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Synthesis of triazole-linked SAM-adenosine conjugates: functionalization of adenosine at N-1 or N-6 position without protecting groups

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Abstract: More than 150 RNA chemical modifications have been identified to date. Among them, methylation of adenosine at the N-6 position (m^6A) is crucial for RNA metabolism, stability and other important biological events. In particular, this is the most abundant mark found in mRNA in mammal cells. The presence of a methyl group at the N-1 position of adenosine (m^1A) is mostly found in ncRNA and mRNA and is mainly responsible for stability and translation fidelity. These modifications are installed by m^6A and m^1A RNA methyltransferases (RNA MTases), respectively. In human, deregulation of m^6A RNA MTases activity is associated with many diseases including cancer. To date, the molecular mechanism involved in the methyl transfer, in particular substrate recognition, remains unclear. We report the synthesis of new SAM-adenosine conjugates containing a triazole linker branched at the N-1 or N-6 position of adenosine. Our methodology does not require protecting groups for the functionalization of adenosine at these two positions. The molecules described here were designed as potential bisubstrate analogues for m^6A and m^1A RNA MTases that could be further employed for structural studies. This is the first report of compounds mimicking the transition state of the methylation reaction catalyzed by m^6A RNA MTases.

Keywords: RNA m^6A methyltransferase; RNA m^1A methyltransferase; bisubstrate analogues; S-adenosyl-L-methionine; 1-N-alkylated adenosine; 1,2,3-triazole; click chemistry; CuAAC

1. Introduction

Among the numerous post-transcriptional modifications of RNA identified to date, methylation is currently one of the most studied [1]. This modification can occur at the terminal cap of RNAs or at internal position, and the methyl group is found on nucleic acid bases or at the 2’ position of the ribose units. The adenine base can be methylated at the C-2 and C-8 atoms as well as at the nitrogen atoms N-1 and N-6 [2,3]. N^6-Methyladenosine (m^6A) is an abundant reversible modification found in all types of RNA, involved in the regulation of RNA metabolism, protein expression or RNA-protein recognition [4-7]. Abnormal methylation process is associated with the development of diseases such as cancers, obesity, infertility [8-10]. Though less studied, the modification at the N-1 site (m^1A) is also reversible and linked to the structural stability and the functions of RNAs [11-12].
Since m^1A can rearrange to m^6A under alkaline conditions by Dimroth rearrangement [13], its presence in mRNA has been detected only recently in mammalian mRNAs [14-16]. The functional consequence of this modification is poorly understood but could impact the regulation and the function of m^6A for some positions in RNA and tRNA in the human transcriptome. However, the unique chemical properties of m^1A, with both a positive charge and a methyl group, potentially allow for a strong effect in terms of RNA structure or protein-RNA interaction [14].

For these two modifications (m^1A and m^6A), the methyl group is introduced enzymatically by RNA methyltransferases (RNA MTases) that catalyze the transfer of the methyl from the cofactor S-adenosyl-L-methionine (SAM) to the nucleotides (Scheme 1). Most m^6A marks in mammals mRNA are written by a dedicated methyltransferase complex involving the heterodimer METTL3/METTL14, targeting the consensus sequence RRACH [17]. However, little is known about the recognition of the RNA targets by this complex [18]. METTL16 has a distinct set of targets for m^1A modification, including the 3’ UTR of MAT2A mRNA and the U6 snRNA, a longer conserved sequence of UACAGAGAA [19-21]. Among human m^6A RNA MTases, only METTL16 has been crystallized with RNA substrate [22]. For m^1A modification of mRNA it’s not clear whether or not specific methylation machinery could also exist. It has been recently hypothesized that the tRNA methyltransferase complex TRMT6/61A could catalyze the methyl transfer on the mRNA [16]. In conclusion, few RNA-bound MTases structures are currently available. This lack of complex structures is due to the difficulties in crystallizing RNA/protein complexes. As a consequence, RNA recognition patterns and methylation reaction mechanisms remain poorly understood.

![Scheme 1](image_url)

**Scheme 1.** RNA MTases-catalyzed methylation of adenosine at the N-6 and N-1 positions.

In this context, we recently described the synthesis of SAM-adenosine conjugates as first transition state analogues for m^6A RNA MTases and their use as tools for structural study [23,24]. We showed that a SAM-adenosine conjugate containing a three-carbon linker tethering the analogue of SAM to the N-6 atom of the adenosine binds the bacterial RNA MTase RlmJ with a conformation close to the real transition state. The structure of this bisubstrate analogue favors the correct positioning of the RNA moiety mimicked by an adenosine and the methionine part of the cofactor into the catalytic site of the MTase. However, a deviation was observed for the positioning of the adenosine in the cofactor part, which is rotated 120° out of the canonical binding pocket for SAH. This deviation indicates that our bisubstrate analogues are not optimal. In this study, we pursue the development of SAM-adenosine conjugates for m^6A RNA MTases and extend our work to the
synthesis of the first potential bisubstrate analogues for m1A RNA MTases by covalently linking an analogue of SAM to the N-6 or N-1 atom of the adenosine substrate, respectively.

N'-alkylation of adenosine derivatives is mainly achieved through aromatic nucleophilic substitution (SnAr) of diverse electrophilic adenosine derivatives [25-37] or by Dimroth rearrangement of 1-N-alkylated adenosines [38-50]. In the context of SnAr, synthetic strategies involving non classic leaving groups were developed using peptide-coupling agents for activation of the amide group of inosine derivatives [51-54]. Another study reports the reduction of N'-acyl-adenosine derivatives with LiAlH4 [55]. Selective N'-alkylation of adenosine derivatives can also be achieved under phase transfer catalysis conditions as described by Arimoto et al. [56]. Two groups used Mitsunobu reaction applied to N'-acetyl-2',3',5'-tri-O-acetyladenosine [57-58] or N'-Boc protected adenosine [59] to regioselectively synthesize N'-alkylated products. Finally, functionalization of 6-chloroadenosine derivatives can be achieved by palladium-catalyzed Buchwald–Hartwig coupling [60-61].

To date, the alkylation reaction at the N-1 position has been principally developed through the N-1 nucleophilic attack of alkyl halides [38-50]. This approach allows for the introduction of methyl and alkyl groups as well as benzyl, or allyl substituents. Propargyl group has been also installed on N'-acetyl-2',3',5'-tri-O-acetyladenosine but in a quite low yield of 14% [49]. In 2005, Terrazas et al. synthesized 1-N-alkylated adenosines by reacting electrophilic inosine with primary amines [62].

In this context, we sought to introduce new chemical modifications at the N-6 and N-1 positions of adenosine to synthesize new SAM-adenosine conjugates. Click chemistry, especially Copper(I)-catalyzed Alkyne-Azide Cycloaddition (CuAAC), is an efficient strategy to rapidly synthesize complex structures. On these bases, we designed new bisubstrate analogues bearing a 1,2,3-triazole ring instead of the alkyl linker to increase rigidity between the mimic of the substrate and the SAM analogue while maintaining an appropriate length between the two entities (Figure 1). The synthetic strategy used to obtain these molecules relies on the efficient introduction of the propargyl group at the N-6 and N-1 positions of adenosine derivatives.

**Figure 1.** (A) Our previous work: Structure of SAM-adenosine conjugates with an alkyl linker [23-24]; (B) Structure of SAM-adenosine conjugates with a 1,2,3-triazole linker synthesized in this study.

2. Results and discussion
2.1. Synthesis of SAM-adenosine conjugates using protecting groups

First, we took advantage of the work of Sekine et al. who used tetrabutylammonium bromide (TBABr) as the phase transfer catalyst to produce a mixture of $N^\circ$ and 1-$N^\circ$-alkylated adenosines from $N^\circ$-benzoylated adenosine [56]. A short study was carried out to investigate the alkylation of $N^\circ$-benzoyl-2',3',5'-tris-O-(tert-butyldimethylsilyl)adenosine 1a [63] with propargyl bromide (Scheme 2). Using tetrabutylammonium hydroxide (TBAOH) instead of TBABr led to the formation of adenosines 2a and 3a in 57 and 28% yield with total conversion of the starting material (Scheme 2).

Analysis of 1D and 2D NMR spectra confirmed the site of alkylation for each regioisomer (Figure 2) [49,56]. The signals for 2-H and 8-H appear at 8.49 and 8.14 ppm for $N^\circ$-alkylated compound 2a while they are upfield at 8.24 and 7.88 ppm in 3a as expected. Moreover, two correlations are observed in the HMBC spectrum of 2a between protons of the methylene group of the propargyl (H$^p$) and C6 and C=O of benzoyl group in the $N^\circ$-regioisomer (Figure 2A). For compound 3a, HMBC experiments show a correlation between 2-H and the carbon of the methylene group (C$^p$) as well as two correlations between the H$^p$ protons and C2 and C6 (Figure 2B). The same reaction conditions applied to compound 1b [23] containing a tert-butoxycarbonyl protecting group at the N-6 position of adenosine afforded 2b and 3b in 76 and 16% yield respectively, the presence of the carbamate function favoring the $N^\circ$ alkylation.

Scheme 2. Synthesis of $N^\circ$ and 1-$N^\circ$-propargylated adenosines.
Azide 6 was synthesized in three steps [23] (Scheme 3). Briefly, removal of TBS group at the 5’ position of 1a led to the alcohol 4 that was mesylated to afford derivative 5 in 82% yield. Finally, treatment of 5 with sodium azide provided azido adenosine 6 in 92% yield.

Scheme 3. Synthesis of azide 6.

CuAAC reactions between adenosines 2a-b and 3a-b and azides 6 or 7 [23] were conducted under classic conditions in the presence of sodium ascorbate and copper sulfate in THF/H2O to afford triazoles 8-11 in 60-89% yield (Scheme 4).

Scheme 4. Synthesis of triazoles 8-11.
The fully deprotected SAM-adenosine conjugate 12 could be obtained from either 9a or 9b (Scheme 5). Compound 9a was successively treated with methylamine, ZnBr$_2$ and cesium fluoride (CsF) to remove the benzoyl-, the Boc- and the TBS groups respectively to give 12 in 5% yield over three steps after HPLC purification. In comparison, a two-step strategy from 9b, followed by HPLC purification, led to the formation of 12 in 4% yield. These results seem to indicate that in the pathway 2, the removal of the two Boc groups is less efficient than the two steps required for the deprotection of the benzoyl and Boc groups in the pathway 1 (Scheme 5).

**Scheme 5.** Synthesis of SAM-adenosine conjugate 12.

We applied the strategy used for the deprotection of compound 9a to compound 11a. Unfortunately, efforts to remove the protecting group of the exocyclic amine were unsuccessful. Indeed, using the same successive steps, we observed the formation of the N-methylated compound 13 in 8% yield as a mixture of E and Z imines (Scheme 6). Other attempts were conducted with bases such as ammonia or potassium carbonate which led to the recovery of the starting material in the first case and to degradation in the second one. As an alternative, we chose to remove only the Boc and the TBS groups in a two-step sequence allowing for the formation of SAM-adenosine conjugate 14 in 13% yield (Scheme 6).

**Scheme 6.** Access to SAM-adenosine conjugates 13 and 14.
By contrast and to our delight, treating derivative 11b with ZnBr₂ and then CsF afforded the fully deprotected SAM-adenosine conjugate 15 in 20% yield over two steps (Scheme 7). Of note, deprotection of compounds 8a and 10a using methylamine and then CsF provided compounds 12 and 14, respectively that could not be properly purified in these particular conditions.

\[\text{Scheme 7. Access to SAM-adenosine conjugate 15.}\]

2.2. Synthesis of SAM-adenosine conjugates without protecting groups

Since our syntheses require numerous steps of protection and deprotection, we reinvestigated the synthetic strategies to develop more efficient approaches to get the SAM-adenosine conjugates. We also sought to introduce regioselectively the propargyl group at the N-6 and N-1 positions. We first modified the synthesis of N-6-conjugate 12. Wan et al. previously reported the amination of unprotected inosine using primary amines in the presence of BOP and DIPEA [53]. Following this methodology, the propargyl group was introduced in one step at the N-6 position of adenosine leading to compound 16 in 71% yield (Scheme 8).

\[\text{Scheme 8. Access to SAM-adenosine conjugate 12.}\]

The unprotected azido partner 17 was prepared following a two-step procedure in 36% yield [64,65]. Then, alkyne 16 and azide 17 were reacted in the presence of copper sulfate and sodium ascorbate in a DMF/H₂O mixture to afford the expected SAM-adenosine conjugate 12 in 36% yield (Scheme 8).

We next investigated the synthesis of 1-propargyladenosine 18 (Scheme 9). Adenosine is known to react with alkyl halides in polar solvents such as DMF or DMA at room temperature, to afford 1-N-alkylated compounds [38,49]. Under these conditions, adenosine was treated with an excess of propargyl bromide in DMF at 50 °C for 24 h and 1-N-propargylated adenosine 18 was obtained in 56% yield (Scheme 9). The synthesis of SAM-adenosine conjugate with a triazole linker connected at the N-1 position was then achieved through the CuAAC between 17 and 18. The reaction was carried out in the presence of sodium ascorbate and copper sulfate to afford the expected conjugate 19 in 53% yield (Scheme 9). Compound 19 corresponds to the protonated and positively charged form of compound 15. This was confirmed by ¹H NMR spectra analysis for both compounds 15 and 19 (See the supporting file for NMR spectra of compounds 15 and 19). The signal for 2-H (H2a)
appears at 8.62 ppm for compound 19 while it is upfield at 8.22 ppm for compound 15 as expected [49].

![Chemical structure](image)

**Scheme 9**. Access to SAM-adenosine conjugate 19.

### 3. Materials and Methods

Reactions were carried out under argon atmosphere and solvents were dried using standard methods and distilled before use. DCM, Pyridine and DMF were dried over calcium hydride and THF over sodium and benzophenone. Unless otherwise specified, materials were purchased from commercial suppliers and used without further purification. TLC was performed using Merck commercial aluminum sheets coated with silica gel 60 F254. Compounds were detected by charring with 10% H2SO4 in ethanol followed by heating. Purification was performed by flash chromatography on silica gel (60 Å, 180-240 mesh; Merck). Preparative HPLC was performed using a HPLC system with a reverse phase C-18 column (250 mm × 21.2 mm) using a solvent system consisting of 50 mM aqueous CH3CN-NH4OAc (linear gradient from 0:100 to 100:0 in 30 min) at a flow rate of 15 mL min-1 and UV detection at 254 nm. The purity of final compounds (>95%) was established by analytical HPLC, which was performed on Macherey Nagel C18 100-5 NUCLEOSIL column (25 mm × 4.6 mm, 5 μm) with UV detection at 214 and 254 nm. NMR spectra were recorded on Bruker spectrometers (Avance II 500 and Avance III HD 4000). Chemical shifts (δ) are reported in parts per million (ppm) and referenced to the residual proton or carbon resonance of the solvents: CDCl3 (δ 7.26), MeOD (δ 3.31), D2O (δ 4.79) or (CD3)2SO (δ 2.50) for 1H and CDCl3 (δ 7.71), MeOD (δ 4.90) or (CD3)2SO (δ 39.52) for 13C. Signals were assigned using 1D (1H and 13C) and 2D (HSQC, COSY and HMBC) experiments. NMR coupling constants (J) are reported in Hertz (Hz) and splitting patterns are indicated as follows: s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublet), m (multiplet). High-resolution mass spectroscopy (HRMS) was recorded with an ion trap mass analyzer under electrospray ionization (ESI) in the negative or positive ionization detection mode. HRMS was performed using Thermo Scientific LTQ Orbitrap XL and Bruker MaXis II ETD spectrometers.

Compounds 2a and 3a: The trisilylated adenosine 1a (1.59 g, 2.22 mmol) and propargyl bromide 4.0 % in toluene (674 μL, 8.88 mmol) were dissolved in DCM (50 mL) and tetrabutylammonium hydroxide (1.78 g, 22.2 mmol) and 1M aqueous NaOH (22.2 mL) were added to the solution. After vigorous stirring at room temperature for 1 h, the reaction mixture was diluted in DCM and washed with brine, dried over MgSO4, and the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (eluent: Cyclohexane/EtOAc 9:1 then 7:3) to provide the desired compounds as white foams (946 mg, 57% for 2a and 475 mg, 28% for 3a). 2a: 1H NMR (500 MHz, CDCl3): δ 8.49 (s, 1H, H2), 8.14 (s, 1H, H8), 7.39-7.37 (m, 2H, H8a), 7.18-7.15 (m, 1H, H8a), 7.06-7.03 (m, 2H, H8a), 5.95 (d, J = 5.9 Hz, 1H, H1'), 5.7 (d, J = 4.9 Hz, 2H, CH2N), 4.54-4.52 (m, 1H), 4.17-4.15 (m, 1H, H3'), 4.03-4.01 (m, 1H, H4'), 3.89 (dd, J = 4.4, 11.4 Hz, 1H, H5'), 3.68 (dd, J = 2.8,
Compounds 2b and 3b: To a stirred solution of compound 1b (1.5 g, 2.11 mmol) in DCM (47.5 mL) was added propargyl bromide (80% in toluene) (0.795 mL, 8.44 mmol), tetrabutylammonium hydroxide (1.68 g, 1.68 mmol) and 1 M aqueous NaOAc (21.1 mL). The reaction mixture was stirred at room temperature for 1 h. The reaction mixture was dissolved in DCM and washed with brine. The organic layer was washed with brine, dried over MgSO₄, filtered and concentrated in vacuo. To a stirred solution of 2c (88.2 (C1), 145.4 (C2), 145.1 (Cq), 88.7 (C=O), 177.2 (C=O), 146.1 (C6), 145.4 (C2), 145.1 (C4), 138.8 (C8), 135.8 (Cq), 132.0 (Cq), 129.9 (2C, Cq), 128.1 (2C, Cq), 122.3 (C5), 88.2 (C1), 85.4 (C4), 76.7 (C=C=CH), 76.1 (C2), 75.9 (C=C=CH), 72.0 (C3), 62.7 (C5), 37.8 (CH₃N), 26.1 (3C, tBu), 25.9 (3C, tBu), 25.8 (3C, tBu), 18.6 (Cq), 18.2 (Cq), 18.0 (Cq), -4.2 (Me₃Si), -4.4 (Me₃Si), -4.5 (Me₃Si), -4.8 (Me₃Si), -5.2 (Me₃Si), -5.3 (Me₃Si). HRMS (ESI) m/z: calc for C₃₇H₃₉N₉O₃S₄ [M + Na⁺]: 774.3878; found: 774.3911.

Compounds 2b and 3b: To a stirred solution of compound 1a (1.42 g, 1.97 mmol) in THF (20 mL) at 0 °C, was added dropwise an aqueous solution of TFA (1:1, 7.55 mL, 98.72 mmol). The solution was stirred at room temperature for 1 h 30. The reaction mixture was neutralized with saturated aqueous NaHCO₃ solution and dissolved in EtOAc. The organic layer was washed with brine, dried over MgSO₄, filtered and concentrated in vacuo. The residue was purified on silica gel chromatography (elucent: Cyclohexane/EtOAc 6:4) to afford compound 4 as a white foam (1.02 g, 86%). ¹H NMR (500 MHz, CDCl₃): δ 8.77 (s, 1H, H₂), 8.81 (s, 1H, H₈), 6.10 (d, J = 4.9 Hz, 1H, H¹), 4.80 (d, J = 2.4 Hz, 2H, CH₂N), 4.64 (t, J = 4.6 Hz, 1H, H²), 4.33 (t, J = 4.0 Hz, 1H, H³), 4.14 (q, J = 3.8 Hz, 1H, H⁴), 4.04 (dd, J = 11.4, 4.1 Hz, 1H, H⁵), 3.80 (dd, J = 11.4, 2.8 Hz, 1H, H⁵), 2.16 (t, J = 2.4 Hz, 2H, C=CH), 1.48 (s, 9H, tBu), 0.96 (s, 9H, tBu), 0.93 (s, 9H, tBu), 0.80 (s, 9H, tBu), 0.15 (s, 3H, Me₃Si), 0.14 (s, 3H, Me₃Si), 0.10 (s, 3H, Me₃Si), 0.09 (s, 3H, Me₃Si), -0.04 (s, 3H, Me₃Si), -0.22 (s, 3H, Me₃Si). ¹³C NMR (126 MHz, CDCl₃): δ 152.7 (C6), 152.5 (C4), 152.4 (C=O), 151.8 (C2), 142.2 (C8), 127.6 (C5), 88.7 (C¹), 85.5 (C⁴), 82.9 (Cq), 79.9 (C=C=CH), 76.1 (C²), 72.0 (C³), 71.2 (C=C=CH), 62.6 (C⁵), 37.3 (CH₃N), 28.1 (3C, tBu), 26.3 (3C, tBu), 26.0 (3C, tBu), 25.8 (3C, tBu), 18.7 (Cq), 18.2 (Cq), 18.0 (Cq), -4.2 (Me₃Si), -4.5 (Me₃Si), -4.6 (Me₃Si), -4.8 (Me₃Si), -5.2 (Me₃Si), -5.2 (Me₃Si). HRMS (ESI) m/z: calc for C₃₇H₃₉N₉O₃S₄ [M + H⁺]: 748,4315; found: 748,4309.
Compound 5: Methanesulfonyl chloride (0.26 mL, 3.33 mmol) in pyridine (10 mL) was added dropwise to a solution of compound 4 (1 g, 1.66 mmol) in pyridine (7 mL) at 0 °C. The reaction mixture was stirred at room temperature for 16 h. The reaction was quenched by addition of water and then diluted in DCM. The organic layer was washed with a saturated solution of NaHCO₃ and brine. The combined organic layer was dried over MgSO₄ and concentrated to dryness. The residue was purified on silica gel chromatography (eluent: Cyclohexane/EtOAc 6:4) and compound 5 was isolated as a white foam (0.92 g, 82%). ¹H NMR (500 MHz, CDCl₃): δ 8.82 (s, 1H, H2), 8.20 (s, 1H, H8), 8.04 (d, J = 7.3 Hz, 2H, H’), 7.62 (t, J = 7.4 Hz, 1H, H’), 7.54 (t, J = 7.6 Hz, 2H, H’), 6.00 (d, J = 4.8 Hz, 1H, H1’), 4.99 (t, J = 4.4 Hz, 1H, H2’), 4.62 (dd, J = 11.2, 4.1 Hz, 1H, H5’), 4.50 (dd, J = 11.2, 4.6 Hz, 1H, H5’), 4.40 – 4.35 (m, 2H, H3’, H4’), 3.03 (s, 3H, CH3), 0.95 (s, 9H, TBS), 0.82 (s, 9H, TBS), 0.15 (s, 3H, MeTBS), 0.13 (s, 3H, MeTBS), 0.00 (s, 3H, MeTBS), -0.20 (s, 3H, MeTBS). ¹³C NMR (126 MHz, CDCl₃): δ 161.3 (C=O), 152.8 (C6), 151.5 (C2), 149.9 (C4), 142.5 (C8), 134.8 (Cq₂), 132.9 (Cq₂), 129.0 (2C, Cq), 127.9 (2C, Cq₃), 123.9 (C5), 89.9 (C1’), 82.4 (C4’), 74.3 (C2’), 72.1 (C3’), 67.9 (C5’), 37.8 (CH3), 25.9 (3C, tBuTBS), 25.8 (3C, tBuTBS), 18.2 (CqTBS), 18.00 (CqTBS), -4.3 (MeTBS), -4.5 (MeTBS), -4.7 (MeTBS), -4.8 (MeTBS). HRMS (ESI) m/z: calcld for C₂₈H₃₅N₂O₅Si: [M + H]: 625.3096; found: 625.3088.

Compound 6: To a stirred solution of compound 5 (920 mg, 1.35 mmol) in DMF (7 mL) was added sodium azide (264 mg, 4.06 mmol) and the mixture was heated at 70 °C for 4 h. The reaction mixture was cooled to room temperature and was dissolved in EtOAc and washed with brine. The organic layer was dried over MgSO₄, concentrated in vacuo and purified on silica gel chromatography (eluent: Cyclohexane/EtOAc 8:2) to afford compound 6 as a white foam (770 mg, 92%). ¹H NMR (500 MHz, CDCl₃): δ 8.80 (s, 1H, H2), 8.28 (s, 1H, H8), 8.03 (d, J = 7.3, 2H, H’), 7.60 (t, J = 7.3, 1H, H’), 7.52 (t, J = 7.8, 2H, H’), 5.98 (d, J = 4.0 Hz, 1H, H1’), 4.88 (t, J = 4.1 Hz, 1H, H2’), 4.31 (t, J = 4.6 Hz, 1H, H3’), 4.23 (q, J = 4.6 Hz, 1H, H4’), 3.80-3.67 (m, 2H, H5’), 0.94 (s, 9H, TBS), 0.84 (s, 9H, tBuTBS), 0.12 (s, 3H, MeTBS), 0.11 (s, 3H, MeTBS), -0.01 (s, 3H, MeTBS), -0.12 (s, 3H, MeTBS). ¹³C NMR (126 MHz, CDCl₃): δ 164.6 (C=O), 152.8 (C6), 151.5 (C2), 149.8 (C4), 142.4 (C8), 133.8 (Cq₂), 132.9 (Cq₂), 129.0 (2C, Cq), 127.9 (2C, Cq₃), 123.9 (C5), 90.1 (C1’), 82.8 (C4’), 74.8 (C2’), 72.3 (C3’), 51.6 (C5’), 25.9 (3C, tBuTBS), 25.8 (3C, tBuTBS), 18.2 (CqTBS), 18.00 (CqTBS), -4.2 (MeTBS), -4.5 (MeTBS), -4.7 (MeTBS), -4.8 (MeTBS). HRMS (ESI) m/z: calcld for C₂₈H₃₅N₂O₅Si: [M + H]: 625.3096; found: 625.3088.

General procedure A for CuAAC reaction: To a solution of alkyn (1 eq) in THF (13 mL/mmol), were successively added azido compound 6 or 7 (1.2 eq), CuSO₄ (0.3 eq, in water 3 mL/mmol) and sodium ascorbate (0.6 eq, in water 3 mL/mmol). The heterogeneous mixture was stirred at room temperature for 16 h. EtOAc was added and the organic layer was washed with brine, dried over MgSO₄, and concentrated in vacuo. The crude was purified by flash chromatography to afford the desired compounds.

Compound 8a: Following the general procedure A for CuAAC, starting from alkyn 2a (200 mg, 0.26 mmol) and azido compound 6 (194 mg, 0.31 mmol) and using Cyclohexane/EtOAc 6:4 as eluent for flash chromatography purification, compound 8a was obtained as a white foam (245 mg, 68%).
Compounds 9a: Following the general procedure A for CuAAC, starting from alkyne 2a (578 mg, 0.77 mmol) and azido compound 7 (277 mg, 0.38 mmol) and using Cyclohexane/EtOAc 7:3 as eluent for flash chromatography purification, compound 9a was obtained as a white foam (384 mg, 60%).

1H NMR (500 MHz, CDCl3): δ 8.75 (s, 1H, H8), 8.51 (s, 1H, H8 or H2), 8.19 (s, 1H, H8 or H2), 8.04 (bs, 1H, NH), 7.92 (bs, 1H, H8 or H2), 7.40 (s, 1H, H H3*), 7.42-7.40 (m, 2H, H2*), 7.23-7.20 (m, 1H, H H5*), 7.11-7.08 (m, 2H, H8*), 5.98 (d, J = 5.6 Hz, 1H, H1a), 5.86 (d, J = 6.2 Hz, 1H, H1b), 5.63 (s, 2H, CHN), 5.04-5.02 (m, 1H, H2b), 4.84 (dd, J = 6.3, 14.2 Hz, 1H, H5b), 4.61-4.57 (m, 2H, H5b and H2a), 4.39-4.38 (m, 1H, H3b), 3.45-3.42 (m, 1H, H4b), 4.25-4.23 (m, 1H, H3a), 4.10-4.08 (m, 1H, H4a), 3.96 (dd, J = 4.4, 11.3 Hz, 1H, H1a), 3.75 (dd, J = 3.0, 11.4 Hz, 1H, H5a), 1.56 (s, 9H, tBuBOC), 0.93 (s, 9H, tBu), 0.91 (s, 9H, tBu), 0.87 (s, 9H, tBu), 0.73 (s, 18H, tBu), 0.11 (s, 3H, MeTBS), 0.10 (s, 3H, MeTBS), 0.08 (s, 6H, MeTBS), 0.02 (s, 3H, MeTBS), -0.08 (s, 3H, MeTBS), -0.11 (s, 6H, MeTBS), -0.43 (s, 6H, MeTBS). 13C NMR (126 MHz, CDCl3): δ 172.1 (C=O), 153.7 (C2 or C8), 153.1 (Cq), 152.8 (Cq), 152.0 (C2 or C8), 151.6 (Cq), 150.4 (C2 or C8), 144.7 (Cq), 143.2 (C8a or C8b), 143.0 (C8a or C8b), 136.0 (Cq) 133.9 (Cq) 132.9 (Cq) 130.9 (Cq), 129.1 (2C, Cq), 129.0 (2C, Cq), 127.9 (2C, Cq), 127.3 (Cq), 124.9 (CH(Tsazole)), 124.2 (Cq), 90.1 (C1b), 88.4 (C1’a), 86.0 (C4’a), 84.1 (C4’b), 75.8 (C2’a), 73.4 (C3’b), 73.3 (C2’a), 72.3 (C3’a), 62.8 (C5’a), 51.7 (C5’b), 44.0 (CH:N), 26.2 (3C, tBu), 26.0 (3C, tBu), 25.9 (3C, tBu), 25.8 (6C, tBu), 18.6 (Cq), 18.2 (Cq), 17.9 (Cq), 17.8 (Cq), -4.3 (Cq), -4.5 (3C, MeTBS), -4.6 (MeTBS), -4.7 (MeTBS), -4.9 (MeTBS), -5.0 (MeTBS), -5.2 (MeTBS), -5.3 (MeTBS). HRMS (ESI) m/z: calc for C37H38N3O9SiS [M + H]+: 1376.7077; found: 1376.7056.

Compounds 10a: Following the general procedure A for CuAAC, starting from alkyne 2b (50 mg, 0.067 mmol) and azido compound 7 (49 mg, 0.08 mmol) and using Cyclohexane/EtOAc 7:3 as eluent for flash chromatography purification, compound 10b was obtained as a white foam (68 mg, 74%). 1H NMR (500 MHz, CDCl3): δ 8.74 (s, 1H, H8b), 8.70 (s, 1H, H8a), 8.38 (s, 1H, NH), 7.90 (s, 1H, H8b), 7.70 (s, 1H, H H3*), 6.07 (dd, J = 4.7 Hz, 1H, H1a), 5.86 (d, J = 6.2 Hz, 1H, H1b), 5.30 (s, 2H, CHN), 5.10 (dd, J = 6.2, 4.3 Hz, H2b), 4.89 (dd, J = 14.3, 6.3 Hz, 1H, H5b), 4.65-4.58 (m, 2H, H5b, H2a), 4.41 (dd, J = 4.3, 2.4 Hz, 1H, H3b), 4.36-4.31 (m, 2H, H4b, H3a), 4.15-4.12 (m, 1H, H4a), 4.05 (dd, J = 11.4, 4.1 Hz, 1H, H5a), 3.79 (dd, J = 11.4, 2.9 Hz, 1H, H5a), 1.57 (s, 9H, tBuBOC), 1.38 (s, 9H, tBu), 0.95 (s, 9H, tBu), 0.93 (s, 9H, tBu), 0.88 (s, 9H, tBu), 0.80 (s, 9H, tBu), 0.74 (s, 9H, tBu), 0.13 (s, 3H, MeTBS), 0.12 (s, 3H, MeTBS), 0.10 (s, 3H, MeTBS), 0.09 (s, 3H, MeTBS), 0.04 (s, 3H, MeTBS), -0.04 (s, 3H, MeTBS), -0.06 (s, 3H, MeTBS), -0.10 (s, 3H, MeTBS), -0.21 (s, 3H, MeTBS), -0.42 (s, 3H, MeTBS). 13C NMR (126 MHz, CDCl3): δ 153.3 (C2b), 153.1 (Cq), 152.9 (Cq), 152.5 (Cq), 151.9 (C2a), 150.6 (Cq), 150.4 (C=O), 149.6 (C=O), 145.8 (Cq), 142.6 (C8b), 142.2 (C8a), 127.7 (Cq), 124.1 (CH(Tsazole)), 122.9 (Cq), 90.1 (C1b), 88.8 (C1’a), 85.3 (C4’a), 84.4 (C4’b), 82.5 (2C, Cq), 75.9 (C2’a), 73.5 (C3’b), 73.1 (C2’a), 72.3 (C3’a), 62.5 (C5’a), 43.4 (CH:N), 28.3 (3C, tBu), 28.0 (3C, tBu), 26.2 (3C, tBu), 26.0 (3C, tBu), 25.9 (3C, tBu), 25.7 (3C, tBu), 18.7 (Cq), 18.2
Compound 10a: Following the general procedure A for CuAAC, starting from alkyne 3a (200 mg, 0.26 mmol) and azido compound 6 (194 mg, 0.31 mmol) and using Cyclohexane/EtOAc 5:2 as eluent for flash chromatography purification, compound 10a was obtained as a white foam (258 mg, 72%).  

1H NMR (500 MHz, CDCl3): δ 8.70 (s, 1H, H2b), 8.26 (s, 1H, H2a), 8.05 (d, J = 7.4 Hz, 2H, Hb), 7.95 (d, J = 7.2 Hz, 2H, Hb), 7.79 (s, 1H, HN), 7.46-7.43 (m, 1H, H2), 7.36-7.32 (m, 2H, Hb), 5.85 (d, J = 4.5 Hz, 1H, H1a), 5.78 (d, J = 5.4 Hz, 1H, H1b), 5.44 (d, J = 14.6 Hz, 1H, CHN), 5.36 (d, J = 14.6 Hz, 1H, CHN), 5.12 (m, 1H, H2b), 4.87 (dd, J = 5.2, 14.3 Hz, 1H, H5b), 4.70 (dd, J = 7.3, 14.2 Hz, 1H, H5b), 4.48-4.47 (m, 1H, H3b), 4.46-4.44 (m, 1H, H2a), 4.37-4.34 (m, 1H, H4b), 4.24-4.23 (m, 1H, H3a), 4.07-4.05 (m, 1H, H4a), 3.88 (dd, J = 4.0, 11.3 Hz, 1H, H5a), 3.71 (dd, J = 3.3, 11.3 Hz, 1H, H5a), 1.57 (s, 9H, tBuN(Me)), 0.91 (s, 9H, tBuN), 0.89 (s, 9H, tBuN), 0.87 (s, 9H, tBuN), 0.80 (s, 9H, tBuN), 0.77 (s, 9H, tBuN), 0.70 (s, 3H, Me), 0.07 (s, 6H, Me), 0.05 (s, 3H, Me), 0.04 (s, 3H, Me), -0.08 (s, 3H, Me), -0.18 (s, 3H, Me), -0.39 (s, 3H, Me). 13CNMR (126 MHz, CDCl3): δ 176.8 (C=O), 152.9 (C2b), 150.3 (2C, Cq), 149.6 (C=O), 146.7 (2C, C2a and Cq), 145.4 (Cq), 142.6 (C8b), 141.9 (Cq), 139.0 (2C8a), 135.6 (Cq), 131.9 (C6b), 129.7 (2C, C8b), 128.1 (2C, C8b), 125.6 (CHq), 122.8 (Cq), 122.6 (Cq), 122.6 (Cq), 90.4 (C1b), 88.5 (C1a), 85.1 (C4a), 83.6 (C4b), 82.5 (Cq), 76.0 (C2a), 73.5 (C3b), 73.5 (C2b), 71.7 (C3a), 62.6 (C5a), 51.9 (C5b), 43.8 (CHN), 28.2 (3C, tBuN), 26.1 (3C, tBuN), 26.0 (3C, tBuN), 25.9 (3C, tBuN), 25.8 (3C, tBuN), 25.80 (3C, tBuN), 18.6 (Cq), 18.2 (Cq), 18.1 (Cq), 18.0 (2C, Cq), 12.4 (Me), 12.0 (4H, tBuN), 4.6 (2C, Me), 4.4 (2C, Me), 4.3 (2C, Me), 4.2 (Me), 4.1 (4H, tBuN), 0.04 (s, 3H, Me), 0.03 (s, 3H, Me), -0.04 (s, 3H, Me), -0.06 (s, 3H, Me), -0.19 (s, 3H, Me), -0.34 (s, 3H, Me). Compound 10a was obtained as a white foam (258 mg, 72%).

Compound 11a: Following the general procedure A for CuAAC, starting from alkyne 3a (45 mg, 0.059 mmol) and azido compound 7 (19 mg, 0.03 mmol) and using Cyclohexane/EtOAc 7:3 as eluent for flash chromatography purification, compound 11a was obtained as a white foam (25 mg, 77%).

1H NMR (500 MHz, CDCl3): δ 8.72 (s, 1H, H2a or H2b), 8.10 (s, 1H, H2a or H2b), 8.10 (s, 1H, H8a or H8b), 8.01 (s, 1H, H8a or H8b), 7.90 (s, 1H, HN), 5.85-5.83 (m, 2H, H1a and H1b), 5.30-5.21 (m, 2H, CHN), 5.17 (dd, J = 5.8, 4.3 Hz, 1H, H2b), 4.92 (dd, J = 14.3, 6.5 Hz, 1H,
H5'b), 4.62 (dd, J = 14.3, 6.5 Hz, 1H, H5'b), 4.48-4.45 (m, 1H, H3'b), 4.39-4.35 (m, 1H, H4'b), 4.32-4.27 (m, 2H, H2'a, H3'a), 4.09-4.06 (m, 1H, H4'a), 3.98 (dd, J = 11.6, 3.4 Hz, 1H, H5'a), 3.75 (dd, J = 11.6, 2.5 Hz, 1H, H8), 1.56 (s, 18H, TBS), 0.92 (s, 9H, TBS), 0.90 (s, 9H, TBS), 0.86 (s, 9H, TBS), 0.76 (s, 9H, TBS), 0.76 (s, 9H, TBS), 0.10 (s, 3H, MeTBS), 0.10 (s, 3H, MeTBS), 0.07 (s, 3H, MeTBS), 0.05 (s, 3H, MeTBS), 0.03 (s, 3H, MeTBS), 0.01 (s, 3H, MeTBS), -0.03 (s, 3H, MeTBS), -0.08 (s, 6H, MeTBS), -0.40 (s, 3H, MeTBS).

1H NMR (126 MHz, CDCl3): δ 153.0 (C2a or C1b), 150.5 (Cq), 150.4 (Cq), 149.6 (C2, C8), 146.9 (CqTBS), 146.5 (C2a or C2b), 144.2 (Cq), 142.7 (Cq), 142.3 (C8a or C8b), 138.1 (C8a or C8b), 125.7 (CHTBS), 123.0 (Cq), 122.3 (Cq), 90.4 (C1'a or C1'b), 88.9 (C1'a or C1'b), 84.1 (C4'a), 83.9 (C4'b), 82.4 (2C, CqTBS), 76.7 (C2'a), 73.5 (C3'b), 73.2 (C2'b), 70.5 (C3'a), 61.7 (C5'a), 51.9 (C5'b), 43.1 (CHN), 28.3 (3C, TBS), 28.3 (3C, TBS), 26.3 (3C, TBS), 26.0 (3C, TBS), 25.9 (6C, CqTBS), 25.8 (3C, TBS), 18.7 (CqTBS), 18.2 (CqTBS), 18.1 (CqTBS), 18.0 (CqTBS), 17.9 (CqTBS), -4.1 (MeTBS), -4.4 (2C, 2 MeTBS), -4.5 (MeTBS), -4.6 (MeTBS), -4.7 (MeTBS), -4.9 (MeTBS), -5.1 (MeTBS), -5.2 (MeTBS), -5.3 (MeTBS). HRMS (ESI) m/z: calcld for C33H31Ni3O13S6: 1368.7601; found: 1368.7611.

Synthesis of compound 12 following pathway 1: Protected compound 9a (768 mg, 0.56 mmol) was dissolved in DCM/MeOH 4:1 (10 mL) and MeNH2: 33% in EtOH (3.6 mL, 28.0 mmol) was added at 0 °C to the solution. The reaction was stirred at room temperature for 16 h, then diluted in DCM and washed with brine. The combined organic layers were dried over MgSO4 and concentrated. The residue was purified by silica gel chromatography (eluent: Cyclohexane/EtOAc 5:5) to provide the debenzyolated compound as a white foam. The residue was then dissolved in DCM (1.4 mL) and ZnBr2 (686 mg, 2.80 mmol) was added. The reaction mixture was vigorously stirred at room temperature for 24 h. Then, water (5.8 mL) was added and the reaction mixture was stirred for 2 additional hours. A work up was performed with DCM and brine. The combined organic layers were dried over MgSO4 and concentrated. The residue was purified by silica gel chromatography (eluent: Cyclohexane/EtOAc 3:7 then DCM/MeOH 9:1) to afford a yellow powder. The resulting compound was engaged in the last deprotection step and was dissolved in MeOH (10 mL) and CsF (8.5 g, 112 mmol) was added. The reaction mixture was stirred at 60 °C for 24 h, concentrated and diluted in water. The residue was purified by HPLC to afford compound 12 as a white foam (17 mg, 5% over 3 steps).

Synthesis of compound 12 following pathway 2: Protected compound 9b (60 mg, 0.046 mmol) was dissolved in DCM (1 mL) and ZnBr2 (58 mg, 0.21 mmol) was added. The reaction mixture was vigorously stirred at room temperature for 24 h. Then, water (2 mL) was added and the reaction mixture was stirred for 2 additional hours. A work up was performed with DCM and brine. The combined organic layers were dried over MgSO4 and concentrated. The residue was purified by silica gel chromatography (eluent: Cyclohexane/EtOAc 2:8) to afford a yellow pale powder. The resulting compound was engaged in the last deprotection step and was dissolved in MeOH (10 mL) before adding CsF (480 mg, 3.2 mmol). The reaction mixture was stirred at 60 °C for 24 h, concentrated and purified by HPLC to afford compound 12 as a white foam (1.2 mg, 4% over 2 steps).

Compound 12: 1H NMR (500 MHz, (CD3)2SO): δ 3.85 (s, 1H, H2 or H8), 8.26 (bs, 1H, NH), 8.23 (s, 1H, H2 or H8), 8.19 (s, 1H, H2 or H8), 8.14 (s, 1H, H2 or H8), 7.81 (s, 1H, Htriazole), 7.28 (bs, 2H, NH2), 5.90-5.89 (m, 2H, H1’a and H1’a), 5.55 (d, J = 5.9 Hz, 1H, OH2’,b), 5.43-5.41 (m, 2H, OH2’a and OH3’b), 5.36-5.34 (m, 1H, OH5’a), 5.16 (d, J = 4.6 Hz, 1H, OH3’a), 4.74-4.67 (m, 4H, H5’b and CHN2), 4.67-4.63 (m, 1H, H2’b), 4.63-4.60 (m, 1H, H2’a), 4.25-4.20 (m, 2H, H4’b and H3’b), 4.16-4.14 (m, 1H, H3’a), 3.97-3.95 (m, 1H, H4’a), 3.69-3.65 (m, 1H, H5’a), 3.58-3.53 (m, 1H, H5’a). 13C NMR (126 MHz, (CD3)2SO): δ 156.1 (Cq), 154.2 (Cq), 152.6 (C2 or C8), 152.2 (C2 or C8), 149.3 (Cq), 148.5 (Cq), 1453 (Cq), 139.9 (C2 or C8), 139.8 (C2 or C8), 123.6 (CHTBS), 119.8 (Cq), 119.2 (Cq), 87.9 (C1’a or C1'b), 87.7 (C1’a or C1'b), 85.8 (C4’a), 82.5 (C4'b), 73.4 (C2’a), 72.5 (C2’b), 71.0 (C3'b), 70.6 (C3'a), 61.6 (C5'a), 51.3 (C5'b), 35.3 (CHN), HRMS (ESI) m/z: calcld for C33H31Ni3O13S6: [M + H]+: 596.2078; found: 596.2065. HPLC purity: 96.3%; tR = 17.8 min (MeCN/H2O 0:100 to 100:0 over 30 min).
Compound 13: Protected compound 11a (400 mg, 0.3 mmol) was dissolved in DCM/Methanol 4:1 (3 mL) and MeNH2 33% in EtOH (1.8 mL, 15.0 mmol) was added at 0 °C to the solution. The reaction was stirred at room temperature for 16 h, then diluted in DCM and washed with brine. The combined organic layers were dried over MgSO4 and concentrated. The residue was purified by silica gel chromatography (eluent: EtOAc/Methanol 9:1) to provide the debenzoylated compound as a white foam. The residue was then dissolved in DCM (1 mL) and ZnBr2 (117 mg, 0.47 mmol) was added. The reaction mixture was vigorously stirred at room temperature for 24 h. Then, water (2 mL) was added and the reaction mixture was stirred for 2 additional hours. A work up was performed with DCM and brine. The combined organic layers were dried over MgSO4 and concentrated. The resulting compound was engaged in the last deprotection step and was dissolved in MeOH (10 mL) and CsF (2.9 g, 19 mmol) was added. The reaction mixture was stirred at 60 °C for 24 h, concentrated and diluted in water. The residue was purified by HPLC to afford compound 13 as a white foam (15 mg, 8% over 3 steps). 1H NMR (500 MHz, CD3OD): δ 8.35 (2s, 1H, H2a), 8.23 (s, 1H, H8a or H8b), 8.13 (2s, 1H, H8a or H8b), 8.03 (2s, 1H, H2b), 7.84 (s, 0.5H, Htriazole), 7.69 (s, 0.5H, Htriazole), 6.01-5.98 (m, 1H, H1’a), 5.95-5.93 (m, 1H, H1’b), 5.32 (2s, 2H, CH2N), H5’b masked in the residual pick of water, 4.64 (J = 5 Hz, 1H, H2’a), 4.47-4.41 (m, 0.5H, H2’b), 4.37-4.31 (m, 1.5H, H3’b and H3’a), 4.14-4.13 (m, 2H, H4’a and H4’b), 3.88-3.85 (m, 1H, H5’a), 3.78-3.74 (m, 1H, H5’b), 3.55 (2s, 3H, CH3). 13C NMR (126 MHz, CD3OD): δ 178.3 (Cq), 163.0 (Cq), 157.3 (Cq), 153.7 (C8a or C8b), 150.4 (Cq), 150.0 (Cq), 149.8 (Cq), 149.4 (Cq), 147.8 (Cq), 147.5 (Cq), 142.0 (C2a or C2b), 126.6 (CHtriazole), 125.7 (CHtriazole), 126.2 (Cq), 120.8 (Cq), 110.0 (C1’b), 90.4 (C1’a), 87.6 (C2’a and C4’b), 83.9 (C3’a), 76.1 (C2’a), 74.2 (C2’b), 72.2 (C3’b), 62.9 (C5’a), 52.5 (C5’b), 45.1 (CH2N), 43.9 (CHN), 37.9 (CH3), 35.0 (CH3). LRMS (ESI) m/z: calcld for C15H12N2O2: [M + H]+: 262.23; found: 262.23. HPLC purity: 95.3%; rt = 14.6 min (MeCN/H2O 0:100 to 100:0 over 30 min).

Compound 14: Compound 11a (107 mg, 0.08 mmol) was dissolved in DCM (1 mL) and ZnBr2 (95 mg, 0.40 mmol) was added. The reaction mixture was vigorously stirred at room temperature for 24 h. Water (2 mL) was added and the reaction mixture was stirred for 2 additional hours. A work up was performed with DCM and brine. The combined organic layers were dried over MgSO4 and concentrated. The residue was purified by silica gel chromatography (eluent: Cyclohexane/EtOAc 2:8) to afford a white foam (32 mg). The purified intermediate was then dissolved in MeOH (5 mL) and CsF (1.2 g, 8 mmol) was added. The reaction mixture was stirred at 60 °C for 24 h, concentrated and diluted in water. The residue was purified by HPLC to afford compound 14 as a white foam (7 mg, 13% over 2 steps). 1H NMR (500 MHz, CD3OD): δ 8.53 (s, 1H, H2 or H8), 8.12 (s, 1H, H2 or H8), 8.06 (s, 1H, H2 or H8), 7.90 (s, 2H, H2 or H8 and Htriazole), 7.83-7.81 (m, 2H, H2b), 7.45-7.42 (m, 1H, H5b), 7.33-7.30 (m, 2H, H2b), 5.96 (d, J = 5.0 Hz, 1H, H1’a), 5.90 (d, J = 5.0 Hz, 1H, H1’b), 5.43 (s, 2H, CH2N), H5’b masked in the residual pick of water, 4.64-4.60 (m, 2H, H2’b and H2’a), 4.47 (t, J = 5.0 Hz, 1H, H3’b), 4.37-4.34 (m, 1H, H4’b), 4.32-4.30 (m, 1H, H3’a), 4.11 (q, J = 3.3 Hz, 1H, H4’a), 3.83 (dd, J = 5.0, 15.0 Hz, 1H, H5’a), 3.74 (dd, J = 5.0, 15.0 Hz, 1H, H5’b). 13C NMR (126 MHz, CD3OD): δ 178.8 (C=O), 157.2 (C2 or C8), 153.7 (C2 or C8), 149.2 (Cq), 148.5 (Cq), 146.9 (Cq), 143.3 (Cq), 141.5 (C2 or C8), 141.2 (Cq), 136.7 (C2 or C8), 133.2 (Cq), 130.6 (Cq), 129.1 (2C, Cq), 129.0 (2C, Cq), 127.0 (CHtriazole), 123.2 (Cq), 120.6 (Cq), 91.0 (C1’a), 90.4 (C1’b), 87.4 (C4’a), 83.4 (C4’b), 76.0 (C2’a), 74.5 (C2’b), 72.2 (C3’b), 72.0 (C3’a), 62.8 (C5’a), 52.4 (C5’b), 45.0 (CH2N). HRMS (ESI) m/z: calcld for C15H12N2O2: [M + H]+: 262.23; found: 262.23. HPLC purity: 97.1%; rt = 19.0 min (MeCN/H2O 0:100 to 100:0 over 30 min).

Compound 15: Protected compound 11b (50 mg, 0.036 mmol) was dissolved in DCM (1 mL) and ZnBr2 (44 mg, 0.18 mmol) was added. The reaction mixture was vigorously stirred at room temperature for 24 h then water (2 mL) was added and the reaction mixture was stirred for 2 additional hours. A work up was performed with DCM and brine. The combined organic layers were dried over MgSO4 and concentrated. The residue was purified by silica gel chromatography (eluent: Cyclohexane/EtOAc 1:9) to afford a yellow pale powder. The resulting compound was
engaged in the last deprotection step and was dissolved in MeOH (10 mL) and CsF (636 mg, 4.2 mmol) was added. The reaction mixture was stirred at 60 °C for 24 h, concentrated and purified by HPLC to afford compound 15 as a white foam (4.3 mg, 20% over 2 steps). 1H NMR (500 MHz, (CD3)2SO): δ 8.27 (s, 1H, H8b), 8.22 (s, 1H, H2a), 8.15 (s, 1H, H2b), 8.14 (s, 1H, H8a), 7.96 (s, 1H, H1'), 7.28 (bs, 2H, NH2), 5.89 (d, J = 5.5 Hz, 1H, H1'b), 5.75 (d, J = 5.9 Hz, 1H, H1'a), 5.28-5.17 (m, 2H, CH2N), 4.74-4.70 (m, 2H, H5b), 4.64-4.61 (m, 1H, H2's), 4.48-4.44 (m, 1H, H2'a), 4.27-4.20 (m, 2H, H3'b and H4'b), 4.10 (d, J = 4.9 Hz, 1H, H3'a), 3.92 (q, J = 3.8 Hz, 1H, H4'a), 3.64 (dd, J = 12.0, 4.0 Hz, 1H, H5'a), 3.53 (dd, J = 12.0, 3.9 Hz, 1H, H5'a). 13C NMR (126 MHz, (CD3)2SO): δ 156.1 (Cq), 153.2 (Cq), 152.6 (C2b), 149.3 (Cq), 148.2 (C2a), 142.5 (Cqtriazo), 141.2 (Cq), 139.8 (C8b), 137.9 (C8a), 124.5 (CH2triazo), 122.8 (Cq), 119.2 (Cq), 87.6 (C1'a or C1'b), 87.6 (C1'a or C1'b), 85.6 (C4'a), 82.3 (C4'b), 73.9 (C2'a), 72.5 (C2'b), 70.9 (C3'b), 70.4 (C3'a), 61.4 (C5'a), 51.4 (C5'a), 41.2 (CH2N). HRMS (ESI) m/z: calcd for C20H23N5O5 [M + H]+: 598.2229; found: 598.2229. HPLC purity: 96.2%; ts = 17.5 min (MeCN/H2O 0:100 to 100:0 over 30 min).

**Compound 16**: Propargyl amine (1.4 µL, 0.031 mmol), BOP (13 mg, 0.031 mmol) and DIPEA (6.8 µL, 0.039 mmol) were added at 0 °C to a solution of inosine (7 mg, 0.026 mmol) in DMF (1 mL). The reaction mixture was stirred at room temperature for 24 h and then concentrated under reduced pressure.

The crude was diluted in water and purified by HPLC to afford compound 16 as a white foam (5.7 mg, 71%). 1H NMR (500 MHz, D2O): δ 8.31 (s, 1H, H8), 8.27 (s, 1H, H2), 6.07 (d, J = 5 Hz, 1H, H1'), H2' masked in the residual pick of water, 4.47-4.45 (m, 1H, H3'), 4.35 (s, 2H, CH2N), 4.33 (q, J = 3.3 Hz, 1H, H5'), 3.97 (dd, J = 5, 11 Hz, 1H, H5'), 3.88 (dd, J = 5, 10 Hz, 1H, H5'), 2.69 (t, J = 2.5 Hz, 1H, C=CH). 13C NMR (126 MHz, D2O): δ = 153.9 (C5), 152.3 (C2), 147.8 (C4), 140.4 (C8), 119.6 (C6), 88.4 (C1'), 85.8 (C4'), 80.3 (C=C-CH2), 73.7 (C2'), 71.9 (C=CH), 70.6 (C3'), 61.5 (C5'), 30.2 (CH2N). HRMS (ESI) m/z: calcd for C20H19N5O5 [M - H]-: 304.1045; found: 304.1048.

**Compound 17**: Pyridine (610 µL, 7.48 mmol) and thionyl chloride (1.4 mL, 18.7 mmol) were added at 0 °C, over 5 min to a solution of adenosine (1 g, 3.74 mmol) in MeCN (10 mL). The reaction mixture was stirred at 0 °C for 3 h before being warmed to room temperature and stirred for 16 h. The resulting precipitate was filtered and dissolved in water/MeOH (5:1) and aqueous ammonia (25%, 2 mL) was added. The reaction mixture was stirred at room temperature for 30 min and the solvent was removed under reduced pressure to provide 5'-chloroadenosine.44 The resulting 5'-chloroadenosine was then solubilized in DMF (5 mL) and sodium azide (1.2 g, 18.7 mmol) was added. The reaction mixture was heated at 80 °C for 5 h, and cooled to room temperature. The excess of sodium azide was removed by filtration and the filtrate purified by flash chromatography (DCM/MeOH 9:1) to give 17 as a white foam (393 mg, 36% over 2 steps). 1H NMR (500 MHz, CD3OD): δ 8.29 (s, 1H, H8), 8.21 (s, 1H, H2), 6.03 (s, 1H, H1'), 4.80-4.78 (m, 1H, H2'), 4.40-4.36 (m, 1H, H4'), 4.27 (q, J = 5 Hz, 0.5H, H3'), 4.18 (q, J = 5 Hz, 0.5H, H3'), 3.94 (dd, J = 10, 5 Hz, 0.5H, H5'), 3.84 (dd, J = 10, 5 Hz, 0.5H, H5'), 3.69-3.62 (m, 1H, H5'). HRMS (ESI) m/z: calcd for C20H23N5O5 [M + H]+: 293.1110; found: 293.1098. Analytical data were in accordance with the literature.65

**General procedure B for CuAAC reaction**: To a solution of alkyne 16 or 18 (1 eq) in DMF (1 mL), were successively added azido compound 17 (1.5 eq), CuSO4·5H2O (0.3 eq, in water 500 µL) and sodium ascorbate (0.6 eq, in water 500 µL). The mixture was stirred at room temperature for 16 h and then concentrated in vacuo. The crude product was purified by HPLC to afford the desired compounds.

**Synthesis of compound 12 following general procedure B for CuAAC**: Following the general procedure B, starting from alkyne 16 (5.7 mg, 0.019 mmol) and azido compound 17 (8.2 mg, 0.027 mmol), compound 12 was obtained as a white foam (4 mg, 36%).

**Compound 18**: Propargyl bromide (80% in toluene) (143 µL, 1.8 mmol) was added to a solution of adenosine (100 mg, 0.37 mmol) in DMF (1 mL) and the reaction mixture was stirred at 50 °C for 24 h. After removal of the solvent under reduced pressure, the crude was diluted in water and purified
by HPLC to afford compound 18 as a white foam (64 mg, 56%). \(^1\)H NMR (500 MHz, D₂O): δ 8.73 (s, 1H, H2), 8.61 (s, 1H, H8), 6.20 (d, J = 5 Hz, 1H, H1'), 5.27 (s, 2H, CH₂N), 4.84-4.82 (t, J = 5 Hz, 1H, H2'), 4.50 (t, J = 5 Hz, 1H, H3'), 4.33-4.31 (m, 1H, H4'), 3.97 (dd, J = 5, 10 Hz, 1H, H5'), 3.90 (dd, J = 5, 10 Hz, 1H, H5'), 3.19 (bs, 1H, CeCH). \(^1^3\)C NMR (126 MHz, D₂O): δ 150.2 (C6), 146.9 (C2), 146.8 (C4), 143.4 (C8), 119.6 (C5), 88.6 (C1'), 85.5 (C4'), 78.7 (C=CH), 74.2 (C2'), 73.1 (C=CH), 70.1 (C3'), 61.1 (C5'), 40.8 (CH₂N). HRMS (ESI) m/z: calcd for C₂₅H₂₃N₅O₂ [M⁺]: 399.1623; found: 399.1623.

**Compound 19:** Following the general procedure B for CuAAC, starting from alkyne 18 (6.3 mg, 0.020 mmol) and azido compound 17 (9.1 mg, 0.03 mmol), compound 19 was obtained as a white foam (6.5 mg, 53%). \(^1\)H NMR (500 MHz, CD₃OD): δ 8.62 (s, 2H, H2a and H8b), 8.14 (s, 1H, H2b), 8.05 (s, 1H, H8a), 7.96 (s, 1H, H₃T₃iazolo), 6.09 (d, J = 5 Hz, 1H, H1' b), 5.95 (d, J = 5 Hz, 1H, H1' a), 5.52 (s, 2H, CH₂N), 4.92-4.96 (m, 1H, H5' b), 5.65 masked in the residual pick of water, 4.76-4.74 (m, 1H, H2'a), 4.62 (t, J = 5 Hz, 1H, H2'b), 4.48 (t, J = 5 Hz, 1H, H3'a), 4.38-4.33 (m, 2H, H3'b and H4'a), 4.15 (q, J = 3.1 Hz, 1H, H4'b), 3.89-3.85 (m, 1H, H5'a), 3.76-3.70 (m, 1H, H5'a). \(^1^3\)C NMR (126 MHz, CD₃OD): δ 157.2 (Cq), 154.0 (C2b), 152.2 (Cq), 150.3 (Cq), 148.4 (C2a), 148.0 (Cq), 144.1 (C8b), 141.7 (C8a), 140.8 (C₃T₃iazolo), 126.7 (CH₃T₃iazolo), 121.3 (Cq), 120.7 (Cq), 91.2 (C1'), 90.4 (C1'b), 87.6 (C4'b), 83.6 (C4'a), 76.5 (C2'b), 74.5 (C2'a), 72.4 (C3'a), 71.8 (C3'b), 62.5 (C5'a), 52.7 (C5'b), 45.9 (CH₂N). HRMS (ESI) m/z: calcd for C₂₅H₂₃N₅O₂ [M + H⁺]: 598.2234; found: 598.2222. HPLC purity: 97.6%; tr = 14.6 min (MeCN/H₂O 0:100 to 100:0 over 30 min).

4. Conclusions

We reported in this study the synthesis of new SAM-adenosine conjugates with a 1,2,3-triazolo linker that covalently links the SAM analogue to the N-6 or N-1 position of the adenosine substrate. The use of protecting groups allowed the formation of the N° and 1-N-conjugates but required numerous steps of protection and deprotection. Revisiting the synthetic strategy, we were able to avoid the steps of protection and deprotection of the hydroxyl and exocyclic amine functions and to propose more straightforward and efficient syntheses. The N° and 1-N conjugates were obtained in 2 steps with overall yield of 26% and 30%, respectively. In addition, we developed an efficient methodology based on CuAAC to get access to conjugates by connecting through a triazole linker an analogue of RNA MTases substrate (an adenosine modified at the N-6 or N-1 position) to a SAM analog cofactor. We think that this approach could be applied for the preparation of modified oligonucleotides to get more complex bisubstrate analogues for the study of m⁶A and m⁷A RNA MTases.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figure S1: title, Table S1: title, Video S1: title.

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References

1. Helm, M.; Motorin, Y. Detecting RNA modifications in the epitranscriptome: predict and validate. *Nat. Rev. Genet.* 2017, 18, 275–291.
2. a) http://mods.rna.albany.edu; b) http://modomics.genesilico.pl.
3. Motorin, Y.; Helm, M. RNA nucleotide methylation. *Wiley Interdiscip. Rev. RNA* 2011, 2, 611-631.
4. Cao, G.C.; Li, H.B.; Yin, Z.N.; Flavell, R.A. Recent advances in dynamic m⁶A RNA modification. *Open Biol.* 2016, 6, 160003.
5. Sergiev, P.V.; Golovina, A.Ya.; Osterman, I.A.; Nesterchuk, M.V.; Sergeeva, O.V.; Chugunova, A.A.; Evratov, S.A.; Andreianova, E.S.; Petlnev, P.I.; Laptev, I.G.; Petriukov, K.S.; Navalayeu, T.I.; Kotelianskiy, V.E.; Bogdanov, A.A.; Dontsova, O.A. N6-Methylated Adenosine in RNA: From Bacteria to Humans. J. Mol. Biol. 2016, 428, 2134-2145.

6. Zhao, B.S.; Roundtree, I.A.; He, C. Post-transcriptional gene regulation by mRNA modifications. Nat. Rev. Mol. Cell Biol. 2017, 18, 31-42.

7. Shi, H.; Wei, J.; He, C. Where, When, and How: Context-Dependent Functions of RNA Methylation Writers, Readers, and Erasers. Mol. Cell. 2019, 74, 640-650.

8. Wei, W.; Ji, X.; Guo, X.; Ji, S. Regulatory role of N6-methyladenosine (m6A) methylation in RNA Processing and Human Diseases. J. Cell Biochem. 2017, 118, 2534-2543.

9. Liu, L.; Wang, Y.; Wu, J.; Liu, J.; Qin, Z.; Fan, H. N6-Methyladenosine: A Potential Breakthrough for Human Cancer. Mol. Ther. Nucleic Acids 2020, 19, 804-813.

10. Dai, D.; Wang, H.; Zhu, L.; Jin, H.; Wang, X. N6-methyladenosine links RNA metabolism to cancer progression. Cell Death and Disease 2018, 9, 124-136.

11. Xiong, X.; Li, X.; Yi, C. N6-methyladenosine methylome in messenger RNA and non-coding RNA. Curr. Opin. Chem. Biol. 2018, 45, 179-186.

12. Oerum, S.; Dégut, C.; Barraud, P.; Tisné, C. m6A Post-Transcriptional Modification in tRNAs. Biomolecules 2017, 7, 20-35.

13. Macon, J.B.; Wolfenden, R. 1-Methyladenosine. Dimroth rearrangement and reversible reduction. Biochim. Biophys. Acta 1968, 7, 3453-3458.

14. Dominissini, D.; Nachtergaele, S.; Moshitch-Moshkovitz, S.; Peer, E.; Kol, N.; Ben-Haim, M.S.; Dai, Q.; Di Segni, A.; Salmon-Divon, M.; Clark, W.C.; Zheng, G.; Pan, T.; Solomon, O.; Eyal, E.; Hershkovitz, V.; Han, D.; Doré, L.C.; Amariglio, N.; Rechavi, G.; He, C. The dynamic N(1)-methyladenosine methylome in eukaryotic messenger RNA. Nature 2016, 530, 441-446.

15. Li, X.; Xiong, X.; Wang, K.; Wang, L.; Shu, X.; Ma, S.; Yi, C. Transcriptome-wide mapping reveals reversible and dynamic N6-methyladenosine methylome. Nat. Chem. Biol. 2016, 12, 311-316.

16. Li, X.; Xiong, X.; Zhang, M.; Wang, K.; Chen, Y.; Zhou, J.; Mao, Y.; Lv, J.; Yi, D.; Chen, X.W.; Wang, C.; Qian, S.B.; Yi, C. Base-Resolution Mapping Reveals Distinct m6A Methylome in Nuclear- and Mitochondrially-Encoded Transcripts. Mol. Cell 2017, 68, 993–1005.

17. a) Dominissini, D.; Moshitch-Moshkovitz, S.; Schwartz, S.; Salmon-Divon, M.; Ungar, L.; Osenberg, S.; Cesarkas, K.; Jacob-Hirsch, J.; Amariglio, N.; Kupiec, M.; Sorek, R.; Rechavi, G. Topology of the human mouse m6A RNA methylomes revealed by m6A-seq. Nature 2012, 485, 201–206; b) Linder, B.; Grozhik, A.V.; Olarereny-George, A.O.; Meydan, C.; Mason, C.E.; Jaffrey, S.R. Single-nucleotide-resolution mapping of m6A and m6Am throughout the transcriptome. Nat. Methods 2015, 12, 767-772; c) Meyer, K.D.; Saletore, Y.; Zumbo, P.; Elemento, O.; Mason, C.E.; Jaffrey, S.R. Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons. Cell 2012, 149, 1635–1646.

18. Meyer, K.D.; Jaffrey, S.R. Rethinking m6A Readers, Writers, and Erasers. Annu. Rev. Cell Dev. Biol. 2017, 33, 319-342.

19. Warda, A.S.; Kretschmer, J.; Hackert, P.; Lenz, C.; Urlaub, H.; Hobartner, C.; Sloan, K.E.; Bohnsack, M.T. Human METTL16 is a N6-methyladenosine (m6A) methyltransferase that targets pre-mRNAs and various non-coding RNAs. Embo Journal 2017, 16, 2004-2014.

20. Pendleton, K.E.; Chen, B.; Liu, K.; Hunter, O.V.; Xie, Y.; Tu, B.P.; Conrad, N.K. The U6 snRNA m6A methyltransferase METTL16 regulates SAM synthetase intron retention. Cell 2017, 169, 824-835.

21. Shima, H.; Matsumoto, M.; Ishigami, Y.; Ebina, M.; Muto, A.; Sato, Y.; Kumagai, S.; Ochiai, K.; Suzuki, T.; Igarashi, K. S-Adenosylmethionine synthesis is regulated by selective N6-adenosine methylation and mRNA degradation involving METTL16 and YTHDC1. Cell Rep. 2017, 21, 3354-3363.

22. Doxtader, K.A.; Wang, P.; Scarborough, A.M.; Seo, D.; Conrad, N.K.; Nam, Y. Structural basis for regulation of METTL16, an S-Adenosylmethionine homeostasis factor. Mol Cell. 2018, 71, 1001-1011.

23. Atdjian, C.; Iannazzo, L.; Braud, E.; Ethève-Quelquejeu, M. Synthesis of SAM-adenosine conjugates for the study of m6A-RNA methyltransferases. Eur. J. Org. Chem. 2018, 4411-4425.

24. Oerum, S.; Catala, M.; Atdjian, C.; Brachet, F.; Ponchon, L.; Barraud, P.; Iannazzo, L.; Droogmans, L.; Braud, E.; Ethève-Quelquejeu, M.; Tisné, C. Bisubstrate analogues as structural tools to investigate m6A methyltransferase active sites. RNA Biol. 2019, 16, 798-808.
25. Fleysher, M.H.; Hakala, M.T.; Bloch, A.; Hall, R.H. Synthesis and biological activity of some N6-alkyladenosines. *J. Med. Chem.* 1968, 11, 717-720.

26. Fleysher, M.H. N6-Substituted adenosines. Synthesis, biological activity, and some structure-activity relations. *J. Med. Chem.* 1972, 15, 187-191.

27. Dolež, K.; Popa, I.; Hauserová, E.; Spichal, L.; Chakrabarty, K.; Novák, O.; Kryštýf, V.; Voller, J.; Holub, J.; Strnad, M. Preparation, biological activity and endogenous occurrence of N6-benzyladenosines. *Bioorg. Med. Chem.* 2007, 15, 3737-3747.

28. Kovalovs, A.; Novosjolova, I.; Bizděna, E.; Bižáne, I.; Skardziute, L.; Kazlauskas, K.; Jursenas, S.; Turks, M. 1,2,3-Triazoles as leaving groups in purine chemistry: a three-step synthesis of N6-substituted-2-triazolyl adenine nucleosides and photophysical properties thereof. *Tetrahedron Lett.* 2013, 54, 850-853.

29. Allerson, C.R.; Chen, S.L.; Verdine, G.L. A Chemical Method for Site-Specific Modification of RNA: The Convertible Nucleoside Approach. *J. Am. Chem. Soc.* 1997, 119, 7423-7433.

30. Miles, R.W.; Samano, V.; Robbins, M.J. Nucleophilic Functionalization of Adenine, Adenosine, Tubercidin, and Formycin Derivatives via Elaboration of the Heterocyclic Amino Group into a Readily Displaced 1,2,4-Triazolyl-4-yl Substituent. *J. Am. Chem. Soc.* 1995, 117, 5951-5957.

31. Véliz, E.A.; Breal, P.A. 6-Bromopurine Nucleosides as Reagents for Nucleoside Analogue Synthesis. *J. Org. Chem.* 2001, 66, 8592-8598.

32. Wolf, J.; Dombos, V.; Appel, B.; Muller, S. Synthesis of guanosine 5′-conjugates and their use as initiator molecules for transcription priming. *Org. Biomol. Chem.* 2008, 6, 899-907.

33. Januchta, W.; Serocki, M.; Dzierzbicka, K.; Cholewinski, G.; Gensicka, M.; Składanowski, A. Synthesis and biological evaluation of novel analogues of batraclycin with synthetic amino acids and adenosine: an unexpected effect on centromere segregation in tumor cells through a dual inhibition of topoisomerase IIA and Aurora B. *RSC Adv.* 2016, 6, 42794-42806.

34. Mahajan, S.; Manetsch, R.; Merkler, D.J.; Stevens Jr., S.M. Synthesis and Evaluation of a Novel Adenosine-Ribose Probe for Global-Scale Profiling of Nucleoside and Nucleotide-Binding Proteins. *PLoS One* 2015, 10, e0115644.

35. Yu, J.; Zhao, L.X.; Park, J.; Lee, H.W.; Sahu, P.K.; Cui, M.; Moss, S.M.; Hammes, E.; Warnick, E.; Gao, Z.G.; Noh, M.; Choi, S.; Ahn, H.C.; Choi, J.; Jacobson, K.A.; Jeong, L.S. N6-Substituted 5′-N-Methylcarbamoyl-4′-selenoadenosines as Potent and Selective A3 Adenosine Receptor Agonists with Unusual Sugar Puckering and Nucleobase Orientation. *J. Med. Chem.* 2017, 60, 3422-3437.

36. Lin, X.; Robins, M.J. Mild and Efficient Functionalization at C6 of Purine 2′-Deoxynucleosides and Ribonucleosides. *Org. Lett.* 2000, 2, 3497-3499.

37. Ottoria, R.; Casati, S.; Baldoli, E.; Maier, J.A.M.; Ciuffreda, P. N6-Alkyladenosines: Synthesis and evaluation of in vitro anticancer activity. *Bioorg. Med. Chem.* 2010, 18, 8396-8402.

38. Jones, J.W.; Robins, R.K. Purine nucleosides. III. Methylation study of certain naturally occurring purine nucleosides. *J. Am. Chem. Soc.* 1963, 85, 193-201.

39. Brookes, P.; Dipple, A.; Lawley, P.D. The preparation and properties of some benzylated nucleosides. *J. Chem. Soc. (C)* 1968, 2026-2028.

40. Leonard, N.J.; Fujii, T. The synthesis of compounds possessing k inetin activity. The use of a blocking group at the 9-position of adenine for the synthesis of 1-substituted adenines. *Proc. Natl. Acad. Sci. U. S. A.* 1964, 51, 73-75.

41. Brookes, P.; Lawley, P.D. The methylation of adenosine and adenyl acid. *J. Chem. Soc.* 1960, 539-545.

42. Coddington, A. The preparation of 6-N-methyldeoxyadenosine. *Biochim. Biophys. Acta.* 1962, 59, 472-474.

43. Leonard, N.J.; Achmatowicz, S.; Loeppky, R.N.; Carraway, K.L.; Grimm, W.A.H.; Szweykowska, A.; Hamzi, H.Q.; Skoog, F. Development of cytokinin activity by rearrangement of 1-substituted adenosines to 6-substituted aminopurines: inactivation by N6,1-cyclization. *Proc. Natl. Acad. Sci. U. S. A.* 1966, 56, 709-716.

44. Robins, M.J.; Trip, E.M. Nucleic acid related compounds. 6. Sugar-modified N6-(3-methyl-2-butenyl)adenosine derivatives, N6-benzyl analogs, and cytokinin-related nucleosides containing sulfur or formycin. *Biochemistry* 1973, 12, 2179-2187.

45. Grimm, W.A.H.; Leonard, N.J. Synthesis of the “Minor Nucleotide” N6-(γγ-Dimethylallyl) adenosine 5′-Phosphate and Relative Rates of Rearrangement of 1- to N6-Dimethylallyl Compounds for Base, Nucleoside, and Nucleotide. *Biochemistry* 1967, 6, 3625-3631.
Sample Availability: Samples of the compounds are not available from the authors.

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