

Synthesis and Biological Evaluation of a Highly Constrained Analogue of Methylthioadenosine (MTA)

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ABSTRACT

We describe the synthesis and the antibacterial evaluation 2',N³-cyclonucleoside **3** analogue of MTA that is characterized by the presence of an additional linkage between the heterocyclic ring and the sugar moiety.

Keywords: Synthesis; Biological Evaluation; Antibacterial; Analogue Methylthioadenosine

1. Introduction

During the last few years, physicians have been faced with little solutions to care bacterial infections and in particular those due to the advent of resistant bacteria such as MRSA (methicillin-resistant *Staphylococcus aureus*) [1-3].

Up to now, antibacterial drug discovery has constantly endeavored to propose to the medical community compounds exhibiting a great selectivity towards pathogens without causing any harm to patients [4]. During the recent decades, investigations have been directed to various microbe specific enzymes that played a key role in the metabolism of pathogens [5-7].

Among others, the case of 5'-methylthioadenosine/S-adenosylmethionine nucleosidase is of utmost importance. Indeed, the recovery of 5'-deoxy-5'-methyl-thioadenosine and S-adenosylhomocysteine through the S-adenosylmethionine metabolic pathway is crucial to mammals as well as to various microorganisms for the synthesis of polyamines and the methylation of macromolecules (**Figure 1**) [8-10].

A number of fundamental investigations have disclosed the differences between the metabolic pathways of mammals and those of lower microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Enterococcus faecalis*, *Mycobacterium tuberculosis*, among others. These studies showed that 5'-methyl-

thioadenosine/S-adenosylhomocysteine (MTA/AdoHcy) nucleosidase is specific for many microbes; whereas a corresponding phosphorylase is active in mammals (**Figure 1**). Accordingly, the dual substrate enzyme, MTA/AdoHcy nucleosidase, may be an ideal target for the design of specific antimicrobial drugs [11-13].

To date, some nucleoside analogues of MTA, either naturally derived or fully synthetic, acting as inhibitors of MTA/AdoHcy nucleosidase, have been discovered (**Figure 2**) [13-15].

These nucleoside analogues feature a great variety of chemical structures such as derivatives of acyclic nucleosides, L-nucleosides, carbocyclic nucleosides and many others [16,17].

Herein, we propose the construction of cyclonucleosides that are characterized by the presence of an additional linkage between the heterocyclic ring and the sugar moiety. In this regards, it is noteworthy that cyclonucleosides have been given little attention in view of their application in the medicinal domain [16,17].

Recently, we have defined an efficient access to 2', N³-cyclonucleosides derived from adenosine as shown in **Figure 3** [18].

In continuation of this work, we have now synthesized the 2',N³-cyclonucleoside **3** analogue of MTA. Our consideration was that if this rigidified MTA analogue could be a substrate of MTA/AdoHcy nucleosidase, it might release a 5-methylthioribose unit having an adenine re-

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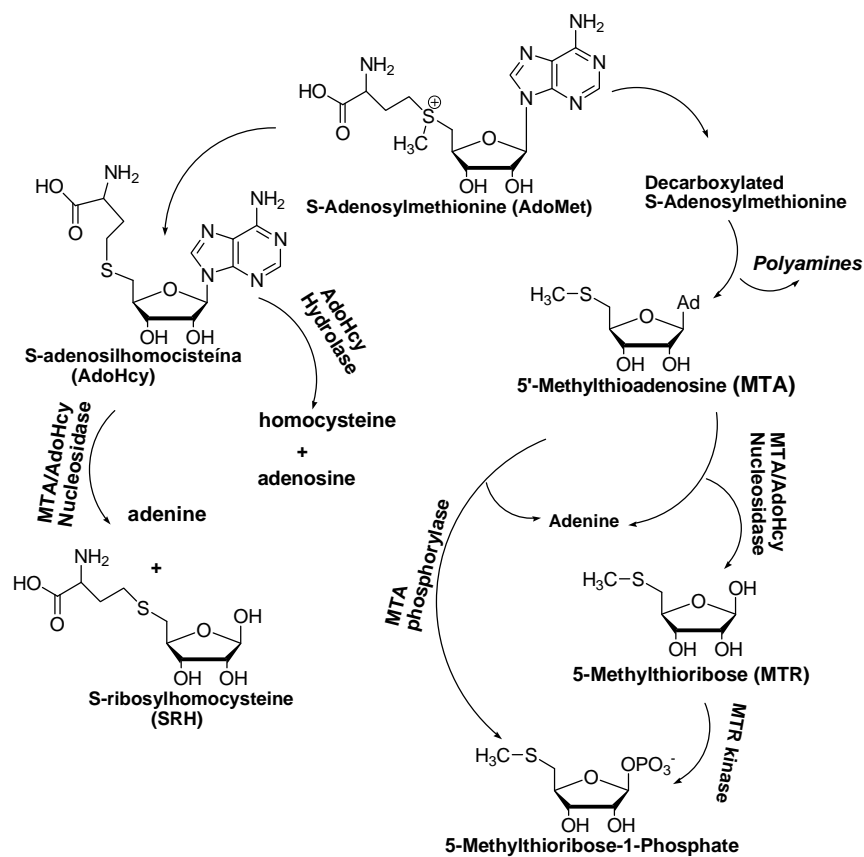


Figure 1. Metabolic pathway of S-adenosylmethionine.

maining attached to the ribose moiety, thus depriving the cell of the necessary MTR (Figures 1 and 4).

2. Experimental Section

2.1. Chemistry

General information. All chemicals were purchased as reagent grade and used without further purification, unless otherwise noted. Acetonitrile was distilled over CaH_2 . DMF was stirred with $\text{Ca}(\text{OH})_2$ and distilled under reduced pressure. Reactions were monitored with CCDS and visualized under UV (254 nm) and/or by exposure at H_2SO_4 20% in ethanol. Column chromatography was performed on silica gel (0.6 - 0.2 mM). ^1H NMR and ^{13}C NMR spectra were recorded on Bruker 300 MHz spectrometer and/or Bruker 500 MHz spectrometer at 25°C . 2D NMR was recorded on Bruker 500 MHz spectrometer at 25°C .

Synthesis.

1,2-*O*-Isopropylidene-3,5-di-*O*-(*p*-toluenesulfonyl)- α -*D*-xylofuranose **8**

To a solution 1,2-*O*-isopropylidene- α -*D*-xylofuranose **7** (5.00 g, 26.30 mmol) in CH_2Cl_2 (70 mL) were added DMAP (10.90 g, 89.38 mmol) and *p*-toluenesulfonyl chloride (13.00 g, 68.35 mmol) at 0°C . The resulting so-

lution was stirred 16 h at RT. Extraction with dichloromethane and evaporation gave a residue that was purified by silica gel column chromatography. Elution with a mixture of hexane/AcOEt (70:30), gave compound **8** as a white solid (9.2 g, 18.45 mmol, 70%) mp 101.0°C - 102.0°C ; ^1H NMR (300 MHz, CDCl_3) δ (ppm), J (Hz): 1.26 and 1.42 (2 s, 6 H, 2 CH_3 , Ip), 2.45 and 2.48 (2 s, 6 H, 2 CH_3 , Ts), 3.99 - 4.05 (dd, 2 H, H-5a and H-5b, $J_1 = 6.5$ and $J_2 = 4.0$), 4.32 (m, 1 H, H-4), 4.67 (d, 1 H, H-2, $J = 3.5$), 4.76 (d, 1 H, H-3, $J = 2.7$), 5.86 (d, 1 H, H-1), 7.33 - 7.41 (m, 4 H, CH, *m*-Ts), 7.71 - 7.80 (m, 4 H, CH, *o*-Ts); ^{13}C NMR (75 MHz, CDCl_3) δ (ppm): 21.8, 21.9 (2 CH_3 , Ts), 26.4 and 26.7 (2 CH_3 , Ip), 66.1 (C-5), 76.4 (C-4), 81.4 (C-3), 83.2 (C-2), 104.9 (C-1), 112.9 (C, Ip), 128.2, 130.1, 130.5, 132.3, 132.5, 145.4, 146.1 (C, Ts).

1,2-*O*-Isopropylidene-5-deoxy-5-thiomethyl-3-*O*-(*p*-toluenesulfonyl)- α -*D*-xylofuranose **9**

To a DMF (15 mL) solution of **8** (8.0 g, 16.05 mmol), was added sodium methylsulfide (1.7 g, 24.26 mmol). The reaction mixture was stirred at reflux for 3h, filtered, extracted with AcOEt and evaporation gave a residue that was purified by silica gel column chromatography. Elution with hexane/AcOEt (9:1) gave compound **9** (4.8 g, 12.84 mmol, 80%) as yellow oil; ^1H NMR (300 MHz, CDCl_3) δ (ppm), J (Hz): 1.22 and 1.42 (2 s, 6 H, 2 CH_3 ,

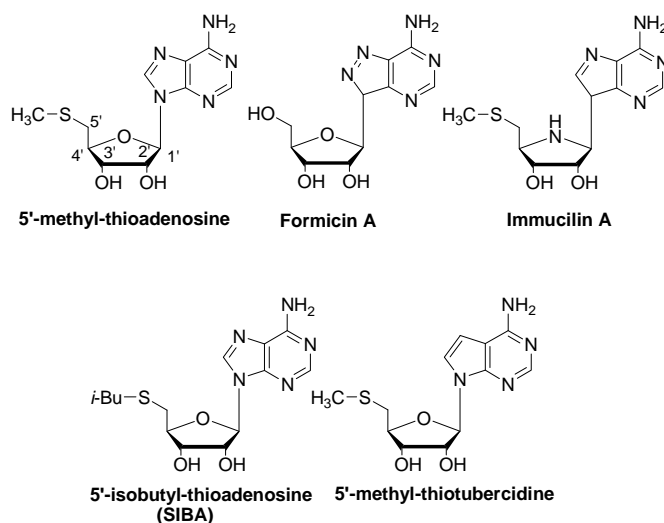


Figure 2. MTA and analogues inhibitors of MTA/AdoHcy nucleosidase.

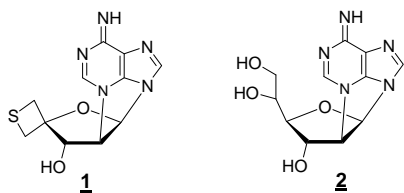


Figure 3. Recent examples of constrained 2',N³-cyclonucleosides.

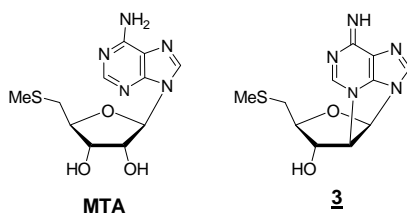


Figure 4. Comparison between MTA and its closer 2',N³-cyclonucleoside (3) analogue.

Ip), 2.00 (s, 3 H, SMe), 2.39 (s, 3 H, CH₃, Ts), 2.43 - 2.48 (dd, 1 H, H-5a), 3.18 - 3.20 (dd, 1 H, H-5b, $J_1 = 6.0$ and $J_2 = 3.0$), 4.24 - 4.29 (m, 1 H, H-4), 4.64 (d, 1H, H-2, $J = 3.0$), 4.78 (s, 1 H, H-3), 5.84 (d, 1 H, H-1), 7.31 (d, 2 H, CH, *m*-Ts, $J = 8.0$), 7.75 (d, 2 H, CH, *o*-Ts); ¹³C NMR (75 MHz, CDCl₃) δ (ppm): 16.2 (CH₃, SMe), 21.6 (CH₃, Ts), 26.1 and 26.5 (2 CH₃, Ip), 31.2 (C-5), 78.7 (C-4), 82.2 (C-3), 82.9 (C-2), 104.6 (C-1), 112.2 (C, Ip), 127.9 (CH, *o*-Ts), 130.1 (CH, *m*-Ts), 132.7 (C-SO₂-O, Ts), 145.5 (C-CH₃, Ts).

1,2-di-O-Acetyl-5-deoxy-5-thiomethyl-3-O-(*p*-toluenesulfonyl)-D-xylofuranose **10**

Sulfuric acid (95% - 98%) (10 drops) was added to a solution of **9** (3.0 g, 8.01 mmol) in a mixture of acetic acid (15 mL) and acetic anhydride (15 mL) under ice bath. After 4 h at RT the mixture was added to water.

The solution was neutralized with aq. NaHCO₃ and extracted with dichloromethane. The dried organic phase was evaporated and the residue purified by silica gel column chromatography. Elution with a mixture of hexane/AcOEt 80:20 gave the mixture **10** (1.61 g, 3.85 mmol, 60%), as a yellow syrup; ¹H NMR (300 MHz, CDCl₃) δ (ppm), J (Hz): 2.05 - 2.13 (m, 18 H, 4 CH₃, 2 Ac and 2 SMe), 2.47 (sl, 6 H, 2 CH₃, 2 Ts), 2.72 - 2.77 (m, 4 H, 2 CH₂S), 4.48 - 4.55 (m, 2 H, 2 H-4), 4.96 (d, 1 H, H-2 α), 5.17 (m, 2 H, H-3 β and H-2 β), 5.30 (s, 1 H, H-3 α), 6.02 (s, 1 H, H-1 β), 6.35 (d, 1 H, H-1 α), 7.37-7.39 (m, 4 H, CH, *m*-Ts), 7.84 - 7.86 (m, 4 H, CH, *o*-Ts); ¹³C NMR (75MHz, CDCl₃) δ (ppm): 16.4, 16.9, 20.1, 20.6, 20.7, 20.9, 21.1 and 21.8 (CH₃, Ac, SMe, CH₃, Ts), 33.1 and 33.3 (2 C-5), 76.0, 78.6, 78.9, 79.9, 80.9 and 82.3 (2 C-2, 2 C-3 and 2 C-4), 93.0 and 98.7 (2 C-1), 128.1 and 128.2 (2 CH, *o*-Ts), 130.1 (CH, *m*-Ts), 133.0 (C-SO₂-O, Ts), 145.7 (C-CH₃, Ts), 168.8, 169.0, 169.1 and 169.3 (4 C=O, Ac).

*N*⁶-Benzoyl-9-[2'-O-acetyl-5'-deoxy-5'-thiomethyl-3'-O-(*p*-toluenesulfonyl)- β -D-xylofuranosyl]-adenine **11**

A suspension of *N*⁶-benzoyladenine (300 mg, 1.25 mmol) in HMDS (4.0 mL) and 0.5 mL of TMSCl was refluxed for 18 h. The reaction was cooled and evaporated to dryness under vacuum. Then **10** (500 mg, 1.20 mmol), in anhydrous acetonitrile (5 mL) containing a catalytic amount of TMSOTf (0.2 mL, 1.10 mmol), was added to the silylated 6-*N*⁶-benzoyladenine under an argon atmosphere.

After stirring for 24 h, TLC analysis (AcOEt) showed conversion of the starting carbohydrate into a lower-running product. The reaction mixture was quenched with a saturated solution of NH₄Cl (3 mL) and stirred for 30 min, diluted with ether (30 mL) and poured in aq. NaHCO₃ (5 mL).

The organic phase was washed with water (5 mL), dried, filtered and evaporated. The reaction product was purified by silica gel column chromatography. Elution with a mixture of hexane/AcOEt 9:1, to give 180 mg of compound **11**, as a brown amorphous solid 40% yield; mp: 107.1°C - 109.3°C; ¹H NMR (300 MHz, CDCl₃) δ (ppm), *J* (Hz): 2.16 and 2.17 (m, 6 H, CH₃, Ts and SMe), 2.38 (s, 3 H, CH₃, Ts), 2.81 - 2.97 (qd, 2 H, H-5'a and H-5'b, *J*₁ = 13.0, *J*₂ = 6.0), 4.50 - 4.55 (m, 1 H, H-4'), 5.15 (d, 1 H, H-2', *J* = 3.7), 5.38 (s, 1 H, H-3'), 6.20 (d, 1 H, H-1'), 7.23 (d, 2 H, CH, *m*-Ts, *J* = 7.0), 7.53 (t, 2 H, *m*-Bz, *J* = 7.0), 7.57-7.60 (m, 1 H, CH, *p*-Bz), 7.69 (d, 2 H, CH, *o*-Ts, *J* = 8.0), 8.05 (d, 2 H, *o*-Bz, *J* = 7.0), 8.20 (s, 1 H, H-2), 8.67 (s, 1 H, H-8), 9.35 (bs, 1 H, N-H); ¹³C NMR (750MHz, CDCl₃) δ(ppm): 16.8 (SMe), 20.8 (CH₃, Ac), 21.9 (CH₃, Ts), 31.9 (C-5'), 79.2 (C-4'), 80.6 (C-2'), 82.2 (C-3'), 87.9 (C-1'), 122.8 (C-5), 127.9 and 128.1 (CH, *m*-Bz and CH, *o*-Ts), 129.1 (CH, *o*-Bz), 130.1 and 130.2 (CH, *m*-Ts), 132.6 (C-C(=O)-Ph, Bz), 133.1 (CH, *p*-Bz), 133.5 (C-SO₂-O, Ts), 141.1 (C-8), 146.2 (C-CH₃, Ts), 149.4 (C-4), 151.3 (C-6), 152.7 (C-2), 165.1 (C=O, Bz), 168.8 (C=O, Ac). ESI-MS: [M + Na]⁺ 620.2; Anal. Calcd. for C₂₇H₂₇N₅O₇S₂Na.

2',*N*³-cyclo-9-[5'-deoxy-5'-*S*-methyl-β-D-arabinofuranosyl]-adenine **3**

A solution of **11** (200 mg, 0.34 mmol) in a mixture of MeOH (6 mL) and 25% NH₄OH in water (3 mL) was stirred at room temperature for 18 h. The solution was evaporated to dryness. The residue was purified by silica gel column chromatography. Elution with a mixture of CH₂Cl₂/MeOH 70:30 gave 83.40 mg of **3**, 89.23% yield; ¹H NMR (500 MHz, CDCl₃) δ (ppm): *J* (Hz): 2.09 (s, 3 H, SMe), 2.68 - 2.80 (m, 2 H, H-5'a and H-5'b), 4.19 (s, 1 H, H-2'), 4.45 - 4.47 (m, 2 H, H-3' and H-4'), 5.86 (sl, 2 H, O-H and N-H), 6.17 (s, 1 H, H-1'), 7.96 (s, 1 H, H-8), 8.35 (s, 1 H, H-2); ¹³C NMR (100 MHz, CDCl₃) δ (ppm): 16.3 (SMe), 35.3 (C-5'), 58.0 (C-2'), 59.9 (C-3'), 79.9 (C-4'), 84.5 (C-1'), 120.5 (C-5), 140.1 (C-8), 149.5 (C-4), 153.3 (C-2), 155.8 (C-6); ESI-MS: [M + Na]⁺ 302.0; Anal. Calcd. for C₁₁H₁₃N₅O₂SNa (302.1). HMRS: [M + Na]⁺ 302.0694; Anal. Calcd. for C₁₁H₁₃N₅O₂SNa (302.0682).

2.2. Biology

Antibacterial activity.

As Broth Dilution Method [19] recommended by the Clinical Laboratory Standard Institute, the antibacterial activity of the compound in question was investigated *in vitro* against eight ATCC strains (American Type Culture Collection): 4 Gram negative (*Klebsiella pneumoniae* ATCC 13866, *Salmonella typhi* ATCC 19430, *Escherichia coli* ATCC 11229, *Pseudomonas aeruginosa* ATCC 27853 and 4 Gram positive (*Staphylococcus epidermidis*

ATCC 12228, *Staphylococcus aureus* ATCC 25923, *Micrococcus lentus* ATCC 10240, and *Enterococcus faecalis* ATCC 51299).

Microorganisms were cultured aerobically in broth Triptych Soy Agar (Difco) at 35.5°C for 24 h for inoculation, each was adjusted to 0.5 McFarland turbidity (1.5 × 10⁸ CFU/ml) with sterile saline (NaCl 0.85%). The compound tested **3** was dissolved in DMSO for the purchase of two different concentration (1.79 mM and 11.81 mM) and solutions were diluted with Mueller Hinton Broth (Difco) to obtain concentrations 0.023 mM and 1.833 mM in a final volume of 3 mL. The tubes were inoculated with 100 mM of bacterial culture and incubated aerobically at 35.5°C/24 h. The experiment was done in triplicate. Control experiments were performed using only DMSO.

3. Results and Discussion

3.1. Chemistry

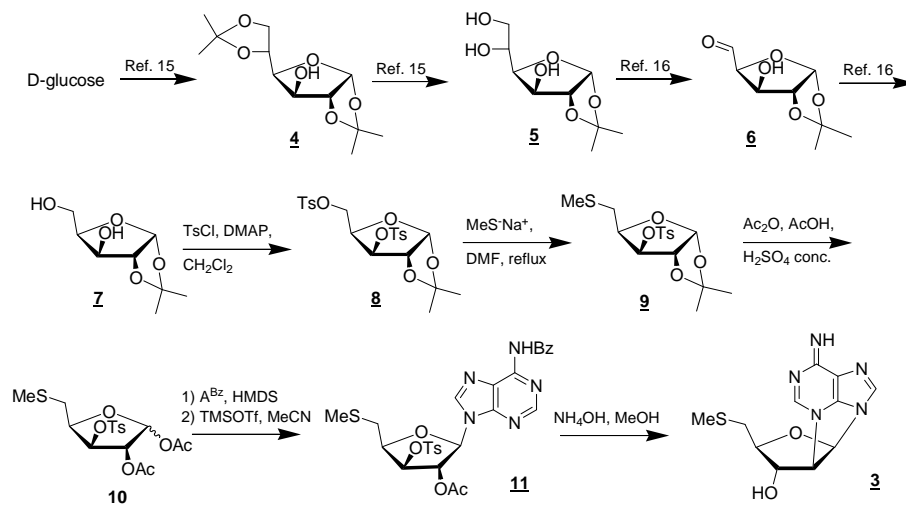
Synthesis and characterization of 2',N³-cyclo-5'-deoxy-5'-methyl-thioadenosine 3 (Scheme 1)

First, diisopropylidene glucose was selectively hydrolyzed to give the known triol **5** [18]. After an oxidative cleavage step, the resulting aldehyde **6** was reduced with NaBH₄ to give diol **7** [20]. A solution of this compound in dichloromethane was treated with 3 eq. of *p*-toluenesulphonyl chloride in the presence of DMAP to provide the ditosylated compound **8**.

A subsequent treatment of compound **8** with sodium methylsulfide in DMF at 120°C led to derivative **9**. The spectral data (NMR) confirmed that compound **9** was monotosylated in agreement with the presence of one methyl singlet at δ 2.39 ppm, whereas the methyl singlet at δ 2.00 ppm was due to the presence of the MeS-group at the C-5 position of ribose that is confirmed by viewing the new pattern of the signals ascribed to the methylene at C-5.

In the next step, compound **9** was engaged in the necessary transformations to continue with the introduction of *N*⁶-benzoyladenine at the anomeric position using the Vorbrüggen's method. Thus, removal of the isopropylidene group and *in situ* acetylation of the resulting diol was accomplished using a mixture of acetic anhydride/acetic acid containing a catalytic amount of concentrated sulfuric acid. This procedure furnished the diacetate **10** as a 1:1 mixture of *α/β* diastereoisomers.

Then, the mixture **10** was combined with a previously silylated *N*⁶-benzoyladenine in the presence a TMS-triflate in acetonitrile under argon atmosphere to provide nucleoside **11**. Due to the formation of an intermediate acyloxonium, there is no need to separate the *α/β* diastereoisomers of mixture **10**, the final product being the *β*-isomer, exclusively. The spectral data (NMR) of the



Scheme 1. Reaction pathway for the synthesis of the 2', N^3 -cyclonucleoside (**3**) analogue of MTA.

protected nucleoside **11** are in agreement with the proposed structure as shown by the presence of the H-1' sugar doublet signal at δ 6.20 ppm and the H-2 singlet signal of adenine at δ 8.20 ppm.

Finally, as expected from our previous report, the treatment of precursor **11** in a solution of $\text{NH}_4\text{OH}/\text{MeOH}$ 1:3 at room temperature gave rise to a cyclization reaction together with adenine debenzoylation leading to compound **3** in 60% yield. The structure of cyclonucleoside **3** was confirmed by the interpretation of its MS and NMR data, including HMBC and NOESY spectra (Table 1).

All compounds have been characterized by IR and NMR spectroscopy. When needed, we utilized 2D NMR spectroscopy.

3.2. Bioassays

Antibacterial activity.

The antibacterial activity of 2', N^3 -cyclo MTA **3** was further evaluated *in vitro* against eight bacteria, in which four Gram positive (*Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 51299 and *Micrococcus lentus* ATCC 10240) and four Gram negatives (*Escherichia coli* ATCC 11229, *Salmonella typhi* ATCC 19430, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae* ATCC 13866). Overall, the MIC ranged between 0.023 mM and 1.833 mM.

Compound **3** showed activity at concentrations above 1.0 mM for all tested bacteria strains, minimal inhibitory concentrations were not determined because the compound precipitated in the culture media above 1.0 mM.

4. Conclusion

Compound 2', N^3 -cyclonucleoside (**3**) analogue of MTA

Table 1. Data of ^1H , ^{13}C , HMBC and NOESY for compound **3**.

Position	^{13}C δ (3)	^1H δ (3) (mult, J in Hz)	HMBC ^1H - ^{13}C (3)	NOESY ^1H - ^1H (3)
2	153.3	8.35 (s; 1H)	4 and 6	-
4	149.5	-	-	-
5	120.5	-	-	-
6	155.8	-	-	-
8	140.1	7.96 (s; 1H)	4 and 5	1'
1'	84.5	6.17 (d; 1H; 5.6)	3', 4', 8 and 4	4' and 8
2'	58.0	4.19 (d; 1H; 5.6)	4'	5'b
3'	59.9	4.45 - 4.47 (m; 2H)	1'	5'a and 5'b
4'	80.0	4.45 - 4.47 (m; 2H)	2'	1', 5'a and 5'b
5'	35.3	3.00 (dd; 2H; 2.1; 9.6)	3'; 4' and MeS	2', 3', 4' and MeS

^aThe experiment was performed at 500 MHz for ^1H and 100 MHz for ^{13}C in CDCl_3 and TMS as internal (δ 0.00 ppm).

belonging to the little explored class of adenine 2', N^3 -cyclonucleosides has been synthesized. Preliminary biological investigations showed the compound to manifest a promising anti-bacterial activity against a number of representative bacterial strains. These results are promising since the constrained nucleoside appeared to be non-toxic in human MRC-5 cells.

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