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Supplementary Information

Solvent- dependent regioselectivity of 2'-deoxyadenosine alkylation by 9-aminomethyl derivative of the SN38

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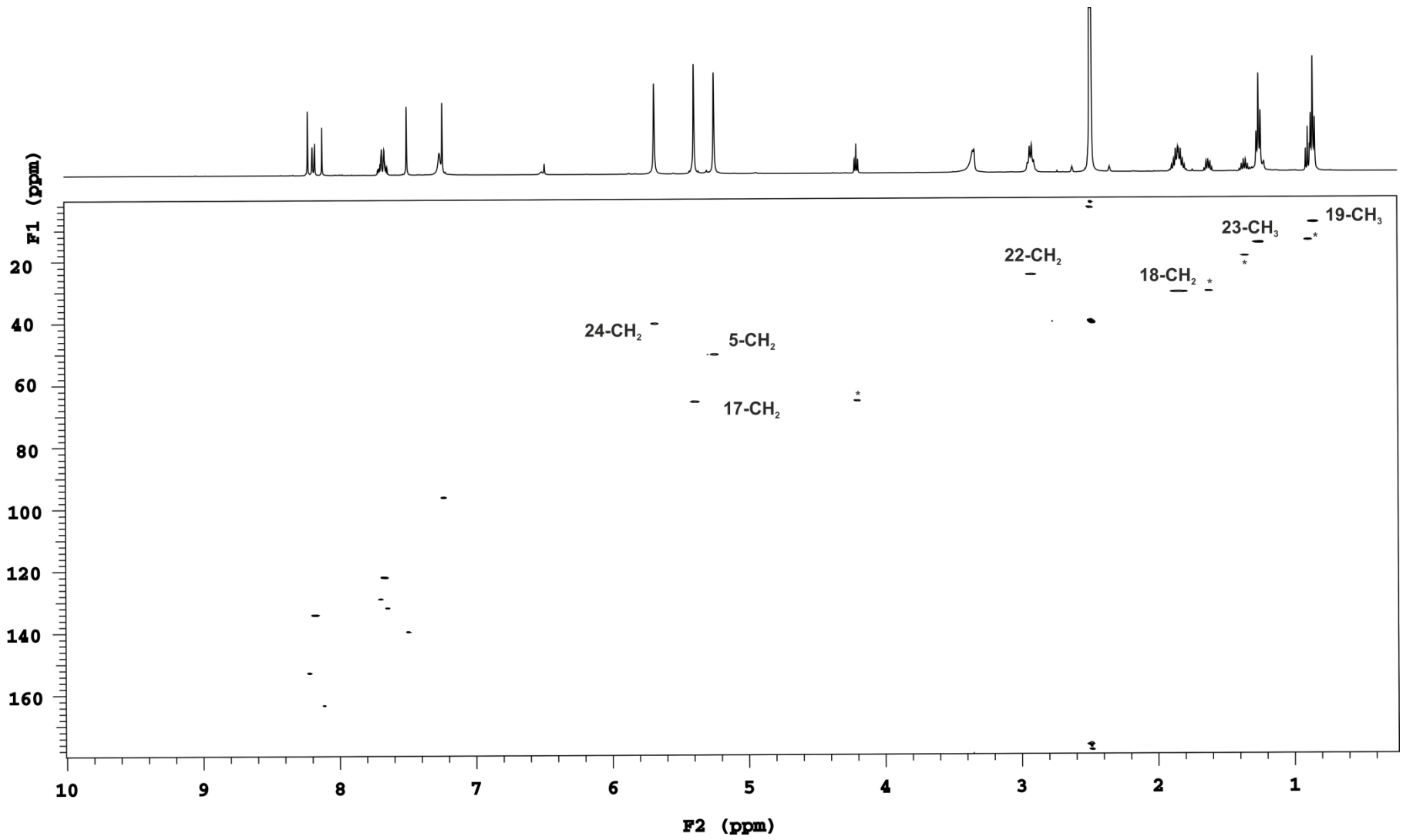

Fig. S1 HSQC spectrum of N9 dA adduct in DMSO-d_6 solution *-means impurities

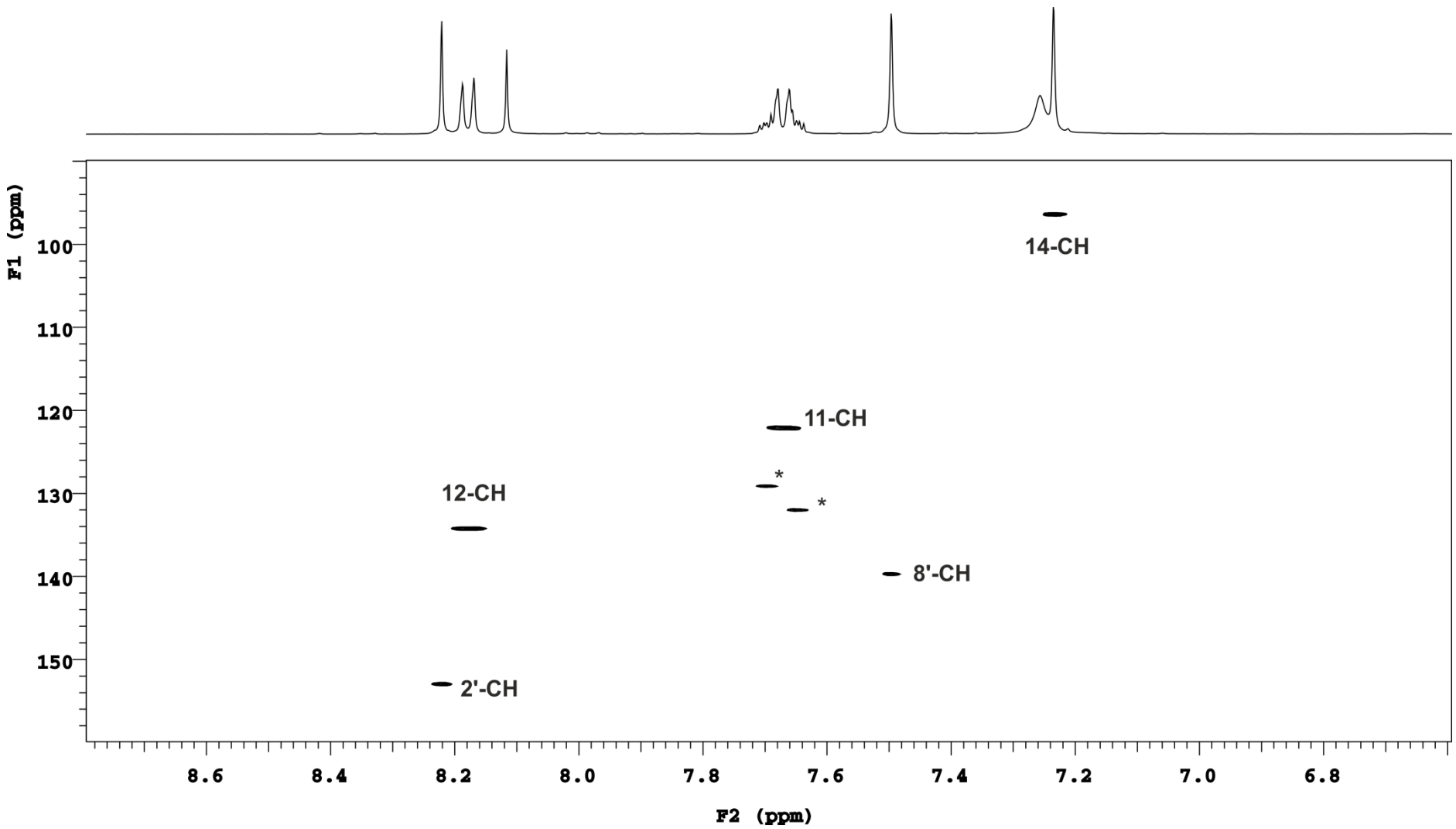

Fig. S2 Partial HSQC spectrum of N9 dA adduct in DMSO-d_6 solution *-means impurities

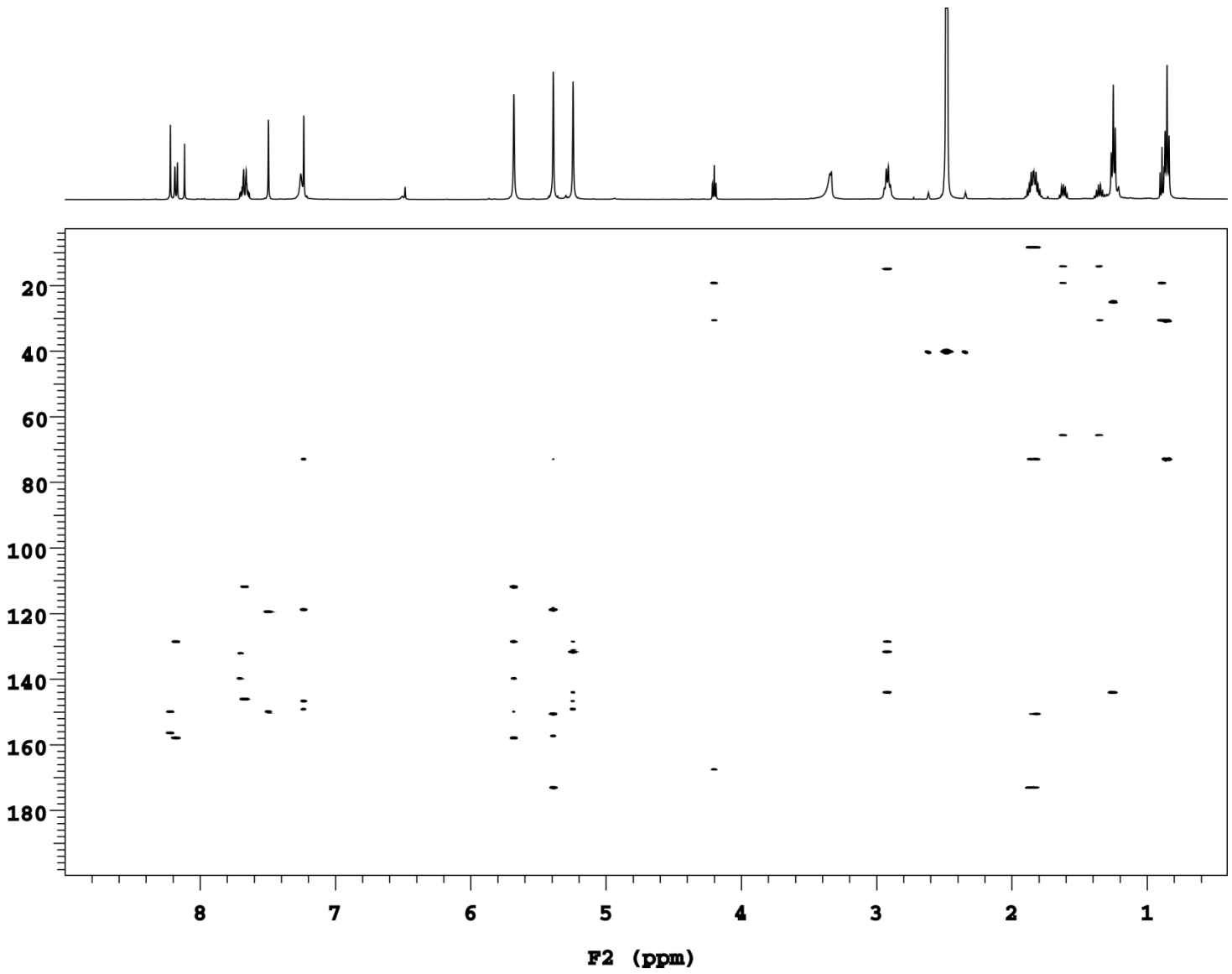

Fig. S3 HMBC spectrum of N9 dA adduct in DMSO-d_6 solution

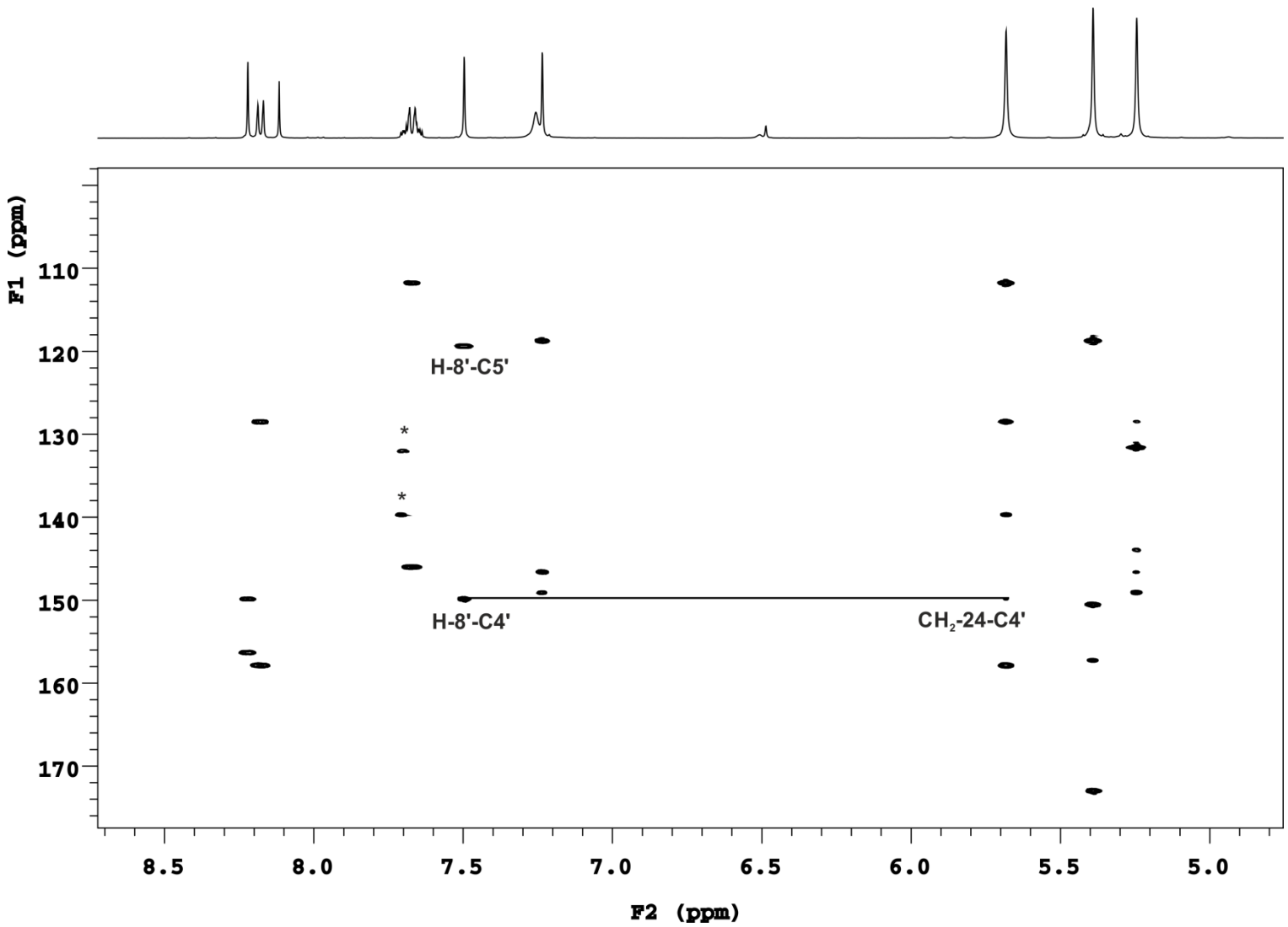

Fig. S4 Partial HMBC spectrum of N9 dA adduct in DMSO-d_6 solution *-means impurities

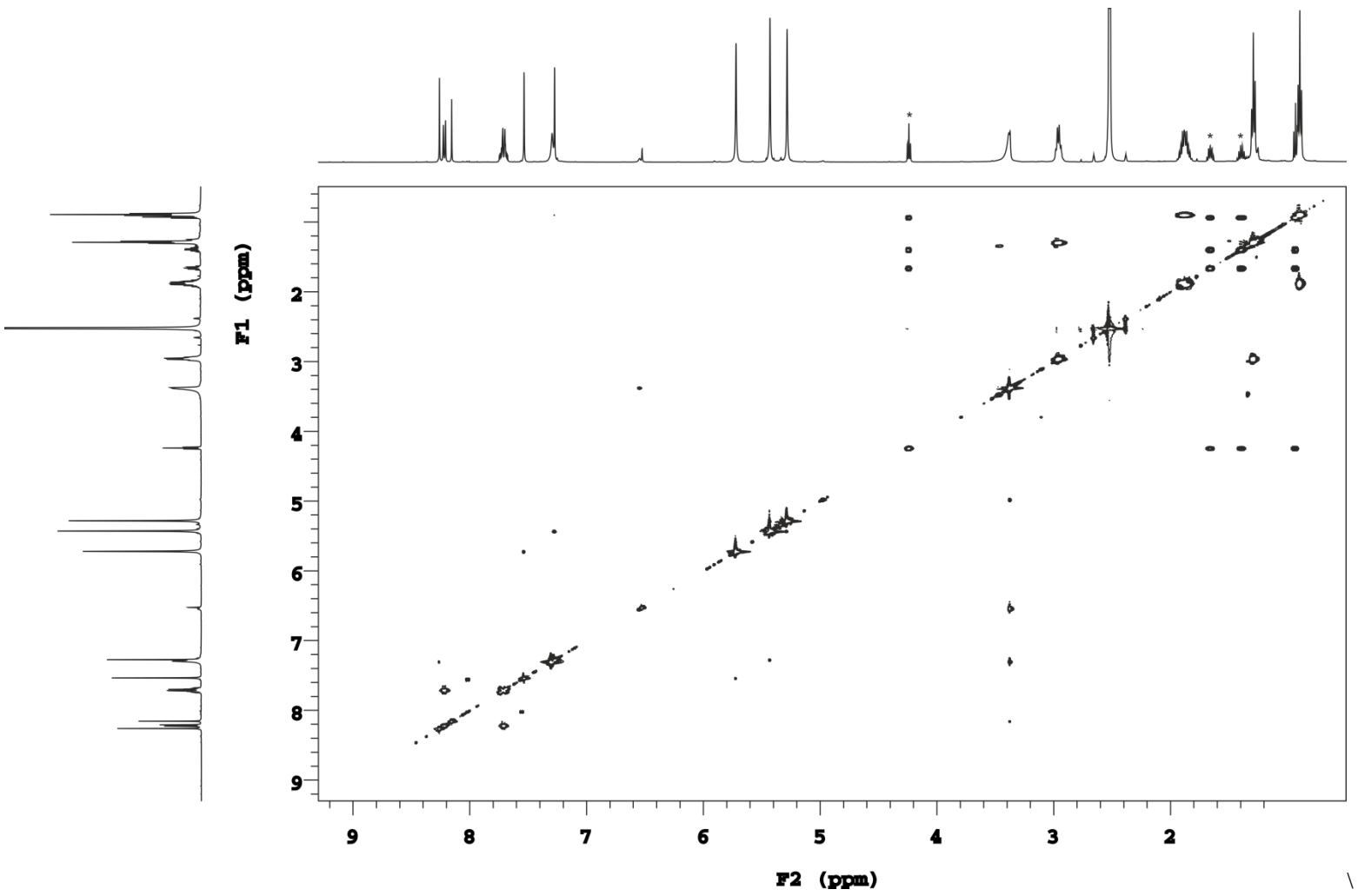

Fig. S5 2D TOCSY spectrum of N9 dA adduct in DMSO-d_6 solution *-means impurities

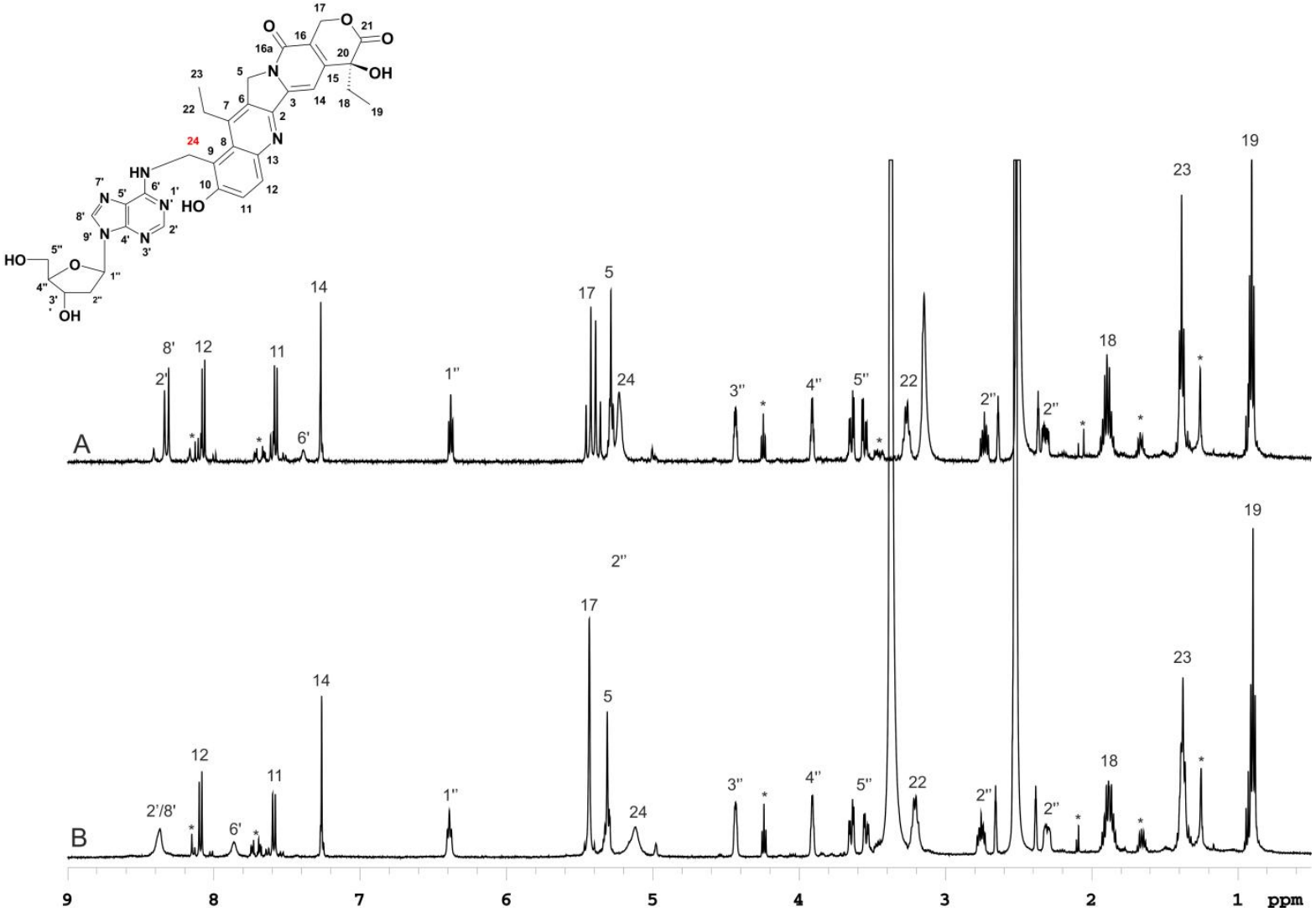

Fig. S6 Full ^{1}H NMR spectra of N6 dA adduct in DMSO-d_6 : A- at 70 ^{o}C; B- at 25°C. *-means impurities

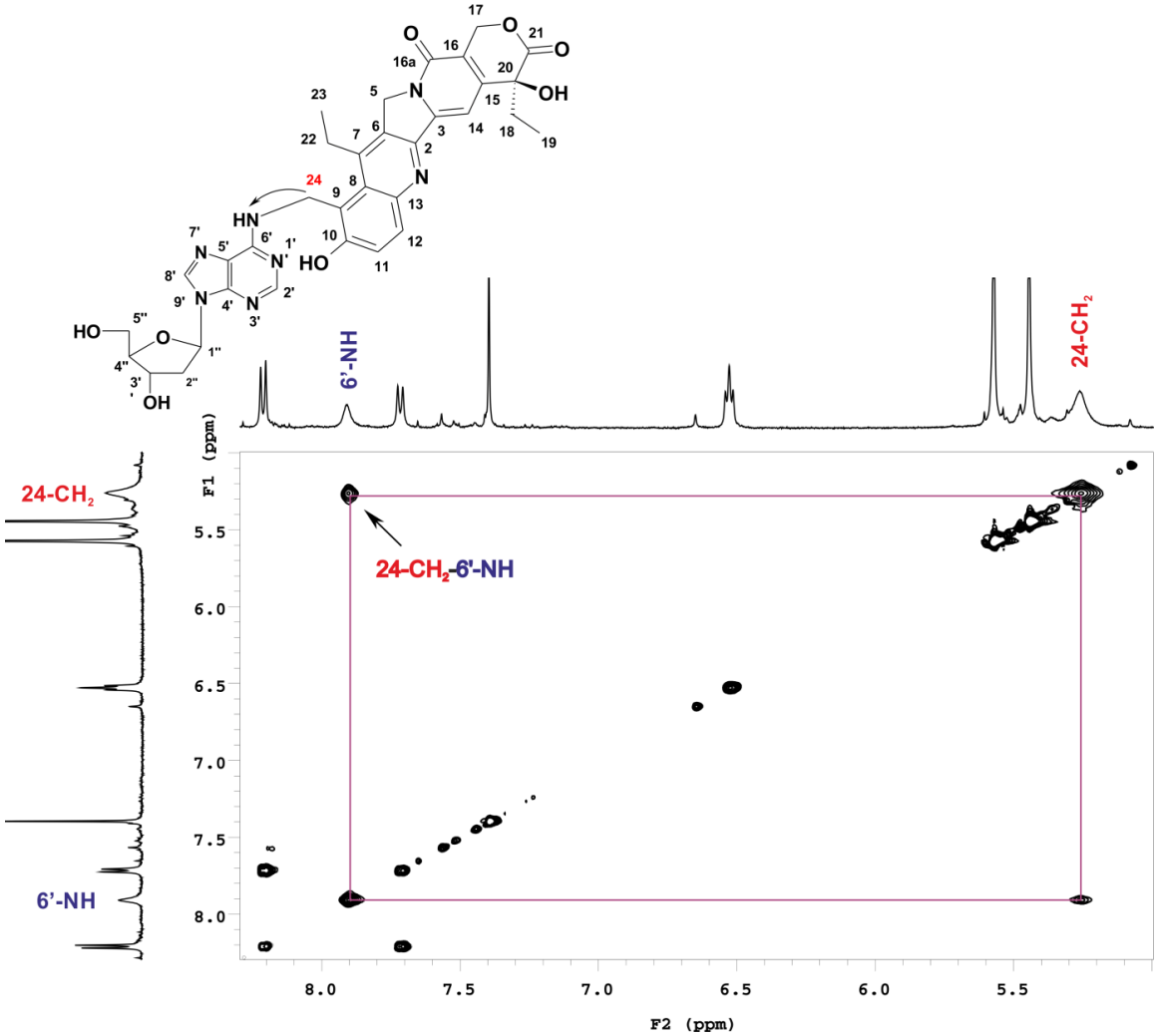

Fig. S7 2D TOCSY of N6'-dA adduct in DMSO-d_6 solution at 25°C. The correlation $24CH_2$-6'-NH cross-peak is marked on both sides of the diagonal

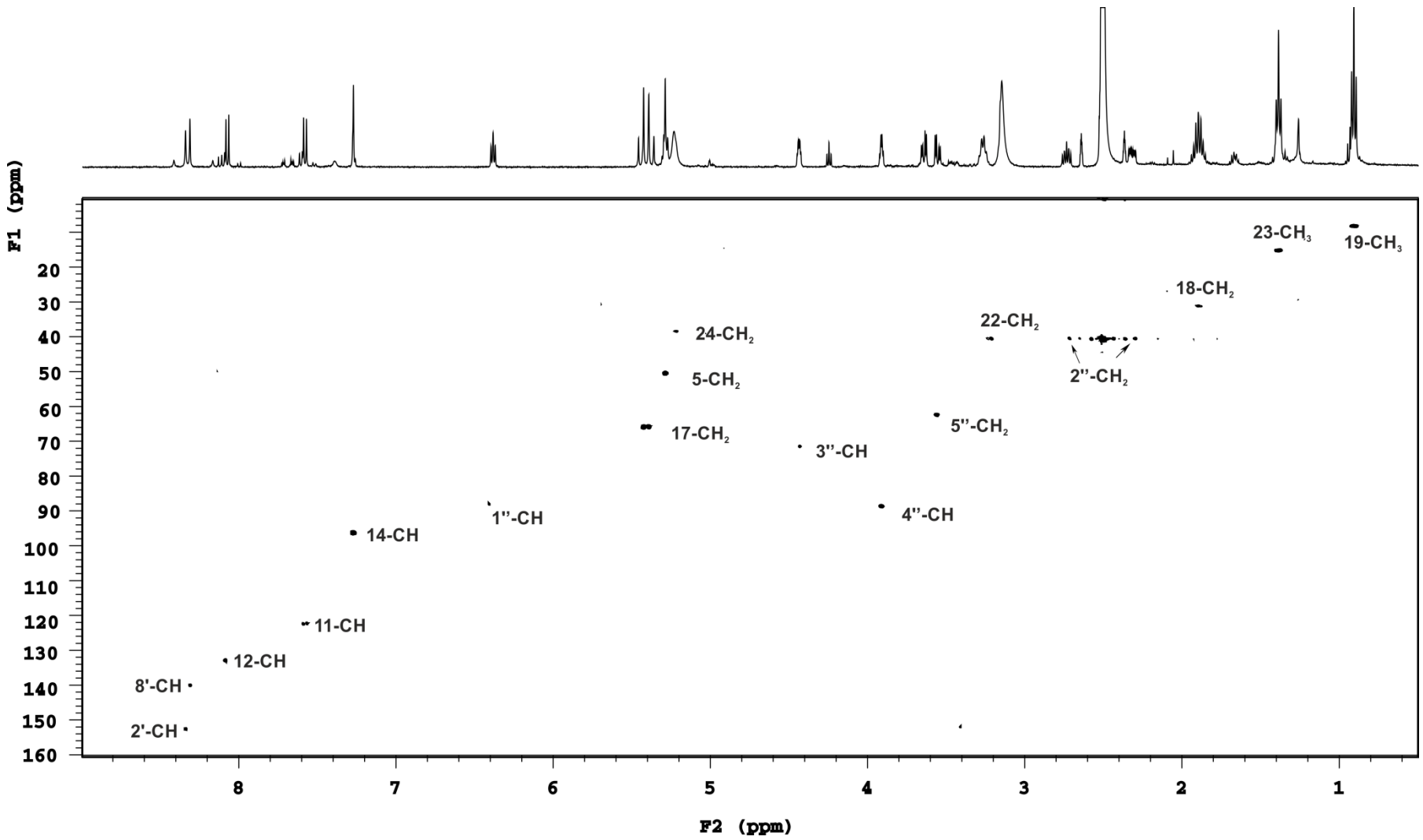

Fig. S8 2D HSQC of N6'-dA adduct in DMSO-d_6 solution at 70°C.

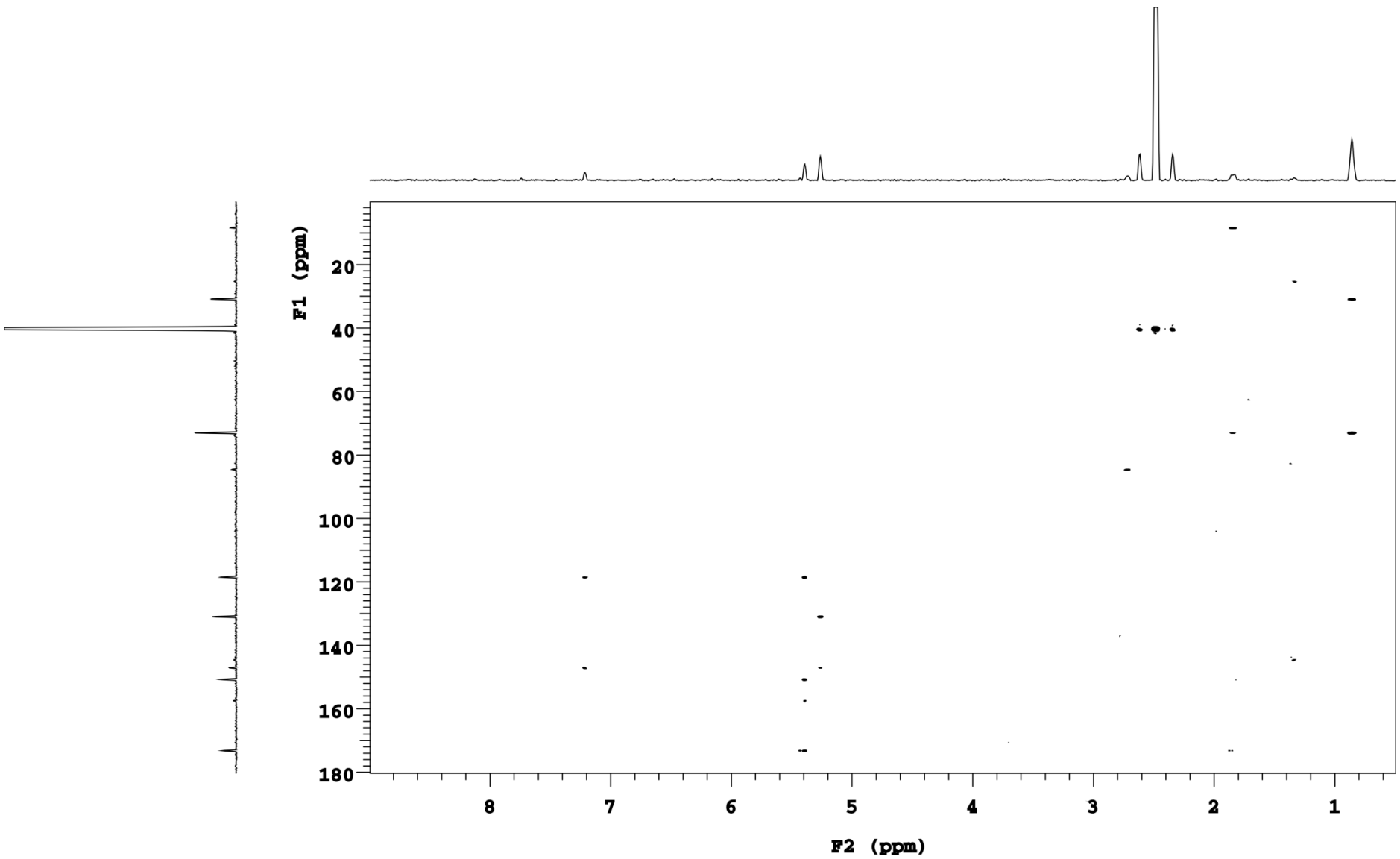

Fig. S9 2D HMBC of N6'-dA adduct in DMSO-d_6 solution at 25 ^{o}C.

EXPERIMENTAL

2'-Deoxyadenosine hydrate was purchased from Aldrich and used without further purification (purity grade $\geq$ 99%). HPLC-grade solvents were used in the chromatography experiments.

Synthesis of the 2'-deoxyadenosine adduct with 7-ethyl-9-(N-morpholino)methyl-10-hydroxycamptothecin hydrochloride in DMSO

A solution of 2'-deoxyadenosine hydrate (1.29 mg, 4. 8 x 10^{-3} mmol) in DMSO-d_6 (300 μl) was added to a solution of 7-ethyl-9-(*N*-morpholino)methyl-10-hydroxycamptothecin hydrochloride (2.53 mg, 4. 8 x10^{-3}mmol) in DMSO-d_6 (300 μl). The final reaction solution contained 8 mM both dA and SN38 derivative (pH=4.75). The progress of reaction was monitored by ^{1}H NMR and LC-MS technique. The reaction was carried out at room temperature and finished after 72 hours. The reaction mixture was purified using HPLC on an RP-C18 LPH column (150 mm x 10 mm) using as a mobile phase 0.1% HCOOH aqueous solution (A) and MeCN (B), in the following gradient: 0-15 min 5 → 50% B, 15 min 50% B. Flow rate of the mobile phase: 3 ml/min. The course of the chromatography was monitored using UV detection at a wavelength of 260 nm and 365 nm. Product of reaction N9-adduct was collected and was lyophilized. (Yield 11 %).

^{1}H NMR δ (DMSO-*d_6*) 500 MHz: : 0.85 (t, J=7.3 Hz, 3H), 1.26 (t, J=7.8 Hz, 3H), 1.84 (m, 2H), 2.93 (q, J=7.8 Hz, 2H), 5.25 (s, 2H), 5.39 (s, 2H), 5.70 (s, 2H), 7.24 (s, 1H), 7.59 (bs, 1H) 7.64 (d, J=9.1 Hz ,1H), 8.18 (d, J=9.1 Hz, 1H), 8.27 (s, 1H).**^{13}C NMR** δ (DMSO-*d_6)* 125 MHz; HSQC/ HMBC : 8.2, 14.8, 25.1, 30.8, 40.7, 50.5, 65.8, 73.0, 96.4, 111.7, 118.9, 119.6, 122.1, 128.6, 131.6, 134.4, 140.3, 144.0 , 146.0, 146.7, 149.2, 149.9, 150.6 , 152.9, 156.3, 158.0, 157.3, 173.0. UV (in DMSO) nm: 272, 297, 337, 371, 389. **IR** (in DMSO, BaF_2, d=96,8μm) cm^{-1}: 1219, 1461, 2542, 2910, 3346. **HR-MS** (ESI): calculated mass for $C_{28}H_{26}N_7O_5[M+H]^+$: 540.1995, measured 540.1988.

Synthesis of the 2'-deoxyadenosine adduct with 7-ethyl-9-(N-methylo)methyl-10-hydroxycamptothecin hydrochloride in DMSO

A solution of 2'-deoxyadenosine hydrate (2.67 mg, 9.9 x 10^{-3} mmol) in DMSO-d_6 (300 μl) was added to a solution of 7-ethyl-9-(*N*-methylo)methyl-10-hydroxycamptothecin hydrochloride (8.8 mg, 4. 8 x10^{-3}mmol) in DMSO-d_6 (300 μl). The final reaction solution contained 16.5 mM of dA and 14.7 mM of SN38 derivative. The progress of reaction was monitored by ^{1}H NMR.. The reaction was carried out at room temperature and finished after 70 hours. The reaction mixture was purified using HPLC on an RP-C18 LPH column (150 mm x 10 mm) using as a mobile phase 0.1% HCOOH

aqueous solution (A) and MeCN (B), in the following gradient: 0-15 min 5 → 50% B, 15 min 50% B. Flow rate of the mobile phase: 3 ml/min. The course of the chromatography was monitored using UV detection at a wavelength of 260 nm and 365 nm. Product of reaction N9-adduct was collected and was lyophilized.(Yield 12 %).

***Synthesis of the 2'-deoxyadenosine adduct with 7-ethyl-9-(N-morpholino)methyl-10-hydroxycamptothecin hydrochloride* in water buffer at pH=7**

To a solution of 2'-deoxyadenosine hydrate (0.97 mg, 3. 6 x 10^{-3} mmol) in buffered water (25mM NaCl/25mM K_3PO_4 ,650 µl D_2O) at pH=7 was added 7-ethyl-9-(*N*-morpholino)methyl-10-hydroxycamptothecin hydrochloride (3.12 mg, 6. 6 x10^{-3}mmol). The final reaction solution contained 5.5 mM of dA and 10mM of SN38 derivative (pH=7,11). The progress of reaction was monitored by ^{1}H NMR. The reaction was carried out at room temperature and finished after 52 hours. The reaction mixture was purified using HPLC on an RP-C18 LPH column (150 mm x 10 mm) using as a mobile phase 0.1% HCOOH aqueous solution (A) and MeCN (B), in the following gradient: 0-15 min 5 → 50% B, 15 min 50% B. Flow rate of the mobile phase: 3 ml/min. The course of the chromatography was monitored using UV detection at a wavelength of 260 nm and 365 nm. Product of reaction N6-adduct was collected and was lyophilized.(Yield 13 %).

***Synthesis of the 2'-deoxyadenosine adduct with 7-ethyl-9-(N-methylo)methyl-10-hydroxycamptothecin hydrochloride* in water buffer at pH=7**

To a solution of 2'-deoxyadenosine hydrate (3.00 mg, 0.011 mmol) in buffered water (25mM NaCl/25mM K_3PO_4 ,2 ml D_2O) at pH=7 was added 7-ethyl-9-(*N*-methylo)methyl-10-hydroxycamptothecin hydrochloride (5.30 mg, 0.011 mmol). The final reaction solution contained 5.5 mM both dA and SN38 derivative (pH=7,0). The progress of reaction was monitored by ^{1}H NMR. The reaction was carried out at room temperature and finished after 60 hours. The reaction mixture was purified using HPLC on an RP-C18 LPH column (150 mm x 10 mm) using as a mobile phase 0.1% HCOOH aqueous solution (A) and MeCN (B), in the following gradient: 0-15 min 5 → 50% B, 15 min 50% B. Flow rate of the mobile phase: 3 ml/min. The course of the chromatography was monitored using UV detection at a wavelength of 260 nm and 365 nm. Product of reaction N6-adduct was collected and was lyophilized.(Yield 15 %).

^{1}H NMR δ (DMSO-d_6) 500 MHz: 0.90 (t, J=7.3 Hz, 3H), 1.39 (t, J=7.5 Hz, 3H), 1.90 (m, 2H),2.32 (m,1H), 2.73 (m,1H), 3.27 (q, J=7.5 Hz, 2H),3.56 (m,1H), 3.64 (m,1H),3.90 (bs, 1H), 4.43 (bs, 1H), 5.22 (bs, 2H),5.30 (s, 2H), 5.38 (s, 2H), 6.38 (t, J=7.1 Hz, 1H)), 7.27 (s, 1H)) 7.58 (d, J=9.1 Hz ,1H), 8.08 (d, J=9.1 Hz, 1H), 8.31 (s, 1H), 8.34 (s, 1H).**^{13}C NMR** δ (DMSO-d_6) 125 MHz; HSQC/ HMBC : 8.2, 15.2 , 25.2, 31.1, 38.3, 40.4, 50.3, 62.5, 65.8, 71.3, 73.3, 84.9, 88.5,

96.3, 118.9 , 122.4, 131.4, 132.9, 140.0 , 144.9 ,147.3, 147.4 , 151.0, 152.6 , 157.8, 173.4 (7 carbon atoms not assigned). UV (in H_2O) nm: 227, 280, 324, 337, 358. **IR** (in DMSO, BaF_2, d=96,8μm) cm^{-1}: 1163, 1461, 1661, 2543, 3231, 3406. **HR-MS** (ESI): calculated mass for $C_{33}H_{32}N_7O_8$ $[M-H]^-$: 654.2310, measured 654.2312.

NMR experiments

All NMR experiments for N9-dA and N6-dA adduct in DMSO-d_6 solution were recorded at 298 K and 343 K on Varian VNMRS - 500 (500MHz) spectrometer equipped with a 5-mm Z-SPEC Nalorac IDG 500-5HT gradient probe. TSPA-d_4 was added to monitor chemical shifts.
For 1D acquisition the parameters were as follows: ^1H spectral width 12000 Hz and 48K data points, 1-2s low power presaturation of residual water signal.

The 2D experiments were performed according to the following conditions:

TOCSY [1,2] – spectral widths 6000 Hz in both dimensions, 1024 complex points in t_2, 400 increments in t_1, 8 scans per increment, relaxation delay 1s and spin-lock times 40 ms and 80 ms.
HSQC [3] spectral widths 6000 Hz in t_2 and 22600 Hz in t_1, 1024 points in t_2, 400 increments in t_1,64 scans per increment, relaxation delay of 1 s and $^1J(C,H)$ = 146 Hz.

$^1H/^{13}C$-HMBC [4] spectral widths 6000 Hz in t_2 and 22600 Hz in t_1, 1024 points in t_2, 256 increments in t_1,128 scans per increment, relaxation delay of 1 s.

The data were processed with linear prediction in t_1 followed by zero-filling in both dimensions. Gaussian weighting functions were applied in both domains prior to Fourier transformation.

Computational details

Geometry optimizations to energy minima of all of the considered compounds were achieved by DFT calculations at the B3LYP/6-31+G(d,p) level of theory, using the B3LYP hybrid functional as implemented in the Gaussian 09 (revision A.01) program package. The polarizable continuum model (PCM) using the integral equation formalism variant (IEFPCM) was applied in calculations performed in water.[5-10]

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