5'-Methylthioadenosine Nucleosidase is Implicated in Playing a Key Role in the Modified Futalosine Pathway for Menaquinone Biosynthesis in *Campylobacter jejuni*

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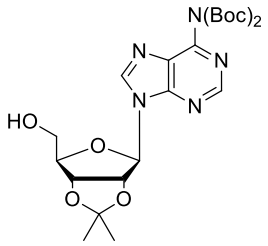
Content:

Materials and General Methods

Chemicals were purchased from Sigma-Aldrich and used without further purification unless otherwise noted. Dry solvents were distilled fresh, using CaH_2 (CH_2Cl_2 and MeOH) or Na/benzophenone (THF) as drying agents. ^1H NMR spectra were recorded on Bruker AV300 or AV400 spectrometers. Chemical shifts are reported in ppm with residual undeuterated solvents as the internal standard (referenced to 7.26 ppm for $CDCl_3$, and 4.79 ppm for D_2O). Mass spectra were obtained on a Waters Micromass LCT mass spectrometer using electrospray ionization (ESI-MS).

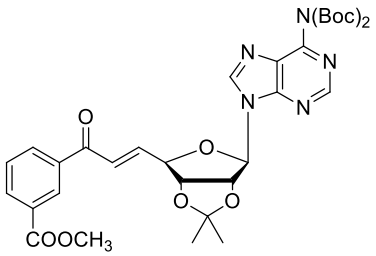
Synthetic Procedures

N^6,N^6-Bis(*tert*-butoxycarbonyl)-2',3'-*O*-isopropylideneadenosine (2)



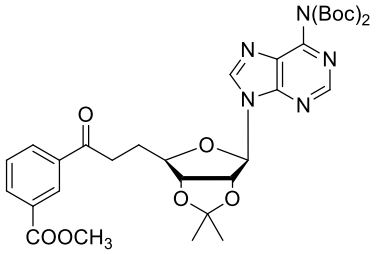
N^6,N^6-Bis(*tert*-butoxycarbonyl)-2',3'-*O*-isopropylideneadenosine (**2**) was synthesized according to the procedure previously described[1]. The overall yield from adenosine over four steps was 81%. ^1H NMR (400 MHz, $CDCl_3$) δ_H 1.38 (3H, s), 1.46 (18H, s), 1.65 (3H, s), 3.81 (1H, m), 3.98 (1H, d, J = 12.7 Hz), 4.54 (1H, d, J = 1.2 Hz), 5.11 (1H, dd, J= 6.0, 1.1 Hz), 5.21 (1H, t, J = 5.6 Hz), 5.37 (1H, dd, J = 10.9, 1.8 Hz), 5.95 (1H, d, J = 4.7 Hz), 8.14 (1H, s), 8.83 (1H, s); ESI-MS for $C_{23}H_{33}N_5O_8$ [M+Na^+] calcd 530, found 530.

Compound 3



To a stirred solution of N^6,N^6-Bis(*tert*-butoxycarbonyl)-2',3'-*O*-isopropylideneadenosine (**2**) (760 mg, 1.5 mmol) and 2-(3-methoxycarbonylphenyl)-2-oxoethyltriphenyl-phosphorane[2] (2.6 g, 6.0 mmol, 4 equiv.) in dry CH_2Cl_2 (15 mL) was added Dess-Martin periodinane (1.9 g, 4.5 mmol, 3 equiv.) at 0 °C. The mixture was allowed to stir at room temperature for 12 h. After dilution with 30 mL EtOAc, the mixture was filtered. The filtrate was subsequently washed with a mixture of saturated $Na_2S_2O_3$ solution and saturated $NaHCO_3$ solution (1:1) followed by water and brine. It was then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by column chromatography on silica gel (Et_2O/CH_2Cl_2 1:10) to yield **3** (397 mg, 40%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.42 (3H, s), 1.44 (18H, s), 1.67 (3H, s), 3.94 (3H, s), 4.96 (1H, t, *J* = 4.1 Hz), 5.20 (1H, dd, *J* = 6.3, 4.0 Hz), 5.51 (1H, dd, *J*= 6.3, 2.0 Hz), 6.23 (1H, d, *J* = 2.1 Hz), 7.01 (1H, dd, *J* = 15.5, 1.1 Hz), 7.13 (1H, dd, *J* = 15.4, 5.0 Hz), 7.52 (1H, t, *J* = 7.8 Hz), 7.94 (1H, d, *J* = 7.8 Hz), 8.17 (1H, s), 8.21 (1H d, *J* = 7.8 Hz), 8.49 (1H, s), 8.83 (1H, s); ESI-MS for $C_{33}H_{39}N_5O_{10}$ [M+Na^+] calcd 688, found 688.

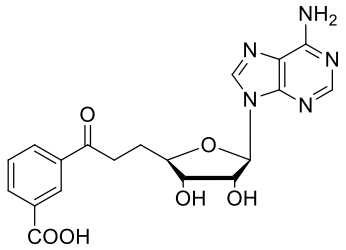
Compound 4



Compound **3** (397 mg, 0.6 mmol) was stirred with 10% palladium on charcoal (50 mg) in methanol (10 mL) at room temperature under 1 atm of hydrogen gas for 12 h. The reaction mixture was filtered through a pad of celite and concentrated to afford a yellow glass. Purification of the crude product on a silica gel column (Et_2O/CH_2Cl_2 1:10) yielded **4** (358 mg, 90%) as a colorless glass. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.40 (3H, s), 1.46 (18H, s), 1.62 (3H, s), 2.22 (2H, m), 3.12 (2H, m), 3.95 (3H, s), 4.32 (1H, m), 4.94 (1H, dd, *J* = 6.5, 4.1 Hz), 5.48 (1H, dd, *J* = 6.5, 2.5 Hz), 6.10 (1H, d, *J* = 2.5 Hz), 7.52 (1H, t, *J*

= 7.8 Hz), 8.09 (1H, d, *J* = 7.8 Hz), 8.14 (1H, s), 8.21 (1H, d, *J* = 7.7 Hz), 8.56 (1H, s), 8.86 (1H, s); ESI-MS for $C_{33}H_{41}N_5O_{10}$ [M+Na^+] calcd 690, found 690.

6-Amino-6-deoxyfutalosine



Compound **4** (358 mg, 0.54 mmol) was dissolved in 50% trifluoroacetic acid in CH_2Cl_2-H_2O (4:1, 5 mL). The reaction was allowed to stir at room temperature for 2.5 hours before solvents were evaporated. The crude product was purified by column chromatography on silica (CH_2Cl_2/MeOH, 20:1) to afford 6-amino-6-deoxyfutalosine methyl ester as a white solid. The methyl ester was subsequently dissolved in 0.2 M LiOH (5 mL), and was stirred at room temperature for 1 h before pH was adjusted to 7 with 1M HCl. Removal of solvent afforded the crude product, which was subsequently purified using a Waters Sep-Pak C18 column to give 6-amino-6-deoxyfutalosine as a white solid (121 mg, 54%).[1] ^{1}H NMR (300 MHz, D_2O) δ_H 2.30 (2H, m), 3.12 (2H, m), 4.27 (1H, m), 4.44 (1H, t, *J* = 5.0 Hz), 4.92 (1H, t, *J* = 5.0 Hz), 5.93 (1H, d, *J* = 4.8 Hz), 7.49 (1H, t, *J* = 8.0 Hz), 7.94 (1H, d, *J* = 7.9 Hz), 8.09 (2H, m), 8.20 (1H, s), 8.31 (1H, s); ESI-MS for $C_{19}H_{19}N_5O_6$ [M+Na^+] calcd 436, found 436.

1. Bioorganic & Medicinal Chemistry, **17**, 6641-6650 (2009)

2. Tetrahedron Lett. **51**, 6463-6465 (2010)

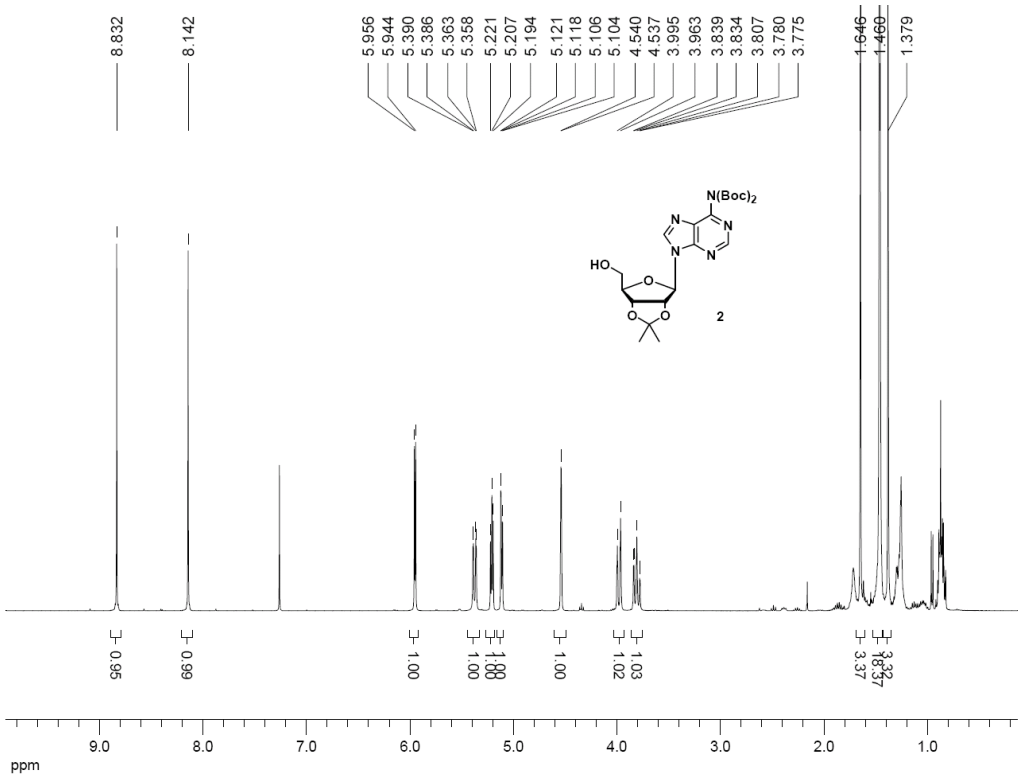


Figure S1. ^{1}H NMR spectrum of compound **2** ($CDCl_3$, 400 MHz).

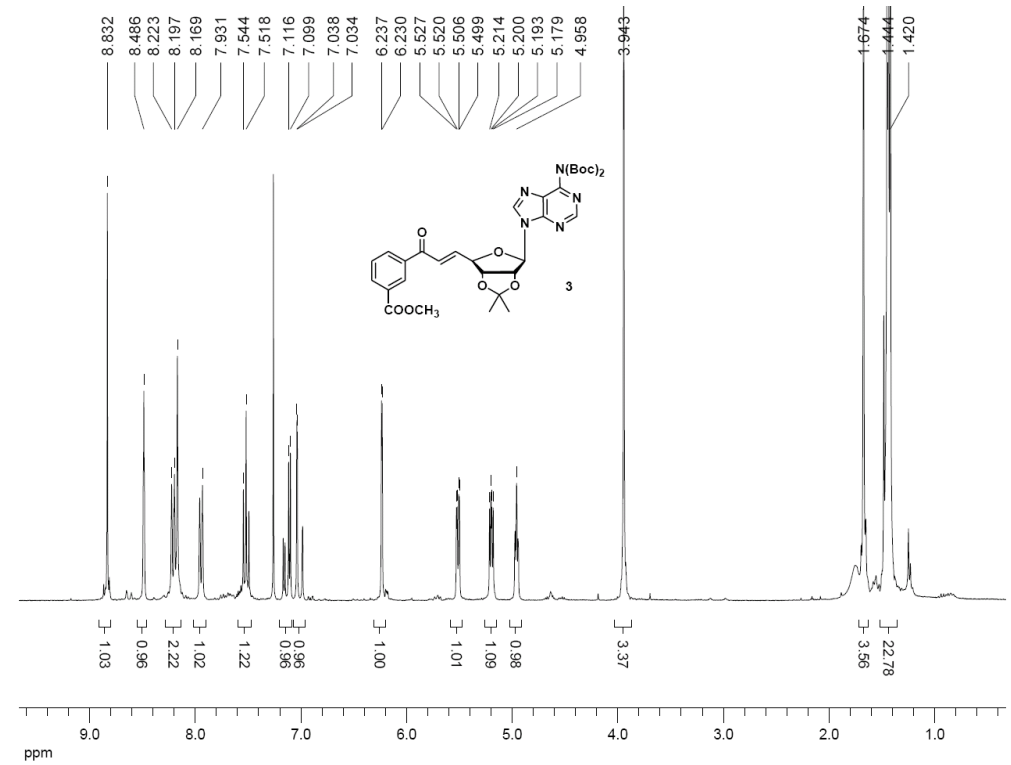


Figure S2. ^{1}H NMR spectrum of compound **3** ($CDCl_3$, 300 MHz).

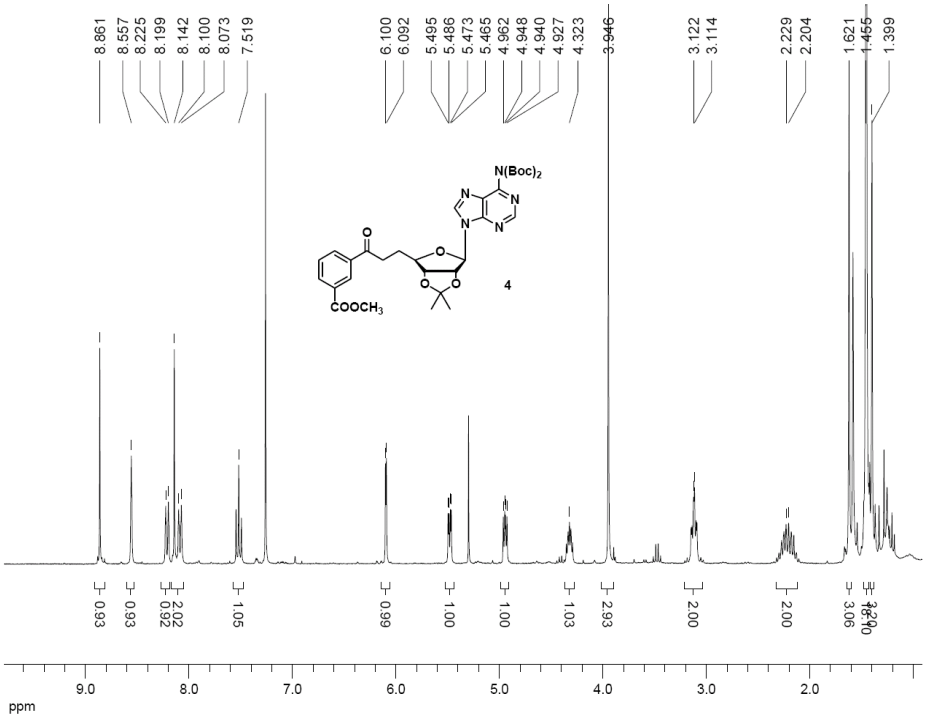


Figure S3. ^{1}H NMR spectrum of compound **4** ($CDCl_3$, 300 MHz).

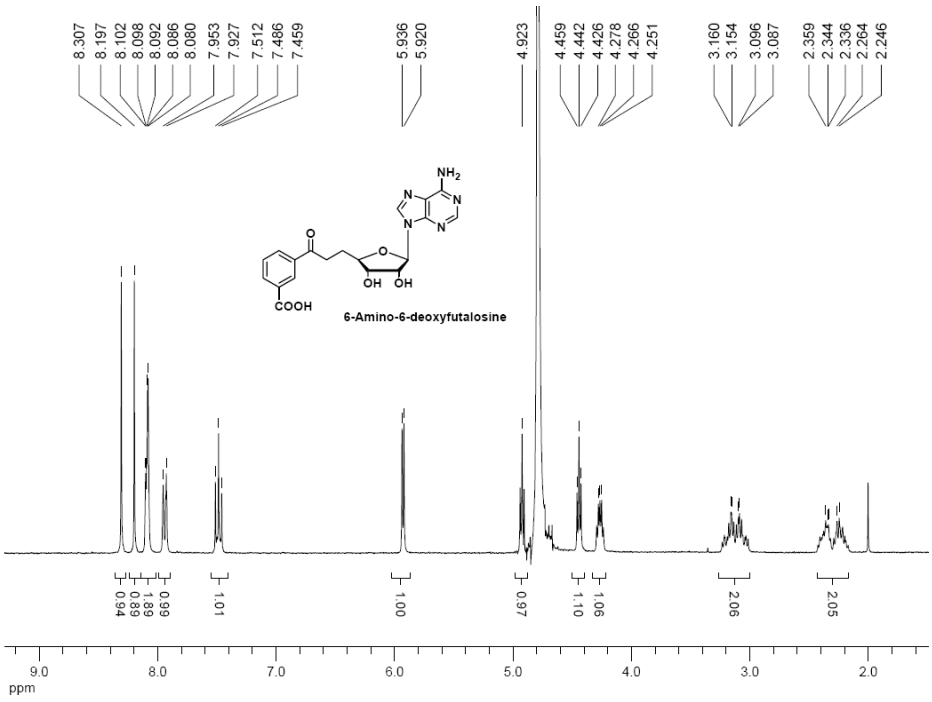


Figure S4. ^{1}H NMR spectrum of 6-amino-6-deoxyfutalosine (D_2O, 300 MHz).

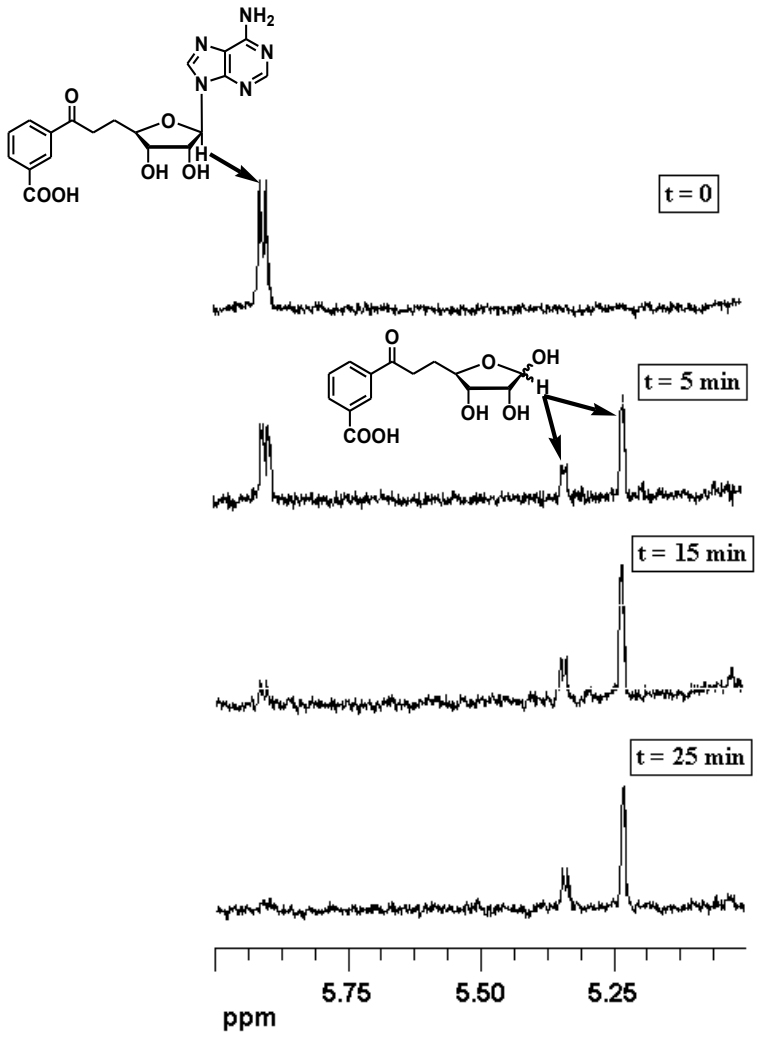


Figure S5. ^{1}H NMR spectral time course showing the conversion of 6-amino-6-deoxyfutalosine into de-hypoxanthine futalosine (DHFL). The signals corresponding to the anomeric ribose proton are displayed.

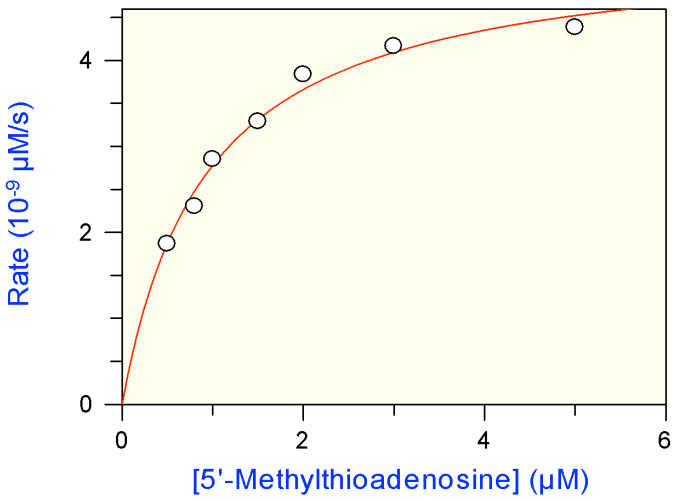


Figure S6. Plot of rate versus substrate concentration for the *C. jejuni* MTAN-catalyzed hydrolysis of 5'-methylthioadenosine. The data has been fitted to the Michaelis-Menten equation.

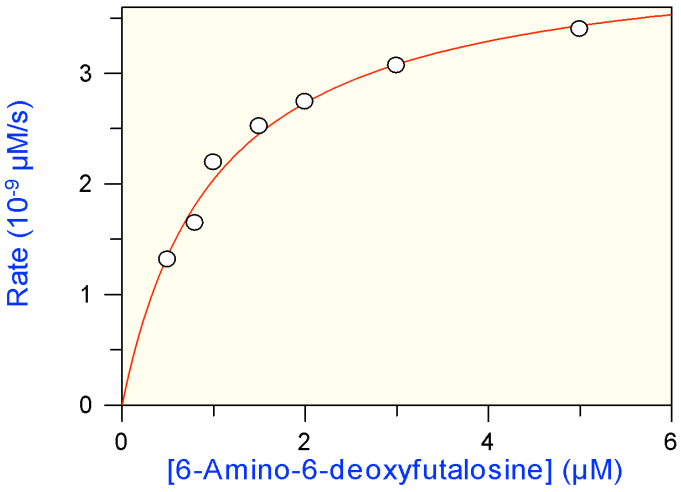


Figure S7. Plot of rate versus substrate concentration for the *C. jejuni* MTAN-catalyzed hydrolysis of 6-amino-6-deoxyfutalosine. The data has been fitted to the Michaelis-Menten equation.