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A reinvestigated mechanism of ribosylation of adenine under silylating conditions

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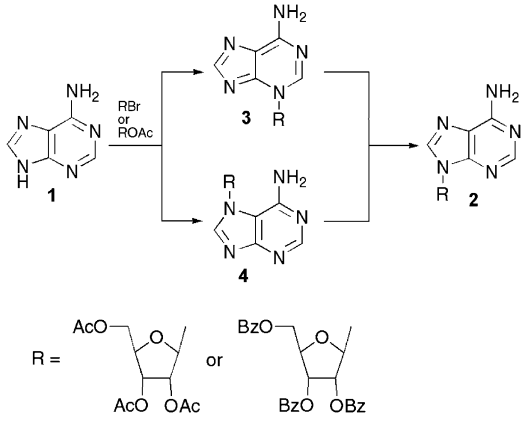
Abstract—The mechanism of chemical synthesis of adenosine has been reinvestigated. Depending on the reaction conditions and the presence of N^6-protecting groups, ribosylation of adenine proceeds via different kinetic products: 3-riboadenine in strongly acidic media, 7-ribosylated derivative in the silyl method, and 1-(β-D-ribofuranosyl)adenine when applying N^6-acyladenine and silylating conditions.

1. Introduction

It has been known for a century that ribosylation of adenine (**1**) in the presence of acidic catalysts gives 9-(β-D-ribofuranosyl)adenine, i.e., adenosine (**2**), one of the basic components of ribonucleic acids (Scheme 1). However, the literature data on the mechanism of ribosylation and the structure of a kinetically controlled product of ribosylation seem to be rather confusing. The first proposed mechanism[1] postulates an initial formation of 3-(β-D-ribofuranosyl)adenine (isoadenosine; **3**), and in fact, compounds of the type **3** have been isolated from reaction mixtures and their structures have been fully confirmed.[2,3] In line with that observation, the following sequence of events has been established: (i) initial ribosylation at N3, (ii) second ribosylation at N9 with the formation of 3,9-bis-ribosyladenine, and (iii) its decomposition to the stable 9-regioisomer (**2**). This mechanism has been extended for glycosylation reactions of all purine bases and may be found in every handbook on nucleoside chemistry. More recently, however, it has been shown that only N7 and N9 atoms can serve as glycosyl donors or acceptors in glycosylation and transglycosylation reactions in the guanine series.[4,5]



Scheme 1.

On the other hand, there are some literature reports on isolation of protected derivatives of 7-(β-D-ribofuranosyl)adenine (**4**) as kinetic products in the ribosylation of adenine.[6–10] The first synthesis of 7-riboadenine was presented in 1971: a direct coupling of bis(trimethylsilyl)-N^6-benzoyladenine and 1-bromo-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose in the presence of $HgBr_2$ reportedly gave the 7-isomer, in addition to the predominant amount of N^6-benzoyladenosine.[6] Quite similar results were obtained when $SnCl_4$ was used as a catalyst.[7,8] More recently, it has been reported that glycosylation of persilylated N^6-benzoyladenine with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-L-ribofuranose in the presence of trimethylsilyl triflate (TMSOTf) leads to a mixture of 7- and 9-regioisomers.[9] Interestingly, 7-riboadenine (**4**) can be formed not only under the Vorbrüggen's conditions[10] of ribosylation: compound **4** has been obtained in the fusion reaction of N^6-benzyladenine and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose performed in nitrophenols, and the kinetic nature of the 7-regiosiomer has been demonstrated for the first time.[11] On the basis of those literature reports, we may assume that there is an alternative mechanism of ribosylation of adenine, different than the generally accepted 3→9

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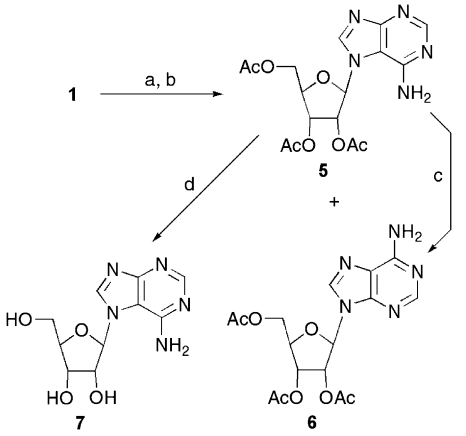
pathway of glycosylation. In this case, the kinetically controlled 7-regiosiomer of adenine would be transformed to adenosine (**2**) via a 7,9-bis-ribosyladenine intermediate, as it has been documented for 6-oxopurine bases: hypoxanthine[12] and guanine.[4,5,13]

2. Results and discussion

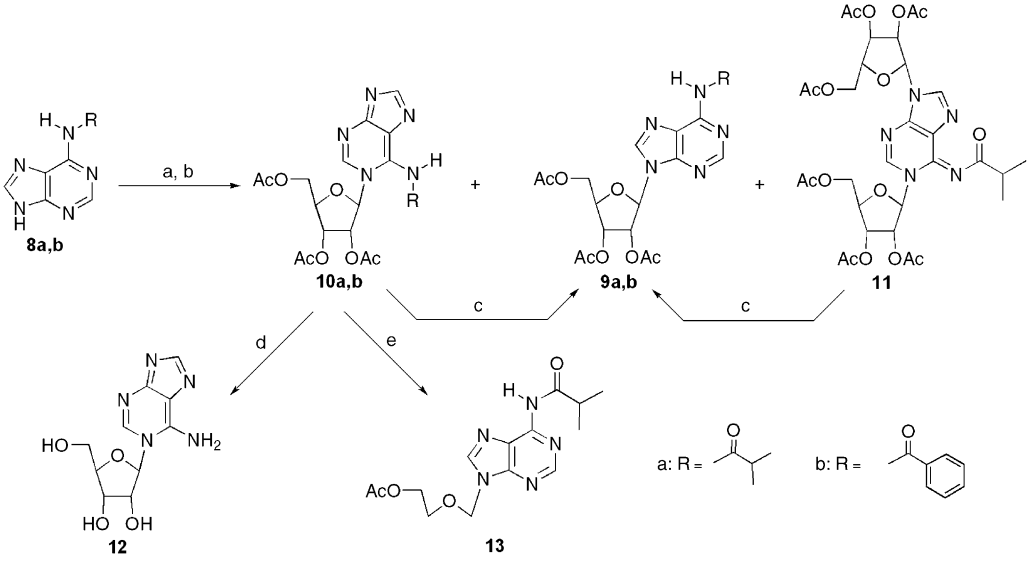
In the course of our systematic reinvestigation on mechanisms of the *N*-glycosylic bond formation, we performed a series of experiments to establish the factors responsible for either 3→9 or 7→9 mechanism in the ribosylation of adenine. This time we focused our attention on the silyl method, the most common synthetic procedure at present. Thus, adenine (**1**) was silylated with hexamethyldisilazane (HMDS) and then subjected to ribosylation with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose in the presence of trimethylsilyl triflate (TMSOTf) (Scheme 2). After 80 min the reaction mixture contained 36% of the 7-isomer (**5**; isolated by column chromatography), along with a smaller amount (less than 10%) of triacetyladenosine (**6**). When heating was continued for a longer time, the 9-regioisomer **6** was the only remaining product. Similarly, the isolated product **5** could be quantitatively isomerized to **6** in the presence of *p*-toluenesulfonic acid on refluxing in chlorobenzene. This shows clearly that 7-riboadenine is a kinetic product in the ribosylation of adenine. Most probably, the 7→9 transglycosylation proceeds via a 7,9-bis-ribosyl intermediate, likewise in the guanine series, but the reaction equilibrium is totally shifted toward the 9-regioisomer (**6**). The product **5** was deprotected with aqueous ammonia in methanol to give 7-(β-D-ribofuranosyl)-adenine (**7**), identical in all respects with the sample obtained in the presence of $SnCl_4$.[7]



Scheme 2. Reagents and conditions: (a) $(NH_4)_2SO_4$, HMDS, reflux, 2.5 h; (b) CH_3CN, 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose, TMSOTf, rt, 80 min; (c) *p*-TsOH, chlorobenzene, 150 °C, 2.5 h; and (d) 25% NH_4OH, MeOH, rt, 30 min.

Considering the above-mentioned result as well as the literature data,[6,7] we could expect a similar initial 7-ribosylation in the reaction of N^6-acylated derivatives of adenine, performed according to the Vorbrüggen's procedure. The reaction sequence is shown in Scheme 3. N^6-Isobutyryl (**8a**) and N^6-benzoyladenine (**8b**) were silylated with *N*,*O*-bis-trimethylsilylacetamide (BSA), and then subjected to ribosylation with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose in the presence of TMSOTf. Surprisingly, the reaction gave, along with the 9-ribo products (**9a**,**b**), new products of the structure of 1-regioisomers (**10a**,**b**) in the yield of 33% and 30%, respectively. In addition, a careful chromatographic separation in the isobutyryl series allowed to isolate a minor reaction product, the 1,9-bis-ribosyl derivative **11** (5%). The deprotection of **10a** with methanolic ammonia gave 1-(β-D-ribofuranosyl)adenine (**12**), a new regioisomer of naturally occurring adenosine.

Compounds **10a**,**b** underwent isomerization to the respective 9-regioisomers (**9a**,**b**) after a prolonged reaction time, and this proves the kinetic nature of the 1-regioisomers of adenosine. Furthermore, the isolated N^6-isobutyryl derivative (**10a**) underwent an almost quantitative conversion to **9a** under transglycosylation conditions (refluxing in



Scheme 3. Reagents and conditions: (a) BSA/CH_3CN, Ar, 60 °C, 30 min; (b) 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose, TMSOTf, 60–75 °C, 3 h; (c) *p*-TsOH, chlorobenzene, 60–150 °C, 10–150 min; (d) NH_3/MeOH, 25 °C, 24 h; and (e) $AcOCH_2CH_2OCH_2OAc$, *p*-TsOH, chlorobenzene, reflux, 4 h.

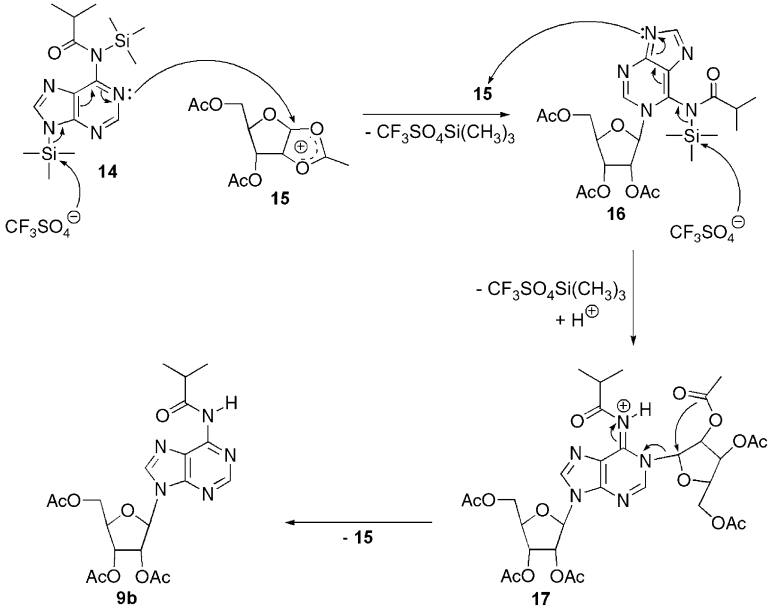
chlorobenzene in the presence of *p*-toluenesulfonic acid). Under the same conditions, the 1,9-bis-ribofuranosyl intermediate (**11**) was decomposed to a 6:1 mixture of the respective 9- and 1-regioisomers (**9a** and **10a**). Finally, the reaction of **10a** with 2-acetoxyethyl acetoxymethyl ether[14,15] resulted in the formation of acycloadenosine derivative **13**, and this is evidence for an intermolecular course of the 1→9 isomerization.

Taking into account these experimental facts, we can propose a new mechanism of the ribosylation in the N^6-acyl series (Scheme 4), shown here for the N^6-isobutyryl derivatives. In the first step, 9,N^6-bis-trimethylsilylated substrate (**14**) reacts with the acyloxonium sugar cation (**15**), generated from tetraacetylribose.[10] The position N9, an ultimate site of ribosylation, is already blocked by the TMS group. Therefore, the initial ribosylation may take place at any alternative site, e.g., N1, N3, or N7. As presented above, the N1-position is ribosylated first, perhaps due to a limited access to N7 in the presence of the two N^6-substituents, and this leads to the kinetically controlled 1-ribonucleoside **16** (isolated as a desilylated derivative **10a**). Compound **16** then undergoes an intermolecular transglycosylation to regain the thermodynamically preferred aromatic system, corresponding to the most stable N-9-H tautomer of adenine. Thus, a second ribosylation at N9 gives the 1,9-bis-ribosyl intermediate **11**, which after protonation (structure **17**) undergoes a decomposition to the final 9-regioisomer **9a**, with liberation of the acyloxonium cation **15**. The 1→9 isomerization is irreversible. It is worthy to note that quite a similar mechanism can be drawn for an isomeric structure of the bis-trimethylsilyl substrate **14**, in which the second TMS group would be attached not to N6, but to oxygen atom of the N^6-acyl substituent, as it has been proposed recently.[10] In that case, explanation of the observed regioselectivity would be even more convincing. Interestingly, the formation of 1-(β-D-ribofuranosyl)adenine as a possible kinetic intermediate has been anticipated by Vorbrüggen and Höfle,[16] but this has never been proved experimentally.

All compounds were fully characterized by the ^{1}H and ^{13}C NMR (1D & 2D, NOE) techniques and analytical methods. Table 1 presents the first comparison of ^{13}C NMR spectra of adenosine and all its regioisomers. In particular, the data for 7-riboadenine (**7**) are in good agreement with those published for related compounds.[17,18] Both isomeric nucleosides, 1- and 7-(β-D-ribofuranosyl)adenine (**12** and **7**, respectively) gave crystals suitable for X-ray diffraction and their crystal structures have been determined (Fig. 1).[19] In the crystals of **12**, there are two symmetry independent molecules, denoted A and B, showing no significant differences in their geometrical parameters. Interestingly, both regioisomeric nucleosides in their crystal structures adopt conformations, which enable the formation of a three-center intramolecular N–H···O hydrogen bond between the amino group of the base and O1′ and O5′ from the ribose moiety (Fig. 2). To form such a bond, the isomers must adopt different sugar conformations: 1-riboadenine (**12**) adopts an envelope C2′-*endo* form, while 7-riboadenosine (**7**) occurs in crystal as a C2′-*endo*-C1′-*exo* conformer.

3. Conclusion

The mechanism of ribosylation in the adenine series evidently depends on reaction conditions. While application of 1-halosugars in strongly acidic media favors the 3→9 pathway, the use of 1-*O*-acetylated sugars and Lewis acids in the silyl approach results in either 7→9 or 1→9 glycosylation sequence. In the latter method, a comparison of the data obtained in this work and those reported in the literature allows us to formulate some general rules, which may be useful in the synthesis of regioisomers of adenosine. The use of Lewis acid catalysts like $HgBr_2$[6] or $SnCl_4$[7,8] results in the formation of 7-(β-D-ribofuranosyl)adenine as



Scheme 4. A proposed mechanism for the 1→9 ribosylation pathway.

Table 1. Comparison of the ^{13}C NMR spectra of adenosine and its regioisomers (151 MHz, DMSO-d_6, TMS)

Compounds	C2	C4	C5	C6	C8	C1′	C2′	C3′	C4′	C5′
Adenosine	152.42	149.06	119.38	156.19	139.96	87.92	73.44	70.70	85.93	61.71
3-(β-D-Ribofuranosyl)adenine	143.53	147.14	120.68	155.69	151.28	94.95	72.12	71.01	87.82	61.85
7-(β-D-Ribofuranosyl)adenine (**7**)	152.61	160.69	110.05	151.49	144.41	89.18	74.82	68.78	86.16	60.32
1-(β-D-Ribofuranosyl)adenine (**12**)	140.43	148.58	118.88	154.42	150.53	92.14	72.66	69.82	86.19	60.43

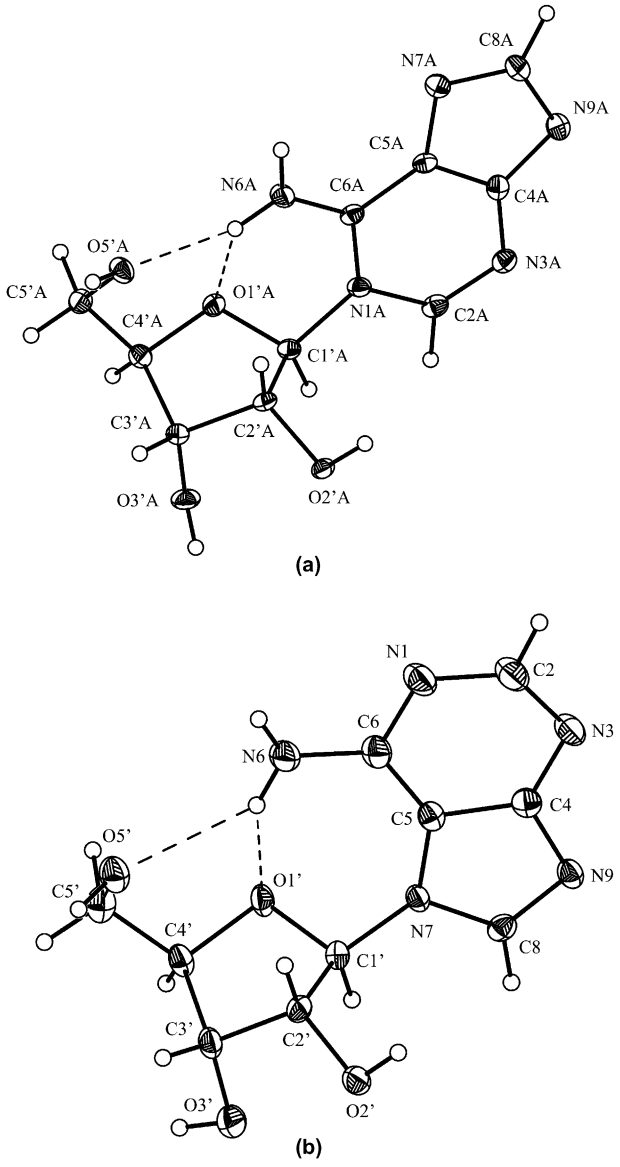


Figure 1. Ortep drawings of (a) **12**, molecule A, and (b) **7** at 50% probability level with atom numbering. Intramolecular hydrogen bonds are shown as dashed lines.

a kinetically controlled product, no matter whether the N^6-exocyclic amino group is protected or not. However, when the Vorbrüggen catalyst (TMSOTf) is applied, the presence of N^6-protection is crucial for the course of reaction: the ribosylation of persilylated derivatives of adenine without the N^6-acyl protection leads to the formation of 7-(β-D-ribofuranosyl)adenine, while N^6-acylated substrates are directly ribosylated in the position N1. To our knowledge, this is the first synthesis of the so far unknown 1-(β-D-ribofuranosyl) adenine, and that compound can now be obtained in a reasonable yield. The approach presented here should prove useful

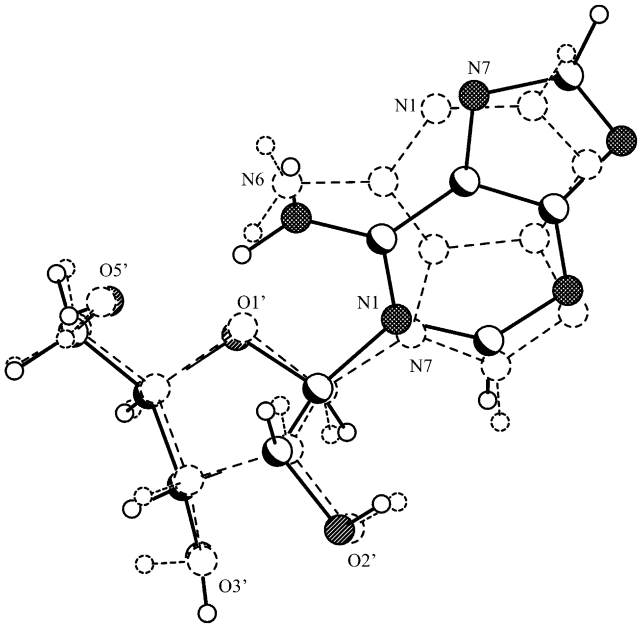


Figure 2. A superposition of the two isomers (**12** and **7**) in the conformations adopted in their crystal structures.

in the synthesis of related analogs of potential biological activity and in the study of base-pairing properties on the oligonucleotide level.

4. Experimental

4.1. General

3-(β-D-Ribofuranosyl)adenine for comparative study was obtained according to Leonard and Laursen.[2] N^6-Benzoyladenine (**8b**) was prepared according to the published procedure.[20] Melting points were determined on a Laboratory Devices Mel-Temp II micromelting points apparatus and are uncorrected. UV spectra were measured on a Beckman DU-65 spectrophotometer. The optical rotations were measured with a Perkin–Elmer 243B polarimeter. The infrared spectra were determined in KBr with a Bruker IFS 66v/s spectrophotometer. ^1H (300 MHz) and ^{13}C NMR (75.5 MHz) spectra were recorded on a Varian Unity 300 FT NMR 300 MHz spectrometer with tetramethylsilane as an internal standard, and chemical shifts are reported in δ-values (ppm). 2D ^1H and ^{13}C NMR (151 MHz) were recorded on a Bruker Avance 600 MHz spectrometer. The following NMR techniques were applied for structural assignment of the obtained compounds: COSY, HMQC, HMBC, TOCSY, and NOE. Mass spectra were taken on an AMD-604 spectrometer using the LSIMS technique (Cs^+, 12 keV; in NBA). Elemental analyses were performed on a Perkin–Elmer 240 Elemental Analyzer. TLC was conducted

on Merck silica gel F_{254} 60 plates using the following solvent systems (measured by volume): A, chloroform/methanol (9:1); B, toluene/ethanol (4:1); C, isopropanol/concd NH_4OH/water (7:1:2). For preparative short-column chromatography Merck TLC gel H 60 was used.

4.2. Crystal data

The diffraction data were collected at 130 K with a Kuma-CCD diffractometer, CrysAlis CCD, and CrysAlis RED. Version 1.171. Oxford Diffraction, using graphite monochromated Mo Kα radiation. The structures were solved by direct methods with the program SHELXS-97[21] and refined by full-matrix least-squares method on F2 with SHELXL-97.[22]

4.2.1. 7-(β-D-Ribofuranosyl)adenine 7 (CCDC 297135). $C_{10}H_{13}N_5O_4$, orthorhombic, space group $P2_12_12$, a=8.2006(7), b=17.8347(14), c=7.7064(7) Å, V=1346.04(19) Å^3, Z=4, d_x=1.575 g cm^{-3}, T=130 K. Data were collected for a crystal with dimensions 0.2×0.2×0.02 mm^3. Final R indices for 1021 reflections with $I>2\sigma(I)$ and 177 refined parameters are: R_1=0.0346, wR_2=0.0765 (R_1=0.0442, wR_2=0.0799 for all 1177 data).

4.2.2. 1-(β-D-Ribofuranosyl)adenine 12 (CCDC 297134). $C_{10}H_{13}N_5O_4$, monoclinic, space group $P2_1$, a=6.7737(4), b=16.7201(8), c=9.7134(4) Å, β=90.895(4)°, V=1099.98(10) Å^3, Z=4, d_x=1.614 g cm^{-3}, T=130 K. Data were collected for a crystal with dimensions 0.5×0.2×0.2 mm^3. Final R indices for 2232 reflections with $I>2\sigma(I)$ and 369 refined parameters are: R_1=0.0240, wR_2=0.0620 (R_1=0.0251, wR_2=0.0620 for all 2314 data).

4.3. 7-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)adenine (5) and 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)adenine (6)

To a stirred suspension of adenine (**1**; 200 mg, 1.48 mmol) in HMDS (6 mL) was added $(NH_4)_2SO_4$ (10 mg, 0.087 mmol) and the mixture was refluxed under argon for 2.5 h. The resulting solution was concentrated to an oil, dissolved in dry acetonitrile (6 mL), and treated with tetraacetylribose (470 mg, 1.47 mmol) and TMSOTf (267 μL, 1.47 mmol). After stirring at rt for 80 min, the reaction mixture was evaporated to a white solid foam. The products were isolated by SiO_2 column chromatography in a gradient of $CHCl_3$/MeOH (from 95:5 to 9:1) to give (in order of elution) the 9-isomer **6** (31 mg, 5.3%); R_f 0.48(A), 0.32(B) and the 7-isomer **5** (208 mg, 36%) as a solid foam: R_f 0.37(A), 0.10(B); λ_{max} (MeOH) 245 (sh), 274 nm; ^{1}H NMR ($CDCl_3$): 2.04, 2.10, 2.16 (3s, 3×3H), 4.36 (m, 3H), 5.47 (dd, 1H, J=4.5, 6.4 Hz), 5.59 (t, 1H, J=6.5 Hz), 5.70 (br s, 2H), 6.02 (d, 1H, J=6.6 Hz), 8.14 (s, 1H), 8.54 (s, 1H); ^{13}C NMR (DMSO-d_6): 20.11, 20.32, 20.39, 62.43, 68.74, 72.49, 79.65, 86.41, 110.19, 143.94, 151.30, 152.89, 160.43, 169.10, 169.48, 169.99; HRMS: calcd for $C_{16}H_{20}N_5O_7$ (M+H): m/z 394.1362, found: 394.1347.

4.4. 7-(β-D-Ribofuranosyl)adenine (7)

A solution of **5** (122 mg, 0.310 mmol) in methanol (4 mL) was treated with 25% NH_4OH (2 mL). After 30 min at rt the solvent was evaporated to obtain a white solid, which was crystallized from MeOH (60 mg, 72%): mp 207–210 °C (lit. 211–212[7] and 246[23]); R_f 0.57 (C; adenosine 0.65); $[\alpha]_D^{20}$ −93.6 (c 0.25, H_2O; lit. −100[7]); λ_{max} (MeOH) 245 (sh), 270 nm; ν_{max} 3500–2600 (br), 3443, 3345, 3246, 1636, 1593, 1561, 1520, 1489, 1447, 1408, 1303, 1116, 1057, 990, 881 cm^{-1}; ^{1}H NMR (DMSO-d_6): 3.68 (m, 2H), 3.99 (q, 1H, J=5.0 Hz), 4.07 (q, 1H, J=6.0 Hz), 4.12 (m, 1H), 5.31 (d, 1H, J=4.2 Hz), 5.36 (t, 1H, J=4.8 Hz), 5.62 (d, 1H, J=6.0 Hz), 5.82 (d, 1H, J=7.2 Hz), 6.99 (s, 2H), 8.22 (s, 1H), 8.52 (s, 1H). Anal. calcd for $C_{10}H_{13}N_5O_4$ (267.25): C, 44.94; H, 4.90; N, 26.21. Found: C, 44.86; H, 4.79; N, 26.17.

4.5. *N*^{6}-Isobutyryladenine (8a)

Adenine (6.7 g, 49.58 mmol) (vacuum dried) was stirred in isobutyric anhydride (160 mL) at 70 °C (oil bath temperature) for 4 h. TLC analysis showed the presence of two products, mono- and disubstituted ones. The mixture was then refluxed in absolute methanol (170 mL) until the disubstituted product was completely decomposed (2 h). The solvent was evaporated, and the resulting syrup was crystallized from ethanol. The product was recrystallized from boiling ethanol to give 9.15 g of white crystals (90%): mp 231–233 °C; R_f 0.38(A), 0.23(B); λ_{max} (MeOH) 281, 291 nm; ^{1}H NMR (DMSO-d_6): 1.17 (d, 6H, J=6.9 Hz), 2.92 (septet, 1H, J=6.9 Hz), 8.39 (s, 1H), 8.63 (s, 1H), 11.15 (s, 1H), 12.21 (s, 1H); HRMS: calcd for $C_9H_{12}N_5O$ (M+H): m/z 206.1041, found: 206.1032.

4.6. *N*^{6}-Isobutyryl-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)adenine (9a), *N*^{6}-isobutyryl-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)adenine (10a), and *N*^{6}-isobutyryl-1,9-bis-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)adenine (11)

An anhydrous suspension of **8a** (0.90 g, 4.38 mmol) and 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (1.79 g, 5.63 mmol) in dry acetonitrile (20 mL) was washed with argon for 30 min, then BSA (1.64 g, 8.09 mmol) was added. The mixture was stirred at 75 °C for 30 min until a clear solution was obtained. TMSOTf (0.48 g, 2.11 mmol) was then added and the mixture was stirred at 75 °C for 3 h. After cooling down to rt the obtained solution was diluted with CH_2Cl_2 (180 mL) and extracted with cold saturated solution of $NaHCO_3$ (150 mL). The organic layer was dried over Na_2SO_4, evaporated to a yellow solid foam and then chromatographed on a SiO_2 column in a CH_3Cl/CH_3CN gradient (from 2:1 to 1:1) to give (in order of elution): compound **11**, an oil: 0.16 g, 5%; R_f 0.80(A), 0.50(B); $[\alpha]_D^{20}$ +20.7 (c 0.31, MeOH); λ_{max} (MeOH) 293 nm; ν_{max} 3600–2850 (br), 1755, 1685, 1629, 1547, 1531, 1508, 1464, 1430, 1374, 1229 (br), 1097, 1047, 1017, 904, 757 cm^{-1}; ^{1}H NMR ($CDCl_3$): 1.22, 1.24 (2d, 6H, J=6.9 Hz), 2.08, 2.09, 2.10, 2.12, 2.14, 2.20 (6s, 6×3H), 2.75 (septet, 1H, J=6.9 Hz), 4.41–4.45 (m, 6H), 5.33 (dd, 1H, J=5.4, 7.8 Hz), 5.60 (m, 2H), 5.88 (t, 1H, J=5.4 Hz), 6.03 (d, 1H, J=5.4 Hz), 6.27 (d, 1H, J=2.4 Hz), 7.81 (s, 1H), 8.35 (s, 1H); ^{13}C NMR ($CDCl_3$): 18.97, 19.06, 20.21, 20.31, 20.35, 20.44, 20.49, 20.73, 37.41, 62.43, 62.76, 68.96, 69.88, 72.23, 72.42, 78.76, 79.54, 85.83, 90.04, 120.84, 139.87, 141.73, 143.35, 146.22, 169.13, 169.26, 169.40, 169.43, 170.23, 170.30, 187.60; HRMS: calcd for $C_{31}H_{40}N_5O_{15}$ (M+H): m/z 722.2521, found: 722.2538; the 9-isomer **9a**, an oil: 0.21 g, 10%; R_f 0.70(A), 0.46(B); λ_{max} (MeOH) 272 nm; the 1-isomer **10a**, a white

solid: 0.66 g, 33%; mp 159–161 °C (40% EtOH); R_f 0.54(A), 0.45(B); $[\alpha]_D^{20}$ +86.5 (*c* 0.27, MeOH); λ_{max} (MeOH) 313 nm; ν_{max} 3500–2850 (br), 1750, 1651, 1599, 1504, 1427, 1375, 1365, 1243, 1231, 1215, 1115, 1099, 1063, 1018 cm^{-1}; ^1H NMR ($CDCl_3$): 1.17 (d, 3H, *J*=7.0 Hz), 1.19 (d, 3H, *J*=7.0 Hz), 2.06, 2.18, 2.24 (3s, 3×3H), 2.65 (septet, 1H, *J*=7.0 Hz), 4.42 (dd, 1H, *J*=3.0, 12.6 Hz), 4.51–4.55 (m, 2H), 5.34 (dd, 1H, *J*=4.8, 8.4 Hz), 5.63 (d, 1H, *J*=4.8 Hz), 6.65 (s, 1H), 8.13 (s, 1H), 8.82 (s, 1H), 12.49 (br s, 1H); ^{13}C NMR ($CDCl_3$): 19.55, 19.84, 20.26, 20.30, 20.72, 39.51, 61.05, 67.67, 74.51, 78.86, 90.31, 114.27, 141.53, 142.17, 148.02, 157.05, 168.93, 169.20, 170.23, 188.87; HRMS: calcd for $C_{20}H_{26}N_5O_8$ (M+H): *m/z* 464.1781, found: 464.1809. Anal. calcd for $C_{20}H_{25}N_5O_8$ (463.45): C, 51.83; H, 5.44; N, 15.11. Found: C, 51.75; H, 5.23; N, 15.01.

4.7. N^6-Benzoyl-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)adenine (9b) and N^6-benzoyl-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)adenine (10b)

A similar experimental procedure as described for synthesis of **10a** was applied in the N^6-benzoyl series. Reaction of **8b** (0.60 g, 2.50 mmol), tetraacetylribose (0.955 g, 3.00 mmol), BSA (1.006 g, 4.90 mmol), and TMSOTf (0.280 g, 1.30 mmol) was carried out at 60 °C for 3 h to give, after chromatographic separation, the 9-isomer **9b**, an oil: 0.099 g, 8%; R_f 0.80(A), 0.50(B); λ_{max} (MeOH) 232, 280 nm, and the 1-isomer **10b**, an oil: 0.377 g, 30%; mp 202–205 °C (EtOH); R_f 0.62(A), 0.49(B); λ_{max} (MeOH) 228, 332 nm; ν_{max} 3600–2900 (br), 1749, 1745, 1641, 1599, 1557, 1500, 1487, 1423, 1373, 1315, 1287, 1230, 1118, 1095, 1058 cm^{-1}; ^1H NMR ($CDCl_3$): 2.07, 2.08, 2.25 (3s, 3×3H), 4.41–4.60 (m, 3H), 5.38 (dd, 1H, *J*=5.1, 8.1 Hz), 5.64 (dd, 1H, *J*=2.1, 5.1 Hz), 6.70 (d, 1H, *J*=2.1 Hz), 7.42 (m, 2H), 7.52 (m, 1H), 8.17 (s, 1H), 8.22 (m, 2H), 8.90 (s, 1H), 12.65 (br s, 1H); ^{13}C NMR ($CDCl_3$): 20.31, 20.41, 20.80, 61.26, 68.09, 74.90, 79.29, 89.93, 114.61, 128.03, 128.17, 129.75, 137.25, 141.95, 142.20, 148.81, 157.60, 168.88, 169.21, 170.24, 175.18; HRMS: calcd for $C_{23}H_{24}N_5O_8$ (M+H): *m/z* 498.1625, found: 498.1655.

4.8. 1-(β-D-Ribofuranosyl)adenine (12)

A solution of **10a** (0.222 g, 0.48 mmol) in saturated methanolic ammonia (10 mL) was stirred at 25 °C for 24 h. The solvent was evaporated to a white solid, which was stirred in $CHCl_3$/MeOH (1:1, 5 mL) for 2 h. The precipitate was filtered off to give 0.120 g (93%) of **12**. An analytical sample was crystallized from water, mp>178 °C (decomp.); R_f 0.53 (C); $[\alpha]_D^{20}$ −23.5 (*c* 0.14, H_2O); λ_{max} (MeOH) 228, 275 nm; ν_{max} 3550–2300 (br), 3407, 3339, 3189, 1679, 1623, 1561, 1557, 1476, 1450, 1358, 1310, 1117, 1071, 907, 863 cm^{-1}; ^1H NMR (D_2O): 4.04 (dd, 1H, *J*=3.6, 12.6 Hz), 4.07 (dd, 1H, *J*=2.4, 12.6 Hz), 4.49 (dd, 1H, *J*=3.6, 51 Hz), 4.79 (t, 1H, *J*=6.0 Hz), 6.15 (d, 1H, *J*=6.0 Hz), 8.21 (s, 1H), 8.60 (s, 1H). Anal. calcd for $C_{10}H_{13}N_5O_4$ (267.25): C, 44.94; H, 4.90; N, 26.21. Found: C, 44.74; H, 4.69; N, 26.15.

4.9. Transglycosylation reactions

4.9.1. N^6-Isobutyryl-9-[(2-acetoxyethoxy)methyl]adenine (13). An anhydrous solution of **10a** (150 mg, 0.32 mmol), 2-acetoxyethyl acetoxymethyl ether[14] (253 μL, 1.62 mmol), and *p*-toluenesulfonic acid monohydrate (6.2 mg, 0.032 mmol) in chlorobenzene (7.5 mL) was refluxed for 4 h. The solvent was removed in vacuo and the residue was subjected to SiO_2 column chromatography in a CH_3Cl/CH_3OH gradient (from 98:2 to 9:1) to yield **13** as an oil, 57 mg (55%); R_f 0.60(A), 0.34(B); λ_{max} (MeOH) 273 nm; ν_{max} 3400–2800 (br), 3262, 1736, 1729, 1721, 1686, 1605, 1589, 1580, 1454, 1266, 1237, 1220, 1050, 761 cm^{-1}; ^1H NMR (DMSO-d_6): 1.13 (d, 6H, *J*=6.9 Hz), 1.92 (s, 3H), 2.94 (septet, 1H, *J*=6.9 Hz), 3.74 (m, 2H), 4.07 (m, 2H), 5.68 (s, 2H), 8.61 (s, 1H), 8.68 (s, 1H), 10.67 (s, 1H); HRMS: calcd for $C_{14}H_{20}N_5O_4$ (M+H): *m/z* 322.1515, found: 322.1510.

4.9.2. Isomerization of 5 to 6. A suspension of protected 7-isomer **5** (4.2 mg, 0.01 mmol) and *p*-toluenesulfonic acid monohydrate (0.4 mg, 0.002 mmol) in chlorobenzene (0.4 mL) was stirred at 150 °C for 2.5 h to give a product identical with an authentic sample of **6** (>90%; TLC, ^1H NMR).

4.9.3. Isomerization of 10a to 9a. A sample of **10a** (6.0 mg, 0.013 mmol) was stirred with *p*-toluenesulfonic acid (0.25 mg, 0.0013 mmol) in chlorobenzene (1 mL) at 60 °C for 2 h. TLC analysis showed the formation of **9a** (ca. 90%), and traces of **8a**.

4.9.4. Decomposition of 11. A sample of 1,9-bis-ribofuranosyl derivative **11** (90.0 mg, 0.12 mmol) was refluxed with *p*-toluenesulfonic acid (2.2 mg, 0.012 mmol) in chlorobenzene (5 mL) for 10 min. After this time TLC analysis showed a mixture of **9a** and **10a** in a ratio 6:1, respectively. The structure of products was confirmed after their chromatographic separation (TLC, ^1H NMR, UV).

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