

General Procedures. All air or water sensitive reactions were performed in flame dried glassware under a positive pressure of argon. All air or water sensitive materials were transferred in oven-dried syringes unless otherwise specified. Commercial solvents and reagents were used without further purification with the exception of CH_2Cl_2 , CH_2Br_2 , CH_3CN , and pyridine which were distilled under nitrogen from calcium hydride and toluene which was distilled from molten sodium under nitrogen. Water was purified on a MILLI-Q™ Water System.

HPLC Analyses were performed on a Beckman 421A HPLC system. The HPLC was equipped with a SSI detector (model 500) with output to both a Linear chart recorder (model 285), and a Hewlett Packard integrator (model 3390A). UV monitoring was at 260 nm. Solvent gradients were run at 10 mL/min for preparative separations (Rainin, 300 Å, C_{18} , 250 mm x 21.4 mm column) and 1 mL/min for analytical separations (Alltech, 5- μm , C_{18} , 250 mm x 4.6 mm Econosphere column). Solvent gradients were as follows: Gradient A: solvent A: 10 mM ammonium acetate (pH 7.0); solvent B: acetonitrile; isocratic 92% A for 7 min, 10 min linear gradient to 70% A, 5 min linear gradient to 40% A, 5 min linear gradient to 30% A, 5 min linear gradient to 92% A. Gradient B: solvent A: 10 mM ammonium acetate (pH 7.0); solvent B: acetonitrile; 50 min linear gradient from 98% A to 75% A, isocratic 75% A for 10 min, 10 min linear gradient to 98% A.

Compound 1. Tetraol **4** (108 mg, 0.155 mmol) was dissolved in a mixture of 1.6 mL formamide, 1.6 mL EtOH, 1.0 mL MeOH, 0.4 mL EtOAc, and 1.2 mL 1,4-cyclohexadiene. A slow stream of argon was bubbled through the solution for 5 min followed by addition of Pd black (25 mg). The reaction was sonicated briefly to disperse the Pd and stirred for 16 h. The Pd was removed by centrifugation and the supernatant dried to a volume of ~ 1 mL. The crude reaction mixture was purified by HPLC using gradient A to yield 50.1 mg (63%) of **1** as a white solid. ^1H NMR (D_6 -DMSO, 500 MHz): δ 2.32 (ddd, 2H), 2.70 (ddd, 2H), 3.52 (dd, 2H), 3.64 (dd, 2H), 3.84 (ddd, 2H), 4.62 (bs, 2H), 4.93 (bs, 2H), 5.45 (bs, 2H), 6.22 (dd, 2H), 8.15 (s, 2H); ES-LRMS: m/e 518.6 ($\text{M}+\text{H}^+$).

Compound 2. Triphenylphosphine (6.7 g, 25.4 mmol) and 3', 5'-*tert*-butyldimethylsilyl deoxyguanosine¹ (6.3 g, 12.7 mmol) were placed in a dried flask under vacuum for 15 minutes. The flask was refilled with argon and dry dioxane (220 ml) and benzyl alcohol (2.6 ml, 25.4 mmol) were added via syringe. To the resulting white suspension was added diethylazodicarboxylate (4.0 ml, 25.4 mmol) slowly via syringe. After a period of 15 minutes a homogeneous solution was obtained and the reaction was allowed to stir an additional hour before it was concentrated *in vacuo* to yield a yellow oil. The resulting oil was dissolved in diethylether (75 ml) and allowed to stand at -20 °C for 1 hour. The resulting white solid was removed by vacuum filtration and washed with cold ether. The filtrate was concentrated *in vacuo* and chromatographed on silica gel (2% methanol/methylene chloride) to give 3.65 g of **2** as a white solid. Impure fractions were further purified by dissolving them in 1 ml of methanol for every 30 mg of compound. An equal volume of water was then added and the white precipitate collected by vacuum filtration to yield an additional 1.3 g of **2** for a total yield of 67%. ¹H NMR (CDCl₃, 500 MHz): δ 0.069 (s, 3H), 0.073 (s, 3H), 0.095 (s, 6H), 0.90 (s, 9H), 0.91 (s, 9H), 2.34 (ddd, 1H), 2.56 (ddd, 1H), 3.75 (dd, 1H), 3.81 (dd, 1H), 3.97 (ddd, 1H), 4.58 (dd, 1H), 5.54 (d, 1H), 5.57 (d, 1H), 6.32 (dd, 1H), 7.28-7.35 (m, 3H), 7.49 (d, 2H), 7.90 (s, 1H); FAB-HRMS: m/e 585.31732 (M+H⁺).

Compound 3. Amine **2** (3.7 g, 6.2 mmol) was dried by concentration from anhydrous toluene (50 ml) *in vacuo*. Antimony (III) bromide (3.2 g, 8.7 mmol) was then added to the flask containing the dried amine and the system was purged with argon. Anhydrous methylene bromide (75 ml) was cooled to 0°C and added via cannula with stirring to the reaction vessel which was also maintained at 0°C. When solvent addition was complete, the reaction was cooled to -10 °C and *tert*-butylnitrite (2.6 ml, 21.7 mmol) was added slowly via syringe. The reaction was stirred for 1 hour at -10°C before it was poured into a solution of 10 g sodium bicarbonate in 250 ml of crushed ice/water. The resulting white precipitate was filtered off and the filtrate was extracted 3 times with methylene chloride. The combined organic extracts were dried over magnesium sulfate, filtered,

and concentrated *in vacuo*. The crude oil was purified by silica gel chromatography (1% methanol/methylene chloride) to obtain 2.4 g (60%) of **3** as a light-yellow oil. ¹H NMR (CDCl₃, 500 MHz): δ 0.087 (s, 6H), 0.10 (s, 6H), 0.90 (s, 9H), 0.91 (s, 9H), 2.43 (ddd, 1H), 2.60 (ddd, 1H), 3.77 (dd, 1H), 3.89 (dd, 1H), 3.99 (ddd, 1H), 4.62 (dd, 1H), 5.62 (d, 1H), 5.65 (d, 1H), 6.42 (dd, 1H), 7.32-7.38 (m, 3H), 7.54 (d, 2H), 8.22 (s, 1H); FAB-HRMS: m/e 648.2153 (M+H⁺).

Silyl protected derivative of compound 4. Amine **2** (100 mg, 0.170 mmol), bromide **3** (122 mg, .188 mmol), tris(dibenzylideneacetone)-dipalladium(0) (Pd₂dba₃) (2 mg, 0.002 mmol), (R)-(+)-2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (BINAP) (4 mg, 0.006 mmol), and sodium *tert*-butoxide (23 mg, .24 mmol) were placed in a dry flask. The flask was purged with argon for 5 min before 1 mL of toluene was added. The reaction was then stirred under argon at 80 °C for 4 hours. At this time, the flask was cooled to room temperature and diluted with diethylether (5 mL). After filtering through a plug of celite, the crude reaction was concentrated *in vacuo* and purified on a silica gel column (1% MeOH/CH₂Cl₂) to obtain 87 mg (40%) of pure compound as a white solid. ¹H NMR (CDCl₃, 500 MHz): δ 0.057 (s, 12H), 0.064 (s, 6H), 0.069 (s, 6H), 0.89 (s, 18H), 0.90 (s, 18H), 2.33 (ddd, 2H), 2.61 (ddd, 2H), 3.72 (dd, 2H), 3.82 (dd, 2H), 3.89 (ddd, 2H), 4.58 (dd, 2H), 5.71 (d, 2H), 5.74 (d, 2H), 6.45 (dd, 2H), 7.28-7.34 (m, 6H), 7.53 (d, 4H), 7.81 (s, 1H), 8.09 (s, 2H); FAB-HRMS: m/e 1153.6220 (M+H⁺).

Compound 4. (42 mg, 0.036 mmol) was dissolved in 1 mL THF. 180 μL of 1.0M tetrabutylammonium fluoride in THF was then added to the reaction mixture and it was stirred for 30 min. The crude reaction mixture was concentrated *in vacuo* and purified by preparative TLC (20% methanol/methylene chloride) to yield 21 mg (85%) of **4** as a white solid. ¹H NMR (CDCl₃, D₆-DMSO, 500 MHz): δ 2.34 (ddd, 2H), 2.83 (ddd, 2H), 3.61 (ddd, 2H), 3.71 (ddd, 2H), 3.99 (bd, 2H), 4.50-4.54 (m, 6H), 5.56 (s, 4H), 6.37 (dd, 2H), 7.18-7.24 (m, 6H), 7.39 (d, 4H), 7.73 (s, 1H), 7.96 (s, 2H); FAB-HRMS: m/e 697.2628 (M+H⁺).

5' Bis-Trityl Derivative of Compound 4. Tetraol **4** (140 mg, 0.200 mmol) was azeotropically dried by concentration from 5 mL of pyridine. To the dried nucleoside was added 4,4'-dimethoxytrityl chloride (168 mg, 0.500 mmol) and 5 mL pyridine and the reaction was stirred under argon for 3.5 h at which time 2 mL of methanol was added and the reaction was dried *in vacuo*. The crude reaction mixture was purified by column chromatography on silica gel (5% MeOH/5% Et₃N/CH₂Cl₂) to yield 206 mg (79%) of the desired compound as a white solid. ¹H NMR (CDCl₃, 500 MHz): δ 2.6 (ddd, 2H), 2.88 (ddd, 2H), 3.24 (dd, 2H), 3.36 (dd, 2H), 3.75 (s, 12H), 4.12 (ddd, 2H), 4.63 (dd, 2H), 5.64 (d, 2H), 5.66 (d, 2H), 6.56 (dd, 2H), 6.76 (d, 8H), 7.15-7.53 (m, 28H), 7.81 (s, 1H), 7.90 (s, 2H); FAB-LRMS: m/e 1303 (M+H⁺).

Compound 5. The bis trityl compound (150 mg, 0.115 mmol) was dissolved in 1 mL pyridine. Allyl 1-benzotriazolyl carbonate (50 mg, 0.23 mmol) was added and the solution stirred under argon for 2 days at which time it was concentrated *in vacuo* and purified on a silica gel column (2.5% MeOH/5% Et₃N/CH₂Cl₂) to yield 48 mg (30%) of **5** as a white solid. Unreacted starting material was recovered and recycled. ¹H NMR (CDCl₃, 500 MHz): δ 2.49 (ddd, 1H), 2.68 (dd, 1H), 2.96-3.02 (m, 2H), 3.23 (dd, 1H), 3.33-3.37 (m, 2H), 3.44 (dd, 1H), 3.73 (s, 3H), 3.74 (s, 3H), 3.74 (s, 3H), 4.08 (dd, 1H), 4.26 (bs, 1H), 4.62-4.66 (m, 2H), 5.28 (t, 2H), 5.34-5.41 (m, 2H), 5.72-5.64 (m, 4H), 5.88-5.95 (m, 1H), 6.44 (dd, 1H), 6.53 (dd, 1H), 6.73 (d, 4H), 6.78 (dd, 4H), 7.11-7.44 (m, 26H), 7.52 (d, 2H), 7.86 (s, 1H), 7.90 (s, 1H), 7.91 (s, 1H); FAB-LRMS: m/e 1387 (M+H⁺).

Compound 6. Compound **5** (46 mg, 0.033 mmol) and diisopropylammonium tetrazolide (3.6 mg, 0.023 mmol) were placed in a flask and purged with argon. 2 mL CH₂Cl₂ and O-2-cyanoethyl N,N,N',N'-tetraisopropylphosphoramidite (21 μL, 0.066 mmol) were added via syringe and the reaction was stirred under argon for 16 hours before it was concentrated and purified on a silica gel column (5% EtOAc/0.5% Et₃N/CH₂Cl₂) to yield 35 mg (67%) of **6** as a

light yellow oil (mixture of diastereomers). ^1H NMR (CDCl_3 , 500MHz): δ 1.07 (d, 6H), 1.12 (d, 6H), 1.16 (d, 6H), 1.16 (d, 6H), 2.55 (t, 2H), 2.61 (t, 2H), 2.66 (dd, 2H), 2.84-2.88 (m, 1H), 2.94-2.98 (m, 1H), 3.30-3.41 (m, 4H), 3.52-3.63 (m, 4H), 3.73 (s, 3H), 3.73 (s, 3H), 3.74 (s, 3H), 3.74 (s, 9H), 3.75 (s, 6H), 4.10-4.24 (m, 2H), 4.59 (d, 1H), 4.66-4.71 (m, 1H), 5.28 (d, 4H), 5.35-5.42 (m, 4H), 5.64-5.80 (m, 8H), 5.86-5.95 (m, 2H), 6.45 (dd, 2H), 6.52 (dd, 2H), 6.77 (d, 16H), 7.16-7.42 (m, 52H), 7.53 (t, 4H), 7.75 (s, 1H), 7.78 (s, 1H), 7.92 (s, 2H), 7.94 (s, 1H), 7.95 (s, 1H); ^{31}P $\{^1\text{H}\}$ NMR (CDCl_3 , 200MHz): δ 148.99 (s), 149.17 (s); FAB-LRMS: m/e 1589 ($\text{M}+\text{H}^+$).

Compound 7. The bis trityl compound (25 mg, 0.019 mmol) and diisopropylammonium tetrazolide (4 mg, 0.027 mmol) were placed in a flask and purged with argon. 1.5 mL CH_2Cl_2 and O-2-cyanoethyl N,N,N',N'-tetraisopropylphosphoramidite (18 μL , 0.057 mmol) were added via syringe and the reaction was stirred under argon for 16 hours before it was concentrated and purified on a silica gel column (10% EtOAc/0.5% $\text{Et}_3\text{N}/\text{CH}_2\text{Cl}_2$) to yield 21 mg (65%) of **6** as a light yellow oil (mixture of diastereomers). ^1H NMR (CDCl_3 , 500MHz): δ 1.06 (d, 6H), 1.12 (d, 6H), 1.16 (d, 6H), 1.16 (d, 6H), 2.40 (t, 2H), 2.48-2.53 (m, 2H), 2.53 (t, 2H), 2.59-2.62 (m, 2H), 2.80-2.84 (m, 4H), 3.29-3.38 (m, 8H), 3.50-3.64 (m, 8H), 3.73 (s, 6H), 3.74 (s, 12H), 3.75 (s, 6H), 4.13 (d, 2H), 4.17 (d, 2H), 4.66-4.72 (m, 4H), 5.67-5.76 (m, 8H), 6.47 (dd, 4H), 6.77 (t, 16H), 7.15-7.40 (m, 48H), 7.51 (d, 4H), 7.75 (t, 4H), 7.94 (s, 2H), 7.96 (s, 2H); ^{31}P $\{^1\text{H}\}$ NMR (CDCl_3 , 200MHz) δ 149.90 (s), 150.03 (s); FAB-LRMS: m/e 1704 ($\text{M}+\text{H}^+$).

Synthesis of Cross-Linked DNA (Scheme IIA). Phosphoramidite **6** (17 mg, 10.7 μmol) was azeotropically dried by concentration from CH_3CN (200 μL) *in vacuo*. After removal of the DMT group from the residue 3' to the cross-link site, the solid support (1 μmol) was washed with CH_3CN and removed from the DNA synthesizer. The solid support cartridge was fitted with two syringes - one containing phosphoramidite **6** dissolved in 100 μL CH_3CN and the other containing 100 μL of 0.4M tetrazole in CH_3CN . These two solutions were passed back and forth through the

cartridge at a rate of ~30 times/min for 5 min. The cartridge was then returned to the DNA synthesizer and the synthesis resumed with the oxidation step. Standard synthesis was continued until the DMT group was removed from the last base. At this time, the synthesis was stopped and the solid support was treated with acetic anhydride and N-methylimidazole to cap the 5' hydroxyls. The solid support was removed from the cartridge and placed in a microfuge tube with THF (300 μL), butylamine (6 μL) and $\text{Pd}[\text{PPh}_3]_4$ (2 mg, 1.7 μmol). The tube was nutated for 24 hours before the beads were isolated by centrifugation, rinsed with THF, and returned to the synthesizer. The synthesis was then continued using reverse phosphoramidites and an extended coupling time of 10 min. Standard procedures were used for cleaving the DNA from the resin and removal of the amine protecting groups. The benzyl groups were removed and the crude DNA was purified by DPAGE to obtain ~10% yield of cross-link based on optical density measurements.

Synthesis of Cross-Linked DNA (Scheme IIB). Phosphoramidite **7** (9 mg, 5.3 μmol) was azeotropically dried by concentration from CH_3CN (200 μL) *in vacuo*. After removal of the DMT group from the residue 3' to the cross-link site, the solid support (2.5 μmol) was removed from the DNA synthesizer. The solid support cartridge was fitted with two syringes - one containing phosphoramidite **7** dissolved in 200 μL CH_3CN and the other containing 200 μL of 0.4M tetrazole in CH_3CN . These two solutions were passed back and forth through the cartridge at a rate of ~30 times/min over a period of 10 min. 200 μL of a 1.0M solution of 2-cyanoethanol in CH_3CN and 200 μL of the tetrazole solution were then passed back and forth through the cartridge at the same rate for 1 min to quench any unreacted phosphoramidite. The cartridge was then returned to the DNA synthesizer and the synthesis resumed with the oxidation step. Standard synthesis and deprotection protocols were used for the remainder of the synthesis. The benzyl groups were removed and the crude DNA was purified by DPAGE to obtain ~10% yield of cross-link based on optical density measurements.

Removal of O⁶-Benzyl Protecting Groups in DNA. Each 2.5 μmol synthesis was dried down in a 1.5 mL microfuge tube and ethanol precipitated by dissolving in 100 μL 0.3M NaOAc and diluting with 700 μL cold ethanol. The DNA pellet was collected by centrifugation, dried *in vacuo*, and dissolved in 400 μL formamide followed by addition of 400 μL EtOH, 120 μL EtOAc, and 240 μL 1,4-cyclohexadiene. After degassing the solution with a slow stream of argon, Pd black (5 mg) was added and the tube was sonicated for 1 hour and allowed to stand overnight. Pd was removed by centrifugation and the supernatant dried *in vacuo*. Removal of the benzyl groups was verified by HPLC analysis (gradient A) of the enzymatic hydrolysate (For example See Figure 1).

Enzymatic Digestions. 0.2 O.D. of DNA was dissolved in 20 μL of water and placed in a 1.5 mL microfuge tube. 3 μL of 10X dephosphorylation buffer (Boehringer Mannheim, 0.5M Tris-HCl, 1 mM EDTA, pH 8.5), 4 μL (4 units) of calf intestinal phosphatase (Boehringer Mannheim), 2 μL (1 unit) of snake venom phosphodiesterase I (Amersham), and 1 μL (200 units) of S1 Nuclease (Amersham) were added sequentially to the DNA. The microfuge tube was allowed to stand at least one hour at 37°C before analysis.

Purification of Cross-Linked DNA. The DNA was dissolved in 98% formamide/10 mM EDTA loading buffer and purified on 20 20% denaturing polyacrylamide gels (17 x 15 x .15 cm). The cross-link band was cut from the gel, the gel slice crushed, and the DNA eluted by soaking overnight in elution buffer (500 mM ammonium acetate pH 7.0, 10 mM MgCl₂, and 10 mM Na₂EDTA). The DNA was desalted on a Waters Sep-Pak C₁₈ cartridge. For NMR analysis, the gel-purified DNA was further purified by HPLC using gradient B. The purified cross-link was run through a Sephadex G-15 (Sigma) column (1.5 x 23 cm) eluting with H₂O prior to NMR analysis. Yield of pure cross-link for NMR analysis was 3% based on optical density measurements.

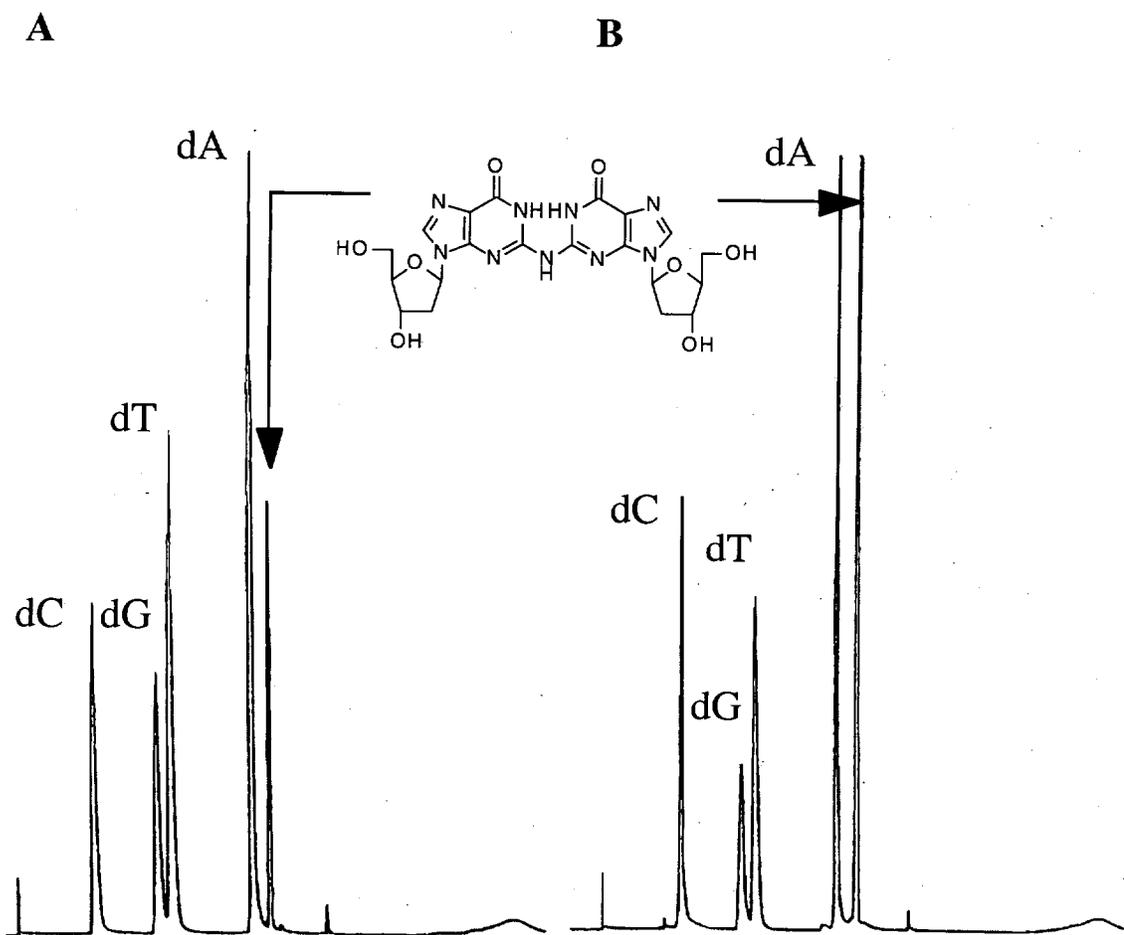
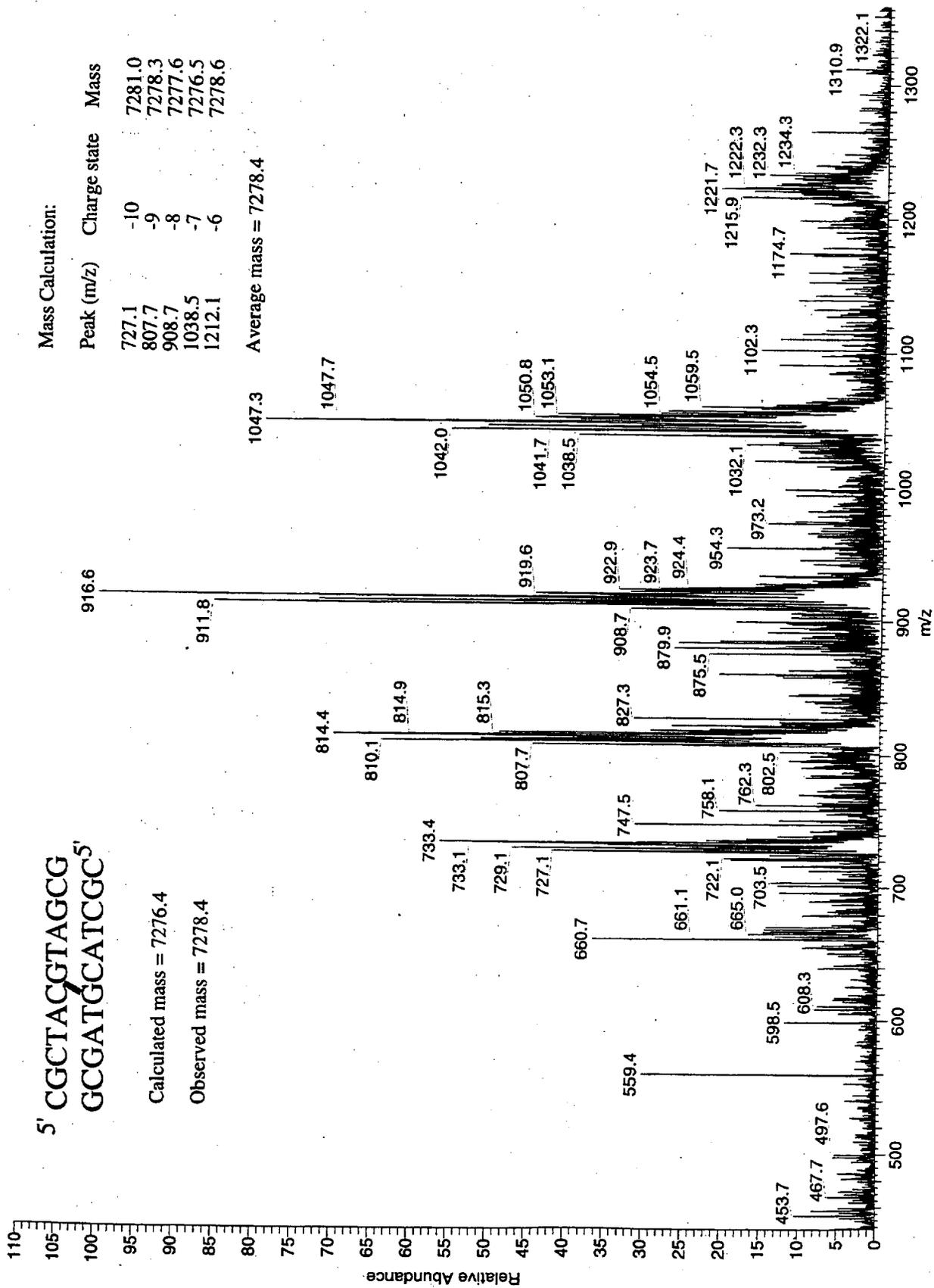


Figure 1. HPLC trace of an enzymatic digest of purified cross-link 1. (5' CATACGTATG underline indicates cross-link site) A) Digest of purified cross-link. B) Digest of cross-link + co-injection of authentic lesion.

6 S



5' CGCTACGTAGCG
 GCGATGCATCGC 5'

Calculated mass = 7276.4
 Observed mass = 7278.4

Mass Calculation:

Peak (m/z)	Charge state	Mass
727.1	-10	7281.0
807.7	-9	7278.3
908.7	-8	7277.6
1038.5	-7	7276.5
1212.1	-6	7278.6

Average mass = 7278.4

01 5

10

5' GCATCCGGATGC
CGTAGGCCCTACG 5'
DNA I

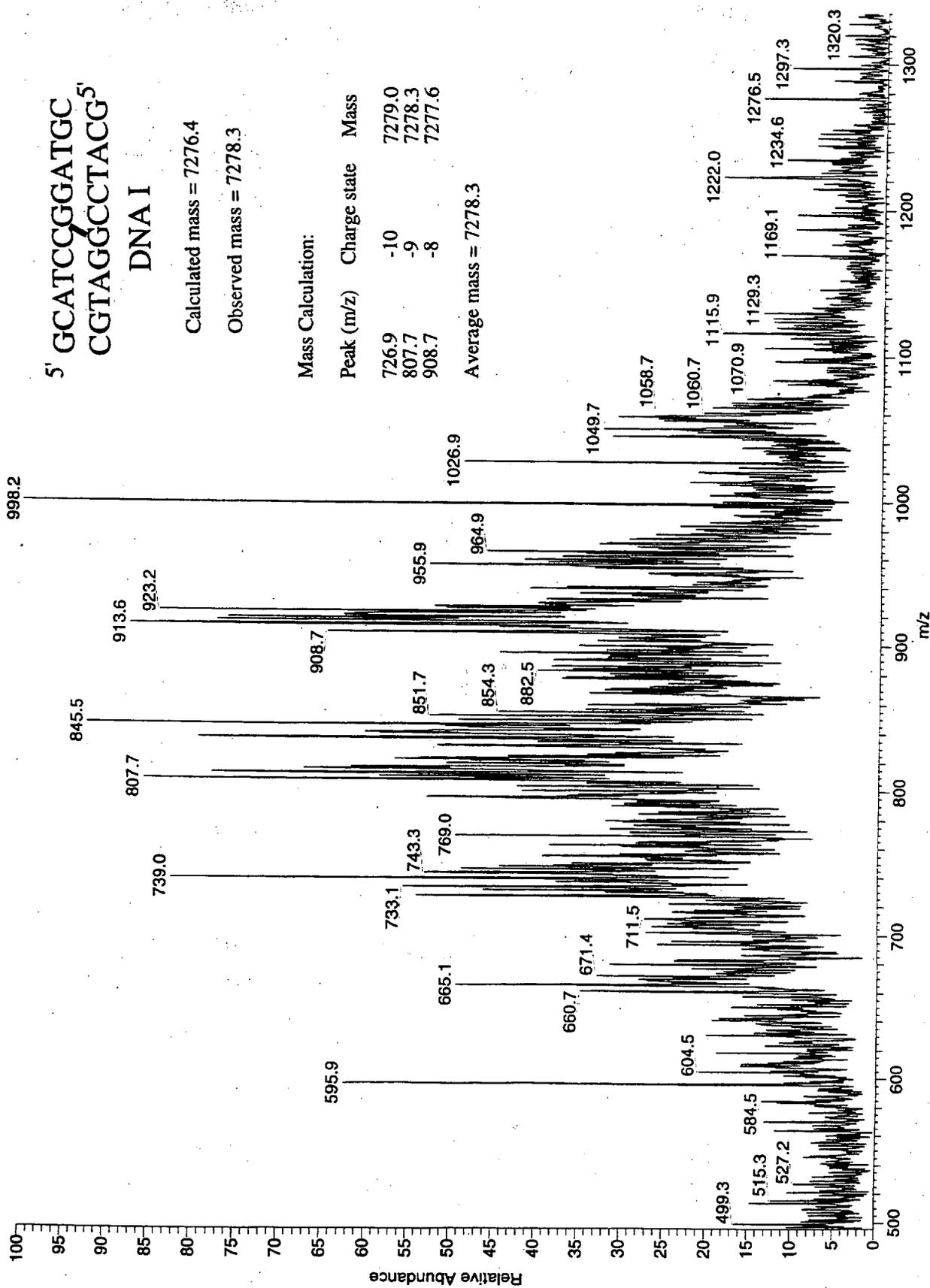
Calculated mass = 7276.4

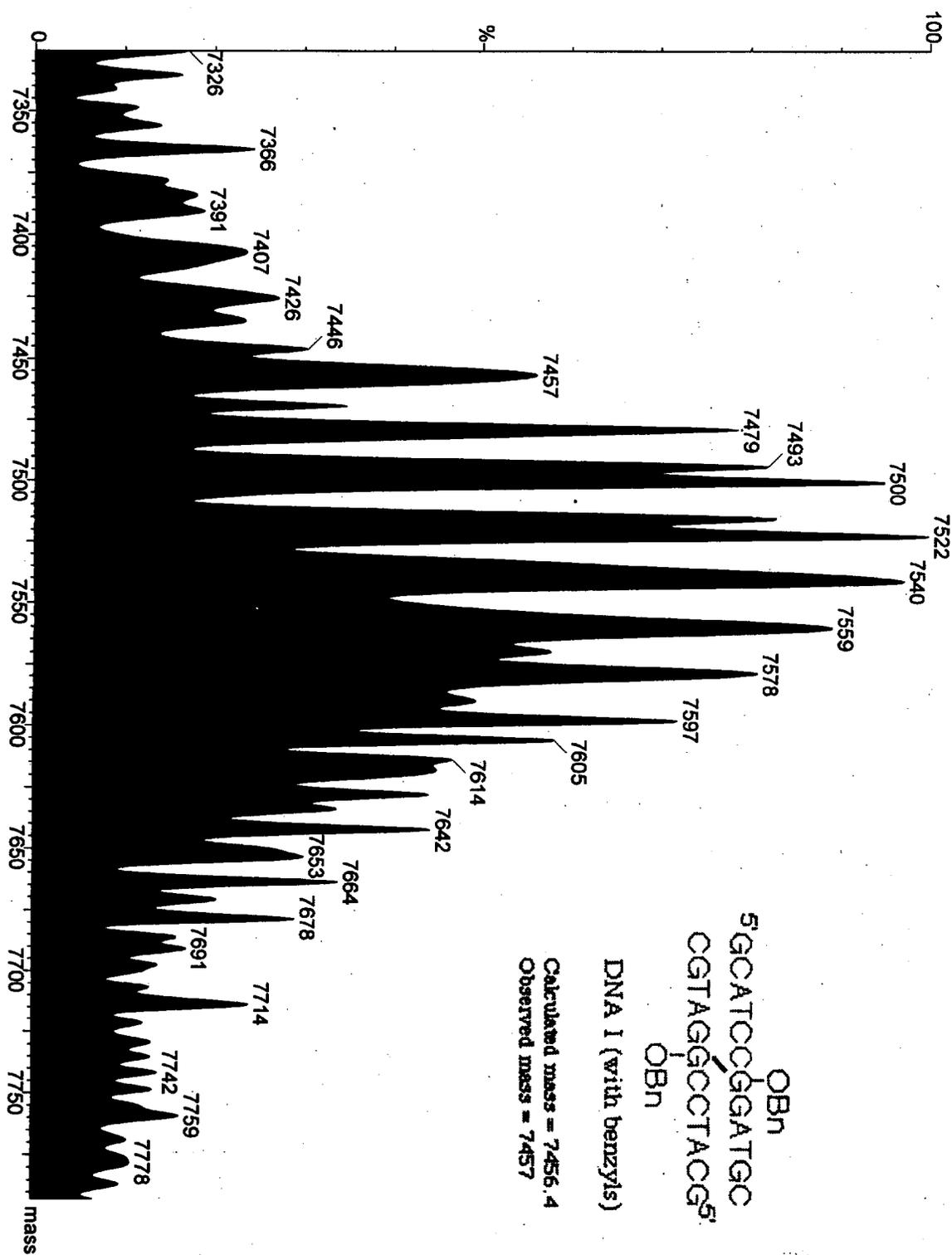
Observed mass = 7278.3

Mass Calculation:

Peak (m/z)	Charge state	Mass
726.9	-10	7279.0
807.7	-9	7278.3
908.7	-8	7277.6

Average mass = 7278.3





¹ Synthesized following the procedure of Oglive, K.K. *Can. J. Chem.*, **1973**, *51*, 3799-3807.