stannyl)-1,3-dithiane, **1h**, to less positive values and a 1-eV lowering of its lowest ionization potential compared with 1,3-dithiane.[21]

Acknowledgment. We gratefully acknowledge support of this work by the U.S. Public Health Service, National Institutes of Health, Grant No. HL15104. We thank Professor Dennis Lichtenberger and Dr. Mark Jatcho for measurement of the PES of **1h**.

(21) A very large, geometry-dependent stabilization of positive charge on a β-carbon by a carbon–tin bond has been reported: Lambert, J. B.; Wang, G.; Teramura, D. H. *J. Org. Chem.* **1988**, *53*, 5422–5428.

Site-Specific Adduct Formation in Oligomeric DNA Using a New Protecting Group

Francis Johnson,* Ivan Habus, Robert G. Gentles, Shinya Shibutani, Hong-Chang Lee, Charles R. Iden, and Robert Rieger

Department of Pharmacological Sciences
School of Medicine, Health Sciences Center
State University of New York at Stony Brook
Stony Brook, New York 11794-8651

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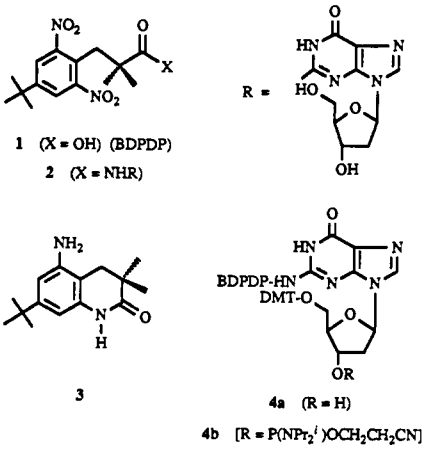
The synthesis of oligomeric DNA containing site-specifically-modified 2′-deoxynucleoside residues, which are considered to be mutagenic and/or carcinogenic lesions, is a topic of intense current interest.[1] Almost all of the methods available for such DNA syntheses involve a presynthetic strategy in which the modified base is synthesized in a protected form and then introduced into an oligomeric chain either by solution-based methods[2] or by an automated resin-based procedure.[3] Only one general *selective* postsynthetic strategy is known. This elegant method[4] involves the incorporation of a 2-fluoro-2′-deoxyinosine residue, whose fluorine atom sub equently can be replaced by treating the oligomeric DNA with an appropriate nucleophile.

We would now like to report a second approach which involves the use of a new protecting group. In this communication our strategy is demonstrated by the selective postsynthetic introduction of a single 8-fluorenylamino group into oligomers containing two 2′-deoxyguanosine residues. The protecting group that we have devised for this strategy is based on 3-(4-*tert*-butyl-2,6-dinitrophenyl)-2,2-dimethylpropionic acid (BDPDP, **1**), a compound that may be regarded as a phenyl-substituted pivalic acid. As a protecting group for the synthesis of oligomeric DNA, it carries a number of advantages: (a) it is easy to prepare,[5] (b) all three natural amino-containing 2′-deoxynucleosides implicit in DNA are easily derivatized by it,[9] (c) its amide derivatives are resistant to hydrolysis in basic solution because of its pivalate-like structure, (d) it is easily detached from the heterocyclic base by reduction[10] at neutral pH, liberating **3** by an internal ring closure reaction,[11] and lastly (e) it confers additional lipophilic character on the DNA, thus making the separation and purification of the desired 4,4-dimethoxytrityl (DMT) oligomer quite easy because it is the last peak to be eluted during chromatographic separation.



As a demonstration of the strategy, the BDPDP derivative of 2′-deoxyguanosine was first converted to the DMT phosphoroamidite **4b** by standard procedures.[12] This monomer was utilized with high coupling efficiency in a synthesis of two pentadecamers having compositions **5** and **6** in which dG* represents a deoxyguanosinyl residue protected by BDPDP. The other nucleosides that needed protection (dA and dG) during the synthesis were incorporated using the commercially-available phenoxyacetyl-protected forms[12c] of their DMT phosphoramidites.

5′-d(AATTG*TATAAGATAT)-3′ (**5**)

5′-d(AATTGTATAAG*ATAT)-3′ (**6**)

5′-d(AATTGTATAAGATAT)-3′ (**7**)

In the critical synthetic step, namely, the release and deprotection of the oligomer from the CPG resin support, it was found that treatment with 29% aqueous ammonia at 20 °C for 45–60 min was sufficient to remove the phenoxyacetyl groups[12c,13] while more than 80% of the BDPDP group was retained. The enhanced lipophilicity of the desired oligomers made them easy to separate,

* Corresponding address: Department of Chemistry, SUNY at Stony Brook, Graduate Chemistry Building, Stony Brook, NY 11794-3400.

(1) (a) Beland, F. A.; Kadlubar, F. F. *Environ. Health Perspect.* **1985**, *62*, 19. (b) Westra, J. G.; Kriek, E.; Hittenhausen, H. *Chem. Biol. Interact.* **1976**, *15*, 149. (c) Shibutani, S.; Gentles, R. G.; Iden, C. R.; Johnson, F. *J. Am. Chem. Soc.* **1990**, *112*, 5667. (d) Visser, A.; Westra, J. G. *Carcinogenesis* **1981**, *2*, 737. (e) Rio, P.; Bazgar, S.; Long, M. *Carcinogenesis* **1982**, *3*, 225. (f) Fuchs, R. P. P.; Schwartz, N.; Daune, M. P. *Nature* **1981**, *294*, 657. (g) Hetlich, R. H.; Djuric, Z.; Zhuo, Z.; Fullerton, N. F.; Casciano, D. A.; Breland, F. A. *Environ. Mol. Mutagen.* **1988**, *11*, 167. (h) Maher, V. M.; Yang, J.-L.; Mah, M. L.; McCornick, J. *Mutat. Res.* **1989**, *220*, 83. (i) Basu, A. K.; Essigmann, J. M. *Chem. Res. Toxicol.* **1988**, *1*, 1. (j) Johnson, D. L.; Reid, T. M.; Lee, M.-S.; King, C. M.; Romano, L. J. *Biochemistry* **1986**, *25*, 449. (k) Johnson, D. L.; Reid, T. M.; Lee, M.-S.; King, C. M.; Romano, L. J. *Carcinogenesis* **1987**, *8*, 619. (l) Shibutani, S.; Gentles, R. G.; Johnson, F.; Grollman, A. P. *Carcinogenesis* **1991**, *12*, 813.

(2) Ikehara, M.; Ohtsuka, E.; Uesugi, S.; Tanaka, T. In *Chemistry of Nucleosides and Nucleotides*; Townsend, L. B., Ed.; Plenum Press: New York, 1988; pp 283–367 and references cited therein.

(3) Kaplan, B. E.; Itakura, K. In *Synthesis and Applications of DNA and RNA*; Narang, S. A., Ed.; Academic Press: Orlando, FL, 1987; pp 9–45 and references cited therein.

(4) Harris, C. M.; Zhou, L.; Strand, E. A.; Harris, T. M. *J. Am. Chem. Soc.* **1991**, *113*, 4238.

(5) The synthesis of **1** is easily accomplished by a three-step procedure. 4-*tert*-Butylbenzyl bromide, prepared using the method of Mitchel and Iyer,[6] when added to the dianion of isobutyric acid[7] affords 3-(4-*tert*-butylphenyl)-2,2-dimethylpropionic acid (72% yield). When the latter is nitrated with $NO_2^+BF_4^-$ in acetonitrile,[8] **1** is obtained in 94% yield.

(6) Mitchell, R. H.; Iyer, V. S. *Synlett* **1989**, 55.

(7) (a) Creger, P. L. *J. Am. Chem. Soc.* **1967**, *89*, 2500. (b) Creger, P. L. *Ibid.* **1970**, *92*, 1396. (c) Creger, P. L. *Ibid.* **1970**, *92*, 1397.

(8) *Organic Syntheses*; Baumgarten, H. E., Ed.; John Wiley and Sons: New York, 1973; Collect. Vol. V, pp 480–485.

(9) Ti, G. S.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1982**, *104*, 1316.

(10) (a) Knecht, B. *Ber. Dtsch. Chem. Ges.* **1903**, *36*, 166. (b) Sachs, F.; Sichel, E. *Ber. Dtsch. Chem. Ges.* **1904**, *37*, 1861. (c) Ho, T.-L.; Wong, C. M. *Synthesis* **1974**, 45. (d) Somei, M.; Kato, K.; Inoue, S. *Chem. Pharm. Bull.* **1980**, *28*, 2515. (e) Stanovnik, B.; Tisler, M.; Polanc, S.; Gracner, M. *Synthesis* **1978**, 65.

(11) Other protecting groups that are released in a similar manner have been reported: (a) Holley, R. W.; Holley, A. D. *J. Am. Chem. Soc.* **1952**, *74*, 3069. (b) Johnson, D. A.; Panetta, C. A.; Smith, R. R. *J. Org. Chem.* **1966**, *31*, 2560. (c) Panetta, C. A. *J. Org. Chem.* **1969**, *34*, 2773. (d) Entwistle, I. D. *Tetrahedron Lett.* **1979**, 555.

(12) (a) Barone, A. D.; Tang, J. Y.; Caruthers, M. H. *Nucleic Acids Res.* **1984**, *12*, 4051. (b) Sinha, N. D.; Biernat, J.; MacManus, J.; Koster, H. *Nucleic Acids Res.* **1984**, *12*, 4539. (c) Schulhof, J. C.; Molko, D.; Teoule, R. *Nucleic Acids Res.* **1987**, *15*, 397.

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

0002-7863/92/1514-4923$03.00/0

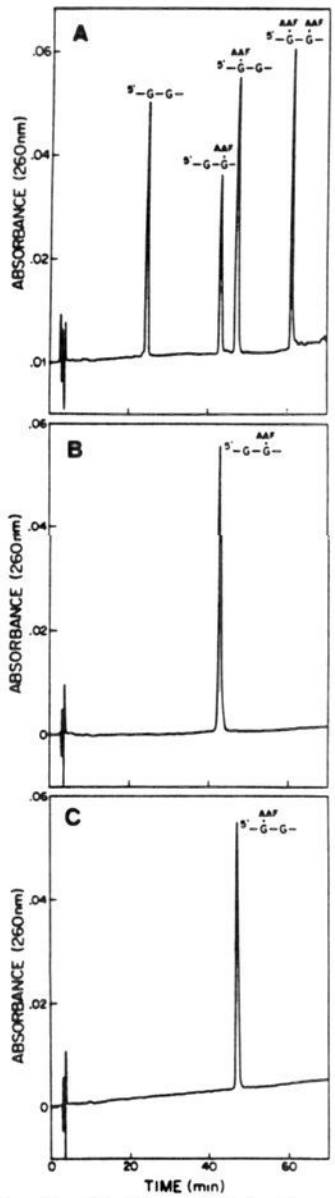


Figure 1. HPLC profile of AAF-modified oligodeoxynucleotides. The unmodified oligodeoxynucleotide **7** or protected oligodeoxynucleotide **5** or **6** (A) was allowed to react with AAAF in 10 mM sodium citrate buffer (pH 6.85). After removal of the protecting group from **8** or **9**, each AAF-modified oligomer, **10** (B) or **11** (C), was subjected to HPLC using a reverse-phase column, μBondapak C-18 (0.30 × 30 cm). The column was eluted with a linear gradient of 0.05 M triethylamine acetate buffer (pH 7.0) containing 10–15% acetonitrile over 45 min and then continuously with 15–30% acetonitrile over 45 min at a flow rate 1.0 mL/min.

with yields 50–60% of that obtained in the case of the standard oligomer **7**, after chromatographic purification at the pre- and post-DMT-removal stages.

Treatment of each of these oligomers with 2-(*N*-acetoxy-*N*-acetylamino)fluorene under solvolytic conditions[1j-l] then led to the monoaminoarylated products **8** and **9** in the cases of **5** and **6**, respectively, but to a mixture of mono- and disubstituted products **10**, **11**, and **12**, respectively, in the case of **7**.

5′-d(AATTG*TATAAG(8-AAF)ATAT)-3′ (**8**)

5′-d(AATTG(8-AAF)TATAAG*ATAT)-3′ (**9**)

5′-d(AATTGTATAAG(8-AAF)ATAT)-3′ (**10**)

5′-d(AATTG(8-AAF)TATAAGATAT)-3′ (**11**)

5′-d(AATTG(8-AAF)TATAAG(8-AAF)ATAT)-3′ (**12**)

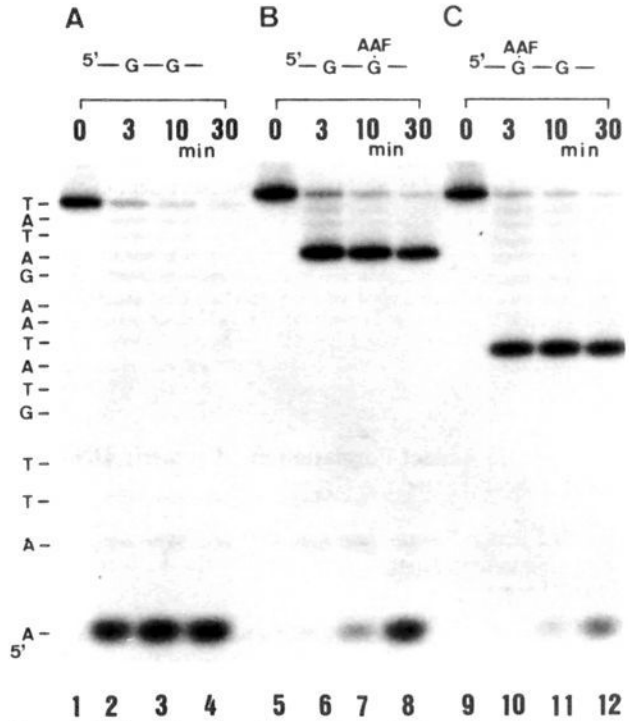


Figure 2. Polyacrylamide gel electrophoresis of enzymatically-digested unmodified and AAF-modified oligodeoxynucleotides. The unmodified oligodeoxynucleotide **7** (A) or AAF-modified oligodeoxynucleotide **10** (B) or **11** (C) was labeled with ^{32}P as described in the supplementary material. The 5′-^{32}P-labeled oligodeoxynucleotide (1.0 pmol) was digested with 1.0×10^{-5} unit of venom phosphodiesterase I in 100 mM Tris-HCl buffer (pH 8.0) for 3, 10, and 30 min at 25 °C, heated at 95 °C for 3 min, and then subjected to electrophoresis on 20% polyacrylamide gel (35 cm) for 6 h at 1200 V.

When either **8** or **9** was submitted to reduction with an excess of Ti^{3+} ion in an aqueous buffered medium (pH 7.0) under anaerobic conditions, complete removal of the BDPDP group occurred within 5 min, without causing deacetylation of the AAF residue. Purification of the products by HPLC then gave in each case a 70% yield of a material identical with **10** (t_R 42.8 min) (from **8**) or **11** (r_R 47.2 min) (from **9**). No material corresponding to **12** (t_R 61.1 min) or to other isomers[14] could be detected (Figure 1). Ultraviolet spectroscopy revealed the presence of a single AAF residue in each of these oligomers (**10** and **11**), results in accord with the HPLC retention times. Proofs of composition of oligomers **10** and **11** were obtained by the enzymatic partial degradation of ^{32}P-radiolabeled (5′-terminus) samples[15] using snake venom phosphodiesterase. PAGE analysis of the products revealed (Figure 2) that digestion was retarded at the fourth or tenth phosphate linkage of **10** and **11**, respectively, indicating a bulky residue to be present at the corresponding fifth or eleventh position of the chains.

Currently we are investigating the use of this protecting group to allow site-specific functionalization of the oligomeric chain while it is still attached to the resin support.

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Supplementary Material Available: Listings of synthetic procedures and spectral data for all new compounds (8 pages). Ordering information is given on any current masthead page.

(14) It is well-known that normal DNA is aminoarylated on the 2′-deoxyguanosine residue at the 8 (major) and N^2 (minor) positions by the hydroxylamines derived from the carcinogenic amines.[1j-l] In our studies we only observed the 8-substituted products.

(15) Maniatis, T.; Fritsch, E. F.; Sambrook, J. F. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1982.