

# CORRELATING STRUCTURAL PROPERTIES OF N7-ADDUCTED GUANINE BASES AND GLYCOSIDIC BOND CLEAVAGE IN DNA



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## ABSTRACT

A series of compounds have been modeled to gain insight into the relationship between electrophiles that bind to the N7-position of guanine and the subsequent rate of glycosidic bond cleavage. The compounds modeled have had the  $T_{1/2}$  of depurination measured on native DNA, and the parameters of this kinetic event have been presented in a recent review<sup>3</sup>. The compounds were modeled as their deoxyguanosine analogs with geometry restrictions on the deoxyribose ring to mimic the geometrical environment in the DNA polymer. After obtaining geometric and electronic properties from PM3, semi-empirical calculations, correlations between calculated properties and experimental  $T_{1/2}$  of depurination were sought. The calculated length of the glycosidic bond correlated poorly when all compounds were included,  $R^2 = 0.044$ . Better correlations were seen when compounds were grouped by the size of their ligands,  $R^2 = 0.418$  for medium sized ligands. The molecular volume of the ligand was shown to have a significant correlation to the  $T_{1/2}$  of depurination,  $R^2 = 0.520$ . Also, the aqueous solvation energy for a ligand correlated to the rate of glycosidic bond cleavage,  $R^2=0.599$ . These results imply that the steric environment imposed at the N7-position by electrophilic addition to guanine influences the subsequent rate of depurination more than electronic effects.

## INTRODUCTION

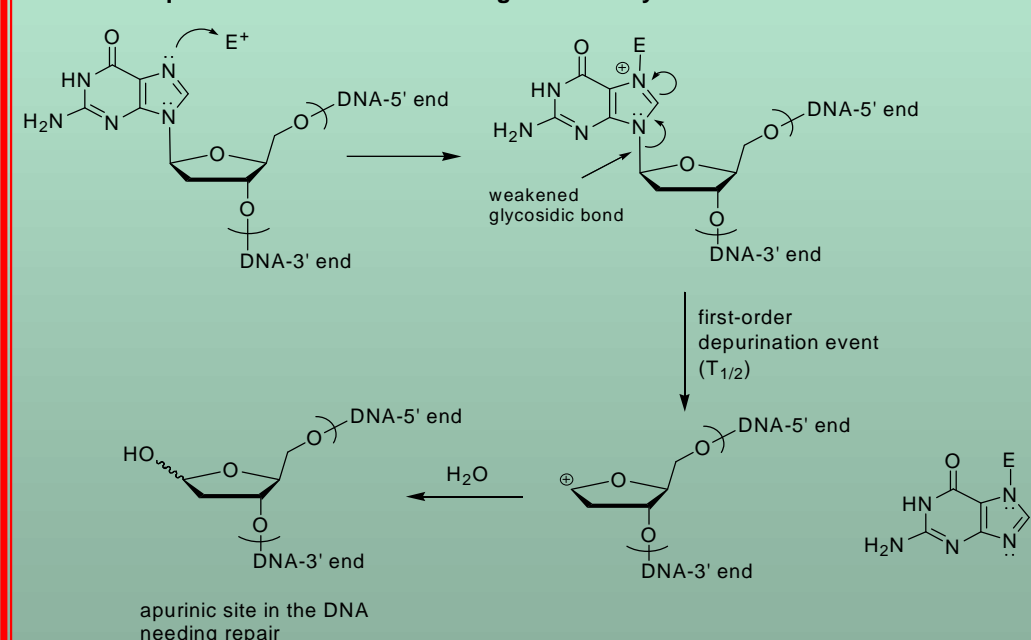
The initiation of cancer can be caused by chemical modification to DNA. If these chemical modifications occur at critical genes, and are left unrepaired, mutations leading to tumor formation are possible. Chemical modification of DNA can lead to stable DNA adducts that remain bonded to the DNA polymer, or depurinating adducts that detach from the DNA by cleavage of the glycosidic bond connecting the base to the sugar backbone. Depurinating adducts create an apurinic site in the DNA which must be repaired. Depurination occurs naturally in DNA at a rate of approximately 10,000 sites per cell per day. Although the formation of apurinic sites is a naturally occurring phenomenon, when too many apurinic sites are created, the cell's ability to repair these sites can become overwhelmed and lead to the misreplication of the DNA. Mutations induced by the polycyclic aromatic hydrocarbons, potent environmental carcinogens, have been demonstrated to occur via formation of apurinic sites on the H-ras gene, an oncogenic site related to mouse skin papillomas<sup>1</sup>. In addition, depurinating adducts have been detected in breast cancer tissue exposed to estrogen quinones, endogenous compounds known to form predominately depurinating adducts<sup>2</sup>.

Chemical modification at the N7-position of guanine by electrophiles causes the glycosidic bond between the base and the sugar to weaken (Figure 1). The unimolecular process for depurination varies with the nature of the electrophile. Methylated N-7 guanine occurs naturally in the DNA of some species and is stable compared to other N-7 adducted guanines. Recently, a review of N7-adducted guanines and the  $T_{1/2}$  for depurination in DNA has been collated<sup>3</sup>. The ability of cells to repair apurinic sites would be a function of how quickly these sites are generated. Thus, electrophiles that induce the depurinating event quickly would be more genotoxic (damaging to the DNA) than electrophiles that form more stable guanine adducts.

What structural features of the N7-adduct guanine base would cause cleavage of the glycosidic bond to occur faster or slower? We sought to investigate this question by modeling the structural and electronic features of a representative group of known N7-adduct guanine compounds (Figure 1) made available by a recent review<sup>3</sup>. Molecular modeling can accurately predict the structure and certain electronic aspects of molecules. One structural feature hypothesized to correlate to the value of  $T_{1/2}$  was the length of the glycosidic bond. As bonds weaken, they grow longer in length. Thus adducts that impose a longer glycosidic bond might be expected to depurinate faster. Also, greater charge localization on the N7 atom may prompt the electron flow depicted in Figure 1 to occur more readily. This work constitutes investigating correlations between the  $T_{1/2}$  of N7-adducted guanines and their structural and electronic properties.

1. Chakravarti D, Pelling J.C., Cavaliere E.L., and Rogan E.G. (1995) Relating Aromatic Hydrocarbon-Induced DNA Adducts and c-H-ras Mutations in Mouse Skin Papillomas: The Role of Apurinic Sites. *Proc. Natl. Acad. Sci. USA*, 92, 10422-10426.
2. Stack, D. E.; Byun, J.; Gross, M. L.; Rogan, E. G. and Cavaliere, E. L. (1996) "Molecular Characteristics of Catechol Estrogen Quinones in Reactions with Deoxyribonucleosides." *Chemical Research in Toxicology* 9, 851-859. And: Cavaliere, E. L.; Stack, D. E.; Devanesan, P. D.; Todorovic, R.; Dwivedy, I.; Higginbotham, S.; Johansson, S. L.; Pail, K. D.; Gross, M. L. (1997) Molecular origin of cancer: catechol estrogen-3,4-quinones as endogenous tumor initiators. *Proc. Natl. Acad. Sci. USA*, 92, 10422-10426.
3. Gates K.S., Nooner T. and Sanjay Dutta, S. (2003) Biologically relevant chemical reactions of N7-alkylguanine residues in DNA. *Chemical Research in Toxicology* 17, 839-856.

Figure 1: Chemical Modification at the N7-Position of Guanine by Electrophiles and Subsequent Unimolecular Cleavage of the Glycosidic Bond.



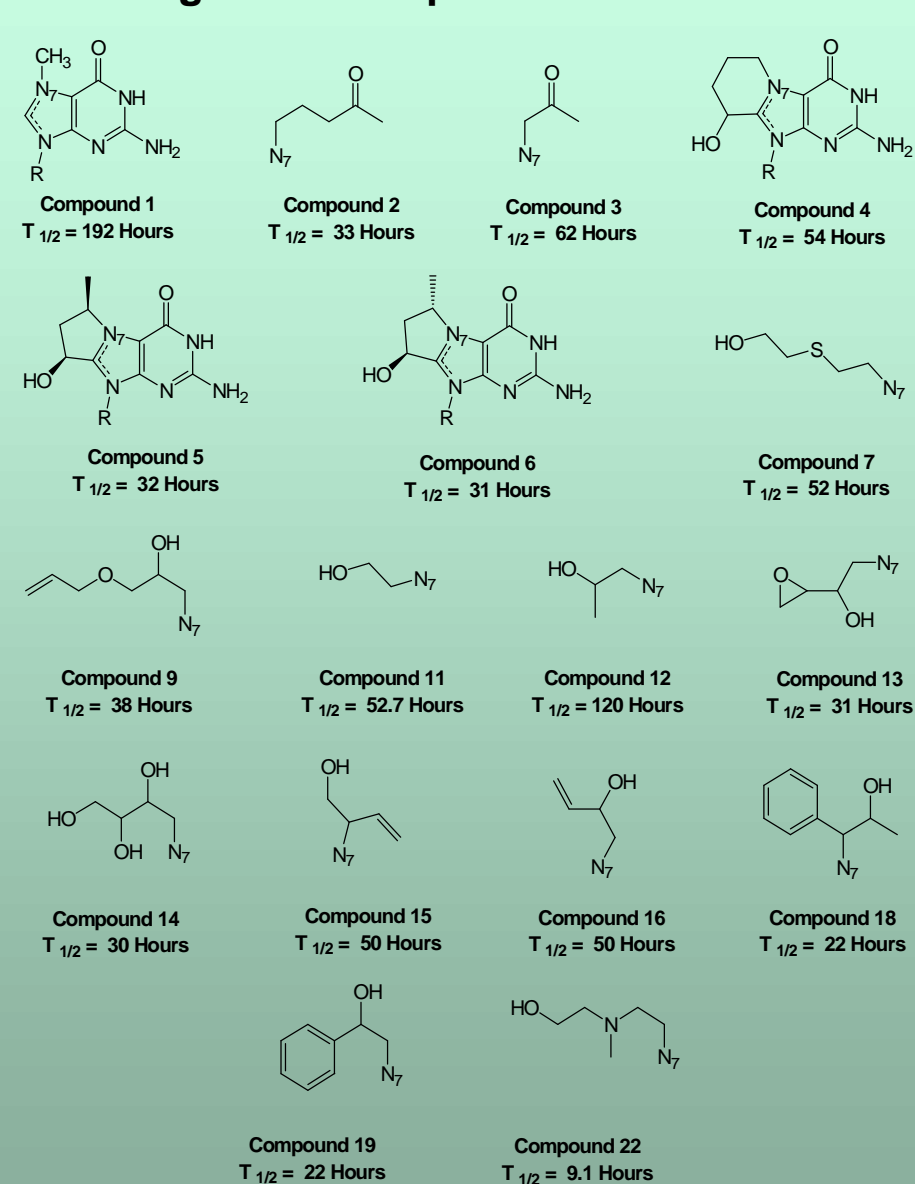
## METHODS

Structures were modeled by first constructing the compounds in Figure 2 using ChemDraw (version 7, CambridgeSoft), exporting to Chem3-D (version 7, CambridgeSoft) and saving the resulting files in the .pdb format. The .pdb files were then imported into Spartan (version 5.1, Wavefunction). Initial studies indicated that the length of the glycosidic bond was dependent on the dihedral angle between the DNA base and the deoxyribose ring system. Unconstrained minimized structures had dihedral angles that differed substantially from that found in native DNA. Therefore, a sample of deoxyguanosine was obtained by extracting a dG structure from a known DNA plasmid. Protein Data Bank structure 1N14 (unmodified) with the sequence (GCTAAGGAAGCC) was used, and the highlighted guanine was extracted from the structure using BioMedCACHe (version 6.0 Fujitsu Corp.). The extracted dG structure was then imported into Spartan, and all atoms in the deoxyribose ring were constrained, with the exception of the C1' carbon and its corresponding hydrogen. Using this extracted dG structure, all other compounds in Figure 2 were constructed and collated using the spreadsheet feature in Spartan. Geometry optimization using the PM3 Hamiltonian was conducted. Atomic charges were calculated using Mulliken, natural and electrostatic charges as implemented by the Spartan package. These charges, along with glycosidic bond distances were imported into the Spartan spreadsheet. The spreadsheet was then exported from Spartan to use in Excel. In the Excel spreadsheet,  $T_{1/2}$  from reference 3 were added, and the data was mined for correlations.

Determination of molecular volume for the N7-ligands was conducted by replacing the dG structure with a hydrogen, collating the structures into a Spartan spreadsheet, calculating the PM3 geometry, and implementing the Spartan molecular volume spreadsheet function. Molecular volumes are calculated using an electron density cut-off of 0.01 electrons/Å<sup>3</sup>. Ligands forming cyclic structures (Compounds 4, 5 and 6) were constrained to cyclic forms during the molecular volume calculations. This data was exported into Excel as before, and correlations investigated. AM1-SM2 solvation energies were calculated using the method of Cramer and Truhlar<sup>4</sup>.

<sup>4</sup> Cramer C.J.; Truhlar D.G. AM1-SM2 and PM3-SM3 parameterized SCF solvation models for free energies in aqueous solution. *Journal of computer-aided molecular design* 1992, 6(6), 629-66.

Figure 2: Compounds Modeled



SMALL GROUP (Vol. <90) Compounds: 1, 3, 11, 12  
MEDIUM GROUP (Vol. 104-114) Compounds: 2, 4, 5, 6, 13, 15, 16  
LARGE GROUP (Vol. >129) Compounds: 7, 9, 14, 18, 19, 22

Figure 3: Half-Life vs. Distance, Compounds, All

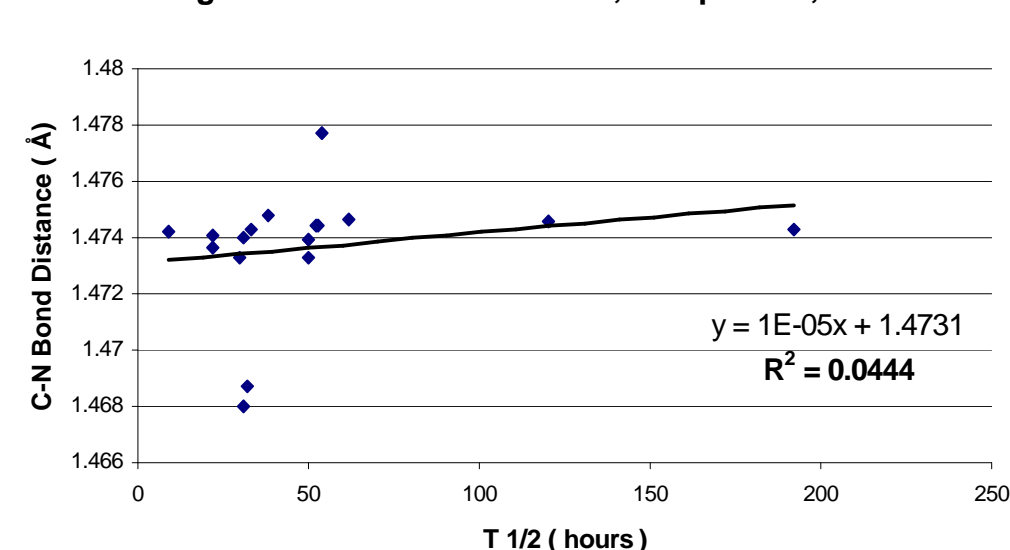


Figure 4: Half-Life to Electrostatic Charge of N7, All

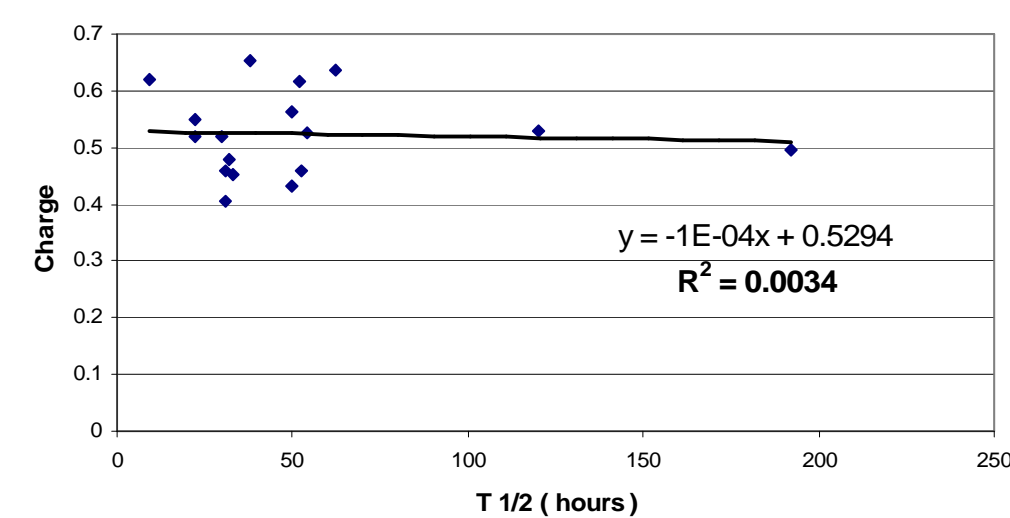


Figure 5: Half-Life to Electrostatic Charge of N7, Compounds: 5, 11, 14, 15, 18, 19, 22

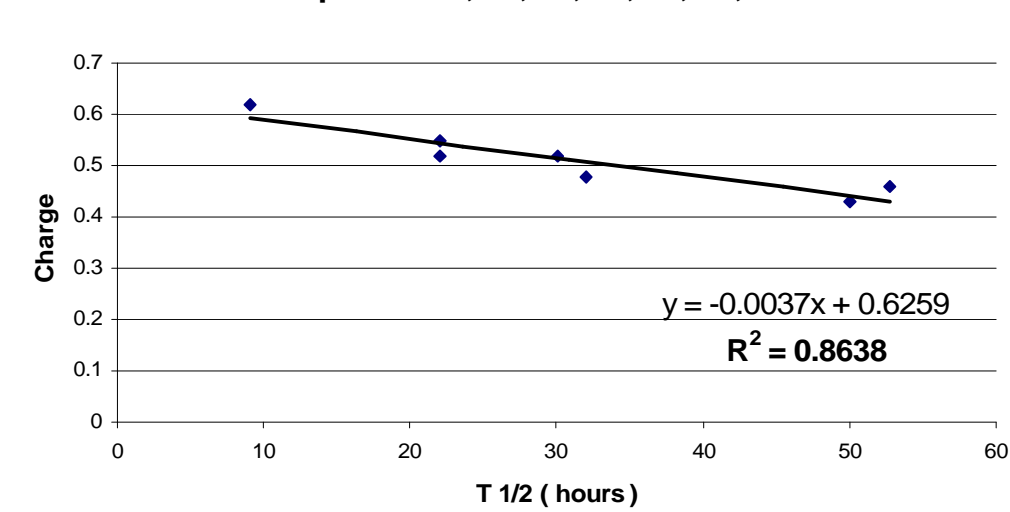


Figure 6: Half-Life to C-N9: Small Volumes

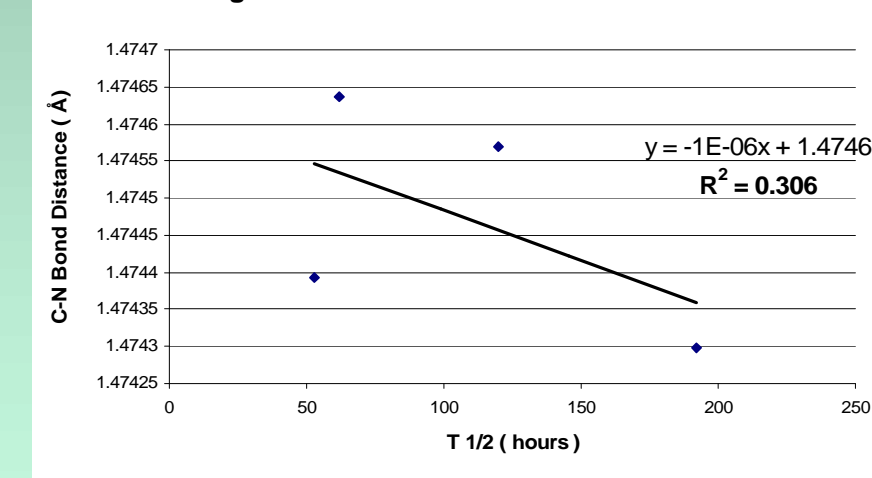


Figure 7: Half-Life to C-N9 Distance: Medium Volumes

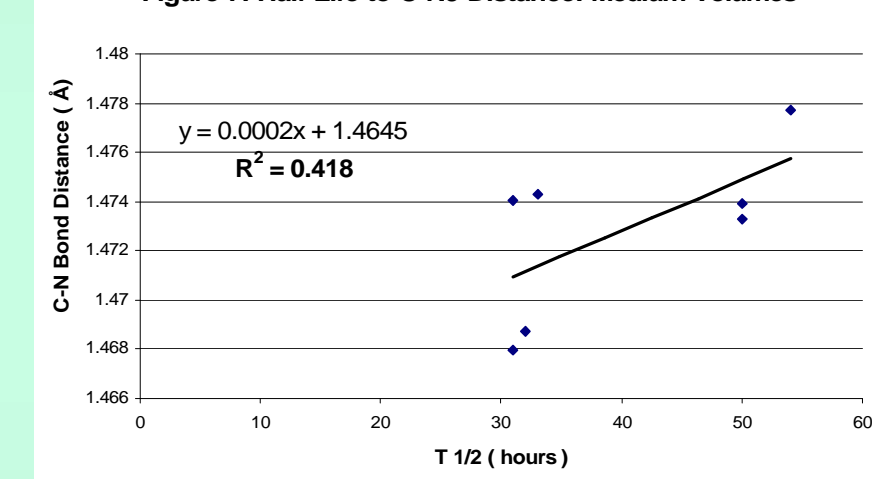


Figure 8: Half-Life to C-N9 Distance: Large Volumes

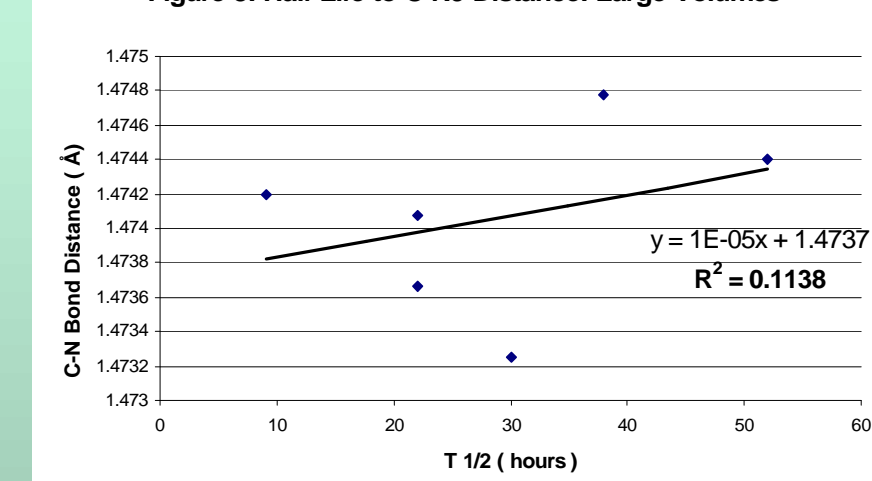


Figure 9: Half-Life to Ligand Volume, All Compounds

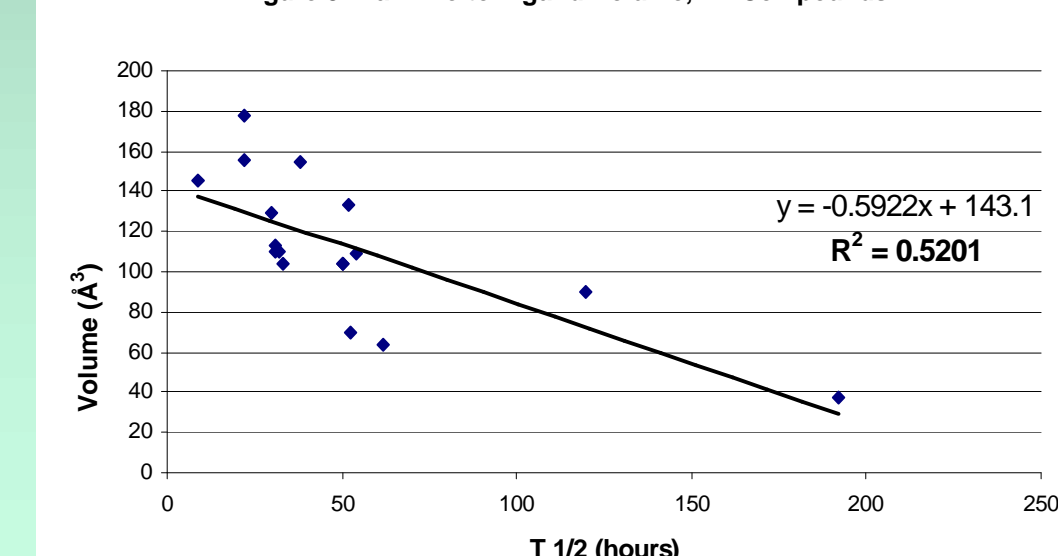
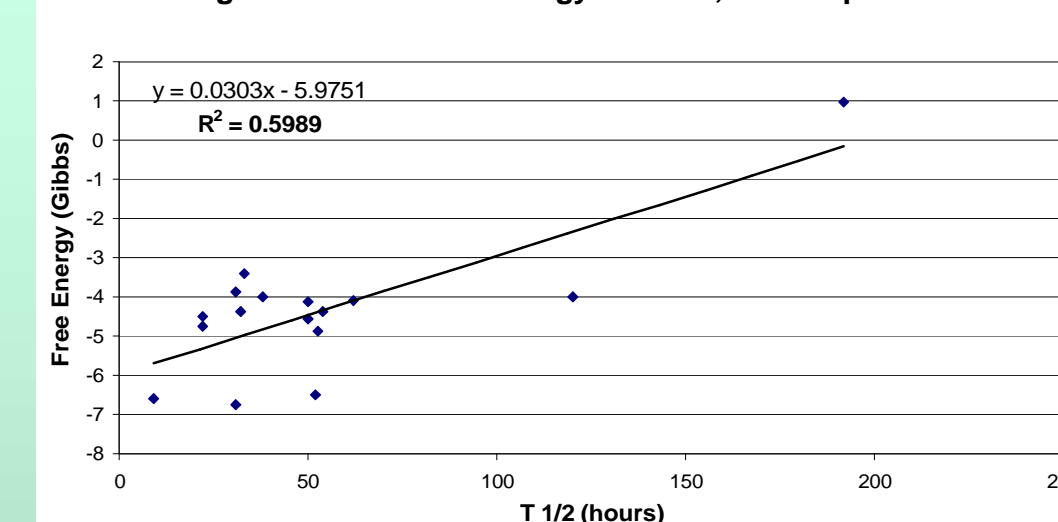


Figure 10: Solvation Energy vs. T 1/2, All Compounds



## RESULTS AND DISCUSSION

Figure 2 displays the compounds used for this study. These structures were chosen from reference 3 since the half-lives were measured under similar conditions (there were more compounds listed in reference 3 than shown in Figure 2). All half-lives were measured on native DNA under physiological pH. The compounds were modeled as their deoxyguanosine analogs. In order to mimic the conformation of the sugar ring as it exists in DNA, all atoms in the sugar ring, except the C1' and its corresponding hydrogen, were frozen during geometry optimization (see Methods section). Figure 3 shows essentially no correlation between the glycosidic bond distance (N9-C1') and the rate of depurination. When other electronic factors, such as atomic charge at N7 (see Figure 4), were investigated, no correlations were found using the complete set of compounds in Figure 2. Attempts to mine the calculated data for significant correlations to half-life times resulted in sets of compounds from Figure 2 displaying some correlations. For example, Figure 5 shows a grouping of seven compounds whose electrostatic charge at N7 correlates well with the rate of depurination. Other groups were found using glycosidic bond distances, charge at N9, or charge at C8. At first, it seemed these groupings were random, but further analysis seemed to indicate groups were based on the size of the ligand attached to the N7-position. Figures 6 – 8 show that when the compounds in Figure 2 are grouped according to the molecular volume of the ligand, the correlation between glycosidic bond distance and sugar loss improves. Figure 9 shows that there is a significant correlation between the size of the ligand attached to the N7-position of guanine and rate of depurination in the DNA. The larger ligands produce a faster rate of glycosidic bond cleavage when compared to the smaller ligands. Thus, steric size may be a better indicator of the genotoxicity of an electrophile not the electronic effects it may impose on the glycosidic bond. A larger ligand could cause a local distortion of the DNA placing added strain of the glycosidic linkage attaching the base to the DNA polymer.

The N7-position of guanine is one of the more nucleophilic sites in the DNA due to the electron rich nature of the guanine base, and because the N7-position is exposed in the major groove of the DNA. A small ligand, such as a methyl group, could exist in the major groove without causing steric strain to the sugar polymer. Other factors that could cause a ligand to impart strain would be its tendency to associate with the aqueous environment outside the DNA. This property can be modeled by calculating the solvation energy of the ligand. Figure 10 shows a significant correlation between the polarity of the ligand, as measured by its solvation energy, and the rate of depurination. The more polar ligands may also produce interactions with neighboring phosphate groups, via hydrogen bond donors, also causing steric stress to the glycosidic bond.

## CONCLUSIONS

The length of the glycosidic bond does not correlate to the rate of depurination with the compounds modeled in Figure 2. Other electronic effects, such as electrostatic charge at the N7-position of guanine also correlate poorly to the kinetics of depurination. When compounds of similar size were compared, electronic correlations could be found. The size of the ligand attached to the N7-position seems to be a better predictor to the rate of depurination than does the ligand's electronic influence on the guanine ring. Other interactions, such as the affinity the ligand has to the outside aqueous environment of the DNA may also cause weakening of the glycosidic bond. In summary, with the compounds modeled in this study, steric effects seem to predominate over electronic effects.

## ACKNOWLEDGEMENTS

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