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Chemo-enzymatic Synthesis of a New Type of Enantiomerically Pure Carbocyclic Nucleoside Analogues with Strong Inhibitory Effects on Terminal Deoxynucleotidyl Transferase

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Abstract—The synthesis of enantiomerically pure carbocyclic adenosine derivatives which have been prepared based on the kinetic resolution of a *trans*-2-(hydroxymethyl)cyclopentanol derivative is described. Their corresponding triphosphates were evaluated as inhibitors of DNA polymerase β, terminal deoxynucleotidyl transferase (TdT), telomerase, *Escherichia coli* DNA polymerase I and reverse transcriptase of human immunodeficiency virus. Surprisingly, the triphosphate of (1*S*,2*R*)-1-(6-aminopurin-9-yl)-2-(hydroxymethyl)cyclopentane [(1*S*,2*R*)-**6**] and its enantiomer (1*R*,2*S*)-**6** emerged as strong inhibitors of TdT ($K_i = 0.5$ and 1.9 mM, K_{mapp} dATP = 40 mM), whereas the activities of all other enzymes tested proved to be unaffected.

Introduction

Carbocyclic nucleoside analogues, particularly in their enantiomerically pure form, are of increasing interest in organic synthesis due to their biological properties.[1] Compounds of this type are important as potential antiviral or antineoplastic agents as well as inhibitors of several enzymes. Carbocyclic nucleosides are hydrolytically and enzymatically more stable than their natural parent compounds.

The use of biotransformations for the synthesis of enantiomerically pure compounds has been established as an important tool in organic synthesis during the past decade.[2] Among the biocatalysts used in organic synthesis lipases have been applied most frequently because they are easy to handle, available from many sources and accept a broad range of substrates.[3]

Enantiomerically pure intermediates for the synthesis of carbocyclic nucleoside analogues have been prepared using different approaches of asymmetric synthesis including biocatalytic processes. Particularly, the application of lipase-catalyzed kinetic resolutions or asymmetrizations of suitable building blocks has received increasing attention at present.[4]

In continuation of our recent work on the chemo-enzymatic synthesis of enantiomerically pure carbocyclic nucleoside analogues[5] it was our aim to use an intermediate which we very recently have been applied as starting material for the synthesis of the antibiotic β-aminoacid cispentacin in both enantiomeric forms.[6] Synthesis of nucleoside analogues with the *cis*-(2-hydroxymethyl)cyclopentyl residue seems to be promising because replacement of the sugar moiety of nucleosides by strange substituents afforded biologically interesting compounds. For example, the mono-

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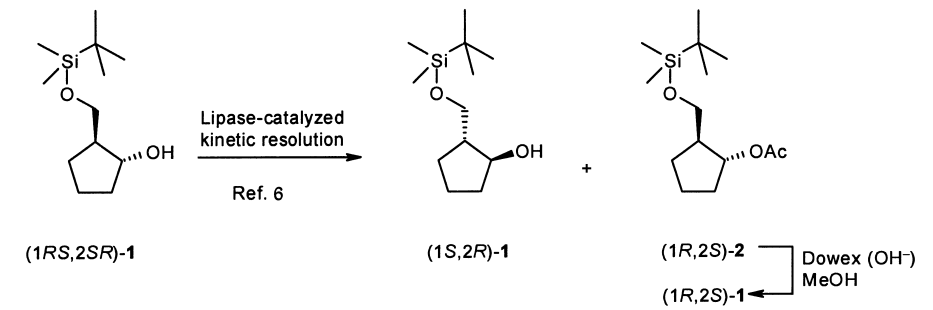
saccharide part can be replaced by the *cis*-1,2-bis(hydroxymethyl)cyclohexane skeleton furnishing analogues with antiviral properties as very recently demonstrated.[7]

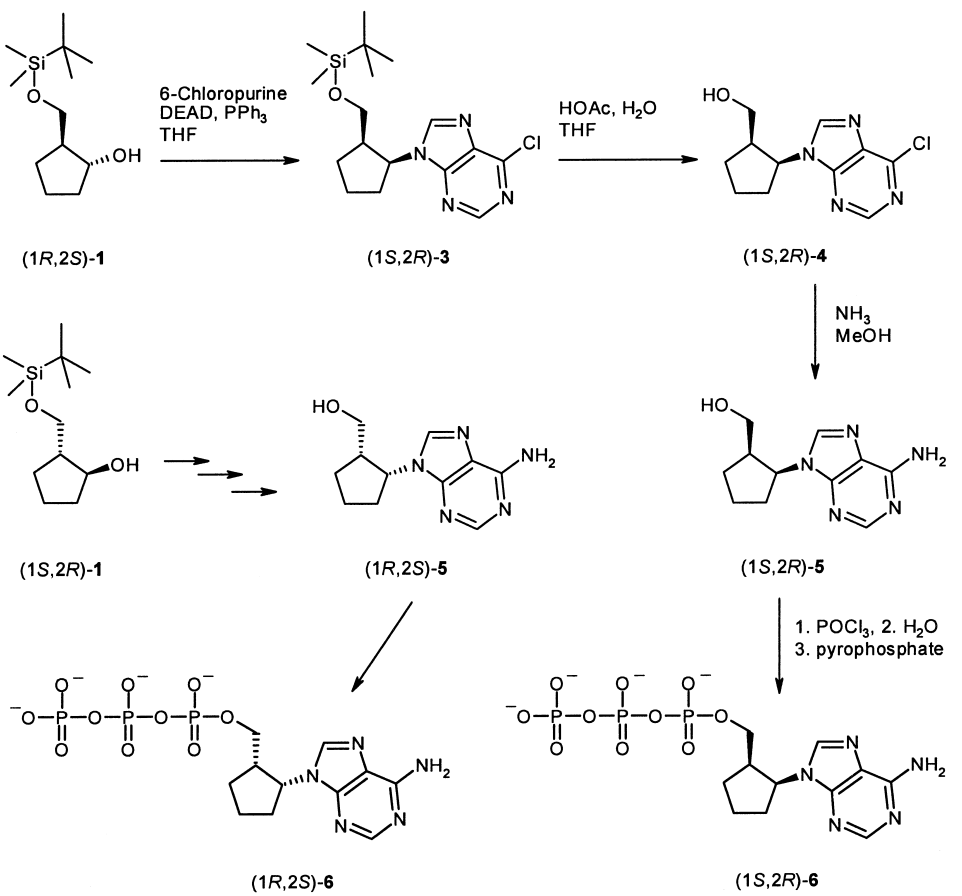
Results and Discussion

Racemic *trans*-2-(hydroxymethyl)cyclopentanol[8] and the corresponding silyloxy alcohol (1RS,2SR)-**1**[6] (Scheme 1) have been successfully resolved into their enantiomers by enzyme-catalyzed transesterification with vinyl acetate in organic solvents in the presence of lipase from *Pseudomonas cepacia* (lipase PS from Amano).

Scheme 2 depicts the reaction sequence for the preparation for both enantiomeric carbocyclic nucleoside analogues (1*S*,2*R*)-**5** and (1*R*,2*S*)-**5** based on (1*R*,2*S*)-**1** and (1*S*,2*R*)-**1,** respectively. Reaction of the *trans*-silyloxy



Scheme 1.



Scheme 2.

alcohol (1*R*,2*S*)-**1** (*ee* 95%) with 6-chloropurine under Mitsunobu conditions[9] in the presence of diethyl azodicarboxylate and triphenyl phosphine yielded under inversion of the configuration at the stereogenic center after chromatographic separation of the reaction mixture the *cis*-chloropurine derivative (1*S*,2*R*)-**3** in a yield of 69% in enantiomerically pure form as shown by HPLC.[10] One-pot deprotection of the primary hydroxy group and ammonolysis of the chloropurine (1*S*,2*R*)-**3** to furnish (1*S*,2*R*)-**5** failed. Therefore, (1*S*,2*R*)-**3** was first desilylated with water/acetic acid in THF to afford the hydroxymethyl purine (1*S*,2*R*)-**4** in 95% yield. Subsequent ammonolysis of (1*S*,2*R*)-**4** with ammonia in methanol at 100 °C in a sealed steel bomb afforded the adenine derivative (1*S*,2*R*)-**5** in 86% yield.

The corresponding enantiomeric adenine derivative (1*R*,2*S*)-**5** was prepared in the analogues manner starting from the enantiomeric silyloxy alcohol (1*S*,2*R*)-**1**.

In order to test their inhibitory properties toward some DNA polymerases the adenosine analogues (1*S*,2*R*)-**5** and (1*R*,2*S*)-**5** were converted into their triphosphates (1*S*,2*R*)-**6** and (1*R*,2*S*)-**6** (Scheme 2), respectively, using standard procedures.[11]

The triphosphates of the enantiomeric adenosine analogues (1*R*,2*S*)-**6** and (1*S*,2*R*)-**6** were investigated for their inhibitory activity against cellular DNA polymerase β, terminal deoxynucleotidyl *trans*ferase (TdT), telomerase, *Escherichia coli* DNA polymerase I, and the reverse *trans*criptase of human immunodeficiency virus (HIV-RT).

Surprisingly, both enantiomers of **6** proved to be strong inhibitors of TdT, whereas the activity of all other DNA polymerases tested was not significantly influenced by **6** at concentrations up to 100 μM.

Using dATP as substrate for TdT the concentrations required for a 50% inhibition (ID_{50}) of the enzyme was 0.82 μM for (1*R*,2*S*)-**6** and 2.7 μM for (1*S*,2*R*)-**6**. The Lineweaver–Burk plots indicate a competitive nature of the inhibition of TdT as demonstrated in Figure 1 for (1*R*,2*S*)-**6** and dATP as substrate. Unlike the other tested DNA polymerases the synthesizing activity of TdT is not template directed and consequently all of the dNTP substrates compete with each other for binding to TdT. Therefore, the inhibitory effects of (1*R*,2*S*)-**6** and (1*S*,2*R*)-**6** on TdT were assessed for each of the four dNTPs. The kinetic data are summarized in Table 1.

The K_{mapp}/K_i ratios indicated a 16- to 124-fold higher affinity of (1*R*,2*S*)-**6** and a 6- to 21-fold higher affinity of (1*S*,2*R*)-**6** for the binding site of TdT compared with the normal substrates. Furthermore, these results clearly indicate a higher affinity of the triphosphate (1*R*,2*S*)-**6** for the enzyme. This is striking because the latter compound is more similar to the unnatural L-dATP whereas (1*S*,2*R*)-**6** corresponds to the natural D-dATP. In order to estimate the ability of TdT to incorporate the analogues at the 3′-hydroxy terminus of 5′-^{32}P-oligo(dT)$_{15}$, the products of the reaction were subjected to gel electrophoresis and audioradiography. However, no indication for an incorporation of the compounds into nascent DNA by TdT could be found (data not shown).

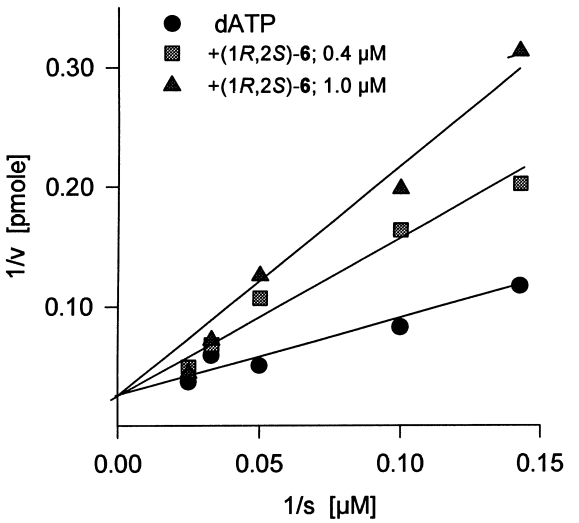


Figure 1. Lineweaver–Burk plot of the effects of (1*R*,2*S*)-**6** on the activity of TdT in the presence of increasing concentrations of the substrate [^{3}H]-dATP.

Although the structure of the newly synthesized adenosine analogue has a completely changed sugar substitute the presented data demonstrate that their enantiomeric triphosphates are active and selective inhibitors of TdT. The ability of the template independent TdT to recognize carbocyclic analogues has been demonstrated recently for cD4A.[14] In contrast, these compounds have been described to be incorporated into a primer by TdT

Table 1. Inhibitory effects of (1*S*,2*R*)-**6** and (1*R*,2*S*)-**6** on purified TdT from calf thymus[a]

	K_{mapp}	K_i(1*R*,2*S*)-**6**	K_i(1*S*,2*R*)-**6**
dATP	40 μM	0.5 μM	1.9 μM
dGTP	34 μM	1.4 μM	5.5 μM
dCTP	26 μM	1.6 μM	3.8 μM
dTTP	29 μM	0.2 μM	3.3 μM

[a]The enzyme was assayed with oligo(dT)$_{15}$ as primer and one of the four dNTPs as substrate. The K_{mapp} and K_i values were calculated from the replots of the slopes of Lineweaver–Burk regression lines. Mean values of at least two separate experiments are shown.

and HIV-RT. The further changed cyclopentyl ring of **6** might cause the higher selectivity for TdT and the loss of any activity to HIV-RT.

Conclusion

In conclusion we have realized a stereocontrolled chemo-enzymatic synthesis of a novel type of enantiomerically pure carbocyclic adenosine analogues which, surprisingly, are strong and selective competitive inhibitors of TdT. The affinity of the synthetic compounds (1*R*,2*S*)-**6** and (1*S*,2*R*)-**6** for TdT is higher compared with the natural dNTPs and shows a significant difference between both enantiomers.

Experimental

All reactions were followed by TLC on glass plates coated with a 0.25 mm layer of silica gel. Compounds were visualized with a 3.5% solution of molybdatophosphoric acid in ethanol and/or by UV light. HPLC on chiral phases was carried out on a Merck–Hitachi system consisting of L-6200A Pump, L-4000 UV Detector and Chromato-Integrator D-2500 and ion-exchange or RP–HPLC on a Kontron liquid chromatograph. Flash chromatography was performed with silica gel 60 (0.040–0.063 mm). ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ if not otherwise indicated on the Varian instruments UNITYplus-500 or -300 at 500 or 300 and 125 or 75 MHz, respectively. APCI and FABMS were recorded on the Autospec VG and MALDI-TOF mass spectra on a VG Tofspec (Fisons Instruments). Optical rotations were measured on a Perkin-Elmer 241 polarimeter, and are given in units of 10^{-1} deg cm^2 g^{-1}.

The used DNA polymerase β derived from Mobitec, Göttingen, TdT, the *E. coli* DNA polymerase I, and HIV-RT were from Boehringer, Mannheim. Activities of the DNA polymerase β, *E. coli* DNA polymerase I, and HIV-RT were measured as described[12] using activated DNA. TdT was tested with oligo(dT)$_{15}$. The telomerase activity was assayed in HL-60 cell-lyosate according to the method of Piatyszek et al.[13] The dATP concentration was 10 μM for all polymerases; in case of TdT also the concentrations of TTP, dCTP and dGTP were 10 μM.

(1*S*,2*R*)-1-(6-Chloropurin-9-yl)-2-(*tert*-butyldimethylsilyloxymethyl)cyclopentane [(1*S*,2*R*)-3]. A solution of the silyloxy alcohol (1*R*,2*S*)-**1** (1.0 g, 4.34 mmol), 6-chloropurine (1.34 g, 8.7 mmol) and triphenyl phosphine (2.28 g, 8.7 mmol) in dry THF (40 mL) was treated under ice-cooling and argon with a solution of diethyl azodicarboxylate (1.51 g, 8.7 mmol) in THF (10 mL). After stirring for 10 min the cooling bath was removed and stirring was continued for 2.5 h at room temperature. The solvent was removed under reduced pressure and the residue was purified by flash chromatography on silica (100 g, 20×3.6 cm) with hexane/ethyl acetate (20:1) to furnish the chloropurine derivative (1*S*,2*R*)-**3** (1.10 g, 69%). The crude crystalline product shows an *ee* > 99% as demonstrated by HPLC.[10]: mp 40–42 °C (*n*-hexane); $[\alpha]_D^{20}$ −54.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR: −0.18 (s, 6 H), 0.75 (s, 9 H), 1.70–1.87 (m, 2 H), 1.90–2.18 (m, 2 H), 2.25–2.40 (m, 4 H), 2.52 (m, 1 H), 3.29 (d, *J* = 5 Hz, 2 H), 5.14 (q, *J* = 8 Hz, 1 H), 8.20 (s, 1 H), 8.71 (s, 1 H); ^{13}C NMR (−5.83, −5.78, 18.05, 22.88, 25.74, 26.91, 29.71, 31.23, 44.36, 57.99, 62.46, 131.54, 145.15, 151.53, 152.20; MS (APCI): *m*/*z* 367 (M^+ + H, 100%); calcd C 55.60, H 7.42, N 15.27, found C 55.63, H 7.52, N 15.23.

(1*S*,2*R*)-1-(6-Chloropurin-9-yl)-2-(hydroxymethyl)cyclopentane [(1*S*,2*R*)-4]. The chloropurine derivative (1*S*,2*R*)-**3** (960 mg, 2.6 mmol) was dissolved in acetic acid:water:THF (40 mL, 3:1:1) and stirred for 5 h at 50 °C. The solvents were removed under reduced pressure and co-distilled with toluene. The residue was purified by flash chromatography on silica (50 g, 20×2.2 cm) with ethyl acetate as eluent to yield the desilylated purine derivative (1*S*,2*R*)-**4** (624 mg, 95%): mp 130–132 °C (ethyl acetate/*n*-hexane); $[\alpha]_D^{20}$ −46.3 (*c* 1.0, MeOH); ^{1}H NMR: 1.41 (m, 1 H), 1.90–2.23 (m, 3 H), 2.25–2.63 (m, 3 H), 2.84 (t, *J* = 5 Hz, 1 H), 3.40–3.54 (m, 2 H), 5.20 (m, 1 H), 8.10 (s, 1 H), 8.76 (s, 1 H); ^{13}C NMR: 22.25, 25.46, 30.91, 47.91, 57.88, 60.97, 131.50, 144.39, 151.60, 152.21; MS (FAB, magic bullet): *m*/*z* 253 (M^+ + H, 100%), 219 (10), 155 (53); calcd C 52.28, H 5.18, N 22.17, found C 52.26, H 5.18, N 22.19.

(1*S*,2*R*)-1-(6-Aminopurin-9-yl)-2-(hydroxymethyl)cyclopentane [(1*S*,2*R*)-5]. A solution of (1*S*,2*R*)-**4** (620 mg, 2.45 mmol) in methanol saturated with NH_3 (50 mL) was heated at 100 °C for 4 h in a sealed steel bomb. The solvent was removed under reduced pressure and the residue was purified by flash chromatography on silica gel with ethyl acetate: EtOH (4:1) to afford (1*S*,2*R*)-**5** (495 mg, 86%): mp 171–172 °C (MeOH); $[\alpha]_D^{20}$ −59.5 (*c* 1.0, MeOH); ^{1}H NMR (DMSO-d_6): 1.58–1.68 (m, 2 H), 1.70–2.05 (m, 2 H), 2.21 (m, 1 H), 2.35 (m, 1 H), 2.95 (m, 2 H), 4.41 (t, *J* = 5 Hz, 1 H), 4.94 (q, *J* = 7 Hz, 1 H), 7.17 (s, 2 H), 8.09 (s, 1 H), 8.13 (s, 1 H); ^{13}C NMR (DMSO-d_6): 22.18, 27.07, 30.22, 45.24, 56.07, 60.37, 118.48, 139.98, 149.80, 152.04, 155.86; MS (FAB, magic bullet): *m*/*z* 234 (M^+ + H, 100 %), 205 (5), 152 (20), 163 (60), 119 (25); calcd C 56.64, H 6.48, N 30.02, found C 56.57, H 6.42, N 30.00.

(1*S*,2*R*)-1-(6-Aminopurin-9-yl)-2-(hydroxymethyl)cyclopentane triphosphate [(1*S*,2*R*)-6]. A suspension of (1*S*,2*R*)-**5** (70 mg, 0.3 mmol) in triethyl phosphate

(1.5 mL) was treated at 0 °C with phosphoryl trichloride (92 mg, 0.6 mmol) and stirred overnight. Then the reaction was quenched with water (0.5 mL) and neutralized with triethyl amine. The resulting mixture was purified by ion-exchange chromatography on a Fractogel TSK DEAE-650 (M) (Merck) column (2×60 cm) using a linear gradient (0.07–0.25 M) of aqueous triethylammonium hydrogen carbonate buffer (pH 7–8). The monophosphate was eluted at the buffer concentration of 0.15–0.2 M. The fractions containing the monophosphate were concentrated under reduced pressure. The obtained monophosphate of (1*S*,2*R*)-**5** was converted into its pyridinium salt by passing through a Dowex-50W X-8 cation-exchange resin column in the pyridinium form. Subsequently, to the solution of the pyridinium salt tributylamine (0.5 mL) was added to furnish the corresponding tributylammonium salt which was dried by repeated evaporation of anhydrous pyridine and then *N*,*N*-dimethyl formamide (DMF) under reduced pressure. The solution of the anhydrous tributylammonium salt in DMF (1.5 mL) was treated with 1,1′-carbonylbis(imidazole) (160 mg, 1 mmol). After 1 h methanol (70 μL) was added, and the reaction mixture was kept for 5 min. Then tributylammonium pyrophosphate (918 mg, 1 mmol; prepared from pyridinium pyrophosphate by addition of four equivalents of tributylamine) in DMF (2 mL) was added to the stirred solution. The reaction mixture was kept overnight at room temperature and then evaporated to dryness. The triphosphate (1*S*,2*R*)-**6** was isolated by anion-exchange chromatography on a Fractogel TSK DEAE-650 (M) (Merck) column (2×60 cm) using a linear gradient (0.05–0.5 M) of triethylammonium hydrogen carbonate buffer as eluent. (1*S*,2*R*)-**6** was eluted at buffer concentrations of 0.2–0.26 M. The fractions containing (1*S*,2*R*)-**6** were pooled and concentrated under reduced pressure. Residual triethylammonium hydrogen carbonate was removed by repeated co-evaporation with deionized water.

The purity of (1*S*,2*R*)-**6** was controlled by ion-exchange HPLC on a WAX-column (Du Pont, Wilmington) using the system buffer A, K_2HPO_4/KH_2PO_4 (pH 7.0, 0.02 M) and buffer B, K_2HPO_4/KH_2PO_4 (pH 7.0, 1 M) as eluent with a 35 min linear gradient from 100% to 50% buffer A with a flow rate of 1.5 mL/min. Retention time of (1*S*,2*R*)-**6**: 30.4 min.[15] Purity of (1*S*,2*R*)-**6** was further controlled by reversed-phase HPLC on a Spherisorb 50 DS 2 column using the following system: buffer A, aqueous triethylammonium acetate (pH 7.0, 0.1 M) and solvent B, acetonitrile/water (95:5, v/v), linear gradient from 100% to 90% buffer A, flow rate 1 mL/min. (1*S*,2*R*)-**6** used in the biochemical evaluation was separated from possible contaminations by the same RP HPLC system. Retention time of (1*S*,2*R*)-**6**: 17.2 min.[15] MS: MALDI-TOF [matrix: saturated solution of 2,4,6-trihydroxy acetophenone (0.05 M) diammoniumhydrogen citrate/50% acetonitrile): 471.8 (M^+-H), calcd 473 for $C_{11}H_{18}N_5O_{10}P_3$ (free acid).

Starting from the enantiomerically pure silyloxy alcohol (1*S*,2*R*)-**1** the enantiomers of the second series have been prepared as described above.

(1*R*,2*S*)-1-(6-Chloropurin-9-yl)-2-(*tert*-butyldimethylsilyloxymethyl)cyclopentane [(1*R*,2*S*)-3]. Mp 40–42 °C, $[\alpha]_D^{20}$ +53.8 (*c* 1.0, $CHCl_3$).

(1*R*,2*S*)-1-(6-Chloropurin-9-yl)-2-(hydroxymethyl)cyclopentane [(1*R*,2*S*)-4]. Mp 130–132 °C (ethyl acetate/*n*-hexane); $[\alpha]_D^{20}$ +45.7 (*c* 1.0, MeOH).

(1*R*,2*S*)-1-(6-Aminopurin-9-yl)-2-(hydroxymethyl)cyclopentane [(1*R*,2*S*)-5]. Mp 171–172 °C (MeOH); $[\alpha]_D^{20}$ +60.5 (*c* 1.0, MeOH).

(1*R*,2*S*)-1-(6-Aminopurin-9-yl)-2-(hydroxymethyl)cyclopentane triphosphate [(1*R*,2*S*)-6]. Anion-exchange chromatography: retention time: 29.8 min,[15] RP-chromatography: retention time: 17.4 min,[15] MALDI-TOF MS: 471.9 (M^+-H).

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10. Chromatographic data: stationary phase: Chiralcel OJ® (250×4.6 mm); mobile phase: *n*-heptane:2-propanol (99:1); flow rate: 1 mL/min; temperature: 22 °C; UV-detection (254 nm): first eluted: (1*S*,2*R*)-**3**: $R_{t1} = 10.91$ min, $k'_1 = 2.90$, second eluted (1*R*,2*S*)-**3**: $R_{t2} = 13.46$ min, $k'_2 = 3.81$; $k'_2/k'_1 = 1.31$, Res. = 5.89.

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15. The retention times of the enantiomers should be equal, however the differences found are in the normal range of error of the method.