

Synthesis of 2'-Deoxy-2'-C- α -methylpurine Nucleosides

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Abstract: 2'-Deoxy-2'-C- α -methylribonucleosides provide valuable biochemical probes with which to study RNA structure and function. Using methyl 2-acetoxymethyl-3,5-di-*O*-(*tert*-butyldimethylsilyl)-D-ribofuranoside (**1**) as a glycosylating agent, we achieved in four steps an improved synthesis of 2'-deoxy-2'-C- α -methyladenosine (**8**) and the first synthesis of 2'-deoxy-C- α -methylguanosine (**9**) in 25% and 17% overall yield, respectively.

Key words: nucleosides, deoxygenation, glycosylation, deoxy-methyladenosine, deoxymethylguanosine

Ribonucleic acids (RNA) adopt complex three-dimensional architectures that mediate biological functions. These architectures and their accompanying functions depend inextricably on the presence of the 2'-hydroxyl group. This integral component of the RNA backbone influences ribose conformation and helix geometry, mediates tertiary interactions via metal ion coordination and hydrogen bonding, and provides a scaffold for the hydration network that accompanies the folded molecule.¹ Understanding RNA biology therefore hinges on identification of the residues that bear important hydroxyl groups and elucidation of the underlying mechanisms by which those hydroxyl groups impart function.

2'-C- α -Methylnucleosides, aside from their potential biotechnological and therapeutic value,² serve as valuable analogues by which to investigate how hydroxyl groups engender functional behavior within RNA molecules. (1) They complement deoxynucleosides as analogues with which to evaluate the effect of eliminating the hydrogen bond capacity of the hydroxyl group (Figure 1a).³ (2) They impart unique chemical diversity to an array of 2'-modified nucleoside analogues, empowering the use of quantitative structure activity relationships (QSAR) in the analysis of RNA biology (Figure 1b).⁴ (3) They serve as 'deoxynucleoside controls' for 2'- α -hydroxymethylnucleosides, thereby revealing the specific functional contribution of a hydroxyl group upon extending it away from the ribose ring by one methylene unit (Figure 1c). The effect of hydroxymethyl nucleoside substitution on an RNA-mediated process relative to that of methyl nucleoside substitution gives a measure of whether a 2'-hydroxyl group imparts its functional contribution via inductive effects or through-space interactions with solvent

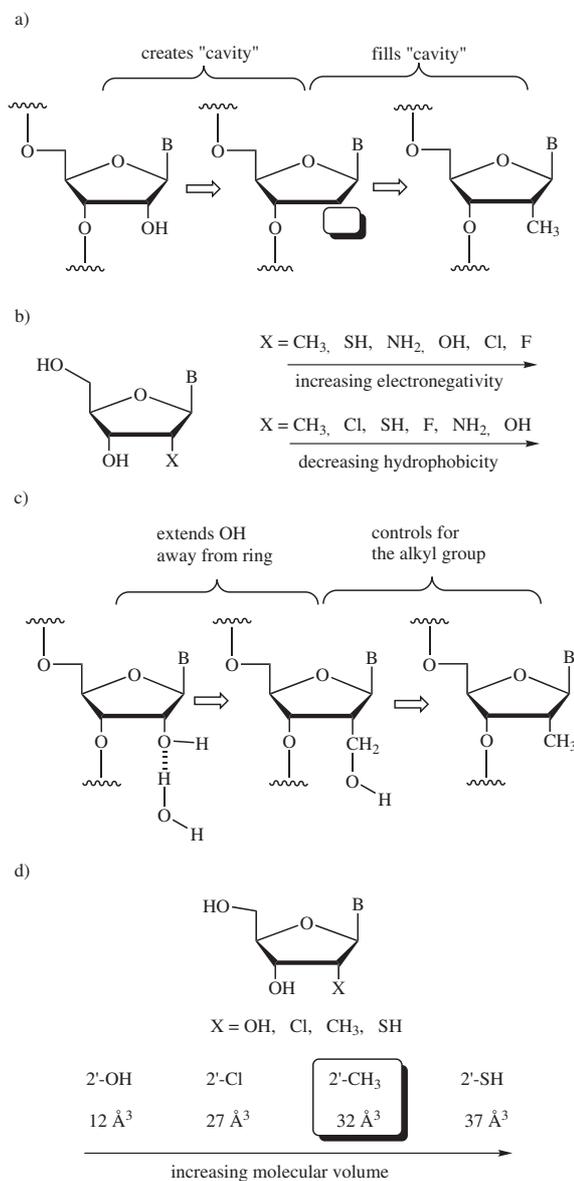


Figure 1 Applications for 2'-C- α -methylnucleosides in the study of RNA structure and function. a) Probing the importance of the 2'-hydroxyl group's space-filling capacity. b) Quantitative structure-activity relationship analysis. c) Probing the importance of solvent interactions with the 2'-hydroxyl group. d) Probing the spatial environment (the packing density) surrounding a 2'-hydroxyl group within a structured RNA. See text for further description and explanation.

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(Figure 1c).⁴ (4) Together with 2'-chloro and 2'-mercapto-nucleosides, 2'-methylnucleosides form a series of analogues that span a narrow range (~10 Å³) of molecular volumes so as to provide a sensitive measure of the spatial environment (the packing density) surrounding a 2'-hydroxyl group within a structured RNA molecule. The resulting packing density metric offers a strategy to evaluate the functional validity of crystallographic and biochemical RNA structure models (Figure 1d).⁵

The preceding experimental approaches for the study of RNA biology require access to analogues of the four natural nucleosides, adenosine, guanosine, cytidine, and uridine. We previously reported efficient syntheses of the pyrimidine nucleosides: 2'-deoxy-2'-C- α -methyluridine and 2'-deoxy-2'-C- α -methylcytidine.⁶ Here we report the synthesis of the purine nucleoside analogues: 2'-deoxy-2'-C- α -methyladenosine and 2'-deoxy-2'-C- α -methylguanosine.

The literature contains no report of the synthesis of 2'-deoxy-2'-C- α -methylguanosine and only one report of the synthesis of 2'-deoxy-2'-C- α -methyladenosine.⁷ Novak et al. obtained 2'-deoxy-2'-C- α -methyladenosine from the reaction of 2- α -methyl-3,5-di-*O*-(4-methylbenzoyl)ribofuranosyl chloride with chloromercuri-*N*-benzoyladenine, followed by removal of the protecting groups. The glycosylation reaction gave a mixture of anomers, and no information about yield or selectivity was provided.⁷ Moreover, the synthesis of the glycosylating agent, 2- α -methyl-3,5-di-*O*-(4-methylbenzoyl)ribofuranosyl chloride, required six steps from 2-*C*- β -methyl-D-ribonolactone and gave no stereoselectivity, rendering this published synthesis unattractive for accessing 2'-deoxy-2'-C- α -methylpurine nucleosides.

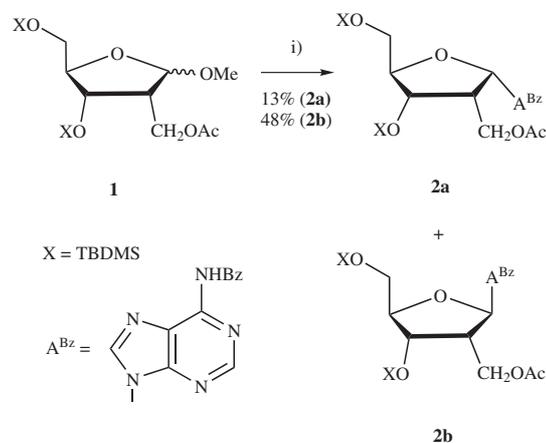
To synthesize 2'-deoxy-2'-C- α -methylpurine nucleosides, we considered adapting approaches used previously for the synthesis of 2'-deoxy-2'-C- α -methylpyrimidine nucleosides. These approaches include both a linear strategy involving nucleoside transformation and a convergent strategy involving glycosylation of the pyrimidine nucleobases with the appropriate modified sugar derivative. The linear strategy from nucleosides usually lacks efficiency, as the desired 2'- α -methyl isomers emerge as minor products.^{8,9} The convergent strategy using modified sugar reagents generally has proven more efficient than nucleoside transformation, as glycosylation of persilylated nucleobases gives the desired 1'- β -isomers predominantly.¹⁰

Recently we exploited the glycosylation strategy to develop an efficient synthesis of 2'-C- α -methyl-2'-deoxypyrimidine nucleosides via radical deoxygenation of 2'-C- α -hydroxymethylnucleosides.⁶ We accessed the hydroxymethylnucleosides directly and efficiently from the readily prepared ribose derivatives, methyl 2- α -acetoxymethyl-3,5-di-*O*-(4-chlorobenzyl)-2-deoxy-D-ribofuranoside and methyl 2- α -acetoxymethyl-3,5-di-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-D-ribofuranoside (**1**). These reagents glycosylate pyrimidines with high β -selectivity.⁶ Here we

report the practical utility of this approach for the synthesis of the purine analogues.

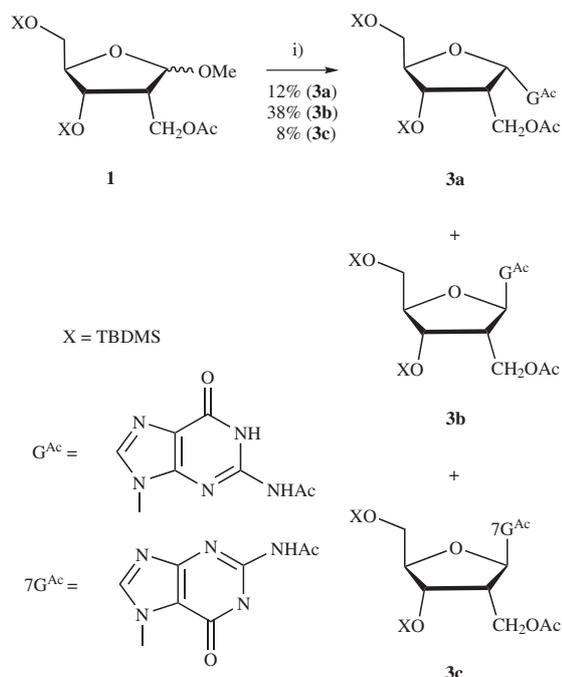
Sugar reagent **1** glycosylates persilylated uracil or cytosine in acetonitrile in the presence of SnCl₄ to give the corresponding nucleoside derivatives in about 60% yield with β/α selectivity of 94:6 and 90:10, respectively.⁶ However, the analogous glycosylation reaction of persilylated *N*⁶-benzoyladenine or *N*²-acetylguanine gives complex results, presumably due to reaction at both the N-7 and N-9 positions. Consequently, we employed two previously established experimental strategies to enhance the yield of the N-9 isomer.¹¹ (1) We used trimethylsilyl triflate as a catalyst in combination with a nonpolar solvent, either 1,2-dichloroethane or toluene; and (2) we conducted the reactions at reflux temperature to favor formation of the thermodynamically stable product (N-9 isomer).

In the presence of trimethylsilyl triflate, *N*⁶-benzoyl bis(trimethylsilyl)adenine reacted with **1** to give only the N-9 isomer (Scheme 1). However, the yield and anomeric ratio depended on the conditions, including reaction temperature and time (Table 1). Reaction in refluxing toluene (~110 °C) or *p*-xylene (~138 °C) resulted in decomposition of the products (entries 1 and 2). In contrast, reaction in refluxing dichloroethane for 1 hour gave the N-9 isomer in 61% yield with β/α anomeric ratio of ~3.7:1 (entry 3). Allowing the reaction to proceed at room temperature for 2 or 42 hours before initiating reflux decreased the stereoselectivity (entries 4 and 5). Refluxing for 19 hours resulted in product decomposition, reducing the yield of **2b** to only 8% (entry 6).



Scheme 1 Reagents and conditions: i) *N*⁶-benzoyl bis(trimethylsilyl)adenine (2.0 equiv), 1,2-dichloroethane, TMSOTf (1.0 equiv), reflux, 1 h

Compared to persilylated *N*⁶-benzoyladenine, the persilylated *N*²-acetylguanine reacted with **1** to produce the desired isomer with even weaker regio- and stereoselectivity. In dichloroethane at room temperature with SnCl₄ as a catalyst, the reaction yielded *N*⁷-isomer **3c** predominantly. However, reaction in refluxing dichloroethane for one hour using trimethylsilyl triflate (1.0 equiv) as a catalyst gave the best results, producing the desired **3b**



Scheme 2 Reagents and conditions: i) N^6 -acetyl tris(trimethylsilyl)guanine (1.5 equiv), 1,2-dichloroethane, TMSOTf (1.0 equiv), reflux, 1 h

Table 1 Glycosylation of N^6 -Benzoyl Bis(trimethylsilyl)adenine with Reagent 1

Run ^a	Solvent	Reaction conditions	Yield (%) ^b	2b/2a ^c
1 ^{d,e}	<i>p</i> -xylene	reflux, 2 h	decomposed	–
2 ^{d,e,f}	toluene	reflux, 3 h	decomposed	–
3	$\text{ClCH}_2\text{CH}_2\text{Cl}$	reflux, 1 h	61	3.7:1
4 ^e	$\text{ClCH}_2\text{CH}_2\text{Cl}$	0 °C to r.t., 2 h; then reflux, 1 h	–	2.9:1
5 ^e	$\text{ClCH}_2\text{CH}_2\text{Cl}$	0 °C to r.t., 42 h; then reflux, 1 h	–	2.1:1
6	$\text{ClCH}_2\text{CH}_2\text{Cl}$	reflux, 19 h	8	2b only

^a All experiments were carried out with N^6 -benzoyl bis(trimethylsilyl)adenine (2.0 equiv) in the presence of trimethylsilyl triflate (1.0 equiv) under argon.

^b Isolated yield.

^c Ratio was estimated by ^1H NMR of the crude product.

^d Ratio not calculated, and product was decomposed.

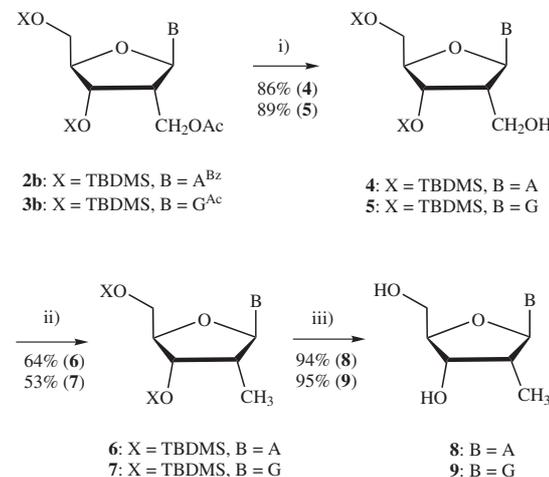
^e Product not isolated.

^f Reaction was incomplete, and product was partially decomposed.

in 38% yield along with smaller amounts of **3a** (12%) and **3c** (8%) (Scheme 2). As described for N^6 -benzoyladenine above, refluxing in higher boiling solvents such as *p*-xylene decomposed the product.

To transform **2b** and **3b** to the corresponding 2'- C - α -methylnucleosides, we first treated them with ammonia in methanol to obtain 3',5'-di-*O*-(*tert*-butyldimethylsilyl)-2'-

deoxy-2'-hydroxymethylpurines **4** and **5** in 86 and 89% yields, respectively (Scheme 3). Reaction of **4** and **5** with phenyl chlorothionoformate followed by reaction with tributyltin hydride in refluxing benzene produced the methylnucleoside derivatives **6** and **7** in 64 and 53% yields, respectively. Desilylation gave the final product 2'-deoxy-2'- C - α -methyladenine (**8**) and 2'-deoxy-2'- C - α -methylguanosine (**9**) in 94 and 95% yield, respectively.



Scheme 3 Reagents and conditions: i) NH_3 , MeOH, 0–4 °C, 72 h for **4**; 0 °C to r.t., 24 h for **5**; ii) 1) PhOCSCl , DMAP, CH_2Cl_2 , r.t., 3–5 h; 2) $\text{Bu}_3\text{SnH/AIBN}$, benzene, reflux, 5–7 h; iii) TBAF, THF, r.t., 3.5–12 h

We confirmed the structures of **2a** and **2b** by NOESY experiments. For **2a**, 1'-H ($\delta = 6.70$) exhibited a stronger NOE with 2'-H ($\delta = 3.20$) than with 2'- CH_2 ($\delta = 3.95$ – 3.89); additionally, 8-H ($\delta = 8.50$) exhibited stronger NOEs with 2'- CH_2 ($\delta = 3.95$ – 3.89) and 4'-H ($\delta = 4.45$) than with 1'-H ($\delta = 6.70$) or 2'-H ($\delta = 3.20$). We also observed a strong NOE between 2'-H ($\delta = 3.20$) and 3'-H ($\delta = 4.56$). These results suggest that **2a** has the α -configuration at the 1'-carbon. In contrast, the NOESY spectra for **2b** exhibited a pattern consistent with the β -configuration; we observed that 1'-H ($\delta = 6.27$) exhibited a stronger NOE with 2'- CH_2 ($\delta = 4.18$) than with 2'-H ($\delta = 3.24$). Conversely, the 8-H ($\delta = 8.28$) exhibited a strong NOE with 2'-H ($\delta = 3.24$) and no NOE with 2'- CH_2 ($\delta = 4.38$, 4.18). We also observed a strong NOE between 2'-H ($\delta = 3.24$) and 3'-H ($\delta = 4.55$). These results suggest that **2b** has the β -configuration at the 1'-carbon. Comparison of the ^{13}C NMR data for the C-4, C-5 and C-8 carbons of **2a** and **2b** with 7- and 9-methyladenine supported our regiochemical assignment of **2a** and **2b** as 9-glycosyladenines.¹²

Analogously, we confirmed the structures of **3a** and **3c** by NOESY experiments. For **3a**, we observed that 1'-H ($\delta = 6.28$) exhibited a stronger NOE cross peak with 2'-H ($\delta = 3.05$) than with 2'- CH_2 ($\delta = 4.02$, 3.83), and that 3'-H ($\delta = 4.48$) exhibited strong NOE cross peaks with 2'-H ($\delta = 3.05$) and 5'-H ($\delta = 3.70$, 3.58). These results suggest that **3a** has the α -configuration at 1'-carbon. For **3c**, we observed that 1'-H ($\delta = 6.46$) exhibited stronger NOE cross

peaks with 2'-CH₂ (δ = 4.42, 4.28) than with 2'-H (δ = 2.95); we also observed strong NOE cross peaks between 8-H (δ = 8.25) and 2'-H (δ = 2.95). These results suggest that **3c** has the β -configuration at the 1'-carbon. Comparison of the ¹³C NMR data for the C-4, C-5, and C-8 carbons of **3a**, **3b** and **3c** with *N*²-acetyl-2',3',5'-tri-*O*-acetyl-7- and 9-guanosine supported our regiochemical assignments of **3a** and **3b** as 9-glycosylguanines and **3c** as a 7-glycosylguanine.¹³ We confirmed the structure of **3b** indirectly by the NOESY spectra of the final product: 2'-deoxy-2'-*C*- α -methylguanosine (**9**). We observed that 1'-H (δ = 5.64) exhibited a stronger NOE cross peak with 2'-CH₃ (δ = 0.90) than with 2'-H (δ = 2.76). We also observed a strong NOE between 8-H (δ = 7.84) and 2'-H (δ = 2.76), but no NOE between 8-H (δ = 7.84) and 2'-CH₃ (δ = 0.90), 3'-H (δ = 4.24), 4'-H (δ = 4.03), or 5'-H (δ = 3.65). These results support our assignment of compound **9** as the β -nucleoside.

In summary, we have established synthetic access to 2'-deoxy-2'-*C*- α -methylpurine nucleosides starting from the glycosylating agent, methyl 3,5-di-*O*-(*tert*-butyldimethylsilyl)-2-*acetoxy*ribofuranoside. As expected, this reagent and its precursor exhibit greater stereo- and regioselectivity with pyrimidines than with purines. Nevertheless, we have optimized conditions that give the 2'-deoxy-2'-*C*- α -methylpurine nucleosides in yields that are practically useful. Moreover, our overall strategy offers both the methyl and hydroxymethyl analogues of the four natural nucleosides in a highly convergent manner from a common sugar reagent. These analogues may be converted either to the corresponding phosphoramidite for site-specific incorporation by solid phase synthesis or to the corresponding nucleoside triphosphates (or α -thiotriphosphates) for incorporation into RNA by transcription.

¹H and ¹³C NMR spectra were recorded on GE 500, Bruker 500 or Bruker 400 MHz NMR spectrometer. ¹H chemical shifts are reported in δ (ppm) relative to tetramethylsilane and ¹³C chemical shifts δ (ppm) relative to the solvent used. High Resolution Mass spectra were obtained from the Department of Chemistry, University of California at Riverside on the VG-ZAB instrument. Methyl 2-*acetoxy*methyl-3,5-di-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-D-ribofuranoside (**1**) was prepared according to our reported procedure.⁶

2'-C- α -Acetoxymethyl-3',5'-di-*O*-(*tert*-butyldimethylsilyl)-*N*⁶-benzoyl- α -adenosine (2a**) and 2'-C- α -Acetoxymethyl-3',5'-di-*O*-(*tert*-butyldimethylsilyl)-*N*⁶-benzoyl- β -adenosine (**2b**)**

A mixture of *N*⁶-benzoyladenine (478 mg, 2.0 mmol), 1,1,1,3,3,3-hexamethyldisilazane (5.0 mL) and pyridine (2.5 mL) was stirred under argon at reflux for 3 h to obtain a clear solution. The solution was evaporated to dryness, and the residue was dried under vacuum overnight. The persilylated adenine was dissolved into anhyd 1,2-dichloroethane (10 mL) under argon, and compound **1** (448 mg, 1.0 mmol) was added, followed by slow addition of TMSOTf (0.18 mL, 1.0 mmol). The mixture was then refluxed for 1 h. TLC showed that **1** was completely consumed. The mixture was cooled and quenched with sat. aq NaHCO₃. The organic phase was separated, and the aqueous phase was extracted with CH₂Cl₂. The organic layers were combined, washed with brine and dried (MgSO₄). After solvent was removed, the residue was purified by silica gel chromatography,

eluting with 40% EtOAc in hexane, to give compound **2a** (87 mg, 13%, the slow isomer) and **2b** (317 mg, 48%, the fast isomer).

2a

¹H NMR (CDCl₃/TMS): δ = 9.22 (br s, 1 H, 6-NH), 8.78 (s, 1 H, 2-H), 8.50 (s, 1 H, 8-H), 8.01 (d, 2 H, *J* = 7.2 Hz), 7.56 (m, 1 H), 7.49 (t, 2 H, *J* = 7.7 Hz), 6.70 (d, 1 H, *J* = 7.4 Hz, 1'-H), 4.56 (m, 1 H, 3'-H), 4.45 (m, 1 H, 4'-H), 3.95–3.89 (m, 2 H, 2'-CH₂), 3.74 (dd, 1 H, *J* = 3.3, 10.9 Hz, 5'-H), 3.63 (m, 1 H, 5'-H), 3.20 (m, 1 H, 2'-H), 1.90 (s, 3 H), 0.92 (s, 9 H), 0.91 (s, 9 H), 0.166 (s, 3 H), 0.115 (s, 3 H), 0.100 (s, 3 H), 0.095 (s, 3 H).

¹³C NMR (CDCl₃): δ = 170.2, 164.7, 152.6, 151.7, 149.3, 142.8, 133.8, 132.6, 128.7, 127.8, 122.2, 90.2, 85.2, 73.7, 63.5, 58.9, 46.7, 25.8, 25.7, 20.6, 18.3, 18.0, -4.7, -5.3, -5.4, -5.6.

HRMS: *m/z* calcd for C₃₂H₅₀N₅O₆Si₂ [MH⁺]: 656.3300; found: 656.3321.

2b

¹H NMR (CDCl₃/TMS): δ = 9.44 (br s, 1 H, 6-NH), 8.74 (s, 1 H, 2-H), 8.28 (s, 1 H, 8-H), 7.99 (d, 2 H, *J* = 7.4 Hz), 7.54 (t, 1 H, *J* = 7.3 Hz), 7.45 (t, 2 H, *J* = 7.6 Hz), 6.27 (d, 1 H, *J* = 8.5 Hz, 1'-H), 4.55 (m, 1 H, 3'-H), 4.38 (dd, 1 H, *J* = 7.4, 11.2 Hz, 2'-CH₂), 4.18 (dd, 1 H, *J* = 7.2, 11.2 Hz, 2'-CH₂), 4.09 (m, 1 H, 4'-H), 3.88 (dd, 1 H, *J* = 4.7, 11.2 Hz, 5'-H), 3.75 (dd, 1 H, *J* = 2.7, 11.2 Hz, 5'-H), 3.24 (m, 1 H, 2'-H), 1.72 (s, 3 H), 0.91 (s, 9 H), 0.90 (s, 9 H), 0.101 (s, 3 H), 0.094 (s, 3 H), 0.088 (s, 3 H), 0.080 (s, 3 H).

¹³C NMR (CDCl₃): δ = 170.4, 164.6, 152.7, 151.8, 149.5, 141.6, 133.7, 132.8, 128.8, 127.9, 123.2, 88.1, 86.6, 73.5, 63.3, 60.2, 48.2, 25.9, 25.7, 20.5, 18.4, 18.0, -4.6, -5.1, -5.3, -5.5.

HRMS: *m/z* calcd for C₃₂H₅₀N₅O₆Si₂ [MH⁺]: 656.3300; found: 656.3292.

2'-C- α -Acetoxymethyl-3',5'-di-*O*-(*tert*-butyldimethylsilyl)-*N*²-acetyl- α -guanosine (3a**), 2'-C- α -Acetoxymethyl-3',5'-di-*O*-(*tert*-butyldimethylsilyl)-*N*²-acetyl- β -guanosine (**3b**) and 2'-C- α -Acetoxymethyl-3',5'-di-*O*-(*tert*-butyldimethylsilyl)-*N*²-acetyl- β -(*N*-7)-guanosine (**3c**)**

Persilylated *N*²-acetylguanine was prepared by the reaction of *N*²-acetylguanine (580 mg, 3.0 mmol) with hexamethyldisilazane (10 mL) in anhyd pyridine (4.0 mL) as described for the persilylation of *N*⁶-benzoyladenine in the synthesis of **2a** and **2b**. Under argon, the persilylated guanine was dissolved into anhyd 1,2-dichloroethane (20 mL), and **1** (896 mg, 2.0 mmol) and TMSOTf (0.36 mL, 2.0 mmol) were added. The mixture was then refluxed for 1 h, allowed to cool, and then quenched with sat. aq NaHCO₃. The organic phase was separated, and the aqueous phase was extracted with CH₂Cl₂. The organic layers were combined, washed with brine and dried (MgSO₄). After removal of the solvent, the residue was purified by silica gel chromatography, eluting with 2.5% MeOH in CHCl₃, to give compound **3a** (152 mg, 12% yield, the slow isomer), **3b** (462 mg, 38% yield, the middle isomer), and **3c** (100 mg, 8% yield, the fast isomer).

3a

¹H NMR (CDCl₃/TMS): δ = 9.73 (br s, 1 H), 8.12 (s, 1 H, 8-H), 6.28 (d, 1 H, *J* = 7.2 Hz, 1'-H), 4.48 (m, 1 H, 3'-H), 4.41 (m, 1 H, 4'-H), 4.02 (m, 1 H, 2'-CH₂), 3.83 (dd, 1 H, *J* = 8.8, 10.8 Hz, 2'-CH₂), 3.70 (m, 1 H, 5'-H), 3.58 (m, 1 H, 5'-H), 3.05 (m, 1 H, 2'-H), 2.28 (s, 3 H), 1.94 (s, 3 H), 0.90 (s, 9 H), 0.88 (s, 9 H), 0.139 (s, 3 H), 0.084 (s, 3 H), 0.079 (s, 3 H), 0.076 (s, 3 H).

¹³C NMR (CDCl₃): δ = 172.2, 170.3, 155.9, 148.2, 147.4, 138.9, 120.4, 90.2, 85.1, 73.3, 63.6, 58.5, 46.8, 25.8, 25.7, 24.4, 20.7, 18.3, 18.0, -4.7, -5.4.

HRMS: *m/z* calcd for C₂₇H₄₈N₅O₇Si₂ [MH⁺]: 610.3092; found: 610.3119.

3b

$^1\text{H NMR}$ (CDCl_3/TMS): δ = 10.15 (br s, 1 H), 7.92 (s, 1 H, 8-H), 5.91 (d, 1 H, J = 8.4 Hz, 1'-H), 4.48 (m, 1 H, 3'-H), 4.25 (m, 1 H, 2'-CH₂), 4.15 (m, 1 H, 2'-CH₂), 4.00 (m, 1 H), 3.73 (m, 2 H, 5'-H), 2.95 (m, 1 H, 2'-H), 2.25 (s, 3 H), 1.77 (s, 3 H), 0.86 (s, 9 H), 0.83 (s, 9 H), 0.06–0.02 (m, 12 H).

$^{13}\text{C NMR}$ (CDCl_3): δ = 172.1, 170.4, 156.0, 148.4, 147.4, 137.0, 120.8, 87.8, 85.9, 73.1, 63.2, 59.9, 48.7, 25.7, 25.5, 24.2, 20.4, 18.2, 17.8, -4.8, -5.3, -5.6, -5.7.

HRMS: m/z calcd for $\text{C}_{27}\text{H}_{48}\text{N}_5\text{O}_7\text{Si}_2$ [MH^+]: 610.3092; found: 610.3108.

3c

$^1\text{H NMR}$ (CDCl_3/TMS): δ = 11.51 (br s, 1 H), 8.25 (s, 1 H, 8-H), 6.46 (d, 1 H, J = 7.7 Hz, 1'-H), 4.51 (m, 1 H, 3'-H), 4.42 (m, 1 H, 2'-CH₂), 4.28 (m, 1 H, 2'-CH₂), 4.07 (m, 1 H, 4'-H), 3.90 (m, 1 H, 5'-H), 3.79 (m, 1 H, 5'-H), 2.95 (m, 1 H, 2'-H), 2.40 (s, 3 H), 1.86 (s, 3 H), 0.923 (s, 9 H), 0.917 (s, 9 H), 0.12 (s, 3 H), 0.11 (s, 3 H), 0.10 (s, 3 H), 0.09 (s, 3 H).

$^{13}\text{C NMR}$ (CDCl_3): δ = 173.4, 170.4, 156.8, 152.8, 148.2, 141.6, 111.4, 88.5, 87.7, 72.7, 63.0, 60.1, 50.2, 25.9, 25.6, 24.6, 20.6, 18.3, 17.9, -4.7, -5.1, -5.5, -5.6.

HRMS: m/z calcd for $\text{C}_{27}\text{H}_{48}\text{N}_5\text{O}_7\text{Si}_2$ [MH^+]: 610.3092; found: 610.3114.

3',5'-Di-*O*-(*tert*-butyldimethylsilyl)-2'-C- α -hydroxymethyl- β -adenosine (4)

Ammonia was bubbled into a solution of **2b** (114 mg, 0.17 mmol) in MeOH (10 mL) at 0 °C for 30 min. The flask was sealed and refrigerated (0–4 °C) for 3 d. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 7.5% MeOH in CHCl_3 , to give compound **4** as a white solid powder (76 mg, 86%).

$^1\text{H NMR}$ (CDCl_3/TMS): δ = 8.23 (s, 1 H), 8.06 (s, 1 H), 6.39 (br s, 2 H), 6.30 (d, 1 H, J = 7.4 Hz), 4.60 (dd, 1 H, J = 2.2, 5.9 Hz), 4.06 (m, 1 H), 3.93 (m, 2 H), 3.86 (dd, 1 H, J = 5.1, 11.1 Hz), 3.73 (dd, 1 H, J = 3.6, 11.1 Hz), 3.02 (m, 1 H), 0.91 (s, 9 H), 0.88 (s, 9 H), 0.112 (s, 3 H), 0.109 (s, 3 H), 0.06 (s, 6 H).

$^{13}\text{C NMR}$ (CDCl_3): δ = 155.6, 152.7, 149.3, 139.0, 119.8, 87.7, 87.6, 74.2, 62.9, 59.0, 50.9, 25.9, 25.7, 18.3, 17.9, -4.7, -5.1, -5.5, -5.6.

HRMS: m/z calcd for $\text{C}_{23}\text{H}_{44}\text{N}_5\text{O}_4\text{Si}_2$ [MH^+]: 510.2932; found: 510.2925.

3',5'-Di-*O*-(*tert*-butyldimethylsilyl)-2'-C- α -hydroxymethyl- β -guanosine (5)

Ammonia was bubbled into a solution of **3b** (158 mg, 0.26 mmol) in MeOH (15 mL) at 0 °C for 30 min. The flask was sealed and kept at r.t. for 24 h. TLC showed that **3b** was completely consumed. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 10% MeOH in CHCl_3 , to give compound **5** as a white solid powder (121 mg, 89%).

$^1\text{H NMR}$ (CDCl_3/TMS): δ = 7.76 (s, 1 H), 6.48 (br s, 2 H), 6.08 (d, 1 H, J = 6.4 Hz), 4.57 (m, 1 H), 4.00–3.80 (m, 3 H), 3.80–3.65 (m, 2 H), 2.94 (m, 1 H), 0.91 (s, 9 H), 0.87 (s, 9 H), 0.12 (s, 3 H), 0.11 (s, 3 H), 0.08 (s, 3 H), 0.07 (s, 3 H).

$^{13}\text{C NMR}$ (CDCl_3): δ = 158.9, 153.7, 151.1, 135.8, 116.9, 87.2, 86.8, 73.7, 62.8, 59.1, 50.4, 25.9, 25.7, 18.4, 18.0, -4.8, -5.0, -5.4, -5.5.

HRMS: m/z calcd for $\text{C}_{23}\text{H}_{43}\text{N}_5\text{O}_5\text{Si}_2\text{Na}$ [MNa^+]: 548.2700; found: 548.2700.

3',5'-Di-*O*-(*tert*-butyldimethylsilyl)-2'-C- α -methyl- β -adenosine (6)

To a solution of **4** (94 mg, 0.19 mmol) and DMAP (147 mg, 1.2 mmol) in anhyd CH_2Cl_2 (10 mL) at 0 °C was added phenyl chlorothionoformate (28 μL , 0.20 mmol). After stirring the mixture at r.t. for 3 h, the mixture was diluted with EtOAc, and the EtOAc layer was washed consequently with sat. aq NaHCO_3 and brine. The organic layer was dried (MgSO_4). The solvent was removed, and the residue was dried under vacuum and used directly in the next step without purification. To the solution of dried crude thionocarbonyl ester in benzene (15 mL) under argon, were added Bu_3SnH (0.30 mL, 1.1 mmol) and AIBN (10 mg, 0.060 mmol). The mixture was refluxed under stirring for 5 h. The solvent was removed and the residue was purified by silica gel chromatography, eluting with 50% EtOAc in hexane, to give compound **6** as a white foam (58 mg, 64%).

$^1\text{H NMR}$ (CDCl_3/TMS): δ = 8.34 (s, 1 H), 8.08 (s, 1 H), 6.06 (br s, 2 H), 6.01 (d, 1 H, J = 8.4 Hz), 4.36 (m, 1 H), 4.06 (m, 1 H), 3.85 (dd, 1 H, J = 4.8, 10.8 Hz), 3.75 (dd, 1 H, J = 3.2, 11.2 Hz), 2.82 (m, 1 H), 1.05 (d, 3 H, J = 6.8 Hz), 0.920 (s, 9 H), 0.917 (s, 9 H), 0.09 (s, 12 H).

$^{13}\text{C NMR}$ (CDCl_3): δ = 155.5, 153.0, 150.1, 139.0, 119.8, 89.3, 87.7, 74.9, 63.5, 43.9, 26.0, 25.8, 18.4, 18.1, 9.3, -4.6, -4.9, -5.4, -5.5.

HRMS: m/z calcd for $\text{C}_{23}\text{H}_{44}\text{N}_5\text{O}_3\text{Si}_2$ [MH^+]: 494.2983; found: 494.2987.

3',5'-Di-*O*-(*tert*-butyldimethylsilyl)-2'-C- α -methyl- β -guanosine (7)

To a solution of **5** (245 mg, 0.47 mmol) and DMAP (374 mg, 3.1 mmol) in anhyd CH_2Cl_2 (10 mL) at 0 °C was added phenyl chlorothionoformate (71 μL , 0.51 mmol). After stirring the mixture at r.t. for 5 h, the reaction was quenched with MeOH (0.50 mL). The mixture was diluted with EtOAc, and the EtOAc layer was consequently washed with sat. aq NaHCO_3 and brine. The organic layer was dried (MgSO_4). The solvent was removed, and the residue was dried under vacuum overnight. The dried crude thionocarbonyl ester was dissolved into benzene (20 mL) under argon, and Bu_3SnH (0.67 mL, 2.5 mmol) and AIBN (20.0 mg, 0.12 mmol) were added. The mixture was refluxed under stirring for 7 h. The solvent was removed and the residue was purified by silica gel chromatography, eluting with 6% MeOH in CH_2Cl_2 to give compound **7** as a white foam (126 mg, 53%).

$^1\text{H NMR}$ (CDCl_3/TMS): δ = 7.75 (s, 1 H), 6.52 (br s, 1 H), 5.77 (d, 1 H, J = 4.0 Hz), 4.34 (m, 1 H), 4.03 (m, 1 H), 3.76 (m, 2 H), 2.72 (m, 1 H), 1.10–0.85 (m, 21 H), 0.20–0.01 (m, 12 H).

$^{13}\text{C NMR}$ (CDCl_3): δ = 159.2, 153.9, 152.0, 135.5, 117.3, 88.2, 87.5, 74.9, 63.5, 43.0, 26.0, 25.8, 18.4, 18.1, 9.3, -4.6, -4.8, -5.3, -5.5.

HRMS: m/z calcd for $\text{C}_{23}\text{H}_{43}\text{N}_5\text{O}_4\text{Si}_2\text{Na}$ [MNa^+]: 532.2751; found: 532.2753.

2'-C- α -Methyl- β -adenosine (8)

To a solution of **6** (58.0 mg, 0.12 mmol) in THF (15 mL) was added TBAF (1.0 M in THF, 0.50 mL, 0.50 mmol). The mixture was stirred at r.t. for 12 h. The solvent was removed and the residue was purified by silica gel chromatography, eluting with 12.5% MeOH in CHCl_3 to give the product as a white solid (30 mg, 94%).

$^1\text{H NMR}$ (D_2O): δ = 8.07 (s, 1 H), 7.90 (s, 1 H), 5.71 (d, 1 H, J = 9.0 Hz), 4.24 (m, 1 H), 4.05 (m, 1 H), 3.66 (m, 2 H), 2.68 (m, 1 H), 0.86 (d, 3 H, J = 6.9 Hz).

$^{13}\text{C NMR}$ (D_2O): δ = 155.2, 152.1, 148.2, 140.1, 118.7, 89.8, 87.3, 73.6, 62.0, 42.9, 7.8.

HRMS: m/z calcd for $C_{11}H_{16}N_5O_3$ [MH⁺]: 266.1253; found: 266.1266.

2'-C- α -Methyl- β -guanosine (9)

To a solution of **7** (42.0 mg, 0.082 mmol) in THF (10 mL) was added TBAF (1.0 M in THF, 0.40 mL, 0.40 mmol). The mixture was stirred at r.t. for 3.5 h. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 15% MeOH in CHCl₃ to give the product as a white solid (22 mg, 95%).

¹H NMR (D₂O/CD₃OD): δ = 7.81 (s, 1 H, 8-H), 5.61 (d, 1 H, J = 9.2 Hz, 1'-H), 4.21 (m, 1 H, 3'-H), 4.00 (m, 1 H, 4'-H), 3.65 (m, 2 H, 5'-H), 2.71 (m, 1 H, 2'-H), 0.88 (d, 3 H J = 7.2 Hz, 2-CH₃).

¹³C NMR (D₂O/CD₃OD): δ = 159.1, 153.9, 151.3, 137.6, 116.5, 89.0, 86.9, 73.5, 61.9, 42.2, 7.7.

HRMS: m/z calcd for $C_{11}H_{15}N_5O_4Na$ [MNa⁺]: 304.1022; found: 304.1026.

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- (12) The ¹³C NMR data of the adenine ring are assigned as follows: For **2a** (CDCl₃): δ = 152.6 (C-6), 151.7 (C-2), 149.3 (C-4), 142.8 (C-8), 122.2 (C-5) and **2b** (CDCl₃): δ = 152.7 (C-6), 151.8 (C-2), 149.5 (C-4), 141.6 (C-8), 123.2 (C-5); cf. experimental section. For 7-methyladenine (DMSO-*d*₆): δ = 159.82 (C-4), 152.31 (C-2), 151.91 (C-6), 145.94 (C-8), 111.77 (C-5) and 9-methyladenine (DMSO-*d*₆): δ = 155.98 (C-6), 152.50 (C-2), 149.94 (C-4), 141.47 (C-8), 118.72 (C-5); see: Chenon, M.-T.; Pugmire, R. J.; Grant, D. M.; Panzica, R. P.; Townsend, L. B. *J. Am. Chem. Soc.* **1975**, *97*, 4627.
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