10.1071/CH09645_AC
CSIRO 2010
Australian Journal of Chemistry 2010, 63(6), 873-876

SUPPORTING INFORMATION

9-(5′-deoxy-5′-thio-β-D-xylofuranosyl)adenine disulfide from the southern Australian marine sponge *Trachycladus laevispirulifer*: the first natural occurrence of a nucleoside disulfide

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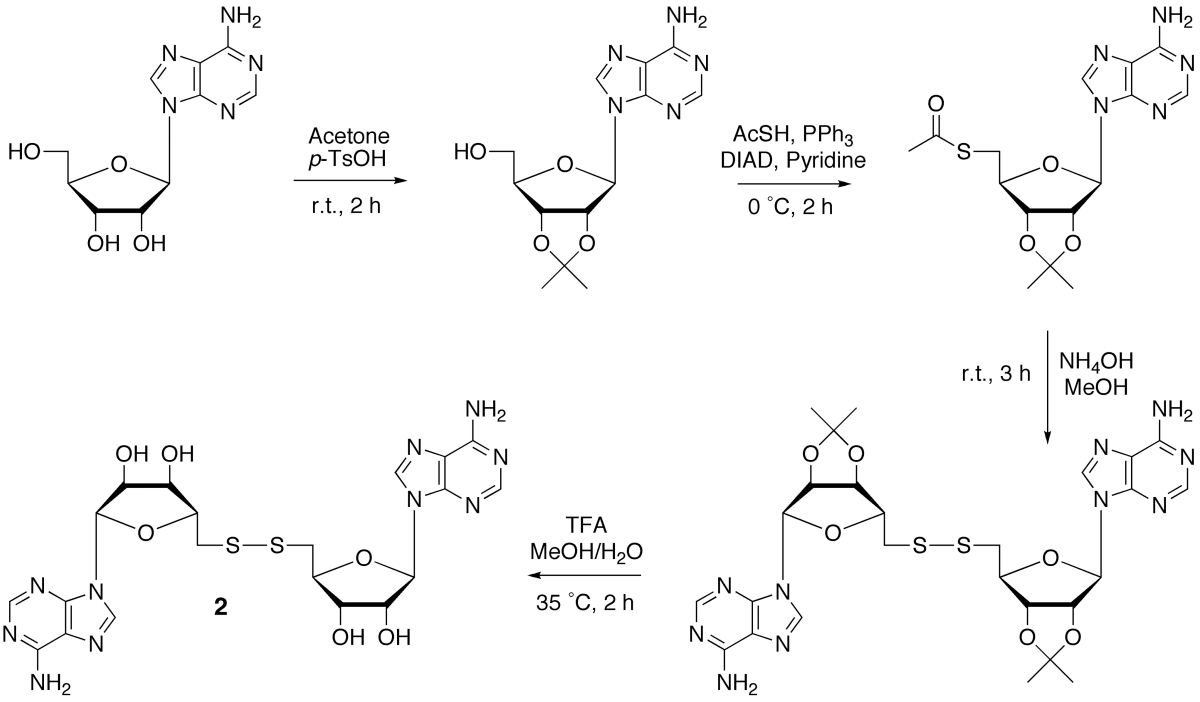
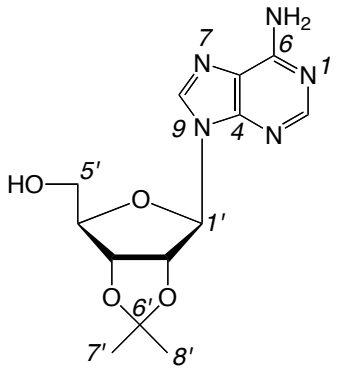
Sponge Taxonomy

Specimen CMB-03397 was described as follows: Colour in life: orange. Growth form erect, arborescent, branches cylindrical, dichotomously branching with rounded club-shaped ends, 5 – 10 mm diameter. Texture spongy, flexible but difficult to tear. Oscules discrete and scattered over branches. Surface opaque, optically smooth, wrinkled in places. Spicules include oxeas curved, telescoped and occasionally stylote (300 – 580 × 7 – 10 μm); microscleres are spinispirae spined, c-shaped or double spiral (5 – 8 μm). Ectosome a distinct cortex, 100 μm thick, of densely packed spinispirae. Choanosome has a condensed axial region of fibres cored by oxeas becoming plumose tracts coring fibres in the extra-axial region. Collagen abundant extra-axially and filled with spinispirae. Specimen CMB-03397 was identified as Class: Demospongiae, Order: Hadromerida, Family: Trachycladidae, Genus and species: *Trachycladus laevispirulifer* (Carter, 1879b), and a voucher sample was deposited with Museum Victoria (Reg No. MVF147424).



Scheme 1. Synthesis of adenosine disulfide (**2**)

Synthesis of adenosine isopropylidene



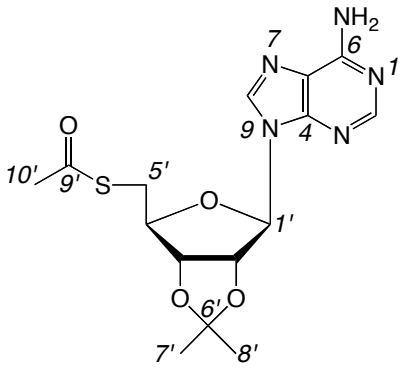
Adenosine isopropylidene was synthesised using a modified version of the method reported by Schmitt and Tampé.[1] A solution of adenosine (1.07 g, 4.00 mmol) and *p*-toluenesulfonic acid (2.06 g, 12.0 mmol) in acetone (25 mL) was stirred at room temperature for 2 h. The reaction mixture was then diluted with aqueous sodium hydrogen carbonate (1 M; 100 mL) and extracted with DCM (3 × 100 mL). The DCM layers were combined, dried over anhydrous magnesium sulfate and reduced to dryness *in vacuo* yielding adenosine isopropylidene as a white solid (0.43 g, 35%). ESIMS *m/z* 308 ($[M+H]^+$), 306 ($[M-H]^-$).

Table S1. NMR data (600 MHz, DMSO-d_6) for adenosine isopropylidene

Position	δ_C	δ_H, m (*J* in Hz)	COSY	^{1}H-^{13}C HMBC
2	152.7	8.14, s		4, 6
4	148.8			
5	119.1			
6	156.1			
8	139.9	8.33, s		4, 5
1′	89.6	6.11, d (3.1)	2′	4, 8, 3′, 4′
2′	83.2	5.33, dd (6.1, 3.1)	1′, 3′	4′
3′	81.4	4.95, dd (6.1, 2.6)	2′, 4′	1′, 4′
4′	86.3	4.20, ddd (5.1, 5.1, 2.6)	3′, 5′a/b	1′, 2′
5′a	61.6	3.55, ddd (11.6, 5.1, 5.1)	4′, 5′b, 5′-OH	3′, 4′
5′b		3.50, ddd (11.6, 5.4, 5.1)	4′, 5′a, 5′-OH	3′, 4′
6′	113.0			
7′	27.1	1.53, s		6′, 8′
8′	25.2	1.31, s		6′, 7′
5′-OH		5.23, dd (5.4, 5.1)	5′a/b	5′
6-NH_2		7.33, br s		

[1] L. Schmitt, R. Tampé, *J. Am. Chem. Soc*. **1996**, *118*, 5532-5543.

Synthesis of adenosine isopropylidene thioacetate



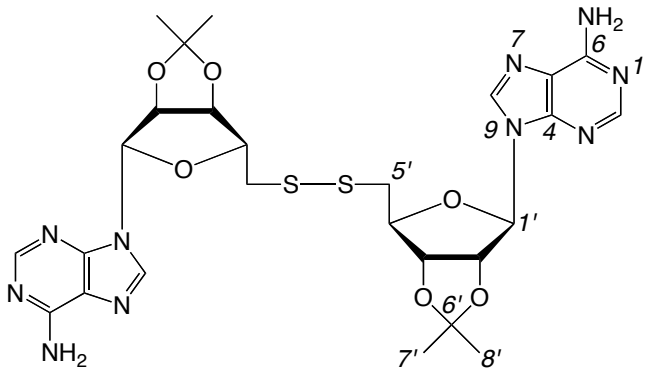
Adenosine isopropylidene thioacetate was synthesised using a modified version of the method reported by Pignot *et al*.[2] A solution of adenosine isopropylidene (31 mg, 0.10 mmol) and thioacetic acid (18 μL, 0.25 mmol) in anhydrous pyridine (1 mL) was cooled to 0 °C and a solution of triphenylphosphine (63 mg, 0.24 mmol) and diisopropyl azodicarboxylate (DIAD; 44 μL, 0.23 mmol) in anhydrous pyridine (1 mL) was added dropwise, with vigorous stirring, over 30 min. The reaction mixture was allowed to return to room temperature over 30 min and was then stirred at room temperature for 1 h. The solvent was removed from the reaction mixture under a stream of nitrogen and the residue was loaded onto a silica gel column equilibrated with 96:4 DCM:MeOH. The column was developed isocratically with 96:4 DCM:MeOH and 12 fractions were collected. Fractions 6-11 were combined (TLC) and reduced to dryness *in vacuo*, yielding adenosine isopropylidene thioacetate as a colourless oil (27 mg, 74%). ESIMS *m/z* 365 ($[M+H]^+$), 363 ($[M-H]^-$).

Table S2. NMR data (600 MHz, DMSO-d_6) for adenosine isopropylidene thioacetate

Position	δ_C	δ_H, m (*J* in Hz)	COSY	^{1}H-^{13}C HMBC
2	152.5	8.17, s		4, 6
4	148.7			
5	119.1			
6	155.9			
8	140.1	8.32, s		4, 5
1′	89.0	6.16, d (2.4)	2′	4, 8, 3′, 4′
2′	83.2	5.48, dd (6.3, 2.4)	1′, 3′	4′
3′	83.0	4.94, dd (6.3, 3.1)	2′, 4′	1′, 4′
4′	84.7	4.14, ddd (7.2, 6.9, 3.1)	3′, 5′a/b	1′, 2′
5′a	30.8	3.20, dd (13.7, 7.2)	4′, 5′b	3′, 4′, 9′
5′b		3.09, dd (13.7, 6.9)	4′, 5′a	3′, 4′, 9′
6′	113.5			
7′	26.9	1.50, s		6′, 8′
8′	25.1	1.30, s		6′, 7′
9′	194.6			
10′	30.5	2.32, s		9′
6-NH_2		7.41, br s		5

[2] M. Pignot, G. Pljevaljcic, E. Weinhold, *Eur. J. Org. Chem.* **2000**, *3*, 549-555.

Synthesis of adenosine isopropylidene disulfide

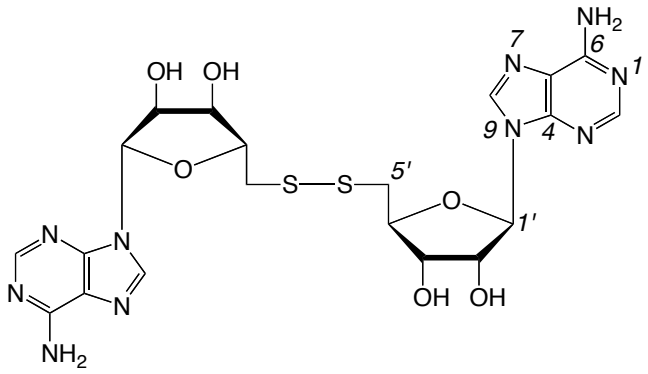


A solution of adenosine isopropylidene (25 mg, 68 μmol) and aqueous ammonium hydroxide (30%; 1 mL) in methanol (1 mL) was stirred at room temperature for 3 h. The reaction mixture was reduced to dryness under a stream of nitrogen, yielding adenosine isopropylidene disulfide as a colourless oil (22 mg, quant.). ESIMS *m/z* 645 ($[M+H]^+$), 643 ($[M-H]^-$).

Table S3. NMR data (600 MHz, DMSO-d_6) for adenosine isopropylidene disulfide

Position	δ_C	δ_H, m (*J* in Hz)	COSY	^{1}H-^{13}C HMBC
2	152.7	8.15, s		4, 6
4	148.7			
5	119.2			
6	156.1			
8	140.1	8.29, s		4, 5
1′	89.3	6.16, d (2.2)	2′	4, 8, 3′, 4′
2′	83.2	5.49, dd (6.3, 2.2)	1′, 3′	4′
3′	83.2	5.00, dd (6.3, 2.7)	2′, 4′	1′, 4′
4′	84.7	4.32, ddd (7.4, 7.1, 2.7)	3′, 5′a/b	1′, 2′
5′a	40.0	3.03, dd (13.7, 7.4)	4′, 5′b	3′, 4′
5′b		2.95, dd (13.7, 7.1)	4′, 5′a	3′, 4′
6′	113.3			
7′	26.8	1.52, s		6′, 8′
8′	25.1	1.31, s		6′, 7′
6-NH_2		7.35, br s		5

Synthesis of adenosine disulfide (2)



A solution of adenosine isopropylidene disulfide (25 mg, 39 μmol) and aqueous trifluoroacetic acid (33%; 5 mL) in methanol (2 mL) was stirred at 35 °C for 2 h. The reaction mixture was reduced to dryness *in vacuo* and the residue was recrystallised from water, yielding adenosine disulfide (**2**) as a white powder (19 mg, 88%). ESIMS *m/z* 565 ($[M+H]^+$), 563 ($[M-H]^-$).

Table S4. NMR data (600 MHz, DMSO-d_6) for adenosine disulfide (**2**)

Position	δ_C	δ_H, m (*J* in Hz)	COSY	^{1}H-^{13}C HMBC
2	152.6	8.14, s		4, 6
4	149.4			
5	119.2			
6	156.1			
8	140.0	8.33, s		4, 5
1′	87.4	5.88, d (6.0)	2′	4, 8, 3′
2′	72.5	4.78, ddd (6.0, 6.0, 5.4)	1′, 3′, 2′-OH	1′, 4′
3′	72.5	4.16, ddd (5.4, 4.8, 3.4)	2′, 4′, 3′-OH	
4′	82.6	4.10, ddd (7.8, 5.6, 3.4)	3′, 5′a/b	2′
5′a	41.0	3.16, dd (13.7, 5.6)	4′, 5′b	3′, 4′
5′b		3.08, dd (13.7, 7.8)	4′, 5′a	3′, 4′
6-NH_2		7.28, br s		5
2′-OH		5.52, d (6.0)	2′	1′, 3′
3′-OH		5.39, d (4.8)	3′	2′, 4′

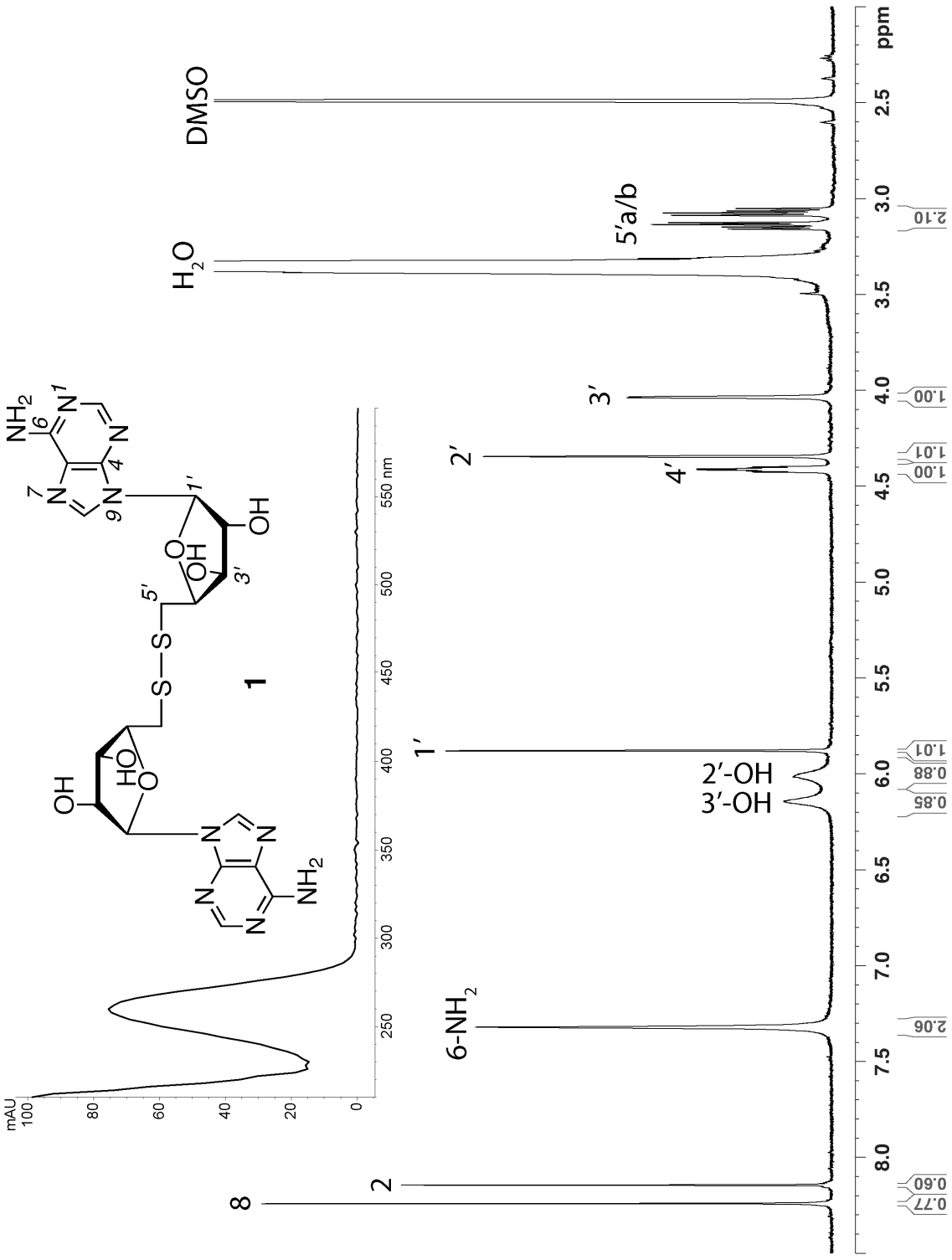


Figure S1. ^{1}H-NMR spectrum (600 MHz, DMSO-d_6) and UV-vis spectrum of 9-(5′-deoxy-5′-thio-β-D-xylofuranosyl)adenine disulfide (**1**).

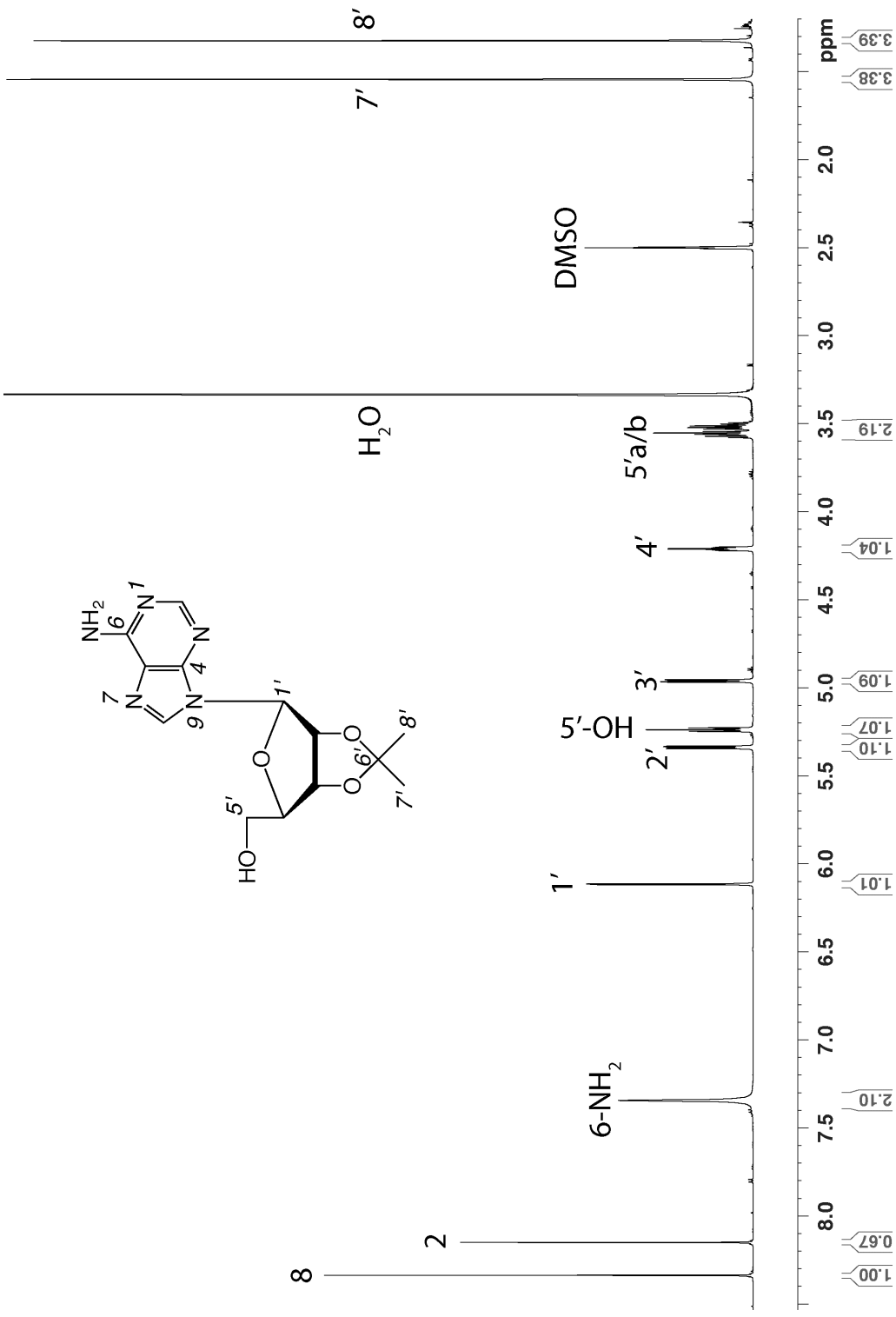


Figure S2. ^{1}H-NMR spectrum (600 MHz, DMSO-d_6) of adenosine isopropylidene

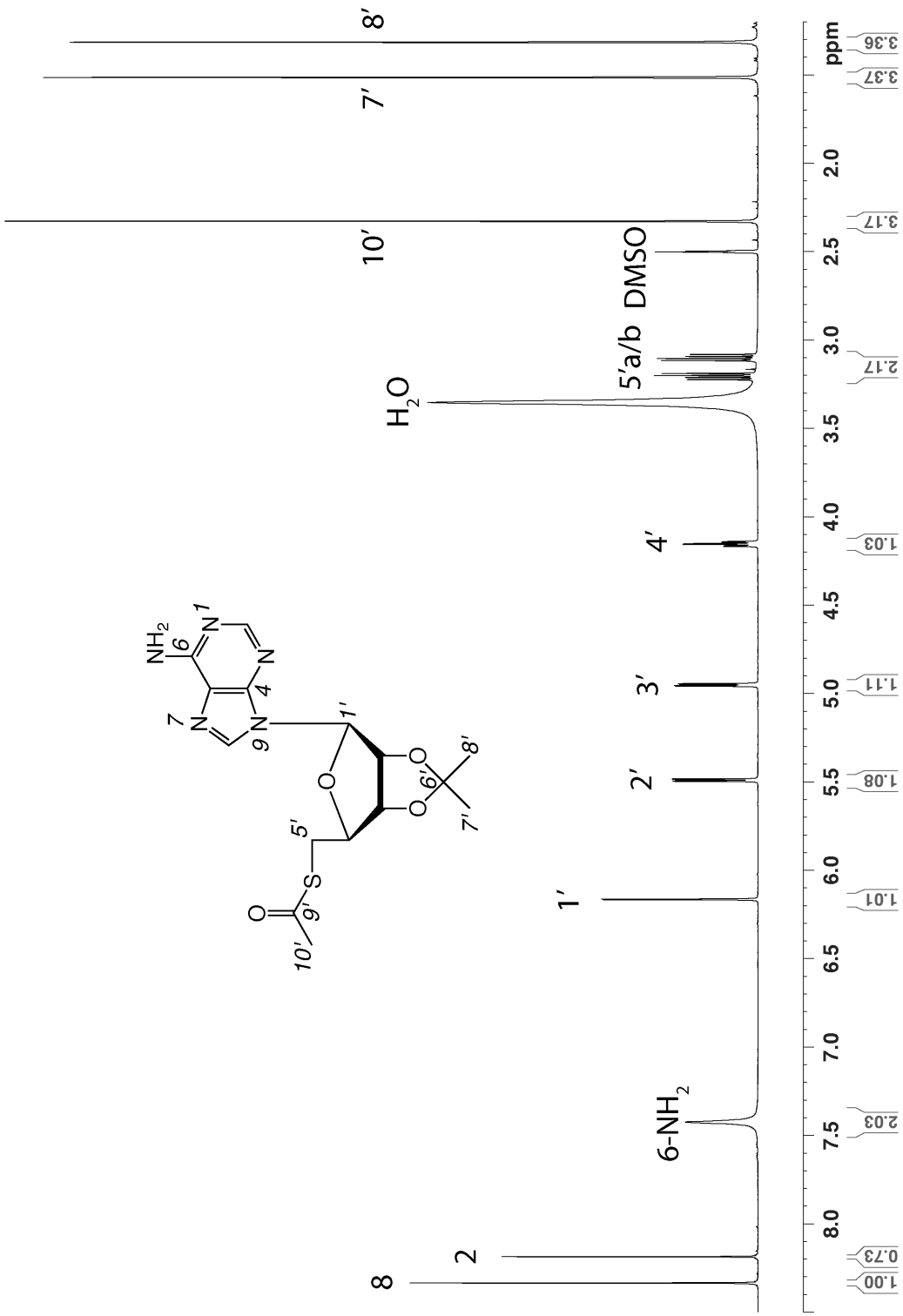


Figure S3. ^{1}H-NMR spectrum (600 MHz, DMSO-d_6) of adenosine isopropylidene thioacetate

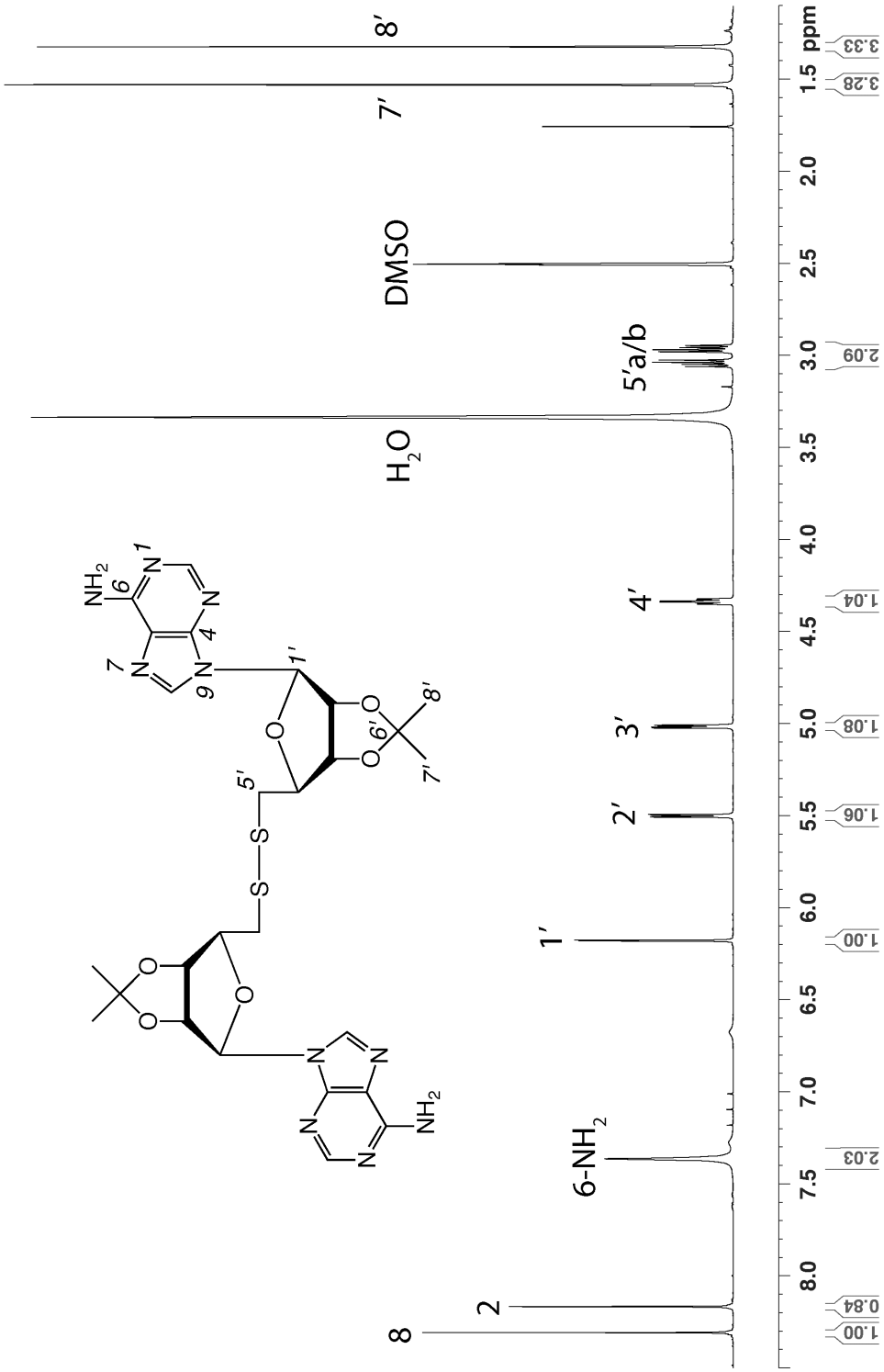


Figure S4. ^{1}H-NMR spectrum (600 MHz, DMSO-d_6) of adenosine isopropylidene disulfide

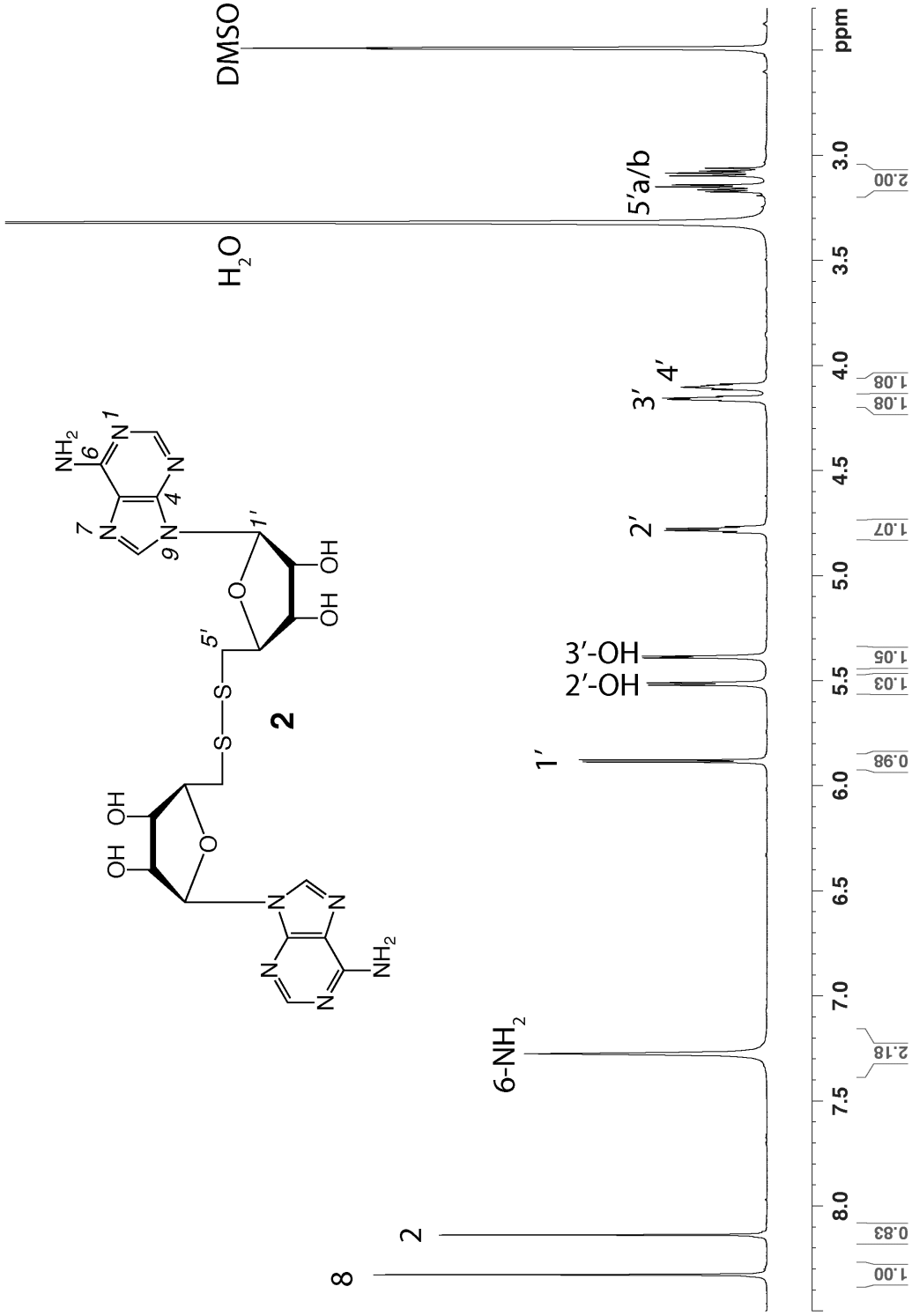


Figure S5. ^{1}H-NMR spectrum (600 MHz, DMSO-d_6) of adenosine disulfide (**2**)

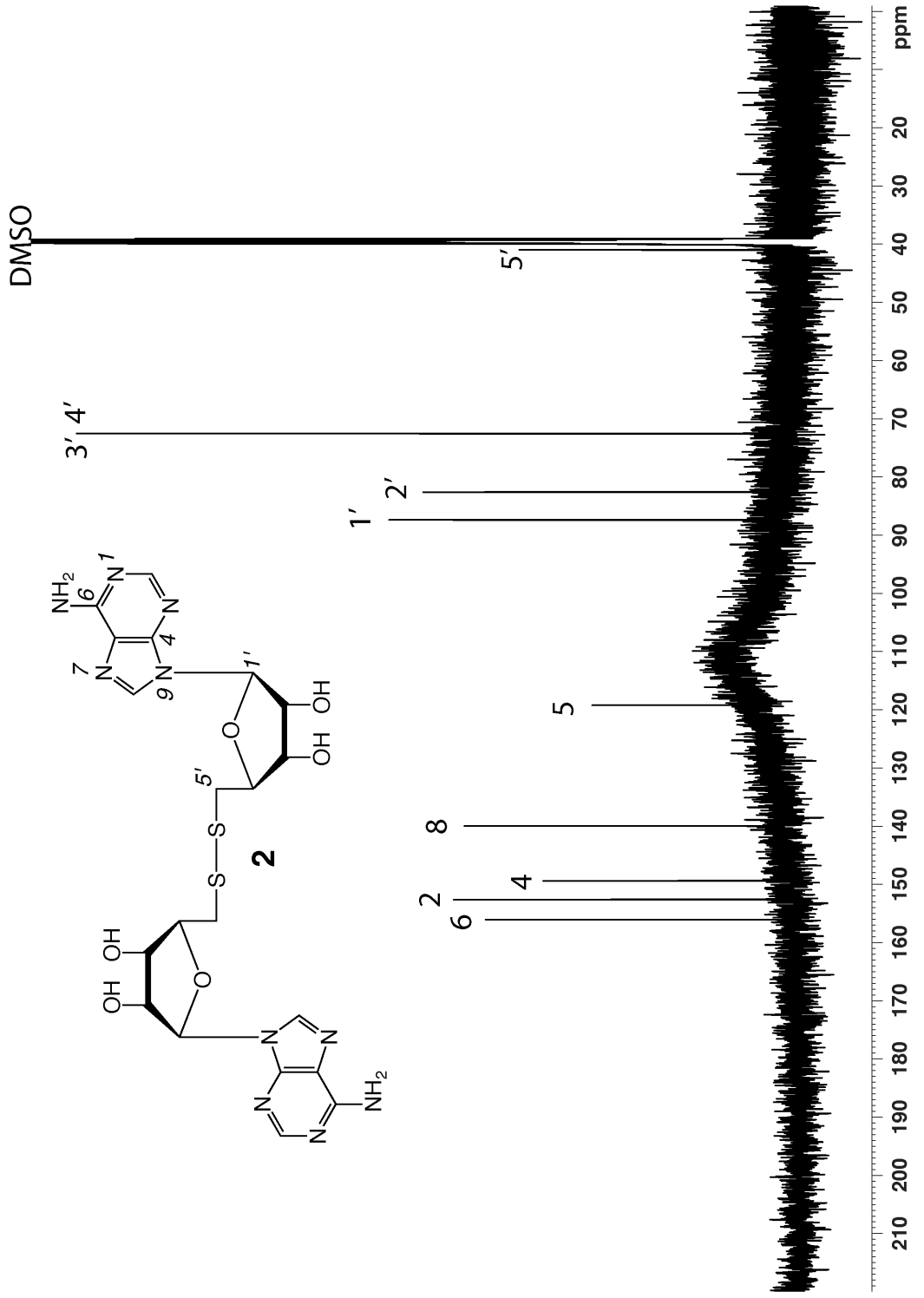


Figure S6. ^{13}C-NMR spectrum (150 MHz, DMSO-d_6) of adenosine disulfide (**2**)

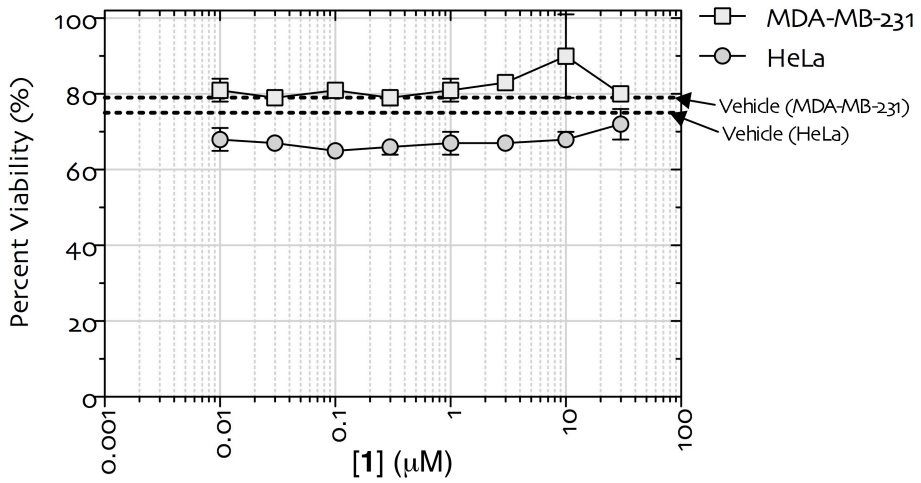


Figure S7. *In vitro* cytotoxicity of 9-(5′-deoxy-5′-thio-β-D-xylofuranosyl)adenine disulfide (**1**) to human breast (MDA-MB-231) and cervical (HeLa) adenocarcinoma cell lines, as measured by MTT assay.

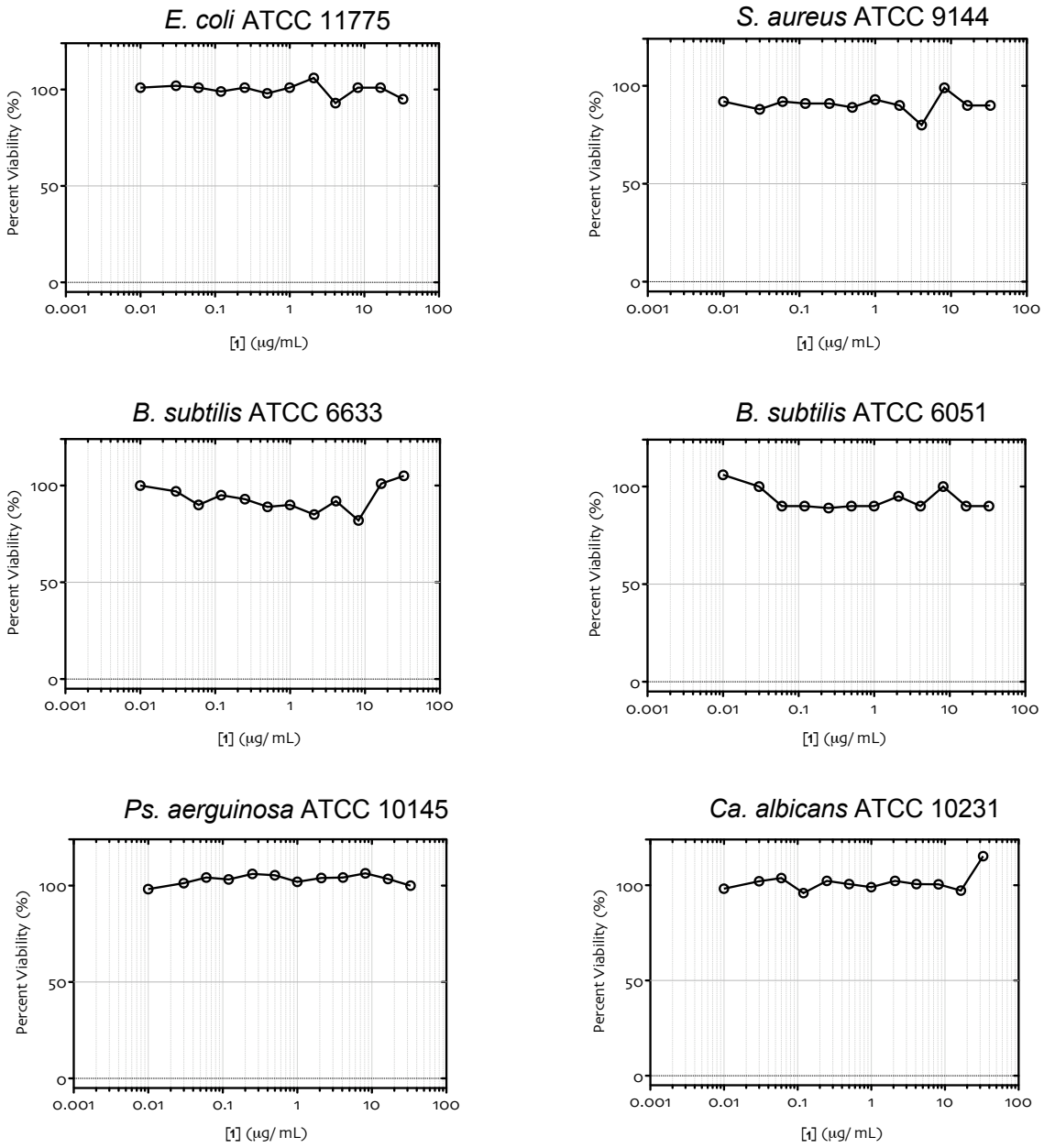


Figure S8. *In vitro* susceptibility of various microorganisms to 9-(5′-deoxy-5′-thio-β-D-xylofuranosyl)adenine disulfide (**1**).