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SUPPLEMENTARY INFORMATION

Isolation of 5'-O-sulfamyladenosine and related 3'-O-*b*-glucosylated adenosines from the nucelocidin producer *Streptomyces calvus*

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General experimental procedures

Room temperature refers to 18-25 °C. All evaporations and concentrations were performed under reduced pressure (*in vacuo*) by Büchi Rotavapor R-200. All reagents were purchased from Sigma Aldrich, Alfa Aesar or Fluorochem, were of synthetic grade and were used directly, without any further purification.

All microbiological experiments were carried out in laminar flow hood, using standard sterile techniques. Glassware, consumables and medium for biological operations were sterilised by autoclaving, flaming or wiping with 75% ethanol before use. Sterilised consumables were used as supply. Bacterial cultures were incubated in a temperature-control incubator (New Brunswick Scientific). Centrifugation of 20 mL to 1 L was processed by Beckman Avanti centrifuge. A Hettich Mikro 200 bench-top centrifuge was used for micro-centrifugation.

NMR spectra were recorded at 298 K on Bruker AV-III HD 700 instrument. ^{1}H and ^{13}C NMR spectra were recorded in deuterated solvent as the lock and the residual solvent as the internal standard. ^{19}F NMR spectra were recorded using $CFCl_3$ as an external reference. Chemical shifts are reported in parts per million (ppm) and coupling constants (*J*) are reported in Hertz (Hz). The abbreviations for the multiplicity of the proton, carbon and fluorine signals are as follows: s singlet, d doublet, dd doublet of doublets, ddd doublet of doublet of doublets, t triplet, dt doublet of triplets, q quartet, m multiplet, br s broad singlet.

The reference compounds were obtained synthetically or enzymatically. The synthesis of **7**, 3'-O-(β-D-Glucopyranosyl)-adenosine, followed the method developed previously in this laboratory. [1] The synthesis of **6**, 5'-O-Sulfamoyl-adenosine followed the method reported by F. Egami and N. Takahashi. [2] **8**, 5'-O-Sulfamoyl-3'-O-(β-D-glucopyranosyl)-adenosine was prepared enzymatically from synthetic 5'-O-sulfamoyl-adenosine via NucGT [1] and progress was validated by ^{1}H NMR.

HPLC analysis was achieved with a Shimazu LC-20A system. High resolution electrospray ionisation mass spectra were obtained on a ThermoFisher Excalibur Orbitrap spectrometer operating in positive or negative ion modes, from solutions in MeOH/H_2O, at the Mass Spectrometry facility at the University of St Andrews.

Growth of *Streptomyces calvus* on solid media

Streptomyces calvus was grown on solid ISP4 agar plates made by dissolving soluble starch (10 g), calcium carbonate (2 g), ammonium sulphate (2 g), sodium chloride (1 g), dipotassium phosphate (1 g), magnesium sulphate heptahydrate (1 g), ferrous sulphate (1 mg), manganese chloride (1 mg), zinc sulphate (1 mg), agar (2 %) in deionised water (to 1 L). The ISP4-agar medium was then sterilised in an autoclave before use. The plates were maintained at 30 °C incubation for 14 to 21 days. The spores were collected by means of sterilised cotton swabs and stored in a 25% glycerol solution at -80 °C. The petri dishes, after harvesting the spores, were stored at 4 °C for future use.

Seed culture of *Streptomyces calvus*

The seed culture was performed in TSB liquid medium composed of 3 % tryptone soy broth. The seed culture of *S. calvus* was obtained by inoculating 50 µL spores into 50 mL TSB, and the culture was allowed to grow at 28 °C for 2 days (in a 250 mL conical flask, shaking at 180 rpm).

Fermentation culture

A mass of the mycelium of *S. calvus* was obtained by inoculating a sterilised defined medium (100 mL in 500 mL conical flask) with the seed culture obtained as described above (inoculate 2 mL per 100 mL) and the culture was allowed to grow at 28 °C, and at 180 rpm for 8 days. The defined medium (1 L) was composed of tap water, corn steep liquor (12.5 g), mannitol (10 g), sodium chloride (2 g), diammonium phosphate

(2 g), monopotassium phosphate (1.5 g), magnesium sulphate heptahydrate (0.25 g), Hoagland's salt solution (1 mL), potassium fluoride solution (7.5 mL, 0.5 M).

The Hoagland's salt solution contained deionised water (1 L), manganese(II) chloride tetrahydrate (0.389 g), phosphorous acid (0.611 g), copper(II) sulfate (0.056 g), ammonium molybdate tetrahydrate (0.056 g), nickel(II) sulfate hexahydrate (0.056 g), zinc sulfate heptahydrate (0.056 g), aluminium sulfate (0.056 g), stannous chloride dihydrate (0.028 g), cobalt(II) nitrate hexahydrate (0.056 g), titanium dioxide (0.056 g), lithium chloride (0.028 g), potassium iodide (0.028 g) and potassium bromide (0.028 g). Sterilised by autoclaving.

Extraction and purification of the metabolites 6-8

The cultures were monitored using LC-MS. After 6 to 11 days incubation, the cells were discarded by centrifugation and the supernatant was extracted by adsorption onto charcoal/Celite (5 g per 1000 mL). The charcoal and Celite were mixed at a ratio of 1:2. The mixture, which was stirred in the supernatant for 1 hour to adsorb metabolitess, was collected by filtration and then washed with 100 mL acetone. The acetone was then dried in vacuo, and the residue was re-dissolved in water and analyzed by HPLC

A Phenomenex C-18 Luna Semi-preparation column (25cm) was used. Mobile phase A used MiliQ water with 0.05% (V/V) TFA and mobile phase B used acetonitrile with 0.05% (V/V) TFA. The crude extract from the *S. calvus* culture was separated by gradient elution: 0-5 min, 0% mobile phase B; 18 min, 95% mobile phase B; 23 min, 100% mobile phase B; 25 min, 0% mobile phase b; 35 min, 0% mobile phase B; flow rate is 2.5 mL/min; detection wave length: 254 nm. Reference compounds were used to navigate the HPLC traces of the natural products. The fractions eluted at 9 to 10 min (fraction 1) and 12 to 13 min (fraction 2) were collected. Then the fractions were separated using the same HPLC column but under isocratic elution: 0-15 min, 10% mobile phase B, to obtain the pure compounds.

HPLC and HRMS analysis of the 3 natural products.

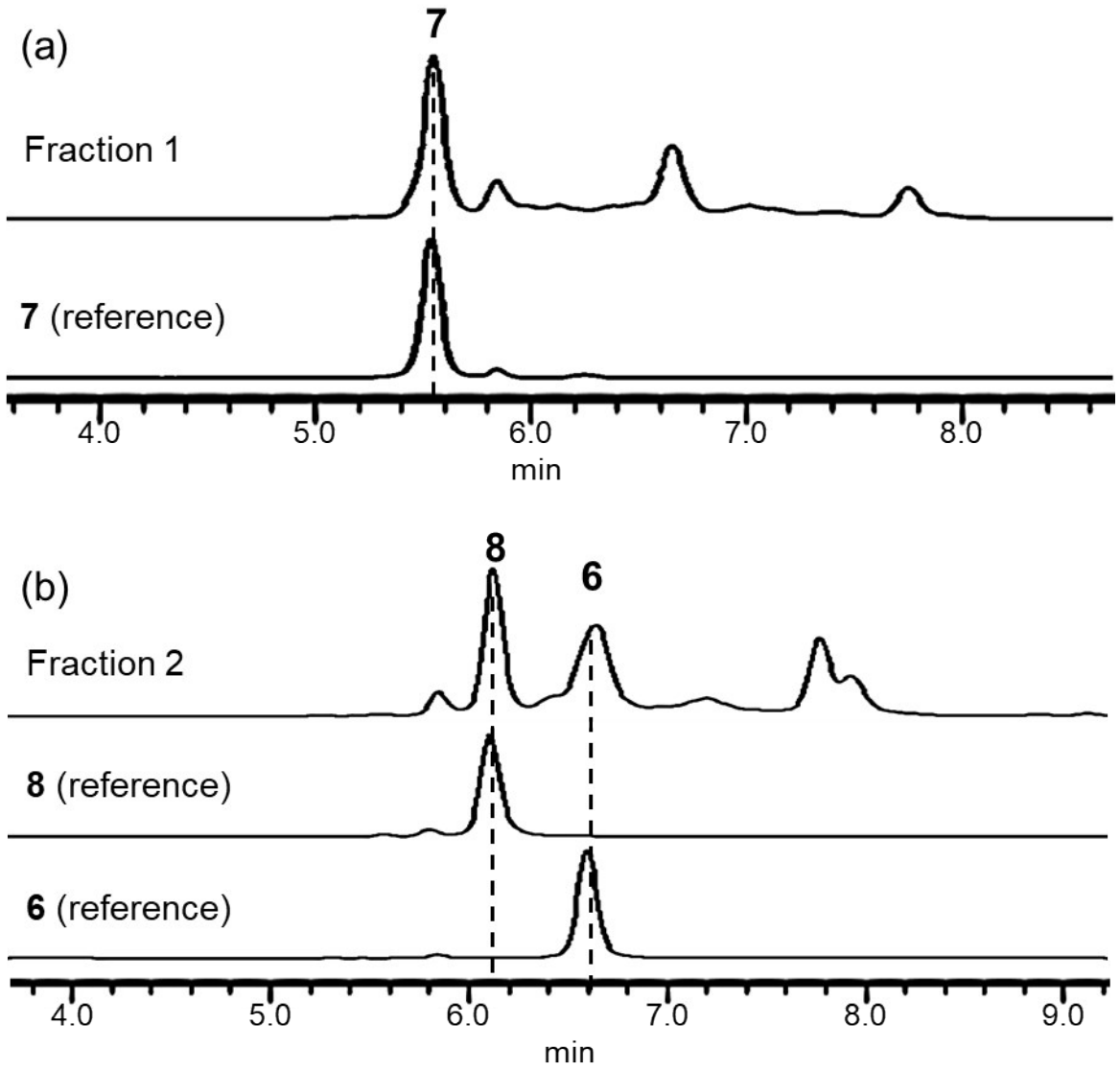


Figure S1. HPLC traces of purified and reference compounds. (a) F1: the second HPLC separation of Fraction 1; **7**: Synthetic 3'-O-(β-D-glucopyranosyl)-adenosine. (b) F2: the second HPLC separation of Fraction 2; **8**: Enzymatically derived 5'-O-sulfamoyl-3'-O-(β-D-glucopyranosyl)-adenosine; **6**: Synthetic 5'-O-sulfamoyl-adenosine.

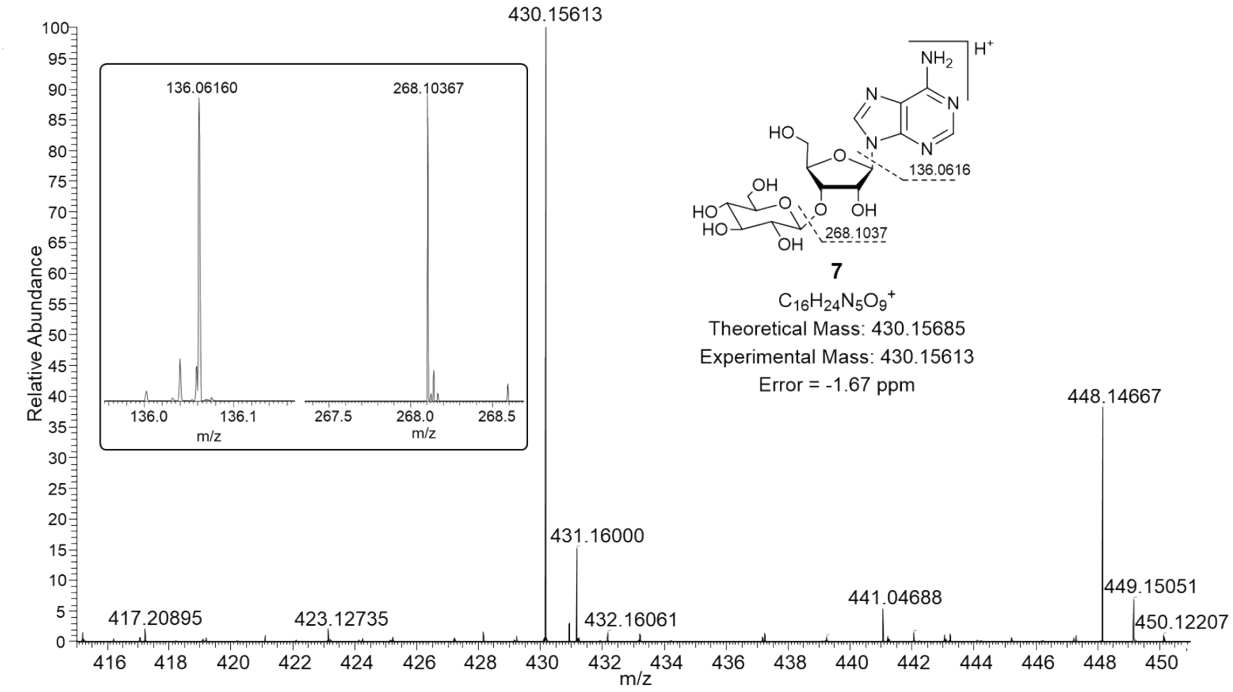


Figure S2. LC-HRMS and MS^2 (shown in box) analysis of **7**, the calculated $[M+H]^+$ = 430.15685 amu, the experimental $[M+H]^+$ = 430.15613 amu, error = -1.67 ppm.

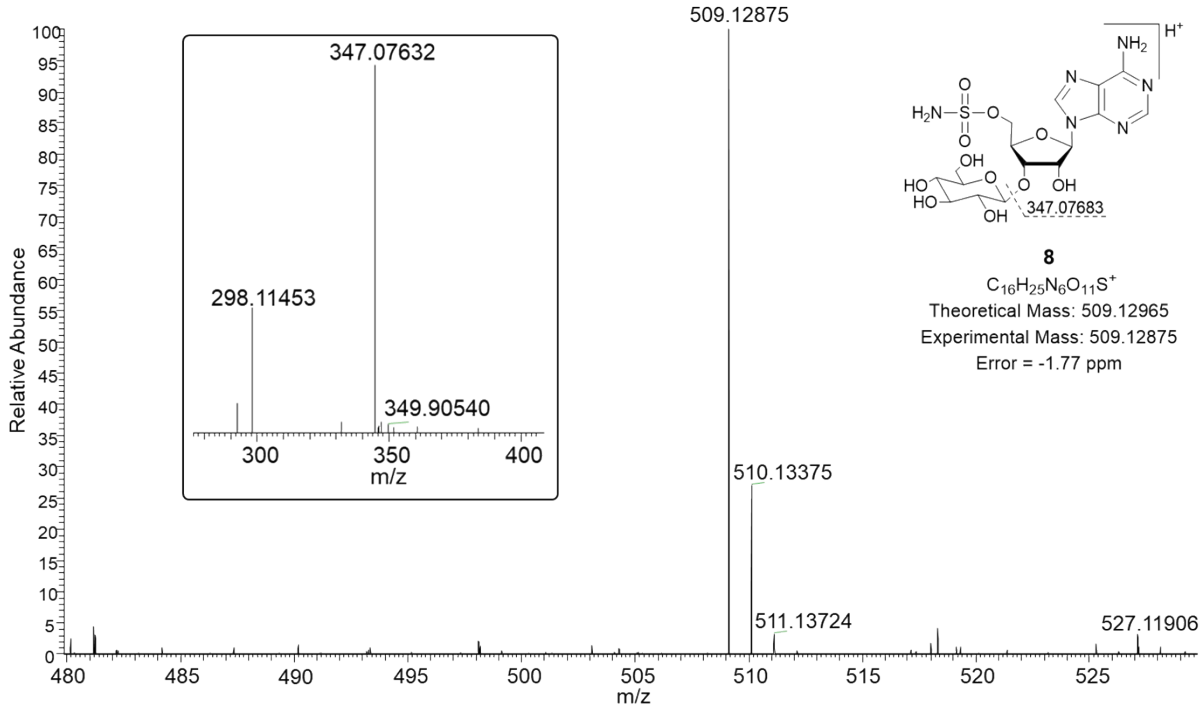


Figure S3. LC-HRMS and MS^2 (shown in box) analysis of **8**, the calculated $[M+H]^+$ = 509.12965 amu, the experimental $[M+H]^+$ = 509.12875 amu, error = -1.77 ppm.

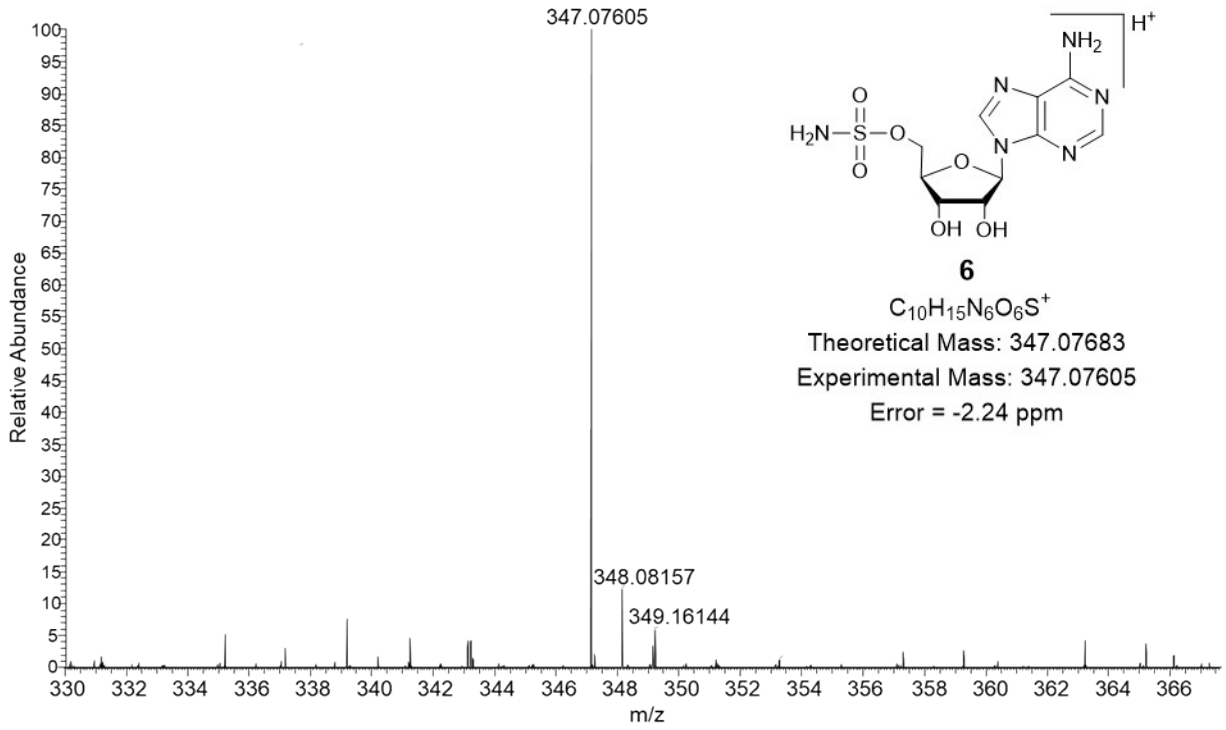
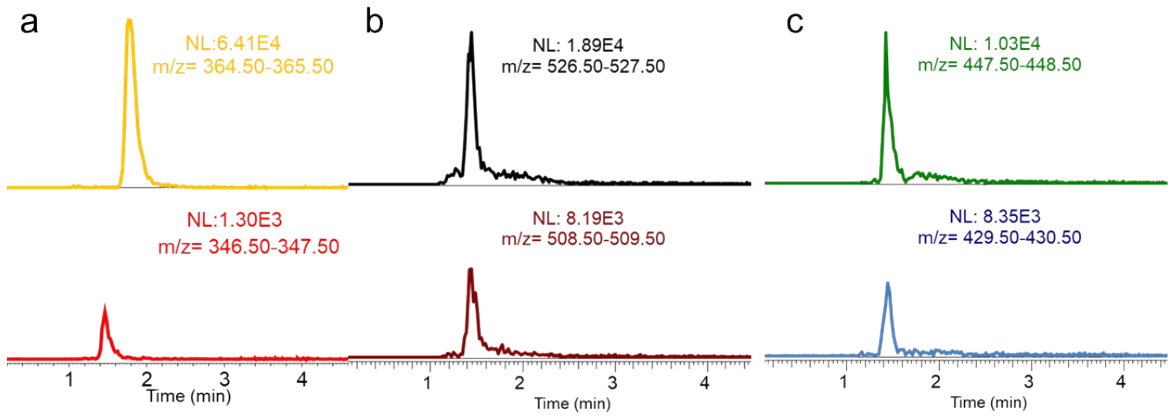


Figure S4. LC-HRMS analysis of **6**, the calculated $[M+H]^+$ = 347.07683 amu, the experimental $[M+H]^+$ = 347.07605 amu, error = -2.24 ppm.



d

Molecular ions	**1 : 6**	**3 : 8**	**2 : 7**
Average ratio	7 : 1	12 : 5	2 : 1

Figure S5. The ratios of fluorometabolites **1-3** /defluorometabolites **6-8**. (a) The ion intensities (normalized level, NL) of **1** and **6** extracted from total ions; (b) The ion intensities (normalized level, NL) of **3** and **8** extracted from total ions; (c) The ion intensities (normalized level, NL) of **2** and **7** extracted from total ions; (d) The average ratios of fluorometabolites/defluorometabolites.

Table S1. The LC-MS data used for the calculation of the ratios.

Sample	Ion intensity (normalized level, NL)								
	1	**6**	**1:6**	**3**	**8**	**3:8**	**2**	**7**	**2:7**
S1	1.8	0.202	8.91089	2.09	1.11	1.88288	0.34	0.224	1.5179
S2	4.34	0.573	7.57417	1.7	0.585	2.90598	0.67	0.322	2.0807
S3	1.92	0.487	3.94251	2.18	1.24	1.75806	0.684	0.166	4.1205
S4	6.41	1.3	4.93077	3.04	1.19	2.55462	0.469	0.257	1.8249
S5	2.8	0.505	5.54455	1.05	0.673	1.56018	0.944	0.653	1.4456
S6	1.71	0.372	4.59677	2.52	0.592	4.25676	0.743	0.409	1.8166
S7	2.76	0.243	11.358	1.39	0.633	2.19589	0.608	0.396	1.5354
S8	1.17	0.142	8.23944	1.56	0.69	2.26087	0.545	0.267	2.0412
Average	2.8638	0.478	6.88714	1.9413	0.839	2.42191	0.625	0.3368	2.0479
Ratio			7:1			12:5			2:1

^{1}H-NMR analysis of 6, 7and 8.

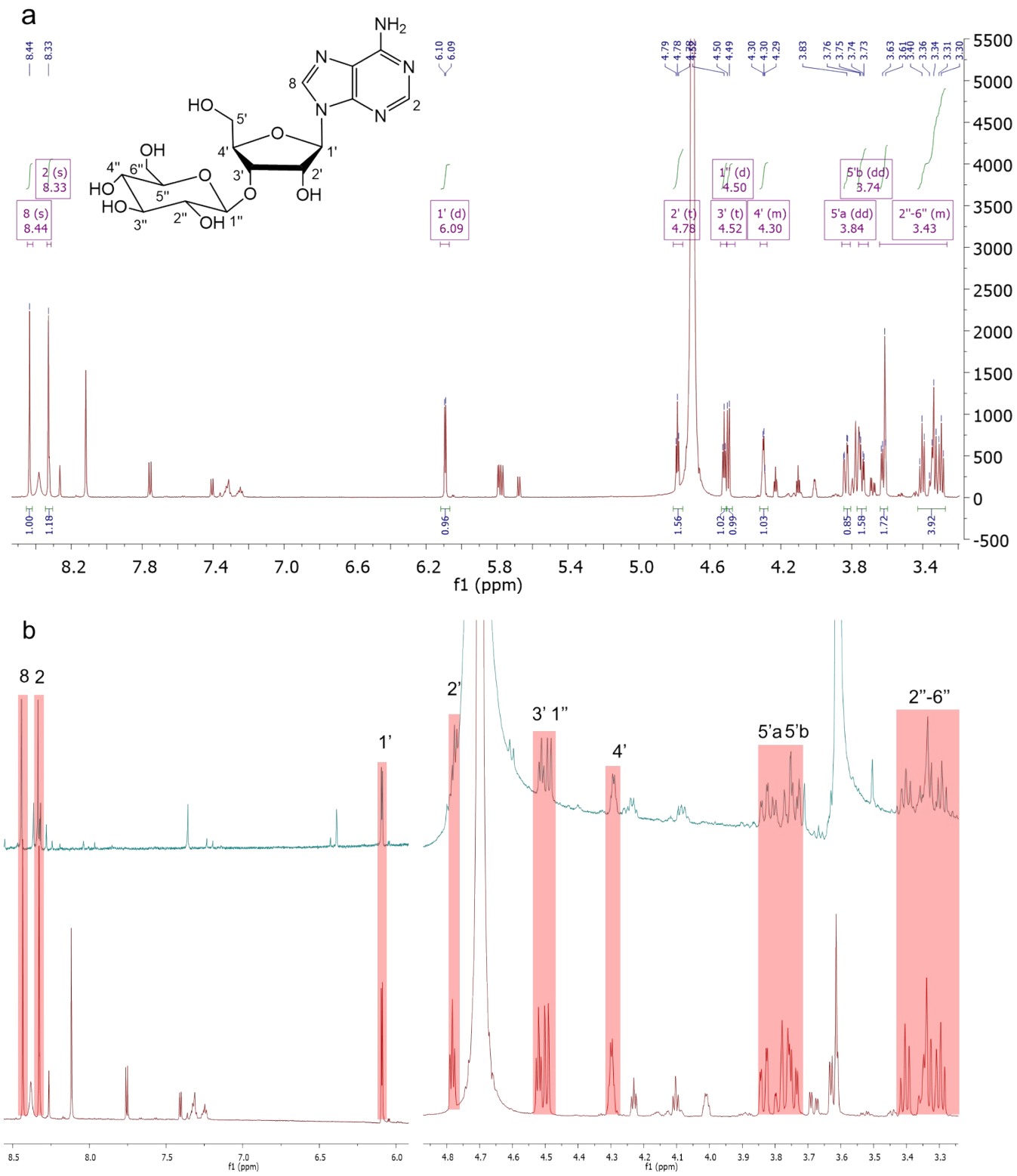


Figure S6. (a) ^{1}H NMR of **7** from *S. calvus* culture (700 MHz, D_2O) and **(b)** comparison of ^{1}H NMR of **7** (natural) and reference compound (700 MHz, D_2O).

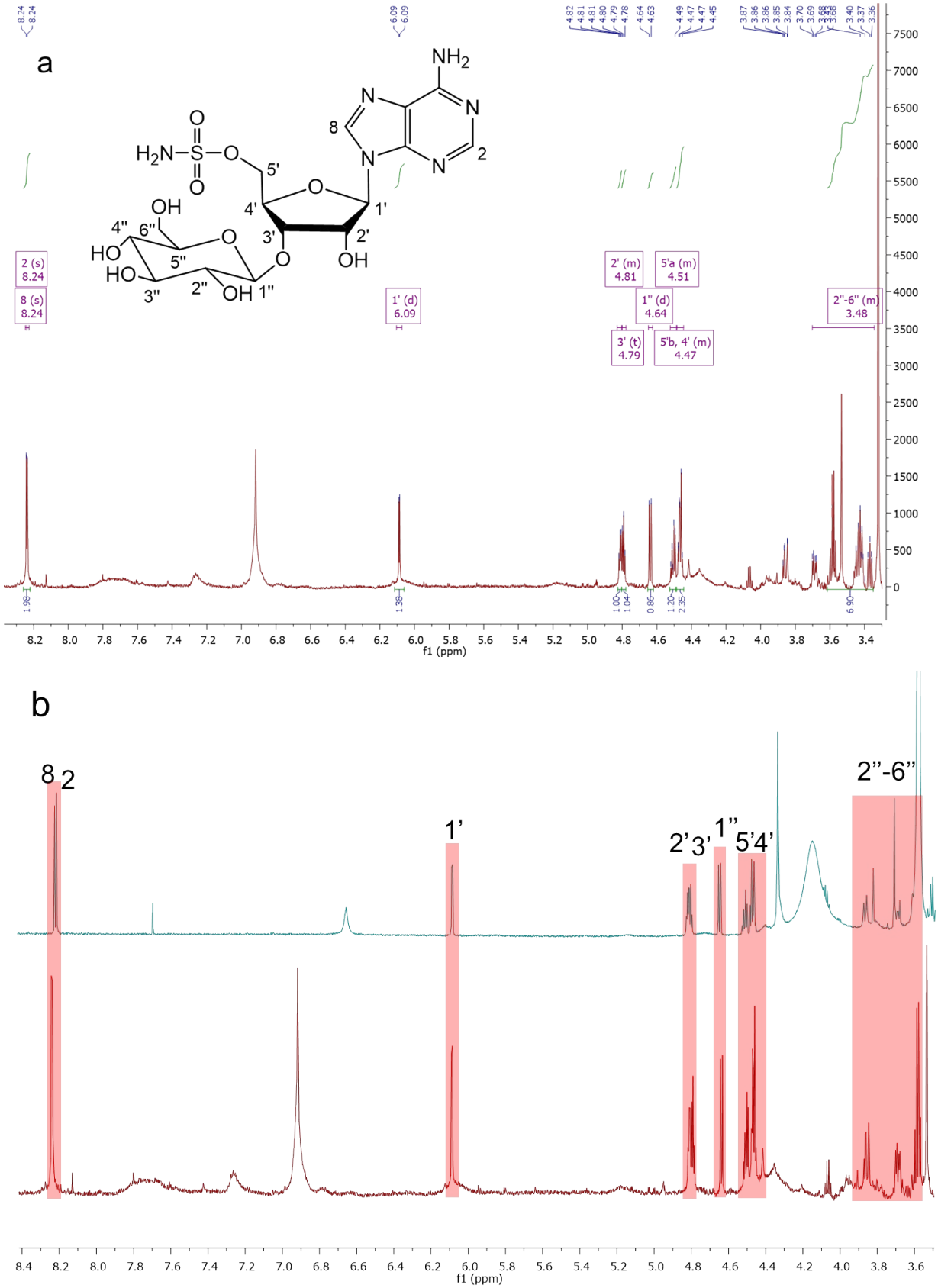


Figure S7. (a) ^{1}H NMR of **8** from *S. calvus* culture (700 MHz, Acetone-d_6) and **(b)** comparison of ^{1}H NMR of **8** (natural) and reference compound (enzymatic) (700 MHz, Acetone-d_6).

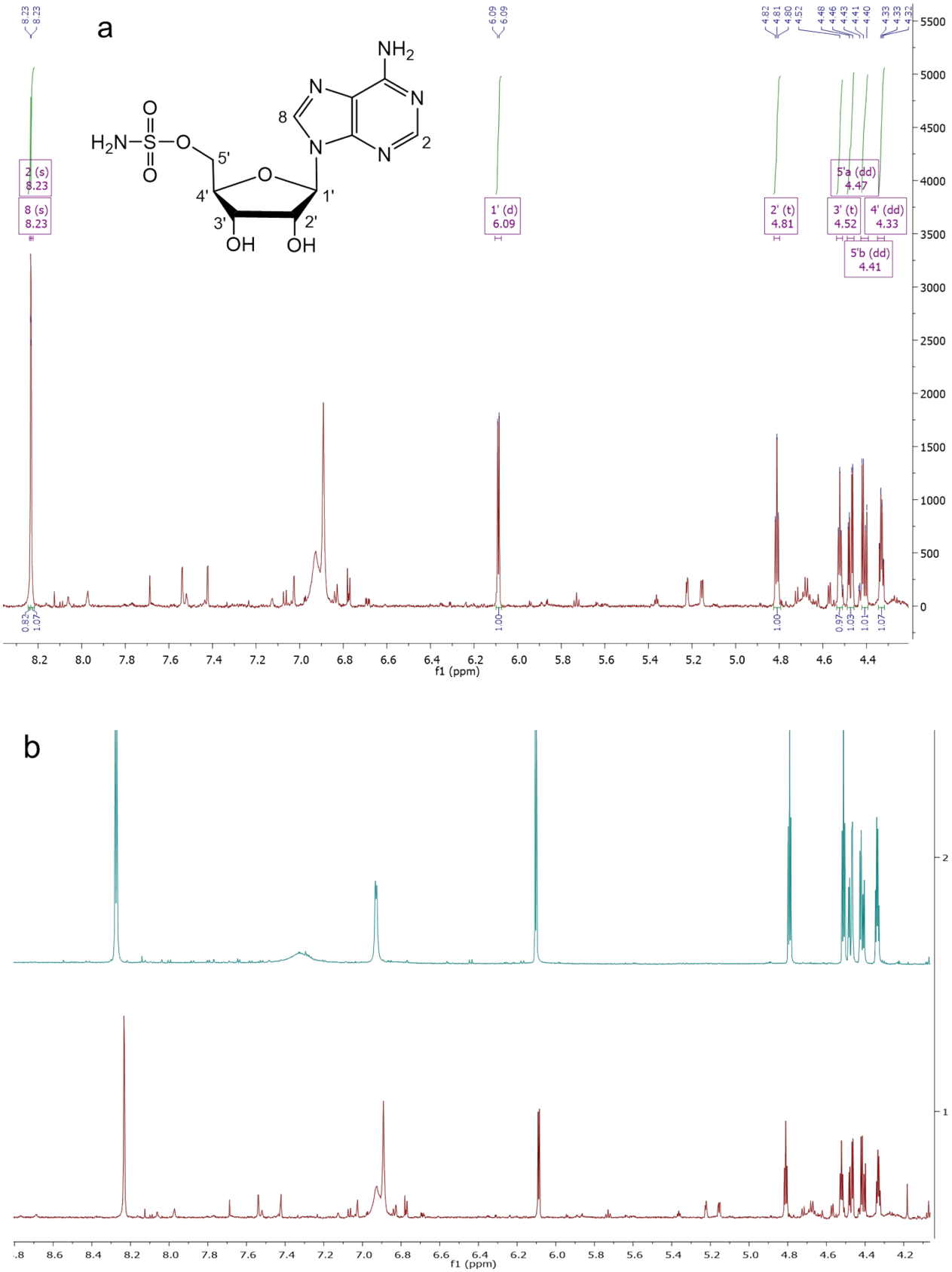


Figure S8. (a) ^{1}H NMR of **6** from *S. calvus* culture (700 MHz, Acetone-d_6) and **(b)** comparison of ^{1}H NMR of **6** (natural) and reference compound (synthetic) (700 MHz, Acetone-d_6).

Kinetic study of NucGT

Enzymatic activity was assayed at 37 °C by monitoring the production using analytical HPLC (Shimadzu SPD-20A detector at 254 nm coupled with SIL-20A HT autosampler). The glucosyltransferase (0.5 mg/mL) was incubated at various concentrations of adenosine or **6** with 100 mM $MgCl_2$ and a saturating concentration of UDP-glucose (17.7 mM) in Tris-HCl buffer (50 mM, pH 7.8), in a final volume of 0.25 mL. An aliquot (100 µL) was denaturalized with Phenol/Chloroform at various time points (1 min or 2 mins) and then instantly cooling on ice. Precipitated protein was then removed by centrifugation (13000 rpm, 10 min at 4 °C) and the sample was filtered with a PTFE filter (0.22 µm, Fisherbrand). The eluant was injected into analytical HPLC to determine the level of the products against a standard curve. Each sample was injected three times to obtain the average value. Kinetic parameters were obtained by Michaelis-Menten fitting of the initial velocity against substrate concentrations using OriginPro 2018.

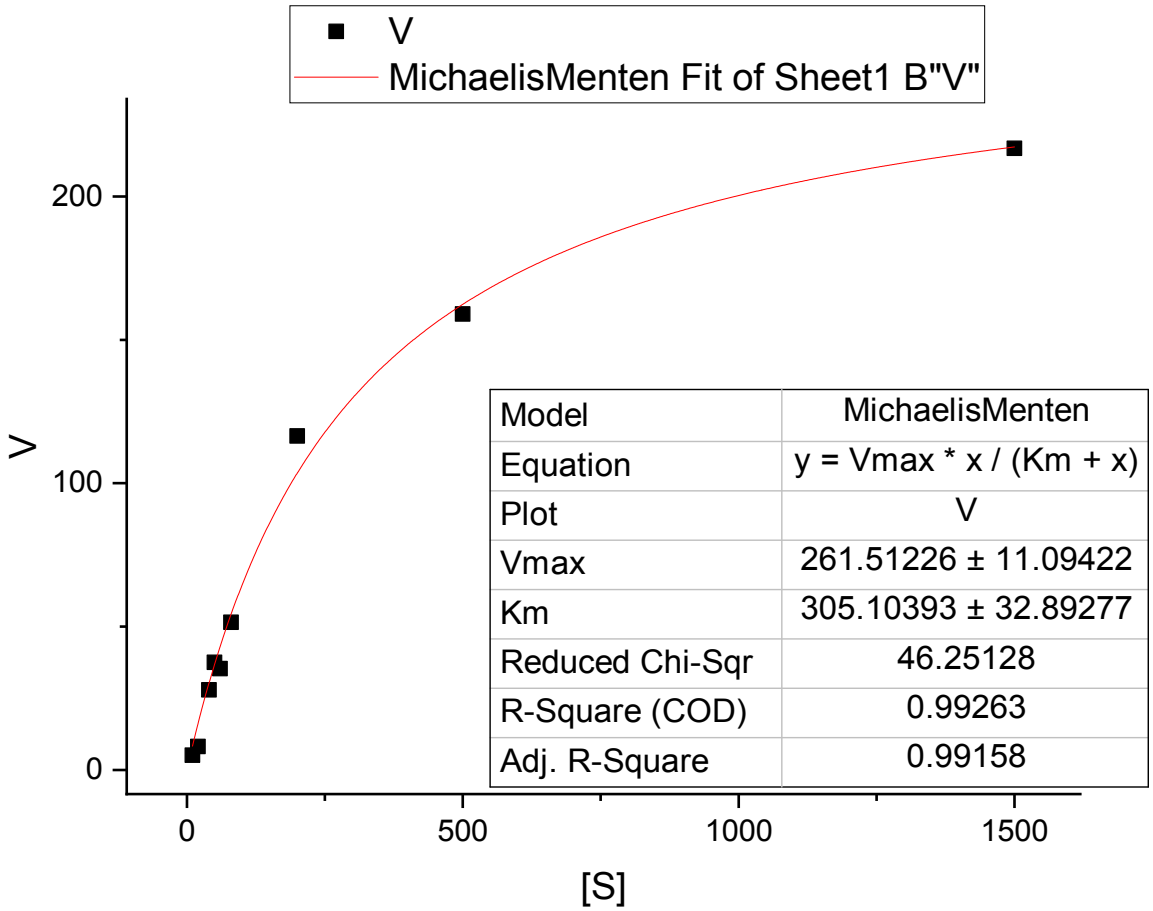


Figure S9. Michaelis–Menten kinetics curve of NucGT with adenosine to generate **7**.

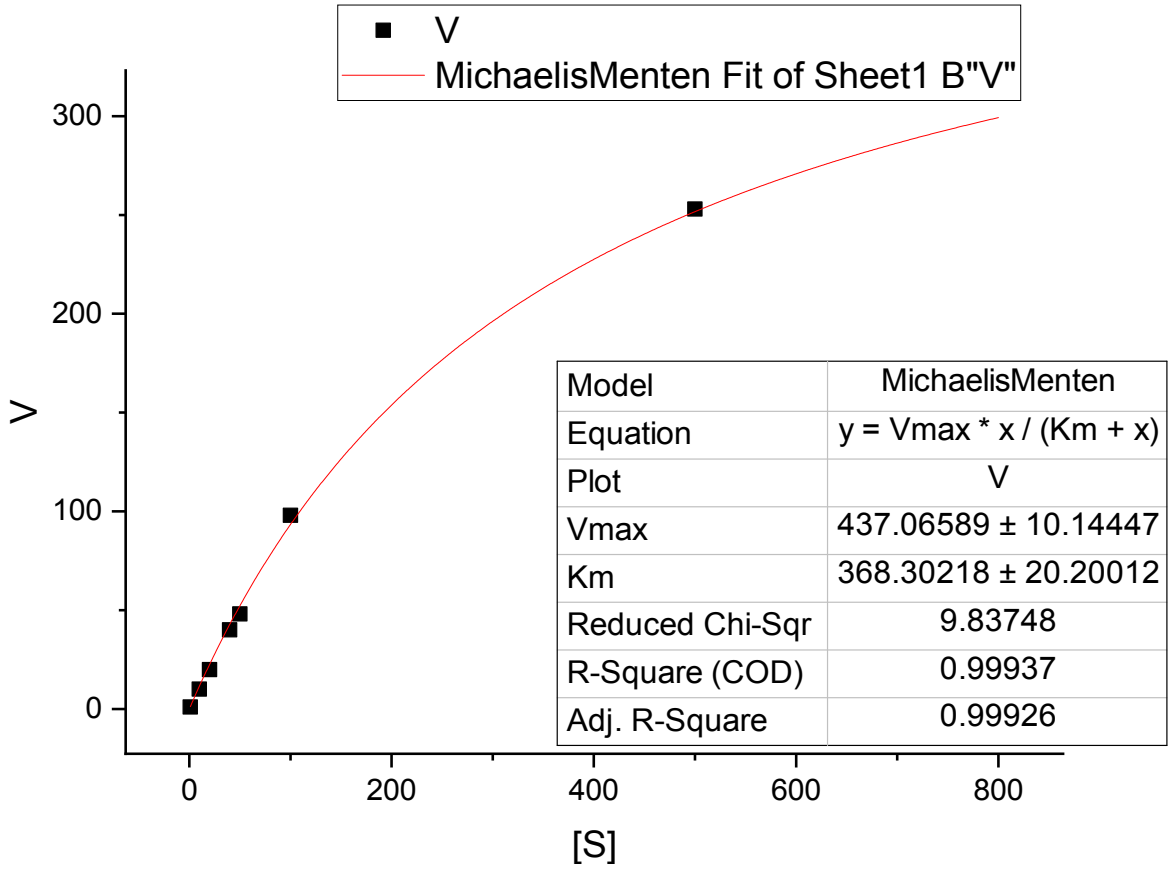


Figure S10. Michaelis–Menten kinetics curve of NucGT with **6** to generate **8**.

Table S2. kinetic data of NucGT with adenosine or 5'-O-sulfamyladenosine **6**

	Km (μM)	Vmax (μM·min^{-1})	kcat (S^{-1})	kcat/Km (S^{-1}·M^{-1})
Adenosine	305.104 ± 32.893	261.512 ± 11.094	7.68633	25192.57
6	368.302 ± 20.200	437.066 ± 10.144	12.84619	34879.51

References

1. X. Feng, D. Bello, P. Lowe, J. Clark, D. O'Hagan, *Chem. Sci.*, 2019,**10**, 9501-9505.

2. F. Egami and N. Takahashi, *Bull. Chem. Soc. Jpn.*, 1955, **28**, 666-668.