A Study of Reactions of α,β-Unsaturated Carbonyl Compounds with Deoxyguanosine[1]

Fung-Lung Chung,* Kalpana R. Roy, and Stephen S. Hecht

Division of Chemical Carcinogenesis, Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, New York 10595

Received March 23 1987

The reactions under mild conditions of deoxyguanosine with the mutagenic α,β-unsaturated carbonyl compounds methyl vinyl ketone and 2-cyclohexen-1-one were investigated. Major products from these reactions were isolated by reverse-phase high-performance liquid chromatography. The structures of the adducts were characterized by UV, proton NMR, and mass spectra and compared to those formed from reactions of acrolein and crotonaldehyde with deoxyguanosine. Analogous to the major acrolein adducts, the major adducts from reaction of deoxyguanosine with methyl vinyl ketone were a pair of equilibrating cyclic 1,N^2-propanodeoxyguanosine diastereomers 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5,6,7,8-tetrahydro-6-hydroxy-6-methylpyrimido[1,2-*a*]purin-10(3*H*)-one (**6** and **7**). The interconversion of these adducts was probably due to Schiff's base formation followed by rapid hydration resulting in isomerization at C-6. Two pairs of diastereomeric adducts were formed from the reaction of deoxyguanosine with 2-cyclohexen-1-one. The structures of these adducts were assigned as a pair of tetracyclic deoxyguanosine adducts, 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5,6,7,8,9,10-hexahydro-6-hydroxy-6,10-methano[1,3]diazocino[1,2-*a*]purin-12(3*H*)-one (**10** and **11**), and a pair of open-chain N^2-substituted adducts, 2′-deoxy-*N*-(3-oxocyclohexyl)guanosine (**12** and **13**). Characteristic UV patterns were noted at pH 13 for these adducts and the regiochemistry of their formation via Michael addition is discussed.

α,β-Unsaturated carbonyl compounds are widespread in the human environment.[2] These compounds readily react with nucleophilic biological macromolecules. As a result, many of them are toxic and mutagenic. Acrolein (**1**), crotonaldehyde (**2**), methyl vinyl ketone (**3**), and 2-cyclohexen-1-one (**4**) are mutagenic toward *Salmonella typhimurium* without metabolic activation.[2,3] Crotonaldehyde is tumorigenic in rats.[4] These results strongly suggest that α,β-unsaturated carbonyl compounds are capable of interacting with cellular DNA. We have previously shown that acrolein and crotonaldehyde react with DNA under physiological conditions forming structurally unique cyclic 1,N^2-propanodeoxyguanosine adducts.[5,6] Because positions 1 and N^2 of guanine are involved in hydrogen bonding in the α-helical structure of DNA, it is plausible that the formation of these adducts could be partially responsible for the genotoxicities of these compounds.

Mechanistically, the cyclic deoxyguanosine adducts of acrolein or crotonaldehyde are formed via Michael addition at the olefinic carbon by either N-1 or the exocyclic NH_2 of guanine followed by 1,2-addition at the aldehydic group by the exocyclic NH_2 or N-1, respectively. In view of their facile formation and potential biological significance, we have investigated the generality of adduct formation from other α,β-unsaturated carbonyl compounds. In the present study, we have chosen methyl vinyl ketone (**3**) and 2-cyclohexen-1-one (**4**), in order to characterize the reactivity of representative ketones toward deoxyguanosine. We have characterized the major adducts formed in these reactions and have compared their structures with those obtained from acrolein, crotonaldehyde, and other related compounds. The structural features influencing the reactivity of these α,β-unsaturated carbonyl compounds in forming deoxyguanosine adducts are also discussed.

Chart I

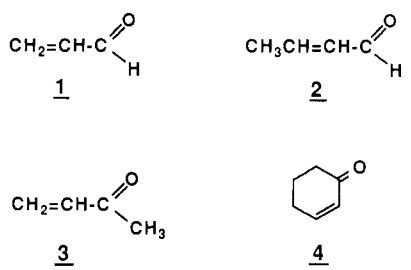


Table I. Ultraviolet Spectral Data for Deoxyguanosine and Guanine Adducts of Methyl Vinyl Ketone and 2-Cyclohexen-1-one

	λ_{max} (nm)		
adduct no.	pH 1.0	pH 7.0	pH 13.0
6 and **7**	259, 278 (sh)	258, 272 (sh)	257.2, 272 (sh)
8	253, 275 (sh)	251, 271, (sh)	280, 261
9	257, 279 (sh)	254.5, 280 (sh)	285, 261.5
10 and **11**	259.5	257.5	259.5
14	255, 280 (sh)	251, 274 (sh)	284, 261
12 and **13**	260	254, 274 (sh)	260
15	251	247, 277 (sh)	277, 253 (sh)

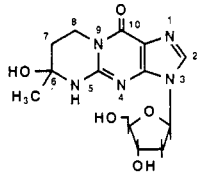
Results and Discussion

Reaction of methyl vinyl ketone with deoxyguanosine occurred readily at 37 °C in pH 7 buffer. After 24 h of incubation, two adducts were isolated by HPLC in 5–10% yield based on deoxyguanosine. These two adducts interconverted. This behavior closely resembled that observed for the acrolein adducts formed by Michael addition of N-1 of guanine at the olefinic carbon followed by ring closure at the aldehydic carbon by the exocyclic NH_2 (see **5**, Figure 1).[5] These data and the UV spectra (Table I) suggested that these adducts were the diastereomeric 1,N^2-disubstituted deoxyguanosine derivatives **6** and **7**.[4,5] The corresponding enantiomeric guanine base adducts **8** obtained upon acid hydrolysis of **6** and **7** showed a characteristic UV pattern of a 1,N^2-cyclic guanine derivative. Furthermore, the proton NMR spectra of the guanine adducts **8** were essentially identical with those of the acrolein adducts **5** except that the resonance due to the methine proton at C-6 of the acrolein adducts was replaced

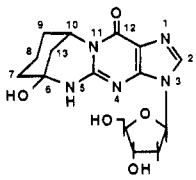
(1) A Study of Chemical Carcinogenesis. 104.
(2) Marnett, L. J.; Hurd, H. K.; Hollstein, M. C.; Levin, D. E.; Esterbauer, H.; Ames, B. N. *Mutat. Res.* **1985**, *148*, 25.
(3) Lutz, D.; Eder, E.; Neudecker, T.; Henschler, D. *Mutat. Res.* **1982**, *93*, 305.
(4) Chung, F.-L.; Tanaka, T.; Hecht, S. S. *Cancer Res.* **1986**, *46*, 1285.
(5) Chung, F.-L.; Young, R.; Hecht, S. S. *Cancer Res.* **1984**, *44*, 990.
(6) Chung, F.-L.; Hecht, S. S. *Cancer Res.* **1983**, *45*, 1230.

0022-3263/88/1953-0014$01.50/0

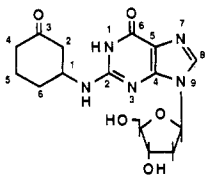
Chart II



6 and 7

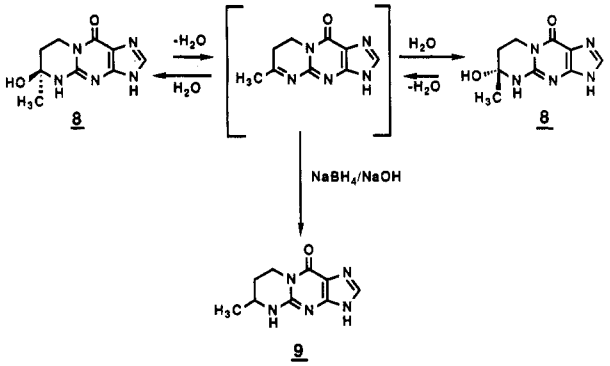


10 and 11



12 and 13

Scheme I



8 8 9

by a methyl singlet at 1.50 ppm. On the basis of these results, the structures of **6** and **7** were assigned as two equilibrating diastereomers of 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5,6,7,8-tetrahydro-6-hydroxy-6-methylpyrimido[1,2-*a*]purin-10(3*H*)-one (**6** and **7**). These assignments were further confirmed by the *m/e* 203 ($M^+ - H_2O$) base peak in the MS of the corresponding guanine adducts **8** obtained by acid hydrolysis of **6** and **7**. Upon treatment with $NaOH/NaBH_4$, guanine adducts **8** were apparently dehydrated to a Schiff base and subsequently reduced to form **9**. The proton NMR spectrum of **9** featured a methyl doublet at 1.15 ppm, indicating that the reduction had occurred. Entirely analogous results were observed for the corresponding acrolein adduct **5**.[5] This result also supports the structure of **8**. The facile dehydration of methyl vinyl ketone adducts **6**, **7**, or **8** yields a Schiff base intermediate which undergoes covalent hydration, producing an equilibrium mixture of the two diastereomers **6** and **7** or enantiomers **8**. An analogous hydration of a C=N in a triazine ring of a structurally related guanosine derivative was previously reported.[7]

The reaction of 2-cyclohexen-1-one (**4**) with deoxyguanosine proceeded at a slower rate at 37 °C and pH 7.0 as compared to the reaction of methyl vinyl ketone. After 72 h of incubation, four adducts **10–13** were formed in a total yield less than 0.2%. Their relative amounts were **10:11:12:13** = 1:1:2:2. To obtain a sufficient quantity of these adducts, the reaction temperature was raised to 100 °C. After 24 h, this reaction gave a 2.4% yield of adducts based on deoxyguanosine. The four adducts, **10–13**, were isolated by HPLC and characterized spectroscopically.

(7) Anderson, G. L.; Rizkalla, B. H.; Broom, A. D. *J. Org. Chem.* **1974**, *39*, 937.

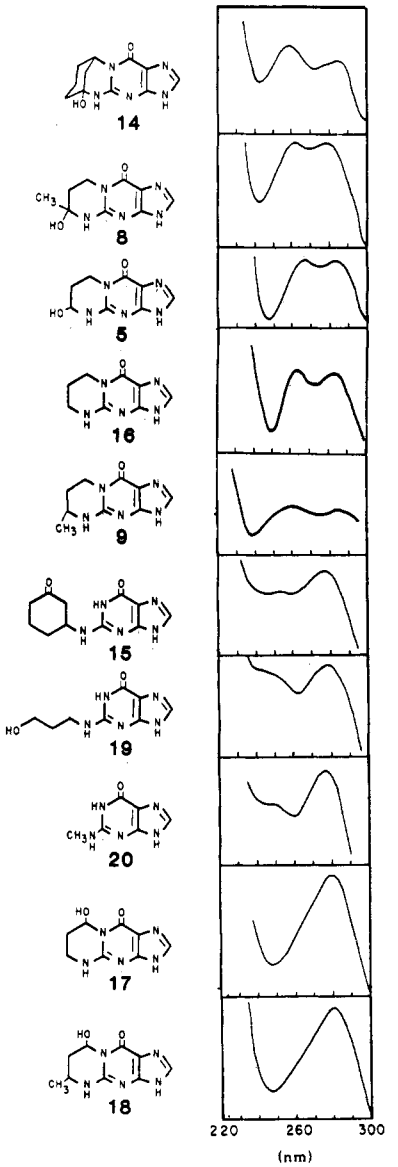


Figure 1. UV spectra of 1- and N^2- or N^2-substituted guanine adducts in aqueous pH 13 medium.

The early eluting adducts **10** and **11** were virtually identical in their UV, MS, and proton NMR spectra. Upon acid hydrolysis, **10** and **11** each gave the corresponding guanine adduct **14** (Figure 1) which had identical UV and proton NMR spectra and HPLC retention times. However, their CD spectra were mirror images with a maximum at 248 nm. Therefore, adducts **10** and **11** were a pair of diastereomers and adducts **14** were a pair of enantiomers. Analogous results were obtained with the later eluting adducts **12** and **13**. The corresponding guanine adducts **15** (Figure 1) from acid hydrolysis were identical in all respects but had opposite CD spectra. The californium-252 fission fragment MS of adducts **10** and **11** or adducts **12** and **13** gave identical molecular ions at *m/e* 386.3 (M^+ + Na) which are indicative of the addition of one 2-cyclohexen-1-one molecule to deoxyguanosine. High-resolution MS of adducts **10** and **11** failed to give the molecular ion due to low volatility. Adducts **10** and **11** were therefore derivatized with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) in the presence of a catalytic amount of pyridine at 90 °C for 2 h. Negative ion chemical ionization high-resolution MS of the TMS derivatives of **10** and **11** gave a molecular ion at *m/e* 578.2668 ($M^+ - H$). This value is consistent with the elemental composition $C_{16}H_{21}N_5O_5$

after correction for the 3TMS – H groups (M_r of $C_{16}H_{21}N_5O_5$ + 3TMS – H is 578.2639). A proton NMR of adducts **10** and **11** showed, aside from the protons of deoxyguanosine, a multiplet between 1.2 and 2.0 ppm which was attributed to the protons of the cyclohexyl ring and a singlet of 5.05 ppm which was assigned to the methine proton at C_{10}. Two exchangeable protons at 7.85 ppm and 6.55 ppm were assigned to the amino proton at N-5 and the hydroxy proton at C_6, respectively. Taken together, these data support the tetracyclic structures of a pair of diastereomers of 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5,6,7,8,9,10-hexahydro-6-hydroxy-6,10-methano[1,3]diazocino[1,2-*a*]purin-12(3*H*)-one (**10** and **11**). The product from acid hydrolysis of adducts **10** and **11** had the characteristic UV absorption of a 1,N^2-cyclic guanine derivative and possessed a proton NMR spectrum almost superimposable on that of adducts **10** and **11** except for the deoxyribosyl protons. Contrary to the corresponding acrolein or methyl vinyl ketone adducts, adducts **10** and **11** were resistant to dehydration by base treatment and they did not seem to interconvert. This is probably due to the presence of the bridgehead hydroxy gorup at the C-6 position in these adducts. Similar results were obtained with certain lactamols containing bridgehead hydroxy groups. They were found to be stable under severe hydrolytic conditions including refluxing in 40% potassium hydroxide.[8]

The proton NMR spectra of adducts **12** and **13** showed aliphatic protons in the region between 1.70 ppm and 2.60 ppm. These resonances appeared at lower field than those in adducts **12** and **13**, suggesting that the carbonyl group of the cyclohexyl group was retained. COSY spectra of adducts **12** and **13** clearly demonstrated the coupling of the C-1 methine proton at 4.16 ppm with the exchangeable N-2 amino proton at 6.58 ppm, with the C-2 protons adjacent to the carbonyl group at 2.42 ppm and 2.56 ppm, and with the C-6 protons at 1.70 ppm. It also indicated the coupling between the C-5 protons at 1.90 ppm and 2.05 ppm to the C-4 protons at 2.30 ppm and to the C-6 protons. These results indicated that adducts **12** and **13** were a pair of diastereomers of 2′-deoxy-*N*-(3-oxocyclohexyl)-guanosine. The open structures of adducts **12** and **13** were further verified by $NaBH_4$ reduction which yielded the corresponding hydroxy derivative as indicated by an upfield chemical shift of the cyclohexyl protons in the proton NMR. In contrast, $NaBH_4$ treatment of the adducts **10** and **11** resulted in their quantitative recovery. This result is also consistent with the assigned cyclic structures of adducts **10** and **11**.

In basic medium, several interesting UV patterns were noted for these structurally related guanine adducts. As shown in Figure 1, double maxima at about 280 nm and 260 nm appear to be unique to the methyl vinyl ketone guanine adducts **8** and acrolein guanine adducts **5** in which the hydroxy group resides at the C-6 carbon adjacent to N-5. Similar double maxima with slight bathochromic shifts to about 285 nm were also common to the reduced cyclic 1,N^2-propanoguanine adducts of methyl vinyl ketone **9** and acrolein **16** and to the 2-cyclohexen-1-one guanine adduct **14** in which the hydroxy resides at the C-6 bridgehead carbon. In comparison, acrolein adducts **17** and crotonaldehyde adducts **18** with the hydroxy group attached to the C-8 carbon adjacent to N-9 showed a single maximum at 280 nm. N^2-Substituted open-chain guanine adducts such as **19** and **20** have maxima at 278 nm and 250 nm (sh). These UV characteristics are consistent with those of adduct **15** of 2-cyclohexen-1-one and provide further support for the assigned open structures. These UV spectral features, observed at pH 13, appear to be unique to various 1- and/or N^2-substituted guanine adducts and are useful as supportive evidence for their structural elucidation.

Our previous studies have demonstrated that in the reaction of acrolein with deoxyguanosine at 37 °C and pH 7, the major adducts are formed by the addition of N-1 of guanine to the olefinic carbon followed by addition of the aldehyde at the exocyclic NH_2, whereas the minor adducts are formed from the opposite direction of ring-closure.[5] These results suggest that for unsubstituted α,β-unsaturated aldehydes the initial Michael addition by N-1 of guanine is favored. However, under identical conditions the only crotonaldehyde adducts formed were derived from initial addition by N^2.[6] These results clearly demonstrated the steric effect of the methyl group which precludes the addition of N-1 to the substituted olefinic carbon. Space-filling models of 1,N^2-propanodeoxyguanosine adducts with a methyl group at C-8 show a high degree of crowding between O^6 and the methyl group, which agrees well with the experimental observations. Based on these results, it is not surprising that the predominant methyl vinyl ketone cyclic adducts isolated were generated from initial Michael addition by N-1 of guanine. Furthermore, the formation of N^2 open-chain adducts **12** and **13** as major products and the cyclic adducts **10** and **11** as minor products in the reaction of deoxyguanosine with 2-cyclohexen-1-one suggests that after 1,4-addition by either the N-1 or exocyclic NH_2 group, the cyclization at the ketone group is more facile at exocyclic NH_2 than at N-1. These results are in agreement with the previous observation that open-chain N^2 adducts were the major products from the reaction of 1-(3-pyridyl)-2-buten-1-one with deoxyguanosine.[9] Interestingly, the disubstituted 3-methyl-2-cyclohexen-1-one failed to react with deoxyguanosine at 37 °C, under reflux in pH 7 phosphate buffer or DMF, presumably due to steric hindrance and the electron-releasing effect of the methyl group.

In addition to their presence in the environment, some of the α,β-unsaturated carbonyl compounds are formed as metabolites in vivo. Acrolein has been shown to be a decomposition product of the polyamine spermine[10] and is a major metabolite of cyclophosphamide,[11] a widely used chemotherapeutic drug. Crotonaldehyde is a metabolite of *N*-nitrosopyrrolidine, a known liver carcinogen.[12] *trans*-4-Hydroxy-2-nonenal, a product of lipid peroxidation,[13] and its homologues *trans*-4-hydroxy-2-hexenal, a product from metabolism of the pyrrolizidine alkaloid senecionine,[14] have recently been shown to form structurally related adducts with deoxyguanosine.[15,16] The facile reaction of these compounds with deoxyguanosine

(8) Rodig, O. R.; Johnston, N. J. *J. Org. Chem.* **1969**, *34*, 1942.

(9) Hecht, S. S.; Lin, D.; Chuang, J.; Castonguay, A. *J. Am. Chem. Soc.* **1986**, *108*, 1292.

(10) (a) Russo, A.; Pritchell, J. B.; DeGraff, W.; Friedman, N.; Gamson, J. *Cancer Res.* **1985**, *45*, 4910. (b) Alarcon, R. A. *Arch. Biochem. Biophys.* **1970**, *137*, 365.

(11) Gurtoo, H. L.; Hopkens, J. H.; Sharma, S. D. *Cancer* Res. **1981**, *41*, 3584.

(12) Wang, M. Y.; Chung, F.-L.; Hecht, S. S. *Proc. Am. Assoc. Cancer Res.* **1987**, *28*, 128.

(13) (a) Benedetti, A.; Comporti, M.; Esterbauer, H. *Biochem. Biophys. Acta* **1980**, *620*, 281. (b) Benedetti, A.; Pompella, A.; Fulceri, R.; Romani, A.; Comporti, M. *Biochem. Biophys. Acta* **1986**, *876*, 658.

(14) Segall, H. J.; Wilson, D. N.; Dallas, J. L.; Haddon, W. F. Science (*Washington, D.C.*) **1985**, *229*, 472.

(15) Winter, C. K.; Segall, H. J.; Haddon, W. F. *Cancer Res.* **1986**, *46*, 5682.

(16) Chung, F.-L.; Sodum, R. S. *Proc. Am. Assoc. Cancer Res.* **1987**, *28*, 98.

implicates the potential role of these adducts in the mutagenicity or possible tumorigenicity of these compounds. While the formation of these adducts in vivo and their biological significance have yet to be demonstrated, the characterization in vitro is useful in providing insights into their mechanisms of formation and in assessing their potential genotoxic effects.

Experimental Section

Proton NMR spectra were recorded on either a Bruker WH-300 spectrometer at 300 MHz (Bruker Instruments, Inc., MA) or a Nicolet NT-300 spectrometer at 300 MHz (Rockefeller University, NY) with DMSO-d_6 as solvent and Me_4Si as internal standard. COSY experiments were performed on the Bruker spectrometer. High-resolution chemical ionization and fission fragmentation MS were obtained with a VG-11-250 spectrometer and a home-made spectrometer, respectively, at the Rockefeller University Mass Spectrometric Biotechnology Resource, NY. UV spectra were recorded on a Cary Model 118 spectrometer. CD spectra were measured with a AVIV Spectrophotometer 60DS (Rockefeller University, NY). HPLC was performed with a Waters Associates Model ALC/GPC-204 equipped with a Model 6000A solvent delivery system, a Model 660 solvent programmer, and a Model 440 UV visible detector at 254 nm.

Reaction of Methyl Vinyl Ketone and Deoxyguanosine. Methyl vinyl ketone (1.7 g, 24.3 mmol) was added to 75 mL of phosphate buffer at pH 7.0 containing deoxyguanosine (0.3 g, 1.1 mmol). The mixture was incubated at 37 °C with shaking. After 24 h, the incubation mixture was extracted with $CHCl_3$ (3 × 20 mL) to remove excess methyl vinyl ketone. The aqueous layer was concentrated to 15 mL and allowed to stand at room temperature for 2 h; during this time a solid precipitated. The solid was filtered and the filtrate was collected and analyzed by HPLC on a Whatman Partisil ODS-3 Magnum 9 column programmed from 0% to 20% MeOH in H_2O in 50 min, using curve 6 and a flow rate of 5 mL/min. Two major deoxyguanosine products eluted at 41 and 43 min. Another product, formed in a relative yield of 60–70% of the main cyclic adducts, eluted at 48 min. This product appeared to be contaminated with impurities and showed UV and proton NMR spectra of a guanine derivative. This product was resistant to acid hydrolysis. Due to the impurities, the structure of this product has not been fully characterized. The deoxyguanosine adducts eluting at 41 and 43 min were treated with 0.1 N HCl at 90 °C for 45 min. Hydrolysis was quantitative. The resulting hydrolysate was neutralized with 0.1 N NaOH before analysis by HPLC. The methyl vinyl ketone guanine adducts **8** were purified on a Whatman Partisil ODS-3 Magnum 9 column programmed from 20% to 50% MeOH in H_2O in 50 min, using curve 8 at a flow rate of 5 mL/min. Both adducts eluted in 15 min. Proton NMR of **8**: 1.50 (s, 3, CH_3), 1.75, 1.95 (m, 2, C-7-H_2), 3.50, 4.40 (m, 2, C-8-H_2), 5.70 (br s, 1, C-6-OH), 7.70 (s, 1, C-2-H), 8.00 (s, 1, N-5-H), 12.50 (br s, 1, N-3-H).

Reaction of 2-Cyclohexen-1-one and Deoxyguanosine. To a solution of deoxyguanosine (0.5 g, 1.9 mmol) in 100 mL of phosphate buffer at pH 7.0 was added 2 mL of 2-cyclohexen-1-one (1.98 g, 20.1 mmol). This solution was heated at 100 °C. After 24 h, the reaction mixture was extracted with $CHCl_3$ (3 × 10 mL). The aqueous layer was evaporated in vacuo to dryness. The residue was extracted with 1:1 MeOH/EtOH (2 × 10 mL). The insoluble material was filtered. The filtrate was combined and evaporated together with a small amount of silica gel (1.5 g) in vacuo to dryness. The residue was applied to a dry-packed silica gel column for preliminary purification. Fractions containing products were collected and concentrated. The final purification was carried out by HPLC using a Whatman ODS-3 Magnum 9 column with elution by 80:20 H_2O/MeOH at a flow rate of 5 mL/min. The combined yields based on deoxyguanosine of adducts **10** and **11**, and adducts **12** and **13** were 0.8% and 1.6%, respectively. Proton NMR of **10** and **11**: 1.20–2.00 (m, 8, C-7,8,9,13-H_8), 2.20 (m, 1, C-2′-H), 3.57 (m, 2, C-5′-H_2), 3.81 (m, 1, C-4′-H), 4.38 (br s, 1, C-3′-H), 4.94 (t, 1, C-5′-OH), 5.05 (s, 1, C-10-H), 5.30 (s, 1, C-3′-OH), 6.14 (t, 2, C-1′-H_2), 6.55 (br s, 1, C-6-OH), 7.85 (s, 1, N-5-H), 7.90 (s, 1, C-2-H). Proton NMR of **12** and **13**: 1.70 (t, 2, C-6-H_2), 1.90, 2.05 (m, 2, C-5-H_2), 2.20, 2.67 (m, 2, C-2′-H_2), 2.30 (t, 2, C-4-H_2), 2.42, 2.56 (m, 2, C-2-H_2), 3.50 (m, 2, C-5′-H_2), 3.79 (m, 1, C-4′-H), 4.16 (m, 1, C-1-H), 4.37 (m, 1, C-3′-H), 6.13 (t, 1, C-1′-H), 6.58 (d, 1, N^2-H), 7.92 (s, 1, C-8-H), 10.38 (br s, 1, N-1-H).

Acid hydrolysis of adducts **10** and **11** or adducts **12** and **13** was performed as described above to give **14** or **15**, respectively. Using the same chromatographic conditions as described above, the guanine adducts **14** and **15** were eluted at 27 min and 32 min. Proton NMR of **14**: 1.20–2.00 (m, 8, C-7,8,9,13-H_8), 5.05 (s, 1, C-10-H), 6.25 (br s, 1, C-6-OH), 7.60 (s, 1, C-2-H), 7.80 (br s, 1, N-5-H). NMR of **15**: 1.70–2.56 (m, 8, C-2,4,5,6-H_8), 4.10 (m, 1, C-3-H), 6.80 (br s, 1, N^2-H), 7.67 (s, 1, C-8-H).

$NaBH_4$ Reduction of Adduct 8. Adduct **8** (5 mg, 0.02 mmol) obtained from acid hydrolysis of **6** and **7** was treated with excess $NaBH_4$ (20 mg) in 0.5 N NaOH (2 mL) in a Reacti-vial at 90 °C for 40 min. The reaction mixture was cooled to room temperature and neutralized with 0.5 N HCl. The product **9** was isolated by HPLC as described for the methyl vinyl ketone guanine adducts. The retention time of **9** was 26 min. Proton NMR of **9**: 1.15 (d, 3, CH_3), 1.50, 2.00 (m, 2, C-7-H_2), 3.55, 4.20 (m, 2, C-8-H_2), 3.60 (m, 1, C-6-H), 7.46 (br s, 1, N-5-H), 7.60 (s, 1, C-2-H).

$NaBH_4$ Reduction of Adduct 15. Adduct **15** (4 mg, 0.016 mmol) obtained from acid hydrolysis of **12** and **14** was treated with an excess of $NaBH_4$ (15 mg) at room temperature for 3 h. The reaction mixture was neutralized with 0.1 N HCl and analyzed by HPLC using the method described for adducts **12** and **13**. The only product eluted at 44 min. Proton NMR: 1.00–1.38 (m, 4, C-4,5-H_4), 1.62–2.20 (m, 4, C-2,6-H_4), 3.46 (m, 1, C-3-H), 3.62 (m, 1, C-1-H), 7.53 (s, 1, C-8-H).

Acknowledgment. This work was supported by Grant No. CA 43159 from the National Cancer Institute. We thank Drs. A. Bencsath and B. Chait of the Rockefeller University Mass Spectrometric Biotechnology Resource for the MS and F. Picart of the same university for the proton NMR spectra and for providing facilities for CD measurment. We also thank Bruker Instruments, Inc., MA, for the COSY spectra.

Registry No. **3**, 78-94-4; **4**, 930-68-7; **6**, 111291-80-6; **7**, 111291-81-7; (*R*)-**8**, 111323-80-9; (*S*)-**8**, 111291-82-8; **9**, 111291-88-4; **10**, 111291-83-9; **11**, 111407-34-2; **12**, 111291-84-0; **13**, 111291-85-1; **14**, 111291-86-2; **15**, 111291-87-3; H-dGuo-H, 961-07-9.