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A new approach to the synthesis of optically active alkylated adenine derivatives

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Abstract—A new synthesis of chiral acyclic nucleoside and nucleotide analogues starting from D(−)- or L(+)-riboses was proposed. Antiviral properties of the synthesized compounds towards the pox virus family were evaluated.

1. Introduction

Acyclic nucleoside and nucleotide analogues are currently used as antiviral drugs possessing the broad spectrum of activities.[1–3] These compounds are targeted at inhibition of viral DNA polymerases and require intracellular phosphorylation to become active. Additionally, adenine derivatives of this type can inhibit *S*-adenosyl-L-homocysteine hydrolase (SAH hydrolase) another enzyme essential for the viral life cycle.[4,5]

The reported syntheses of 3-hydroxy-2-phosphonylmethoxypropyl (HPMP) derivatives involve either alkylation of purine or pyrimidine bases with an acyclic fragment containing a phosphonomethyl group[6] or phosphonomethylation of the corresponding acyclic nucleoside analogue.[7] Although the preparation of (*R*,*S*)-3-(adenin-9-yl)-2-hydroxypropanoic acid [(*R*,*S*)-AHPA] has been described,[8] chiral AHPA isomers have only been obtained as byproducts in the process of the synthesis of eritadenine and its derivatives.[9] A major approach to the synthesis of (*S*)-9-(2,3-dihydroxypropyl)adenine [(*S*)-DHPA] is based on the adenine coupling of with (*S*)-1,2-*O*-isopropylidene-3-tosyl-*sn*-glycerol.[10] In addition, (*S*)-DHPA and its analogues can be obtained by oxidation of the propenyladenine double bond to the vicinal diol system,[11] but this procedure did not allow an optically active product.[10] All these methods suffer serious disadvantages. In particular, they are multi-step and laborious and are targeted at the synthesis of the analogues of one type.

We describe herein the synthesis of optically active (*S*)- and (*R*)-9-(3-hydroxy-2-oxy-((*R*,*S*)-1-methoxy-2-hydroxyethyl)propyl)adenines (**1a** and **1b**), which can be used as key intermediates in the synthesis of HPMPA and its analogues and (*S*)-DHPA-like nucleoside analogues. The proposed uniform scheme allows the preparation of both acyclic nucleosides and nucleotides.

2. Chemistry

The starting compounds for the synthesis of synthons of *S*-series (**1a**) and *R*-series (**1b**), which are commonly used for the synthesis of various acyclic nucleoside analogues, were obtained from commercially available L(+)-ribose (**2a**) and D(−)-ribose (**2b**), respectively (Fig. 1).

Ribose (**2a**) was methylated with methanol in the presence of hydrogen chloride obtained in situ by the reaction of acetyl chloride with methanol (Scheme 1). In

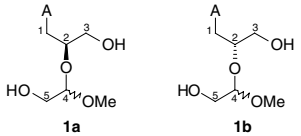

Figure 1. Key intermediates **1a** and **1b**.

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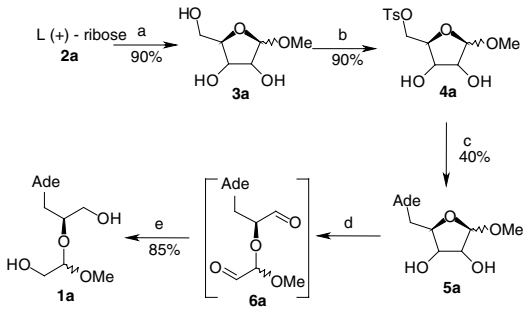


Scheme 1. Reagents and conditions: (a) AcCl/MeOH, rt, 16 h; (b) TsCl/Py, 4 °C, 16 h; (c) Ade^-/DMF, 80 °C, 5 days; (d) $NaIO_4$, 4 °C, 1 h; (e) $NaBH_4$, 4 °C, 3 h.

addition to α,β-methylribofuranoside (**3a**), approximately 10% of α,β-methylribopyranoside was formed. The crude reaction mixture was treated with tosyl chloride in pyridine at 4 °C to give 5-tosyl(methyl)riboside (**4a**) in a yield of 90%. The distribution of the reaction mixture between chloroform and a saturated $NaHCO_3$ solution allowed the isolation of 90% of tosylate (**4a**). A key stage was the coupling of adenine with 5-tosylriboside (**4a**) under anhydrous conditions at 80 °C in the presence of NaH. Unreacted tosylate (**4a**) was removed by the distribution of the reaction mixture between chloroform and water. An excess of adenine was removed using ion-exchange chromatography on Dowex 1 (OH^-). The subsequent column chromatography on LiChroprep RP-8 afforded the target (L)-9-(α,β-1-methyl-5-ribofuranosyl)adenine (**5a**) in a yield of 40%.

The diol system of riboside (**5a**) was oxidized with an aqueous sodium periodate solution (4 °C, 1 h). The inorganic salts were precipitated with ethanol, and the supernatant containing dialdehyde (**6a**) was treated with an aqueous solution of $NaBH_4$ to give the optically active (*S*)-9-(3-hydroxy-2-oxy-[(*R*,*S*)-1-methoxy-2-hydroxyethyl)propyl]adenine (**1a**). Compound **1a** was isolated using ion-exchange chromatography on Dowex 50 (NH_4^+) in a good yield (85%). The total yield of chiral **1a** relative to commercially available L-ribose (**2a**) achieved 28%.

The synthesis of **1b** was performed from D(−)-ribose using the scheme similar to the synthesis of **1a** (Scheme 1). The total yield of compound **1b** was varied from 15% to 30% and depended on the nature of the base used in the reaction of adenine coupling with 5-tosylriboside (**4b**). For example, the yields of the product (**5b**) were 20% in the presence of potassium bis(trimethylsilylamide) (K-HMDS) and 40% in the presence of NaH or a 1:5 mixture of Cs_2CO_3–K_2CO_3.

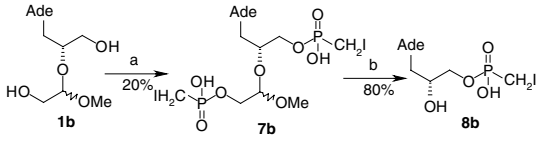
According to the NMR analysis, compounds **1a** and **1b** are diastereomers differing in the configuration at the C2 atom. Chemical shifts of the proton resonances at C4 and the methoxy group of **1a** were observed at 4.17 and 3.21 ppm, respectively, whereas in the case of **1b** they were 4.43 and 2.64 ppm, respectively. In addition, the resonances of the C3 protons also differed in their positions and shapes. For example, in compound **1a** this proton resonance was observed as two doublet of doublets at 3.03 and 3.19 ppm with $J_{3,2}$ 4.7 Hz and $J_{3a,3b}$ 11.9 Hz, whereas for **1b** it appeared as a doublet at 3.35 ppm with $J_{3,2}$ 5.0 Hz. This may imply the lack of equivalence of the C3 protons in the *S*-isomer (**1a**) and their equivalence in the *R*-isomer (**1b**).[12]

The resulting chiral **1a** and **1b** were used for the synthesis of both nucleotide-like HPMPA analogues and nucleoside-like analogues.

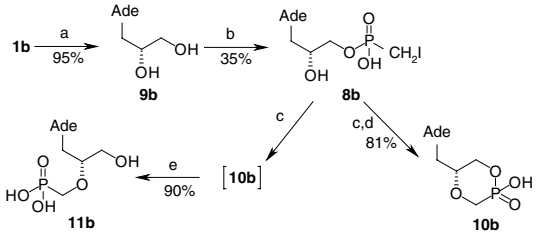
Alkylation of compound **1b** with an excess of iodomethylphosphonic acid in pyridine in the presence of DCC resulted in phosphonate **7b**, which was isolated in a 20% yield by successive ion-exchange chromatography on DEAE-Spheron and reverse-phase chromatography on LiChroprep RP-18. Compound **7b** was hydrolyzed with 60% formic acid for 24 h at 37 °C to give 3′-iodomethylphosphonate of (*R*)-DHPA (**8b**) in an 80% yield. It is worth noting that no hydrolysis of **7b** was observed in the presence of Dowex 50 (H^+) for 12 h at room temperature.

Since the overall yield of 3′-iodomethylphosphonate (**8b**) was low, we used another method of preparation (Scheme 3). Synthon **1b** was either treated with 60% formic acid for 24 h at 37 °C or with Dowex 50 (H^+) for 6 h to give (*R*)-DHPA (**9b**) nearly quantitatively. The target product **9b** was eluted from Dowex 50 (H^+) with 1 M aqueous ammonia with more than 95% purity. It should be mentioned that the acid-induced cleavage of the diacetal residue of diphosphonate **7b** (Scheme 2) proceeded much more slowly than that of compound **1b**. The synthesis of (*S*)-DHPA (**9a**) was carried out from compound **1a** according to Scheme 3. The ^1H NMR spectral data for the (*S*)- and (*R*)-isomers (**9a** and **9b**) were identical.[13]

Chirality of **9a** and **9b** was confirmed by the data of circular dichroism. As was expected, the spectral pat-



Scheme 2. Reagents and conditions: (a) $ICH_2P(O)(OH)_2$/DCC/Py, rt, 28 h; (b) H^+, 24 h.



Scheme 3. Reagents and conditions: (a) H^+, 6 h; (b) $ICH_2P(O)(OH)_2$/DCC/Py, 16 h; (c) NaH/DMF, 6 h; (d) H_2O, 3 h; (e) glacial AcOH, 3 h.

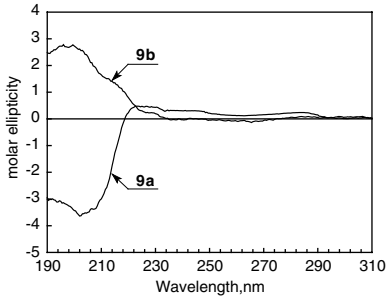


Figure 2. CD spectra of (*S*)-DHPA (**9a**) and (*R*)-DHPA (**9b**) at 20 °C in water.

terns of these compounds were observed as curves with negative and positive elipticity, respectively (Fig. 2).

The reaction of (*R*)-DHPA (**9b**) with 1.5 equiv of iodomethylphosphonic acid in the presence of DCC in pyridine resulted in iodomethylphosphonate **8b** (35%). When treated with 5 equiv NaH in DMF for 6 h, compound **8b** underwent an intermolecular cyclization to give derivative **10b**. The treatment of the reaction mixture with glacial acetic acid allowed the isolation of *cyclo*-HPMPA in a yield of about 90%. If the reaction mixture was treated with water, the isolated product was (*R*)-HPMPA. Its yield after chromatographic purification exceeded 90%. The corresponding (*S*)-*cyclo*-HPMPA (**10a**) and (*S*)-HPMPA (**11a**) were obtained in a similar fashion from (*S*)-DHPA (**9a**).

The structures of the synthesized compounds were confirmed by NMR spectroscopy data and coincided with the published data.[14] Figure 3 presents CD spectral patterns of compounds **11a** and **11b**.

We also synthesized some nucleoside analogues starting from chiral synthons **1a** and **1b**. First, we tried oxidation of compound **1b** with potassium permanganate (pH 13). However, since the yield of the target (*R*)-3-(adenin-9-yl)-2-oxy-((*R*,*S*)-1-methoxy-1-carboxy)propanoic acid (**12b**) only was 10%, we used another approach (Scheme 4). Oxidation of (L)-9-(α,β-1-methyl-5-ribofuranosyl)adenine (**5a**) with aqueous solution of $NaIO_4$ resulted in dialdehyde **6a**, which was further oxidized under alkaline conditions (pH 13) with either $RuCl_3$ or $KMnO_4$. The yield of acid **12a** was 60% in both cases, and isomeric **12b** was obtained in a similar yield.

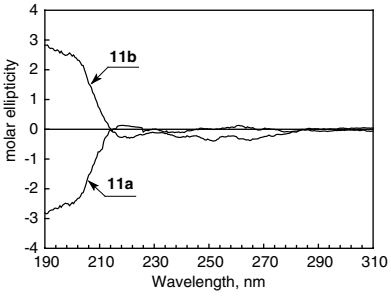
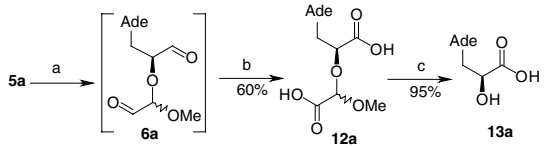


Figure 3. CD spectra of (*S*)-HPMPA (**11a**) and (*R*)-HPMPA (**11b**) at 20 °C in water.



Scheme 4. Reagents and conditions: (a) $NaIO_4$, 4 °C, 1 h; (b) $KMnO_4$ or $RuCl_3$ (pH 13), 12 h; (c) Dowex 50 (H^+), 12 h.

The NMR spectral patterns for **12a** and **12b** were virtually identical, with the exception of the resonances of methoxy groups. In particular, for **12a** the chemical shift of these protons was observed at 3.08 ppm, whereas in the case of **12b** it was at 2.64 ppm.[15] After acid hydrolysis of **12a** or **12b**, optically active (*S*)- or (*R*)-3-(adenin-9-yl)-2-hydroxypropanoic acid (**13a** or **13b**, respectively) were obtained. In particular, in the presence of 60% HCOOH (24 h, 20 °C) compounds **12a** and **12b** were hydrolyzed to give about 70% of **13a** or **13b**, respectively, whereas treatment of **12a** or **12b** with Dowex 50 (H^+) (12 h, 20 °C) yielded these products in nearly quantitative yields. The products were eluted with a 1 M ammonia solution from a Dowex 50 (H^+) column, and the purity exceeded 95%. The NMR spectra for the isomers agreed well with that described for (*R*)-AHPA.[9]

3. Biology

All of the synthesized compounds were evaluated as potential antiviral agents and were tested in Vero cell cultures against vaccinia virus (VV), monkeypox (MPV) and cowpox (CPV) viruses. The antiviral activity of the compounds under study is shown in Table 1. It can be mentioned that the activities against VV and CPV of **9a** and **11a** synthesized by us were very close to those described earlier for these analogues.[2,16]

In addition, compounds **1b**, **9a** and **13a** inhibited the replication of small pox virus (strain India 3a) in Vero cells with IC_{50} values of 62, 41 and 91 μM, respectively. They did not exhibit toxicity up to 500 μM.

To summarize, we have developed a new approach to the synthesis of chiral (both *S*- and *R*-) 9-(3-hydroxy-2-oxy-((*R*,*S*)-1-methoxy-2-hydroxyethyl)-propyl)adenine (**1a** and **1b**) starting from commercially available D(−)-

Table 1

Compound	IC_{50}, μM			CC_{50}, μM
	VV	MPV	CPV	
9a	34	54	49	>500
9b	>500	>500	>500	>500
10a	41	48	61	>350
10b	>350	>350	>350	>350
11a	12.5	22	13	>300
11b	>300	>300	>300	>300

IC_{50}—concentration, at which the virus replication was inhibited by 50%; CC_{50}—concentration, at which cell viability was reduced by 50%.

or L(+)-riboses, which allowed the preparation of a number of acyclic nucleoside and nucleotide analogues. Some of these analogues showed antiviral properties in cell cultures infected with the viruses of the pox family.

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13. **9a** and **9b**: (D_2O) δ 7.87 (s, 1H, H-8 Ade), 7.84 (s, 1H, H-2 Ade), 4.13 (dd, 1H, H-1_a, $J_{1a,1b} = 14.2$ Hz and $J_{1a,2} = 1.6$ Hz), 3.97 (dd, 1H, H-1_b, $J_{1b,2} = 8.1$ Hz), 3.89–3.94 (m, 1H, H-2), 3.42 (dd, 1H, H-3_a, $J_{3a,3b} = 11.8$ Hz and $J_{3a,2} = 4.6$ Hz), 3.53 (dd, 1H, H-3_b, $J_{3b,2} = 4.6$ Hz).
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