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A Novel Synthesis of S[6]-Cyanoethyl-2'-deoxy-6-thioguanosine and its Incorporation into Triple Helix Forming Oligonucleotides[1]

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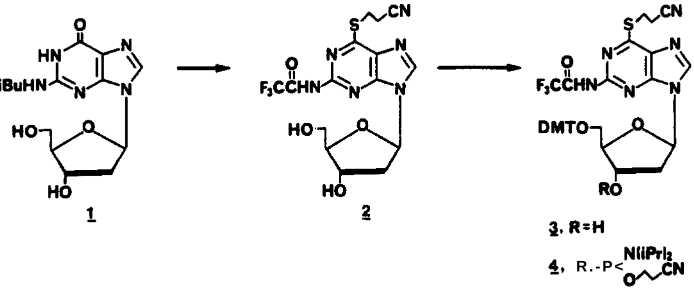
ABSTRACT: **A simple and expeditious synthesis of S[6]-cyanoethyl-2'-deoxy-6-thioguanosine from 2'-deoxyguanosine has been accomplished in good yield and incorporated into several triple helix forming oligonucleotides using the solid-phase phosphoramidite chemistry.**

Recently it has been shown that in the presence of divalent metal ions, short guanine-rich oligonucleotides can bind to specific sites in duplex DNA to form triple helices[2-4] at physiological pH. Evidence has also been provided to show 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 target protein. Therefore, the potential therapeutic significance of these triplex forming oligonucleotides (TFOs) is obvious.

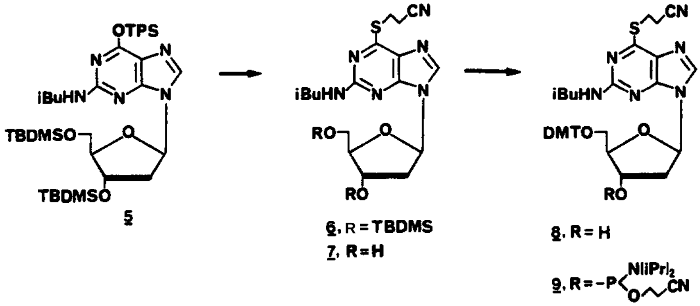
The preponderance of guanine residues in these TFOs can promote self-association and lead to stable G-tetrads[7-10] by the formation of eight H-bonds and by coordination of the four O^6 atoms of guanine with alkali metal ions bound to the center of the quadruplex. Chemical modification of the guanine moiety that disrupts the multiple hydrogen bonding of the G-tetrad can reduce or eliminate its formation. Replacement of all or some of the guanine residues in these TFOs with 6-thioguanine is expected to reduce self-association by interfering with the coordination of alkali metal and by reducing the strength of H-bonds to that position. In addition, the increased atomic size of sulfur relative to oxygen may lead to significant steric repulsion in the tetrad. However, this modification is not expected to affect the groups believed to be involved in the H-bonding of GGC triplets. We now report a novel and simple synthesis of protected 2'-deoxy-6-thioguanosine (S^6-dGuo) and its incorporation into several TFOs using the automated solid-phase phosphoramidite chemistry.

Although a few reports have appeared describing the synthesis of S^6-dGuo containing oligonucleotides,[11-13] none of these syntheses considered protecting the thione group of S^6-dGuo, which is of paramount importance to prevent oxidative hydrolysis that is expected in sulfur-containing nucleotides. Our own attempts to use 5'-O-dimethoxytrityl-2'-deoxy-6-thioguanosine-3'-phosphoramidite in the solid-phase oligonucleotide synthesis resulted not only in lower coupling yields but also in the formation of multiple products. Therefore, we decided to protect the thione functionality of S^6-dGuo with a cyanoethyl group, since the cyanoethyl group can be readily removed under mild alkaline conditions. During the course of the present work, Christopherson and Broom[14] reported the synthesis of oligonucleotides containing S^6-dGuo using the cyanoethyl group for the protection of the thione moiety. Their approach involved the synthesis of S-protected building block from the preformed S^6-dGuo.



In our first approach, N^2-isobutyryl-2'-deoxyguanosine[15] (1) was transformed to the corresponding 6-pyridyl intermediate using trifluoroacetic anhydride in pyridine[16]. Since the pyridyl group attached to the 6-position of purine being very susceptible to nucleophilic displacement,[17] the 6-pyridyl intermediate was reacted with 2-mercaptopropionitrile. This one-flask, two-step procedure gave a homogeneous product (85% yield), which was identified as S^6-cyanoethyl-N^2-trifluoroacetyl-2'-deoxy-6-thioguanosine[18] (2). The 5'-hydroxyl group of 2 was selectively protected as the 4,4'-dimethoxytrityl ether by the treatment with dimethoxytrityl chloride in pyridine. The pure product 3 was isolated in a 76% yield after silica gel column chromatography. Compound 3 was conveniently converted into the corresponding 3'-phosphoramidite[19] (4) by reaction with 2-cyanoethyl-N,N,N',N'-tetraisopropylphosphoro-diamidite in the presence of tetrazole and diisopropylamine in dichloromethane. The yield of pure 4 after silica gel column chromatography was 83%.

In this approach, during the preparation of 2, the N^2-isobutyryl group was displaced by a trifluoroacetyl moiety. Although the exact mechanism of this displacement is unknown, it may be due to the high reactivity of trifluoroacetic anhydride, which is reacting with the basic NI-I functionality displacing the isobutyryl group.



Although the phosphoramidite **4** was found to be suitable for solid-phase DNA synthesis, this methodology limits the possibility of using a very labile protecting group on the exocyclic amino function. In view of this we have also synthesized the fully protected **S^6-dGuo** building block ***via*** a **6-O-mesitylenesulfonyl** intermediate. Thus, treatment of **N^2-isobutyryl-6-O-triisopropylbenzenesulfonyl-3',5'-O-bis(*tert*-butyldimethylsilyl)-2'-deoxyguanosine**[20] **(5)** with 1-methylpyrrolidine (10 eq) and **2-mercaptopropionitrile** (10 eq) in dichloromethane for 2 h at room temperature gave **S^6-(2-cyanoethyl)thio** ether **(6)** in a 89% yield. Selective removal of the sugar protecting groups using HF/tetrabutylammonium fluoride in dry pyridine afforded **N^2-isobutyryl-S^6-cyanoethyl-2'-deoxythioguanosine** (7), mp **164°C**. Although this approach allows the usage of any type of protecting groups for the amino function, in the present study it is demonstrated only by using a isobutyryl group. Compound **7** was converted to the corresponding **5'-O-dimethoxytrityl** derivative **(8)** by the conventional procedure, which on treatment with **2-cyanoethyl-N,N,N',N'-tetraisopropylphosphorodiamidite** in the presence of tetrazole and diisopropylamine gave the target building block [21] **(9)**.

TFOs containing **S^6-dGuo** residues were prepared with a **stepwise** coupling efficiency of **>98%** by coupling **9** three times to the solid support and increasing the reaction time for an additional 30 seconds on a ABI 3808 DNA synthesizer. Deprotection was performed with concentrated **NH_4OH** and the TFO was purified by ion-exchange HPLC using a Q-Sepharose column (Pharmacia). The purified product was desalted and analyzed on a 20% denaturing polyacrylamide gel after labeling with **^{32}P-ATP** using polynucleotide kinase. Unsubstituted oligonucleotide was used as the standard to compare its mobility and purity.

A series of **TFOs** were prepared based on the following model sequence, which was designed for in ***vitro*** testing of triplex formation

5'...d-GGGGTTGGGGGGTTGGGGGTTGGGGG...3'-NH_2

The test TFO is a 26-mer containing 20 **dGuo** and 6 T residues. The **TFOs containig S^6-dGuo** residues exhibit a characteristic UV absorption at 345 **nm.** Three, 11 and all 20 **dGuo** residues were replaced by **S^6-dGuo** units. The ratio of absorptions at 345 and 260 nm is consistent with the increasing number of **S^6-dGuo** residues, e.g. the TFO containing three **S^6-** residues had a ratio of 0.13 and for 11 and 20 residues, the ratios were 0.6 and 1.8, respectively.

In conclusion, this communication describes a simple and novel synthetic strategy which can be used for the synthesis of **TFOs** containing **S^6-dGuo** without the possibility of expected oxidative hydrolysis of the thione function.

References and Notes

1. This research was supported in part by the National Institutes of Health, National Cancer Institute, SBIR Grant **#1** R43 **CA59020-01**
2. Cooney, M.; Czernuszewicz, G.; Postel, E. H.; Flint, S. **J.;** Hogan, M. E. *Science,* 1988,242, 456459.
3. Durland, **R.** H.; Kessler, D. J.; **Gunnell,** S.; Duvic, M.; Pettitt, B. M.; Hogan, M.E. Biochemistry, **1991,** ***30,*** 9246-9255.
4. **Pilch,** D. S.; Levenson, C.; Shafer, R. H. ***Biochemistry,*** **1991,** ***30,*** **6081-6087.**
5. Birg, F.; Praseuth, D.; **Zerial,** A.; Thuong, N.; **Asseline,** U.; **LeDoan,** T.; Helene, C. Nucleic *Acids Res.*, **1990,** ***18,*** 2901-2908.
6. **Maher,** L. J.; Dervan, P.B.; **Wold,** B. J. Biochemistry, **1990,** ***29,*** 8820-8826.
7. Sen, D.; Gilbert, W. *Nature*, **1988,** ***334,*** **364-366.**

a. Sundquist, W. I.; **Klug,** A. Nature, 1989,342, 825829.

9. Jin, R.; Breslauer, K. J.; Jones, **R.** A.; Gaffney, **B.** L. Science, 1998,250, 543-546.
10. Kim, **J.;** Cheong, C.; Moore, P. B. *Nature*, **1991,** ***351,*** **331-332.**
11. Bronstein, S. M.; Richardson, F. C.; Skopek, T. R.; Swenberg, J. A. ***Proc.*** Am. *Assoc. Cancer Res.*, **1988,** ***29,*** 1401.
12. Rappaport, H. P. Nucleic Acids Res., ***1988,*** ***16,*** 7253-7267.
13. Richardson, F. C.; Bronstein, S. M. Intl. Patent Publication, No. **WO8909,** 778 (Oct. 19, 1989).
14. Christopherson, M. S.; Broom, A. D. Nucleic Acids *Res.*, 1991, ***19,*** 5719-5724.
15. Ti, G. S.; Gaffney, **B.** L.; Jones, R. A. ***J.*** Am. ***Chem. Soc.,*** 1982,104, 1316-1319.
16. **Fathi,** R.; **Goswami,** B.; **Kung,** P-P.; Gaffney, B. L.; Jones, R. A. *Tetrahedron* ***Lett.,*** **1990,** ***31,*** 319-322.
17. Kung, P-P.; Jones, **R.** A. *Tetrahedron* ***Lett.,*** **1991,** ***32,*** 39193922.
18. Compound **2** had mp of **168-169°C** (dec.). ^1H NMR ($DMSO$-d_6): δ 2.27-2.52 (m, 1 H, $C_{2''}H$), 2.69-2.84 (m, 1 H, $C_{2'}H$), 3.15 (t, J = 6.6 **Hz,** 2 H, $-CH_2CN$), 3.58 (m, 4 H, $C_{5'}CH_2$ and SC&-), 3.85 (m, 1 H, $C_{4'}H$), 4.44 (m, 1 H, $C_{3'}H$), 4.84 (br s, 1 H, $C_{5'}OH$), 5.33 (br s, 1 H, $C_{3'}OH$), 6.38 (t, J = 6.4 Hz, 1 H, $C_{1'}H$), 8.69 (s, 1 H, C_8H) and 12.17 (s, 1 H, CONK). **Anal**. Calcd. for $C_{15}H_{15}F_3N_6O_4S$. 0.25 H_2O: C, 41.24; H, 3.56; N, 19.26; F, 13.06. Found: C, 41.08; H, 3.43; N, 18.94; F, 13.05.
19. ^1H NMR (CD_3CN) : δ 1.00-1.35 [m, 6H, $-CH(CH_3)_2$], 1.95 [m, 6 H, $-CH(CH_3)_2$], 2.40-2.80 (m, 2 H, $C_{2'}H$ and $C_{2''}H$), 3.03 (t, 2 H, CH_2), 3.10-3.70 (m, 4 H, $C_{5'}CH_2$ and CH_2), 3.73 (s, 6 H, 2 OCH_3), 4.20 (m, 1 H, $C_{4'}H$), 4.85 (m, 1 H, $C_{3'}H$), 6.37 (t, J = 6.4 Hz, 1 H, $C_{1'}H$), 6.60-7.40 (m, 13 H, DMT) and 8.18 (s, 1 H, C_8H); ^{31}P NMR (CD_3CN) : δ 149.38 ppm. **Anal.** Calcd. for $C_{45}H_{50}F_3N_8O_7SP$: C, 57.80; H, 5.39; N, 11.98; S, 3.43. Found: C, 57.51; H, 5.74; N, 12.01; S, 3.30.
20. Gaffney, B. L.; Marky, L. A.; Jones, R. A. *Tetrahedron*, ***1984,*** ***40,*** **3-13.**
21. ^1H NMR (CD_3CN) : δ 1.15 (m, 18 H, isopropyl and isobutyryl protons), 1.95 (m, 1 H, $COCH$<), **2.40-2.90** (m, 2 H, $C_{2'}H$ and $C_{2''}H$), 3.00-3.90 (m, 6 H, $C_{5'}CH_2$ and $-CH_2$-CH_2), 3.73 (s, 6 H, 2 OCH_3), 4.20 (m, 1 H, $C_{4'}H$), 4.89 (m, 1 H, $C_{3'}H$), 6.34 (t, J = 6.40 **Hz,** 1 H, $C_{1'}H$), 6.67-7.38 (m, 13 H, **DMT),** 8.10 (s, 1 H, C_8H) and 8.75 (br s, 1 H, NH). ^{31}P NMR (CD_3CN): δ 149.29 **ppm.**

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