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1 Article

2 Synthesis of triazole-linked SAM-adenosine 3 conjugates: functionalization of adenosine at N-1 or 4 N-6 position without protecting groups

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

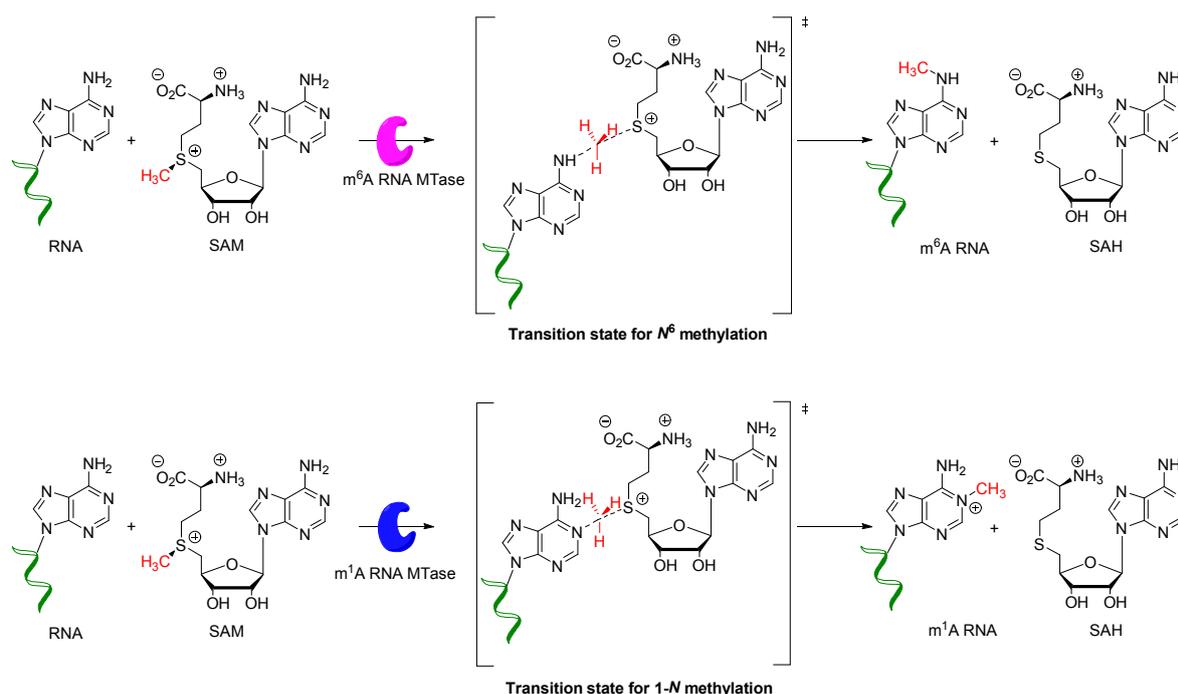
29 **Keywords:** RNA m^6A methyltransferase; RNA m^1A methyltransferase; bisubstrate analogues;
30 S-adenosyl-L-methionine; 1-N-alkylated adenosine; 1,2,3-triazole; click chemistry; CuAAC
31

32 1. Introduction

33 Among the numerous post-transcriptional modifications of RNA identified to date,
34 methylation is currently one of the most studied [1]. This modification can occur at the terminal cap
35 of RNAs or at internal position, and the methyl group is found on nucleic acid bases or at the 2'
36 position of the ribose units. The adenine base can be methylated at the C-2 and C-8 atoms as well as
37 at the nitrogen atoms N-1 and N-6 [2,3]. N^6 -Methyladenosine (m^6A) is an abundant reversible
38 modification found in all types of RNA, involved in the regulation of RNA metabolism, protein
39 expression or RNA-protein recognition [4-7]. Abnormal methylation process is associated with the
40 development of diseases such as cancers, obesity, infertility [8-10]. Though less studied, the
41 modification at the N-1 site (m^1A) is also reversible and linked to the structural stability and the
42 functions of RNAs [11-12].

43 Since m¹A can rearrange to m⁶A under alkaline conditions by Dimroth rearrangement [13], its
 44 presence in mRNA has been detected only recently in mammalian mRNAs [14–16]. The functional
 45 consequence of this modification is poorly understood but could impact the regulation and the
 46 function of m¹A for some positions in rRNA and tRNA in the human transcriptome. However, the
 47 unique chemical properties of m¹A, with both a positive charge and a methyl group, potentially
 48 allow for a strong effect in terms of RNA structure or protein–RNA interaction [14].

49 For these two modifications (m¹A and m⁶A), the methyl group is introduced enzymatically by
 50 RNA methyltransferases (RNA MTases) that catalyze the transfer of the methyl from the cofactor
 51 S-adenosyl-L-methionine (SAM) to the nucleotides (Scheme 1). Most m⁶A marks in mammals
 52 mRNA are written by a dedicated methyltransferase complex involving the heterodimer
 53 METTL3/METTL14, targeting the consensus sequence RRACH [17]. However, little is known about
 54 the recognition of the RNA targets by this complex [18]. METTL16 has a distinct set of targets for
 55 m⁶A modification, including the 3' UTR of MAT2A mRNA and the U6 snRNA, a longer conserved
 56 sequence of UACAGAGAA [19–21]. Among human m⁶A RNA MTases, only METTL16 has been
 57 crystallized with RNA substrate [22]. For m¹A modification of mRNA it's not clear whether or not
 58 specific methylation machinery could also exist. It has been recently hypothesized that the tRNA
 59 methyltransferase complex TRMT6/61A could catalyze the methyl transfer on the mRNA [16]. In
 60 conclusion, few RNA-bound MTases structures are currently available. This lack of complex
 61 structures is due to the difficulties in crystallizing RNA/protein complexes. As a consequence, RNA
 62 recognition patterns and methylation reaction mechanisms remain poorly understood.
 63



64
 65 **Scheme 1.** RNA MTases-catalyzed methylation of adenosine at the N-6 and N-1 positions.
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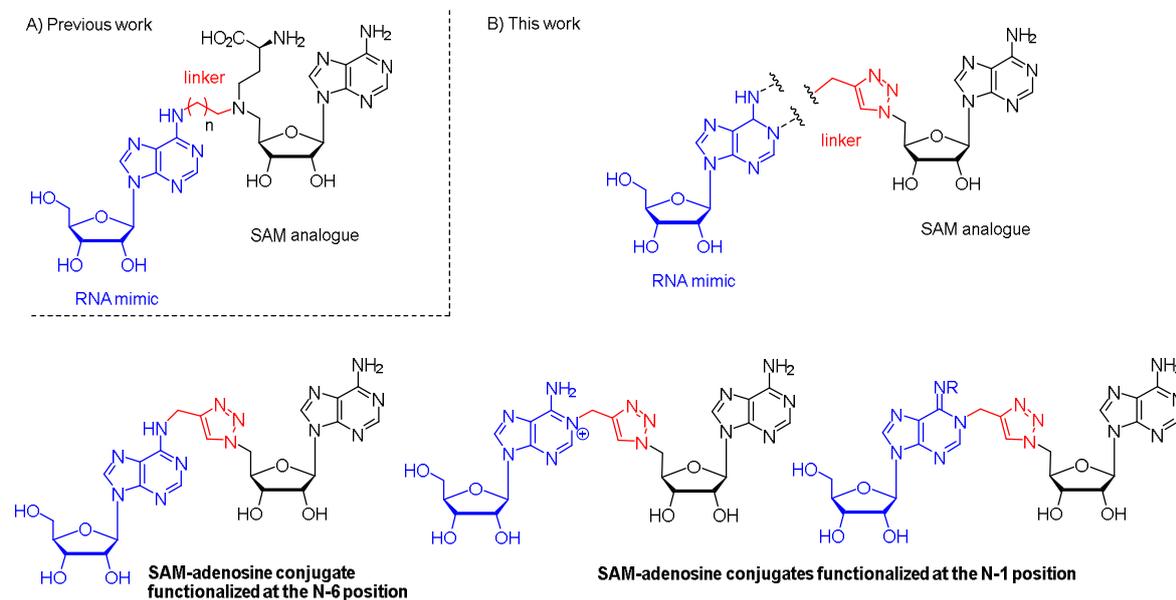
67 In this context, we recently described the synthesis of SAM-adenosine conjugates as first
 68 transition state analogues for m⁶A RNA MTases and their use as tools for structural study [23,24].
 69 We showed that a SAM-adenosine conjugate containing a three-carbon linker tethering the analogue
 70 of SAM to the N-6 atom of the adenosine binds the bacterial RNA MTase RlmJ with a conformation
 71 close to the real transition state. The structure of this bisubstrate analogue favors the correct
 72 positioning of the RNA moiety mimicked by an adenosine and the methionine part of the cofactor
 73 into the catalytic site of the MTase. However, a deviation was observed for the positioning of the
 74 adenosine in the cofactor part, which is rotated 120° out of the canonical binding pocket for SAH.
 75 This deviation indicates that our bisubstrate analogues are not optimal. In this study, we pursue the
 76 development of SAM-adenosine conjugates for m⁶A RNA MTases and extend our work to the

77 synthesis of the first potential bisubstrate analogues for m¹A RNA MTases by covalently linking an
 78 analogue of SAM to the N-6 or N-1 atom of the adenosine substrate, respectively.

79 N⁶-alkylation of adenosine derivatives is mainly achieved through aromatic nucleophilic
 80 substitution (S_NAr) of diverse electrophilic adenosine derivatives [25-37] or by Dimroth
 81 rearrangement of 1-N-alkylated adenosines [38-50]. In the context of S_NAr, synthetic strategies
 82 involving non classic leaving groups were developed using peptide-coupling agents for activation of
 83 the amide group of inosine derivatives [51-54]. Another study reports the reduction of
 84 N⁶-acyl-adenosine derivatives with LiAlH₄ [55]. Selective N⁶-alkylation of adenosine derivatives can
 85 also be achieved under phase transfer catalysis conditions as described by Arimoto *et al.* [56]. Two
 86 groups used Mitsunobu reaction applied to N⁶-acetyl-2',3',5'-tri-O-acetyladenosine [57-58] or N⁶-Boc
 87 protected adenosine [59] to regioselectively synthesize N⁶-alkylated products. Finally,
 88 functionalization of 6-chloroadenosine derivatives can be achieved by palladium-catalyzed
 89 Buchwald–Hartwig coupling [60-61].

90 To date, the alkylation reaction at the N-1 position has been principally developed through the
 91 N-1 nucleophilic attack of alkyl halides [38-50]. This approach allows for the introduction of methyl
 92 and alkyl groups as well as benzyl, or allyl substituents. Propargyl group has been also installed on
 93 N⁶-acetyl-2',3',5'-tri-O-acetyladenosine but in a quite low yield of 14% [49]. In 2005, Terrazas *et al.*
 94 synthesized 1-N-alkylated adenosines by reacting electrophilic inosine with primary amines [62].

95 In this context, we sought to introduce new chemical modifications at the N-6 and N-1 positions
 96 of adenosine to synthesize new SAM-adenosine conjugates. Click chemistry, especially
 97 Copper(I)-catalyzed Alkyne-Azide Cycloaddition (CuAAC), is an efficient strategy to rapidly
 98 synthesize complex structures. On these bases, we designed new bisubstrate analogues bearing a
 99 1,2,3-triazole ring instead of the alkyl linker to increase rigidity between the mimic of the substrate
 100 and the SAM analogue while maintaining an appropriate length between the two entities (Figure 1).
 101 The synthetic strategy used to obtain these molecules relies on the efficient introduction of the
 102 propargyl group at the N-6 and N-1 positions of adenosine derivatives.
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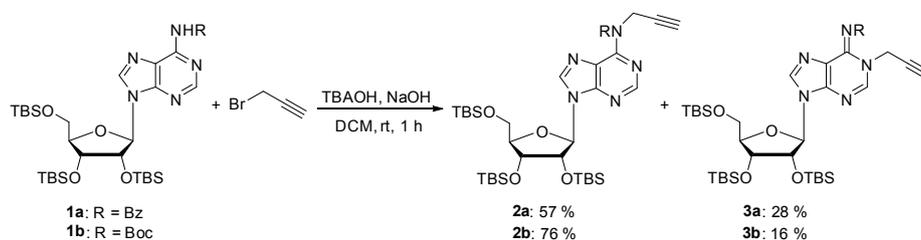
106 **Figure 1.** (A) Our previous work: Structure of SAM-adenosine conjugates with an alkyl linker [23-24]; (B)
 107 Structure of SAM-adenosine conjugates with a 1,2,3-triazole linker synthesized in this study.

108 2. Results and discussion

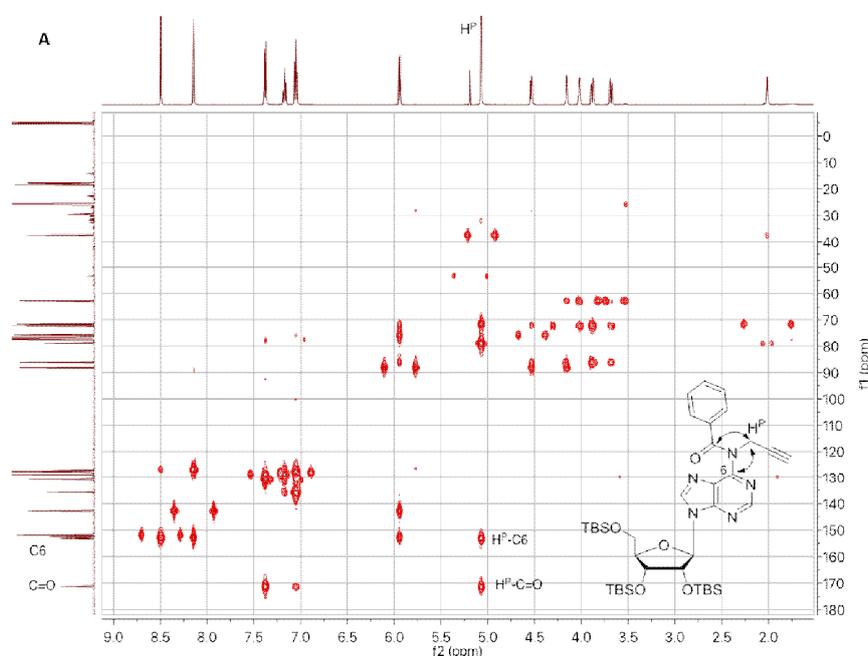
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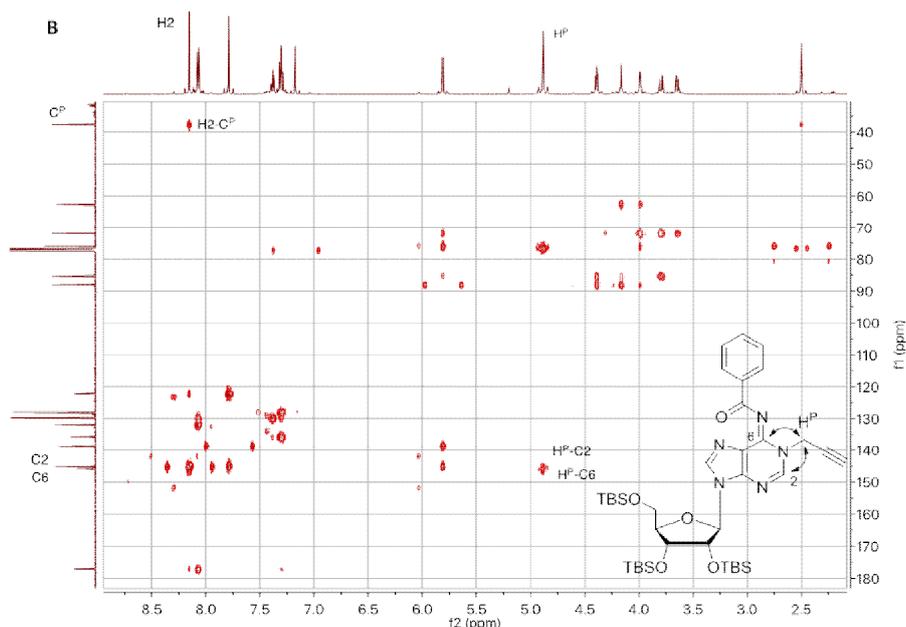
110 2.1. Synthesis of SAM-adenosine conjugates using protecting groups

111 First, we took advantage of the work of Sekine *et al.* who used tetrabutylammonium bromide
 112 (TBABr) as the phase transfer catalyst to produce a mixture of *N*⁶ and 1-*N*-alkylated adenosines from
 113 *N*⁶-benzoylated adenosine [56]. A short study was carried out to investigate the alkylation of
 114 *N*⁶-benzoyl-2',3',5'-tris-*O*-(*tert*-butyldimethylsilyl)adenosine **1a** [63] with propargyl bromide
 115 (Scheme 2). Using tetrabutylammonium hydroxide (TBAOH) instead of TBABr led to the formation
 116 of adenosines **2a** and **3a** in 57 and 28% yield with total conversion of the starting material (Scheme 2).
 117 Analysis of 1D and 2D NMR spectra confirmed the site of alkylation for each regioisomer (Figure 2)
 118 [49,56]. The signals for 2-H and 8-H appear at 8.49 and 8.14 ppm for *N*⁶-alkylated compound **2a**
 119 while they are upfield at 8.24 and 7.88 ppm in **3a** as expected. Moreover, two correlations are
 120 observed in the HMBC spectrum of **2a** between protons of the methylene group of the propargyl
 121 (*H*^P) and C6 and C=O of benzoyl group in the *N*-6 regioisomer (Figure 2A). For compound **3a**,
 122 HMBC experiments show a correlation between 2-H and the carbon of the methylene group (*C*^P) as
 123 well as two correlations between the *H*^P protons and C2 and C6 (Figure 2B). The same reaction
 124 conditions applied to compound **1b** [23] containing a *tert*-butoxycarbonyl protecting group at the
 125 *N*-6 position of adenosine afforded **2b** and **3b** in 76 and 16% yield respectively, the presence of the
 126 carbamate function favoring the *N*⁶ alkylation.



Scheme 2. Synthesis of *N*⁶ and 1-*N*-propargylated adenosines.

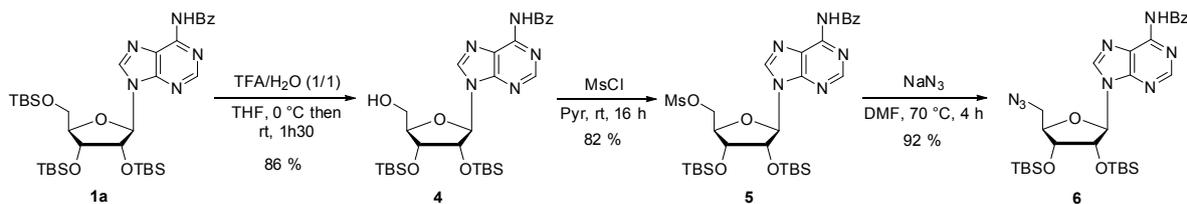




131

132 **Figure 2.** 2D HMBC spectra (500 MHz, CDCl_3): (A) N^6 -propargyladenosine derivative **2a**. (B)
 133 1-propargyladenosine derivative **3a**.

134 Azide **6** was synthesized in three steps [23] (Scheme 3). Briefly, removal of TBS group at the 5'
 135 position of **1a** led to the alcohol **4** that was mesylated to afford derivative **5** in 82% yield. Finally,
 136 treatment of **5** with sodium azide provided azido adenosine **6** in 92% yield.

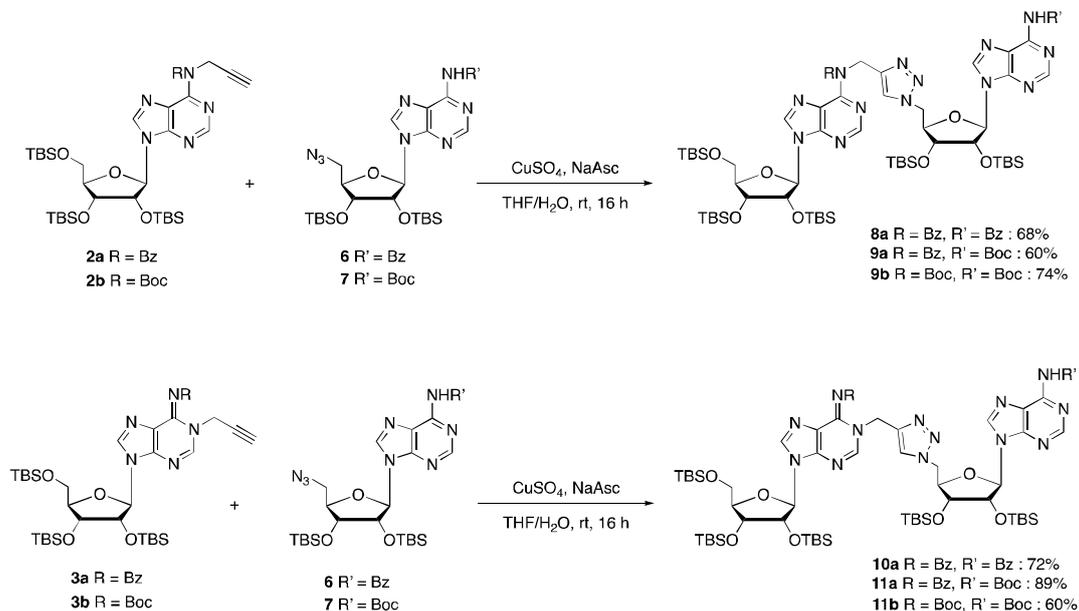


138

139

Scheme 3. Synthesis of azide **6**.

140 CuAAC reactions between adenosines **2a-b** and **3a-b** and azides **6** or **7** [23] were conducted
 141 under classic conditions in the presence of sodium ascorbate and copper sulfate in THF/ H_2O to
 142 afford triazoles **8-11** in 60-89% yield (Scheme 4).

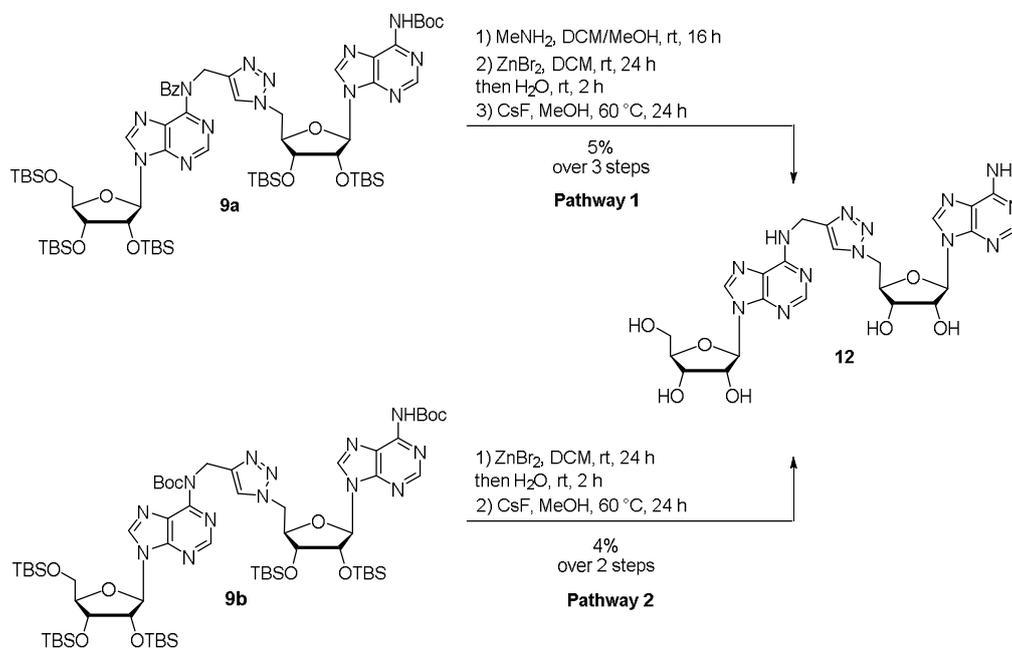


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Scheme 4. Synthesis of triazoles **8-11**.

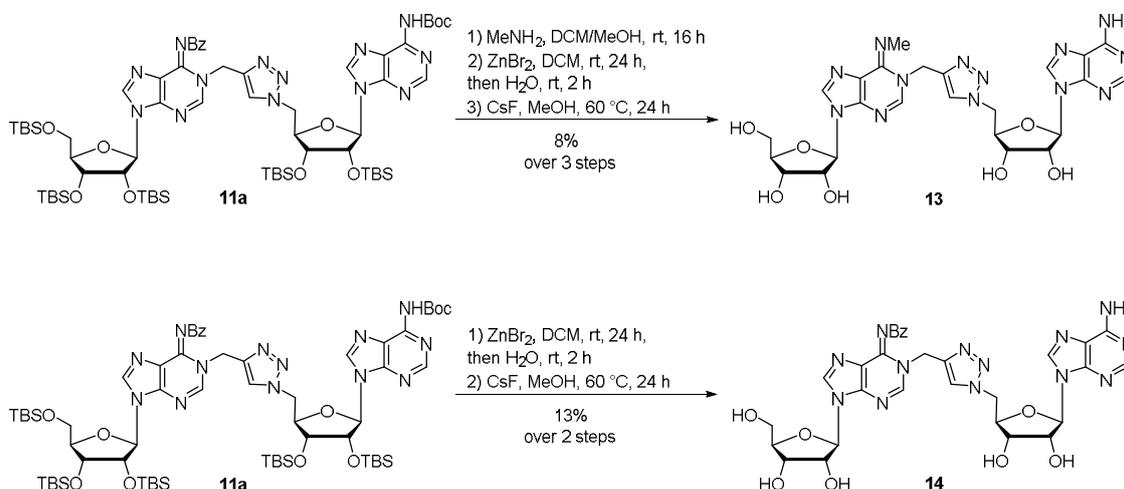
145 The fully deprotected SAM-adenosine conjugate **12** could be obtained from either **9a** or **9b**
 146 (Scheme 5). Compound **9a** was successively treated with methylamine, ZnBr₂ and cesium fluoride
 147 (CsF) to remove the benzoyl-, the Boc- and the TBS groups respectively to give **12** in 5% yield over
 148 three steps after HPLC purification. In comparison, a two-step strategy from **9b**, followed by HPLC
 149 purification, led to the formation of **12** in 4% yield. These results seem to indicate that in the pathway
 150 2, the removal of the two Boc groups is less efficient than the two steps required for the deprotection
 151 of the benzoyl and Boc groups in the pathway 1 (Scheme 5).



152
153 **Scheme 5.** Synthesis of SAM-adenosine conjugate **12**.

154 We applied the strategy used for the deprotection of compound **9a** to compound **11a**.
 155 Unfortunately, efforts to remove the protecting group of the exocyclic amine were unsuccessful.
 156 Indeed, using the same successive steps, we observed the formation of the *N*-methylated compound
 157 **13** in 8% yield as a mixture of *E* and *Z* imines (Scheme 6). Other attempts were conducted with bases
 158 such as ammonia or potassium carbonate which led to the recovery of the starting material in the
 159 first case and to degradation in the second one. As an alternative, we chose to remove only the Boc
 160 and the TBS groups in a two-step sequence allowing for the formation of SAM-adenosine conjugate
 161 **14** in 13% yield (Scheme 6).

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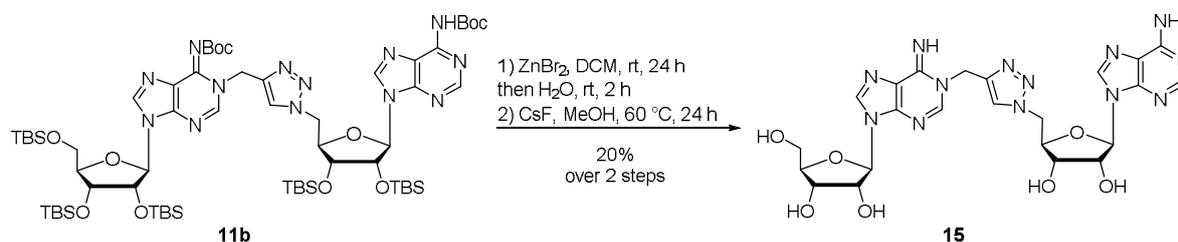


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Scheme 6. Access to SAM-adenosine conjugates **13** and **14**.

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

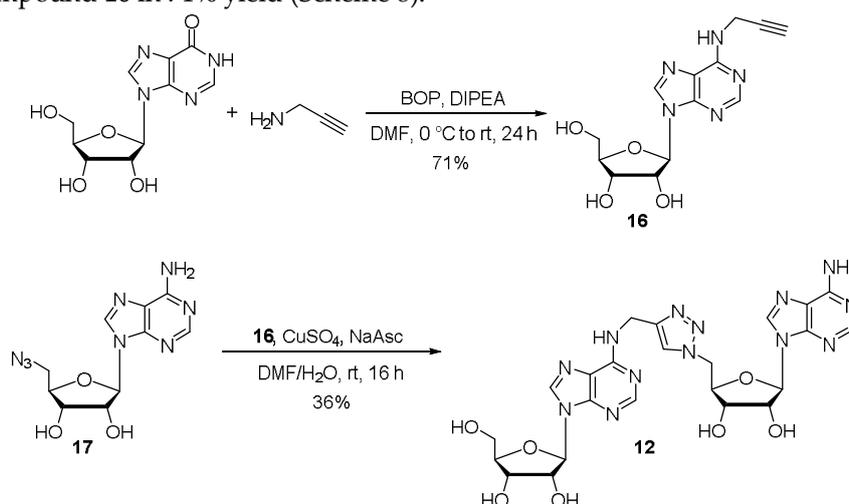


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Scheme 7. Access to SAM-adenosine conjugate **15**.

171 2.2. Synthesis of SAM-adenosine conjugates without protecting groups

172 Since our syntheses require numerous steps of protection and deprotection, we reinvestigated
 173 the synthetic strategies to develop more efficient approaches to get the SAM-adenosine conjugates.
 174 We also sought to introduce regioselectively the propargyl group at the N-6 and N-1 positions. We
 175 first modified the synthesis of N⁶-conjugate **12**. Wan *et al.* previously reported the amination of
 176 unprotected inosine using primary amines in the presence of BOP and DIPEA [53]. Following this
 177 methodology, the propargyl group was introduced in one step at the N-6 position of adenosine
 178 leading to compound **16** in 71% yield (Scheme 8).



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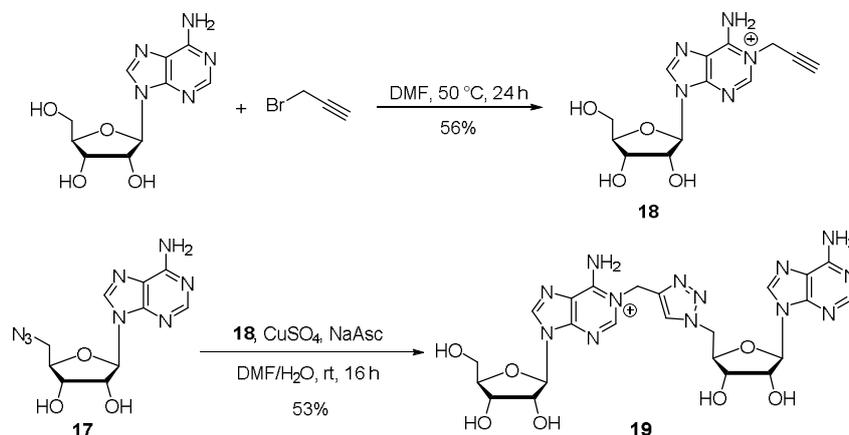
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Scheme 8. Access to SAM-adenosine conjugate **12**.

181 The unprotected azido partner **17** was prepared following a two-step procedure in 36% yield
 182 [64,65]. Then, alkyne **16** and azide **17** were reacted in the presence of copper sulfate and sodium
 183 ascorbate in a DMF/H₂O mixture to afford the expected SAM-adenosine conjugate **12** in 36% yield
 184 (Scheme 8).

185 We next investigated the synthesis of 1-propargyladenosine **18** (Scheme 9). Adenosine is known
 186 to react with alkyl halides in polar solvents such as DMF or DMA at room temperature, to afford
 187 1-N-alkylated compounds [38,49]. Under these conditions, adenosine was treated with an excess of
 188 propargyl bromide in DMF at 50 °C for 24 h and 1-N-propargylated adenosine **18** was obtained in
 189 56% yield (Scheme 9). The synthesis of SAM-adenosine conjugate with a triazole linker connected at
 190 the N-1 position was then achieved through the CuAAC between **17** and **18**. The reaction was
 191 carried out in the presence of sodium ascorbate and copper sulfate to afford the expected conjugate
 192 **19** in 53% yield (Scheme 9). Compound **19** corresponds to the protonated and positively charged
 193 form of compound **15**. This was confirmed by ¹H NMR spectra analysis for both compounds **15** and
 194 **19** (See the supporting file for NMR spectra of compounds **15** and **19**). The signal for 2-H (H_{2a})

195 appears at 8.62 ppm for compound **19** while it is upfield at 8.22 ppm for compound **15** as expected
 196 [49].



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

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3. Materials and Methods

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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 77.16), MeOD (δ 49.0) 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 80 % in toluene (674 μL, 8.88 mmol) were dissolved in DCM (50 mL) and tetrabutylammonium hydroxyde (1.78 g, 2.22 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, H^{Bz}), 7.18-7.15 (m, 1H, H^{Bz}), 7.06-7.03 (m, 2H, H^{Bz}), 5.95 (d, *J* = 5.9 Hz, 1H, H_{1'}), 5.7 (d, *J* = 4.9 Hz, 2H, CH₂N), 4.54-4.52 (m, 1H, H_{2'}), 4.17-4.15 (m, 1H, H_{3'}), 4.03-4.01 (m, 1H, H_{4'}), 3.89 (dd, *J* = 4.4, 11.4 Hz, 1H, H_{5'}), 3.68 (dd, *J* = 2.8,

233 11.2 Hz, 1H, H5'), 2.01 (t, $J = 4.7$ Hz, 1H, C≡CH), 0.85 (s, 9H, *t*Bu^{TBS}), 0.83 (s, 9H, *t*Bu^{TBS}), 0.64 (s, 9H,
234 *t*Bu^{TBS}), 0.02 (s, 3H, Me^{TBS}), 0.01 (s, 3H, Me^{TBS}), 0.00 (s, 6H, 2 Me^{TBS}), -0.19 (s, 3H, Me^{TBS}), -0.51 (s, 3H,
235 Me^{TBS}). ¹³C NMR (126 MHz, CDCl₃): δ 171.4 (C=O), 153.2 (C6), 152.8 (C4), 151.9 (C2), 142.8 (C8), 135.9
236 (Cq^{Bz}), 130.9 (C^{Bz}), 128.9 (2C, C^{Bz}), 127.9 (2C, C^{Bz}), 127.0 (C5), 88.2 (C1'), 86.2 (C4'), 79.0 (C≡CH), 75.8
237 (C2'), 72.4 (C3'), 71.7 (C≡CH), 62.8 (C5'), 37.5 (CH₂N), 26.1 (3C, *t*Bu^{TBS}), 25.9 (3C, *t*Bu^{TBS}), 25.7 (3C,
238 *t*Bu^{TBS}), 18.6 (Cq^{TBS}), 18.1 (Cq^{TBS}), 17.9 (Cq^{TBS}), -4.3 (Me^{TBS}), -4.4 (Me^{TBS}), -4.5 (Me^{TBS}), -5.0 (Me^{TBS}), -5.2
239 (Me^{TBS}), -5.3 (Me^{TBS}). HRMS (ESI) *m/z*: calcd for C₃₈H₆₁N₅NaO₅Si₃ [M + Na]⁺: 774.3878; found:
240 774.3904. **3a**: ¹H NMR (500 MHz, CDCl₃): δ 8.24 (s, 1H, H2), 8.16-8.15 (m, 2H, H^{Bz}), 7.88 (s, 1H, H8),
241 7.48-7.45 (m, 1H, H^{Bz}), 7.40-7.37 (m, 2H, H^{Bz}), 5.90 (d, $J = 5.0$ Hz, 1H, H1'), 4.97 (t, $J = 2.8$ Hz, 2H,
242 CH₂N), 4.48-4.47 (m, 1H, H2'), 4.26-4.24 (m, 1H, H3'), 4.08-4.06 (m, 1H, H4'), 3.88 (dd, $J = 4.0, 11.3$ Hz,
243 1H, H5'), 3.73 (dd, $J = 3.4, 11.4$ Hz, 1H, H5'), 2.59 (t, $J = 2.5$ Hz, 1H, C≡CH), 0.92 (s, 9H, *t*Bu^{TBS}), 0.89 (s,
244 9H, *t*Bu^{TBS}), 0.81 (s, 9H, *t*Bu^{TBS}), 0.09 (s, 3H, Me^{TBS}), 0.08 (s, 3H, Me^{TBS}), 0.06 (s, 3H, Me^{TBS}), 0.05 (s, 3H,
245 Me^{TBS}), -0.03 (s, 3H, Me^{TBS}), -0.19 (s, 3H, Me^{TBS}). ¹³C NMR (126 MHz, CDCl₃): δ 177.2 (C=O), 146.1 (C6),
246 145.4 (C2), 145.1 (C4), 138.8 (C8), 135.8 (Cq^{Bz}), 132.0 (C^{Bz}), 129.9 (2C, C^{Bz}), 128.1 (2C, C^{Bz}), 122.3 (C5),
247 88.2 (C1'), 85.4 (C4'), 76.7 (C≡CH), 76.1 (C2'), 75.9 (C≡CH), 72.0 (C3'), 62.7 (C5'), 37.8 (CH₂N), 26.1
248 (3C, *t*Bu^{TBS}), 25.9 (3C, *t*Bu^{TBS}), 25.8 (3C, *t*Bu^{TBS}), 18.6 (Cq^{TBS}), 18.2 (Cq^{TBS}), 18.0 (Cq^{TBS}), -4.2 (Me^{TBS}), -4.4
249 (Me^{TBS}), -4.5 (Me^{TBS}), -4.8 (Me^{TBS}), -5.2 (Me^{TBS}), -5.3 (Me^{TBS}). HRMS (ESI) *m/z*: calcd for
250 C₃₈H₆₁N₅NaO₅Si₃ [M + Na]⁺: 774.3878; found: 774.3911.
251

252 **Compounds 2b and 3b**: To a stirred solution of compound **1b** (1.5 g, 2.11 mmol) in DCM (47.5 mL)
253 was added propargyl bromide (80% in toluene) (0.795 mL, 8.44 mmol), tetrabutylammonium
254 hydroxide (1.68 g, 1.68 mmol) and 1M aqueous NaOH (21.1 mL). The reaction mixture was stirred at
255 room temperature for 1 h. The reaction mixture was dissolved in DCM and washed with brine. The
256 organic layer was dried over MgSO₄, concentrated *in vacuo* and purified on silica gel
257 chromatography (eluent: Cyclohexane/EtOAc 9:1 then 7:3) to afford the desired compounds as white
258 foams (1.2 g, 76% for **2b** and 0.251 g, 16% for **3b**). **2b**: ¹H NMR (500 MHz, CDCl₃): δ 8.77 (s, 1H, H2),
259 8.41 (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'),
260 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, $J =$
261 11.4, 2.8 Hz, 1H, H5'), 2.16 (t, $J = 2.4$ Hz, 1H, C≡CH), 1.48 (s, 9H, *t*Bu^{Boc}), 0.96 (s, 9H, *t*Bu^{TBS}), 0.93 (s,
262 9H, *t*Bu^{TBS}), 0.80 (s, 9H, *t*Bu^{TBS}), 0.15 (s, 3H, Me^{TBS}), 0.14 (s, 3H, Me^{TBS}), 0.10 (s, 3H, Me^{TBS}), 0.09 (s, 3H,
263 Me^{TBS}), -0.04 (s, 3H, Me^{TBS}), -0.22 (s, 3H, Me^{TBS}). ¹³C NMR (126 MHz, CDCl₃): δ 152.7 (C6), 152.5 (C4),
264 152.4 (C=O), 151.8 (C2), 142.2 (C8), 127.60 (C5), 88.7 (C1'), 85.5 (C4'), 82.9 (Cq^{Boc}), 79.9 (C≡CH), 76.1
265 (C2'), 72.0 (C3'), 71.2 (C≡CH), 62.6 (C5'), 37.3 (CH₂N), 28.1 (3C, *t*Bu^{Boc}), 26.3 (3C, *t*Bu^{TBS}), 26.0 (3C,
266 *t*Bu^{TBS}), 25.8 (3C, *t*Bu^{TBS}), 18.7 (Cq^{TBS}), 18.2 (Cq^{TBS}), 18.0 (Cq^{TBS}), -4.2 (Me^{TBS}), -4.5 (Me^{TBS}), -4.6 (Me^{TBS}),
267 -4.8 (Me^{TBS}), -5.2 (Me^{TBS}), -5.2 (Me^{TBS}). HRMS (ESI) *m/z*: calcd for C₃₆H₆₆N₅O₆Si₃ [M + H]⁺: 748,4315;
268 found 748.4304. **3b**: ¹H NMR (500 MHz, CDCl₃): δ 8.22 (s, 1H, H2), 8.12 (s, 1H, H8), 5.91 (d, $J = 3.3$ Hz,
269 1H, H1'), 4.83 (d, $J = 2.7$ Hz, 2H, CH₂N), 4.36-4.30 (m, 2H, H2', H3'), 4.11-4.08 (m, 1H, H4'), 3.99 (dd, $J =$
270 11.6, 3.4 Hz, 1H, H5'), 3.78 (dd, $J = 11.6, 2.5$ Hz, 1H, H5'), 2.56 (t, $J = 2.6$ Hz, 1H, C≡CH), 1.60 (s, 9H,
271 *t*Bu^{Boc}), 0.94 (s, 9H, *t*Bu^{TBS}), 0.92 (s, 9H, *t*Bu^{TBS}), 0.86 (s, 9H, *t*Bu^{TBS}), 0.12 (s, 3H, Me^{TBS}), 0.11 (s, 3H,
272 Me^{TBS}), 0.10 (s, 3H, Me^{TBS}), 0.08 (s, 3H, Me^{TBS}), 0.02 (s, 3H, Me^{TBS}), -0.04 (s, 3H, Me^{TBS}). ¹³C NMR (126
273 MHz, CDCl₃): δ 161.0 (C=O), 147.1 (C6), 145.1 (C2), 144.2 (C4), 138.2 (C8), 122.0 (C5), 88.7 (C1'), 84.5
274 (C4'), 80.9 (Cq^{Boc}), 76.8 (C≡CH), 76.7 (C2'), 76.4 (C≡CH), 70.9 (C3'), 61.9 (C5'), 37.0 (CH₂N), 28.3 (3C,
275 *t*Bu^{Boc}), 26.3 (3C, *t*Bu^{TBS}), 26.0 (3C, *t*Bu^{TBS}), 25.9 (3C, *t*Bu^{TBS}), 18.7 (Cq^{TBS}), 18.2 (Cq^{TBS}), 18.1 (Cq^{TBS}), -4.1
276 (Me^{TBS}), -4.6 (2C, Me^{TBS}), -4.7 (Me^{TBS}), -5.2 (Me^{TBS}), -5.3 (Me^{TBS}). HRMS (ESI) *m/z*: calcd for
277 C₃₆H₆₆N₅O₆Si₃ [M + H]⁺: 748,4315; found 748.4309.
278

279 **Compound 4**: To a stirred solution of compound **1a** (1.42 g, 1.97 mmol) in THF (20 mL) at 0 °C,
280 was added dropwise an aqueous solution of TFA (1:1, 7.55 mL, 98.72 mmol). The solution was
281 stirred at room temperature for 1 h 30. The reaction mixture was neutralized with saturated aqueous
282 NaHCO₃ solution and dissolved in EtOAc. The organic layer was washed with brine, dried over
283 MgSO₄, filtered and concentrated *in vacuo*. The residue was purified on silica gel chromatography
284 (eluent: Cyclohexane/EtOAc 6:4) to afford compound **4** as a white foam (1.02 g, 86%). ¹H NMR (500

285 MHz, CDCl₃): δ 8.83 (s, 1H, H₂), 8.13 (s, 1H, H₈), 8.07-8.03 (m, 2H, H^{Bz}), 7.65-7.60 (m, 1H, H^{Bz}),
286 7.56-7.51 (m, 2H, H^{Bz}), 5.88 (d, J = 7.6 Hz, 1H, H_{1'}), 5.03 (dd, J = 7.6, 4.5 Hz, 1H, H_{2'}), 4.35 (d, J = 4.5
287 Hz, 1H, H_{3'}), 4.20 (d, J = 1.7 Hz, 1H, H_{4'}), 3.98 (dd, J = 13.1, 1.8 Hz, 1H, H_{5'}), 3.74 (dd, J = 13.0, 1.6 Hz,
288 1H, H_{5'}), 0.96 (s, 9H, *t*Bu^{TBS}), 0.75 (s, 9H, *t*Bu^{TBS}), 0.14 (s, 3H, Me^{TBS}), 0.13 (s, 3H, Me^{TBS}), -0.12 (s, 3H,
289 Me^{TBS}), -0.60 (s, 3H, Me^{TBS}). ¹³C NMR (126 MHz, CDCl₃): δ 164.4 (C=O), 152.4 (C₆), 150.6 (C₂), 150.5
290 (C₄), 143.4 (C₈), 133.7 (C_q^{Bz}), 133.1 (C^{Bz}), 129.1 (2C, C^{Bz}), 127.9 (2C, C^{Bz}), 124.5 (C₅), 91.4 (C_{1'}), 89.7
291 (C_{4'}), 74.2 (C_{2'}), 74.0 (C_{3'}), 63.1 (C_{5'}), 25.9 (3C, *t*Bu^{TBS}), 25.8 (3C, *t*Bu^{TBS}), 18.2 (C_q^{TBS}), 17.9 (C_q^{TBS}), -4.4
292 (Me^{TBS}), -4.4 (Me^{TBS}), -4.5 (Me^{TBS}), -5.7 (Me^{TBS}). HRMS (ESI) m/z : calcd for C₂₉H₄₆N₅O₅Si₂ [M + H]⁺:
293 600.3032; found: 600.3022.

294

295 **Compound 5:** Methanesulfonyl chloride (0.26 mL, 3.33 mmol) in pyridine (10 mL) was added
296 dropwise to a solution of compound **4** (1 g, 1.66 mmol) in pyridine (7 mL) at 0 °C. The reaction
297 mixture was stirred at room temperature for 16 h. The reaction was quenched by addition of water
298 and then diluted in DCM. The organic layer was washed with a saturated solution of NaHCO₃ and
299 brine. The combined organic layer was dried over MgSO₄ and concentrated to dryness. The residue
300 was purified on silica gel chromatography (eluent: Cyclohexane/EtOAc 6:4) and compound **5** was
301 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),
302 8.04 (d, J = 7.3 Hz, 2H, H^{Bz}), 7.62 (t, J = 7.4 Hz, 1H, H^{Bz}), 7.54 (t, J = 7.6 Hz, 2H, H^{Bz}), 6.00 (d, J = 4.8 Hz,
303 1H, H_{1'}), 4.99 (t, J = 4.4 Hz, 1H, H_{2'}), 4.62 (dd, J = 11.2, 4.1 Hz, 1H, H_{5'}), 4.50 (dd, J = 11.2, 4.6 Hz, 1H,
304 H_{5'}), 4.40 – 4.35 (m, 2H, H_{3'}, H_{4'}), 3.03 (s, 3H, CH₃), 0.95 (s, 9H, *t*Bu^{TBS}), 0.82 (s, 9H, *t*Bu^{TBS}), 0.15 (s,
305 3H, Me^{TBS}), 0.13 (s, 3H, Me^{TBS}), 0.00 (s, 3H, Me^{TBS}), -0.20 (s, 3H, Me^{TBS}). ¹³C NMR (126 MHz, CDCl₃): δ
306 168.3 (C=O), 152.8 (C₆), 151.5 (C₂), 149.9 (C₄), 142.5 (C₈), 134.8 (C_q^{Bz}), 132.9 (C^{Bz}), 129.0 (2C, C^{Bz}),
307 127.9 (2C, C^{Bz}), 123.9 (C₅), 89.9 (C_{1'}), 82.4 (C_{4'}), 74.3 (C_{2'}), 72.1 (C_{3'}), 67.9 (C_{5'}), 37.8 (CH₃), 25.9 (3C,
308 *t*Bu^{TBS}), 25.8 (3C, *t*Bu^{TBS}), 18.2 (C_q^{TBS}), 18.00 (C_q^{TBS}), -4.3 (Me^{TBS}), -4.5 (Me^{TBS}), -4.7 (Me^{TBS}), -4.8 (Me^{TBS}).
309 HRMS (ESI) m/z : calcd for C₃₀H₄₈N₅O₇Si₂ [M + H]⁺: 678.2807; found: 678.2788.

310

311 **Compound 6:** To a stirred solution of compound **5** (920 mg, 1.35 mmol) in DMF (7 mL) was
312 added sodium azide (264 mg, 4.06 mmol) and the mixture was heated at 70 °C for 4 h. The reaction
313 mixture was cooled to room temperature and was dissolved in EtOAc and washed with brine. The
314 organic layer was dried over MgSO₄, concentrated *in vacuo* and purified on silica gel
315 chromatography (eluent: Cyclohexane/EtOAc 8:2) to afford compound **6** as a white foam (770 mg,
316 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^{Bz}), 7.60 (t, J
317 = 7.3, 1H, H^{Bz}), 7.52 (t, J = 7.8, 2H, H^{Bz}), 5.98 (d, J = 4.0 Hz, 1H, H_{1'}), 4.88 (t, J = 4.1 Hz, 1H, H_{2'}), 4.31 (t,
318 J = 4.6 Hz, 1H, H_{3'}), 4.23 (q, J = 4.6 Hz, 1H, H_{4'}), 3.80-3.67 (m, 2H, H_{5'}), 0.94 (s, 9H, *t*Bu^{TBS}), 0.84 (s, 9H,
319 *t*Bu^{TBS}), 0.12 (s, 3H, Me^{TBS}), 0.11 (s, 3H, Me^{TBS}), -0.01 (s, 3H, Me^{TBS}), -0.12 (s, 3H, Me^{TBS}). ¹³C NMR (126
320 MHz, CDCl₃): δ 164.6 (C=O), 152.8 (C₆), 151.5 (C₂), 149.8 (C₄), 142.4 (C₈), 133.8 (C_q^{Bz}), 132.9 (C^{Bz}),
321 129.0 (2C, C^{Bz}), 127.9 (2C, C^{Bz}), 123.90 (C₅), 90.1 (C_{1'}), 82.8 (C_{4'}), 74.8 (C_{2'}), 72.3 (C_{3'}), 51.6 (C_{5'}), 25.9
322 (3C, *t*Bu^{TBS}), 25.8 (3C, *t*Bu^{TBS}), 18.2 (C_q^{TBS}), 18.0 (C_q^{TBS}), -4.2 (Me^{TBS}), -4.5 (Me^{TBS}), -4.6 (Me^{TBS}), -4.7
323 (Me^{TBS}). HRMS (ESI) m/z : calcd for C₂₉H₄₅N₅O₄Si₂ [M + H]⁺: 625.3096; found 625.3088.

324

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

331

332 **Compound 8a:** Following the general procedure A for CuAAC, starting from alkyne **2a** (200 mg,
333 0.26 mmol) and azido compound **6** (194 mg, 0.31 mmol) and using Cyclohexane/EtOAc 6:4 as eluent
334 for flash chromatography purification, compound **8a** was obtained as a white foam (245 mg, 68%).
335 ¹H NMR (500 MHz, CDCl₃): δ 8.84 (s, 1H, H_{2a} or H_{2b}), 8.52 (s, 1H, H_{2a} or H_{2b}), 8.21 (s, 1H, H_{8a} or
336 H_{8b}), 8.02 (bs, 3H, H_{8a} or H_{8b} and H^{Bz}), 7.81 (s, 1H, H^{triazole}), 7.60 (bs, 1H, H^{Bz}), 7.53 (bs, 2H, H^{Bz}), 7.42

337 (d, $J = 7.1$ Hz, 2H, H^{Bz}), 7.22 (t, $J = 7.4$ Hz, 1H, H^{Bz}), 7.10 (t, $J = 7.6$ Hz, 2H, H^{Bz}), 5.99 (d, $J = 5.6$ Hz, 1H,
338 H1'a), 5.90 (bs, 1H, H1'b), 5.64 (s, 2H, CH₂N), 5.07 (bs, 1H, H2'b), 4.89–4.81 (m, 1H, H5'b), 4.65–4.58
339 (m, 2H, H5'b and H2'a), 4.43 (bs, 1H, H3'b), 4.36 (bs, 1H, H4'b), 4.26–4.24 (m, 1H, H3'a), 4.11–4.08 (m,
340 1H, H4'a), 3.97 (dd, $J = 11.3, 4.3$ Hz, 1H, H5'a), 3.76 (dd, $J = 11.3, 2.9$ Hz, 1H, H5'a), 0.94 (s, 9H, *t*Bu^{TBS}),
341 0.92 (s, 9H, *t*Bu^{TBS}), 0.89 (s, 9H, *t*Bu^{TBS}), 0.76 (s, 9H, *t*Bu^{TBS}), 0.74 (s, 9H, *t*Bu^{TBS}), 0.11 (s, 3H, Me^{TBS}), 0.11
342 (s, 3H, Me^{TBS}), 0.09 (s, 3H, Me^{TBS}), 0.09 (s, 3H, Me^{TBS}), 0.04 (s, 3H, Me^{TBS}), -0.05 (s, 3H, Me^{TBS}), -0.08 (s,
343 3H, Me^{TBS}), -0.11 (s, 3H, Me^{TBS}), -0.37 (s, 3H, Me^{TBS}), -0.42 (s, 3H, Me^{TBS}). ¹³C NMR (126 MHz, CDCl₃): δ
344 172.2 (2C, C=O), 164.5 (Cq), 153.7 (Cq), 152.8 (Cq), 152.0 (C2a or C2b), 151.5 (Cq), 150.1 (C2a or C2b),
345 144.7 (Cq^{Triazole}), 143.2 (C8a or C8b), 143.0 (C8a or C8b), 136.0 (Cq^{Bz}) 133.9 (Cq^{Bz}) 132.9 (C^{Bz}), 130.9
346 (C^{Bz}), 129.1 (2C, C^{Bz}), 129.0 (2C, C^{Bz}), 127.9 (4C, C^{Bz}), 127.30 (Cq), 124.9 (CH^{Triazole}), 124.2 (Cq), 90.1
347 (C1'b), 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),
348 51.7 (C5'b), 44.0 (CH₂N), 26.2 (3C, *t*Bu^{TBS}), 26.0 (3C, *t*Bu^{TBS}), 25.9 (3C, *t*Bu^{TBS}), 25.8 (6C, *t*Bu^{TBS}), 18.6
349 (Cq^{TBS}), 18.2 (Cq^{TBS}), 18.1 (Cq^{TBS}), 17.9 (Cq^{TBS}), 17.8 (Cq^{TBS}), -4.3 (Me^{TBS}), -4.5 (3C, Me^{TBS}), -4.6 (Me^{TBS}),
350 -4.7 (Me^{TBS}), -4.9 (Me^{TBS}), -5.0 (Me^{TBS}), -5.2 (Me^{TBS}), -5.3 (Me^{TBS}). HRMS (ESI) m/z : calcd for
351 C₆₇H₁₀₆N₁₃O₉Si₅ [M + H]⁺: 1376.7077; found 1376.7056.

352

353 **Compound 9a**: Following the general procedure A for CuAAC, starting from alkyne **2a** (578 mg,
354 0.77 mmol) and azido compound **7** (277 mg, 0.38 mmol) and using Cyclohexane/EtOAc 7:3 as eluent
355 for flash chromatography purification, compound **9a** was obtained as a white foam (384 mg, 60%).
356 ¹H NMR (500 MHz, CDCl₃): δ 8.75 (s, 1H, H8 or H2), 8.51 (s, 1H, H8 or H2), 8.19 (s, 1H, H8 or H2),
357 8.04 (bs, 1H, NH), 7.92 (bs, 1H, H8 or H2), 7.80 (s, 1H, H^{Triazole}), 7.42–7.40 (m, 2H, H^{Bz}), 7.23–7.20 (m,
358 1H, H^{Bz}), 7.11–7.08 (m, 2H, H^{Bz}), 5.98 (d, $J = 5.6$ Hz, 1H, H1'a), 5.86 (d, $J = 6.2$ Hz, 1H, H1'b), 5.63 (s, 2H,
359 CH₂N), 5.04–5.02 (m, 1H, H2'b), 4.84 (dd, $J = 6.3, 14.2$ Hz, 1H, H5'b), 4.61–4.57 (m, 2H, H5'b and H2'a),
360 4.39–4.38 (m, 1H, H3'b), 4.35–4.32 (m, 1H, H4'b), 4.25–4.23 (m, 1H, H3'a), 4.10–4.08 (m, 1H, H4'a), 3.96
361 (dd, $J = 4.4, 11.3$ Hz, 1H, H5'a), 3.75 (dd, $J = 3.0, 11.4$ Hz, 1H, H5'a), 1.56 (s, 9H, *t*Bu^{Boc}), 0.93 (s, 9H,
362 *t*Bu^{TBS}), 0.91 (s, 9H, *t*Bu^{TBS}), 0.87 (s, 9H, *t*Bu^{TBS}), 0.73 (s, 18H, *t*Bu^{TBS}), 0.11 (s, 3H, Me^{TBS}), 0.10 (s, 3H,
363 Me^{TBS}), 0.08 (s, 6H, Me^{TBS}), 0.02 (s, 3H, Me^{TBS}), -0.08 (s, 3H, Me^{TBS}), -0.11 (s, 6H, Me^{TBS}), -0.43 (s, 6H,
364 Me^{TBS}). ¹³C NMR (126 MHz, CDCl₃): δ 172.1 (C=O), 153.7 (C2 or C8), 153.1 (Cq), 152.8 (Cq), 152.0 (C2
365 or C8), 150.6 (Cq), 150.4 (Cq), 149.6 (Cq), 144.7 (Cq), 142.9 (C2 or C8), 142.6 (C2 or C8), 135.9 (Cq),
366 130.9 (C^{Bz}), 129.0 (2C, C^{Bz}), 127.9 (2C, C^{Bz}), 127.3 (Cq^{Bz}), 124.9 (CH^{Triazole}), 122.9 (Cq), 89.8 (C1'b), 88.3
367 (C1'a), 86.0 (C4'a), 84.2 (C4'b), 82.4 (Cq^{Boc}), 75.8 (C2'a), 73.4 (C3'b), 73.1 (C2'b), 72.2 (C3'a), 62.7 (C5'a),
368 51.7 (C5'b), 44.0 (CH₂N), 28.3 (3C, *t*Bu^{Boc}), 26.2 (3C, *t*Bu^{TBS}), 25.9 (3C, *t*Bu^{TBS}), 25.8 (3C, *t*Bu^{TBS}), 25.7 (6C,
369 *t*Bu^{TBS}), 18.6 (Cq^{TBS}), 18.2 (Cq^{TBS}), 18.1 (Cq^{TBS}), 17.9 (2C, Cq^{TBS}), -4.3 (Me^{TBS}), -4.5 (3C, 3 Me^{TBS}), -4.6
370 (Me^{TBS}), -4.8 (Me^{TBS}), -5.0 (Me^{TBS}), -5.1 (Me^{TBS}), -5.2 (Me^{TBS}), -5.3 (Me^{TBS}). HRMS (ESI) m/z : calcd for
371 C₆₅H₁₀₈N₁₃O₁₀Si₅ [M - H]⁻: 1370.7188; found: 1370.7132.

372

373 **Compound 9b**: Following the general procedure A for CuAAC, starting from alkyne **2b** (50 mg,
374 0.067 mmol) and azido compound **7** (49 mg, 0.08 mmol) and using Cyclohexane/EtOAc 7:3 as eluent
375 for flash chromatography purification, compound **9b** was obtained as a white foam (68 mg, 74%). ¹H
376 NMR (500 MHz, CDCl₃): δ 8.74 (s, 1H, H2b), 8.70 (s, 1H, H2a), 8.38 (s, 1H, H8a), 8.00 (s, 1H, NH), 7.90
377 (s, 1H, H8b), 7.70 (s, 1H, H^{Triazole}), 6.07 (d, $J = 4.7$ Hz, 1H, H1'a), 5.86 (d, $J = 6.2$ Hz, 1H, H1'b), 5.30 (s,
378 2H, CH₂N), 5.10 (dd, $J = 6.2, 4.3$ Hz, 1H, H2'b), 4.89 (dd, $J = 14.3, 6.3$ Hz, 1H, H5'b), 4.65–4.58 (m, 2H,
379 H5'b, H2'a), 4.41 (dd, $J = 4.3, 2.4$ Hz, 1H, H3'b), 4.36–4.31 (m, 2H, H4'b, H3'a), 4.15–4.12 (m, 1H, H4'a),
380 4.03 (dd, $J = 11.4, 4.1$ Hz, 1H, H5'a), 3.79 (dd, $J = 11.4, 2.9$ Hz, 1H, H5'a), 1.57 (s, 9H, *t*Bu^{Boc}), 1.38 (s, 9H,
381 *t*Bu^{Boc}), 0.95 (s, 9H, *t*Bu^{TBS}), 0.93 (s, 9H, *t*Bu^{TBS}), 0.88 (s, 9H, *t*Bu^{TBS}), 0.80 (s, 9H, *t*Bu^{TBS}), 0.74 (s, 9H,
382 *t*Bu^{TBS}), 0.13 (s, 3H, Me^{TBS}), 0.12 (s, 3H, Me^{TBS}), 0.10 (s, 3H, Me^{TBS}), 0.09 (s, 3H, Me^{TBS}), 0.04 (s, 3H,
383 Me^{TBS}), -0.04 (s, 3H, Me^{TBS}), -0.06 (s, 3H, Me^{TBS}), -0.10 (s, 3H, Me^{TBS}), -0.21 (s, 3H, Me^{TBS}), -0.42 (s, 3H,
384 Me^{TBS}). ¹³C NMR (126 MHz, CDCl₃): δ 153.4 (C2b), 153.1 (Cq), 152.9 (Cq), 152.5 (Cq), 151.9 (C2a), 150.6
385 (Cq), 150.4 (C=O), 149.6 (C=O), 145.8 (Cq^{Triazole}), 142.6 (C8b), 142.2 (C8a), 127.7 (Cq), 124.1 (CH^{Triazole}),
386 122.9 (Cq), 90.1 (C1'b), 88.8 (C1'a), 85.3 (C4'a), 84.4 (C4'b), 82.5 (2C, Cq^{Boc}), 75.9 (C2'a), 73.5 (C3'b),
387 73.1 (C2'b), 71.8 (C3'a), 62.5 (C5'a), 51.8 (C5'b), 43.4 (CH₂N), 28.3 (3C, *t*Bu^{Boc}), 28.0 (3C, *t*Bu^{Boc}), 26.2
388 (3C, *t*Bu^{TBS}), 26.0 (3C, *t*Bu^{TBS}), 25.9 (3C, *t*Bu^{TBS}), 25.8 (3C, *t*Bu^{TBS}), 25.7 (3C, *t*Bu^{TBS}), 18.7 (Cq^{TBS}), 18.2

389 (Cq^{TBS}), 18.1 (Cq^{TBS}), 18.0 (Cq^{TBS}), 17.9 (Cq^{TBS}), -4.2 (Me^{TBS}), -4.4 (Me^{TBS}), -4.5 (Me^{TBS}), -4.5 (Me^{TBS}), -4.6
390 (Me^{TBS}), -4.7 (Me^{TBS}), -4.8 (Me^{TBS}), -5.1 (Me^{TBS}), -5.2 (Me^{TBS}), -5.3 (Me^{TBS}). HRMS (ESI) m/z: calcd for
391 C₆₃H₁₁₄N₁₃O₁₁Si₅ [M + H]⁺: 1368.7601; found 1368.7601.

392

393

394 **Compound 10a**: Following the general procedure A for CuAAC, starting from alkyne **3a** (200 mg,
395 0.26 mmol) and azido compound **6** (194 mg, 0.31 mmol) and using Cyclohexane/EtOAc 5:5 as eluent
396 for flash chromatography purification, compound **10a** was obtained as a white foam (258 mg, 72%).
397 ¹H NMR (500 MHz, CDCl₃): δ 8.70 (s, 1H, H₂b), 8.26 (s, 1H, H₂a), 8.05 (d, J = 7.4 Hz, 2H, H^{Bz}), 7.95 (d,
398 J = 7.2 Hz, 2H, H^{Bz}), 7.79 (s, 2H, H₈a and H₈b), 7.74 (s, 1H, H^{Triazole}), 7.62 (t, J = 7.3 Hz, 1H, H^{Bz}), 7.54 (t,
399 J = 7.5 Hz, 2H, H^{Bz}), 7.40 (t, J = 7.3 Hz, 1H, H^{Bz}), 7.31 (t, J = 7.6 Hz, 2H, H^{Bz}), 5.85-5.80 (m, 2H, H₁'a and
400 H₁'b), 5.43-5.31 (m, 2H, CH₂N), 5.13-5.07 (m, 1H, H₂'b), 4.85 (dd, J = 14.2, 4.8 Hz, 1H, H₅'b), 4.73 (dd,
401 J = 14.2, 7.2 Hz, 1H, H₅'b), 4.50 (t, J = 3.8 Hz, 1H, H₃'b), 4.44 (t, J = 4.4 Hz, 1H, H₂'a), 4.40-4.36 (m, 1H,
402 H₄'b), 4.23 (t, J = 4.3 Hz, 1H, H₃'a), 4.05 (q, J = 3.9 Hz, 1H, H₄'a), 3.86 (dd, J = 11.4, 4.1 Hz, 1H, H₅'a),
403 3.69 (dd, J = 11.4, 3.4 Hz, 1H, H₅'a), 0.91 (s, 9H, *t*Bu^{TBS}), 0.90 (s, 9H, *t*Bu^{TBS}), 0.86 (s, 9H, *t*Bu^{TBS}), 0.80 (s,
404 9H, *t*Bu^{TBS}), 0.79 (s, 9H, *t*Bu^{TBS}), 0.10 (s, 3H, Me^{TBS}), 0.07 (s, 3H, Me^{TBS}), 0.06 (s, 3H, Me^{TBS}), 0.05 (s, 3H,
405 Me^{TBS}), 0.04 (s, 3H, Me^{TBS}), 0.03 (s, 3H, Me^{TBS}), -0.04 (s, 3H, Me^{TBS}), -0.06 (s, 3H, Me^{TBS}), -0.19 (s, 3H,
406 Me^{TBS}), -0.34 (s, 3H, Me^{TBS}). ¹³C NMR (126 MHz, CDCl₃): δ 177.1 (C=O), 164.7 (C=O), 152.7 (C₂b), 151.2
407 (C_q), 150.1 (C_q), 146.7 (C₂a), 146.6 (C_q), 145.2 (C_q), 143.1 (C₈b), 141.9 (C_q^{Triazole}), 138.8 (C₈a), 135.8
408 (C_q^{Bz}), 133.8 (C_q^{Bz}), 133.0 (C^{Bz}), 131.9 (C^{Bz}), 129.8 (2C, C^{Bz}), 129.0 (2C, C^{Bz}), 128.1 (4C, C^{Bz}), 125.6
409 (CH^{Triazole}), 124.2 (C_q), 122.5 (C_q), 90.5 (C₁'b), 88.5 (C₁'a), 85.1 (C₄'a), 83.5 (C₄'b), 76.0 (C₂'a), 73.5
410 (C₃'b), 73.4 (C₂'b), 71.7 (C₃'a), 62.6 (C₅'a), 51.8 (C₅'b), 43.7 (CH₂N), 26.2 (3C, *t*Bu^{TBS}), 26.0 (3C, *t*Bu^{TBS}),
411 25.9 (3C, *t*Bu^{TBS}), 25.8 (3C, *t*Bu^{TBS}), 25.80 (3C, *t*Bu^{TBS}), 18.6 (C_q^{TBS}), 18.2 (C_q^{TBS}), 18.1 (C_q^{TBS}), 18.0 (2C,
412 C_q^{TBS}), -4.2 (Me^{TBS}), -4.3 (Me^{TBS}), -4.4 (Me^{TBS}), -4.5 (Me^{TBS}), -4.6 (2C Me^{TBS}), -4.7 (Me^{TBS}), -4.9 (Me^{TBS}), -5.2
413 (Me^{TBS}), -5.3 (Me^{TBS}). HRMS (ESI) m/z: calcd for C₆₇H₁₀₆N₁₃O₉Si₅ [M + H]⁺: 1376.7077; found 1376.7060.

414

415 **Compound 11a**: Following the general procedure A for CuAAC, starting from alkyne **3a** (45 mg,
416 0.059 mmol) and azido compound **7** (19 mg, 0.03 mmol) and using Cyclohexane/EtOAc 7:3 as eluent
417 for flash chromatography purification, compound **11a** was obtained as a white foam (37 mg, 89%).
418 ¹H NMR (500 MHz, CDCl₃): δ 8.67 (s, 1H, H₂b), 8.30 (s, 1H, H₂a), 7.97 (m, 2H, H^{Bz}), 7.85 (s, 1H, H₈a),
419 7.78 (s, 1H, H₈b), 7.77 (s, 1H, H^{Triazole}), 7.46-7.43 (m, 1H, H^{Bz}), 7.36-7.32 (m, 2H, H^{Bz}), 5.85 (d, J = 4.5 Hz,
420 1H, H₁'a), 5.78 (d, J = 5.4 Hz, 1H, H₁'b), 5.44 (d, J = 14.6 Hz, 1H, CH₂N), 5.36 (d, J = 14.6 Hz, 1H,
421 CH₂N), 5.12 (m, 1H, H₂'b), 4.87 (dd, J = 5.2, 14.3 Hz, 1H, H₅'b), 4.70 (dd, J = 7.3, 14.2 Hz, 1H, H₅'b),
422 4.48-4.47 (m, 1H, H₃'b), 4.46-4.44 (m, 1H, H₂'a), 4.37-4.34 (m, 1H, H₄'b), 4.24-4.23 (m, 1H, H₃'a),
423 4.07-4.05 (m, 1H, H₄'a), 3.87 (dd, J = 4.0, 11.3 Hz, 1H, H₅'a), 3.71 (dd, J = 3.3, 11.3 Hz, 1H, H₅'a), 1.57
424 (s, 9H, *t*Bu^{Boc}), 0.91 (s, 9H, *t*Bu^{TBS}), 0.89 (s, 9H, *t*Bu^{TBS}), 0.87 (s, 9H, *t*Bu^{TBS}), 0.80 (s, 9H, *t*Bu^{TBS}), 0.77 (s,
425 9H, *t*Bu^{TBS}), 0.08 (s, 3H, Me^{TBS}), 0.07 (s, 6H, Me^{TBS}), 0.05 (s, 3H, Me^{TBS}), 0.04 (s, 3H, Me^{TBS}), 0.02 (s, 3H,
426 Me^{TBS}), -0.04 (s, 3H, Me^{TBS}), -0.08 (s, 3H, Me^{TBS}), -0.18 (s, 3H, Me^{TBS}), -0.39 (s, 3H, Me^{TBS}). ¹³C NMR (126
427 MHz, CDCl₃): δ 176.8 (C=O), 152.9 (C₂b), 150.3 (2C, C_q), 149.6 (C=O), 146.7 (2C, C₂a and C_q), 145.4
428 (C_q), 142.6 (C₈b), 141.9 (C_q^{Triazole}), 139.0 (C₈a), 135.6 (C_q), 131.9 (C^{Bz}), 129.7 (2C, C^{Bz}), 128.1 (2C, C^{Bz}),
429 125.6 (CH^{Triazole}), 122.8 (C_q), 122.6 (C_q), 90.4 (C₁'b), 88.5 (C₁'a), 85.1 (C₄'a), 83.6 (C₄'b), 82.5 (C_q^{Boc}),
430 76.0 (C₂'a), 73.5 (C₃'b), 73.3 (C₂'b), 71.7 (C₃'a), 62.6 (C₅'a), 51.9 (C₅'b), 43.8 (CH₂N), 28.2 (3C, *t*Bu^{Boc}),
431 26.1 (3C, *t*Bu^{TBS}), 26.0 (3C, *t*Bu^{TBS}), 25.9 (3C, *t*Bu^{TBS}), 25.8 (3C, *t*Bu^{TBS}), 25.7 (3C, *t*Bu^{TBS}), 18.6 (C_q^{TBS}), 18.2
432 (C_q^{TBS}), 18.1 (C_q^{TBS}), 18.0 (C_q^{TBS}), 17.9 (C_q^{TBS}), -4.2 (Me^{TBS}), -4.3 (Me^{TBS}), -4.4 (Me^{TBS}), -4.5 (Me^{TBS}), -4.6
433 (Me^{TBS}), -4.7 (2C, Me^{TBS}), -5.0 (Me^{TBS}), -5.2 (Me^{TBS}), -5.3 (Me^{TBS}). HRMS (ESI) m/z: calcd for
434 C₆₅H₁₀₈N₁₃O₁₀Si₅ [M - H]⁻: 1370.7188; found: 1370.7138.

435

436 **Compound 11b**: Following the general procedure A for CuAAC, starting from alkyne **3b** (50 mg,
437 0.067 mmol) and azido compound **7** (49 mg, 0.08 mmol) and using Cyclohexane/EtOAc 6:4 as eluent
438 for flash chromatography purification, compound **11b** was obtained as a white foam (54 mg, 60%).
439 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
440 H₈b), 8.01 (s, 1H, NH), 7.93 (s, 1H, H₈a or H₈b), 7.90 (s, 1H, H^{Triazole}), 5.85-5.83 (m, 2H, H₁'a and
H₁'b), 5.30-5.21 (m, 2H, CH₂N), 5.17 (dd, J = 5.8, 4.3 Hz, 1H, H₂'b), 4.92 (dd, J = 14.3, 6.5 Hz, 1H,

441 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
442 (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, $J = 11.6, 2.5$
443 Hz, 1H, H5'a), 1.56 (s, 18H, $t\text{Bu}^{\text{Boc}}$), 0.92 (s, 9H, $t\text{Bu}^{\text{TBS}}$), 0.90 (s, 9H, $t\text{Bu}^{\text{TBS}}$), 0.86 (s, 9H, $t\text{Bu}^{\text{TBS}}$), 0.85 (s,
444 9H, $t\text{Bu}^{\text{TBS}}$), 0.76 (s, 9H, $t\text{Bu}^{\text{TBS}}$), 0.10 (s, 3H, Me^{TBS}), 0.10 (s, 3H, Me^{TBS}), 0.07 (s, 3H, Me^{TBS}), 0.05 (s, 3H,
445 Me^{TBS}), 0.03 (s, 3H, Me^{TBS}), 0.01 (s, 3H, Me^{TBS}), -0.03 (s, 3H, Me^{TBS}), -0.08 (s, 6H, Me^{TBS}), -0.40 (s, 3H,
446 Me^{TBS}). ^{13}C NMR (126 MHz, CDCl_3): δ 153.0 (C2a or C2b), 150.5 (Cq), 150.4 (Cq), 149.6 (2C, C=O),
447 146.9 (Cq^{Triazole}), 146.5 (C2a or C2b), 144.2 (Cq), 142.7 (Cq), 142.3 (C8a or C8b), 138.1 (C8a or C8b),
448 125.7 (CH^{Triazole}), 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),
449 82.4 (2C, Cq^{Boc}), 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 (CH₂N),
450 28.3 (3C, $t\text{Bu}^{\text{Boc}}$), 28.3 (3C, $t\text{Bu}^{\text{Boc}}$), 26.3 (3C, $t\text{Bu}^{\text{TBS}}$), 26.0 (3C, $t\text{Bu}^{\text{TBS}}$), 25.9 (6C, $t\text{Bu}^{\text{TBS}}$), 25.8 (3C, $t\text{Bu}^{\text{TBS}}$),
451 18.7 (Cq^{TBS}), 18.2 (Cq^{TBS}), 18.1 (Cq^{TBS}), 18.0 (Cq^{TBS}), 17.9 (Cq^{TBS}), -4.1 (Me^{TBS}), -4.4 (2C, 2 Me^{TBS}), -4.5
452 (Me^{TBS}), -4.6 (Me^{TBS}), -4.7 (Me^{TBS}), -4.9 (Me^{TBS}), -5.1 (Me^{TBS}), -5.2 (Me^{TBS}), -5.3 (Me^{TBS}). HRMS (ESI) m/z :
453 calcd for $\text{C}_{63}\text{H}_{114}\text{N}_{13}\text{O}_{11}\text{Si}_5$ [$\text{M} + \text{H}$]⁺: 1368.7601; found 1368.7611.

454

455 *Synthesis of compound 12 following pathway 1:* Protected compound **9a** (768 mg, 0.56 mmol) was
456 dissolved in DCM/MeOH 4:1 (10 mL) and MeNH_2 33% in EtOH (3.6 mL, 28.0 mmol) was added at 0
457 °C to the solution. The reaction was stirred at room temperature for 16 h, then diluted in DCM and
458 washed with brine. The combined organic layers were dried over MgSO_4 and concentrated. The
459 residue was purified by silica gel chromatography (eluent: Cyclohexane/EtOAc 5:5) to provide the
460 debenzoylated compound as a white foam. The residue was then dissolved in DCM (1.4 mL) and
461 ZnBr_2 (686 mg, 2.80 mmol) was added. The reaction mixture was vigorously stirred at room
462 temperature for 24 h. Then, water (5.8 mL) was added and the reaction mixture was stirred for 2
463 additional hours. A work up was performed with DCM and brine. The combined organic layers
464 were dried over MgSO_4 and concentrated. The residue was purified by silica gel chromatography
465 (eluent: Cyclohexane/EtOAc 3:7 then DCM/MeOH 9:1) to afford a yellow powder. The resulting
466 compound was engaged in the last deprotection step and was dissolved in MeOH (10 mL) and CsF
467 (8.5 g, 112 mmol) was added. The reaction mixture was stirred at 60 °C for 24 h, concentrated and
468 diluted in water. The residue was purified by HPLC to afford compound **12** as a white foam (17 mg,
469 5% over 3 steps).

470 *Synthesis of compound 12 following pathway 2:* Protected compound **9b** (60 mg, 0.046 mmol) was
471 dissolved in DCM (1 mL) and ZnBr_2 (58 mg, 0.21 mmol) was added. The reaction mixture was
472 vigorously stirred at room temperature for 24 h. Then, water (2 mL) was added and the reaction
473 mixture was stirred for 2 additional hours. A work up was performed with DCM and brine. The
474 combined organic layers were dried over MgSO_4 and concentrated. The residue was purified by
475 silica gel chromatography (eluent Cyclohexane/EtOAc 2:8) to afford a yellow pale powder. The
476 resulting compound was engaged in the last deprotection step and was dissolved in MeOH (10 mL)
477 before adding CsF (480 mg, 3.2 mmol). The reaction mixture was stirred at 60 °C for 24 h,
478 concentrated and purified by HPLC to afford compound **12** as a white foam (1.2 mg, 4% over 2
479 steps).

480 *Compound 12:* ^1H NMR (500 MHz, $(\text{CD}_3)_2\text{SO}$): δ 8.35 (s, 1H, H2 or H8), 8.26 (bs, 1 H, NH), 8.23 (s,
481 1H, H2 or H8), 8.19 (s, 1H, H2 or H8), 8.14 (s, 1H, H2 or H8), 7.81 (s, 1H, H^{Triazole}), 7.28 (bs, 2H, NH₂),
482 5.90-5.89 (m, 2H, H1'a and H1'b), 5.55 (d, $J = 5.9$ Hz, 1H, OH2'b), 5.43-5.41 (m, 2H, OH2'a and
483 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 CH₂N),
484 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,
485 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). ^{13}C NMR (126 MHz,
486 $(\text{CD}_3)_2\text{SO}$): δ 156.1 (Cq), 154.2 (Cq), 152.6 (C2 or C8), 152.2 (C2 or C8), 149.3 (Cq), 148.5 (Cq), 145.3
487 (Cq), 139.9 (C2 or C8), 139.8 (C2 or C8), 123.6 (CH^{Triazole}), 119.8 (Cq), 119.2 (Cq), 87.9 (C1'a or C1'b),
488 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),
489 51.3 (C5'b), 35.3 (CH₂N). HRMS (ESI) m/z : calcd for $\text{C}_{23}\text{H}_{26}\text{N}_{13}\text{O}_7$ [$\text{M} - \text{H}$]⁻: 596.2078; found: 596.2065.
490 HPLC purity: 96.3%; $t_{\text{R}} = 17.8$ min (MeCN/H₂O 0:100 to 100:0 over 30 min).

491

492 **Compound 13:** Protected compound **11a** (400 mg, 0.3 mmol) was dissolved in DCM/MeOH 4:1 (5
493 mL) and MeNH₂ 33% in EtOH (1.8 mL, 15.0 mmol) was added at 0 °C to the solution. The reaction
494 was stirred at room temperature for 16 h, then diluted in DCM and washed with brine. The
495 combined organic layers were dried over MgSO₄ and concentrated. The residue was purified by
496 silica gel chromatography (eluent: EtOAc/MeOH 9:1) to provide the debenzoylated compound as a
497 white foam. The residue was then dissolved in DCM (1 mL) and ZnBr₂ (117 mg, 0.47 mmol) was
498 added. The reaction mixture was vigorously stirred at room temperature for 24 h. Then, water (2
499 mL) was added and the reaction mixture was stirred for 2 additional hours. A work up was
500 performed with DCM and brine. The combined organic layers were dried over MgSO₄ and
501 concentrated. The resulting compound was engaged in the last deprotection step and was dissolved
502 in MeOH (10 mL) and CsF (2.9 g, 19 mmol) was added. The reaction mixture was stirred at 60 °C for
503 24 h, concentrated and diluted in water. The residue was purified by HPLC to afford compound **13**
504 as a white foam (15 mg, 8% over 3 steps). ¹H NMR (500 MHz, CD₃OD): δ 8.35 (2s, 1H, H2a), 8.23 (s,
505 1H, H8a or H8b), 8.13 (2s, 1H, H8a or H8b), 8.03 (2s, 1H, H2b), 7.84 (s, 0.5H, H^{Triazole}), 7.69 (s, 0.5H,
506 H^{Triazole}), 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
507 residual pick of water, 4.79-4.75 (m, 0.5H, H2'b), 4.71-4.68 (m, 0.5H, H2'b), 4.61 (t, J = 5 Hz, 1H, H2'a),
508 4.47-4.41 (m, 0.5H, H3'b), 4.37-4.31 (m, 1.5H, H3'b and H3'a), 4.14-4.13 (m, 2H, H4'a and H4'b),
509 3.88-3.85 (m, 1H, H5'a), 3.78-3.74 (m, 1H, H5'a), 3.56 (2s, 3H, CH₃). ¹³C NMR (126 MHz, CD₃OD): δ
510 178.3 (Cq), 163.0 (Cq), 157.3 (Cq), 153.7 (C8a or C8b), 150.4 (Cq), 150.0 (Cq), 149.8 (Cq), 149.4 (Cq),
511 147.8 (Cq), 147.5 (Cq), 142.0 (C2a or C2b), 126.6 (CH^{Triazole}), 125.7 (CH^{Triazole}), 126.2 (Cq), 120.8 (Cq),
512 90.9 (C1'b), 90.4 (C1'a), 87.6 (2C, C4'a and C4'b), 83.9 (C3'a), 76.1 (C2'a), 74.2 (C2'b), 72.2 (C3'b), 62.9
513 (C5'a), 52.5 (C5'b), 45.1 (CH₂N), 43.9 (CH₂N), 37.9 (CH₃), 35.0 (CH₃). LRMS (ESI) m/z: calcd for
514 C₂₄H₃₀N₁₃O₇ [M + H]⁺: 612.23; found: 612.33. HPLC purity: 95.3%; rt = 14.6 min (MeCN/H₂O 0:100 to
515 100:0 over 30 min).
516

517 **Compound 14:** Compound **11a** (107 mg, 0.08 mmol) was dissolved in DCM (1 mL) and ZnBr₂ (95
518 mg, 0.40 mmol) was added. The reaction mixture was vigorously stirred at room temperature for 24
519 h. Water (2 mL) was added and the reaction mixture was stirred for 2 additional hours. A work up
520 was performed with DCM and brine. The combined organic layers were dried over MgSO₄ and
521 concentrated. The residue was purified by silica gel chromatography (eluent: Cyclohexane/EtOAc
522 2:8) to afford a white foam (52 mg). The purified intermediate was then dissolved in MeOH (5 mL)
523 and CsF (1.2 g, 8 mmol) was added. The reaction mixture was stirred at 60 °C for 24 h, concentrated
524 and diluted in water. The residue was purified by HPLC to afford compound **14** as a white foam (7
525 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),
526 8.06 (s, 1H, H2 or H8), 7.90 (s, 2H, H2 or H8 and H^{Triazole}), 7.83-7.81 (m, 2H, H^{Bz}), 7.45-7.42 (m, 1H,
527 H^{Bz}), 7.33-7.30 (m, 2H, H^{Bz}), 5.96 (d, J = 5.0 Hz, 1H, H1'a), 5.90 (d, J = 5.0 Hz, 1H, H1'b), 5.43 (s, 2H,
528 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
529 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,
530 J = 5.0, 15.0 Hz, 1H, H5'a), 3.74 (dd, J = 5.0, 15.0 Hz, 1H, H5'a). ¹³C NMR (126 MHz, CD₃OD): δ 178.8
531 (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
532 C8), 141.2 (Cq), 136.7 (C2 or C8), 133.2 (Cq), 130.6 (C^{Bz}), 129.1 (2C, C^{Bz}), 129.0 (2C, C^{Bz}), 127.0
533 (CH^{Triazole}), 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
534 (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: calcd for
535 C₃₀H₃₂N₁₃O₈ [M + H]⁺: 702.2497; found: 702.2470. HPLC purity: 97.1%; tr = 19.0 min (MeCN/H₂O 0:100
536 to 100:0 over 30 min).
537

538 **Compound 15:** Protected compound **11b** (50 mg, 0.036 mmol) was dissolved in DCM (1 mL) and
539 ZnBr₂ (44 mg, 0.18 mmol) was added. The reaction mixture was vigorously stirred at room
540 temperature for 24 h then water (2 mL) was added and the reaction mixture was stirred for 2
541 additional hours. A work up was performed with DCM and brine. The combined organic layers
542 were dried over MgSO₄ and concentrated. The residue was purified by silica gel chromatography
543 (eluent Cyclohexane/EtOAc 1:9) to afford a yellow pale powder. The resulting compound was

544 engaged in the last deprotection step and was dissolved in MeOH (10 mL) and CsF (636 mg, 4.2
545 mmol) was added. The reaction mixture was stirred at 60 °C for 24 h, concentrated and purified by
546 HPLC to afford compound **15** as a white foam (4.3 mg, 20% over 2 steps). ¹H NMR (500 MHz,
547 (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,
548 H^{Triazole}), 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,
549 2H, CH₂N), 4.74-4.70 (m, 2H, H5'b), 4.64-4.61 (m, 1H, H2'b), 4.48-4.44 (m, 1H, H2'a), 4.27-4.20 (m, 2H,
550 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,
551 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
552 (Cq), 152.6 (C2b), 149.3 (Cq), 148.2 (C2a), 142.5 (Cq^{Triazole}), 141.2 (Cq), 139.8 (C8b), 137.9 (C8a), 124.5
553 (CH^{Triazole}), 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
554 (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*:
555 calcd for C₂₃H₂₈N₁₃O₇ [M + H]⁺: 598.2229; found: 598.2229. HPLC purity: 96.2%; *t_R* = 17.5 min
556 (MeCN/H₂O 0:100 to 100:0 over 30 min).
557

558 **Compound 16**: Propargyl amine (1.4 μL, 0.031 mmol), BOP (13 mg, 0.031 mmol) and DIPEA (6.8
559 μL, 0.039 mmol) were added at 0 °C to a solution of inosine (7 mg, 0.026 mmol) in DMF (1 mL). The
560 reaction mixture was stirred at room temperature for 24 h and then concentrated under reduced
561 pressure. The crude was diluted in water and purified by HPLC to afford compound **16** as a white
562 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,
563 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* =
564 3.3 Hz, 1H, H4'), 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,
565 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
566 (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
567 (ESI) *m/z*: calcd for C₁₃H₁₄N₅O₄ [M - H]⁻: 304.1045; found: 304.1048.
568

569 **Compound 17**: Pyridine (610 μL, 7.48 mmol) and thionyl chloride (1.4 mL, 18.7 mmol) were
570 added at 0 °C, over 5 min to a solution of adenosine (1 g, 3.74 mmol) in MeCN (10 mL). The reaction
571 mixture was stirred at 0 °C for 3 h before being warmed to room temperature and stirred for 16 h.
572 The resulting precipitate was filtered and dissolved in water/MeOH (5:1) and aqueous ammonia
573 (25%, 2 mL) was added. The reaction mixture was stirred at room temperature for 30 min and the
574 solvent was removed under reduced pressure to provide 5'-chloroadenosine.⁶⁴ The resulting
575 5'-chloroadenosine was then solubilized in DMF (5 mL) and sodium azide (1.2 g, 18.7 mmol) was
576 added. The reaction mixture was heated at 80 °C for 5 h, and cooled to room temperature. The excess
577 of sodium azide was removed by filtration and the filtrate purified by flash chromatography
578 (DCM/MeOH 9:1) to give **17** as a white foam (393 mg, 36% over 2 steps). ¹H NMR (500 MHz,
579 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,
580 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
581 (dd, *J* = 10, 5 Hz, 0.5H, H5'), 3.69-3.62 (m, 1H, H5'). HRMS (ESI) *m/z*: calcd for C₁₀H₁₃N₈O₃ [M + H]⁺:
582 293.1110; found: 293.1098. Analytical data were in accordance with the literature.⁶⁵
583

584 **General procedure B for CuAAC reaction**: To a solution of alkyne **16** or **18** (1 eq) in DMF (1 mL),
585 were successively added azido compound **17** (1.5 eq), CuSO₄ (0.3 eq, in water 500 μL) and sodium
586 ascorbate (0.6 eq, in water 500 μL). The mixture was stirred at room temperature for 16 h and then
587 concentrated *in vacuo*. The crude product was purified by HPLC to afford the desired compounds.
588

589 **Synthesis of compound 12 following general procedure B for CuAAC**: Following the general
590 procedure B, starting from alkyne **16** (5.7 mg, 0.019 mmol) and azido compound **17** (8.2 mg, 0.027
591 mmol), compound **12** was obtained as a white foam (4 mg, 36%).
592

593 **Compound 18**: Propargyl bromide (80 % in toluene) (143 μL, 1.8 mmol) was added to a solution
594 of adenosine (100 mg, 0.37 mmol) in DMF (1 mL) and the reaction mixture was stirred at 50 °C for 24
595 h. After removal of the solvent under reduced pressure, the crude was diluted in water and purified

596 by HPLC to afford compound **18** as a white foam (64 mg, 56%). ¹H NMR (500 MHz, D₂O): δ 8.73 (s,
597 1H, H₂), 8.61 (s, 1H, H₈), 6.20 (d, *J* = 5 Hz, 1H, H_{1'}), 5.27 (s, 2H, CH₂N), 4.84–4.82 (t, *J* = 5 Hz, 1H, H_{2'}),
598 4.50 (t, *J* = 5 Hz, 1H, H_{3'}), 4.33–4.31 (m, 1H, H_{4'}), 3.97 (dd, *J* = 5, 10 Hz, 1H, H_{5'}), 3.90 (dd, *J* = 5, 10 Hz,
599 1H, H_{5'}), 3.19 (bs, 1H, C≡CH). ¹³C NMR (126 MHz, D₂O): δ 150.2 (C₆), 146.9 (C₂), 146.8 (C₄), 143.4
600 (C₈), 119.6 (C₅), 88.6 (C_{1'}), 85.5 (C_{4'}), 78.7 (C≡CH), 74.2 (C_{2'}), 73.1 (C≡CH), 70.1 (C_{3'}), 61.1 (C_{5'}), 40.8
601 (CH₂N). HRMS (ESI) *m/z*: calcd for C₁₃H₁₆N₅O₄ [M]⁺: 306.1196; found: 306.1193.

602

603 **Compound 19**: Following the general procedure B for CuAAC, starting from alkyne **18** (6.3 mg,
604 0.020 mmol) and azido compound **17** (9.1 mg, 0.03 mmol), compound **19** was obtained as a white
605 foam (6.5 mg, 53%). ¹H NMR (500 MHz, CD₃OD): δ 8.62 (s, 2H, H_{2a} and H_{8b}), 8.14 (s, 1H, H_{2b}), 8.05
606 (s, 1H, H_{8a}), 7.96 (s, 1H, H^{Triazole}), 6.09 (d, *J* = 5 Hz, 1H, H_{1'b}), 5.95 (d, *J* = 5 Hz, 1H, H_{1'a}), 5.52 (s, 2H,
607 CH₂N), 4.92–4.96 (m, 1H, H_{5'b}), H_{5'b} masked in the residual pick of water, 4.76–4.74 (m, 1H, H_{2'a}),
608 4.62 (t, *J* = 5 Hz, 1H, H_{2'b}), 4.48 (t, *J* = 5 Hz, 1H, H_{3'a}), 4.38–4.33 (m, 2H, H_{3'b} and H_{4'a}), 4.15 (q, *J* = 3.1
609 Hz, 1H, H_{4'b}), 3.89–3.85 (m, 1H, H_{5'a}), 3.76–3.70 (m, 1H, H_{5'a}). ¹³C NMR (126 MHz, CD₃OD): δ 157.2
610 (C_q), 154.0 (C_{2b}), 152.2 (C_q), 150.3 (C_q), 148.4 (C_{2a}), 148.0 (C_q), 144.1 (C_{8b}), 141.7 (C_{8a}), 140.8
611 (C_q^{Triazole}), 126.7 (CH^{Triazole}), 121.3 (C_q), 120.7 (C_q), 91.2 (C_{1'a}), 90.4 (C_{1'b}), 87.6 (C_{4'b}), 83.6 (C_{4'a}), 76.5
612 (C_{2'b}), 74.5 (C_{2'a}), 72.4 (C_{3'a}), 71.8 (C_{3'b}), 62.5 (C_{5'a}), 52.7 (C_{5'b}), 45.9 (CH₂N). HRMS (ESI) *m/z*:
613 calcd for C₂₃H₂₈N₁₃O₇ [M + H]⁺: 598.2234; found: 598.2222. HPLC purity: 97.6%; *t_R* = 14.6 min
614 (MeCN/H₂O 0:100 to 100:0 over 30 min).

615 4. Conclusions

616 We reported in this study the synthesis of new SAM-adenosine conjugates with a 1,2,3-triazole
617 linker that covalently links the SAM analogue to the N-6 or N-1 position of the adenosine substrate.
618 The use of protecting groups allowed the formation of the N⁶ and 1-N-conjugates but required
619 numerous steps of protection and deprotection. Revisiting the synthetic strategy, we were able to
620 avoid the steps of protection and deprotection of the hydroxyl and exocyclic amine functions and to
621 propose more straightforward and efficient syntheses. The N⁶ and 1-N conjugates were obtained in 2
622 steps with overall yield of 26% and 30%, respectively. In addition, we developed an efficient
623 methodology based on CuAAC to get access to conjugates by connecting through a triazole linker an
624 analogue of RNA MTases substrate (an adenosine modified at the N-6 or N-1 position) to a SAM
625 analog cofactor. We think that this approach could be applied for the preparation of modified
626 oligonucleotides to get more complex bisubstrate analogues for the study of m⁶A and m¹A RNA
627 MTases.

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

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636

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799 **Sample Availability:** Samples of the compounds are not available from the authors.



