

# Synthesis of 2'-O- $\alpha$ -D-ribofuranosyladenosine, monomeric unit of poly(ADP-ribose)

Sergey N. Mikhailov<sup>a,\*</sup>, Irina V. Kulikova<sup>a</sup>, Koen Nauwelaerts<sup>b</sup>, Piet Herdewijn<sup>b</sup>

<sup>a</sup> Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Vavilov strasse 32, Moscow 119991, Russia

<sup>b</sup> Laboratory of Medicinal Chemistry, Rega Institute for Medical Research, K.U. Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

Received 14 November 2007; received in revised form 20 December 2007; accepted 7 January 2008

Available online 11 January 2008

## Abstract

The first chemical synthesis of 2'-O- $\alpha$ -D-ribofuranosyladenosine, monomeric unit of poly(ADP-ribose), has been achieved starting from 3',5'-O-bis protected 9-(2-O- $\alpha$ -D-arabinofuranosyl- $\beta$ -D-ribofuranosyl)-adenine. Configurational inversion of 2'-hydroxyl group of arabinose moiety was performed by oxidation–reduction sequence.

© 2008 Elsevier Ltd. All rights reserved.

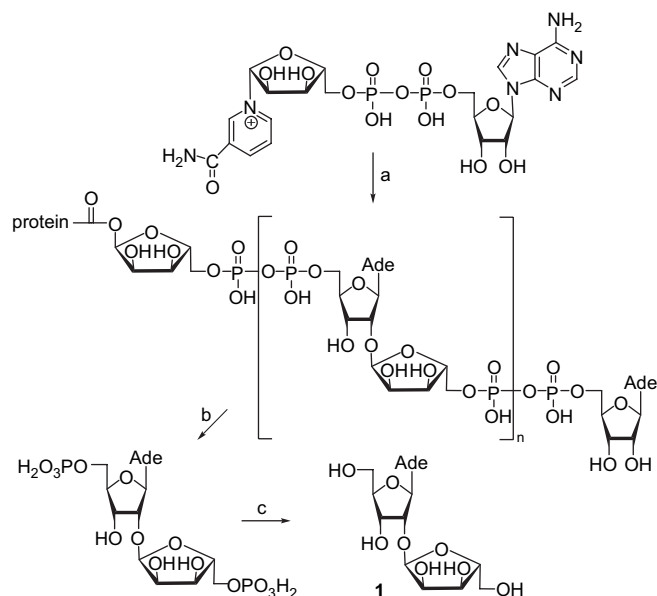
**Keywords:** Poly(ADP-ribose); Monomeric unit; Stereoselective synthesis

## 1. Introduction

Poly-ADP ribosylation is a posttranslational modification of proteins in eukaryotic cells catalysed by poly(ADP-ribose) polymerases (PARPs). These enzymes are responsible for the conversion of nicotinamide adenine dinucleotide (NAD<sup>+</sup>) into a polymer with up to 200–300 residues in length, with simultaneous release of nicotinamide (Scheme 1, route a). The polymer is covalently linked to nuclear proteins through glutamic and aspartic acids. The importance of poly(ADP-ribose) has been established in many cellular processes such as DNA replication, recombination, and repair and cellular differentiation.<sup>1,2</sup> However, a clear and unified picture of its physiological role still remains to be established.

The chemical structure of poly(ADP-ribose) was determined using NMR spectroscopy and enzymatic hydrolysis of the pyrophosphate bond by phosphodiesterase with subsequent enzymatic dephosphorylation with alkaline phosphatase (Scheme 1, routes b and c).<sup>3–5</sup> The development of methods for the synthesis of poly(ADP-ribose) is still a challenging

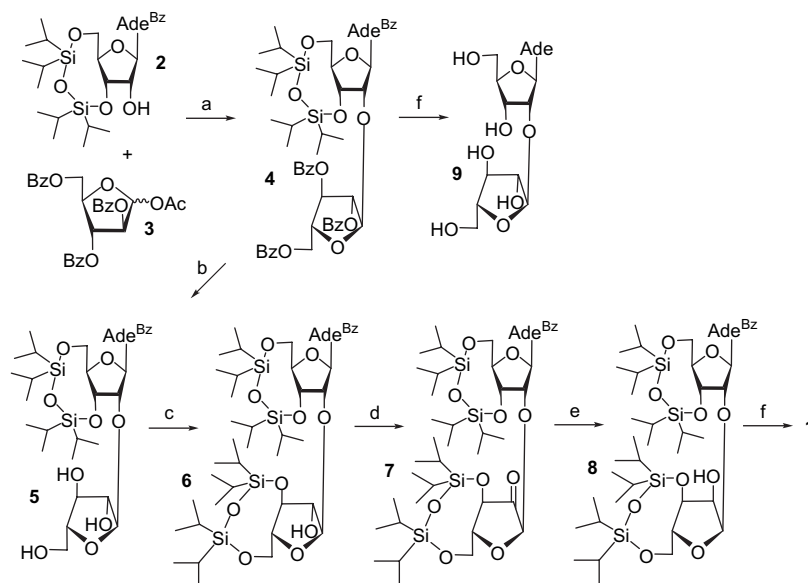
problem and the first obvious step on this way is the synthesis of 2'-O- $\alpha$ -D-ribofuranosyladenosine (**1**).



Scheme 1. Structure, biosynthesis of poly(ADP-ribose) and its enzymatic degradation: (a) poly(ADP-ribose) polymerase; (b) phosphodiesterase; (c) alkaline phosphatase.

\* Corresponding author. Tel.: +07 499 1359733; fax: +07 499 1351405.

E-mail address: [smikh@eimb.ru](mailto:smikh@eimb.ru) (S.N. Mikhailov).



Scheme 2. (a)  $\text{SnCl}_4$ , 0 °C,  $\text{ClCH}_2\text{CH}_2\text{Cl}$ ,  $\text{N}_2$ , 20 h, 62%; (b) 0.1 N  $\text{MeONa}/\text{MeOH}$ , 10 °C, 40 min, 47%; (c)  $\text{ClSi}(\text{i-Pr})_2\text{OSi}(\text{i-Pr})_2\text{Cl}$ , Py, 35 °C, 20 h, 69%; (d)  $\text{DMSO}$ ,  $\text{Ac}_2\text{O}$ , 20 °C, 24 h; (e)  $\text{NaBH}_4$ ,  $\text{EtOH}$ , 0 °C, 1 h, 69% for two steps (d and e); (f)  $\text{NH}_3/\text{MeOH}$ , 20 °C, 2 days;  $\text{Bu}_4\text{NF} \cdot 3\text{H}_2\text{O}$ ,  $\text{THF}$ , 20 °C, 1 h, 93%.

## 2. Results and discussion

Here we report the first chemical synthesis of **1**. The key step is the formation of  $\alpha(1 \rightarrow 2)$  *O*-glycosidic bond between ribofuranose residue and adenosine. The use of *D*-ribose as starting compound defines the formation of 1,2-*cis*-substituted products and usually results in formation of anomeric mixture. We decided to use another approach consisting in the formation of 1,2-*trans*-substituted derivatives of *D*-arabinose with the following configurational inversion of 2'-hydroxyl group.

*O*-Glycosylation of nucleosides with fully acylated pentofuranoses was recently developed.<sup>6–8</sup> Condensation of a small excess of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*D*-arabinofuranose (**3**) with 3',5'-*O*-protected adenosine **2** at 0 °C in 1,2-dichloroethane in the presence of tin tetrachloride results in disaccharide nucleoside **4** (Scheme 2). *O*-Glycosylation proceeded stereospecifically with formation of a  $\alpha$ -glycosidic bond. The presence of a participating 2-*O*-benzoyl group leads exclusively to 1,2-*trans*-ribofuranosides.<sup>9</sup> Selective *O*-debenzylation was achieved using  $\text{MeONa}$  in methanol.

Simultaneous protection of 3',5'-*O*-hydroxyl groups in **5** was achieved using Markiewicz blocking group.<sup>10</sup> The reaction was rather slow at room temperature and it was carried at 35 °C. Configurational inversion of 2'-hydroxyl group of arabinose moiety was performed by Robins oxidation–reduction sequence.<sup>11</sup> The reduction of the carbonyl function with sodium borohydride in **7** gave the inverted 2'-*O*- $\alpha$ -*D*-ribofuranosyl derivative **8** as the main product by attack at the less hindered side of the sugar ring. After deprotection **1** was obtained in good overall yield. The structure of **1** was unambiguously proved by NMR, UV, and mass spectroscopies. For comparison 2'-*O*- $\alpha$ -*D*-arabinofuranosyladenosine (**9**) was prepared after deblocking of disaccharide nucleoside **4**. <sup>1</sup>H NMR spectra of **1**, **9** and earlier prepared 2'-*O*- $\beta$ -*D*-ribofuranosyladenosine (**10**)<sup>12</sup> are presented in Table 1.

To verify the chemical structure of compounds **1**, **9**, and **10** and to derive their conformational properties, several NMR spectra were recorded and all proton and carbon resonances were assigned. NMR spectra of **1** are very close to that presented earlier.<sup>3–5</sup> The differences (Table 1) in the adenosine part of the disaccharide nucleosides **1**, **9**, and **10** are rather small, both in chemical shifts and coupling constants. This translates to a high equivalence in conformation of the adenosine parts of which the riboses mainly adopts a Southern conformation (22% N). In the additional sugar moieties, however, several differences in chemical shift and scalar coupling can be observed. The inversion of configuration at C-2 of the additional sugar residue results in significant change of coupling constants. The *trans* arrangements of  $\text{H1}'\text{--H2}'$  and  $\text{H2}'\text{--H3}'$  in  $\alpha$ -*D*-arabinofuranose in **6** and **9** are changed to *cis* configurations in **8** and **1** of  $\alpha$ -*D*-ribofuranose moieties. This translates to different conformations of the sugar moieties. In compound **10** the  $\beta$ -ribose moiety adopts a Northern conformation. In **9** the  $\alpha$ -arabino moiety occurs as a mixture of Northern and Southern and in **1**, the  $\alpha$ -ribose is in a Southern conformation. For each compound the 2'–1'' glycosidic linkage of the sugar moieties, could be confirmed using a HMBC spectrum in which a clear coupling could be observed between  $\text{C1}''$  and  $\text{H2}'$  and between  $\text{H1}''$  and  $\text{C2}'$  (Fig. 1). The  $\alpha$  configuration of the extra ribose moiety in **1** was confirmed by a ROESY spectrum in which clear crosspeaks could be identified between  $\text{H1}''$  and both  $\text{H2}''$  and  $\text{H3}''$ , indicating *cis* orientation of these protons. The  $\beta$  conformation of the nucleoside sugar moiety of **1** was confirmed by a crosspeak between  $\text{H1}'$  and  $\text{H4}'$  in the ROESY spectrum. No ROESY crosspeaks between  $\text{H8}$  of adenine and any of the sugar protons could be observed.

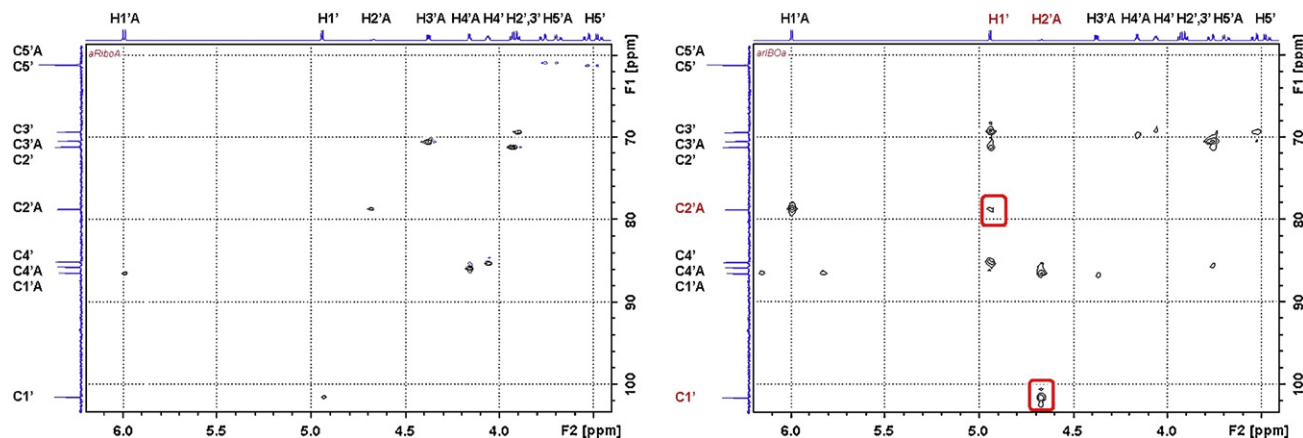
The conformation of **1** was evaluated by ROESY spectroscopy. The intense crosspeak between  $\text{H2}'$  and  $\text{H1}''$ , the crosspeaks between  $\text{H1}''$  and  $\text{H1}'/\text{H3}'$  and the absence of further crosspeaks between protons of the different sugar rings,

Table 1

<sup>1</sup>H NMR (500 MHz) chemical shifts (ppm) and coupling constants (Hz) of 2'-O- $\alpha$ -D-ribofuranosyladenosine (**1**) and related compounds in D<sub>2</sub>O at 27 °C

	Compound <b>10</b>		Compound <b>9</b>		Compound <b>1</b>	
	Moieties					
	Ado	$\beta$ -Rib	Ado	$\alpha$ -Ara	Ado	$\alpha$ -Rib
Chemical shifts						
H-1'	6.01 d	4.95 s	6.01 d	4.85 d	6.03 d	4.98 d
H-2'	4.67 dd	4.02 d	4.68 dd	3.99 dd	4.70 dd	3.97 dd
H-3'	4.44 dd	3.88 dd	4.40 dd	3.80 dd	4.41 dd	3.93 dd
H-4'	4.19 ddd	3.71 ddd	4.18 ddd	3.99 ddd	4.19 ddd	4.09 ddd
H-5'a	3.82 dd	3.21 dd	3.73 dd	3.64 dd	3.80 dd	3.57 dd
H-5'b	3.75 dd	2.64 dd	3.82 dd	3.53 dd	3.72 dd	3.50 dd
H-2	8.24 s		8.23 s		7.97 s	
H-8	8.40 s		8.39 s		8.17 s	
Coupling constants						
1',2'	6.4	<0.5	6.3	1.6	6.2	4.2
2',3'	5.2	4.6	5.1	3.5	5.1	6.2
3',4'	3.2	7.5	3.1	6.0	3.1	2.7
4',5'a	2.7	3.7	3.5	3.3	2.7	3.5
4',5'b	3.6	6.8	2.7	5.8	3.5	4.7
5'a,5'b	-12.8	-12.4	-12.9	-12.4	-12.9	-12.4
% N	22	92	22	51	22	26

Based on scalar coupling constants the percentage North conformation of the sugar residues was calculated.

Figure 1. HSQC and HMBC spectrum of **1**. The HMBC spectrum shows a clear coupling between C1'' and H2' and between H1'' and C2' (both couplings are boxed). This confirms the correct linkage of both rings.

indicate that the disaccharide nucleoside **1**, may occur as an extended molecule.

### 3. Conclusion

The first chemical synthesis of 2'-O- $\alpha$ -D-ribofuranosyladenosine opens new possibilities in search of new potential inhibitors of PARPs. Activation of PARP has been implicated in the pathogenesis of stroke, myocardial ischemia, diabetes, diabetes-associated cardiovascular dysfunction, shock, traumatic central nervous system injury, arthritis, colitis, allergic encephalomyelitis and various other forms of inflammation. Inhibition of PARP by pharmacological agents may prove useful for the therapy of these diseases, as has been shown in pre-clinical animal models.<sup>13,14</sup> Moreover, PARP inhibitors may have additional, potential utility as anticancer agents, radiosensitizers, and antiviral agents.<sup>13–15</sup>

### 4. Experimental

#### 4.1. General

NMR Spectra were recorded on a Bruker AMX-400 and a Bruker Avance II 500 NMR spectrometers at 300 K; chemical shifts  $\delta$  are indicated in parts per million relative to the solvent signals (<sup>1</sup>H and <sup>13</sup>C) or H<sub>3</sub>PO<sub>4</sub> as external standard (<sup>31</sup>P). The coupling constants (*J*) are given in hertz. The signals of **4–6** and **8** were assigned using double resonance techniques, COSY experiments and by comparison with related disaccharide nucleosides. The signals of **1**, **9**, and **10** were assigned by HSQC, HMBC, COSY, and ROESY (tmix: 300 ms) experiments. Analysis of the couplings was performed with Pseurot 6.2.<sup>16</sup> This program uses the Altona–Sundaralingam formalism<sup>17,18</sup> to describe the ring puckering and a generalized Karplus equation developed by

Donders et al.<sup>19</sup> to describe the relation between coupling and dihedral angle. rms was minimized, using a Newton–Raphson minimization.

The UV spectra were recorded on a Cary 300 UV/VIS spectrophotometer (Varian). Mass spectrometry and exact mass measurements of the nucleoside intermediates were performed on a quadrupole/orthogonal-acceleration time-of-flight tandem mass spectrometer (Q-ToF-2, Micromass, Manchester, UK) equipped with a standard electrospray ionization (ESI) interface using positive ion mode. Column chromatography was performed on silica gel (0.063–0.200 mm, Merck). TLC was carried out on Alugram Sil G/UV 254 (Macherey-Nagel) using UV-detection and the following solvent systems (compositions expressed as v/v): methylene chloride (A), methylene chloride–methanol 99:1 (B), methylene chloride–methanol 98:2 (C), methylene chloride–methanol 97:3 (D), methylene chloride–methanol 95:5 (E), methylene chloride–methanol 9:1 (F), methylene chloride–methanol 4:1 (G) and methylene chloride–methanol 1:1 (H).

#### 4.2. *N*<sup>6</sup>-Benzoyl-9-[3,5-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)-2-*O*-(2,3,5-tri-*O*-benzoyl- $\alpha$ -D-arabinofuranosyl)- $\beta$ -D-ribofuranosyl]-adenine (**4**)

To a cool solution (0 °C) of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl- $\beta$ -D-arabinofuranose (1.59 g, 3.2 mmol) in 1,2-dichloroethane (25 ml), tin tetrachloride (0.52 ml, 4.42 mmol) was added under nitrogen and the solution was kept at 0 °C for 10 min. After addition of nucleoside **2** (1.23 g, 2 mmol) the resulting solution was kept at 0 °C for 30 h. Aqueous solution of sodium bicarbonate (10%, 20 ml) was added and the suspension was stirred at 0 °C for 20 min. The suspension was filtered through Hyflo Super Cel, organic layer was separated, washed with water (20 ml), dried over Na<sub>2</sub>SO<sub>4</sub>, and evaporated to dryness. The residue was purified by column chromatography on silica gel (70 g). The column was washed with system A (500 ml) and then eluted with system B to give **4** as a foam. Yield 1.31 g (62%). TLC: *R*<sub>f</sub> 0.49 (system D). LSIMS: (C<sub>55</sub>H<sub>63</sub>N<sub>5</sub>O<sub>13</sub>Si<sub>2</sub>+H) calcd 1058.4039, found 1058.3985. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): 8.96 (br s, 1H, NH), 8.73 (s, 1H, H-8), 8.37 (s, 1H, H-2), 8.12–7.95 (m, 8H, Bz), 7.64–7.41 (m, 12H, Bz), 6.25 (s, 1H, H-1', Ado), 5.83 (s, 1H, H-1' Ara), 5.72 (s, 1H, H-2' Ara), 5.63 (d, 1H, *J*<sub>3',4'</sub>=5.0 Hz, H-3' Ara), 4.87 (ddd, 1H, *J*<sub>4',5'a</sub>=3.4 Hz, *J*<sub>4',5'b</sub>=4.7 Hz, H-4' Ara), 4.79 (dd, 1H, *J*<sub>5'a,5'b</sub>=–12.5 Hz, H-5'a Ara), 4.72 (d, 1H, *J*<sub>2',3'</sub>=4.7 Hz, H-2' Ado), 4.69 (dd, 1H, H-5'b Ara), 4.64 (dd, 1H, *J*<sub>3',4'</sub>=9.3 Hz, H-3' Ado), 4.36–4.23 (m, 2H, H-4',5'a Ado), 4.01 (dd, 1H, *J*<sub>5'b,4'</sub>=2.5 Hz, *J*<sub>5'a,5'b</sub>=–13.4 Hz, 5'b Ado), 1.13–0.85 (m, 28H, *i*-Pr). <sup>13</sup>C NMR (CDCl<sub>3</sub>): 165.90, 165.77, 165.63 and 164.66 (C=O), 153.01 (C-2), 150.81 (C-6), 149.54 (C-4), 140.90 (C-8), 133.95, 133.76, 133.18, 132.83, 130.09, 129.88, 129.16, 128.83 and 128.56 (Bz), 123.69 (C-5), 104.96 (C-1', Ara), 89.16 (C-1', Ado), 82.21 (C-4', Ara), 81.93 (C-4', Ado), 81.47 (C-2', Ara), 79.05 (C-2', Ado), 78.16 (C-3', Ara), 67.92 (C-3', Ado), 63.63 (C-5', Ara), 59.67 (C-5', Ado), 17.59, 17.42, 17.04, 16.86, 13.34,

12.89, 12.84 and 12.57 (*i*-Pr). Anal. Calcd for C<sub>55</sub>H<sub>63</sub>N<sub>5</sub>O<sub>13</sub>Si<sub>2</sub>: C, 62.42; H, 6.00; N, 6.62. Found: C, 62.25; H, 5.80; N, 6.42.

#### 4.3. *N*<sup>6</sup>-Benzoyl-9-[3,5-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)-2-*O*-( $\alpha$ -D-arabinofuranosyl)- $\beta$ -D-ribofuranosyl]-adenine (**5**)

To a solution of nucleoside **4** (2.12 g, 2 mmol) in dioxane (21 ml) 0.3 M sodium methylate in dry methanol (21 ml) was added and the solution was kept for 50 min at 10 °C and then neutralized by addition of 10% acetic acid in methanol to pH 7.0. The resulting solution was concentrated in vacuo to dryness and the residue was partitioned between ethyl acetate (100 ml) and water (40 ml), the organic layer was washed with water (40 ml), dried over Na<sub>2</sub>SO<sub>4</sub>, and evaporated to dryness. The residue was purified on a column with silica gel (50 g). The column was washed with system C (300 ml) and then eluted with system E to give **5** as a foam. Yield 724 mg (47%). *R*<sub>f</sub> 0.39 (system E). LSIMS: (C<sub>34</sub>H<sub>51</sub>N<sub>5</sub>O<sub>10</sub>Si<sub>2</sub>+H) calcd 746.3253, found 746.3253. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): 9.73 (br s, 1H, NH), 8.72 (s, 1H, H-8), 8.34 (s, 1H, H-2), 7.99 (d, 2H, *J*=7.5 Hz, Bz), 7.51 (t, 1H, *J*=7.5 Hz, Bz), 7.43 (t, 2H, *J*=7.5 Hz, Bz), 6.16 (s, 1H, H-1' Ado), 5.60 (s, 1H, H-1' Ara), 4.68 (dd, 1H, *J*<sub>3',2'</sub>=5.0 Hz, *J*<sub>3',4'</sub>=9.3, H-3' Ado), 4.62 (d, 1H, H-2' Ado), 4.18 (br d, 1H, *J*<sub>5'a,5'b</sub>=–13.7, H-5'a Ado), 4.12 (m, 2H, H-2',4' Ara), 4.05 (d, 1H, *J*<sub>3',4'</sub>=9.3, H-3' Ara), 3.97 (m, 1H, H-4' Ado), 3.92 (dd, 1H, *J*<sub>5'b,4'</sub>=2.5, H-5'b Ado), 3.81 (dd, 1H, *J*<sub>5'a,4'</sub>=2.5, *J*<sub>5'a,5'b</sub>=–11.8, H-5'a Ara), 3.76 (dd, 1H, *J*<sub>5'b,4'</sub>=1.9, H-5'b Ara), 1.08–0.88 (m, 28H, *i*-Pr). <sup>13</sup>C NMR (CDCl<sub>3</sub>): 165.20 (C=O), 152.59 (C-2), 150.58 (C-6), 149.52 (C-4), 140.94 (C-8), 132.67, 128.64, and 128.01 (Bz), 123.33 (C-5), 105.67 (C-1', Ara), 88.46 (C-1', Ado), 87.73 (C-4', Ara), 81.94 (C-4', Ado), 78.68 (C-2', Ado), 77.91 (C-2', Ara), 76.11 (C-3', Ara), 67.74 (C-3', Ado), 61.52 (C-5', Ara), 59.43 (C-5', Ado), 17.36, 17.27, 17.19, 16.95, 16.84, 13.78, 13.22, 13.18 and 12.96 (*i*-Pr). Anal. Calcd for C<sub>34</sub>H<sub>51</sub>N<sub>5</sub>O<sub>10</sub>Si<sub>2</sub>: C, 62.42; H, 6.00; N, 6.62. Found: C, 62.24; H, 6.07; N, 6.83.

#### 4.4. *N*<sup>6</sup>-Benzoyl-9-[3,5-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)-2-*O*-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)- $\alpha$ -D-arabinofuranosyl]- $\beta$ -D-ribofuranosyl]-adenine (**6**)

To a solution of nucleoside **5** (540 mg, 0.72 mmol) in pyridine (7 ml) 1,3-dichloro-1,1,3,3-tetraisopropylidisiloxane (0.30 ml, 0.94 mmol) was added and the solution was kept for 24 h at 35 °C, evaporated to dryness and evaporated with toluene (10 ml). The residue was partitioned between methylene chloride (100 ml) and water (40 ml), the organic layer was washed successively with water (40 ml), 10% aqueous solution of sodium bicarbonate (40 ml), water (40 ml), dried over Na<sub>2</sub>SO<sub>4</sub>, evaporated to dryness, and evaporated with toluene (2×10 ml). The residue was purified on a column with silica gel (30 g). The column was washed with system A (200 ml) and then eluted with system B to give **6** as a foam. Yield 496 mg (69%). *R*<sub>f</sub> 0.54 (system C). LSIMS:



(C<sub>46</sub>H<sub>77</sub>N<sub>5</sub>O<sub>11</sub>Si<sub>4</sub>+H) calcd 988.4775, found 988.4737. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): 8.78 (s, 1H, H-8), 8.41 (s, 1H, H-2), 8.06 (d, 2H, *J*=7.5 Hz, Bz), 7.60 (t, 1H, *J*=7.5 Hz, Bz), 7.53 (t, 2H, *J*=7.5 Hz, Bz), 6.18 (s, 1H, H-1' Ado), 5.34 (d, 1H, *J*<sub>1',2'</sub>=1.9 Hz, H-1' Ara), 4.58 (dd, 1H, *J*<sub>3',2'</sub>=4.7 Hz, *J*<sub>3',4'</sub>=9.3, H-3' Ado), 4.51 (d, 1H, H-2' Ado), 4.41 (dd, 1H, *J*<sub>2',3'</sub>=5.6 Hz, H-2' Ara), 4.29–4.18 (m, 3H, H-5'a, 5'b Ado, H-3' Ara), 4.07–4.01 (m, 2H, H-4' Ado, H-4' Ara), 3.94 (dd, 1H, *J*<sub>5'a,4'</sub>=2.8, *J*<sub>5'a,5'b</sub>=−12.5, H-5'a Ara), 3.89 (dd, 1H, *J*<sub>5'b,4'</sub>=4.4, H-5'b Ara), 1.13–0.95 (m, 56H, *i*-Pr). <sup>13</sup>C NMR (CDCl<sub>3</sub>): 164.69 (C=O), 152.95 (C-2), 150.69 (C-6), 149.70 (C-4), 140.97 (C-8), 132.87, 129.00, and 128.05 (Bz), 123.62 (C-5), 106.79 (C-1', Ara), 89.26 (C-1', Ado), 83.38 (C-4', Ara), 82.01 (C-4', Ado), 81.21 (C-2', Ara), 78.94 (C-2', Ado), 78.28 (C-3', Ara), 67.82 (C-3', Ado), 61.87 (C-5', Ara), 59.93 (C-5', Ado), 17.64, 17.59, 17.56, 17.49, 17.43, 17.29, 17.27, 17.24, 17.21, 17.09, 13.65, 13.56, 13.24, 13.11, 13.07, 12.94, 12.85 and 12.79 (*i*-Pr). Anal. Calcd for C<sub>46</sub>H<sub>77</sub>N<sub>5</sub>O<sub>11</sub>Si<sub>4</sub>: C, 55.89; H, 7.85; N, 7.09. Found: C, 55.78; H, 7.81; N, 7.19.

**4.5. N<sup>6</sup>-Benzoyl-9-[3,5-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)-2-O-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)-α-D-ribofuranosyl]-β-D-ribofuranosyl]-adenine (8)**

A solution of nucleoside **6** (471 mg, 0.48 mmol) in dimethyl sulfoxide (3.4 ml) was treated with acetic anhydride (0.22 ml, 2.38 mmol) and the mixture was kept at 20 °C for 22 h. Ethanol (8 ml) was added, the mixture was cooled to 0 °C, and treated with sodium borohydride (72 mg). The mixture was stirred at 0 °C for 1 h and then acetone (1 ml) and saturated aqueous solution of sodium chloride (20 ml) were added. The mixture was extracted with ethyl acetate (50 ml) and the organic layer was washed successively with saturated aqueous solution of sodium chloride (2×20 ml) and water (2×20 ml), dried over Na<sub>2</sub>SO<sub>4</sub>, and evaporated to dryness. The residue was purified on a column with silica gel (30 g). The column was washed with system A (300 ml) and then eluted with system B to give **8** as a foam. Yield 327 mg (69%). *R*<sub>f</sub> 0.60 (system C). LSIMS: (C<sub>46</sub>H<sub>77</sub>N<sub>5</sub>O<sub>11</sub>Si<sub>4</sub>+H) calcd 988.4775, found 988.4758. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): 9.23 (br s, 1H, NH), 8.78 (s, 1H, H-8), 8.37 (s, 1H, H-2), 8.03 (d, 2H, *J*=7.5 Hz, Bz), 7.59 (t, *J*=7.5 Hz, 1H, Bz), 7.51 (t, *J*=7.5 Hz, 2H, Bz), 6.18 (s, 1H, H-1' Ado), 5.47 (d, 1H, *J*<sub>1',2'</sub>=4.4 Hz, H-1' Rib), 4.59 (dd, 1H, *J*<sub>2',3'</sub>=4.7 Hz, H-3' Ado), 4.56 (dd, 1H, H-2' Ado), 4.28 (br d, 1H, *J*<sub>5'a,5'b</sub>=−13.4 Hz, 5'a Ado), 4.24–4.15 (m, 5H, H-2', 3', 4' Rib, H-4', 5'b Ado), 4.02 (m, 2H, H-5'a, 5'b Rib), 1.16–0.88 (m, 56H, *i*-Pr). <sup>13</sup>C NMR (CDCl<sub>3</sub>): 165.26 (C=O), 152.95 (C-2), 150.55 (C-6), 149.96 (C-4), 140.78 (C-8), 132.72, 128.77, and 128.26 (Bz), 114.14 (C-5), 101.92 (C-1', Rib), 89.47 (C-1', Ado), 84.10 (C-4', Rib), 81.91 (C-4', Ado), 80.52 (C-2', Ado), 71.22 (C-2', 3' Rib), 67.74 (C-3', Ado), 64.69 (C-5', Rib), 59.67 (C-5', Ado), 17.55, 17.49, 17.44, 17.39, 17.32, 17.26, 17.18, 17.11, 17.02, 16.93, 14.16, 13.83, 13.62, 13.57, 13.42, 13.22, 12.81, and 12.78 (*i*-Pr). Anal. Calcd for C<sub>46</sub>H<sub>77</sub>N<sub>5</sub>O<sub>11</sub>Si<sub>4</sub>: C, 55.89; H, 7.85; N, 7.09. Found: C, 55.69; H, 7.68; N, 6.83.

**4.6. 9-(2-O-α-D-Ribofuranosyl-β-D-ribofuranosyl)-adenine (1)**

A solution of nucleoside **8** (309 mg, 0.31 mmol) in 5 M ammonia in methanol (15 ml) was kept for 2 days at 20 °C and then concentrated in vacuo to dryness. The residue was partitioned between methylene chloride (60 ml) and water (20 ml), and the organic layer was washed with water (2×20 ml), dried over Na<sub>2</sub>SO<sub>4</sub>, and evaporated to dryness. The residue was dissolved in 0.5 M tetrabutylammonium fluoride trihydrate in tetrahydrofuran (3.8 ml), kept for 1 h at 20 °C. Water (5 ml) and Dowex-50 (Na<sup>+</sup> form, 3 ml) were added, and the suspension was stirred for 20 min. The resin was filtered and washed with water. The combined filtrates were washed with methylene chloride (2×5 ml), evaporated to dryness, and evaporated with ethanol (2×5 ml). The residue was purified by column chromatography on silica gel (25 g, dry application). The column was washed with system F (200 ml) and then eluted with system G. The fractions containing **1** were combined and evaporated in vacuo to dryness. The residue was dissolved in methanol (1 ml), filtered, and evaporated in vacuo to dryness to give **1** as a foam. Yield 117 mg (92%). TLC: *R*<sub>f</sub> 0.32 (system H). UV (pH 7–13): λ<sub>max</sub> 261 nm (ε 14,300); (pH 1): λ<sub>max</sub> 258 nm (ε 13,800). LSIMS: (C<sub>15</sub>H<sub>21</sub>N<sub>5</sub>O<sub>8</sub>+H) calcd 400.1468, found 400.1459. <sup>1</sup>H NMR (600 MHz, see Table 1). <sup>13</sup>C NMR (D<sub>2</sub>O): 154.96 (C-6), 151.99 (C-2), 147.74 (C-4), 140.11 (C-8), 118.41 (C-5), 101.49 (C-1', Rib), 86.41 (C-1', Ado), 85.68 (C-4', Ado), 85.05 (C-4', Rib), 78.71 (C-2', Ado), 71.14 (C-2', Rib), 70.43 (C-3', Ado), 69.32 (C-3', Rib), 61.04 (C-5', Rib), 57.66 (C-5', Ado). Anal. Calcd for C<sub>15</sub>H<sub>21</sub>N<sub>5</sub>O<sub>8</sub>: C, 45.11; H, 5.30; N, 17.54. Found: C, 45.01; H, 5.15; N, 17.34.

**4.7. 9-(2-O-α-D-Arabinofuranosyl-β-D-ribofuranosyl)-adenine (9)**

A solution of nucleoside **4** (417 mg, 0.39 mmol) in 5 M ammonia in methanol (15 ml) was kept for 2 days at 20 °C and then concentrated in vacuo to dryness. The residue was partitioned between methylene chloride (60 ml) and water (20 ml), and the organic layer was washed with water (2×20 ml), dried over Na<sub>2</sub>SO<sub>4</sub>, and evaporated to dryness. The residue was dissolved in 0.5 M tetrabutylammonium fluoride trihydrate in tetrahydrofuran (2.4 ml) and kept for 1 h at 20 °C. Water (5 ml) and Dowex-50 (Na<sup>+</sup> form, 3 ml) were added, and the suspension was stirred for 20 min. The resin was filtered and washed with water. The combined filtrates were washed with methylene chloride (2×5 ml), evaporated to dryness, and evaporated with ethanol (2×5 ml). The residue was purified by column chromatography on silica gel (25 g, dry application). The column was washed with system F (200 ml) and then eluted with system G. The fractions containing **9** were combined and evaporated in vacuo to dryness. The residue was dissolved in methanol (1 ml), filtered, and evaporated in vacuo to dryness to give **9** as a foam. Yield 125 mg (80%). TLC: *R*<sub>f</sub> 0.49 (system H). UV (pH 7–13): λ<sub>max</sub> 261 nm (ε 14,300); (pH 1): λ<sub>max</sub> 258 nm (ε 13,800). LSIMS: (C<sub>15</sub>H<sub>21</sub>N<sub>5</sub>O<sub>8</sub>+H) calcd 400.1468, found 400.1459. <sup>1</sup>H NMR (600 MHz, see Table 1). <sup>13</sup>C NMR

(D<sub>2</sub>O): 154.96 (C-6), 151.97 (C-2), 147.69 (C-4), 140.04 (C-8), 118.42 (C-5), 106.92 (C-1', Ara), 86.43 (C-1', Ado), 85.75 (C-4', Ado), 84.02 (C-4', Ara), 80.30 (C-2', Ara), 78.22 (C-2', Ado), 75.78 (C-3', Ara), 70.24 (C-3', Ado), 61.04 (C-5', Ado), 60.62 (C-5', Ara). Anal. Calcd for C<sub>15</sub>H<sub>21</sub>N<sub>5</sub>O<sub>8</sub>: C, 45.11; H, 5.30; N, 17.54. Found: C, 44.93; H, 5.18; N, 17.29.

## Acknowledgements

Financial support of the Russian Foundation for Basic Research, INTAS, and KUL Research Council is acknowledged.

## References and notes

- Hassa, P. O.; Haenni, S. S.; Elser, M.; Hottiger, M. O. *Microbiol. Mol. Biol. Rev.* **2006**, *70*, 789–829.
- Diefenbach, J.; Bürkle, A. *Cell. Mol. Life Sci.* **2005**, *62*, 721–730.
- Miwa, M.; Saito, H.; Sakura, H.; Saikawa, N.; Watanabe, F.; Matsushima, T.; Sugimura, T. *Nucleic Acids Res.* **1977**, *4*, 3997–4005.
- Inagaki, F.; Miyazawa, T.; Miwa, M.; Saito, H.; Sugimura, T. *Biochem. Biophys. Res. Commun.* **1978**, *85*, 415–420.
- Ferro, A. M.; Oppenheimer, N. J. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 809–813.
- Nauwelaerts, K.; Efimtseva, E. V.; Mikhailov, S. N.; Herdewijn, P. Disaccharide Nucleosides, an Important Group of Natural Compounds. In *Frontiers in Nucleosides and Nucleic Acids*; Schinazi, R. F., Liotta, D. C., Eds.; IHL: Tucker, GA, 2004; pp 187–220.
- Efimtseva, E. V.; Mikhailov, S. N. *Russ. Chem. Rev.* **2004**, *73*, 401–414.
- Efimtseva, E. V.; Kulikova, I. V.; Mikhailov, S. N. *Curr. Org. Chem.* **2007**, *11*, 337–354.
- Efimtseva, E. V.; Bobkov, G. V.; Mikhailov, S. N.; Van Aerschot, A.; Schepers, G.; Busson, R.; Rozenski, J.; Herdewijn, P. *Helv. Chim. Acta* **2001**, *84*, 2387–2397.
- Markiewicz, W. T. *J. Chem. Res., Synop.* **1979**, 24–25.
- Hansske, F.; Madej, D.; Robins, M. J. *Tetrahedron* **1984**, *40*, 125–135.
- Mikhailov, S. N.; Efimtseva, E. V.; Gurskaya, G. V.; Zavodnik, V. E.; De Bruyn, A.; Rozenski, J.; Herdewijn, P. *J. Carbohydr. Chem.* **1997**, *16*, 75–92.
- Southan, G. J.; Szabo, C. *Curr. Med. Chem.* **2003**, *10*, 321–340.
- Jagstap, P.; Szabo, C. *Nature Rev. Drugs Discov.* **2005**, *4*, 421–440.
- Curtin, N. J. *Expert Rev. Mol. Med.* **2005**, *7*, 1–19.
- de Leeuw, F. A. A. M.; Altona, C. *J. Comput. Chem.* **1983**, *4*, 428–437.
- Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc.* **1973**, *95*, 2333–2344.
- Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc.* **1972**, *94*, 8205–8212.
- Donders, L. A.; de Leeuw, F. A. A. M.; Altona, C. *Magn. Reson. Chem.* **1989**, *27*, 556–563.