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Spontaneous 2'-deoxyguanosine alkylation by new generation of Top I inhibitors of camptothecin family

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Electronic Supplementary Information

NMR experiments

500 MHz ^{1}H NMR spectra in H_2O on Varian NMR spectrometers were run using a 12 kHz spectral window, using WATERGATE [1] window sequence for water suppression for H_2O.
(1-2 ms soft pulses) and presaturation for samples in D_2O.
^{1}H/^{13}C-HSQCAD spectra [2] were acquired as the echo-antiecho phase sensitive ^{1}H-^{13}C HSQC (heteronuclear single quantum coherence, adiabatic version) with a relaxation delay of 1.2 s and ^{1}J(C,H) = 135 Hz.
^{1}H/^{13}C-HMBC The gradient selected ^{1}H - ^{13}C HMBC spectra were performed with acquisition time of 0.2 s, ^{1}H - 90° pulse width of 7.8 µs, ^{13}C - 90° pulse width of 11.5 µs, spectral width of 5000 Hz, 2048 data points in the ^{1}H dimension and 25000 Hz, 1024 increments in the ^{13}C dimension, relaxation delay of 1.2 s. The data were acquired as absolute value mode, with 64 transients per t_1 increment. The experiment was optimized for ^{n}J(C,H) = 8 Hz and low pass filter for ^{1}J(C,H) = 140 Hz was used. The data were linear predicted to 2048 points and zero filled to 4096 points in F_1 prior to Fourier transformation.

Pulsed Field Gradient Spin Echo (PFGSE) experiments were performed in H_2O according to the following conditions: 16 spectra were acquired using the BPPSTE[3, 4] (stimulated echo sequence

incorporating bipolar gradients) and DPFGDSTE (with convection compensation) sequences, respectively. The gradient strengths were incremented as a square dependence in the range from 1 to 50 G/cm. The diffusion time (Δ) and the duration of magnetic field gradients (δ) were 300 ms and 2 ms respectively. Other parameters include the following: a sweep width of 12 000 Hz for H_2O, 32K data points, 128 to 4096 transients, depending on sample concentration, and relaxation delay of 2s. The data were processed using Varian DOSY [5] and DECRA procedure [6, 7] from DOSYToolbox. v._0.54_15Mar08 package of M. Nillson.

Alkylation procedure in buffered water, pH 7

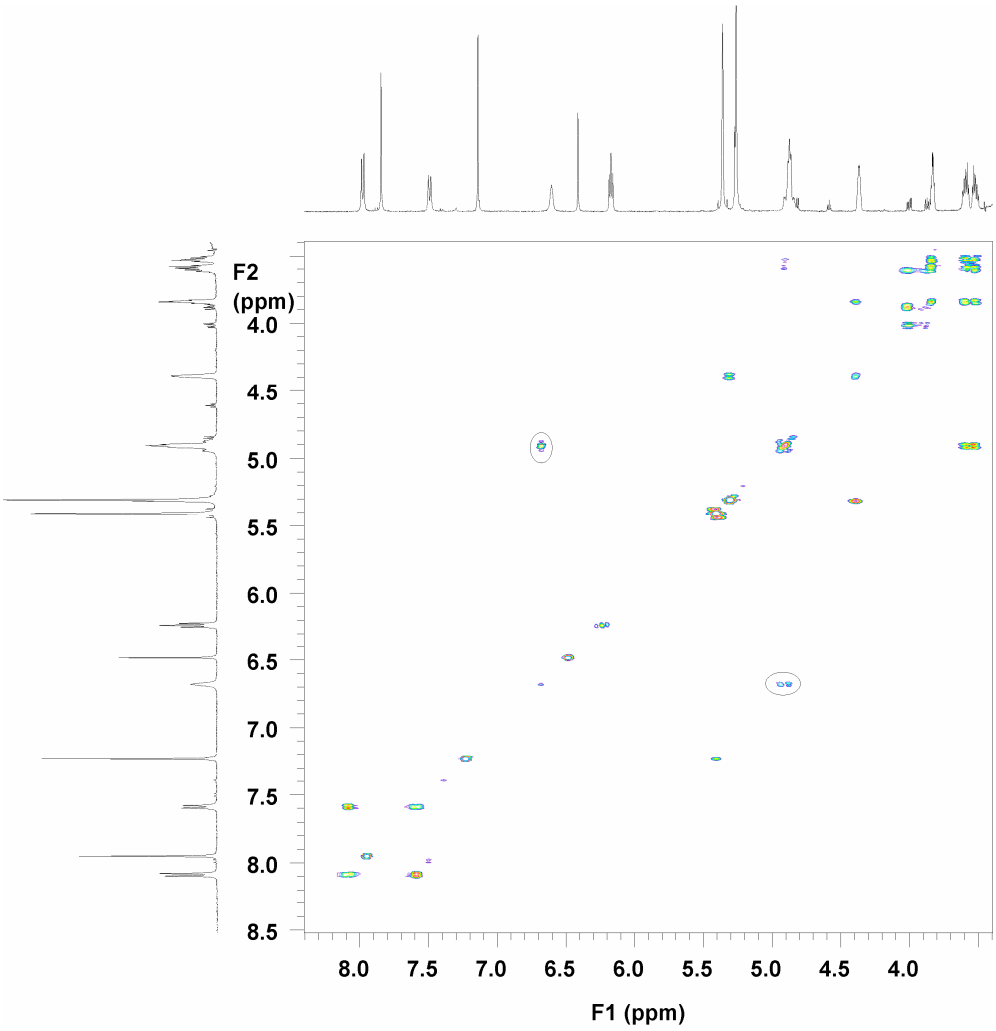

Fig.1S 1H/1H COSY spectrum of a reaction product between 2'-deoxy guanosine and 7-ethyl-9-(N-morpholino)methyl-10-hydroxycamptothecin in buffered water solution at pH 7. Marked crosspeaks define scalar connection between 2-NH_2 of 2'-deoxyguanosine and 24-CH_2- hydrogens of *o*-methylene methide

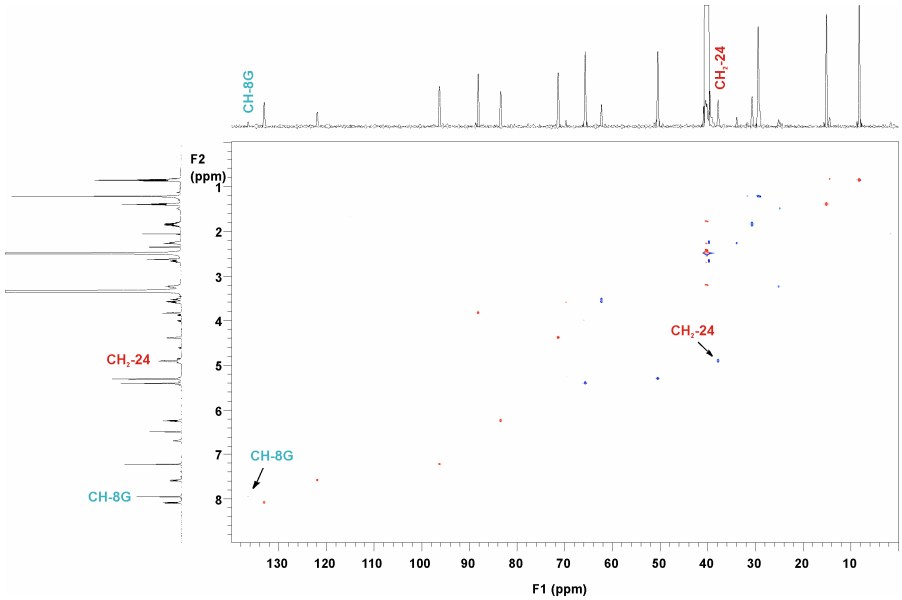

Fig. 2S 1H/13C HSQC spectrum of bioconjugate (see spectrum in Fig. 2) G stands for guanine atom

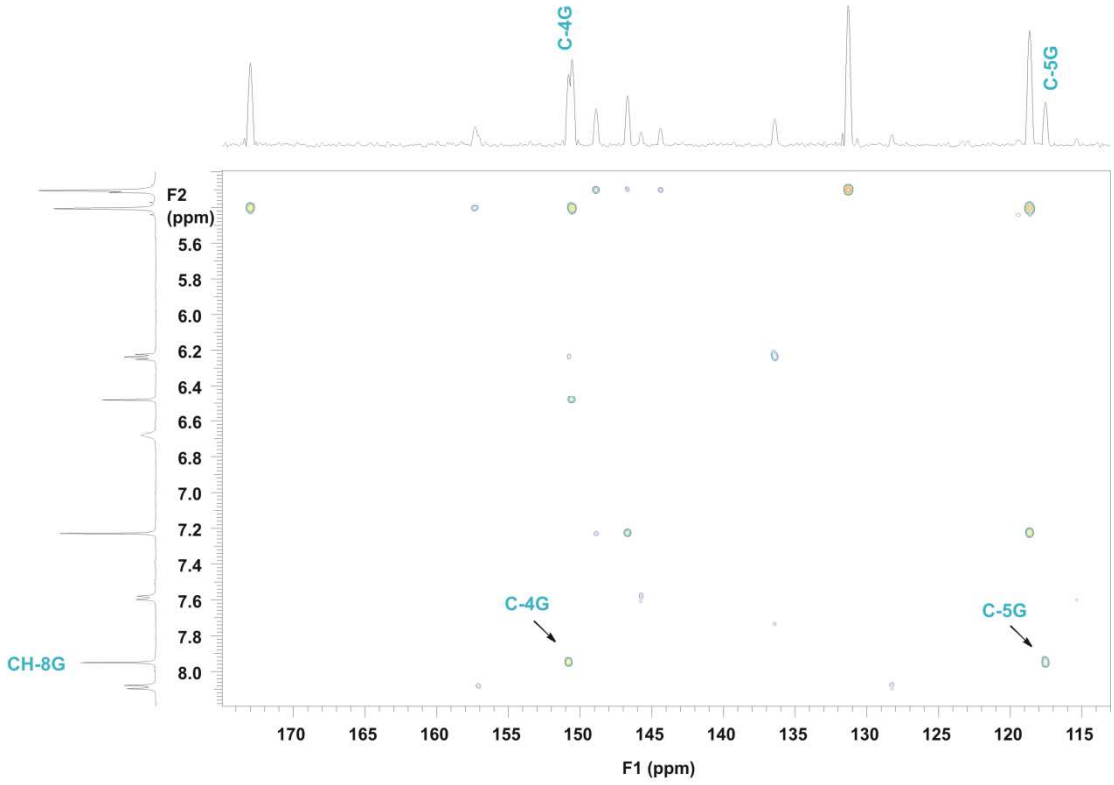

Fig. 3S 1H/13C HMBC spectrum of bioconjugate (see spectrum in Fig. 2)

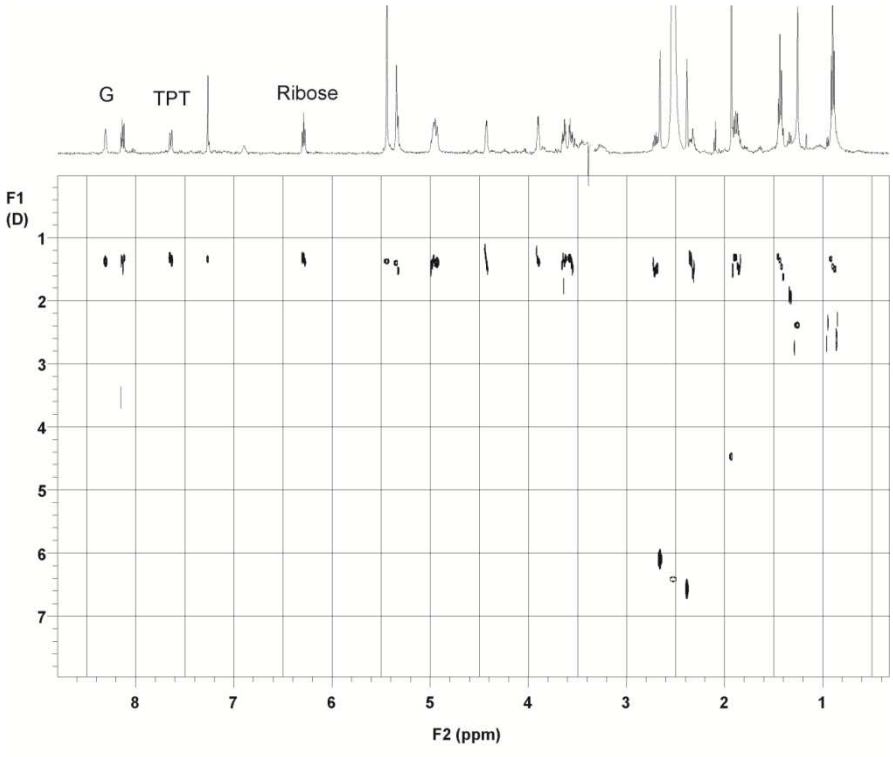

Fig 4S PFGSE spectrum of bioconjugate in DMSO-d6 solution. The signals of all 3 motifs of a structure have the same diffusion coefficient which evidences their covalent binding.

Alkylation procedure in neat water, pH 6

A solution of 2'-deoxyguanosine hydrate (1.07 mg, 3.76 x 10^{-3} mmol) in water (3.76 ml, D_2O) at pH 6 was added to a 7-ethyl-9-(N-morpholino)methyl-10-hydroxycamptothecin hydrochloride (3.97 mg, 7,14x10^{-3}mmol). The final reaction solution contained 1.0 mM dG and 2.0 mM SN38 derivative (adjusted to pH=6 using 5 % NaOD). The progress of reaction was monitored by ^{1}H NMR and LC-MS techniques. The reaction was carried out at room temperature and finished after 24 hours. The reaction mixture was filtered and solid was purified using HPLC on an RP-C18 LPH column (150 mm x 10 mm) using as a mobile phase 10 mM CH_3COONH_4 aqueous solution pH=5.4 (A) and MeCN (B), in the following gradient: 0-30 min 10 → 35% B, 30 min 35% B. Flow rate of the mobile phase: 2.8 ml/min. The course of the chromatography was monitored using UV detection at a wavelength of 260 nm. Product of reaction N2-adduct was collected and was lyophilized.(Yield 6.8 %).

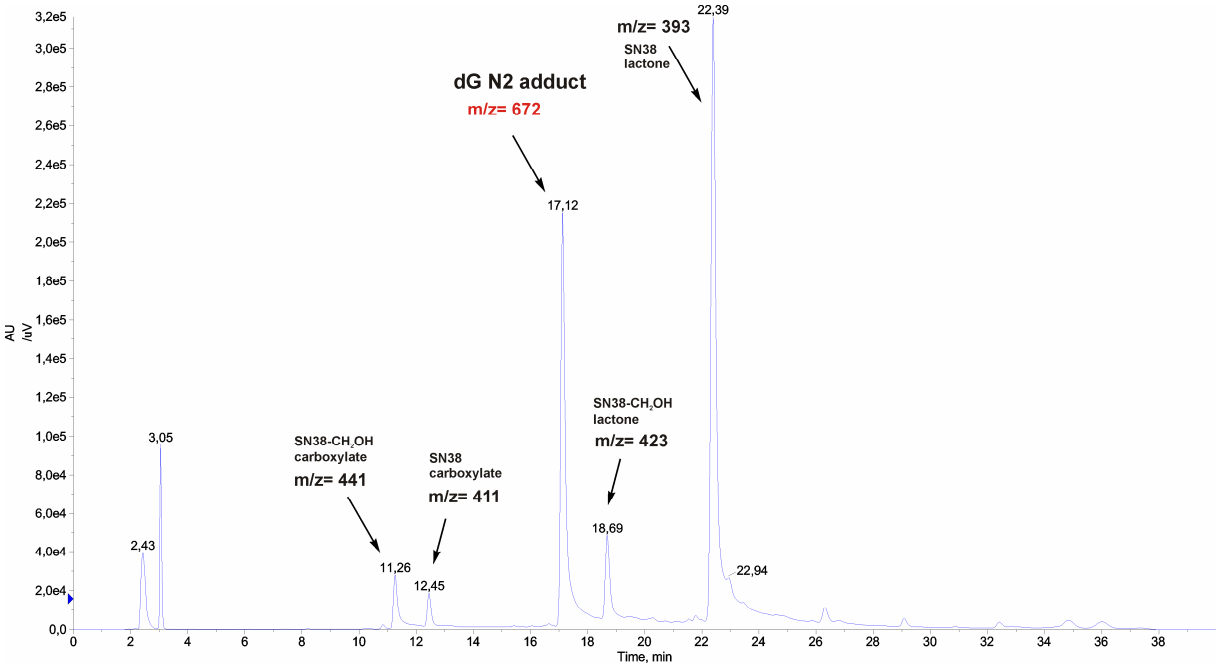

Fig. **5S** LC-MS run of a precipitated solid from the reaction between 2'-deoxyguanosine and 7-ethyl-9-(N-morpholino)methyl-10-hydroxycamptothecin in neat water solution at pH 6. Retention times and m/z (M+1) values are given for each peak.

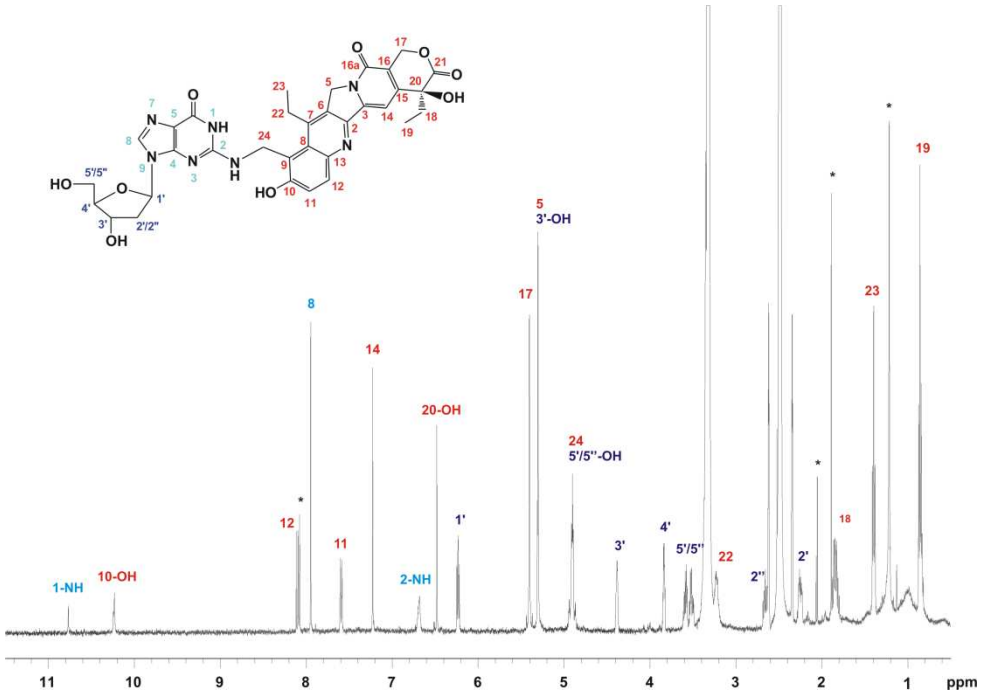

Fig. **6S** 1H NMR run in DMSO-d_6 of a precipitated solid from the reaction between 2'-deoxyguanosine and 7-ethyl-9-(N-morpholino)methyl-10-hydroxycamptothecin in neat water solution at pH 6. MS spectrum shows only lactone form.

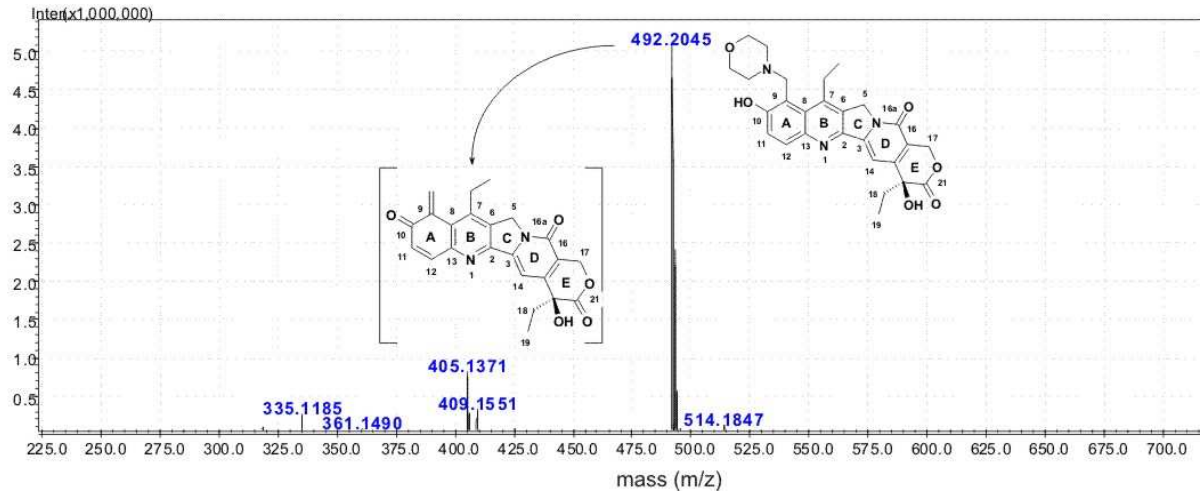

Fig. 7S MS spectrum of a title compound

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