Interdependent Sequence-Selectivity and Diastereoselectivity in the Alkylation of DNA by Decarbamoylmitomycin C.

William Aguilar, Manuel M. Paz, Anayatzinc Vargas**,** Maggie Zheng, Shu-Yuan Cheng and Elise Champeil*****

Abstract: Mitomycin C (MC), an antitumor drug and decarbamoylmitomycin C (DMC) a derivative of MC, alkylate DNA and form deoxyguanosine monoadducts and interstrand crosslinks (ICLs). Interestingly, in mammalian culture cells, MC forms primarily deoxyguanosine adducts with a 1"-*R* stereochemistry at the guanine-mitosene bond (1"-α) whereas DMC forms mainly adducts with a 1"-*S* stereochemistry (1"-β). The molecular basis for the stereochemical configuration exhibited by DMC has been investigated using biomimetic synthesis. Here, we present the results of our studies on the monoalkylation of DNA by DMC. We show that the formation of 1"-β-deoxyguanosine adducts requires bifunctional reductive activation of DMC, and that monofunctional activation only produces 1"-α-adducts. The stereochemistry of the deoxyguanosine adducts formed is also dependent on the regioselectivity of DNA alkylation and on the overall DNA CG content. Additionally, we found that temperature plays a determinant role in the regioselectivity of duplex DNA alkylation by mitomycins: At 0°C, both deoxyadenosine (dA) and deoxyguanosine (dG) alkylation occur whereas at 37 °C, mitomycins alkylate dG preferentially. The new reaction protocols developed in our laboratory to investigate DMC-DNA alkylation raise the possibility that oligonucleotides containing DMC 1"-β-deoxyguanosine adducts at a specific site may be synthesized by a biomimetic approach.

Table of contents **Pages**

TableS1. Frequencies of dG Adducts in Oligonucleotide Substrates under Autocatalytic Conditions (substoechiometric amount of $Na_2S_2O_4$, pH 7.4). **1b** (C1'' *S*) is the stereoisomer of **1a** (C1" *R*).

	Frequency (%) of the major adducts detected			
	MC treatment		DMC treatment	
Duplex Oligonucleotide	**1a**	**1b**	**2a**	**2b**
a: 5'-ATATA**CG**TATAT-3'	14.4%	0%	1.65%	0%
b: 5'-ATATA**GC**TATAT-3'	0.81%	0%	0.18%	0%

Figure S1. Ratio of stereoisomeric adducts **2b**/**2a** formed from the reaction of DMC and 20 O.D. of duplex oligunucleotides 5'-ATATAT**GC**TATAT (blue) and 5'- ATATAT**CG**TATAT (red) under various concentration of DMC. The data represent the average of at least three independent experiments.

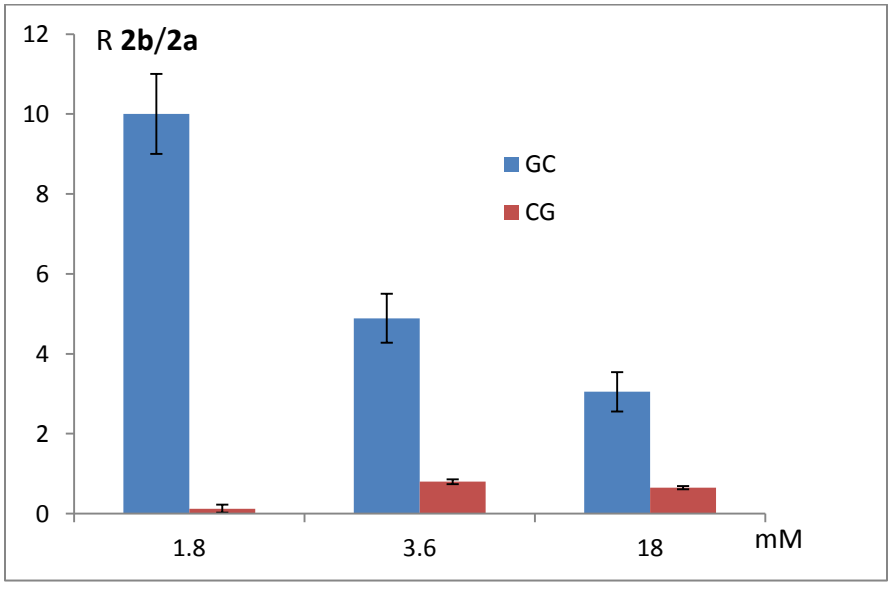


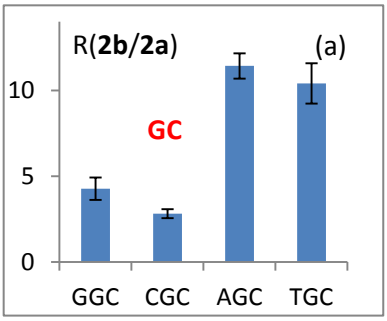
Table S2. Frequencies of dG Adducts in Oligonucleotide Substrates using NADH-cytochrome *c* reductase.

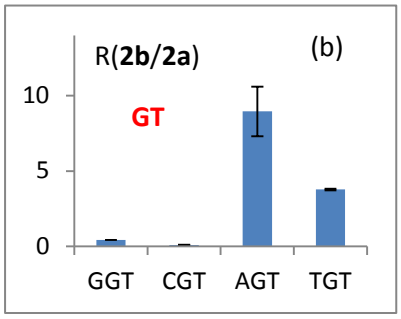
	Frequency (%) of the major adducts detected	
Duplex Oligonucleotide	**2a**	**2b**
a: 5'-ATATA**CG**TATAT-3'	0.84	0
b: 5'-ATATA**GC**TATAT-3'	0.42	0.27

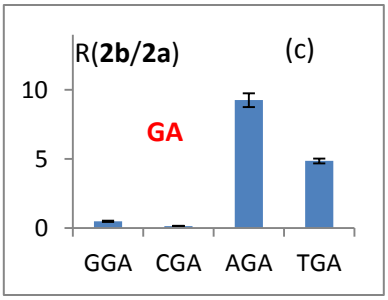
Table S3. Frequency of DNA Adducts Detected in Calf Thymus and *M. Luteus* DNA under Bifunctional Conditions and at Different Temperature and pH. ND=non-detected, NA=non-available.

	Frequencies (%) of adducts detected						
Substrate	**2a**	**2b**	**3a**	**3b**	**4+5**	**6**	**3b/2b**
***M. Luteus* 0°C**	1.43 (±0.03)	0.11 (±0.004)	ND	ND	0.71 (±0.03)	ND	NA
***M. Luteus* RT**	0.59 (±0.05)	0.06 (±0.002)	0.08 (±0.006)	ND	0.23 (±0.05)	0.13 (±0.05)	NA
***M. Luteus* 37°C**	0.22 (±0.02)	0.15 (±0.04)	0.53 (±0.08)	0.07 (±0.02)	nd	0.09 (±0.03)	0.45
CT DNA 0°C	0.72 (±0.008)	1.15 (±0.008)	ND	ND	0.02 (±0.002)	ND	NA
CT DNA RT	0.42 (±0.03)	1.22 (±0.03)	0.11 (±0.008)	0.08 (±0.006)	0.03 (±0.02)	0.09 (±0.03)	0.066
CT DNA 37°C pH 5.8	0.11 (±0.001)	0.56 (±0.03)	0.12 (±0.004)	0.20 (±0.02)	ND	0.09 (±0.02)	0.38
CT DNA 37°C pH 4.5	0.06 (±0.004)	0.22 (±0.005)	0.14 (±0.004)	0.11 (±0.01)	ND	0.10 (±0.01)	0.5
CT DNA 37°C pH 7.5	0.10 (±0.02)	0.22 (±0.002)	0.18 (±0.002)	0.07 (±0.001)	ND	ND	0.32

Figure S2. Ratio of DMC monoadducts formed, R(**2b**/**2a**) formed under bifunctional conditions with various oligonucleotides. The central sequence of each oligonucleotide is indicated on each horizontal axis: (a) oligonucleotides **14**, **15**, **16**, **17**; GpC step (b) oligonucleotides **18**, **19**, **20**, **21**; GpT step (c) oligonucleotides **22**, **23**, **24**, **25**; GpA step (d) oligononucleotides **26**, **27**, **28**, **29**; GpG step.







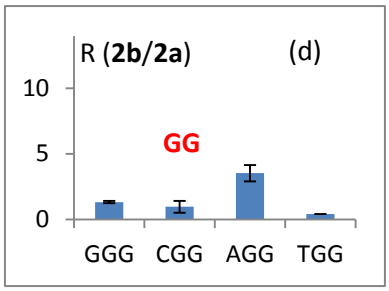


Figure S3. (a) % yields of DMC monoadduct **2b** and of DMC adducts **2a** +**2b** formed under bifunctional conditions with d(ATTATT**GC**TATT).(AATA**GCA**ATAAT) (**17**) and d(ATTATT**IC**TATT).(AATA**GC**AATAAT) (b) Ratio of dG monoadducts formed (R(**2b**/**2a**)) under the same conditions with the same duplex oligonucleotides.

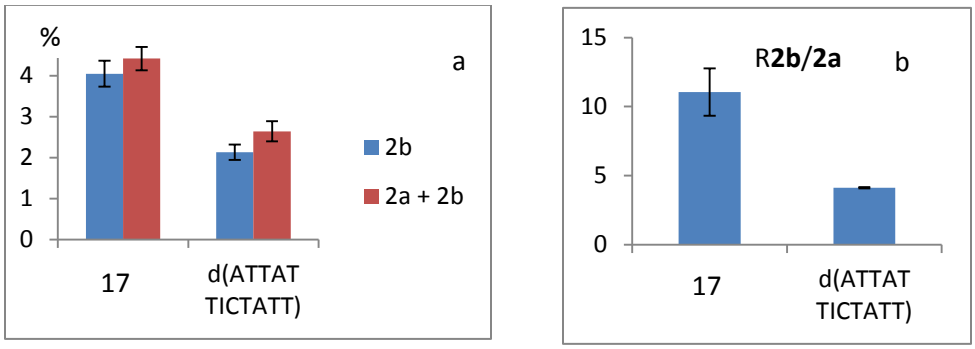


Figure S4. (a) % yields of DMC monoadduct **2b** and of DMC adducts (**2a** +**2b**) formed under bifunctional conditions with d(ATTATT**CG**TATT).(AATA**CG**AATAAT) (**19**) and d(ATTATT**CI**TATT).(AATA**CG**AATAAT) (b) Ratio of dG monoadducts formed (R(**2b**/**2a**)) under the same conditions with the same oligonucleotides.

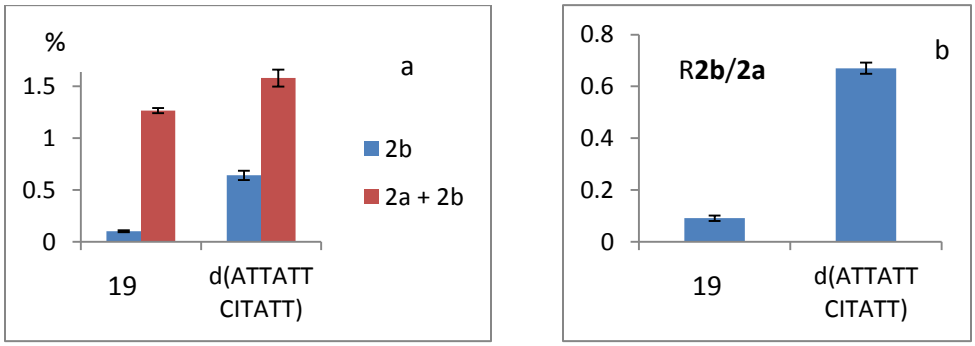
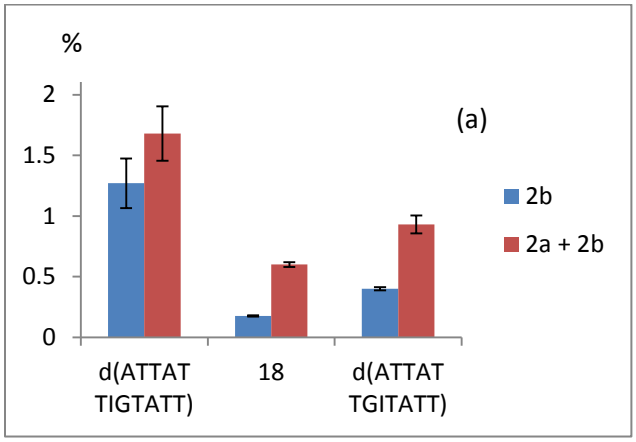
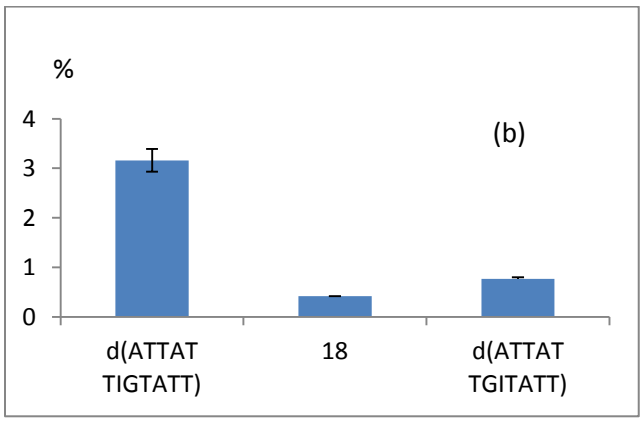


Figure S5. (a) % yields of DMC monoadduct **2b** and of DMC adducts (**2a** +**2b**) formed under bifunctional conditions with duplex (ATTATT**IGT**ATT).(TAATAA**CCA**TAA); oligonucleotide (ATTATT**GGT**ATT).(TAATAA**CCA**TAA) (**18**) and duplex (ATTATT**GIT**ATT).(TAATAA**CCA**TAA) (b) Ratio of dG monoadducts formed (R(**2b**/**2a**)) under the same conditions with the same oligonucleotides.





Supplementary Experimental Section

Calculation of percent yields of Adducts 1a, 2a, 2b, 3a, 3b, 4, 5 and 6: Molar proportions of individual substances in the HPLC profiles were determined by peak area measurements. Peak areas were divided by the appropriate extinction coefficient at the wavelength of the detection; this gave molar proportions. Yields of adducts were determined by relating their molar proportion to the dT peak of the digest serving as internal standard. In the case of *M. Luteus* or calf thymus DNA, the known dT:dG ratio was used for calculating the yield of the adduct based on total dG content of *M. luteus*-DNA or calf thymus-DNA. (*M. luteus* DNA: dT/dG= 0.39; calf thymus DNA: dT/dG=1.38).

Oligonucleotide Alkylation by DMC and MC under Autocatalytic Conditions. 12-mer self-complimentary duplex oligonucleotides (5'-ATATA**CG**TATAT-3'or 5'-ATATA**GC**TATAT-3' ; 10 A_{260} units; 330 µg; corresponding to 0.083 µmol) were mixed with 2.5 µmol of MC or DMC in 100 mM potassium phosphate buffer, pH 7.5, (355 µL) at 0°C in an ice bath. Freshly prepared $Na_2S_2O_4$ solution (1.25 µmol in 10 µL of 100 mM potassium phosphate buffer, pH 7.5) was added at once. After 1 hr at 0°C, the mixture was immediately chromatographed on a 2.5*56 cm Sephadex G-25 column using 20 mM NH_4HCO_3 as eluent. Oligonucleotide containing fractions were lyophilized and digested as described in the experimental part of the manuscript.