Table II. NMR Spectra (δ) of 4a-g

compd	Ar	EtO*CH*	ArCH	CH_2	OCH_2	OCH_2-CH_3	other
4a (*p*-MeO)	7.8-6.6	3.30	1.90	0.9	3.55	1.20	3.70
					3.30	0.96	
4b (*p*-Me)	7.3-6.8	3.20	1.80	0.9	3.49	1.12	2.28
					3.24	0.98	
4c (*p*-H)	7.2-6.9	3.45	1.90	0.9	3.60	1.20	
					3.45	1.00	
4d (*p*-Cl)	7.4-6.8	3.30	1.90	1.1	3.54	1.20	
					3.25	1.00	
4e (*m*-MeO)	7.1-6.4	3.40	1.85	1.0	3.50	1.19	3.73
					3.25	0.93	
4f (*p*-Br)	7.5-6.8	3.28	1.85	0.9	3.50	1.18	
					3.35	0.98	
4g (*m*-Cl)	7.1-6.4	3.39	1.85	1.0	3.55	1.15	
					3.20	0.93	

may be formed directly via nucleophilic attack of **1** accompanied by loss of nitrogen.

Experimental Section

IR spectra were recorded on a Jasco IR-G recording spectrometer. ^1H NMR spectra were determined on a JEOL JNM-MH-100 spectrometer as $CDCl_3$ solutions with an internal $(CH_3)_4Si$ standard. GC-MS spectra were obtained on a Shimadzu GC-MS 1000 spectrometer using a column consisting of silicone OV-17 on Shimalite (4.0 mm × 2.0 m). GC work was done on a Yanagimoto G-180 gas chromatograph using a 4.0 mm × 2.0 m column packed with OV-17 (5%) on 60-80 mesh Diasolid L.

Materials. The aryldiazomethanes **1a-g** were prepared according to literature procedures[18] immediately before use. Authentic samples for identification of reaction products were synthesized as follows. Most of the ethers **2** were conveniently prepared by a Williamson synthesis. Commercially unavailable C-H insertion products were prepared by Grignard reaction of the corresponding ketone with CH_3MgI. Satisfactory spectroscopic data have been obtained for all authentic samples. The cyclopropanes **4** were isolated from the irradiation mixture by GC and identified by NMR and MS. The configuration of the cyclopropanes has been assigned on the basis of the NMR arguments[7a] that the trans-ethoxy protons should be deshielded by the anisotropy of the phenyl ring while the cis-ethoxy protons should show an increased shielding. Equilibration experiments of the two epimeric adducts by treatment with potassium *tert*-butoxide in $(CH_3)_2SO$ also support the assignment. NMR data for *cis*- and *trans*-**4** are given in Table II.

Photochemical Reactions and Analyses. All irradiations were carried out with a Halos 300-W high-pressure mercury lamp with a water-cooled jacket. In a typical procedure, 0.005 mmol of the diazo compounds was added to 2.0 mL of a binary mixture of 2-propanol and ethyl vinyl ether in Pyrex tubes. The sample was then degassed, sealed, and suspended in a transparent Dewar thermostated at the appropriate temperature. Irradiation was generally continued until all of the diazo compound was destroyed. Sensitized experiments were performed under conditions similar to those described above. Usually a 50 M excess of benzophenone to the diazo compound was added to ensure that >95% of the incident light was absorbed by the sensitizer. That addition of benzophenone greatly accelerates the rate of decomposition was noted. Irradiation of oxirane **5** was carried out in a quartz tube. Thermolysis was done in a sealed Pyrex tube. Control experiments exclude possible conversion of the products during the decomposition period and also demonstrate that no reactions occur in the absence of light in the photolysis of **1** and **5** over the temperature range studied.

Product identifications were established either by GC or by GC-MS comparisons using authentic samples. Product distributions were conveniently determined by standard techniques.

Registry No. **1a**, 23304-25-8; **1b**, 23304-24-7; **1c**, 766-91-6; **1d**, 19277-54-4; **1e**, 65864-99-5; **1f**, 73900-14-8; **1g**, 51157-54-1; *cis*-**4a**, 80287-83-8; *trans*-**4a**, 80287-84-9; *cis*-**4b**, 40237-67-0; *trans*-**4b**, 40489-59-6; *cis*-**4c**, 80287-85-0; *trans*-**4c**, 80287-86-1; *cis*-**4d**, 80287-87-2; *trans*-**4d**, 80287-88-3; *cis*-**4e**, 80287-89-4; *trans*-**4e**, 80287-90-7; *cis*-**4f**, 80287-91-8; *trans*-**4f**, 80287-92-9; *cis*-**4g**, 80287-93-0; *trans*-**4g**, 80287-94-1; *cis*-**5**, 1689-71-0; *trans*-**5**, 1439-07-2.

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Synthesis and Characterization of an Oligonucleotide Containing a Carcinogen-Modified Base: O^6-Methylguanine[1]

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Abstract: The synthesis and characterization of the oligomer 5′-dTp(O^6-Me)GpCpA-3′ by the modified triester procedure is described, representing the preparation of a DNA fragment containing a base specifically covalently modified by a carcinogen. With use of the tools of genetic engineering, this tetramer will be substituted for a 5′-TpGpCpA-3′ portion of the DNA of bacterial virus *φX174* in order to study the effect on replication of a well-characterized chemical modification of DNA at an exactly known point. The presence of O^6-methylguanine in the oligomer is shown to inhibit the enzyme activities of snake venom phosphodiesterase and endonuclease P_1.

The formation of chemical carcinogen-DNA adducts may induce changes in DNA base sequence and, ultimately, neoplastic transformation. However, despite the growing body of literature which describes the formation and structure of a number of adducts (such as those derived from aflatoxin B_1,[3] sterigmatocystin,[4] benzo[*a*]pyrene,[5] and acetylaminofluorene[6]), no correlation has been established between the structure of an adduct, its location in a gene, and the mutagenic risk it poses to the cell. Previous studies on the mechanism of mutagenesis have been constrained by the inability to produce a lesion at a particular site, to determine

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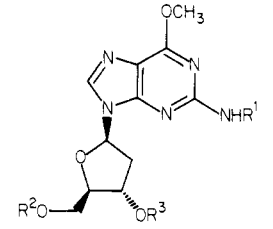
with certainty its chemical structure, and to examine in detail its biochemical consequences.

We have begun a program which takes on these problems and seeks to answer some fundamental questions in chemical carcinogenesis. This report details the synthesis of an oligonucleotide which contains a carcinogen covalently bound to a particular base. This segment will be inserted into a viable viral DNA molecule to serve as a probe for the mutagenic effect of the lesion during DNA replication.

O^6-Methylguanine was chosen as the carcinogen-modified base for four main reasons: (1) the occurrence and persistence of O^6-methylguanine in target tissues of susceptible species treated with chemical carcinogens has been correlated consistently with tumor incidence;[7] (2) since O^6-methylguanine may hydrogen bond to thymine as well as to cystosine, it should be misread as an adenine about one-third of the time[8] (the resulting G–C to A–T transition we predict will not be fatal to our biological system); (3) as demonstrated herein, this covalently modified base is stable to the reaction conditions used in the phosphotriester method of oligonucleotide synthesis; (4) this relatively nonbulky adduct should present minimal problems during insertion of the test segment into a larger piece of DNA using conventional genetic engineering techniques.

The synthesis of the oligonucleotide 5′-dTṗ(O^6-Me)GṗCṗA-3′[9] was accomplished by using the modified triester procedure as described by Miller.[10] O^6-Methyldeoxyguanosine [d(O^6-Me)G] **2** was prepared from the condensation of 2-amino-6-chloropurine with 2-deoxy-3,5-di-*O*-*p*-toluoyl-D-*erythro*-pentosyl chloride,[11] followed by N-acylation, fractional crystallization of the β anomer, and methanolysis.[12] Trisacylation of **2** with benzoyl chloride and



2, $R^1 = R^2 = R^3 = H$
3, $R^1 = R^2 = R^3 = Bz$
4, $R^1 = Bz$, $R^2 = R^3 = H$
5, $R^1 = Bz$, $R^2 = DMTr$, $R^3 = H$
6, $R^1 = Bz$, $R^2 = DMTr$, $R^3 = \dot{p}CE$
7, $R^1 = Bz$, $R^2 = H$, $R^3 = \dot{p}CE$

selective ester hydrolysis of the product **3** under basic conditions[13] provided *N*-benzoyl-O^6-methyldeoxyguanosine **4**. This led to the 5′-protected nucleoside **5** after treatment with dimethoxytrityl chloride in pyridine and 3′-phosphorylation according to Miller[10] afforded the fully protected *p*-chlorophenyl cyanoethyl phosphotriester **6**.

Synthesis of the dimers dDMTrTṗ(O^6-Me)G^{Bz}ṗCE (**8**) and dC^{Bz}ṗA^{Bz}Ac (**9**) using mesitylenesulfonyl tetrazolide (MST)[14] as the condensation reagent proceeded as shown in Scheme I and these were, in turn, linked to give the fully protected tetranucleotide dDMTrTṗ(O^6-Me)G^{Bz}ṗC^{Bz}ṗA^{Bz}Ac (**10**).[15] The observation of

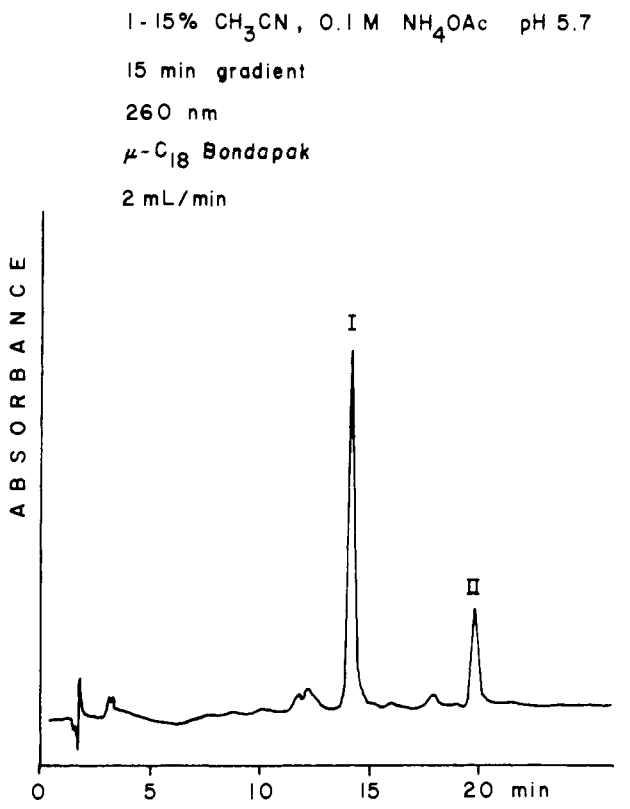
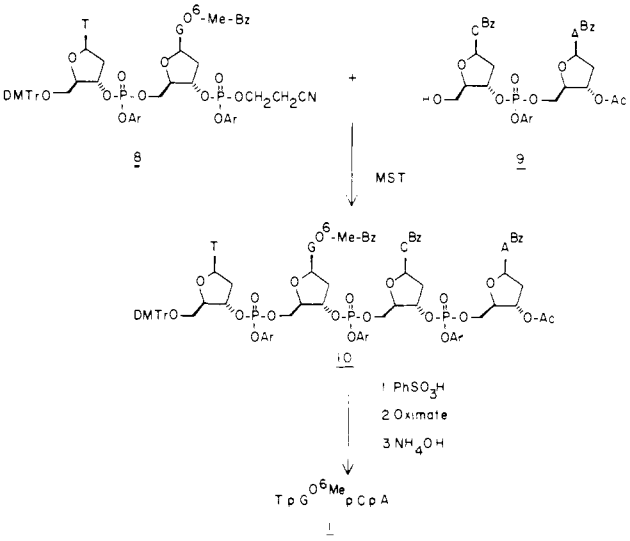


Figure 1. Reversed-phase HPLC chromatogram of the deprotection reaction mixture.

Scheme I



protonated molecular ions for **8**, **9**, and **10** in their field desorption mass spectra confirmed their successful synthesis. After detritylation ($PhSO_3H$) the phosphotriester linkages were deprotected by treatment with the tetramethylguanidinium salt of *p*-nitrobenzaldoxime.[16] A final treatment with aqueous ammonia led

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(9) Abbreviations: d = deoxy; d(O^6-Me)G = O^6-methyldeoxyguanosine; p = ; ṗ = PO_4-*p*-ClC_6H_4; CE = cyanoethyl; DMTr = dimethoxytrityl.
(10) Miller, P. S.; Cheng, D. M.; Dreon, N.; Jayaraman, K.; Kan, L.-s.; Leutsinger, E. E.; Pulford, S. M.; Ts'o, P. O. P. *Biochemistry* **1980**, *19*, 4688–98.
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(15) We found, as did others, that it is not necessary to remove dimethoxytrityl alcohol from the 5′-hydroxyl component prior to phosphate bond formation. Cf. Gough, G. R.; Singleton, C. K.; Weith, H. L.; Gilham, P. T. *Nucleic Acids. Res.* **1979**, *6*, 1557–70.
(16) Chattopadhyaya, J. B.; Reese, C. B. *Nucleic Acids Res.* **1980**, *8*, 2039–53.

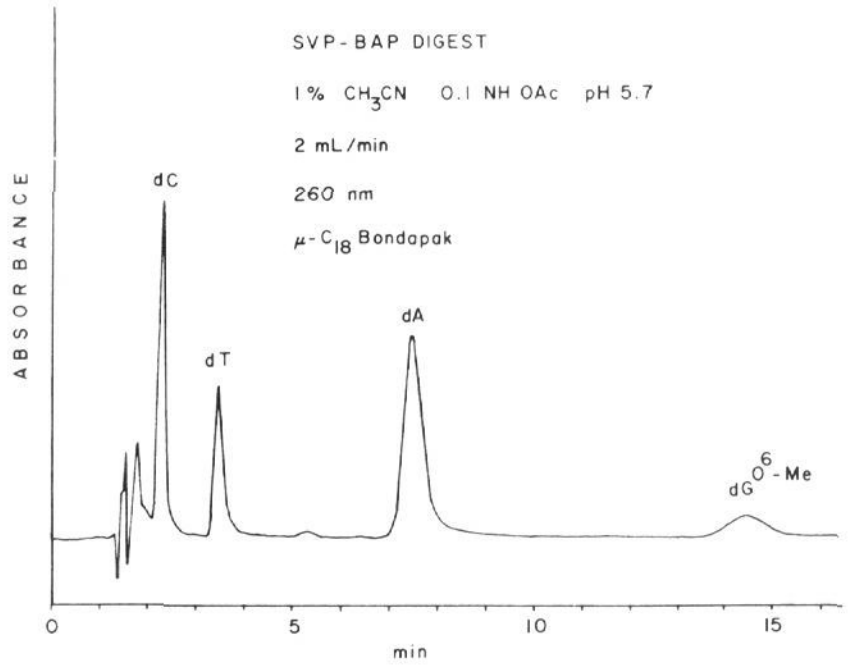

Figure 2. Reversed-phase HPLC chromatogram of the snake venom phosphodiesterase–bacterial alkaline phosphatase digest of tetranucleotide **1**.

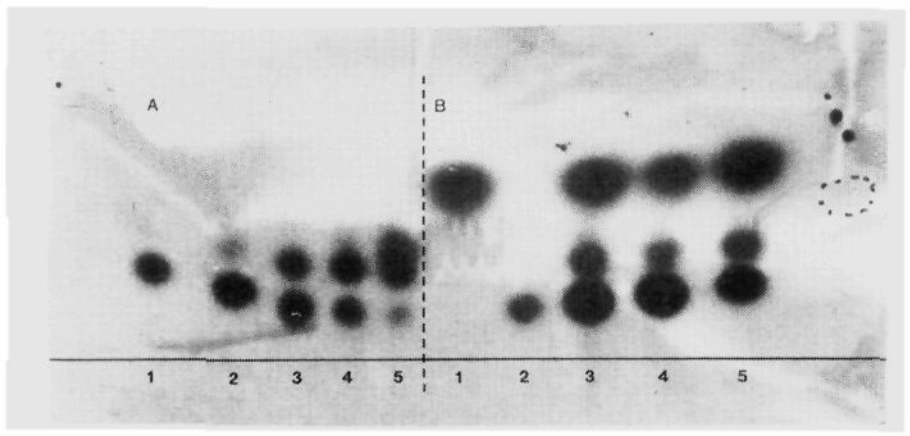

Figure 3. (a) Snake venom phosphodiesterase digestion of tetranucleotide **1** after 5′-end labeling with γ-^{32}P ATP: lane 1, time 0; 2, 2 min at ambient temperature; 3, 10 min; 4, 20 min; 5, an additional 40 min at 37 °C. (b) Endonuclease P_1 digestion of labeled **1**: lane 1, complete digestion after 3 h at 37 °C; 2, starting material; 3, 5-min digestion at ambient temperature with 0.5 μL of enzyme solution; 4, 1.0 μL of solution; 5, 3.0 μL of solution.

to the fully deprotected tetranucleotide. HPLC analysis (Figure 1) on reversed phase[17] revealed a major product (tetranucleotide) and a later eluting impurity.[18] Preparative isolation and C_{18} cartridge desalting gave the final product **1**.

In order to confirm the base composition and sequence of the segment, a small portion of the sample was digested with snake venom phosphodiesterase and bacterial alkaline phosphatase. The resulting component nucleosides were separable by reversed-phase HPLC[19] and were shown by UV spectroscopic properties and retention times to be (in order of elution) dC, dT, dA, and d-(O^6-Me)G (Figure 2). Phosphorylation[20] of another small portion

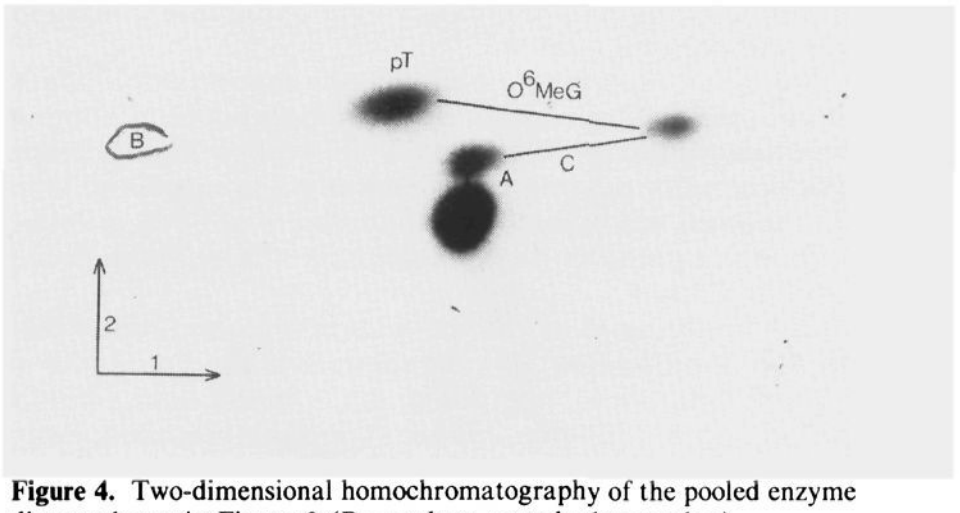

Figure 4. Two-dimensional homochromatography of the pooled enzyme digests shown in Figure 3 (B = xylene cyanole dye marker).

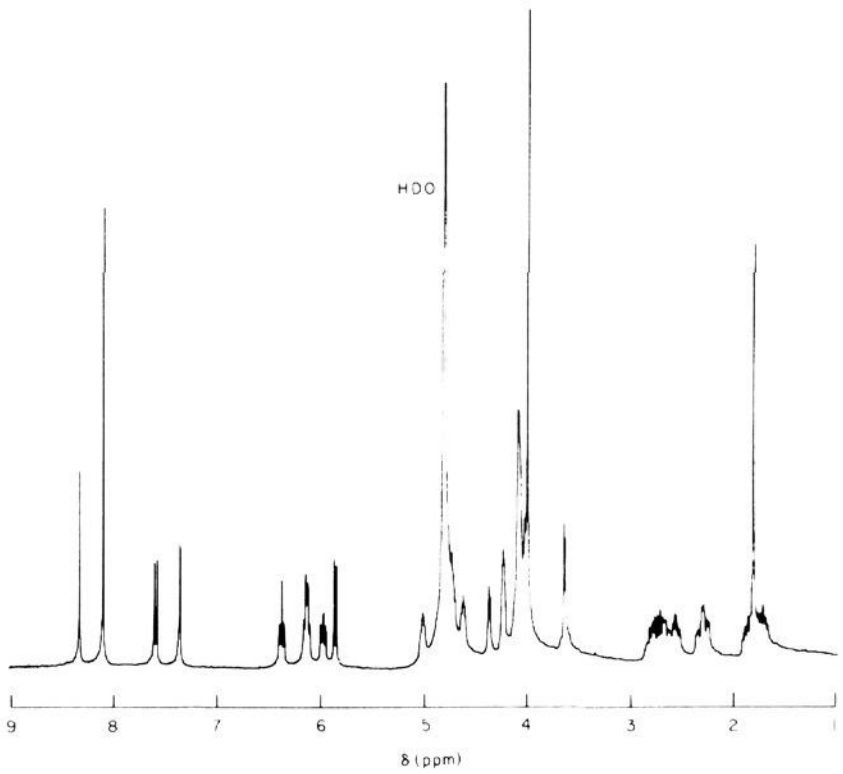

Figure 5. 200-MHz ^{1}H NMR spectrum of the tetranucleotide **1** in D_2O. Shifts are in parts per million downfield from DSS.

of the tetramer with polynucleotide kinase and γ-^{32}P ATP and HPLC purification (a 30-min gradient of 1–15% acetonitrile in 0.1 M ammonium acetate, pH 5.7) gave 5′-end labeled oligomer which was divided into two portions for enzymatic digestion. In one experiment, digestion with snake venom phosphodiesterase was observed over time. Figure 3a (chromatography on DEAE cellulose in 50 mM homomix[20]) shows the rapid appearance of C–A phosphate bond cleavage product at room temperature but a much slower (O^6-Me)G–C bond cleavage, even after 60 min at 37 °C, and no T–(O^6-Me)G hydrolysis in this time. (More vigorous conditions lead to more complete hydrolysis as shown in Figure 2). Digestion of the segment with varying concentrations of endonuclease P_1 for 5 min at room temperature (Figure 3b) effected ready cleavage of all bonds except the (O^6-Me)G–C phosphate linkage which required more vigorous conditions (3 h at 37 °C). Similar inhibitions of nuclease activity by modified bases in RNA[20a,b] and carcinogen-treated DNA[20c] have been noticed elsewhere.

The pooled snake venom phosphodiesterase and endonuclease P_1 partial digests were submitted to homochromatography,[20,21] and the usual mobility shift observed for the second base confirms the presence of a modification (Figure 4).

A 300-MHz ^{1}H NMR spectrum of the tetranucleotide **1** in D_2O is shown in Figure 5. Most notable is the appearance of a sharp three-proton singlet at δ 4.00, corresponding to the O^6-methyl group on guanine. The base proton (δ 7–9) and H-1′ regions (δ 6) clearly show the presence of the four nucleosides.

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(18) The minor product was submitted to enzymatic digestion and HPLC analysis, revealing the presence of dC and dA. The segment is probably not a partially protected tetranucleotide [on (O^6-Me)G] since treatment of this minor product with NH_4OH (55 °C, 18 h) left it intact. Furthermore, the UV spectrum (max 266 nm) is unlike that of the tetranucleotide. This segment may arise from fragmentation of the tetranucleotide since the fully protected tetramer was shown to be free of $dC^{Bz}pA^{Bz}Ac$ prior to deprotection.

(19) Cf. (a) Mischke, C. F.; Wickstrom, E. *Anal. Biochem.* **1980**, *105*, 181–187. (b) Gehrke, C. W.; Kuo, K. C.; Davis, G. E.; Suits, R. D.; Waalkes, T. P.; Borek, E. *J. Chromatogr.* **1978**, *150*, 455–476.

(20) (a) Silberklang, M.; Gillum, A. M.; RajBhandary, U. L. *Nucleic Acids Res.* **1977**, *4*, 4091–4108. (b) Silberklang, M.; Gillum, A. M.; RajBhandary, U. L. *Methods Enzymol.* **1979**, *59*, 58–109. The technique of pooling complementary digests is useful in sequencing RNA's containing modified bases. (c) Boehm, T. L. J.; Drahovsky, D. *Carcinogenesis* **1980**, *1*, 729–731.

(21) Tu, C.-P. D.; Jay, E.; Bahl, C. P.; Wu, R. *Anal. Biochem.* **1976**, *74*, 73–93.

Conclusions

Synthesis of the (O^6-Me)G-containing tetranucleotide **1** represents one step in a program that has the objective of probing the mechanisms of mutagenesis and repair of carcinogen–DNA component adducts. Presently, the tools of genetic engineering are being used to substitute the synthesized tetranucleotide for a 5′-pTpGpCpA-3′ segment of the DNA of the bacterial virus *φX174*. The adduct will thus be located at an exactly known site in the genome, and since O^6-methylguanine is believed to act as both G and A in the replication process,[8] this site will be monitored in progeny DNA molecules for changes in base sequence. A G to A mutation at the site chosen for placement of O^6-methylguanine should on genetic grounds produce viable mutant progeny and at the same time confer a phenotype that can be distinguished from the wild type (i.e., nonmutants). In future work, we hope to combine our developing experience in DNA replication, mutation, and repair with our knowledge of the formation and synthesis of carcinogen–DNA component adducts[3,4,22] to build larger and more complex adducts into DNA at specific points.

Experimental Section

Solvents were either repurified before use or were commerically distilled in glass. Pyridine was distilled from $ClSO_3H$ (1 g/L) and KOH and stored over 4A molecular sieves. Analytical TLC plates were cut from EM 250-μm silica gel 60 F-254 20 × 20 cm plates and compounds were visualized by UV fluorescence quenching and/or spraying with 10% $HClO_4$ for tritylated compounds. "Flash chromatography" was carried out as described by Still.[23] Chromatography of nucleotide digests on DEAE cellulose in 50 mM homomix and two-dimensional homochromatography were performed as described in ref 20. Organic solutions after aqueous workup were dried over anhydrous sodium sulfate and evaporations were performed in vacuo at 35 °C or less. Sep-Pak C_{18} cartridges were purchased from Waters Associates, Milford, MA. HPLC was performed on a Waters Assoc. μ-Bondapak C_{18} column equipped with two M-6000A pumps, a Model 660 solvent programmer, and a variable wavelength UV detector. Combustion analyses were performed by Robertson Laboratory, Florham Park, NJ, and are within ±0.4% of the calculated values. High-resolution EI mass spectra were obtained on a Du Pont CEC-110 photoplate instrument using evaporated AgBr plates (Ionomet). Field desorption mass spectra were obtained at a resolution of M/ΔM of 2000 using carbon emitters in a Varian MAT 731 instrument, source temperature 80–90 °C, emitter currents 15–19 mA, and accelerating voltage 6 or 8 kV. The 300-MHz ^1H NMR spectrum was run on a Bruker WM-300 spectrometer. Nucleosides and snake venom phosphodiesterase (Crotalus adamanteus) were obtained from Sigma Chemical Co., bacterial alkaline phosphatase from Worthington Biochemicals, endonuclease P_1 (Penicillium citrinum) from Yamasa Shoyu (Japan), T4 polynucleotide kinase from Boehringer Mannheim, and γ-^{32}P ATP from New England Nuclear Corporation.

N^2,O^3,O^5-Tribenzoyl-O^6-methyldeoxyguanosine (3). O^6-Methyldeoxyguanosine (**2**) (377 mg, 1.34 mmol)[11,12] was dried by evaporation with pyridine and treated in 10 mL of dry pyridine with benzoyl chloride (0.64 mL, 5.5 mmol) dropwise at 0 °C. After 0.5 h at 0 °C and 2 h at room temperature the reaction mixture was poured into ice-cold aqueous sodium bicarbonate and extracted with 4 × 10 mL of $CHCl_3$. The combined organic layers were washed with water, dried, and evaporated to give 854 mg of foamy gum which was flash-chromatographed with 4:1 EtOAc/hexane to give 620 mg of white foam (78%): UV max (EtOH) 230, 270, 281 (sh) nm (relative ε 2.0:1.0:0.89). The material was used without further purification in the next step.

N^2-Benzoyl-O^6-methyldeoxyguanosine (4). Tribenzoyl-O^6-methyldeoxyguanosine (**3**) (620 mg, 1.04 mmol) was dissolved in 2 mL of pyridine and 3 mL of EtOH and treated with a mixture of 4 mL of 2 N NaOH and 4 mL of EtOH for 5 min at room temperature. The reaction was neutralized with excess pyridinium Dowex 50W-X8 (100–200 mesh). The resin was filtered off and washed with several milliliters of EtOH and pyridine. Evaporation gave 622 mg of a white solid which was preadsorbed onto 4 g of silica gel and flash chromatographed by using 10% MeOH/$CHCl_3$, providing 310 mg of a white solid (77%). Recrystallization from water (10 mg in 2.5 mL) gave a white powder which was dried without heat in vacuo: mp 186–189 °C (corrected); UV max (95% EtOH) 231 nm (ε 18 200), 273 (19 700); high-resolution mass spectrum calcd. for $C_{18}H_{19}N_5O_5$ 386.1420, found 386.1392.

N-Benzoyl-5′-dimethoxytrityl-O^6-methyldeoxyguanosine (5). N-Benzoyl-O^6-methyldeoxyguanosine (**4**) (305 mg, 0.791 mmol) was dissolved in 10 mL of dry pyridine and treated with dimethoxytrityl chloride (281 mg, 0.829 mmol). After 18 h, the reaction mixture was poured into ice-cold aqueous sodium bicarbonate, extracted with 4 × 10 mL of $CHCl_3$, washed with brine, dried, and evaporated to give 646 mg of crude product. Flash chromatography (2% MeOH/$CHCl_3$ + trace pyridine) afforded a yellow oil which was dissolved in a minimum amount of THF and precipitated into 30 mL of cold cyclohexane. Filtration on a medium fritted filter gave 370 mg (68%) of fine white powder: UV max (95% EtOH) 232 nm (ε 34 200), 274 (19 700), 280 (sh, 18 400).

Anal. Calcd for $C_{39}H_{37}N_5O_7$: C, 68.11, H, 5.42; N, 10.18. Found: C, 67.82; H, 5.70; N, 9.96.

N-Benzoyl-O^6-methyldeoxyguanosine 3′-p-Chlorophenyl Cyanoethyl Phosphate 7. N-Benzoyl-5′-dimethoxytrityl-O^6-methyldeoxyguanosine (**5**) (400 mg, 0.582 mmol) was phosphorylated as described by Miller.[10] The crude product was purified by flash chromatography, affording 371 mg of white foam (68%). Since precipitation of a concentrated THF solution of fully protected monomer **6** into hexane gave a gummy product, the material was detritylated without further manipulation.

The fully protected triester monomer **6** (371 mg) was dissolved in 3 mL of $CHCl_3$ and treated with 3 mL of a solution of 2% benzenesulfonic acid monohydrate in 7:3 $CHCl_3$/MeOH for 5 min at room temperature. The solution was poured into 12 mL of a 5% aqueous sodium bicarbonate solution and extracted with 3 × 3 mL of $CHCl_3$. The organic layers were washed with 5 mL of water, dried, filtered, and evaporated. The product was purified by flash chromatography (7% MeOH/$CHCl_3$), giving a colorless foam which was precipitated into 100 mL of hexane, yielding 145 mg of a white powder (40% over two steps): UV max (95% EtOH) 272 nm (ε 19 700), 230 (sh, 18 900), 217 (sh, 31 700).

Anal. Calcd for $C_{27}H_{26}ClN_6O_8P$: C, 51.56, H, 4.17; N, 13.36. Found: C, 51.70; H, 4.36; N, 13.07.

dDMTrTp(O^6-Me)G^{Bz}pCE(8). dDMTrTpCE (55 mg, 70 μmol) was dissolved in 1 mL of pyridine and treated with 0.4 mL each of triethylamine and water. After 0.5 h TLC showed one trityl-positive spot at the origin and the mixture was evaporated twice with pyridine. d-(O^6-Me)G^{Bz}pCE (**7**) (29 mg, 46 μmol) was added, and after threefold evaporation with 2 mL of pyridine, MST (54 mg, 214 μmol), and 2 mL of pyridine were added. After 3 h TLC showed the reaction to be complete and 50% aqueous ice-cold pyridine was added. The mixture was evaporated and the residue was flash chromatographed to give, after hexane precipitation of combined product fractions, 53 mg of white powder (85%): UV max (95% EtOH) 270 nm (ε 31 900), 230 (sh, 48 100); FDMS in $CHCl_3$ solution MH^+, 1345.

dC^{Bz}pA^{Bz}Ac (9). dDMTrC^{Bz}pCE (128 mg, 146 μmol) was deprotected with aqueous triethylamine as described above and condensed with dA^{Bz}Ac[24] (39 mg, 98 μmol) by using MST (111 mg, 440 μmol). After 17 h, the reaction was worked up as before and flash chromatography (5% MeOH/$CHCl_3$) of the resulting 189 mg of crude product afforded 118 mg of a colorless foam. Deprotection without further manipulation using benzenesulfonic acid and purification by flash chromatography (5% MeOH/$CHCl_3$) of the product provided 50 mg of a white powder after precipitation into hexane (57% over two steps): UV max (95% EtOH) 261 nm (ε 37 900); FDMS of tritylated **9** in $CHCl_3$ solution MH^+, 1203.

dDMTrTp(O^6-Me)G^{Bz}pC^{Bz}pA^{Bz}Ac (10). dDMTrTp(O^6-Me)G^{Bz}pCF (**8**) (94 mg, 70 μmol) was deprotected with triethylamine and condensed using MST (58 mg, 230 μmol) with dC^{Bz}pA^{Bz}Ac (**9**) (49 mg, 54 μmol) overnight. After workup TLC showed one trityl-positive spot and the material was purified on two 1000 μm silica gel plates (20 × 20 cm) which had been predeveloped with 5% MeOH/$CHCl_3$ and a trace of pyridine. After two developments with 5% MeOH/$CHCl_3$ the band at R_f 0.24 was eluted with 10% MeOH/$CHCl_3$, providing 80 mg of a colorless foam. Precipitation into hexane gave 77 mg (65%) of white powder: UV max (95% EtOH) 269 nm (ε 57 600), 238 (50 700); FDMS in $CHCl_3$ solution M^+ cluster, 2174–2184 (observed at low resolution).

Detritylation was performed on 7 mg of tetramer, and the product was purified by chromatography on deactivated silica gel[25] (43:2:55 CH_2Cl_2/H_2O/acetone, 250-μm plate), affording 5 mg of white solid, a portion of which was subjected to total deprotection.

5′-dTp(O^6-Me)GpCpA-3′ (1). Detritylated protected tetranucleotide (134 AU_{260}) was dissolved in 0.3 mL of dioxane and p-nitrobenzaldoxime (50 mg, 0.3 mmol), tetramethylguanidine (36 μL, 0.3 mmol), and 0.3 mL of water was added[16]. After being stirred magnetically at room temperature for 23 h, the solution was transferred to a pressure tube with

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2 mL of NH_4OH and heated at 50 °C for 20 h. The orange heterogeneous mixture was evaporated, and the residue was dissolved in 17 mL of H_2O and 4 mL of AcOH (decolorization). Extraction with EtOAc (10, 5, 5 mL) and $CHCl_3$ (5 mL) left an aqueous layer which contained two products by reversed phase HPLC (see text) (15-min gradient, 1-15% CH_3CN in 0.1 M NH_4OAc, pH 5.7, 2 mL/min). After preparative injections and the pooling of fractions, the eluant containing the tetranucleotide **1** (the major peak) was diluted with three volumes of water and passed through a Sep-Pak (prewashed with 80% CH_3CN and H_2O). A 5-mL wash with H_2O removed excess AcOH and NH_4OAc, and the product was eluted with 5 mL of 50% CH_3CN. Lyophilization and solution of the product in water gave 17 AU_{260}: UV max (H_2O) 265 nm, 254 (sh) (relative ϵ 1.0:0.98).

Approximately 4 AU_{260} of the tetramer **1** in 0.5 mL of 0.10 M Tris/2mM $MgCl_2$ pH 8.2 buffer was treated with 80 μg of venom phosphodiesterase, 160 μg of bacterial alkaline phosphates, and 440 μL of buffer to make a total of 1 mL.[20] The reaction was incubated at 37 °C for 17.5 h, 2.5 mL of cold EtOH was added, and after 1 h at −20 °C the proteins were pelleted by centrifugation and the supernatant was evaporated under a stream of argon. The residue was dissolved in 0.5 mL of H_2O and analyzed on a reversed-phase HPLC column (1% CH_3CN, 0.1 M NH_4OAc, pH 5.7, μ-Bondapak C_{18}, 2 mL/min), showing dC (2.3 min), dT (3.6 min), dA (8.6 min), and d(O^6-Me)G (16.5 min) by comparison with authentic standards. The peaks were collected and compared by UV with authentic nucleosides.

5′-Phosphorylation with γ-^{32}P ATP and polynucleotide kinase,[20] HPLC purification (see text), and partial digestion with snake venom phosphodiesterase and endonuclease P_1 (see text) gave a mixture of fragments which were pooled and submitted to homochromatography.[21] Figure 4 shows the expected sequence including an unusual mobility shift for the second base.

^{1}H NMR (D_2O, in ppm downfield from DSS): 8.31 (1 H, s, H8 of A), 8.08 (s, 2, H2 A + H8 (O^6-Me)G), 7.58 (d, 1, J = 7.4 Hz, H6 C), 7.34 (d(m?), 1, J = 1.1 Hz, H6 T), 6.36 (t, 1, J = 6.6 Hz, H1′), 6.14 (t, 1, J = 5.5 Hz, H1′), 6.11 (t, 1, J = 5.5 Hz, H1′), 5.96 (dd, 1, J = 8.5, 5.9 Hz, H1′), 5.84 (d, 1, J = 7.4 Hz, H5 C), 4.4-3.6 (m's, 16, H3′, H4′, H5′). 4.00 (s, 3, OCH_3 of O^6-MeG), 2.9-1.7 (m's, 8, H2′), 1.82 (s, 3, CH_3 T).

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Registry No. **1**, 80228-05-3; **2**, 964-21-6; **3**, 80228-06-4; **4**, 80228-07-5; **5**, 80228-08-6; **6**, 80228-09-7; **7**, 80228-10-0; **8**, 80228-11-1; **9**, 80228-12-2; **10**, 80228-13-3; 2-amino-6-chloropurine, 10310-21-1; 2-deoxy-3,5-di-*O*-*p*-toluoyl-D-*erythro*-pentosyl chloride, 3601-89-6; dDMTrTpCE, 67221-57-2; dDMTrC^{Bz}pCE, 80228-14-4; dA^{Bz}Ac, 25152-95-8.

Total Synthesis of Lycopodium Alkaloids: (±)-Lycopodine, (±)-Lycodine, and (±)-Lycodoline[1]

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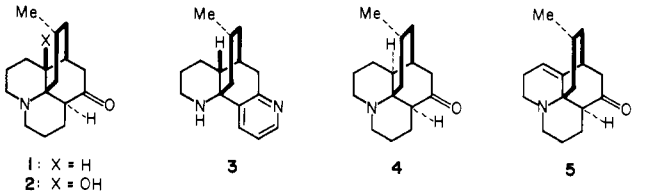
Abstract: Intramolecular Mannich condensation is shown to be a powerful method for the synthesis of lycopodium alkaloids (eq 2). Two syntheses of (±)-lycopodine (**1**) have been developed. In the first (Scheme II), compound **1** is produced in 13 steps from 5-methyl-1,3-cyclohexanedione (16.6% overall yield). In this synthesis, rings A and B are formed in the Mannich cyclization, and ring D is closed by aldol condensation. The alternative lycopodine synthesis (Scheme IV) is more convergent and produces (±)-**1** in only eight operations from the same starting point (13% overall yield). In this synthesis, primary amine **41** is employed in the Mannich reaction, and ring D is closed by intramolecular alkylation of a bromo amine. The synthesis of (±)-lycodine (**3**) also requires eight steps and provides the alkaloid in 13.2% overall yield (Scheme V). This synthesis features an efficient, one-pot conversion of δ,ϵ-unsaturated ketone **46** into pyridine **3**. (±)-Lycodoline (**3**) is produced by an 11-step route in 3.2% overall yield as shown in eq 16, 18, and 22. In this synthesis, the angular hydroxyl is introduced by the stereoselective autoxidation of an octahydroquinoline (eq 16). The Mannich cyclization is completed by a novel method which utilizes the base-catalyzed polymerization of 3-bromo-1-propanol as a method for slow delivery of HBr, thus allowing the reaction to be carried out under essentially neutral conditions (eq 18). The lycodoline synthesis is completed by use of a novel variant of the Oppenauer oxidation (**61** → **62**).

The lycopodium alkaloids are a family of about 100 biogenetically related compounds elaborated by the genus *Lycopodium* (club mosses).[2] The first known,[3] most abundant, and most widely distributed member of the family is lycopodine (**1**). The structure



of lycopodine was established by MacLean in 1960[4] and confirmed

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