Supporting information

Isolation and Rationale for the Formation of Isomeric Decarbamoylmitomycin C-N^6-deoxyadenosine Adducts in DNA

Owen Zacarias[†], William Aguilar[†], Manuel M. Paz[‡], Sergey Tsukanov[†], Maggie Zheng[†], Shu-Yuan Cheng[†], Padmanava Pradhan[Δ] and Elise Champeil[†, §,*]

† John Jay College of Criminal Justice, 524 west 59th street, New-York, NY, 10019, United States

‡ Departamento de Química Orgánica, Facultade de Química, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain

§ Ph.D. Program in Chemistry, The Graduate Center of the City University of New York, New York, NY 10016, United States

[Δ]ppradhan@ccny.cuny.edu, The City College of New York, Marshak Science Building, MR-1024, 160 Convent Avenue, New York, NY 10031. United States

Corresponding Author:

Elise Champeil echampeil@jjay.cuny.edu. John Jay College of Criminal Justice, 524 west 59th street, New York, NY, 10019.

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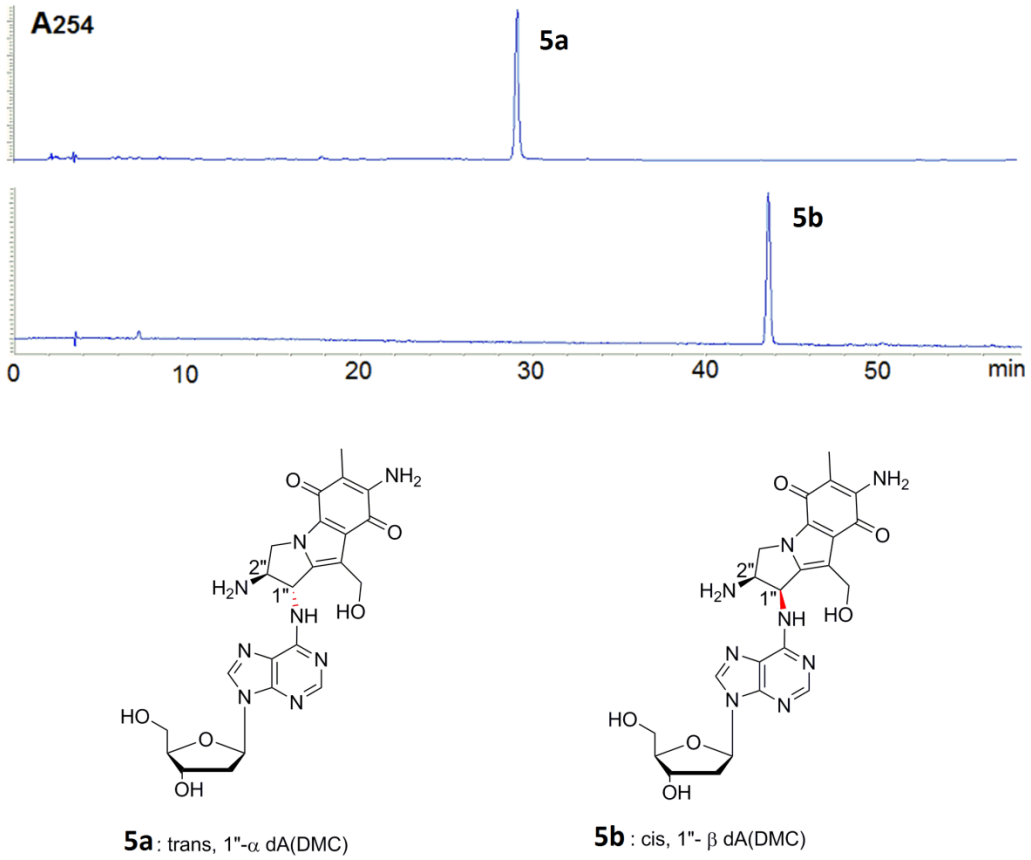


Figure S1: HPLC chromatogram of pure **5a** and **5b** under conditions described in methods.

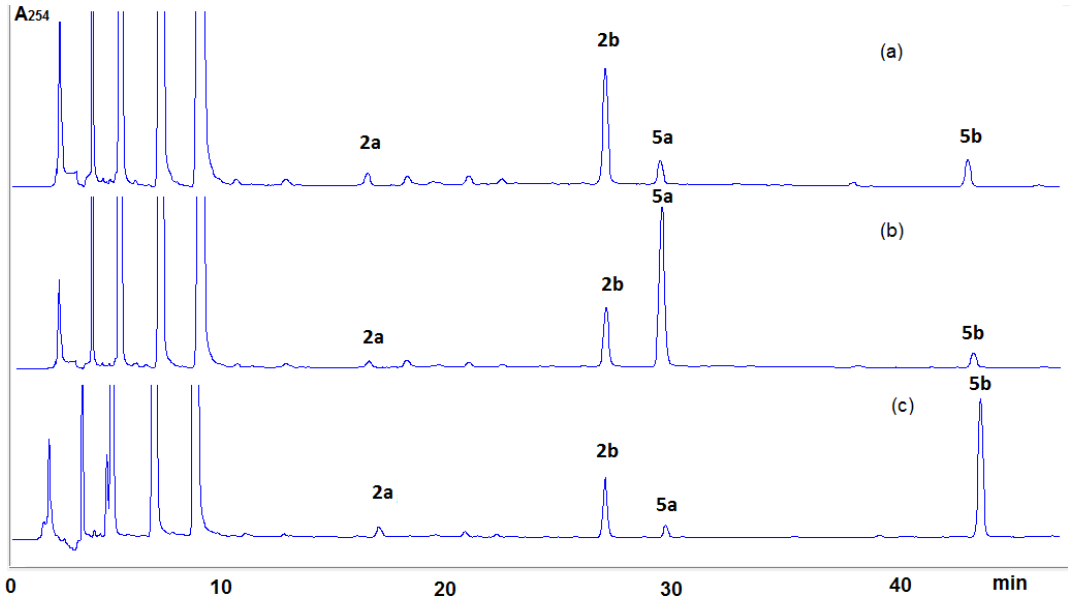


Figure S2. HPLC chromatograms of (a) digest of DMC-oligonucleotide complex formed under DMC bifunctional activation with oligonucleotide **9** (b) co-injection with adduct **5a** (c) co-injection with adduct **5b**.

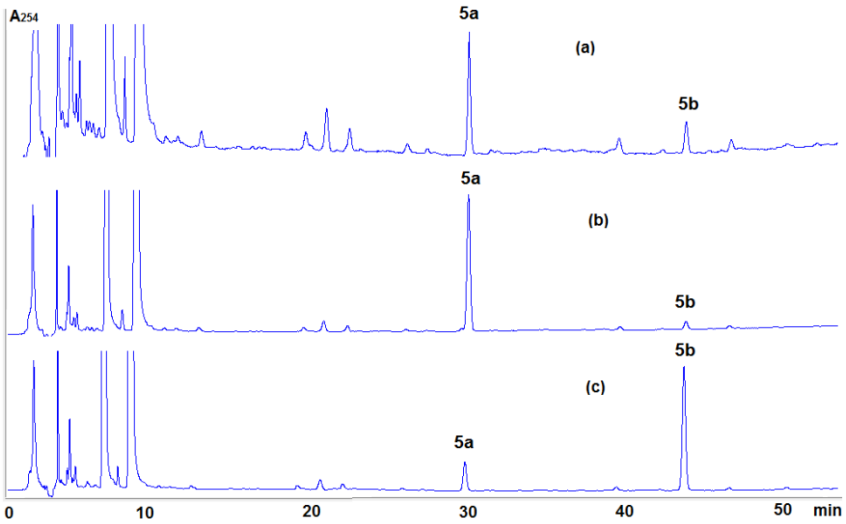
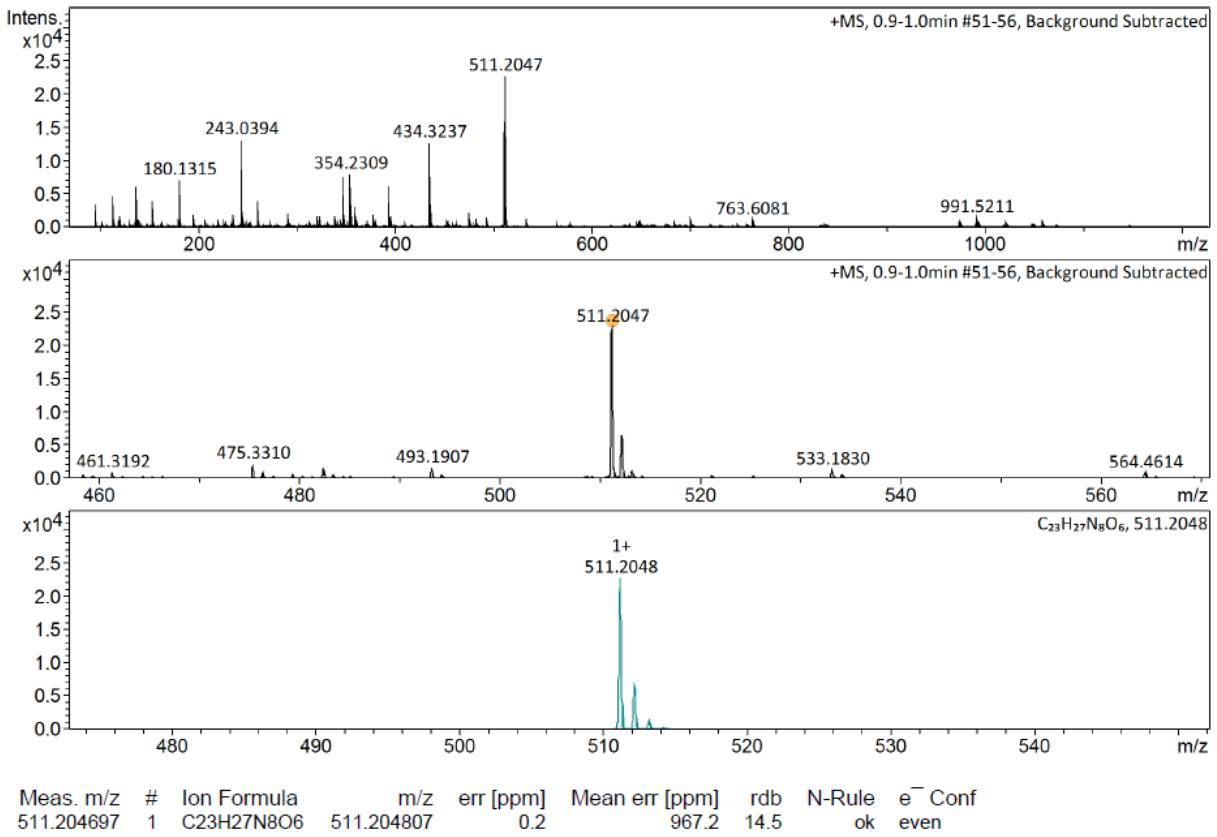
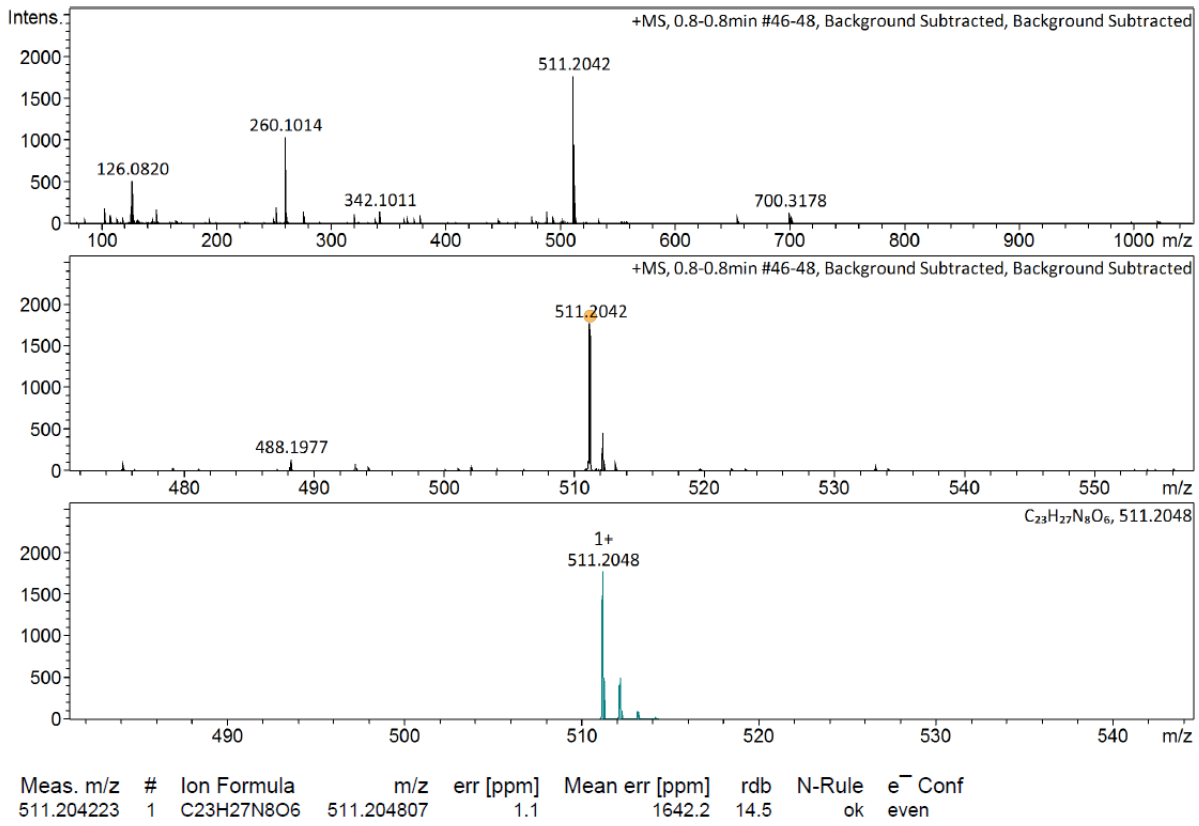


Figure S3. HPLC chromatograms of (a) digest of DMC-oligonucleotide complex formed under DMC bifunctional activation with duplex **6** d(AAAAAAAAAAAA).(TTTTTTTTTTTT) (b) co-injection with **5a** (c) co-injection with adduct **5b**.



Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf
511.204697	1	C23H27N8O6	511.204807	0.2	967.2	14.5	ok	even

Figure S4. HRMS of adduct **5a**.



Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf
511.204223	1	C23H27N8O6	511.204807	1.1	1642.2	14.5	ok	even

Figure S5. HRMS of adduct **5b**.

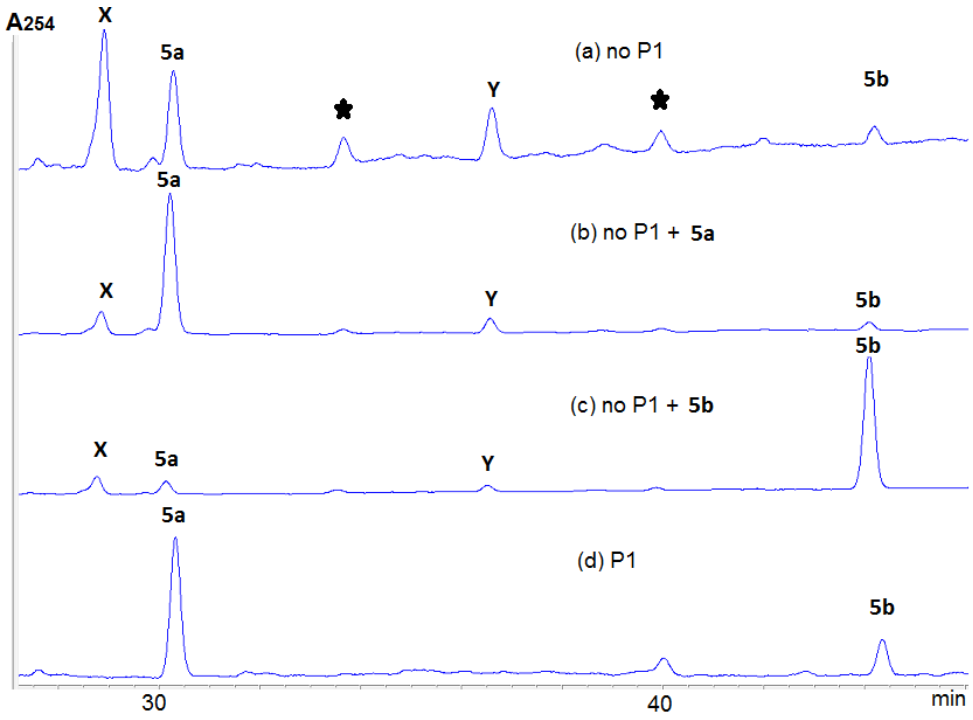


Figure S6: HPLC of (a) digest of duplex **6** d(AAAAAAAAAAAA).(TTTTTTTTTTTT) treated with DMC under bifunctional conditions without nuclease P_1; (b) same sample with co-injection of standard **5a**; (c) same sample with co-injection of standard **5b**; (d) same sample but digest of duplex **6** includes nuclease P_1.

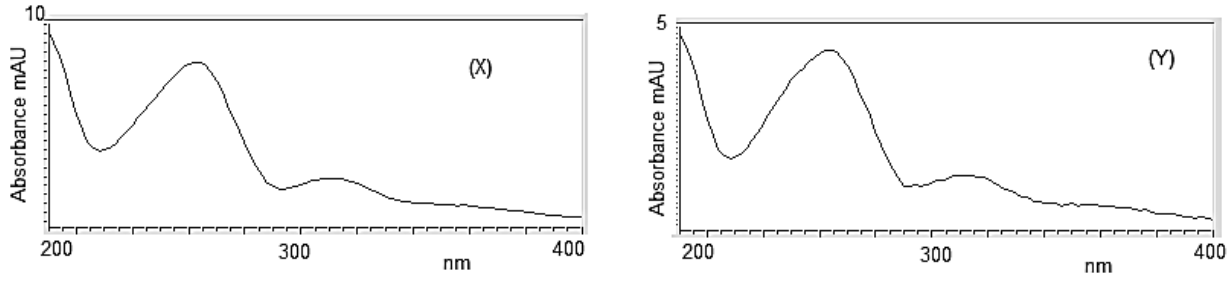


Figure S7: UV spectra of compounds **X** and **Y**.

Table S1. Frequencies of DNA Adducts Detected in Short Oligonucleotides under Bifunctional Conditions at 0°C and 37°C. ND= non-detected, NA=non-available.

Oligonucleotide	Frequencies (%) of adducts detected					
	$dG_{(DMC)}$	**$dA_{(DMC)}$**	**R^a**	**5a**	**5b**	**Log(5a/5b)**
6: 5'-AAAAAAAAAAAA 3'-TTTTTTTTTTTT	na	0.22 (±0.02)	na	0.17 (±0.02)	0.045 (±0.005)	0.47
7: 5'-AAAAAGCAAAAA 3'TTTTTCGTTTTT	3.23 (±0.6)	0.068 (±0.003)	47.5	0.061 (±0.003)	0.007 (±0.007)	0.93
8: 5'-TATATATATATA 3'-ATATATATATAT	na	0.11 (±0.008)	na	0.039 (±0.002)	0.070 (±0.009)	-0.27
9: 5'-TTATTGCAATTA 3'-AATAACGTTAAT	4.77 (±0.15)	0.39 (±0.02)	12.2	0.16 (±0.03)	0.23 (±0.01)	-0.16
10: 5'-TATATGCATATA 3'-ATATACGTATAT	1.22 (±0.3)	0.18 (±0.02)	6.8	0.045 (±0.009)	0.14 (±0.015)	-0.48
11: 5'-GAAAAAAAAAAA 3'-CTTTTTTTTTTT	2.08 (±0.2)	0.08 (±0.004)	26	0.07 (±0.0008)	0.01 (±0.005)	0.68
12: 5'-GAAAAAAAAAGA 3'-CTTTTTTTTTCT	1.06 (±0.01)	0.06 (±0.003)	17.7	0.05 (±0.002)	0.01 (±0.001)	0.60
13: 5'-GAAAAAAAAACA 3'-CTTTTTTTTTGT	2.05 (±0.07)	0.05 (±0.003)	41	0.017 (±0.001)	0.032 (±0.002)	-0.28
14: 5'-AAAAAAAAAAAC 3'-TTTTTTTTTTTG	0.28 (±0.01)	0.021 (±0.002)	13.3	0.021 (±0.002)	ND	NA
15: 5'-AGAAAAAAAAAG 3'-TCTTTTTTTTTC	0.30 (±0.001)	0.01 (±0.001)	30	0.01 (±0.001)	ND	NA
16: 5'-ACAAAAAAAAAG 3'-TGTTTTTTTTTC	1.71 (±0.03)	0.048 (±0.005)	36	1.048 (±0.005)	ND	NA
17: 5'-ATATAGCTATAT 3'-TATATCGATATA	3.95 (±1.1)	0.36 (±0.06)	11.0	0.27 (±0.003)	0.08 (±0.006)	0.53
18: 5'-ATATACGTATAT 3'-TATATGCATATA	2.30 (±0.06)	0.14 (±0.02)	16.4	0.12 (±0.02)	0.019 (±0.003)	0.80
19: 5'-ATTATTGCTATT 3'-TAATAACGATAA	4.41 (±0.25)	0.13 (±0.015)	33.9	0.10 (±0.01)	0.026 (±0.004)	0.59
20: 5'-ATTATCGTTATT 3'-TAATAGCAATAA	1.27 (±0.03)	0.26 (±0.08)	4.9	0.20 (±0.06)	0.058 (±0.02)	0.54
21: 5'-ATTATCGATAT 3'-TAATAGCTATAA	0.64 (±0.01)	0.15	4.3	0.095 (±0.005)	0.05 (±0.002)	0.28
22: 5'-GCAAAAAAAAGC 3'-CGTTTTTTTTCG	1.23 (±0.13)	ND	NA	ND	ND	NA
23: 5'-CGAAAAAAAACG 3'-GCTTTTTTTTGC	0.81 (±0.10)	ND	NA	ND	ND	NA
24: 5'-GAAAAAAAAAAG 3'-CTTTTTTTTTTC	1.12 (±0.15)	ND	NA	ND	ND	NA
25: 5'-AAAAAAAAAAAA	NA	0.071	NA	0.049	0.022	0.65

(single strand)		(±0.008)		(±0.004)	(±0.004)	
26: 5’-GAAAAAAAAAAG (single strand)	ND	0.031 (±0.002)	NA	0.025 (±0.002)	0.006 (±0.0005)	0.62
27: 5’-TTAGG (single strand)	0.054 (±0.0001)	0.064 (±0.002)	0.84	0.050 (±0.002)	0.014 (±0.0005)	0.54
28: 5’-ATTATTGCTATT (single strand)	0.44 (±0.005)	0.27 (±0.01)	1.6	0.19 (±0.015)	0.07 (±0.001)	0.43
29: 5'- ATTATCGTTATT (single strand)	0.42 (±0.03)	0.48 (±0.07)	0.87	0.35 (±0.05)	0.13 (±0.03)	0.43
30: 5'-ATATAGCTATAT (single strand, 37° C)	ND	0.11 (±0.005)	NA	0.076 (±0.003)	0.031 (±0.002)	0.39

[a] R=dG/dA at 0°C.

Figure S8: ^{1}H NMR spectrum of compound **5a**.

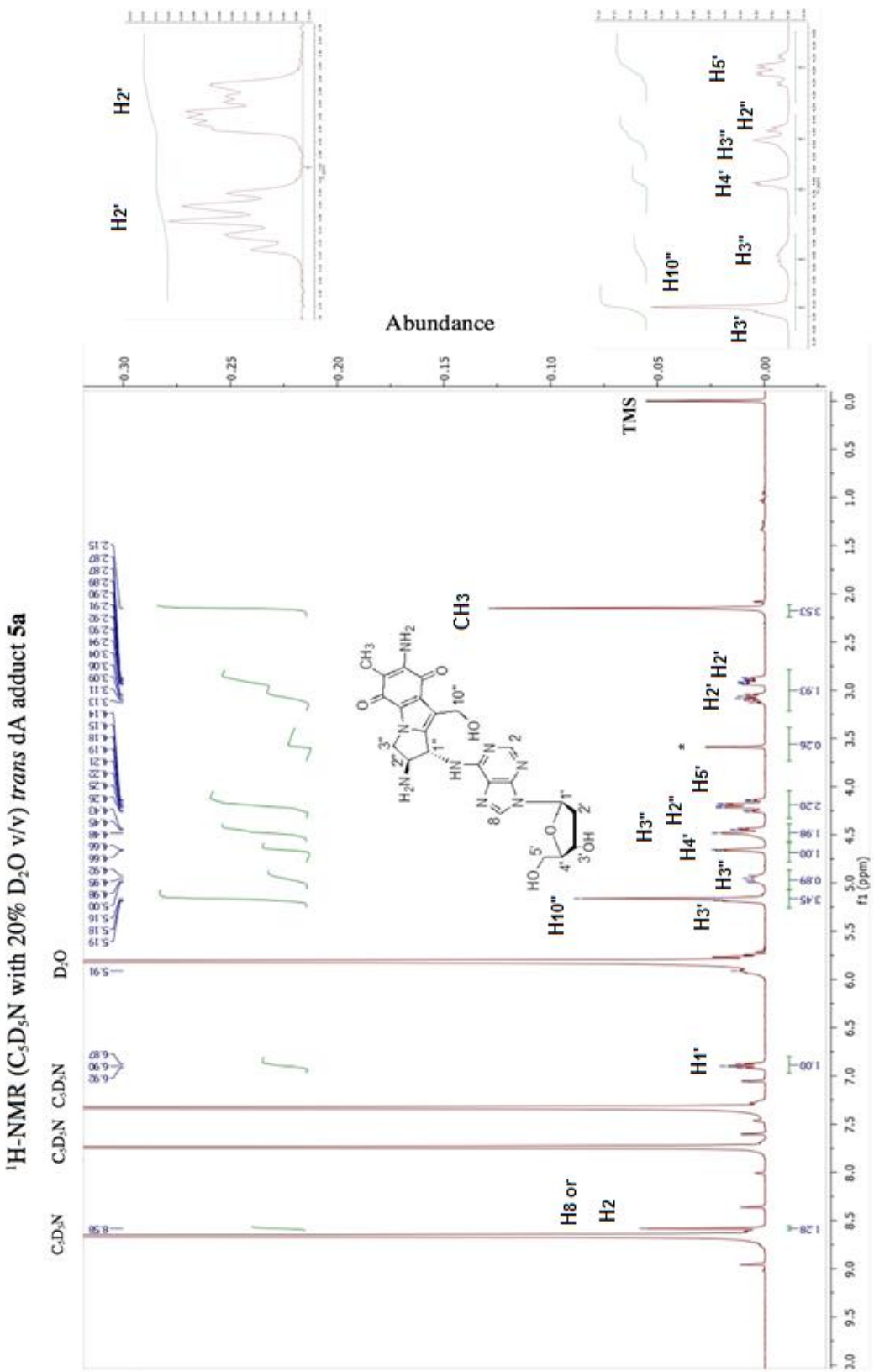


Figure S9: 2-D COSY NMR spectrum of compound **5a**.

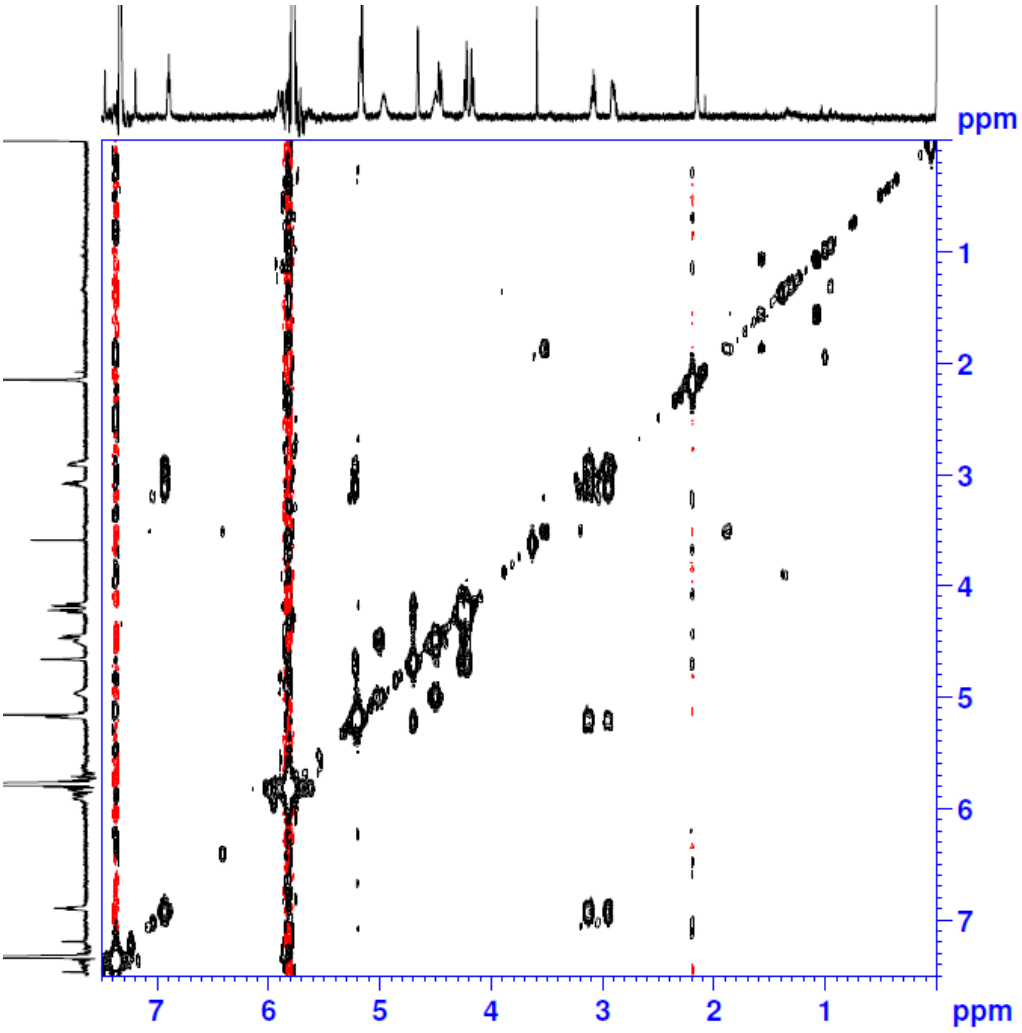


Figure S10: ^{1}H NMR spectrum of compound **5b**.

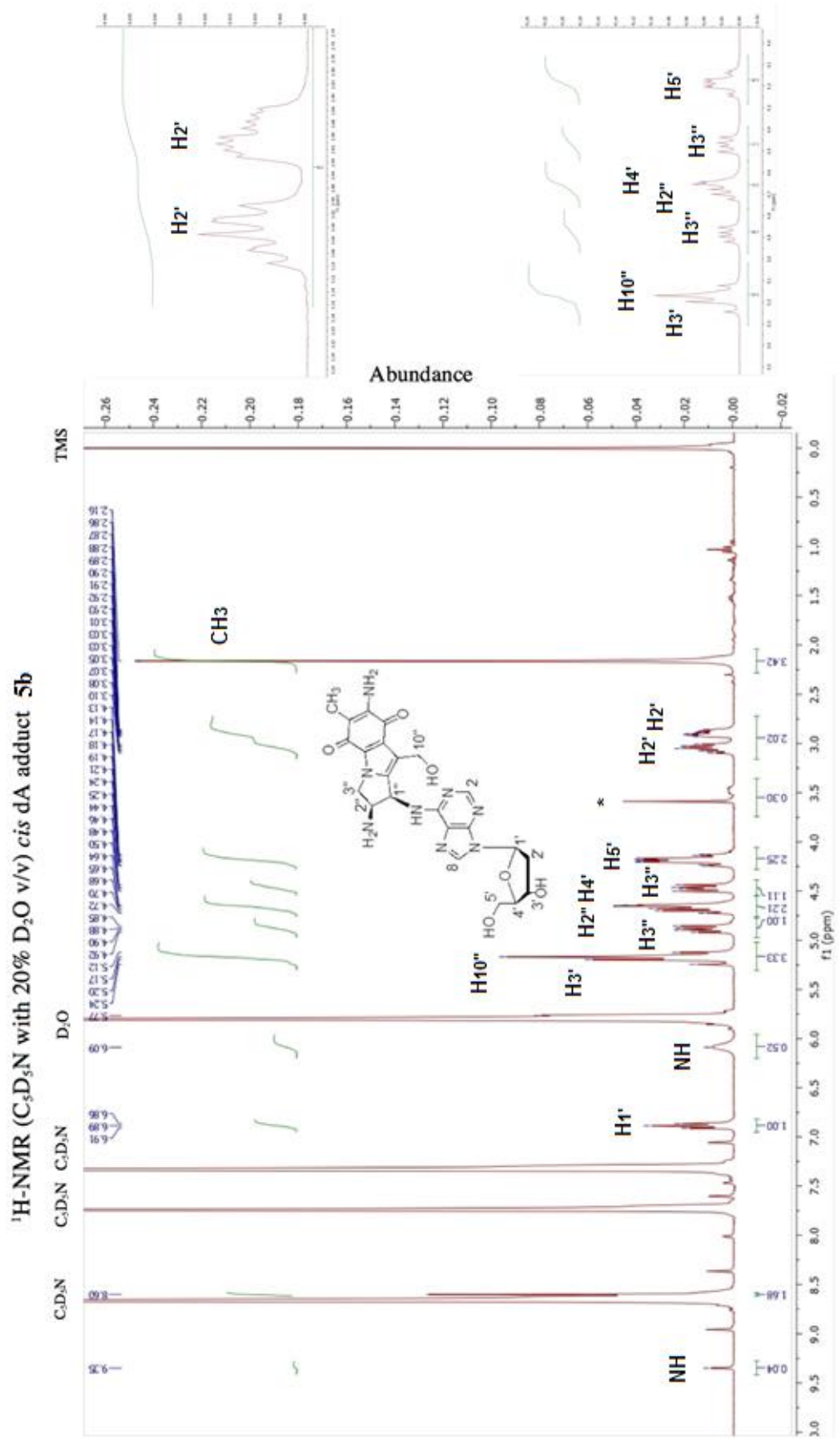


Figure S11: 2-D COSY NMR spectrum of compound **5b**.