Synthesis and Characterization of Oligodeoxynucleotides Containing an N1 β-Hydroxyalkyl Adduct of 2′-Deoxyinosine

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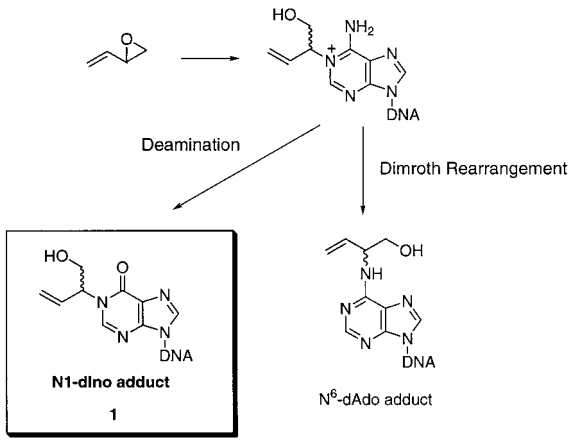
Hydroxyethyl adducts arising by the reactions of simple epoxides at the N1 position of adenine nucleosides can deaminate to give the inosine analogues which, if formed in DNA, are suspected of being highly mutagenic. A method has been developed for synthesis of oligonucleotides containing N1-adducted 2′-deoxyinosines. The 2′-deoxyinosine adduct of 3,4-epoxy-1-butene was prepared from (±)-4-acetoxy-3-bromo-1-butene and tetraisopropyldisiloxanediyl-protected 2′-deoxyinosine with base. The 2′-deoxyinosine derivative was then incorporated into the oligodeoxynucleotide sequence 5′-d(CGGACXAGAAG)-3′ (X = *N*1-(1-hydroxy-3-buten-2-yl)-2′-deoxyinosine).

Introduction

In cells exposed to simple epoxides such as ethylene oxide, 3,4-epoxy-1-butene and styrene oxide, substantial numbers of mutations are observed at adenine sites, but the nature of the lesions producing these mutations is unknown (*1*−*4*). Reaction of simple epoxides with adenine nucleosides occurs largely at the N1 position (*5*−*8*). The N1 β-hydroxyalkyl adducts can undergo Dimroth rearrangement to N^6 adducts (*7*−*10*). However, the resulting N^6 adducts have been shown to be relatively nonmutagenic (*11*, *12*), in contrast to the N^6 adducts of the more bulky diol epoxides of polycyclic aromatic hydrocarbons (*13*−*16*). The N1 β-hydroxyalkyl adducts can also undergo a deamination reaction to form inosine derivatives, particularly when the hydroxy group is on a primary carbon (*17*, *18*); Elfarra has demonstrated this process for the C3 adduct of 3,4-epoxy-1-butene at adenine N1 (Scheme 1) (*8*), and Dipple (*10*, *19*) and Koskinen (*20*) for similar adducts of styrene oxide. Adduction at the terminal carbon of the epoxide is slightly favored for both epoxybutene and styrene oxide; for the former, deaminated products have only been detected for the C3 adduct, whereas for the latter deamination of both α and β N1 dAdo adducts has been observed with the α adduct deaminating more rapidly than the β. Watson−Crick hydrogen bonding is disrupted in the N1-substituted deoxyadenosine derivatives and this potentially plays an important role in mutagenesis and carcinogenesis induced by simple epoxides. Deamination introduces an additional mutagenic feature and such deaminated N1 derivatives have been proposed to be a source of the observed mutations at adenine sites (*8*, *10*, *19*). In this paper, we describe a procedure for synthesis of oligodeoxynucleotides needed to test this hypothesis. 3,4-Epoxy-1-butene, the monoepoxide of butadiene (BD), was chosen as the focus of the present study. The epoxide has the potential to react at C1, C3, and C4, although only the C3 and C4 adducts have been reported. We were particularly interested in adduct **1** resulting from reaction at C3, because this type of adduct has been observed to arise from the reaction of the epoxide with adenosine (*8*) and has been tentatively identified in hydrolysates of CT DNA treated with 3,4-epoxy-1-butene (*21*).

Scheme 1. Reaction of 3,4-Epoxy-1-butene (EB) with Adenine



Experimental Procedures

Materials. Anhydrous solvents were either purchased from Sigma-Aldrich in Sure/Seal bottles or distilled under nitrogen just prior to use. Methylene chloride and pyridine were distilled from calcium hydride and THF from sodium/potassium alloy with benzophenone ketyl as an indicator. Other chemicals were used as purchased without further purification. (*R*)-2-Hydroxy-3-buten-1-yl *p*-tosylate was purchased from Acros Organics.

Chromatography. Thin-layer chromatography was performed with silica gel F254 (Merck) as the adsorbent on glass plates. The chromatograms were visualized under UV (254 nm)

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or by staining with an anisaldehyde/sulfuric acid solution, followed by heating. Column chromatography was performed using silica gel 60, 70-230 mesh (E. Merck). Oligonucleotides were desalted via a Sephadex G-25 column using a Bio-Rad Biologic medium-pressure chromatography system. Vacuum centrifugation was performed on a Jouan RC10.22 instrument equipped with a Titan vapor trap.

HPLC analyses and purifications were carried out on a gradient HPLC (Beckman Instruments; System Gold software) equipped with pump module 125 and photodiode array detector module 168.

Instrumentation. NMR spectra were recorded on a Bruker AC 300 or AM 400 NMR spectrometer.

Low- and high-resolution FAB[1] mass spectra were obtained at the Mass Spectrometry Facility at the University of Notre Dame, Notre Dame, IN. Negative ion MALDI-TOF mass spectra of modified oligonucleotides were obtained in a 3-hydroxypicolinic acid (3-HPA) matrix using ammonium hydrogen citrate (7 mg/mL) to suppress multiple sodium and potassium adducts.

CD spectra were recorded in methanol at 25 °C on a JASCO J-700 spectropolarimeter.

Capillary gel electrophoresis was performed on a Beckman P/ACE 5000 instrument using the manufacturer's ssDNA 100-R gel capillary and Tris-borate-urea buffer. Samples were applied at −10 kV and run at −10 kV at 30 °C.

Synthesis of Modified Nucleosides and Phosphoramidite. *4-Acetoxy-3-bromo-1-butene (**2**) (22, 23).* A solution of hydrogen bromide in glacial acetic acid (1.7 mL, 30 wt. %) was added dropwise to a stirred mixture of 3,4-epoxy-1-butene (500 mg, 7.13 mmol) and glacial acetic acid (12.5 mL). The temperature was kept at 15−20 °C during the addition. After 2 h of stirring at room temperature, the reaction mixture was treated with 25 mL of water followed by extraction with methylene chloride (4 × 50 mL). The combined organic layers were washed with water until the pH of the rinse was that of water, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The oily, colorless residue was purified by flash chromatography on silica gel (hexanes/CH_2Cl_2, 6:4) to give 506 mg of **2** (37% yield). TLC plates were visualized after development by anisaldehyde-sulfuric acid stain. TLC R_f 0.57 (hexanes/CH_2Cl_2, 3:7). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.95 (m, 1H, CH_2═C*H*), 5.35 (d, 1H, J = 16.9 Hz, CH═CH_2 trans), 5.21 (d, 1H, J = 10.1 Hz, CH═CH_2 cis), 4.61 (m, 1H, allylic), 4.33 (m, 2H, CH_2O), 2.09 (s, 3H, CH_3CO). HRMS (FAB$^+$) calcd for $C_6H_{10}BrO_2$ (M + H)$^+$ 192.9864, found 192.9879.

*N1-(1-Acetoxy-3-buten-2-yl)-2′-deoxyinosine (**4a**) and N1-(trans-4-Acetoxy-2-buten-1-yl)-2′-deoxyinosine (**6a**).* Compound **2** (38 mg, 0.2 mmol) was added to a suspension of 2′-deoxyinosine (25.2 mg, 0.1 mmol) and anhydrous K_2CO_3 (20.4 mg, 0.15 mmol) in anhydrous DMF (0.8 mL). After 24 h of stirring under an argon atmosphere at room temperature, an additional equivalent of **2** (22 mg, 0.1 mmol) was added to the reaction and the mixture was allowed to react for an additional 24 h. The products were separated by reversed-phase HPLC on a C8 (*2*) column (250 × 10 mm, Phenomenex) with the following gradient: (A) H_2O and (B) CH_3CN, 8 to 15% B over 5 min, 15 to 20% B over 15 min at a flow rate of 3 mL/min. UV absorbance was monitored at 258 nm. The compounds **6a** and **4a** eluted at 15.4 and 17.8 min, respectively. At that time, the O^6-substituted product **5a** escaped detection since it eluted at much higher acetonitrile content than the other two products. Expanding the gradient revealed the presence of the O^6-product.

*N1-(1-Acetoxy-3-buten-2-yl)-2′-deoxyinosine (**4a**).* Isolated yield 13.8 mg (38%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.34, 8.33 (s, 1H, diastereomeric H2), 8.28, 8.26 (s, 1H, diastereomeric H8), 6.27 (m, 1H, H1′), 6.10 (m, 1H, CH_2═C*H*R), 5.58 (m, 1H, allylic), 5.36 (d, 1H, cis CH_2═CHR, J = 10.5 Hz), 5.26 (d, 1H, trans CH_2═CHR, J = 17.3 Hz), 4.47 (d, 2H, CH_2−O, J = 6.3 Hz), 4.36 (m, 1H, H3′), 3.82 (m, 1H, H4′, hidden under water peak, confirmed with HMQC), 3.52 (m, 2H, H5′, H5″), 2.60 (m, 1H, H2′), 2.31 (m, 1H, H2″), 1.89 (s, 3H, acetyl). ^{13}C NMR (DMSO-d_6, 100.6 MHz) δ 20.7 (CH_3CO), ∼40 (C2′, obscured by DMSO signal), 55.4 (allylic), 61.7 (C5′), 70.9 (C3′), 84.0 (C1′), 88.0 (C4′), 120.6 (CH_2═CHR), 123.5 (C5), 133.0 (CH_2═*C*HR), 139.7 (C8), 147.0 (C2 or C4), 147.3 C4 or C2), 156.3 (C6), 170.8 CH_3CO). HRMS (FAB$^+$) calcd for $C_{16}H_{21}N_4O_6$ (M + H)$^+$ 365.1461, found 365.1482.

*N1-(trans-4-acetoxy-2-buten-1-yl)-2′-deoxyinosine (**6a**).* Isolated yield 5.4 mg (15%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.38 (s, 1H, H2), 8.32 (s, 1H, H8), 6.29 (m, 1H, H1′), 5.91 (m, 1H, OCH_2-C*H*═CH), 5.67 (m, 1H, CH═C*H*-CH_2-N), 5.32 (d, 1H, 3′-OH, J = 4.0 Hz), 4.95 (t, 1H, 5′-OH, J = 5.4 Hz), 4.63 (m, 2H, CH_2N), 4.49 (m, 2H, CH_2O), 4.37 (m, 1H, H3′), 3.85 (m, 1H, H4′), 3.53 (m, 2H, H5′, H5″), 2.61 (m, 1H, H2′), 2.31 (m, 1H, H2″), 1.99 (s, 3H, acetyl). HRMS (FAB$^+$) calcd for $C_{16}H_{21}N_4O_6$ (M + H)$^+$ 365.1461, found 365.1445.

*N1-(1-Hydroxy-3-buten-2-yl)-2′-deoxyinosine (**1**), N1-(cis-4-Hydroxy-2-buten-1-yl)-2′-deoxyinosine, and N1-(trans-4-Hydroxy-2-buten-1-yl)-2′-deoxyinosine.* The general procedure was as follows. Acetyl groups were removed by treatment with saturated methanolic ammonia for 24 h at room temperature. Methanolic ammonia was removed under vacuum and the crude reaction mixture was purified by reversed-phase HPLC on a C8(2) column (250 × 10 mm, Phenomenex) with the following gradient: (A) H_2O and (B) CH_3CN, 8 to 12% B over 5 min, 12 to 22% B over 10 min, 22 to 90% B over 2 min, hold for 4 min, and then 90 to 8% B over 2 min at a flow rate of 3 mL/min. Since **4a** was not resolved from **7a**, the deacetylation process yielded two products: **1** which eluted as a diastereomeric mixture at 12.1 and 12.4 min and *N*1-(*cis*-4-hydroxy-2-buten-1-yl)-2′-deoxyinosine, which eluted at 9.7 min. The deacetylation of **6a** yielded *N*1-(*trans*-4-hydroxy-2-buten-1-yl)-2′-deoxyinosine, which eluted at 8.9 min.

*N1-(1-Hydroxy-3-buten-2-yl)-2′-deoxyinosine (**1**).* Compound **4a** (6.9 mg) afforded after purification 5.6 mg (92% yield) of compound **1**. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.31 (s, 1H, H8), 8.30 (s, 1H, H2), 6.30 (m, 1H, H1′), 6.10 (m, 1H, CH_2═C*H*R), 5.40 (m, 1H, allylic), 5.28 (dd, 1H, cis CH_2═CHR, J_1 = 10.6 Hz, J_2 = 1.3 Hz), 5.19 (dd, 1H, trans CH_2═CHR, J_1 = 17.3 Hz, J_2 = 1.3 Hz), 4.38 (m, 1H, H3′), 3.89−3.76 (m, 3H, H4′, CH_2O), 3.54 (m, 2H, H5′, H5″), 2.62 (m, 1H, H2′), 2.30 (m, 1H, H2″). HRMS (FAB$^+$) calcd for $C_{14}H_{19}N_4O_5$ (M + H)$^+$ 323.1355, found 323.1360.

N1-(cis-4-Hydroxy-2-buten-1-yl)-2′-deoxyinosine. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.38 (s, 1H, H2), 8.31 (s, 1H, H8), 6.31 (m, 1H, H1′), 5.68 (m, 1H, CH═CH), 5.45 (m, 1H, CH═CH), 4.67 (d, 2H, CH_2N, J = 6.9 Hz), 4.37 (m, 1H, H3′), 4.17 (d, 2H, CH_2O, J = 5.5 Hz), 3.83 (m, 1H, H4′), 3.53 (m, 2H, H5′, H5″), 2.63 (m, 1H, H2′), 2.28 (m, 1H, H2″). HRMS (FAB$^+$) calcd for $C_{14}H_{19}N_4O_5$ (M + H)$^+$ 323.1355, found 323.1345.

N1-(trans-4-Hydroxy-2-buten-1-yl)-2′-deoxyinosine. Compound **6a** (2.7 mg) afforded after purification 2.2 mg (92% yield) of *N*1-(4-hydroxy-2-buten-1-yl)-2′-deoxyinosine. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.37 (s, 1H, H2), 8.31 (s, 1H, H8), 6.29 (m, 1H, H1′), 5.73 (m, 2H, trans CH═CH), 4.60 (d, 2H, CH_2N, J = 5.0 Hz), 4.37 (m, 1H, H3′), 3.86 (m, 3H, H4′, CH_2O), 3.53 (m, 2H, H5′, H5″), 2.57 (m, 1H, H2′), 2.28 (m, 1H, H2″). HRMS (FAB$^+$) calcd for $C_{14}H_{19}N_4O_5$ (M + H)$^+$ 323.1355, found 323.1345.

*3′,5′-O-(1,1,3,3-Tetraisopropyl-1,3-disiloxanediyl)-2′-deoxyinosine (**3b**).* 2′-Deoxyinosine (300 mg, 1.20 mmol) was dried by coevaporation with anhydrous pyridine (3 × 10 mL) and then redissolved in pyridine (6 mL). 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane (138 mg, 0.44 mmol) was added dropwise and the reaction mixture was stirred at room temperature under an argon atmosphere for 24 h. The solvents were evaporated to dryness under vacuum and the residue was triturated with water. The precipitate was collected and after drying, purified by flash chromatography on silica gel (CH_2Cl_2/MeOH, 98:2) to

[1] Abbreviations: EB, 3,4-epoxy-1-butene; FAB, fast-atom bombardment mass spectrometry; MALDI-TOF, matrix-assisted laser-desorption time-of-flight mass spectrometry; HRMS, high-resolution mass spectrometry; HMBC, heteronuclear multibond correlation (long-range ^{13}C-^{1}H scalar correlated 2D NMR experiment); HMQC, heteronuclear correlation through multiple-bond quantum coherence; COSY, correlation spectroscopy.

give 564 mg (95% yield) of **3b**. TLC R_f 0.53 (CH_2Cl_2/MeOH, 9:1). ^{1}H NMR (MeOD-d_4, 300 MHz) δ 8.15 (s, 1H, H8), 7.98 (s, 1H, H2), 6.30 (m, 1H, H1′), 5.01 (m, 1H, H3′), 4.03 (m, 2H, H5′, H5″), 2.84 (m, 1H, H2′), 2.62 (m, 1H, H2″), 1.01 (m, 28H, isopropyl). HRMS (FAB$^+$) calcd for $C_{22}H_{39}N_4O_5Si_2$ $(M + H)^+$ 495.2459, found 495.2447.

*N1-(1-Acetoxy-3-buten-2-yl)-3′,5′-O-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)-2′-deoxyinosine (**4b**), O^6-(1-acetoxy-3-buten-2-yl)-3′,5′-O-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)-2′-deoxyinosine (**5b**), and N1-(trans-4-acetoxy-2-buten-1-yl)-3′,5′-O-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)-2′-deoxyinosine (**6b**).* Compound **2** (152 mg, 0.8 mmol) was added to a suspension of **3b** (200 mg, 0.4 mmol) and anhydrous K_2CO_3 (83 mg, 0.6 mmol) in anhydrous DMF (5 mL). After 4 days of stirring under an argon atmosphere at room temperature, the solvent was evaporated under vacuum and the residue was suspended in anhydrous Et_2O. The filtrate was collected and Et_2O was evaporated under reduced pressure. The residue was separated by flash chromatography on silica gel (hexanes/EtOAc, 6:4) to yield 84.9 mg (35% yield) of **4b**, 41.2 mg (17% yield) of **5b** and 39.2 mg (16% yield) of **6b**.

*N1-(1-Acetoxy-3-buten-2-yl)-3′,5′-O-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)-2′-deoxyinosine (**4b**).* TLC R_f 0.49 (EtOAc/hexanes, 9:1). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.30, 8.27 (s, 1H, diastereomeric H2), 8.23, 8.22 (s, 1H, diastereomeric H8), 6.27 (m, 1H, H1′), 6.16 (m, 1H, CH_2═C*H*R), 5.64 (m, 1H, allylic), 5.37 (d, 1H, cis CH_2═CHR, J = 10.6 Hz), 5.28 (d, 1H, trans CH_2═CHR, J = 17.3 Hz), 4.95 (m, 1H, H3′), 4.52 (m, 2H, CH_2O), 3.90 (m, 2H, H5′, H5″), 3.80 (m, 1H, H4′), 2.82 (m, 1H, H2′), 2.57 (m, 1H, H2″), 1.93 (s, 3H, acetyl), 1.01 (m, 28H, isopropyl). HRMS (FAB$^+$) calcd for $C_{28}H_{47}N_4O_7Si_2$ $(M + H)^+$ 607.2983, found 607.2979.

*O^6-(1-Acetoxy-3-buten-2-yl)-3′,5′-O-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)-2′-deoxyinosine (**5b**).* TLC R_f 0.68 (EtOAc/hexanes, 9:1). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.49 (s, 1H, H8), 8.43 (s, 1H, H2), 6.36 (m, 1H, H1′), 6.17 (m, 1H, allylic), 6.03 (m, 1H, CH_2═C*H*R), 5.39 (d, 1H, trans CH_2═CHR, J = 17.3 Hz), 5.29 (d, 1H, cis CH_2═CHR, J = 10.6 Hz), 5.13 (m, 1H, H3′), 4.35 (m, 2H, H5′, H5″), 3.90 (m, 2H, CH_2O), 3.81 (m, 1H, H4′), 2.91 (m, 1H, H2′), 2.59 (m, 1H, H2″), 1.96 (s, 3H, acetyl), 1.01 (m, 28H, isopropyl). HRMS (FAB$^+$) calcd for $C_{28}H_{47}N_4O_7Si_2$ $(M + H)^+$ 607.2983 found, 607.2965.

*N1-(trans-4-Acetoxy-2-buten-1-yl)-3′,5′-O-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)-2′-deoxyinosine (**6b**).* TLC R_f 0.34 (EtOAc/hexanes, 9:1). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.30 (s, 1H, H2), 8.21 (s, 1H, H8), 6.26 (m, 1H, H1′), 5.91 (m, 1H, OCH_2C*H*═CH), 5.68 (m, 1H, CH═C*H*CH_2N), 4.97 (m, 1H, H3′), 4.66 (m, 2H, CH_2O), 4.49 (m, 2H, CH_2N), 3.90 (m, 2H, H5′, H5″), 3.80 (m, 1H, H4′), 2.82 (m, 1H, H2′), 2.57 (m, 1H, H2″), 1.99 (s, 3H, acetyl), 1.01 (m, 28H, isopropyl). HRMS (FAB$^+$) calcd for $C_{28}H_{47}N_4O_7Si_2$ $(M + H)^+$ 607.2983, found 607.3009.

N1-(1-Acetoxy-3-buten-2-yl)-2′-deoxyinosine (4a) via the Disiloxane Pathway. Tetrabutylammonium fluoride (1 M solution in THF, 99 μL) was added dropwise to a solution of **4b** (60 mg, 0.1 mmol) in freshly distilled THF (1.5 mL), and the solution was stirred at room temperature for 5 min. The reaction was quenched with 5% acetic acid (1 mL) and then purified by reversed-phase HPLC on a C8(2) column (250 × 10 mm, Phenomenex) with the following gradient: (A) H_2O and (B) CH_3CN, 8 to 15% B over 5 min, 15 to 20% B over 16 min, 20 to 90% B over 2 min, hold for 4 min, and then 90 to 8% B over 2 min at a flow rate of 3 mL/min. The product eluted at 17.7 min. A total of 27.5 mg (75% yield) of **4a** was isolated. HRMS (FAB$^+$) calcd for $C_{16}H_{21}N_4O_6$ $(M + H)^+$ 365.1461 found, 365.1445. ^{1}H NMR same as described above for **4a**.

5′-O-(4,4′-Dimethoxytrityl)-N1-(1-acetoxy-3-buten-2-yl)-2′-deoxyinosine. Compound **4a** (27.5 mg, 0.075 mmol) was dried by coevaporation with anhydrous pyridine (4 × 5 mL) and then redissolved in 2 mL of pyridine. 4,4′-Dimethoxytrityl chloride (56 mg, 0.16 mmol) was added in three portions over the course of 5 days. The reaction mixture was kept at room temperature under an argon atmosphere. The reaction was quenched with MeOH (0.5 mL), stirred for 15 min and concentrated under vacuum. The oily yellow residue was purified by flash column chromatography on silica gel (CH_2Cl_2:MeOH:pyridine, 98:1.5:0.5 to 96:3.5:0.5) and yielded 39.5 mg (80% yield) of the trityl-protected nucleoside. TLC R_f 0.60 (CH_2Cl_2:MeOH, 9:1). ^{1}H NMR (CD_2Cl_2, 400 MHz) δ 7.93, 7.92 (s, 1H, diastereomeric H2), 7.84 (s, 1H, H8), 7.40 (m, 2H, aromatic), 7.29 (m, 7H, aromatic), 6.83 (m, 4H, aromatic), 6.34 (m, 1H, H1′), 6.05 (m, 1H, CH_2═C*H*R), 5.78 (m, 1H, allylic), 5.45 (dd, 1H, cis CH_2═CHR, J_1 = 10.6 Hz, J_2 = 1.5 Hz), 5.36 (trans CH_2═CHR, partially hidden under the solvent peak), 4.63 (m, 1H, H3′), 4.46 (m, 2H, CH_2O), 4.10 (m, 1H, H4′), 3.78 (s, 6H, CH_3O), 3.34 (m, 2H, H5′, H5″), 2.74 (m, 1H, H2′), 2.48 (m, 1H, H2″), 1.98, and 1.94 (s, 3H, diastereomeric acetyls). HRMS (FAB$^+$) calcd for $C_{37}H_{39}N_4O_8$ $(M + H)^+$ 667.2768, found 667.2772.

*3′-O-[(N,N-Diisopropylamino)-(2-cyanoethyl)-phosphinyl]-5′-O-(4,4′-dimethoxytrityl)-N1-(1-acetoxy-3-buten-2-yl)-2′-deoxyinosine (**8**).* 5′-*O*-(4,4′-Dimethoxytrityl)-*N*1-(1-acetoxy-3-buten-2-yl)-2′-deoxyinosine (37.6 mg, 0.06 mmol) was dried by coevaporation with anhydrous pyridine (4 × 5 mL) and placed under vacuum overnight. Anhydrous 1*H*-tetrazole (5.1 mg, 0.07 mmol) was added to a flame-dried flask and kept under vacuum overnight. Freshly distilled CH_2Cl_2 (2 mL) was added to the flask containing the tetrazole and the resulting solution was transferred to the flask containing tritylated nucleoside, followed by addition of 2-cyanoethyl-*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (25.3 mg, 0.08 mmol). After 3 h, the solvents were removed under reduced pressure and the oily residue was left briefly under vacuum. It was purified by flash column chromatography on silica gel (EtOAc:hexanes:pyridine, 90:9:1) to yield 46.8 mg (95% yield) of the phosphitylated nucleoside **8**. TLC R_f 0.58, 0.50 (EtOAc). ^{1}H NMR (CD_2Cl_2, 300 MHz) δ 7.93, 7.92 (s, 1H, diastereomeric H2), 7.89, 7.97 (s, 1H, diastereomeric H8), 7.40 (m, 2H, aromatic), 7.28 (m, 7H, aromatic), 6.80 (m, 4H, aromatic), 6.33 (m, 1H, H1′), 6.05 (m, 1H, CH_2═C*H*R), 5.78 (m, 1H, allylic), 5.45 (d, 1H, cis CH_2═CHR, J = 10.6 Hz), 5.36 (trans CH_2═CHR, partially hidden under the solvent peak), 4.73 (m, 1H, H3′), 4.46 (m, 2H, CH_2O), 4.28 (m, 1H, H4′), 4.12 (m, 2H, H5′, H5″), 3.78 (s, 3H, CH_3O), 3.77 (s, 3H, CH_3O), 3.50 (m, 2H, isopropyl CH), 3.46 (m, 2H, $POCH_2$), 2.72 (m, 2H, H2′, CH_2-CN), 2.63 (m, 2H, H2″, CH_2CN), 1.97 and 1.92 (s, 3H, diastereomeric acetyls), 1.21 (m, 12H, isopropyl CH_3). ^{31}P NMR (CD_2Cl_2, 121 MHz) δ 149.8 (product) and 14.48 (hydrolyzed material). HRMS (FAB$^+$) calcd for $C_{46}H_{56}N_6O_9P$ $(M + H)^+$ 867.3846, found 867.3874.

*N1-(1-Hydroxy-3-buten-2(S)-yl)-2′-deoxyinosine (**S-1**) and N1-(2(R)-Hydroxy-3-buten-1-yl)-2′-deoxyinosine (**10**).* (*R*)-2-Hydroxy-3-buten-1-yl *p*-tosylate (100 mg, 0.41 mmol) was dissolved in freshly distilled DMSO (0.7 mL) and NaH (20 mg of 60% suspension in oil, 0.49 mmol) was added in one portion. After 15 min, the temperature was raised slowly to 80 °C, and the reaction was then allowed to cool to room temperature. Anhydrous K_2CO_3 (83 mg, 0.60 mmol) and 2′-deoxyinosine (**3a**, 100 mg, 0.40 mmol) were added and the reaction mixture was stirred for 5 days at 45 °C. The products were separated by reverse-phase HPLC on a C8(2) column, (250 × 10 mm, Phenomenex) with the following gradient: (A) H_2O and (B) CH_3CN, 8 to 10% B over 5 min, 10 to 14% B over 10 min, hold at 14% for 1 min, 14 to 90% over 2 min, hold at 90% for 1 min, and then 90 to 8% over 2 min at a flow rate of 3 mL/min. UV absorbance was monitored at 258 nm. ***S*-1** and **10** eluted at 14.5 and 12.4 min, respectively.

*N1-(1-Hydroxy-3-buten-2(S)-yl)-2′-deoxyinosine (**S-1**).* ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.32 (s, 1H, H8), 8.31 (s, 1H, H2), 6.30 (m, 1H, H1′), 6.10 (m, 1H, CH_2═C*H*R), 5.40 (m, 1H, allylic), 5.28 (dd, 1H, cis CH_2═CHR, J_1 = 10.6 Hz, J_2 = 1.2 Hz), 5.19 (d, 1H, trans CH_2═CHR, J = 17.3 Hz), 4.38 (m, 1H, H3′), 3.90−3.73 (m, 3H, H4′, CH_2O), 3.54 (m, 2H, H5′, H5″), 2.63 (m, 1H, H2′), 2.30 (m, 1H, H2″). HRMS (FAB$^+$) calcd for $C_{14}H_{19}N_4O_5$ $(M + H)^+$ 323.1357, found 323.1367. Isolated yield 1.8 mg (1%). NMR signals for the contaminating sodium salt of *p*-toluenesulfonic acid were observed at 2.27, 7.10, and 7.46 ppm.

*N1-(2(R)-Hydroxy-3-buten-1-yl)-2′-deoxyinosine (**10**).* ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.30 (s, 1H, H2), 8.24 (s, 1H, H8), 6.29 (m, 1H, H1′), 5.89 (m, 1H, CH_2═C*H*R), 5.17 (d, 1H, trans CH_2═CHR, J = 17.2 Hz), 5.12 (d, 1H, cis CH_2═CHR, J = 10.5 Hz), 4.38 (m, 1H, H3′), 4.20 (m, 1H, allylic), 4.18 (dd, 1H, CH_2N, J_1 = 13.2 Hz, J_2 = 3.8 Hz), 3.85 (m, 1H, H4′), 3.76 (dd, 1H, CH_2N, J_1 = 13.2 Hz, J_2 = 8.5 Hz), 3.53 (m, 2H, H5′, H5″), 2.62 (m, 1H, H2′), 2.27 (m, 1H, H2″). HRMS (FAB$^+$) calcd for $C_{14}H_{19}N_4O_5$ $(M + H)^+$ 323.1357, found 323.1353. Isolated yield 23.4 mg (17%). NMR signals for the contaminating sodium salt of *p*-toluenesulfonic acid were observed at 2.26, 7.10 and 7.46 ppm.

Preparation of Modified Oligonucleotides. Oligonucleotides (1 μmol scale) were prepared on an Expedite 8909 Nucleic Acid Synthesizer using *tert*-butylphenoxyacetyl-protected 2-cyanoethyl phosphoramidites and adducted phosphoramidite **8**.

Deprotection and Purification of Modified Oligonucleotides. Modified oligonucleotides were cleaved from the beads and deprotected (including the acetyl protection on the adduct moiety) in aqueous ammonia for 16 h at room temperature and purified by reverse-phase HPLC on a C8(2) column (250 × 10 mm, Phenomenex) with the following gradient: (A) 0.1 M ammonium formate and (B) CH_3CN, 1 to 4% B over 5 min, 4 to 6.6% B over 30 min, 6.6 to 90% B over 3 min, hold for 1 min, and then 90 to 1% B over 2 min at a flow rate of 3 mL/min; the diastereomeric oligonucleotides eluted at 25.4 (**9a**) and 28.9 min (**9b**). Mass spectrum: (MALDI-TOF) *m*/*z* calcd for $[M - H]^-$ 3468.7, found 3469.9 (**9a**) and 3468.4 (**9b**).

Enzymatic Hydrolysis. Enzymatic digestion mixtures (0.2−0.5 A_{260} units of oligonucleotide, 20 μL of 0.1 M Tris HCl buffer, pH 9.0, 0.04 units of snake venom phosphodiesterase, 0.4 units of alkaline phosphatase) were incubated at 37 °C overnight, and subsequently analyzed by HPLC on a C8(2) column (250 × 4.6 mm, Phenomenex) with the following gradient: (A) 0.1 M ammonium formate and (B) CH_3CN, 1 to 10% B over 15 min, 10 to 20% B over 5 min, hold for 5 min, and then to 100% B over 10 min at a flow rate of 1 mL/min.

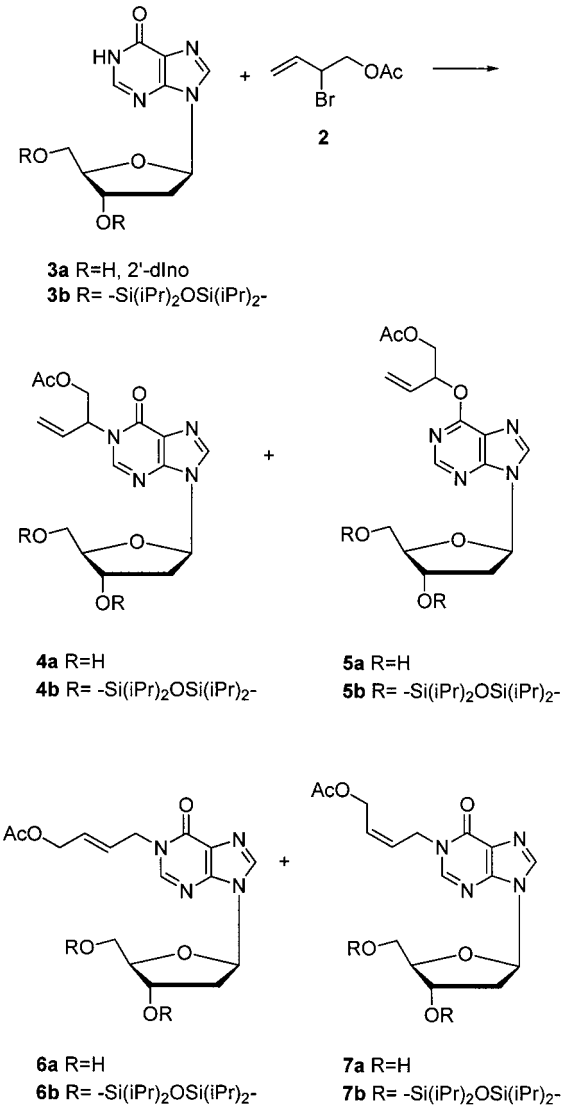
Melting Studies. Modified oligonucleotides and the complementary strand (0.5 A_{260} units each) were dissolved in 1 mL of melting buffer (10 mM Na_2HPO_4/NaH_2PO_4, 1.0 M NaCl, 50 μM Na_2EDTA, pH 7.0). The sample vials were heated to 85 °C and allowed to cool to room temperature. UV measurements were taken at 1 min intervals with a 1 °C/min temperature gradient with observation at 260 nm. The temperature was raised from 5 to 90 °C. Both S (**9a**) and R (**9b**) diastereomers had T_m = 33 °C, while the wild-type duplex had T_m = 55 °C.

Results and Discussion

The most effective strategy for preparation of oligonucleotides containing this lesion involves preparation of the adducted nucleoside and then incorporation of it into the oligonucleotide. The hydroxyl group in the side-chain requires protection for the strategy to be compatible with the phosphoramidite chemistry used in DNA synthesis.

Under the basic conditions employed in this study the anion formed by deprotonation of N1 becomes the main site of reaction with electrophiles (*24*). Consequently, the reaction of 4-acetoxy-3-bromo-1-butene (**2**) with 2′-deoxyinosine (**3a**) was examined (Scheme 2). The reaction was carried out under basic conditions and yielded four products: the desired one (**4a**) arising from attack of N1 of the nucleoside at C3 of the bromoalkene, the *O*-alkyl derivative (**5a**) arising from O^6 of the ambident anion of the nucleoside reacting with C3 of the alkene, and trans and cis adducts **6a** and **7a** arising by S_N2' attack of N1 at C1 of the alkene. Compounds **5a** and **6a** were readily isolated by HPLC, but **4a** and **7a** coeluted; **7a** represented about a 10% contaminant in **4a**. The mixture could be resolved after removal of the acetyl groups but acyl protection of the hydroxyl group was needed for the remaining steps of the oligonucleotide synthesis.

Scheme 2. Synthesis of O-Protected *N*1-(1-Hydroxy-3-buten-2-yl) 2′-Deoxyinosine (4a and 4b) and Related Compounds



As a possible solution to this problem, 2′-deoxyinosine was protected as the 3′,5′-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl) derivative prior to treatment with **2**. Again, the reaction yielded four products (**4b**−**7b**) with **7b** coeluting with **4b**. After removal of the tetraisopropyldisiloxanediyl group and the acetyl group, HPLC showed the contamination of **4b** by **7b** was substantially less than in the previous reaction and was sufficiently small that it could be neglected. The yields of **4b**−**7b** were 33, 17, 16, and <2%, respectively. On this basis, the separation of **4b**, **5b**, and **6b** could be carried out by column chromatography on silica gel. It should be noted that **4b** and **5b** were both irresolvable mixtures of diastereomers. However, two sets of signals for H2 and H8 were discernible in the ^{1}H NMR spectrum of **4b**. Likewise, the diastereomers of **4a** and those of **5a** were unresolved, although two sets of signals for H2, H8, and H1′ were visible in the ^{1}H NMR spectrum of **4a**.

Assignments of product structures were made by 2-D NMR experiments (COSY, HMQC, and HMBC). Structural assignments for **4**−**6** were made primarily on the

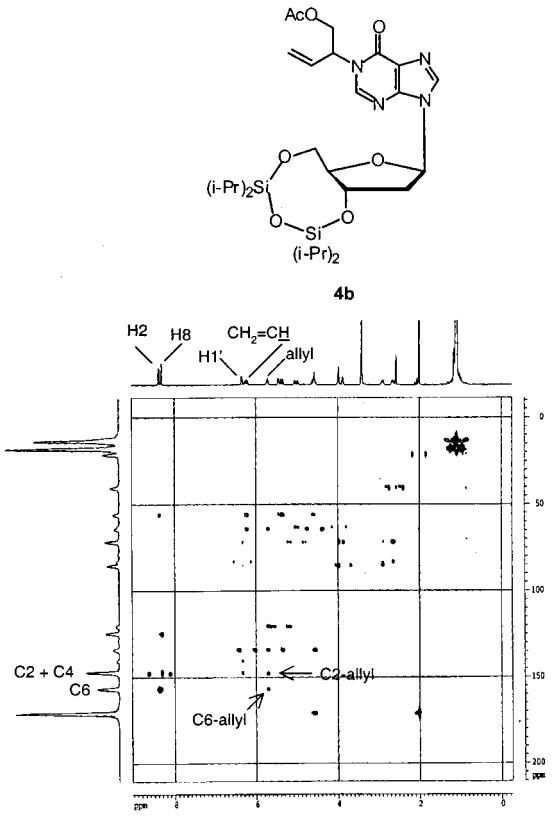
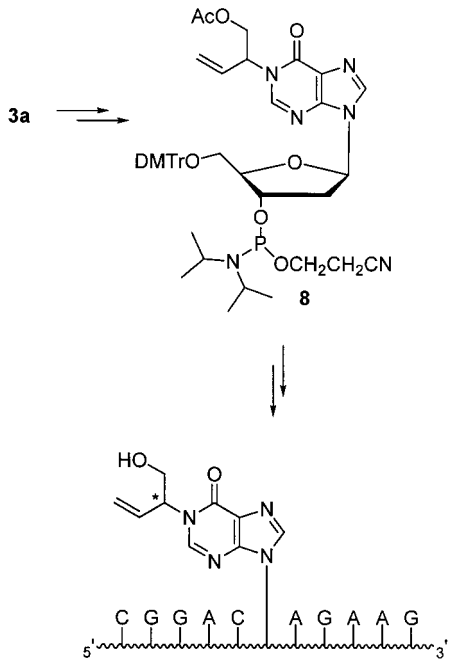


Figure 1. HMBC spectrum of compound **4b**. The ^{13}C spectrum is shown on the vertical axis and the ^{1}H spectrum on the horizontal. Observation of long-range coupling between carbons 2 and 6 and the allylic proton on the side chain allows the site of attachment of the side chain to be assigned as N1 (see discussion in text).

silyl-protected species **4b**, **5b**, and **6b**; however, the spectra of the unprotected analogues **4a**, **5a**, and **6a** were fully concordant with the assigned structures. The site of substitution on the purine ring was determined by HMBC experiments. The N1 substitution pattern for **4b** was elucidated from cross-peaks between H2 of the purine and the allylic carbon of the butadiene moiety and in some cases C2 of the purine and the allylic proton of the substituent (Figure 1). The correlation between C6 of the purine and the allylic proton of the substituent excluded the possibility of **4b** being the N3 adduct. A similar approach was used to confirm the site of substitution of **6b**. Compound **5b** was assigned as the O^6-substituted species. Correlations indicating N1 and N3 substitution were not present for **5b**. The expected correlation between C6 of the purine ring and the allylic proton of the substituent was not observed, most likely due to the broadness of the allylic proton signal; however, the O^6-substitution pattern was deduced from the following observations: (i) The ^{1}H NMR spectra of **5b** and **4b** were almost identical except the chemical shift of the allylic proton **5b** was shifted downfield compared to **4b**, which is consistent with the stronger deshielding effect of oxygen compared to nitrogen. (ii) Compounds **5a,b** were more lipophilic than the N1 adducts **4a**,**b** and **6a**,**b**. (iii) N1 Alkyl derivatives **4b**, **6b**, and **7b** had identical UV spectra whereas the spectrum of the O^6-alkyl derivative **5b** was clearly distinguishable. These observations are consistent with the NMR assignments and provided additional evidence that the structures were assigned correctly.

Scheme 3. Synthesis of 11-mer Oligodeoxyribonucleotides Containing *N*1-(1-Hydroxy-3-buten-2(*S*)-yl)-2′-deoxyinosine (9a) and *N*1-(1-Hydroxy-3-buten-2(*R*)-yl)-2′-deoxyinosine (9b)



9a S diastereomer
9b R diastereomer

Compound **4b** was used without further purification. To prepare a phosphoramidite reagent suitable for use in an automated DNA synthesizer, tetraisopropyldisiloxanediyl protection was removed with tetrabutylammonium fluoride to give **4a**. The 5′ hydroxyl group was protected with the 4,4′-dimethoxytrityl group and the 3′ hydroxyl group phosphitylated with 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite to give phosphoramidite **8**.

The phosphoramidite reagent **8** was then incorporated into an oligonucleotide having the sequence 5′-d(CGGACXAGAAG)-3′, where X represents *N*1-(1-hydroxy-3-buten-2-yl)-2′-deoxyinosine (Scheme 3). Synthesis of the oligonucleotide was performed on a 1 μmol scale. The dGuo, dAdo, and dCyt phosphoramidite reagents and the initial residue on the CPG beads employed *tert*-butylphenoxyacetyl protection on the exocyclic amino groups. Cleavage of the oligomer from the solid support, deprotection of the normal nucleosides, and removal of the acetyl group from the adducted nucleoside could be accomplished in a single step using either 0.1 M NaOH at room temperature for 2 days or ammonium hydroxide at room temperature for 16 h. The two protocols were judged to be equally satisfactory, but ammonia treatment was experimentally more straightforward. The mixture contained diastereomeric adducted oligomers of the desired sequence (**9a** and **9b**) together with failure sequences (Figure 2). The oligonucleotide derived from **7a** was presumed to be present also but was not detected; possibly, it lay under one of the failure sequences. The diastereomeric mixture of adducted oligonucleotides was readily separated by HPLC. This circumvented having to repeat the synthesis with the individual enantiomers of the alkylating agent in order to obtain oligonucleotides containing the individual stereoisomers of the adduct.

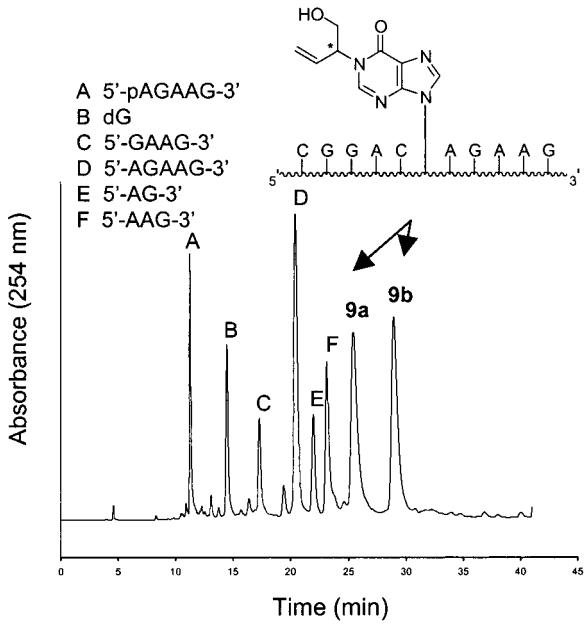


Figure 2. HPLC chromatograms showing the product mixture obtained upon deprotection of oligonucleotides synthesized with phosphoramidite **8**. Peaks were identified by MALDI-TOF mass spectrometry and enzyme digestion. Peaks A−F are failure sequences and peaks **9a** and **9b** are the desired full-length adducted oligonucleotides. HPLC conditions are described in the Experimental Section of the text.

The modified oligonucleotides were purified on reverse-phase HPLC, providing ~12 A_{260} units of each diastereomer from a 1 μmol cassette.

Mass spectra (MALDI) confirmed the molecular weights of the diastereomeric oligonucleotides. The adducted oligonucleotides were characterized by enzymatic digestion to individual nucleosides, followed by identification of the adducts via HPLC coelution with authentic standards (Figure 3). Nucleoside composition was quantified by dividing each peak area from the HPLC profile of the enzyme digest by the appropriate molar extinction coefficient (*25*). The molar extinction coefficient of the modified nucleoside was assumed to be the same as that of 1-methylinosine (*26*). Melting transitions for duplexes formed between the 11-mers and their complements were determined. Whereas the duplex containing the native oligodeoxynucleotide had a T_m of 55 °C, the duplexes containing the inosine adducts were strongly destabilized with a T_m value of 33 °C for both **9a** and **9b**.

To assign the configuration of the diastereomers, the 2(*S*) form of **1** was synthesized from the commercially available 2(*R*)-hydroxy-3-buten-1-yl tosylate (Scheme 4). The tosylate was converted into the (*R*)-3,4-epoxy-1-butene which, in turn, was used to alkylate 2′-deoxyinosine. A one-pot approach was employed to avoid isolation of the volatile epoxide. An initial attempt involved treatment of the tosylate with 2′-deoxyinosine and K_2CO_3 in anhydrous DMF. The condensation proceeded slowly to give only the direct displacement product, 1-(2(*R*)-hydroxy-3-butenyl)-2′-deoxyinosine (**10**). The reaction was repeated using a stoichiometric amount of NaH in DMSO to ensure conversion of the tosylate to the epoxide (*27*), followed by addition of anhydrous K_2CO_3 and 2′-deoxyinosine when the tosylate was no longer present in the reaction mixture (as shown by TLC). The reaction yielded two products in a 10:1 ratio. They

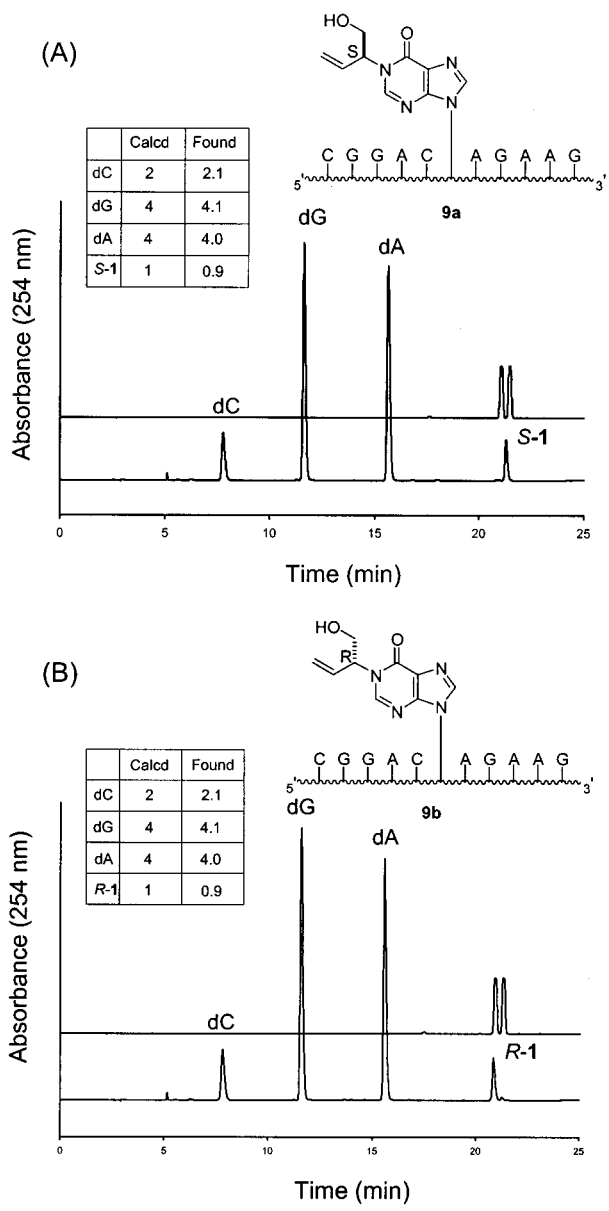


Figure 3. HPLC profile of products obtained from enzymatic digestion of purified adducted oligonucleotides. Inserted boxes show relative nucleoside compositions (calculated and found) for each oligonucleotide. (A) (lower trace) digest of **9a**; (upper trace) nucleoside **1** (diastereomeric mixture); (B) (lower trace) digest of **9b**; (upper trace) nucleoside **1** (diastereomeric mixture). Conditions of hydrolysis and chromatography are described in the text.

were purified by reversed-phase HPLC and characterized by NMR and MS. The major product (**10**) involved attack at the terminal C4 position of the epoxide and the minor one (***S*-1**) resulted from attack at C3. The minor product coeluted on HPLC with the slower moving of the diastereomers of **1** formed by deacetylation of **4a** and had the same ^{1}H NMR (Figure 4). Since the reactions by which the adducts were formed are S_N2 processes, the *R* configuration of the tosylate became an *S* configuration in ***S*-1**. The HPLC retention time of compound ***S*-1** was identical to that of the nucleoside arising from the faster eluting oligonucleotide (**9a**). The CD spectrum of ***S*-1** showed an enhanced negative Cotton effect at 250 nm compared to 2′-deoxyinosine.

In conclusion, we have developed a strategy to synthesize oligodeoxynucleotides containing site-specific

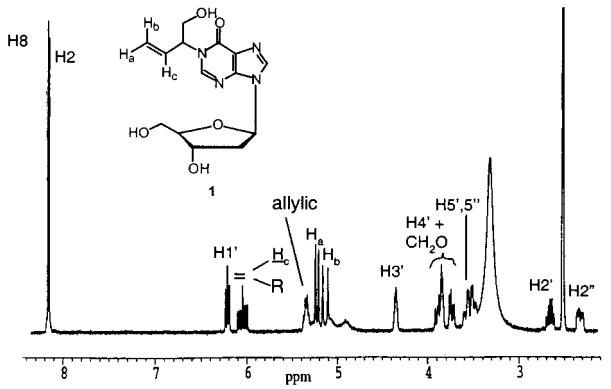
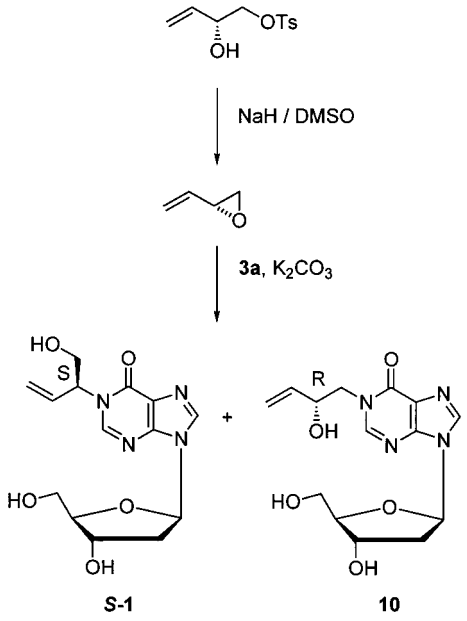


Figure 4. ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of *N*1-(1-hydroxy-3-buten-2-yl)-2′-deoxyinosine (**1**).

Scheme 4. Synthesis of *N*1-(1-Hydroxy-3-buten-2(*S*)-yl)-2′-deoxyinosine and *N*1-(2(*R*)-Hydroxy-3-buten-1-yl)-2′-deoxyinosine



adducts at the N1 position of 2′-deoxyinosine. This strategy should be readily extendable to other N1 adducts such as the C4 adduct of 3,4-epoxy-1-butene and the analogous styrene oxide adducts. In ongoing studies in this laboratory, similar conditions (NaOH in dimethylacetamide) are being used to alkylate the N1 position of 2′-deoxyguanosine. With alkyl halides that have only a single site for reaction with nucleophiles, the method has given excellent yields of the N1 dGuo adduct. The example described here for the adduct of 3,4-epoxy-1-butene is the first site-specific synthesis of an oligodeoxynucleotide containing this type of lesion at a purinic site. Hydroxyalkyl adducts at cytosine N3 are also prone to deamination to give potentially mutagenic uracil derivatives; an oligonucleotide containing a site-specific 3-hydroxyethyldeoxyuridine has been synthesized by Bhanot et al. (*28*) by a route similar to the one reported herein.

The mutagenicity of the N1 β-hydroxyalkyl adducts of deoxyinosine is currently under investigation. It is possible that such adducts may be strongly blocking or readily repaired; to the extent that they are bypassed, it is likely that an incorrect base will be incorporated.

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Supporting Information Available: NMR spectra of nucleosides, circular dichroism spectra of **3a** and **S-1**, and capillary gel electropherograms, MALDI-TOF mass spectra, and melting curves for adducted oligonucleotides **9a** and **9b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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