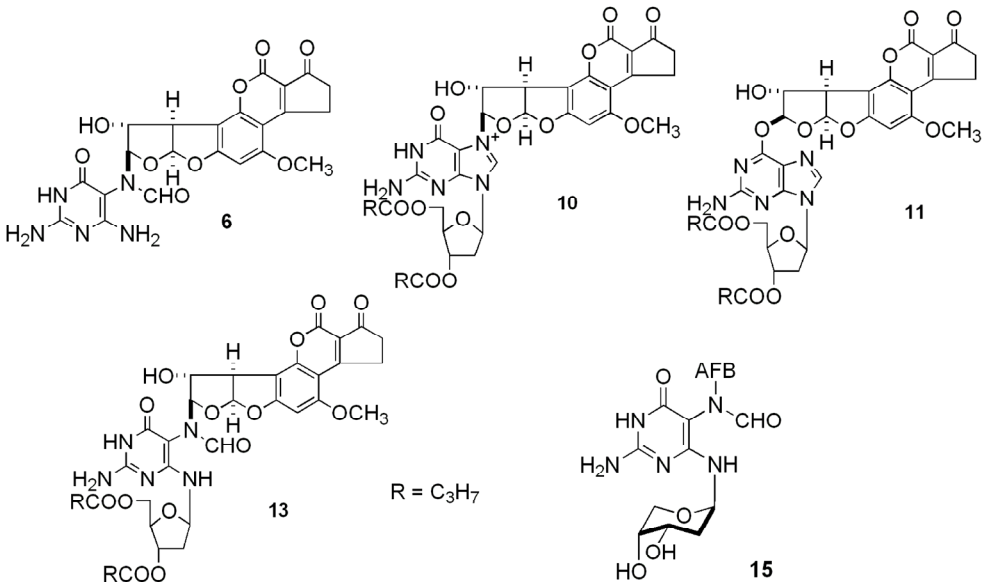
Supporting Information

Unraveling the Aflatoxin-FAPY Conundrum: Structural Basis for Differential Replicative Processing of Isomeric Forms of the Formamidopyrimidine-type DNA Adduct of Aflatoxin B_1

*Kyle L. Brown, James Z. Deng, Rajkumar S. Iyer, Lalitha G. Iyer, Markus W. Voehler, Michael P. Stone, Constance M. Harris and Thomas M. Harris**



Preparative Synthesis and Isolation of 13. The mixture containing **10** was made basic with 10 mL of pH 9.5 buffer (15 mM Na_2CO_3/30 mM $NaHCO_3$) and stirred overnight at ambient temperature. Neutralization using 0.1 M HCl was followed by lyophilization to remove solvents. The residue was dissolved in 4 mL of methanol and clarified by filtration through a 0.45 μm filter. The methanolic solution was fractionated by reverse-phase HPLC (Econosil ODS, 10 μm, 250 x 10 mm, Alltech Associates; 0-67% linear gradient of CH_3CN in water over 40 min; 3.0 mL/min; UV detection at 358 nm). The AFB-FAPY adducts described in this study eluted as a mixture of the major and minor isomers at 33 min. This fraction was collected and lyophilized to afford a mixture of FAPYs A and B in 45-50% combined yield. Separation of the FAPY isomers (**13**-FAPYs A and B) was carried out using reverse-phase HPLC (Econosphere ODS, 5 μm, 250 x 4.6 mm, Alltech Associates; 60% CH_3OH/H_2O; 1.0 mL/min; UV detection at 360 nm). Retention times of FAPY A and FAPY B were 22.4 and 29.8 min, respectively. The isomers were collected over Dry Ice/acetone and lyophilized.

Isolation of 15. The methanolic solution was fractionated by reverse-phase HPLC (Econosil ODS, 10 x 250 mm, 10 μm, Alltech Associates; stepwise gradient of MeOH-H_2O as follows: 20 mL 5% MeOH, 100mL 10% MeOH, 50 mL 15% MeOH, 50 mL 20% MeOH, 50 mL 22% MeOH, 100 mL 25% MeOH and finally 100% MeOH; 3.0 mL/min; UV detection at 360 nM. The poorly resolved AFB-FAPY adducts eluted as a mixture of isomers at 100 and 110 min. These fractions were collected and lyophilized to afford the adducts in a total yield of 40-50%. The 100 min peak, which was the major fraction, was dissolved in 50 mL of 10% aqueous methanol and re-fractionated on the same column with a different gradient (100 mL 10% MeOH, 100mL 15% MeOH, 100 mL 20% MeOH, 100 mL 22% MeOH and finally 100 mL 25% MeOH. Four peaks at retention

times 137, 147, 154 and 156 min were obtained. The major peaks (137 (FAPY **15-A**) and 147 min (FAPY **15**-**B**)) were collected and lyophilized. They were characterized by NMR and MS. FAPY **15-A** was further analyzed by two-dimensional NMR. The 110 minute peak from the first fractionation was similarly separated using the above gradient; it was a mixture of components similar to the original 100 min fraction.

NMR Studies. Routine spectra were recorded on Bruker AC-300 and AM-400 instruments operating at 300.13 MHz and 400.13 MHz, respectively. Two-dimensional spectra were recorded on a Bruker AMX-500 spectrometer at 500.13 MHz. NMR samples of AFB-FAPY nucleoside were prepared as ~0.01 M solution in a mixture of MeOH-d_4/DMSO-d_6 (9:1) except for the ROESY, which was performed in pure DMSO-d_6 to keep the exchangeable protons visible. The [^{15}N] labeled AFB-FAPY nucleoside was prepared as ~0.01 M solution in a mixture of methanol-d_4/DMSO-d_6 (9:1).

^{15}N NMR spectra were recorded at 288 K in MeOD/DMSO-d_6 (9:1) mixtures at 50.9 MHz on a Bruker AMX-500 spectrometer via an INEPT pulse sequence for non-selective polarization transfer, refocused and ^{1}H-decoupled during acquisition. The delay between pulses was set to 16.7 ms to optimize for a $^3J_{NH}$ of 15 Hz. AFB-FAPY nucleoside **13** showed four signals at δ 133.9, 133.6, 131.7, and 130.8. Proton chemical shifts are reported using the residual ^{1}H in MeOH-d_4 as the internal reference (δ 3.30 ppm) or DMSO-d_6 (δ 2.49 ppm) in the ROESY. Carbon shifts are referenced to the CD_3 signal of methanol-d_4 at δ 49.0. The 2D spectra were measured with natural abundance samples and at 15 °C if not described otherwise.

Homonuclear 2D studies were carried out using the following experiments: double quantum filtered COSY,[1] TOCSY 250 ms mixing time,[2] NOESY 800 ms mixing time and ROESY 300 ms mixing time.[3] The acquisition conditions were as followed: $f_1 = f_2 =$

4504 Hz, 2K in the f_2 dimension and 512 increments in f_1, 32 or 64 scans, and the data were processed as 2K x 1K matrices with a shifted sine-bell apodization. The ROESY spectrum was measured at 20 °C in anhydrous DMSO-d_6 to prevent the exchange of the NH protons. A spectral width $f_1 = f_2 = 6024$ Hz was chosen and all the other conditions were used as in the other experiments.

Carbon chemical shifts were obtained via HMQC (Heteronuclear Multiple-Quantum Coherence) or HMBC (Heteronuclear Multiple-Bond Coherence)experiments.[4] 2D HMQC spectra were run using the BIRD sequence to suppress the center signal and with carbon decoupling during acquisition in the TPPI mode.[5] The applied pulse sequence for the HMBC experiment was: relaxation delay - $90°_x(^1H)$ - D_1 - $90°_{f1}(^{13}C)$ - $t_1/2$ - $180°_x(^1H)$ - $t_1/2$ - $90°_{f2}(^{13}C)$ - acquire. The HMBC spectrum was recorded using a delay time D_1 of 60 ms. Both heterocorrelation spectra were 2K covering 4504 Hz in the f_2 dimension and 22,727 Hz in the f_1 dimension and were acquired for 512 increments with 64 scans on the HMQC and 128 scans on the HMBC. The data were processed with 2K in the f_2 dimension and 1K in the f_1 dimension with a shifted sine apodization.

Table S1. ^{13}C Chemical Shifts of AFB_1-FAPY Nucleoside Dibutyrate **13**[a]

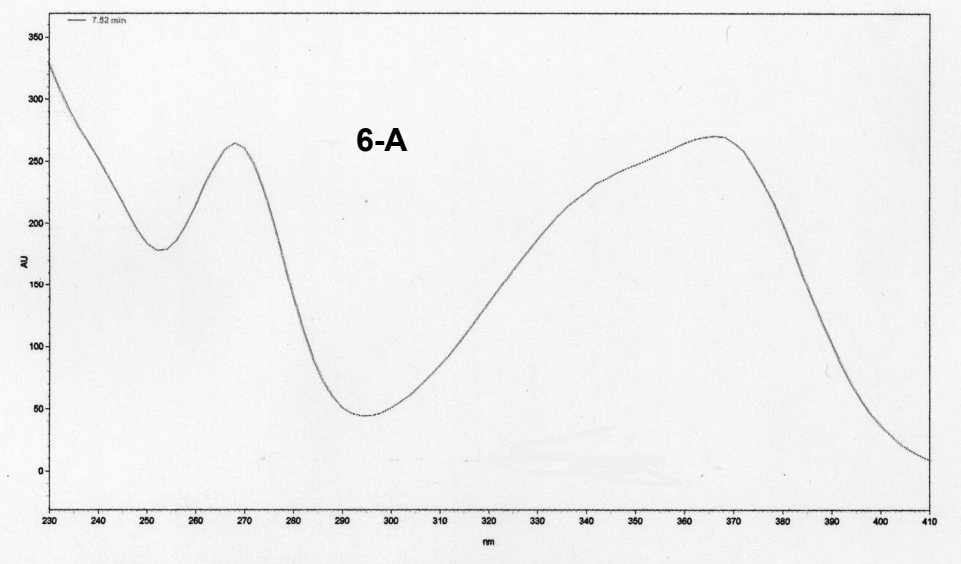
Carbon	FAPY Ia	FAPY Ib	FAPY IIa	FAPY IIb
		Aflatoxin		
C6a	114.23[b]			
C8	95.42	102.93	95.80	103.58
C5	92.13[b]			
C9	75.45	75.22	76.27	76.07
OMe	57.30[b]			
C9a	55.00[b]			
C2	35.94[b]			
C3	30.14[b]			
		Pyrimidine		
CHO	168.0	165.47	168.12	165.68
C5	92.08	89.31	92.27	89.62
		Deoxyribose		
C1'	82.60[b]			
C2'	37.33[b]			
C3'	76.80[b]			
C4'	82.70[b]			
C5'	64.56[b]			
		Butyrates		
CH_3	13.80[b]			
β-CH_2	19.11[b]			
α-CH_2	36.52[b]			

[a] Chemical shifts for ^{13}C signals were determined from HMQC or HMBC 2D spectra recorded in MeOH-d_4/DMSO-d_6. (9:1) mixtures at 290 K. Chemical shifts are in ppm relative to TMS. The chemical shifts are derived from the two-dimensional experiments rather than from a 1-D spectrum. Consequently, their accuracy is subject to data point limitations and signals separated by only a few Hz are not resolved from one another. [b] Only one signal seen for the four FAPY species.

Table S2. ^{1}H and ^{13}C Chemical Shifts of formyl isomers of FAPY Nucleoside **15**[a,b]

Proton	FAPY Aa major	FAPY Ab	Carbon	FAPY Aa major	FAPY Ab minor
			Aflatoxin		
H5	6.39	6.35	C5	91[c]	
H6a	6.45	6.45	C6a	112.9	112.6
OH[e]	6.08	6.07			
H8	6.27		C8	94.6	102.1
H9	4.92	5.08	C9	74.0	73.9
OMe	3.93	3.93	OMe	56.7	
H9a					
			Pyrimidine		
NH1	9.84[e]	9.64[e]			
formyl	7.57	8.33	C5	90.2[d]	87.7[d]
NH2	6.6[e]	6.6[e]			
NH4	6.49[e]	6.52[e]			
H1'	5.12[e]	5.08[e]	C1'	74.6[c]	
H2'	1.89[c]		C2'	33.6[c]	
H3'	3.82[c]		C3'	67.1[c]	
OH3'	4.79[e]	4.72[e]			
H4'	3.43[c]		C4'	66.6[c]	
OH4'	4.53[c,e]				
H5'	3.18[c]		C5'	47.7[c,d]	
H5"	3.53[d]	3.49[d]			

[a] Spectra were recorded in 4:1 mixtures of DMSO-d_6/MeOH-d_4. Chemical shifts are in ppm relative to TMS. [b]Unless otherwise specified, values for proton and carbon chemical shifts were determined from HMQC 2D spectra. [c]Only one signal was seen for both FAPY species. [d]Values determined from HMBC 2D spectra. [e]Values obtained from spectra recorded in DMSO-d_6. AFB H2 and H3 are not tabulated.



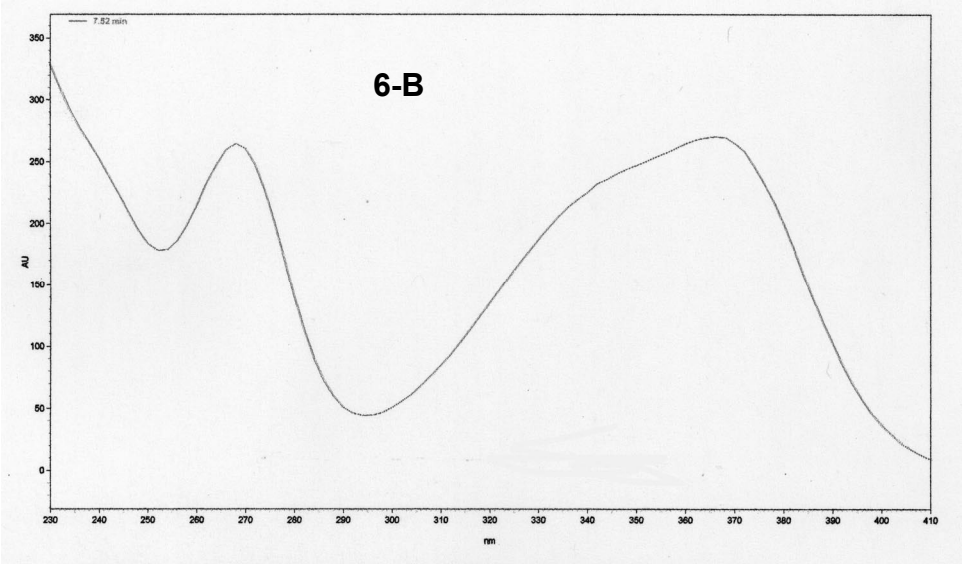


Figure S1. UV spectra (MeOH) of AFB FAPY bases **6-A** (top) and **6-B** (bottom).

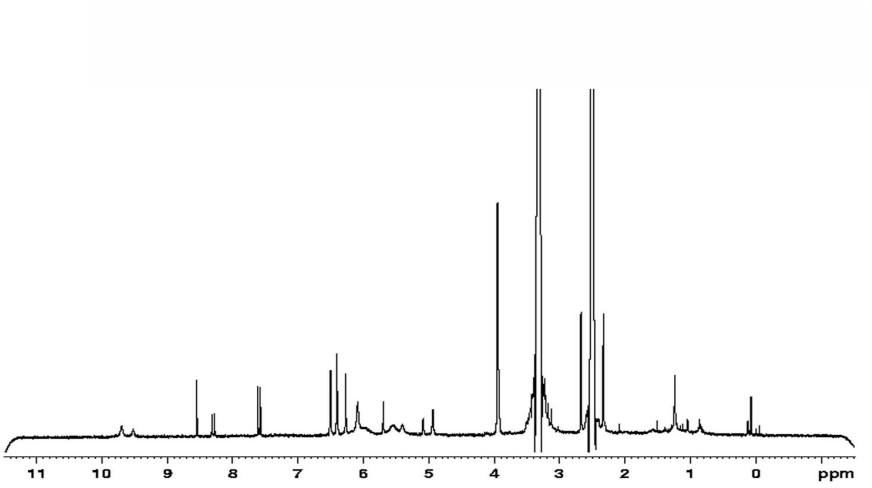


Figure S2. ^{1}H NMR (DMSO-d_6) of FAPY base **6-A.**

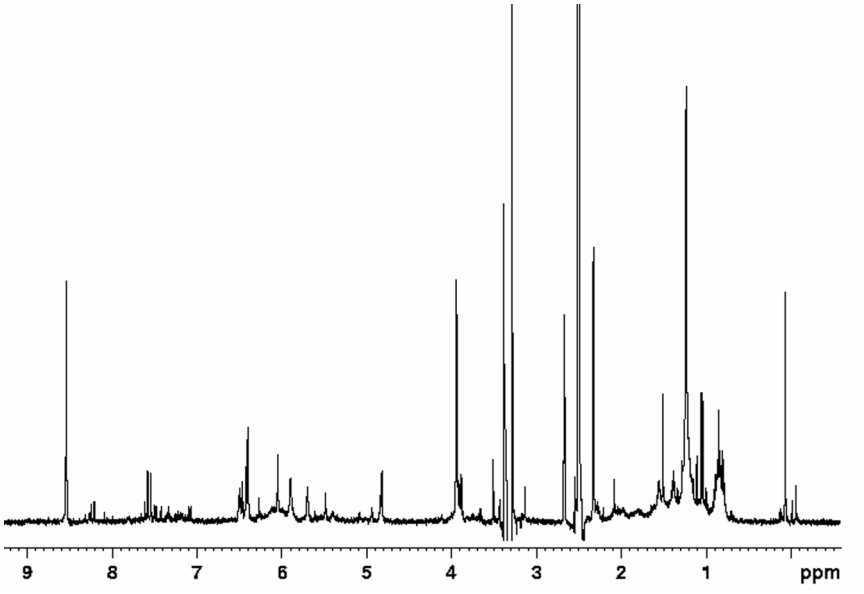


Figure S3. ^{1}H NMR (DMSO-d_6) of FAPY base **6-B.**

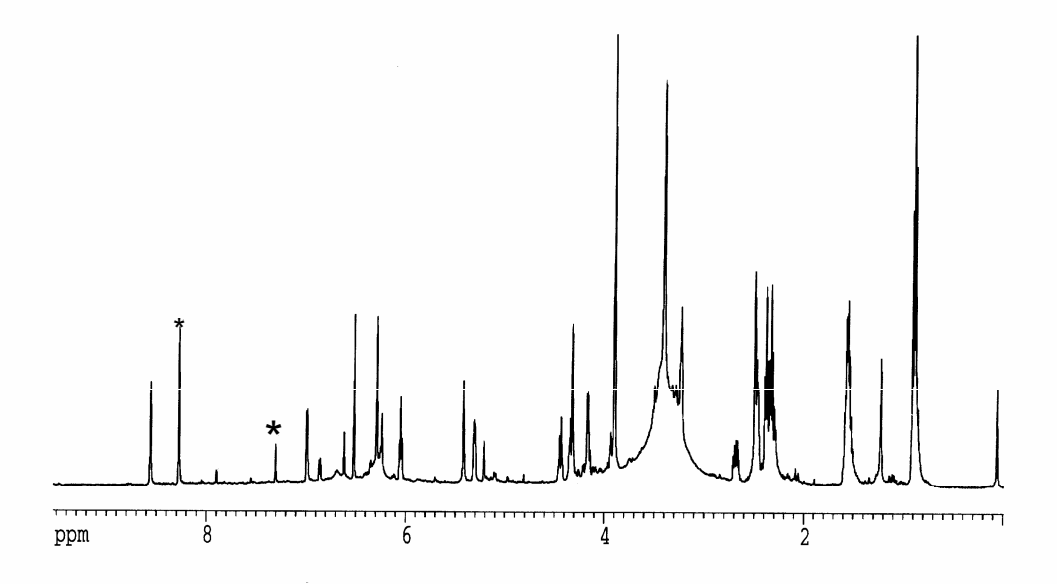


Figure S4. ^{1}H NMR (DMSO-d_6) spectrum of AFB-dGuo N7 adduct **10**. Asterisks indicate impurities.

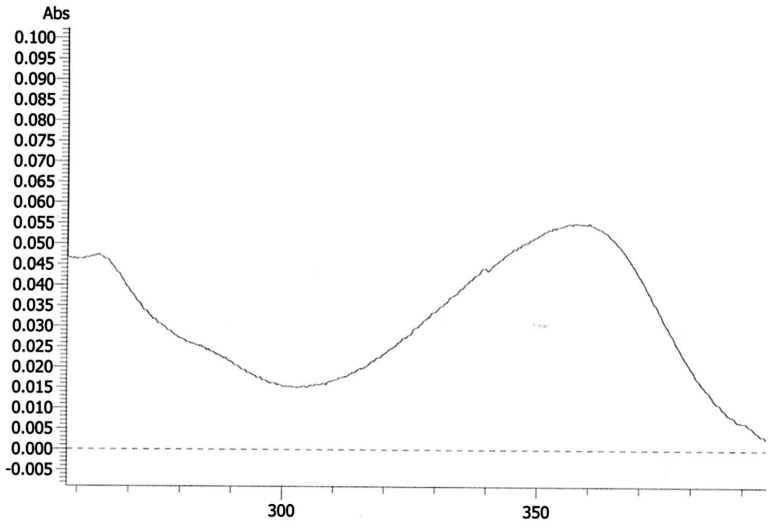


Figure S5. UV (MeOH) spectrum of AFB-dGuo N7 adduct **10**.

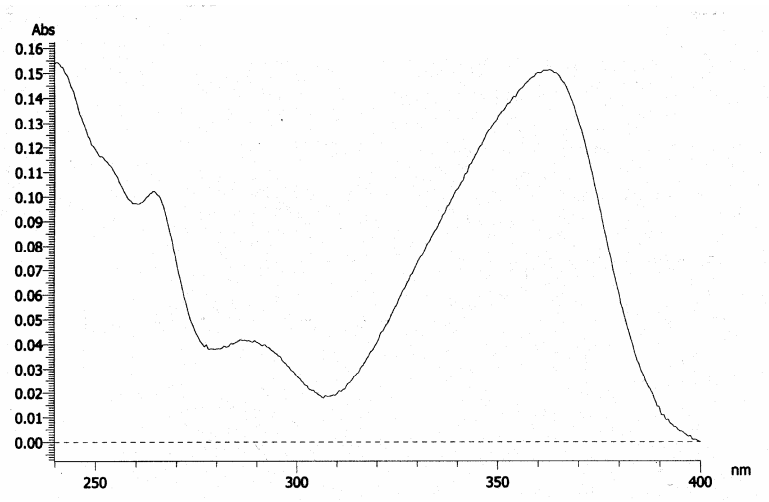


Figure S6. UV spectrum (MeOH) of AFB-O^6 dG 3',5' dibutyrate **11.**

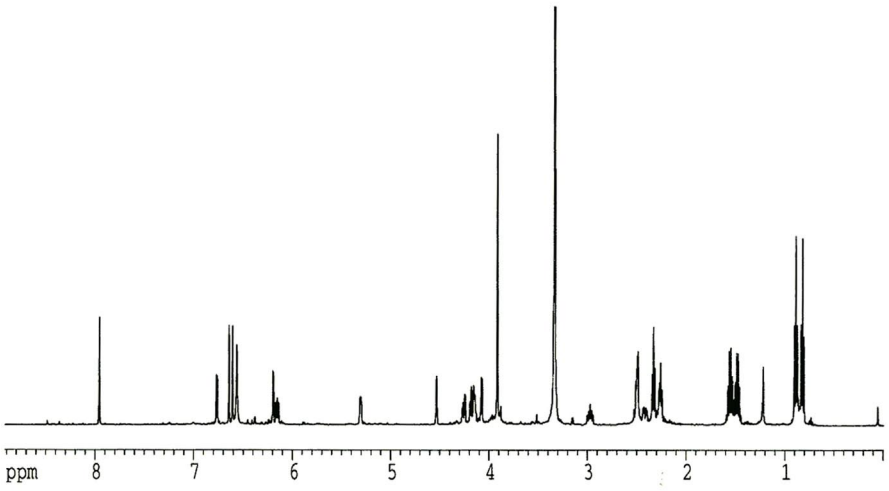
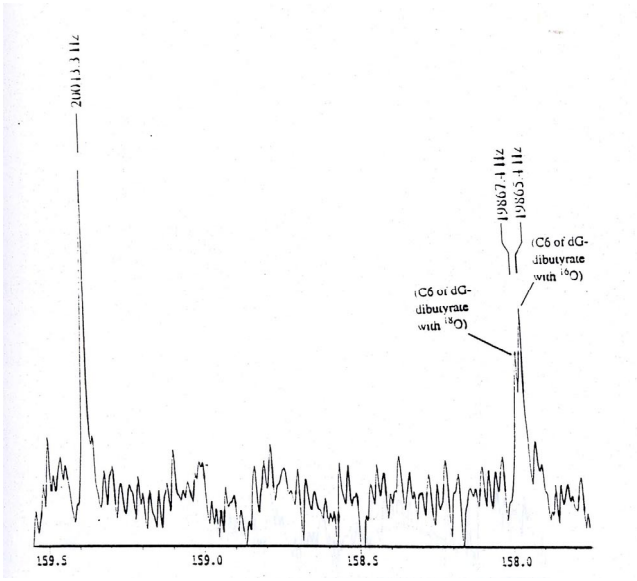


Figure S7. ^{1}H NMR spectrum (DMSO-d_6) of AFB-O^6 dG 3',5' dibutyrate **11.**



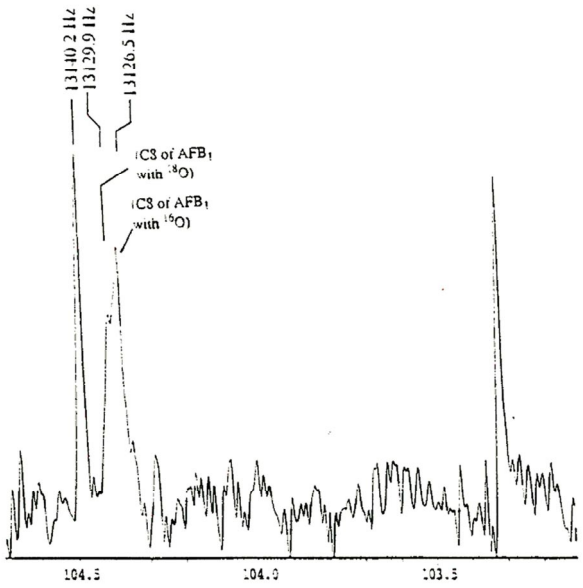


Figure S8. Comparison of the ^{13}C chemical shifts of aflatoxin C6 (upper spectrum) and C8 (lower spectrum) in AFB-O^6 dGuo 3', 5' dibutyrate (**11**) with 6-^{16}O and 6-^{18}O.

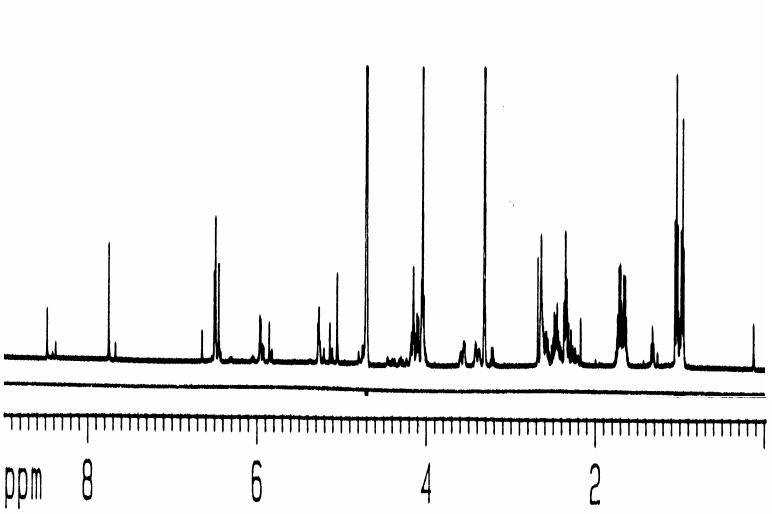


Figure S9. ^{1}H NMR (MeOH-d_4/DMSO-d_6) of AFB-FAPY dibutyrate **13-Aab** with minor amounts of **13-Bab.**

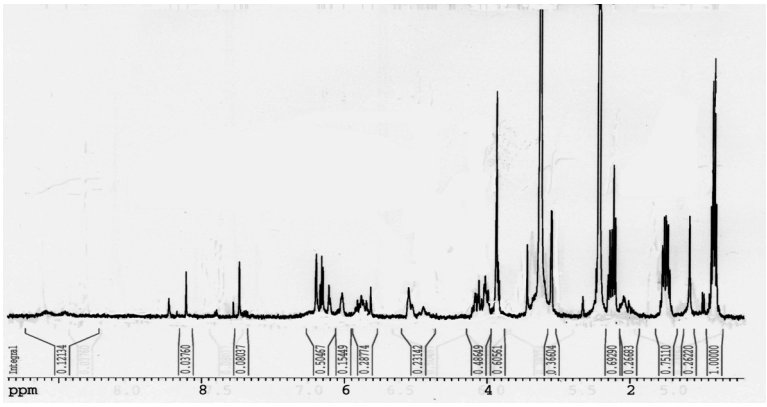


Figure S10. ^{1}H NMR ((MeOH-d_4/DMSO-d_6) of AFB-FAPY dibutyrate **13-Bab** with minor amounts of **13-Aab**

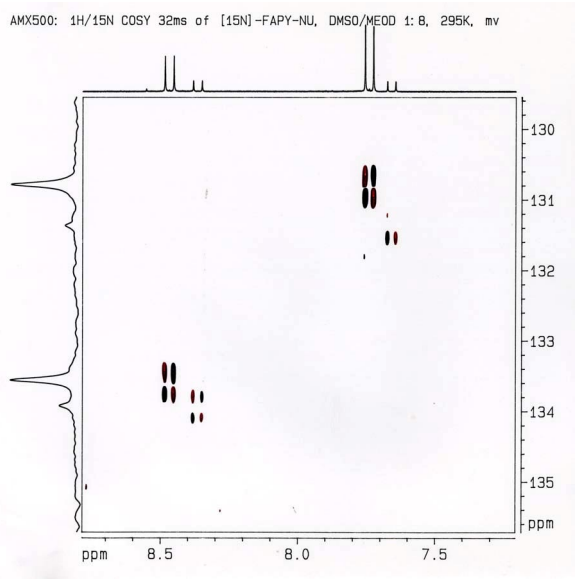


Figure S11. ^{1}H-^{15}N COSY of AFB-FAPY dG dibutyrates

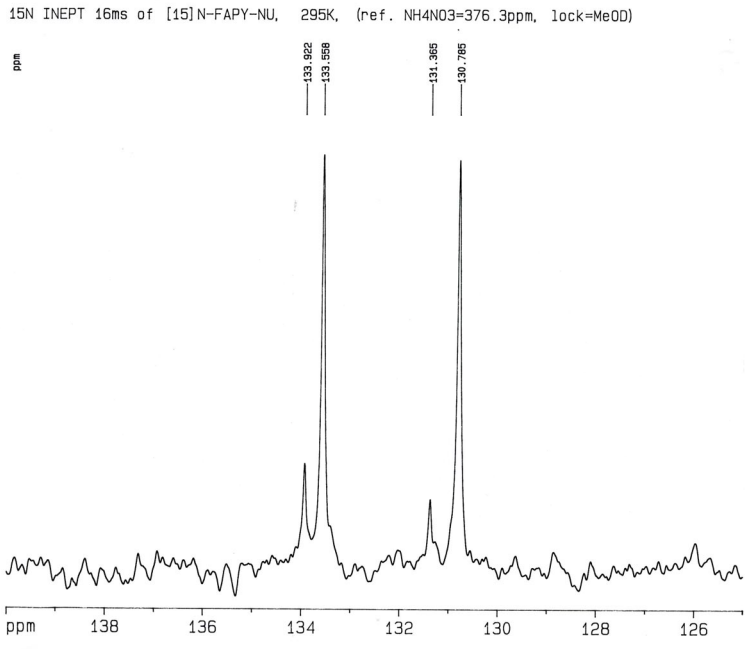
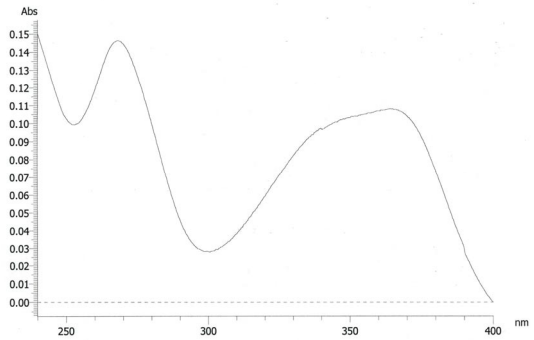


Figure S12. ^{15}N INEPT spectrum of AFB-FAPY dGuo dibutyrates **13**.



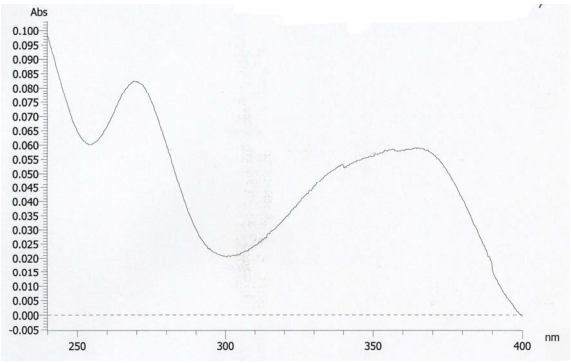


Fig. S13. UV spectra (MeOH) of AFB-FAPY dibutyrates **13-A** (top) **and 13-B** (bottom**).**

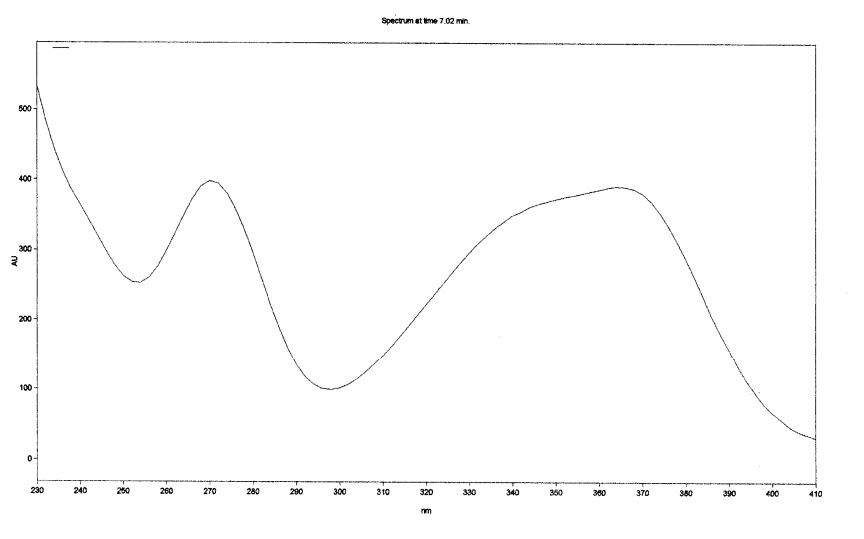


Fig. S14. UV spectrum of AFB-FAPY nucleoside **15**.

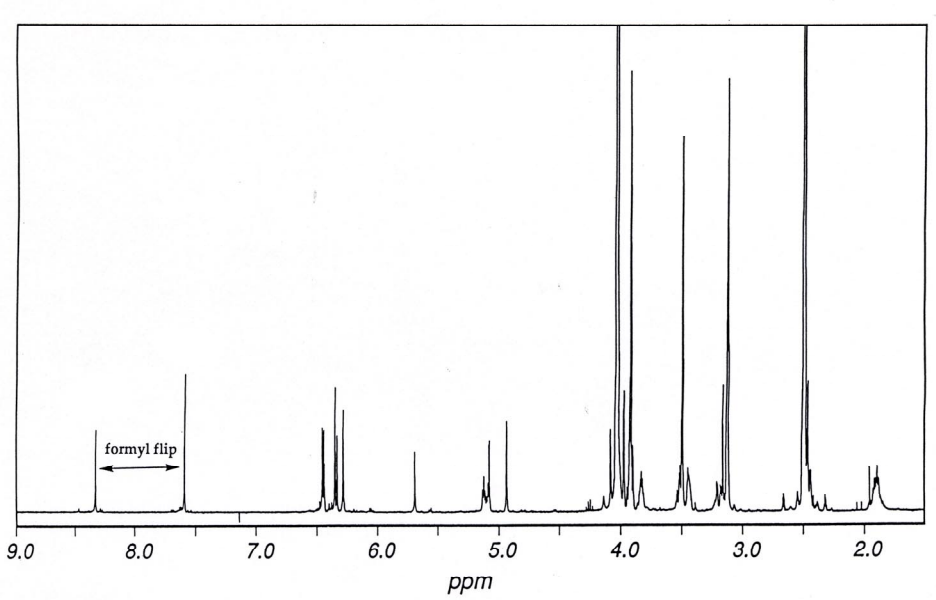


Fig. S15. ^{1}H NMR of deprotected AFB-FAPY nucleoside **15**.

References

1. Rance, M.; Sorensen, O. W.; Leupin, W.; Kogler, H.; Wuthrich, K.; Ernst, R. R. *J. Mag. Res.* **1985**, *61*, 67-80.

2. Bax, A.; Davis, D. G. *J. Mag. Res*. **1985**, *65*, 355-360.

3. Bax, A.; Davis, D. G. *J. Mag. Res.* **1985**, *63*, 207-213.

4. Bax, A.; Griffey, R. H.; Hawkins, B. L. *J. Mag. Res*. **1983**, *55*, 301-315.

5. Bax, A.; Subramanian, S. *J. Mag. Res.* **1986**, *67*, 565-569.

Full text of reference 53 in the article:

53. Cho, Y. J.; Kim, H. Y.; Huang, H.; Slutsky, A.; Minko, I. G.; Wang, H.; Nechev, L. V.; Kozekov, I. D.; Kozekova, A.; Tamura, P.; Jacob, J.; Voehler, M.; Harris, T. M.; Lloyd, R. S.; Rizzo, C. J.; Stone, M. P. *J. Am. Chem. Soc.* **2005,** *127*, 17686-17696.