Chemical Transformation/Derivatization of O^6-Methyl- and O^6-(Hydroxyethyl)guanine for Detection by GC-EC/MS

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In this project we set out to make an important class of DNA adducts, comprising O^6-alkyl and O^6-(hydroxyalkyl)guanines, susceptible to sensitive detection by GC-EC/MS. While existing literature indicated that pentafluorobenzylation would be useful for the ring NH site on these compounds, how to best overcome the polarity of the exocyclic NH_2 and OH groups, without losing the O^6-alkyl moiety, was less clear. Working with O^6-methylguanine and O^6-(2′-hydroxyethyl)guanine as representative analytes, we found that the NH_2 group could be converted into fluoro without loss of the O^6 substituent. For the OH group, a comparison of several derivatives (OR′) led to R′ = *tert*-butyl as the best choice at this stage. The latter work, especially via NMR, also allowed exact structural assignments to be made for the $N7$ and $N9$ pentafluorobenzyl isomeric derivatives that formed. Of these R′ derivatives, the $N7$ isomers migrated slower on silica-TLC, had higher GC retention times, had lower responses by GC-EC/MS, and were preferentially destroyed as the GC column aged. However, the $N9$ isomer was slower on TLC when the OH was not derivatized. This behavior was rationalized using a concept of "polar footprint" for the derivatives. The concept also seemed to explain the puzzling GC-EC/MS behavior of some related compounds in our laboratory. Apparently the polar footprint should be minimized in designing derivatives for trace detection by GC-EC/MS.

INTRODUCTION

Gas chromatography electron capture mass spectrometry (GC-EC/MS) is an attractive technique for detection since it can achieve high sensitivity and specificity. The sensitivity is most impressive (attomole level[1]) for compounds which give an intense, single-ion response by this technique. The first examples were introduced by Hunt and Crow 15 years ago.[2] Relatively few analytes behave in this way.

We are pursuing a general, analytical strategy in which analytes needing sensitive detection are converted into high-response, single-ion electrophores. DNA adducts, the consequence of covalent damage in vivo to DNA, are a class of such analytes. Carcinogens and mutagens tend to produce DNA adducts. The measurement of DNA adducts is therefore of interest especially for human samples to help assess how much of the human burden of cancer and genetic disease arises from exposure of people to DNA-reactive (directly or via metabolites) chemical and physical agents.

For some DNA adducts, such as certain alkyl derivatives of the nucleobases, electrophoric derivatization alone can yield high-response, single-ion electrophores (e.g., ref 3). Other adducts require chemical transformation prior to such derivatization. Hydrazinolysis of amino polyaromatic hydrocarbon adducts,[4] superoxide oxidation of diol epoxide polyaromatic hydrocarbon adducts,[5] and nitrous acid hydrolysis of $N7$-alkylguanine adducts[6] are examples of such chemical transformation.

O^6-Alkylguanines comprise a class of DNA adducts under study in several laboratories (e.g., refs 7–11), including studies of O^6-methylguanine repair.[12-16] This is due to much evidence that this type of lesion tends to be strongly mutagenic as well as carcinogenic.[17,18] For example, O^6-methylguanine is the major mutagenic lesion produced in DNA by simple methylating agents.[19] O^6-Ethylguanine persistence in rat brain vs other rat tissues is consistent with the predominant development of malignant brain tumors in the former tissue upon exposure to the ethylating carcinogen, N-ethyl-N-nitrosourea.[20] O^6-(Hydroxyethyl)guanine is one of the adducts produced by ethylene oxide, a rodent carcinogen that causes chromosomal aberrations in both laboratory animals and humans.[21] Styrene, a possible human carcinogen[22] is metabolized in part to styrene oxide, a known rodent carcinogen,[23] and the latter has been observed to react at the O^6 (and also $N7$ and N^2)

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0003-2700/93/0365-3071$04.00/0

position of guanine in DNA.[24,25] Analogs of these compounds such as propylene oxide[26] may behave similarly. The formation of O^6-alkylguanines may be the rate-limiting step for cancer initiation by a number of chemicals.[27]

While nitrous acid hydrolysis chemically transforms $N7$-alkylguanines into corresponding $N7$-alkylxanthines (leading via subsequent electrophoric derivatization to high-response, single-ion electrophores[6], this strategy cannot be anticipated to work for O^6-alkylguanines, since O^6-alkylxanthines are unknown (apparently they are hydrolytically unstable). Further, O^6-alkylguanines can lose the O^6-alkyl group under some nucleophilic reaction conditions.[28]

Here we report that O^6-alkylguanine adducts can be converted into high-response, single-ion electrophores via chemical transformation with fluoroboric acid/nitrous acid. This forms corresponding 2-fluoro-O^6-alkylhypoxanthines, which then can be electrophore derivatized and detected with high sensitivity by GC-EC/MS. The overall method is demonstrated here for both O^6-methylguanine and O^6-(hydroxyethyl)guanine. For the 2-fluoro-O^6-(2′-hydroxyethyl)hypoxanthine derived from the latter compound, we also report the preparation and testing of a series of ether and ester derivatives to fully optimize the derivatization. This latter work also leads to an exact structural analysis of the final derivatives.

EXPERIMENTAL SECTION

Materials. O^6-Methylguanine and O^6-(2′-hydroxyethyl)guanine were synthesized as described.[3] Tetrafluoroboric acid was purchased from Alfa (Ward Hill, MA). Sodium nitrite, pentafluorobenzyl bromide (PFBzBr), tetrabutylamonium hydrogen sulfate, potassium carbonate, triethylamine, sodium hydroxide, benzoyl chloride, methyl iodide, trimethylacetyl (pivalyl) chloride, boron trifluoride etherate, sodium bicarbonate, *tert*-butyl-2,2,2-trichloroacetimidate (TBTA), anhydrous tetrahydrofuran, ACS-grade acetone, acetonitrile, and dichloromethane were from Aldrich (Milwaukee, WI). Tetramethylsilane (TMS) was used as an internal standard for the ^{1}H NMR measurements. All compositions were volume to volume unless indicated otherwise.

Equipment. Preparative and analytical thin-layer chromatography (TLC) separations were performed with GHLF silica gel Uniplates with fluorescence indicator (Analtech, Newark, DE). Flash column chromatography was performed with silica gel 60 (200–300 mesh) from EM Science (Cherry Hill, NJ). All final products were further purified by HPLC prior to measurement by gas chromatography electron capture mass spectrometry (GC-EC/MS). A Microsorb C_{18} silica reversed-phase column, 10 mm i.d. × 25 cm length, 5-μm particle size (Rainin, Woburn, MA) with a mobile phase of acetonitrile/water (70:30) at 3 mL/min was used for preparative HPLC. The GC-EC/MS equipment has been described before,[1] as has the NMR.[3]

Synthesis. 2-Fluoro-O^6-methylhypoxanthine. To a stirred solution of O^6-methylguanine (6.7 mg, 0.041 mmol) in 0.5 mL of 48% tetrafluoroboric acid solution maintained at 0 °C (ice/water bath) was slowly added 40 μL of 1.45 M sodium nitrite solution (0.058 mmol). After 6 h of stirring at 0 °C, the pH was brought to 7 with 5.0 M sodium hydroxide. The solution was extracted with ethyl acetate, dried over sodium sulfate, filtered, and concentrated under reduced pressure to give a white solid. The reaction product was purified by silica flash column chromatography using ethyl acetate to give 9.6 mg of product: ^{1}H NMR ($CDCl_3$) δ 8.08 (s, 1H), 4.22 (s, 3H).

2-Fluoro-O^6-methyl-N-(7 and 9)-(pentafluorobenzyl)hypoxanthine (4 and 3, respectively). To a stirred solution of 2-fluoro-O^6-methylhypoxanthine (9.6 mg) in 10 mL of acetone/acetonitrile (1:1) was added potassium carbonate (23.7 mg, 0.171 mmol). After 5 min of stirring, pentafluorobenzyl bromide (18 μL, 0.11 mmol) was introduced and stirring was continued for 24 h. The inorganic solid was filtered off (paper), and the filtrate was evaporated. TLC showed complete conversion of starting material to two new products with higher R_f values using ethyl acetate/hexane (1:1), which were purified by preparative TLC using the same solvent system. Product 4: R_f = 0.17; 3.5 mg, 25% yield (from O^6-methylguanine); ^{1}H NMR ($CDCl_3$) δ 8.15 (s, 1H), 5.63 (s, 2H), 4.17 (s, 3H). Product 3: R_f = 0.32; 4.4 mg, 31% yield; ^{1}H NMR ($CDCl_3$) δ 7.93 (s, 1H), 5.46 (s, 2H), 4.19 (s, 3H).

2-Fluoro-O^6-(2′-hydroxyethyl)hypoxanthine. To a stirred solution of O^6-(2′-hydroxyethyl)guanine (218 mg, 1.11 mmol) in 13 mL of 48% tetrafluoroboric acid solution maintained at 0 °C (ice/water bath) was slowly added 1.2 mL of 1.45 M sodium nitrite solution (1.74 mmol). After 4 h of stirring at 0 °C, TLC using methanol/ethyl acetate (1:9) showed the absence of starting material and a new product at a higher R_f. The pH was brought to 7 with 5.0 M sodium hydroxide, and the precipitated product was isolated by filtration (sand/glass wool in a Pasteur pipet), washed with cold water, and eluted with methanol. An ethyl acetate extract of the cold water wash was dried over sodium sulfate and combined with the methanol elution. Evaporation and vacuum drying gave 837 mg of white solid, 10% of which was purified further by silica flash column chromatography using methanol/ethyl acetate (1:9): ^{1}H NMR (CD_3OD) δ 8.29 (s, 1H), 4.65 (t, 2H), 3.97 (t, 2H).

2-Fluoro-O^6-(2′-hydroxyethyl)-N-(7 and 9)-(pentafluorobenzyl)hypoxanthines (6 and 5, respectively). To a stirred solution of 2-fluoro-O^6-(2′-hydroxyethyl)hypoxanthine (830 mg) in 30 mL of acetone/acetonitrile (1:1) was added potassium carbonate (1.75 g 12.7 mmol). After 5 min of stirring, pentafluorobenzyl bromide (2.6 mL, 16.9 mmol) was introduced, and stirring was continued for 24 h. The inorganic solid was filtered off (paper), and the filtrate was evaporated. TLC using ethyl acetate showed complete conversion of starting material to two new products with higher R_f values (0.41 and 0.35, respectively). Silica flash column chromatography gave a white solid (X; 0.104 g, 24.7%) comprising the two products which was used directly for most of the derivatization reactions presented below without further separation.

The ratio of these two isomers, 5 and 6 (see later for structural assignments), was about 1.5:1.0 based on integration of their NCH_2 ^{1}H NMR signals. A pure sample of 6 with R_f = 0.41 was prepared by washing the after-column isomeric mixture with chloroform until the residual solid gave one spot on TLC: ^{1}H NMR (acetone-d_6) δ 8.52 (s, 1H), 5.92 (s, 2H), 4.63 (t, 2H), 3.91 (t, 2H).

The above, combined washing solutions were reduced to dryness and a chloroform extract (50 μL) gave a 90% pure sample of 5 (R_f = 0.35: ^{1}H NMR (acetone-d_6) δ 8.33 (s, 1H), 5.68 (s, 2H), 4.64 (t, 2H), 3.96 (t, 2H).

2-Fluoro-O^6-[2′-(benzoyloxy)ethyl]-$N7$-(pentafluorobenzyl)hypoxanthine (14). To a stirred solution of 6 (4.5 mg, 12 μmol) in 1 mL of dichloromethane were added benzoyl chloride (8 μL, 0.071 mmol) and triethylamine (12 μL, 0.083 mmol) at room temperature. After 20 h, 2 mL of dichloromethane and 2 mL of aqueous saturated sodium bicarbonate were added. The layers were separated, and the aqueous layer was extracted twice with ethyl acetate. The combined organic layer was dried over sodium sulfate, filtered (paper), and concentrated under reduced pressure. TLC using ethyl acetate/hexane (1:1) showed the product at R_f = 0.22, which was purified further by preparative TLC (same solvent) giving 3.9 mg (68.0 %): ^{1}H NMR (acetone-d_6; vs $CDCl_3$ was used below and for the NMR data cited in Table I) δ 8.47 (s, 1H), 7.97 (d, 2H), 7.64 (t, 1H), 7.52 (t, 2H), 5.90 (s, 2H), 4.97 (t, 2H), 4.78 (t, 2H).

2-Fluoro-O^6-[2′-(benzoyloxy)ethyl]-N-(7 and 9)-(pentafluorobenzyl)hypoxanthines (14 and 13, respectively). To a stirred solution of X (the above, unresolved isomeric mixture of 5 and 6; 11.8 mg, 31.2 μmol) in 2 mL of dichloromethane were added benzoyl chloride (22 μL, 0.19 mmol) and then triethylamine (30 μL, 0.22 mmol) at room temperature. After 20 h, 5 mL each of dichloromethane and aqueous saturated sodium bicarbonate solution were added. After shaking, the separated aqueous layer was extracted with ethyl acetate. The combined organic layer

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was dried over sodium sulfate, filtered, concentrated under reduced pressure, and purified by preparative TLC using ethyl acetate/hexane (1:1). Product 14: R_f = 0.22; 4.1 mg, 27.2% yield; ^{1}H NMR ($CDCl_3$) δ 8.08 (s, 1H), 7.98 (d, 2H), 7.57 (t, 1H), 7.41 (t, 2H), 5.63 (s, 2H), 4.95 (t, 2H), 4.81 (t, 2H). Product 13: R_f = 0.42; 9.5 mg, 63.1% yield; ^{1}H NMR ($CDCl_3$) δ 8.02 (d, 2H), 7.94 (s, 1H), 7.52 (t, 1H), 7.41 (t, 2H), 5.46 (s, 2H), 4.96 (t, 2H), 4.75 (t, 2H).

2-Fluoro-*O*^{6}-[2′-(trimethylacetoxy)ethyl]-*N*-(7 and 9)-(pentafluorobenzyl)hypoxanthines (16 and 15, respectively. To a stirred solution of X (11.0 mg, 29.1 μmol) in 3 mL of dichloromethane were added trimethylacetyl chloride (22 μL, 0.17 mmol) and then triethylamine (49 μL, 0.35 mmol) at room temperature. After a 20-h workup as for 4 and 5, two isomer products were obtained. Product 16: R_f = 0.28; 3.3 mg, 24.5% yield; ^{1}H NMR ($CDCl_3$) δ 8.12 (s, 1H), 5.66 (s, 2H), 4.84 (t, 2H), 4.52 (t, 2H), 1.16 (s, 9H). Product 15: R_f = 0.52; 7.8 mg, 58% yield; ^{1}H NMR ($CDCl_3$) δ 7.95 (s, 1H), 5.46 (s, 2H), 4.82 (t, 2H), 4.50 (t, 2H), 1.17 (s, 9H).

2-Fluoro-*O*^{6}-[2′-[(pentafluorobenzyl)oxy]ethyl]-*N*-(7 and 9)-(pentaflurobenzyl)hypoxanthines (8 and 7, respectively). To a stirred solution of X (10.0 mg, 26.4 μmol) in 3 mL of dichloromethane were added tetrabutylammonium hydrogen sulfate (53.9 mg, 0.1586 mmol) and pentafluorobenzyl bromide (80 μL, 53 mmol). After 5 min, 1.1 mL of aqueous 1.0 N potassium hydroxide was added and stirring was continued for 24 h. The reaction mixture was treated with 10 mL of water and 10 mL of dichloromethane. After shaking, the separated aqueous layer was extracted twice with ethyl acetate. The combined organic layer was dried over sodium sulfate, filtered, concentrated under reduced pressure, and then purified further by preparative TLC using ethyl acetate/hexane (1:1). Product 8: R_f = 0.23; 7.1 mg, 48% yield; ^{1}H NMR ($CDCl_3$) δ 8.13 (s, 1H), 5.64 (s, 2H), 4.67 (s, 2H), 4.74 (t, 2H), 3.92 (t, 2H). Product 7: R_f = 0.44; 1.9 mg, 12.9% yield; ^{1}H NMR ($CDCl_3$) δ 7.93 (s, 1H), 5.45 (s, 2H), 4.70 (s, 2H), 4.74 (t, 2H), 3.95 (t, 2H).

2-Fluoro-*O*^{6}-(2′-methoxyethyl)-*N*(7)-(pentafluorobenzyl)hypoxanthine (10). To a stirred solution of X (10.0 mg, 26.4 μmol) in 3 mL of dichloromethane was added 2.6 mL of aqueous 1.0 N potassium hydroxide followed by tetrabutylammonium hydrogen sulfate (53.9 mg, 0.1586 mmol). After 5 min, methyl iodide (107 mL, 1.72 mmol) was added and stirring was continued for 24 h. The reaction mixture was worked up as for 8 and 7, terminating with preparative TLC using ethyl acetate/hexane (7:3). Product 10: R_f = 0.33; 4.4 mg, 42.4% yield; ^{1}H NMR ($CDCl_3$) δ 8.17 (s, 1H), 5.65 (s, 2H), 4.73 (t, 2H), 3.77 (t, 2H), 3.38 (s, 3H). Only a trace of presumably 9 was detected at R_f = 0.41.

2-Fluoro-*O*^{6}-[2′-(*tert*-butyloxy)ethyl]-*N*-(7 and 9)-(pentafluorobenzyl)hypoxanthines (12 and 11, respectively. Taking advantage of a reaction reported by Armstrong et al.,[29] to a stirred solution of X (10.7 mg, 28.3 μmol) in 3 mL of anhydrous tetrahydrofuran, at 0 °C, were added *tert*-butyl-2,2,2-trichloroacetimidate (50 μL, 0.28 mmol) and then 10 μL of boron trifluoride etherate as a catalyst. After 1 h, the reaction mixture was allowed to warm to room temperature and stirring was continued for another 2 h. $NaHCO_3$ (60.0 mg, 0.71 mmol) was added, and the reaction mixture was filtered through a short plug of silica gel/glass wool to remove the solid. Ethyl acetate was used to wash the solid, and the filtrate was evaporated, followed by preparative TLC using ethyl acetate/hexane (1:1). Product 12: R_f = 0.33; 1.9 mg, 15.5% yield; ^{1}H NMR ($CDCl_3$) δ 8.10 (s, 1H), 5.67 (s, 2H), 4.70 (t, 2H), 3.78 (t, 2H), 1.26 (s, 9H). Product 11: R_f = 0.66; 6.6 mg, 53.7% yield); ^{1}H NMR ($CDCl_3$) δ 7.94 (s, 1H), 5.45 (s, 2H), 4.68 (t, 2H), 3.80 (t, 2H), 1.26 (s, 9H).

RESULTS AND DISCUSSION

The main goal of this study was to establish a method by which *O*^{6}-alkylguanine and *O*^{6}-(hydroxalkyl)guanine DNA adducts could be detected with high sensitivity by GC-EC/MS. The scheme shown in Figure 1 summarizes the chemical transformation and derivatization reactions that we studied

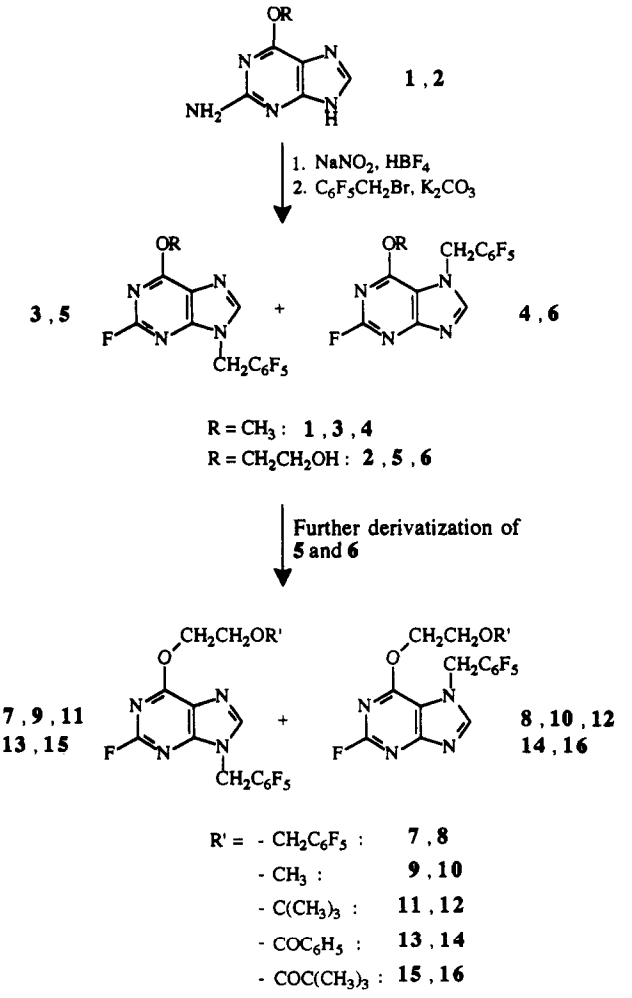

Figure 1. Chemical transformation and derivatization reactions. Compound 9 was not isolated.

to reach this goal. Taking advantage of a known procedure[30] for converting guanine into 2-fluorohypoxanthine by reaction with sodium nitrite and tetrafluoroboric acid (nitrous acid is generated), we found that the problematic 2-NH_2 group of 1 and 2 could be converted into fluoro in high yield. Thus, the reaction manages to avoid a significant hydrolytic loss of the *O*^{6}-alkyl or *O*^{6}-hydroxylalkyl substituent.

Each of the *O*^{6}-substituted hypoxanthine products, from 1 and 2, was next derivatized with pentafluorobenzyl bromide to form corresponding product mixtures 3, 5 and 4, 6, respectively (see Figure 1). This latter derivatization was performed because of its success in converting related nucleobases into products possessing good GC-EC/MS characteristics.[3] The structural assignment for products 3–6 is presented below.

For products 5 and 6, possessing a hydroxy group, further derivatization was needed. We chose to prepare and test several derivatives of this group. As explained below, this approach provided a way to define the *N*7 vs *N*9 location of the pentafluorobenzyl moiety in 3–6. It was expected that this latter moiety would not locate on *N*1 or *N*3, since derivatization at these latter sites would disrupt the aromaticity. We supported this by observing that 1, 3, and 4 all gave an absorption maximum at 253 nm (solution in methanol).

As seen in Figure 1, the hydroxy group in 5 and 6 was converted into three kinds of ethers: pentafluorobenzyl (7,

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Table I. Yield and Physical Properties of Ester and Ether Derivatives of 2-Fluoro-O^6-(2′-hydroxyethyl)-N-(7 and 9)-(pentafluorobenzyl)hypoxanthines and Corresponding O^6-Methylhypoxanthines

compd[g]					NMR (δ)[b]		GC-EC/MS	
no.	R	R′	ring $CH_2C_6F_5$	yield[a] (%)	H (C8)	NCH_2	t_r (min)	RMR[c]
3	CH_3		N9	31[d]	7.93	5.46	3.3	0.25
4	CH_3		N7	25[d]	8.15	5.63	3.7	0.06
5		H	N9	15[e]	8.33	5.68		
6		H	N7	10[e]	8.51	5.92		
7		$CH_2C_6F_5$	N9	13	7.93	5.45	5.2	0.71
8		$CH_2C_6F_5$	N7	48	8.13	5.64	5.9	0.48
9		CH_3[f]	N9					
10		CH_3	N7	25	8.17	5.65	4.2	0.03
11		$C(CH_3)_3$	N9	54	7.94	5.45	4.4	0.16
12		$C(CH_3)_3$	N7	16	8.10	5.67	4.8	0.07
13		COC_6H_5	N9	63	7.94	5.46	6.8	0.10
14		COC_6H_5	N7	27	8.08	5.63	8.0	0.01
15		$COC(CH_3)_3$	N9	58	7.95	5.46	4.8	0.20
16		$COC(CH_3)_3$	N7	25	8.12	5.66	5.2	0.11

[a] Yield starting from a 1.5:1.0 mixture of **5** and **6** unless noted otherwise. [b] Solvent for NMR was acetone-d_6 for **5** and **6** and $CDCl_3$ for the other compounds. [c] For these relative molar response (RMR) values, the reference compound, which was assigned RMR = 1.0 (peak area), was $N1$,$N3$-bis(pentafluorobenzyl)-$N7$-[2′-[(pentafluorobenzyl)oxy]ethyl]xanthine, which has excellent GC-EC/MS characteristics.[1,32] [d] Yield starting from **1**. [e] Yield starting from **2**. [f] This compound was not isolated. [g] See Figure 1.

8), methyl (**9**, **10**), and *tert*-butyl (**11**, **12**). Also, two ester derivatives were prepared: benzoyl (**13**, **14**), and pivalyl (**15**, **16**).

Table I summarizes the yields, selected NMR values, and two GC-EC/MS characteristics (retention and relative molar response) for **3**–**16**. As seen, the chemical shift for the NCH_2 protons is more constant (δ 5.45–5.46) for **3**, **7**, **11**, **13**, and **15** (**9** was not isolated; see below) than for **4**, **8**, **10**, **12**, **14**, and **16** (δ 5.63–5.67). This, along with other correlations (see below), establishes that the former compounds are $N9$-pentafluorobenzyl derivatives, while the latter are corresponding $N7$ derivatives. Only in the $N7$-pentafluorobenzyl derivatives is the NCH_2 moiety near the added R′ substituent. **5** and **6** are not included in this discussion since their NMR spectra were taken in a different solvent.

In the initial ring pentafluorobenzylation reaction of **2**, the $N9$ and $N7$ pentafluorobenzyl derivatives (**5** and **6**, respectively) were formed in ratio of 1.5:1 (based on NMR). This mixed sample was then used directly to form **7**–**16**, leading to the yield data shown in Table I for these compounds. As seen, the relative yields of $N9$ to $N7$ derivatives for this latter series of compounds are consistent with the composition of the starting material except for **7** and **8**. Apparently this ratio in yields is inverted for **7** and **8** (more $N7$ product **8** than $N9$ product **7**) since the reaction leading to their formation (a phase-transfer reaction with pentafluorobenzyl bromide) allows some equilibration between these products via a bis-($N7$,$N9$)-pentafluorobenzyl intermediate. For similar reasons, the phase-transfer reaction of **5**, **6** with methyl iodide gives at most a trace of the $N9$-pentafluorobenzyl product **9** (see Experimental Section): apparently the ease of steric access to the $N9$-pentafluorobenzyl moiety in the phase-transfer reaction leads to its facile removal.

The $N7$ isomers that were available migrate about twice as slow on silica TLC than the $N9$ isomers whenever R′ is relatively nonpolar (**7**, **8**, **11**–**16**; see the R_f values in the Experimental Section), but the $N9$ isomer is slightly slower than $N7$ when R′ is hydroxy (R_f = 0.35 and 0.41 for **5** and **6**, respectively). When analogs of **7** and **8** possessing a 2-NH_2 instead of 2-F moiety were similarly tested, the $N7$ isomer also migrated about 2-fold slower than the $N9$ isomer.[3] In this latter work, isomer assignment was based on electron impact mass spectrometry.

We suggest the mechanism, considering the structural formulas of these compounds (see Figure 1), that the slower compound by TLC in each isomeric pair is the one with a larger "polar footprint". This footprint causes the compound to adsorb more strongly to the polar stationary phase in the TLC separation. For the $N7$ isomers of **3**, **4**, **7**, **8**, and **11**–**16**, this footprint is postulated to consist of the 2-F, $N3$, $N9$ side of the molecule (the bottom side of **3**, **4**, **8**, **10**, **12**, **14** and **16** as presented in Figure 1; this is illustrated for **4** in Figure 3). In contrast, when R′ = H (**5** and **6**), this footprint is suggested to conssist of the O^6-(2′-hydroxyethyl) group and the unsubstituted $N7$ position (the upper side of **5** as presented in Figure 1). Consistent with this (polar compounds tend to be less volatile), the $N7$ isomers of **3**, **4**, **7**, **8**, and **11**–**16** have higher GC retention times than the $N9$ isomers (see Table I).

Relative molar responses (RMRs) by GC/MS must be interpreted with caution since they reflect several events including adsorption losses onto active sites (which can be very specific[31]) in the overall system and complex ionization reactions in the ion source.[1] Nevertheless, a few comments are in order concerning the response values that we have observed for these compounds (see Table I). The RMR is lower for the $N7$ than the corresponding $N9$ isomer for every pair tested. We believe that this behavior (as above) is due to the larger polar footprint of the $N7$ isomers, increasing their susceptibility to adsorption losses. Consistent with our hypothesis that the $N7$ isomers are more prone to adsorption losses, we observe that the response for these isomers tends to decrease more rapidly than for the corresponding $N9$ isomers with column aging (a new column was used to obtain the RMR data shown in Table I). For example, on an older column, the RMRs for **7** and **8** changed from 0.71 and 0.48 (Table I values) to 0.41 and 0.04, respectively.

We speculate that **13** and **14** have unusually low RMRs because they lack thermal stability in the GC-EC/MS due to an $N1$-assisted decomposition of the benzoyl moiety. Apparently the steric bulk of the pivalyl group makes corresponding esters **15** and **16** less susceptible to this event. In prior work involving $N1$,$N3$-bis(pentafluorobenzyl)-5-(hydroxymethyl)uracil, the recovery of a benzoyl ester was not unusual in a GC-EC/MS relative to that of corresponding pentafluorobenzyl and pivalyl derivatives of the 5-hydroxy group.[32] In this latter case, the benzoyl ester moiety is not exposed to an intramolecular nucleophilic site.

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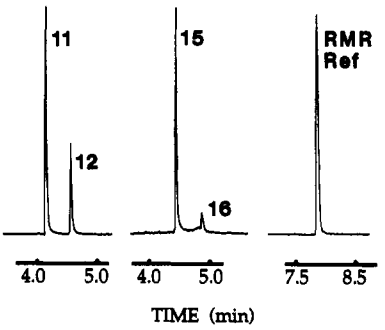

Figure 2. Detection by GC-EC/MS (compound, pg injected, retention time in min, *m/z* monitored, peak height abundance): **11**, 1.16, 4.2, 253, 28 000; **12**, 1.24, 4.6, 253, 11 000; **15**, 1.27, 4.5, 281, 31 000; **16**, 1.31, 4.9, 281, 3000; RMR reference compound (see Table I), 0.273, 7.9, 555, 6000. One-microliter solutions in toluene were on-column injected onto an HP Ultra 1, 25 m length × 0.2 mm i.d. × 0.11 μm film thickness capillary column, and the oven was programmed from 120 to 290 °C at 70 °C/min and then a 7-min hold. Interface from GC to MS, 300 °C; emission current, 300 μA; ion source pressure (methane), 2.0 Torr; ion source *T*, 250 °C; ion source electron energy, 240 eV.

Which derivative is the best one for O^6-hydroxyethylguanine? We favor the *tert*-butyl ethers **11**, **12**, with the pivalyl esters **15**, **16** as a second choice. The equilibration of the *N*-pentafluorobenzyl moiety in ethers **7** and **8** could be difficult to control at a trace level and enriches the less desired (*N*7) isomer **8**. The methyl ether **10** has the liability that **9** is not observed. A low response is observed for the benzoyl derivatives **13**, **14**. The response of the *N*7 pivalyl derivative **16** tends to decrease more with column aging (or sporadically) than the corresponding *tert*-butyl derivative **12**. Figure 2 shows a GC-EC/MS chromatogram of **11**, **12** and **15**, **16** (note the low response for **16**) along with a chromatogram for the reference compound, *N*1,*N*3-bis(pentafluorobenzyl)-*N*7-[2′-[(pentafluorobenzyl)oxy]ethyl]xanthine, which was arbitrarily assigned an RMR of 1. Comparable responses are seen for the preferred derivatives (**11** and **15**) and the reference compound, establishing the high performance of these derivatives.

We chose *N*1,*N*3-bis(pentafluorobenzyl)-*N*7-[2′-[(pentafluorobenzyl)oxy]ethyl]xanthine as an RMR reference compound because it has always given us a high response by GC-EC/MS (e.g., ref 1), even with aging of the GC column. The reason for this was never completely clear to us, but now we believe that the key is the relatively symmetrical substitution of this compound with pentafluorobenzyl groups, minimizing its polar footprint. 5-[[(Pentafluorobenzyl)oxy]-methyl]-N^1,N^3-bis(pentafluorobenzyl)uracil is another symmetrically substituted compound (thereby lacking a significant polar footprint) that we have studied.[32] It too has similarly given a robust high response by GC-EC/MS. 1,3-Bis(pentafluorobenzyl)-*N*7-(2′-methoxyethyl)xanthine is an example of a derivative that gives a puzzling low response by GC-EC/MS.[6] Perhaps this can now be explained by the location of the O^6-keto and flexible methoxy moiety on the same side of this molecule, giving rise to a significant polar footprint. Thus, we suggest that the polar footprint should be minimized in designing derivatives for trace detection by GC-EC/MS. The structures of these compounds and the postulated polar footprints are presented in Figure 3.

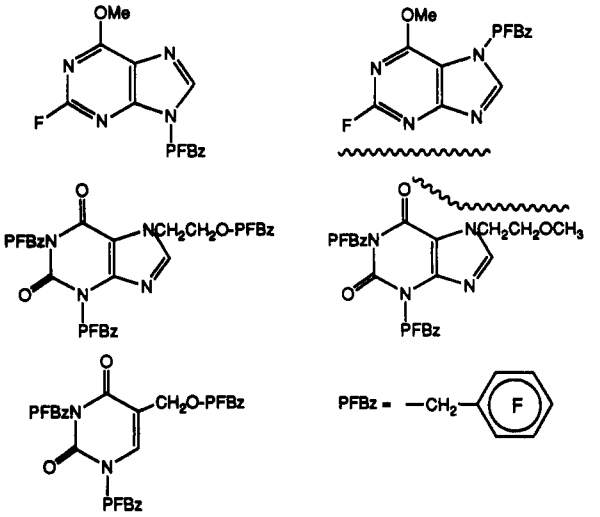

Figure 3. Structures of some compounds that have been tested by GC-EC/MS, with an indication of a postulated "polar footprint" ( ) for two of them.

CONCLUSION AND FUTURE

A derivatization strategy for O^6-alkyl- and O^6-(hydroxyalkyl)guanines prior to their detection by GC-EC/MS has been established. As a side benefit of this study, a concept termed polar footprint emerged which may be useful as a guideline in some cases for derivatization prior to GC. For the future, the methodology needs to be extended to trace amounts of these adducts in biological samples. Success in achieving this in related cases (e.g., refs 21 and 33) increases the likelihood of success here.

ACKNOWLEDGMENT

This work was funded by Grant OH02792 from the National Institute for Occupational Safety and Health, Centers for Disease Control, Grant CN-71 from the American Cancer Society, and NIH Grant ES02109. Contribution No. 594 from the Barnett Institute.

RECEIVED for review April 30, 1993. Accepted July 29, 1993.®

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® Abstract published in *Advance ACS Abstracts*, September 1, 1993.