6.29 (dd, 1 H, J = 5.2 Hz, $C_{1'}H$), 6.51 (br s, 2 H, NH_2), 6.75-7.50 (m, 13 H, *DMT*), 8.02 (s, 1 H, C_8H), 8.38 (t, 1 H, $OCNHCH_2$) and 10.74 (br s, 1 H, N*H*). *Anal.* Calcd. for $C_{33}H_{34}N_6O_7 \cdot 3/4\ CH_3OH$: C, 62.30; H, 5.73; N, 12.92. Found: C, 62.54; H, 5.85; N, 12.59.

4-[1,2-Dideoxy-1-(N^2-isobutyrylguanin-9-yl)-β-D-ribofuranuronamido]-*O*-(4,4'-dimethoxytrityl)ethanol (7). To an ice-cooled solution of 9 (0.18 g, 0.29 mmole) in anhydrous pyridine (4 mL) was added chlorotrimethylsilane (0.36 mL, 2.87 mmoles). After stirring at room temperature for 1.5 h, the reaction mixture was cooled again in an ice-bath and isobutyryl chloride (30.7 μL, 0.29 mmole) was added. The reaction was continued for an additional 2 h, before it was quenched by the addition of water (13 mL). Dichloromethane (100 mL) was added to the reaction mixture and the organic layer was separated and evaporated to dryness. The residue was redissolved in methanol (3 x 50 mL) and evaporated at 40 °C. The product was purified on a silica gel column packed in dichloromethane containing 10% triethylamine. The column was eluted with a mixture of 0-2% methanol:2% triethylamine in dichloromethane. The appropriate homogeneous fractions were pooled and evaporated to dryness. The product was precipitated by the addition of the dichloromethane solution to hexanes to give an amorphous powder, yield 0.16 g (82%); mp 148 - 150 °C. ^{1}H NMR (DMSO-d_6): δ 1.12 [d, 6 H, $COCH(CH_3)_2$], 2.20-2.40 (m, 1 H, $C_{2'}H$), 2.59 - 2.90 (m, 2 H, $C_{2''}H$ and $COCHMe_2$), 2.95 (br s, 2 H, $NHCH_2CH_2$), 3.40 (br s, 2 H, $NHCH_2CH_2O$-), 3.71 (s, 6 H, 2 OCH_3), 4.34 (s, 1 H, $C_{4'}H$), 4.49 (s, 1 H, $C_{3'}H$), 5.73 (s, 1 H, $C_{3'}OH$), 6.37 (dd, 1 H, J = 5.0 Hz and 5.6 Hz, $C_{1'}H$), 6.70 - 7.50 (m, 13 H, *DMT*), 8.20 (t, 1 H, $NHCH_2$), 8.34 (s, 1 H, C_8H), 11.69 (s, 1 H, N*H*) and 12.50 (s, 1 H, N*H*). *Anal.* Calcd. for $C_{37}H_{40}N_6O_8 \cdot CH_3OH$: C, 62.62; H, 6.08; N, 11.53. Found: C, 62.45; H, 5.98; N, 11.59.

3-*O*-(*P*-β-Cyanoethoxy-*N,N*-diisopropylaminophosphinyl-4-[1,2-dideoxy-1-(N^2-isobutyrylguanin-9-yl)-β-D-ribofuranuronamido]-*O*-(4,4'-dimethoxytrityl)ethanol (8). In a similar manner as described for the preparation of **11**, phosphitylation of 7 (0.50 g, 0.72 mmoles) with 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (0.32 mL, 1.45 mmoles) in the presence of *N,N*-diisopropylethylamine (0.5 mL, 2.88 mmoles) in anhydrous dichloromethane (10 mL) gave 0.53 g (82.3%) of the title compound. ^{31}P NMR (CD_3CN): δ 149.25 and 149.95; ^{1}H NMR (CD_3CN): δ 1.20 [m, 6 H, $COCH(CH_3)_2$], 3.75 (s, 6 H, 2 OCH_3), 6.34 (dd, 1 H, J = 5.2 Hz and 5.6 Hz, $C_{1'}H$), 7.90 and 7.89 (2s, 2 H, C_8H of the

isomers) and other protons. *Anal.* Calcd. for $C_{46}H_{57}N_8O_9P{\cdot}CH_3OH$: C, 60.76; H, 6.62; N, 12.06. Found: C, 60.52; H, 6.63; N, 12.18.

5'-Deoxy-5'-(2-hydroxyethylthio)thymidine (13). 3'-*O*-Acetyl-5'-chloro-5'-deoxythymidine[27] (**12**, 6.05 g, 20 mmoles) was dissolved in a mixture of 2 N sodium hydroxide solution (50 mL) and 2-mercaptoethanol (3.12 g, 40 mmoles). The mixture was heated at 80 °C for 1 h. After cooling to O °C, the mixture was acidified (pH 4) with acetic acid. The product that separated was collected by filtration and crystallized from aqueous ethanol to yield 5.14 g (85%) of **13** as needles, mp 118 - 120 °C. ^{1}H NMR (DMSO-d_6): δ 1.80 (s, 3 H, CH_3), 2.05 (m, 2 H, $C_{2'}H$ and $C_{2''}H$), 2.70 (m, 4 H, H_2CSCH_2), 3.56 (m, 2 H, CH_2OH), 3.84 (m, 1 H, $C_{4'}H$), 4.16 (m, 1 H, $C_{3'}H$), 4.80 (t, 1 H, CH_2OH), 5.36 (d, 1 H, $C_{3'}OH$), 6.17 (t, 1 H, J = 6.8 Hz, $C_{1'}H$), 7.50 (s, 1 H, C_6H) and 11.32 (s, 1 H, N*H*). *Anal.* Calcd. for $C_{12}H_{18}N_2O_5S$: C, 47.67; H, 6.00; N, 9.26; S, 10.60. Found: C, 47.67; H, 6.01; N, 9.17; S, 10.82.

5'-Deoxy-5'-(2-*O*-4,4'-dimethoxytritylethylthio)thymidine (14). To a solution of **13** (1.0 g, 3.31 mmoles) in anhydrous pyridine (20 mL) was added 4,4'-dimethoxytrityl chloride (1.33 g, 3.92 mmoles) and the mixture was stirred at room temperature for 2.5 h. The reaction mixture was diluted with dichloromethane (100 mL), the organic layer was separated and washed with 5% sodium hydrogen carbonate solution (50 mL). The aqueous layer was reextracted with dichloromethane (3 x 50 mL). The combined organic layers was dried (Na_2SO_4) and evaporated to dryness. The residue was purified on a silica gel column using 0 to 2.5% methanol in dichloromethane as the eluent. The homogeneous fractions were pooled and evaporated to yield 1.50 g (75%) of **14**, mp 84 - 86 °C. ^{1}H NMR (DMSO-d_6): δ 1.74 (s, 3 H, CCH_3), 2.0 - 2.30 (m, 2 H, $C_{2'}H$ and $C_{2''}H$), 2.65 - 3.0 (m, 4 H, H_2CSCH_2), 3.16 (t, 2 H, OCH_2), 3.74 (s, 6 H, 2 OCH_3), 3.81 (m, 1 H, $C_{4'}H$), 4.15 (m, 1 H, $C_{3'}H$), 5.37 (d, 1 H, $C_{3'}OH$), 6.18 (t, 1 H, J = 5.8 Hz, $C_{1'}H$), 6.75 - 7.45 (m, 13 H, *DMT*), 7.48 (s, 1 H, C_6H) and 11.34 (s, 1 H, N*H*). *Anal.* Calcd. for $C_{33}H_{36}N_2O_7S$. 1/2 CH_3OH: C, 64.82; H, 6.17; N, 4.51. Found: C, 64.60; H, 6.04; N, 4.44.

3'-*O*-(*P*-β-Cyanoethoxy-*N,N*-diisopropylaminophosphinyl)-5'-deoxy-5'-(2-*O*-4,4'-dimethoxytritylethylthio)thymidine (15). In a similar manner as described for the preparation of **11**, phosphitylation of **14** (1.21 g, 2 mmoles) with 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (0.5 mL, 2.3 mmoles) in the

presence of *N,N*-diisopropylethylamine (1.39 mL, 8 mmoles) in anhydrous dichloromethane (8 mL) gave 1.25 g (77.7%) of the title compound. ^{31}P NMR (CD_3CN): δ 149.33; ^1H NMR (CD_3CN): δ 1.0 [m, 12 H, 2 $CH(CH_3)_2$], 1.77 (s, 3 H, CH_3), 2.15 - 2.90 (m, 8 H, H_2CS, $C_{5'}H_2$, $NCCH_2$, $C_{2'}H$ and $C_{2''}H$), 3.20 (m, 2 H, 2 $NCHMe_2$), 3.40 - 3.60 (m, 4 H, OCH_2CH_2), 3.75 (s, 6 H, 2 OCH_3), 4.05 (m, 1 H, $C_{4'}H$), 4.45 (m, 1 H, $C_{3'}H$), 6.18 (t, 1 H, J = 6.8 Hz, $C_{1'}H$), 6.70 - 7.45 (m, 13 H, *DMT*) and 7.46 (s, 1 H, C_6H).

2',5'-Dideoxy-5'-(2-hydroxyethylthio)-3'-*O*-(diphenyl-*tert*-butylsilyl)-N^2-isobutyrylguanosine (17). A solution of 2',5'-dideoxy-5'-iodo-3'-*O*-(diphenyl-*tert*-butylsilyl)-N^2-isobutyrylguanosine[29] (**16**, 2.07 g, 3 mmoles) in anhydrous dioxane (100 mL) was added to a suspension of the sodium salt of 2-mercaptoethanol [the sodium salt was generated by the addition of sodium hydride (80% suspension in oil, 270 mg) to a solution of 2-mercaptoethanol (0.63 mL) in dry dioxane (12 mL) at 0 °C, followed by stirring for 30 min] in anhydrous dioxane. The reaction mixture was stirred at room temperature for 3.5 h. The salt that separated was collected by filtration and washed with dichloromethane (2 x 25 mL). The combined filtrates was evaporated and the product was purified by silica gel chromatography using 0 to 4% methanol in dichloromethane as the eluent, to yield 1.65 g (86.5%) of **17**, mp 110 - 112 °C. ^1H NMR (DMSO-d_6): δ 1.08 (s, 9 H, *tBu*), 1.13 [d, 6 H, $COCH(CH_3)_2$], 2.20 - 2.90 (m, 5 H, $C_{2'}H$, $C_{2''}H$, SCH_2CH_2OH and *CH*), 3.36 (m, 4 H, $C_{5'}H_2$ and SCH_2CH_2OH), 4.01 (m, 1 H, $C_{4'}H$), 4.45 (m, 1 H, $C_{3'}H$), 6.34 (dd, 1 H, J = 5.6 Hz, $C_{1'}H$), 7.50 (s, 5 H, *Ph*), 7.70 (s, 5 H, *Ph*), 8.20 (s, 1 H, C_8H), 11.60 (s, 1 H, N*H*) and 12.10 (s, 1 H, N*H*). *Anal.* Calcd. for $C_{32}H_{41}N_5O_5SSi \cdot 1/2\ CH_3OH$: C, 59.88; H, 6.65; N, 10.74; S, 4.92. Found: C, 59.91; H, 6.63; N, 10.51; S, 5.22.

2',5'-Dideoxy-5'-(2-*O*-4,4'-dimethoxytritylethylthio)-3'-*O*-diphenyl-*tert*-butylsilyl)-N^2-isobutyrylguanosine (18). In a similar manner as described for the preparation of **14**, tritylation of **17** (1.6 g, 2.49 mmoles) with 4,4'-dimethoxytrityl chloride (1.18 g, 3.48 mmoles) in pyridine gave 2.0 g (84.75%) of **18**, mp 102 - 103 °C. ^1H NMR (DMSO-d_6): δ 1.00 (s, 9 H, *tBu*), 1.12 and 1.13 [2d, 6 H, $COCH(CH_3)_2$], 2.10 - 3.10 (m, 8 H, $C_{2'}H$, $C_{2''}H$, $C_{5'}H_2$ and SCH_2CH_2O), 3.70 (s, 6 H, 2 OCH_3), 4.05 (m, 1 H, $C_{4'}H$), 4.45 (m, 1 H, $C_{3'}H$), 6.35 (dd, 1 H, J = 5.6 Hz, $C_{1'}H$), 6.70 - 7.70 (m, 23 H, 2 Ph and *DMT*), 8.15 (s, 1 H, C_8H), 11.61 (s, 1 H, N*H*) and 12.10 (s, 1 H, N*H*). *Anal.* Calcd. for $C_{53}H_{59}N_5O_7SSi$: C, 67.85; H, 6.34; N, 7.46; S, 3.42. Found: C, 67.89; H, 6.35; N, 7.22; S, 3.30.

2',5'-Dideoxy-5'-(2-*O*-4,4'-dimethoxytritylethylthio)-N^2-isobutyryl-guanosine (19). To a solution of **18** (2.2 g, 2.35 mmoles) in dry tetrahydrofuran (15 mL) was added tetrabutylammonium fluoride (1.0 M solution in THF, 7 mL, 7 mmoles). After stirring the reaction mixture at room temperature for 2 h, the solvent was evaporated and the residue was purified by silica gel column chromatography using 0 to 3% methanol in dichloromethane as the eluent, to yield 3.30 g (79.3%) of **19**, mp 106 - 108 °C. ^{1}H NMR (DMSO-d_6)): δ 1.15 [d, 6 H, $COCH(CH_3)_2$], 2.10 - 3.20 [m, 9 H, SCH_2CH_2O, $C_{5'}H_2$, $C_{2'}H$, $C_{2''}H$ and $COCH(CH_3)_2$], 3.70 (s, 6 H, 2 OCH_3), 3.91 (m, 1 H, $C_{4'}H$), 4.30 (m, 1 H, $C_{3'}H$), 5.45 (d, 1 H, $C_{3'}OH$), 6.20 (t, 1 H, J = 6.4 Hz, $C_{1'}H$), 6.80 - 7.45 (m, 13 H, *DMT*), 8.20 (s, 1 H, C_8H), 11.60 (s, 1 H, N*H*) and 12.10 (s, 1 H, N*H*). *Anal.* Calcd. for $C_{37}H_{41}N_5O_7S \cdot 1/2\ CH_3OH$; C, 62.92; H, 6.05; N, 9.78; S, 4.48. Found: C, 63.32; H, 5.96; N, 9.35; S, 4.29.

3'-*O*-(*P*-β-Cyanoethoxy-*N,N*-diisopropylaminophosphinyl)-2',5'-di-deoxy-5'-(2-*O*-4,4'-dimethoxytritylethylthio)-N^2-isobutyrylguanosine (20). In a similar manner as described for the preparation of **11**, phosphitylation of **19** (1.1 g, 1.43 mmoles) with 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (0.38 mL) in the presence of *N,N*-diisopropylethylamine (1.0 mL, 5.76 mmoles) in anhydrous dichloromethane (15 mL) at room temperature gave 1.15 g (89.8%) of the title compound. ^{31}P NMR (CD_3CN): δ 149.21 and 149.38. ^{1}H NMR (CD_3CN): δ 1.10 [m, 12 H, 2 $CH(CH_3)_2$], 2.40 - 3.30 [m, 9 H, SCH_2CH_2, $C_{5'}H_2$, CH_2CN and $CH(CH_3)_2$], 3.50 - 3.80 (m, 4 H, $2CH_2O$), 3.75 (s, 6 H, 2 OCH_3), 4.15 (m, 1 H, $C_{4'}H$), 4.60 (m, 1 H, $C_{3'}H$), 6.18 (t, 1 H, J = 6.8 Hz, $C_{1'}H$), 6.80 - 7.40 (m, 13 H, *DMT*) and 7.85 (s, 1 H, C_8H).

REFERENCES

1. Presented at the 10th International Round Table on Nucleosides, Nucleotides and their Biological Applications, September 16 - 20, **1992**, Park City, Utah, U. S. A., P- 68.
2. Cooney, M.; Czernuszewicz, G.; Postel, E. H.; Flint, S. J.; Hogan, M. E. *Science*, **1988**, *241*, 456.
3. Durland, R. H.; Kessler, D. J.; Gunnell, S.; Duvic, M.; Pettitt, B. M.; Hogan, M. E. *Biochemistry*, **1991**, *30*, 9246.
4. Pilch, D. S.; Levenson, C.; Shafer, R. H. *Biochemistry*, **1991**, *30*, 6081.
5. Birg, F.; Praseuth, D.; Zerial, A.; Thuong, N. T.; Asseline, U.; Le Doan, T.; Helene, C. *Nucleic Acids Res.*, **1990**, *18*, 2901.
6. Maher III, L. J.; Dervan, P. B.; Wold, B. J. *Biochemistry*, **1990**, *29*, 8820.
7. Arnott, S.; Selsing, E. *J. Mol. Biol.*, **1974**, *88*, 509.

8. Miller, P. S.; Yano, J.; Yano, E.; Carroll, C.; Jayaraman, K.; Ts'O, P. O. P. *Biochemistry*, **1979**, *18*, 5134.
9. Bohringer, M. P.; Graff, D.; Caruthers, M. H. *Tetrahedron Lett.*, **1993**, *34*, 2723.
10. Mag, M.; Schmidt, R.; Engels, J. W. *Tetrahedron Lett.*, **1992**, *33*, 7319.
11. Vyle, J. S.; Li, S.; Cosstick, R. *Tetrahedron Lett.*, **1992**, *33*, 3017.
12. Stec, W. J.; Zon, G.; Egan, W.; Stec, B. *J. Am. Chem. Soc.*, **1984**, *106*, 6077.
13. Brill, W. K.-D.; Nielsen, J.; Caruthers, M. H. *J. Am. Chem. Soc.*, **1991**, *113*, 3972.
14. Jones, R. J.; Lin, K. Y.; Milligan, J. F.; Wadwani, S.; Matteucci, *J. Org. Chem.*, **1993**, *58*, 2983; Matteucci, M. *Tetrahedron Lett.*, **1990**, *31*, 2385.
15. Tittensor, J. R. *J. Chem. Soc. (C)*, **1971**, 2656.
16. Cormier, J. F.; Ogilvie, K. K. *Nucleic Acids Res.*, **1988**, *16*, 4583.
17. Musicki, B.; Widlanski, T. S. *Tetrahedron Lett.*, **1991**, *32*, 1267.
18. Vasseur, J. J.; Debart, F.; Sanghvi, Y. S.; Cook, P. D. *J. Am. Chem. Soc.*, **1992**, *114*, 4006.
19. Huie, E. M.; Kirshenbaum, M. R.; Trainor, G. L. *J. Org. Chem.*, **1992**, *57*, 4569.
20. Panneconque, C.; Wigernick, P.; Aerschoft, A. V.; Herdewijn, P. *Tetrahedron lett.*, **1992**, *33*, 7609.
21. Meng, B.; Kawai, S. H.; Wang, W.; Just, G.; Giannaris, P. A.; Damha, M. J. *Angew. Chem. Intl. Ed.* **1993**, *32*, 729; Kawai, S. H.; Wang, P., Giannaris, P. A.; Damha, M. J.; Just, G. *Nucleic Acids Res.*, **1993**, *21*, 1473.
22. Reynolds, R. C.; Crooks, P. A.; Maddry, J. A.; Akhtar, M. S.; Montgomery, J. A.; Secrist III, J. A. *J. Org. Chem.*, **1992**, *57*, 2983.
23. Butterfield, K.; Thomas, E. J. *Synlett.*, **1993**, 411.
24. Horne, D. A.; Dervan, P. B. *J. Am. Chem. Soc.*, **1990**, *112*, 2435.
25. Rao, T. S.; Jayaraman, K.; Revankar, G. R. *Tetrahedron Lett.*, (In press)
26. Moss, G. P.; Reese, C. B.; Schofield, K.; Shapiro, R.; Todd, A. R. *J. Chem. Soc.*, **1963**, 1149.
27. Jones, A. S.; Williamson, A. R.; Winkley, M. W. *Carbohydr. Res.*, **1965**, *1*, 187.
28. Ti, G. S.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.*, **1982** , *104* , 1316.
29. Wang, Y.; Hogankamp, H. P. C.; Long, R. A.; Revankar , G. R.; Robins, R. K. *Carbohydr. Res.*, **1977**, *59*, 449.
30. Sufrin, J. R.; Spiess, A. J.; Kramer, D. L.; Libby, P. R.; Miller, J. T.; Bernacki, R. J.; Lee, Y.; Borchardt, R. T.; Porter, C. W. *J. Med. Chem.*, **1991**, *34*, 2600.
31. Sproat, B. S.; Beijer, B.; Rider, P. *Nucleic Acids Res.*, **1987**, *15* , 6181.
32. Sambrook, J.; Fritsch, E. F.; Maniatis, T. *Molecular Cloning, A Laboratory Manual*, IInd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N. Y., **1989**, p 6.36.

Received 7/21/93
Accepted 9/21/93