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Stereocontrolled Synthesis of the Four Stereoisomeric Diphosphorylphosphonates of Carbocyclic 2',3'-Dideoxy-2',3'-didehydro-5'-noradenosine

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Abstract: A flexible synthesis of the four stereoisomeric enantiomerically pure 5'-nor carbocyclic adenosine analogues **4b**, *ent*-**4b**, **5b** and *ent*-**5b** starting from the common enantio-merically pure allylic monoacetate **3** has been developed. Each of the nucleoside analogues was transformed into the corresponding 4'-phosphonates and subsequently into the diphosphoryl-phosphonates **4e**, *ent*-**4e**, **5e**, and *ent*-**5e**, respectively.

Introduction

The discovery of 3'-azido-3'-deoxythymidine (AZT, zidovudine)[1] as one of the most active compounds for the medicinal treatment of the acquired immunodeficiency syndrome (AIDS) has prompted the synthesis of many nucleoside and nucleotide analogues. AZT is anabolized by host cell kinases to the triphosphate analogue **1** (Figure 1) which is an alternate substrate for the HIV reverse transcriptase (RT) and after incorporation into the growing DNA chain, terminates the DNA synthesis. In the search for compounds possessing better activity and selectivity, much attention has been focussed on the synthesis of 2',3'-dideoxynucleosides[2], the corresponding 2',3'-didehydronucleosides[3], differently modified nucleosides with a restricted conformational flexibility in the carbohydrate moiety[4,5], and the triphosphates (dNTP's) thereof. Amongst many structural types, the carbocyclic nucleosides are of special interest due to their metabolic stability *in vivo*[6] and the high level of antiviral activity demonstrated by some members of this group. A large number of compounds has been tested in racemic form as anti-HIV or as HIV RT inhibitors. In many cases the antiviral activity has been shown to reside in the enantiomer that corresponds the absolute stereochemistry of the natural nucleoside.[7] Very recently it was found that the "unnatural" enantiomer of the carbocyclic nucleotide analogue **2** (Figure 1) is more active as inhibitor of the HIV RT than the "natural" one[8]. Yet, only a few compounds of this type have been obtained enantiomerically pure by a chemical approach.[6] We have been interested in the synthesis of the

stereoisomeric carbocyclic (2',3'-dideoxy-2',3'-didehydro)-5'-noradenosine derivatives and their triphosphate analogues in enantiomerically pure form.

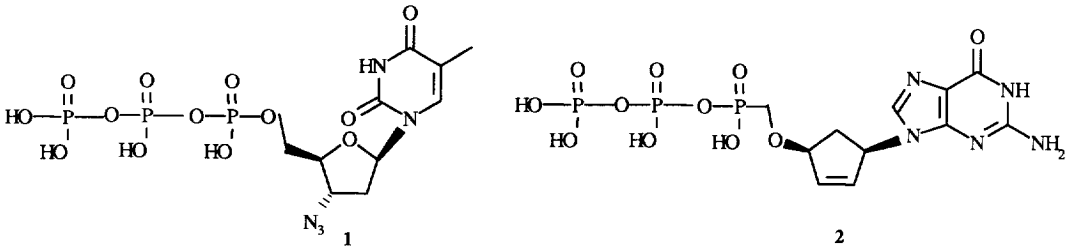


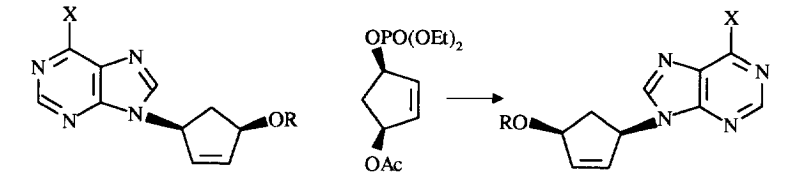
Figure 1

The key feature of our new method consists of regiocontrolled switching the functional groups of the enantiomerically pure allylic acetate **3** and using different types of stereocontrolled nucleophilic substitutions.

Results

Monoacetate **3** can be prepared by lipase-catalyzed transesterification of *cis*-cyclopent-2-ene-1,4-diol[9] or by hydrolysis of the corresponding diacetate with porcine liver esterase.[10] Using the monoacetate **3** (Scheme 1) as starting material, it was possible to prepare the four possible stereoisomeric carbocyclic nucleoside analogues (two enantiomeric pairs of diastereomers) as we reported recently.[11] Reaction of the monoacetate **3** with the sodium salt of 6-chloropurine by Pd(0) catalysis[12] yielded the nucleoside analogue **4a** under retention of the configuration at the stereocentre adjacent to the acetate group. Compound **4a** was identical by its NMR spectra with a sample obtained recently by lipase-catalyzed resolution of the corresponding racemic nucleoside analogues.[13] Ammonolysis of **4a** yielded the adenine derivative **4b** with an overall yield of 42% (rel. to **3**). In contrast, reaction of **3** on Mitsunobu conditions[14] afforded **5a** by inversion of the configuration at the carbon bearing the hydroxy group. Chloropurine **5a** yielded the adenine derivative **5b** by reaction with ammonia.

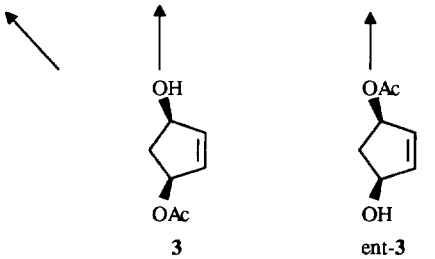
In order to synthesize the nucleoside analogue *ent*-**4b**, we took advantage of the different reactivity of the leaving groups in allylic positions. The reactivity is known to be $OPO(OEt)_2$>OAc>OH.[15,16] Therefore, the monoacetate **3** was transformed into the corresponding diethyl phosphate **6**. By this conversion the reactivity was switched in the subsequent Pd(0)-catalyzed nucleophilic substitution with the sodium salt of 6-chloropurine to give the acetate **7**. Simultaneously to our work, Siddiqi *et al.*[17] used the same approach to prepare 5'-noraristeromycine from *ent*-**3**. But in this case, the removal of the acetyl group was prior to the Pd(0)-catalyzed nucleophilic substitution. Subsequent amination of **7** afforded *ent*-**4b** in 38% yield (rel. to **3**). Silylation of **3** followed by deacetylation gave the cyclopentene **8** which subsequently furnished the nucleoside **9** by Mitsunobu reaction with inversion of the configuration at the reacting stereocentre. Finally, desilylation and subsequent reaction with ammonia gave *ent*-**5b**.



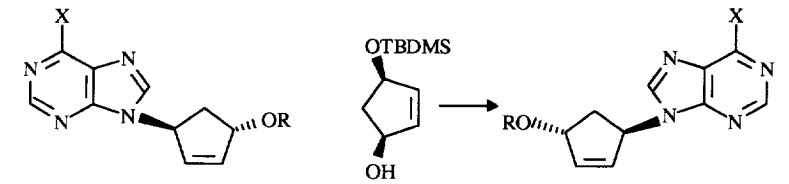
4a: X = Cl, R = Ac
4b: X = NH_2, R = H
4c: X = NH_2, R = $CH_2P(O)(OH)(OEt)$
4d: X = NH_2, R = $CH_2P(O)(OH)_2$

6

7: X = Cl, R = Ac
ent-**4a**: X = Cl, R = H
ent-**4b**: X = NH_2, R = H
ent-**4c**: X = NH_2, R = $CH_2P(O)(OH)(OEt)$
ent-**4d**: X = NH_2, R = $CH_2P(O)(OH)_2$



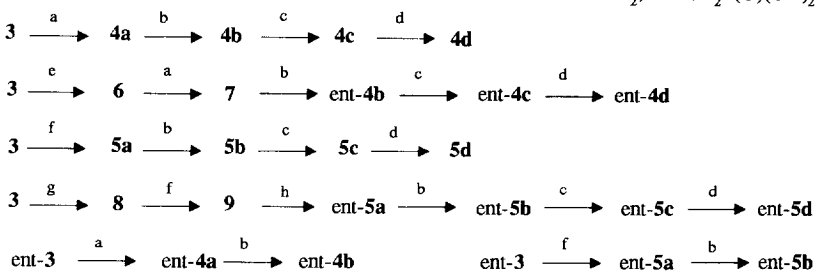
3 ent-**3**



5a: X = Cl, R = Ac
5b: X = NH_2, R = H
5c: X = NH_2, R = $CH_2P(O)(OH)(OEt)$
5d: X = NH_2, R = $CH_2P(O)(OH)_2$

8

9: X = Cl, R = TBDMS
ent-**5a**: X = Cl, R = Ac
ent-**5b**: X = NH_2, R = H
ent-**5c**: X = NH_2, R = $CH_2P(O)(OH)(OEt)$
ent-**5d**: X = NH_2, R = $CH_2P(O)(OH)_2$



a: 6-Chloropurine, NaH, $Pd(PPh_3)_4$; b: MeOH, NH_3; c: NaH, **11**; d: Me_3SiBr, e: $ClP(O)(OEt)_2$, imidazole, 4-DMAP; f: 6-chloropurine, DEAD, PPh_3; g: 1.TBDMS-Cl, imidazole, 4-DMAP; 2. NaOMe; h: TBAF

Scheme 1

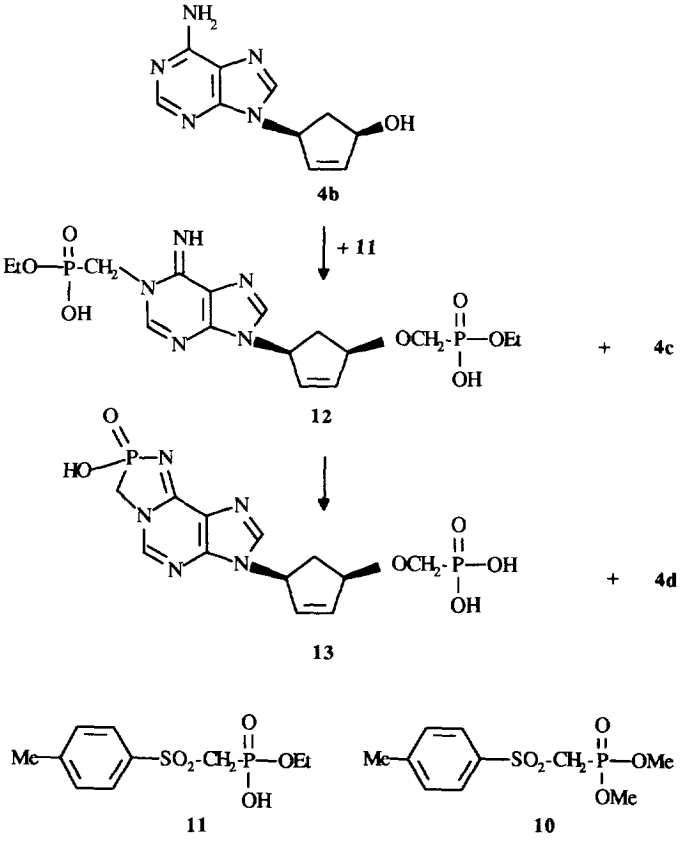
Due to the symmetry of **3**/*ent*-**3** the enantiomeric monoacetate *ent*-**3**[18] can serve as starting material in a similar way. This was demonstrated by the synthesis of *ent*-**4b** and *ent*-**5b**. Reaction of *ent*-**3** by Pd(0)-catalysis with the sodium salt of 6-chloropurine yielded *ent*-**4a** which afforded by amination *ent*-**4b**. Mitsunobu reaction of *ent*-**3** with 6-chloropurine yielded *ent*-**5a** which was converted to *ent*-**5b** by reaction with ammonia. By the latter sequence the structures of *ent*-**4b** and *ent*-**5b** prepared from **3** were confirmed.

The synthesis of the carbocyclic nucleoside analogues **4b** and **5b** as well as their corresponding enantiomers *ent*-**4b** and *ent*-**5b** from one common enantiomerically pure starting material demonstrates the flexibility of our synthetic strategy which allows to switch the reactivity of **3** by functional group interconversion and to prepare all stereoisomers.

In order to test the biological activity of inhibiting a target enzyme like HIV RT, the nucleoside analogues required the activation by corresponding triphosphates. The 5'-nornucleoside analogues lack the primary hydroxy function that cellular enzymes attach to form the corresponding 5'-O-triphosphates *via* the corresponding monophosphates. Several attempts have been made to bypass this crucial phosphorylation step. Recently, it has been shown that the diphosphorylphosphonates of carbocyclic 5'-nornucleoside analogues were active as potent inhibitors of HIV-1 RT.[19] These compounds have been synthesized by diphosphorylation of the corresponding phosphonates.

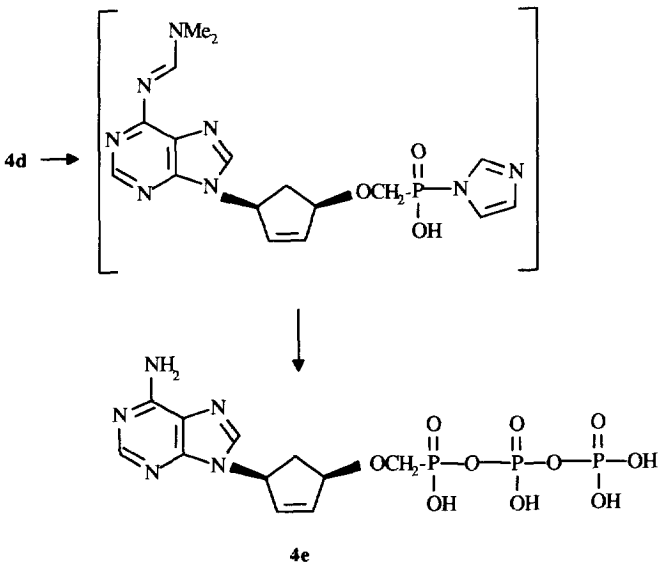
Introduction of the phosphonomethoxy group instead of the secondary hydroxy group required initial protection of the amino group in order to prevent the formation of significant amounts of bis-alkylated material.[20] In our hands the reaction of the sodium salt of **4b** with dimethyl p-tolylsulfonyloxymethanephosphonate (**10**) described by Holy and Rosenberg[21] gave a complex mixture. The target compound **4c** was isolated in 16% yield. The use of monoethyl p-tolylsulfonyloxymethanephosphonate (**11**) proposed recently by Jasko[22] for the alkylation of a primary hydroxy group, allowed to use the unprotected nucleosides **4b**, **5b**, *ent*-**4b** and *ent*-**5b**. Treatment of the sodium salt of the *trans*-nucleoside analogues **5b** or *ent*-**5b** with **11** led to the mono-4'-alkylated products **5c** or *ent*-**5c** (Scheme 1). After deprotection of the phosphonic acid moiety with bromotrimethylsilane the *trans*-4'-phosphonates **5d** and *ent*-**5d** were isolated in 40% yield (rel. to **5b** or *ent*-**5b**).

Phosphonylation of the *cis*-nucleoside analogues **4b** and *ent*-**4b** using the conditions described above showed a different behaviour (Scheme 2). Alkylation of **4b** with **11** led to a mixture of the mono- and bis-alkylated products **4c** and **12**, which could be separated by ion-exchange chromatography. After deprotection of **4c** with bromotrimethylsilane the target phosphonate **4d** was obtained in 35% yield (rel. to **4b**). The FAB-mass spectrum of **12** indicated the presence of two ethyl phosphonylmethylene groups. In addition, a strong UV absorption at 270 nm was found to be typical for a N-1 substituted adenine. In the ^{1}H NMR spectrum of **12** the H-2 signal showed a down-field shift of 0.15 ppm compared to **4b**, **4c** and **4d**. The position of the substituent in the adenine ring was also confirmed by treatment of **12** with bromotri-methylsilane in DMF to furnish the cyclophosphamide **13**, which is an evidence for the N-1 position of the second methyl phosphonic acid substituent. The same transformations carried out with *ent*-**4b** afforded the corresponding enantiomers *ent*-**12** and *ent*-**13**.



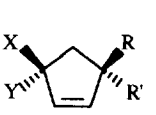
Scheme 2

Initial attempts to transform the phosphonates **4d**, **5d**, *ent*-**4d**, and *ent*-**5d** into the corresponding diphosphorylphosphonates *via* imidazole derivatives failed because of their poor solubility in DMF. In order to overcome this problem the NH_2-group of **4d** was protected with the dimethylaminomethylene group. Then the usual phosphorylation procedure was used (Scheme 3). Deprotection with ammonia gave the triphosphate analogue **4e** which was isolated by ion-exchange chromatography and repurified by low pressure reversed phase chromatography.



Scheme 3

The same transformations on the other phosphonate stereoisomers **5d**, *ent*-**4d**, and *ent*-**5d** afforded the corresponding diphosphorylphosphonates **5e**, *ent*-**4e** and *ent*-**5e**, respectively (Fig. 2).



ent-**4e**: R = Ade, R' = H
X = $OCH_2P_3O_9H_4$, Y = H
5e: R = H, R' = Ade
X = $OCH_2P_3O_9H_4$, Y = H
ent-**5e**: R = Ade, R' = H
X = H, Y = $OCH_2P_3O_9H_4$

Figure 2

All triphosphate analogues were homogeneous according to HPLC and spectral data. The substrate properties of the four carbocyclic nucleoside 5'-triphosphate analogues were evaluated regarding the reverse transcriptases of HIV and avian myoblastosis virus (AMV) and the terminal deoxynucleotidyl transferase (TdT). The *cis*-configurated compounds **4e** and *ent*-**4e** were good substrates for the RT's of HIV and AMV. The *trans*-compounds **5e** and *ent*-**5e** were not recognized by these enzymes, but all four diphosphorylphosphonates analogues are substrates of TdT.[23]

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Experimental

Unless stated otherwise, the reactions were carried out using freshly distilled solvents at anhydrous conditions in an argon atmosphere. Tetrahydrofuran (THF) was distilled from sodium wire and benzophenone. The glassware was dried overnight in an oven at 100°C. Reactions were monitored by thin-layer chromatography (TLC) using glass plates or plastic foils coated with a 0.25 mm layer of silica gel 60-F_{254}. Compounds were visualized by irradiation with an UV-lamp, or by spraying with a solution of 10% H_2SO_4 in ethanol, and with a solution of 3.5% of molybdato phosphoric acid in ethanol, respectively. For column chromatography silica gel 60 (0.040-0.063 mm), DEAE-Toyopearl 650 M (Toyosoda, Japan), siliconized silica gel LiChroprep RP-8 (0.040-0.063 mm), or LiChroprep RP-18 (0.025-0.040 mm) (Merck) were used. HPLC was carried out on a Silasorb column C-18 (0.006 mm, 4 x 150 mm). Elution was performed with a linear gradient of acetonitrile in 0.05 M KH_2PO_4 from 0 to 26% during 20 min (flow rate: 0.8 ml/min). Optical rotations were measured with the polarimeter 241 (Perkin-Elmer). UV-spectra were recorded on a Specord M 40 (Carl Zeiss Jena). Electron impact mass spectra were run on the GC/MS-Datensystem HP 5985B. FAB-mass spectra were recorded on a Kratos MS 50TC mass spectrometer (Samples were mixed with glycerol on the probe tip. Xenon was used for the fast atom gun at 8 keV.). The ^1H NMR spectra were recorded on a MSL 400 (Bruker) or a XL 400 (Varian) spectrometer at 400 MHz using DMSO-d_6 or D_2O as solvents. The ^{31}P NMR spectra were recorded on a Varian 400 spectrometer at 161.9 MHz with H_3PO_4 as external standard. The ^{13}C NMR spectra were recorded on a MSL 400 (Bruker) or a CFT 20 (Bruker) spectrometer at 100 or 50 MHz. All chemical shifts are reported in δ values and the coupling constants (*J*) are quoted in Hz.

(1'S,4'R)-9-(4'-Hydroxycyclopent-2'-enyl)adenine (**4b**).

6-Chloropurine (0.930 g, 6 mmol) and sodium hydride (80% suspension in mineral oil, 0.144 g, 6 mmol) were added to dry THF (7 ml). The reaction mixture was stirred at room temperature for 1.5 h followed by the addition of triphenylphosphine (PPh_3) (0.787 g, 0.3 mmol), tetrakis(triphenylphosphine)palladium (0.280 g, 0.25 mmol), and a solution of **3** (0.710 g, 5 mmol) in THF (3 ml). The resulting mixture was stirred at 50°C for 6 h. Then the solvent was removed under reduced pressure to provide a dark oil which was dissolved in CH_2Cl_2 (15 ml) and filtered through Celite. After evaporation of the solvent the residue was purified by column chromatography (4 x 25 cm) on silica gel with $CHCl_3$-MeOH (98:2) as eluent to yield **4a** (0.625 g). UV (MeOH) λ_{max}: 264.8, 209.7 nm. MS: 236, 238 (M^+) and 155, 157 ($B^+ + 1$). **4a** was dissolved in methanol saturated with NH_3 at 0°C, sealed in a bomb and heated (100°C) for 3 h. The solvent was evaporated under reduced pressure and the residue was purified by chromatography with $CHCl_3$-MeOH (98:2) as eluent to yield **4b** (0.455 g, 42% rel. to **3**). M.p. 196-197°C (acetone). UV (MeOH) λ_{max}: 261.4 (13 300), 212.2 nm (14 812). MS: 217 (M^+), 200 ($M^+ - OH$), 135 (B^+). $[\alpha]_D^{20}$ −110.0 (*c* 0.56, MeOH). ^1H NMR (DMSO-d_6): 8.09 (s, 1H, H-2), 8.02 (s, 1H, H-8), 7.24 (s, 2H, NH_2), 6.13 (m, 1H, H-3'), 5.92 (d, 1H, J = 4, H-2'), 5.52 (d, 1H, J = 7, OH), 5.39 (m, 1H, H-1'), 4.66 (br s, 1H, H-4'), 2.84 (m, 1H, ^2J = 14.8, H-5'b), 1.68 (m, 1H, H-5'a). ^{13}C NMR (DMSO-d_6): 155.87 (C6), 152.25 (C2), 149.11 (C4), 140.14 (C8), 138.80 (C3'), 131.02 (C2'), 118.99 (C5), 74.45 (C4'), 58.58 (C1'), 38.15 (C5'); calcd. C: 55.29, H: 5.10, N: 32.34, found C: 55.10, H: 5.15, N: 32.53.

(1'S,4'S)-9-(4'-Hydroxycyclopent-2'-enyl)adenine (**5b**).

A suspension of Ph_3P (0.598 g, 2.28 mmol) and 6-chloropurine (0.364 g, 2.28 mmol) in THF (15 ml) was treated at room temperature with diethyl azodicarboxylate (DEAD) (0.38 ml, 2.28 mmol) for 1 h. A solution of **3** (0.267 g, 1.9 mmol) in THF (5 ml) was added and next the mixture was stirred at room temperature overnight. After evaporation of the solvent under reduced pressure, the mixture was chromatographed with ethyl acetate - hexane (3:2) as eluent to yield **5a** contaminated with Ph_3PO. UV (MeOH) λ_{max}: 265.1, 208.9 nm. MS: 277, 279 (M^+), 218, 220 ($M^+ - OAc$), 155, 157 (B^+). Compound **5a** was treated with ammonia in methanol as described above. After evaporation of the solvent under reduced pressure the residue was purified by column chromatography to yield **5b** (0.169 g, 41% rel. to **3**). M.p. 208-209°C (EtOH), $[\alpha]_D^{20}$ – 208.8 (*c* 0.85, MeOH), UV (MeOH) λ_{max}: 260.4 (14 355), 212.6 nm (14 224), MS: 218 ($M^+ + 1$), 217 (M^+), 200 ($M^+ - OH$), 136 ($B^+ + 1$). ^1H NMR (DMSO-d_6): 8.09 (s, 1H, H-2), 7.94 (s, 1H, H-8), 7.18 (s, 2H, NH_2), 6.13 (dd, 1H, J = 5.5, 1.8, H-2' or H-3'), 5.96 (dd, 1H, H-2' or H-3'), 5.66 (m, 1H, H-1'), 5.03 (H_2O and 4'-OH overlapping), 2.19 (m, 2H, H-5'). ^{13}C NMR (DMSO-d_6): 156.09 (C6), 152.47 (C2), 149.33 (C4), 140.36 (C8), 139.00 (C3'), 131.24 (C2'), 119.20 (C5), 74.67 (C4'), 58.80 (C1'), 38.35 (C5'); calcd. C: 55.29, H: 5.10, N: 32.34, found C: 55.40, H: 5.20, N: 32.67.

(1'R,4'S)-9-(4'-Hydroxycyclopent-2'-enyl)adenine (*ent*-**4b**)

Method A. The procedure mentioned above for the preparation of **4b** from **3** was used to prepare *ent*-**4b** from *ent*-**3**. Yield 45% related to *ent*-**3**.

Method B. A solution of cyclopentene **3** (0.280 g, 2 mmol), imidazole (0.280 g, 4 mmol) and 4-dimethylaminopyridine (0.060 g, 0.5 mmol) in dry acetonitrile (20 ml) was treated with diethyl chlorophosphate (1.5 ml, 3 mmol). The mixture was stirred at room temperature for 48 h and then filtered. The solvent was evaporated under reduced pressure and the residue was dissolved in diethyl ether (20 ml), washed with water (3 x 5 ml) and dried with Na_2SO_4. Evaporation to dryness yielded crude **6**. [MS: 219 (M^+ - HOAc), 191 (M^+ – HOAc – Et), 162 (M^+ – HOAc – 2 Et), 127 (M^+ – OAc – 2 OEt)]. The diethyl phosphate **6** was treated with the sodium salt of 6-chloropurine [prepared from 6-chloropurine (0.341 g, 2.2 mmol) and a suspension of sodium hydride (80% suspension in mineral oil, 0.053 g, 2.2 mmol)] by Pd(0) catalysis as mentioned before for **4b**. The nucleoside analogue **7** (0.110 g, 25 % rel. to **3**) was obtained as colorless crystals. M.p. 147-150° C (MeOH), MS: 279, 277 (M^+), 218, 220 ($M^+ - OAc$), 155, 157 (B^+). Ammonolysis of **7** as described above afforded *ent*-**4b** (0.110 g, 25% rel. to **3**). M.p. 192-193°C (acetone), $[\alpha]_D^{20}$ +122.0 (*c* 0.56, MeOH); calcd. C: 55.29, H: 5.10, N: 32.34, found C: 55.47, H: 5.05, N: 32.20. UV, MS, ^1H, and ^{13}C NMR spectra were identical with those of **4b**.

(1'R,4'R)-9-(4'-Hydroxycyclopent-2'-enyl)adenine (*ent*-**5b**)

Method A. Reaction of the cyclopentene *ent*-**3** (0.240 g, 1.7 mmol) with 6-chloropurine (0.308 g, 2 mmol) under Mitsunobu conditions and subsequent ammonolysis gave *ent*-**5b** (0.158 g, 43% rel to *ent*-**3**) as described for the preparation of **5b** from **3**.

Method B. A solution of the cyclopentene **3** (1.1 g, 7.75 mmol) in acetonitrile (10 ml) was treated with *tert*-butyldimethylchlorosilane (1.5 g, 10 mmol), 4-dimethylaminopyridine (0.06 g, 0.5 mmol) and imidazole (1.36 g, 20 mmol). The mixture was stirred at room temperature for 3 h. After filtration the organic solvents

were removed under reduced pressure. The residue was dissolved in diethyl ether (25 ml), washed with water (3 x 5 ml) and dried with Na_2SO_4. The solvent was evaporated and the residue treated with a 0.5 M solution of NaOMe in MeOH (pH 9). After 6 h the mixture was neutralized with Dowex 50 (H^+) to give the compound **8**. Reaction of **8** with 6-chloropurine using Mitsunobu conditions afforded **9** which was purified by flash chromatography with ethyl acetate-hexane (1:2) as eluent. UV (MeOH) λ_{max}: 264.3, 211.2 nm, MS: 350, 352 (M^+), 155, 157 ($B^+ + 1$). Treatment of **9** with a 1.0 M solution of tetrabutylammonium fluoride in THF for 4 h at room temperature and subsequent ammonolysis as described above led to *ent*-**5b** (0.540 g, 25% rel. to **3**). M.p. 207-209°C (EtOH), $[\alpha]_D^{20}$ +222.1 (*c* 0.59, MeOH); calcd. C: 55.29, H: 5.10, N: 32.34, found C: 55.20, H: 5.32, N: 32.42. UV, MS, ^1H, and ^{13}C NMR spectra were identical with those of **5b**.

(1'S,4'S)-9-[4'-(Phosphonomethoxy)cyclopent-2'-enyl]adenine (**5d**).

A solution of the nucleoside analogue **5b** (110 mg, 0.5 mmol) in DMF (5 ml) was treated with sodium hydride (80% suspension in mineral oil, 43 mg, 1.5 mmol) and the toluenesulfonate **11** (442 mg, 1.5 mmol). The mixture was stirred at room temperature for 24 h and then neutralized with acetic acid. The solvent was evaporated under reduced pressure and the residue purified on a Toyopearl DEAE (HCO_3^-) column (5 x 40 cm). The column was washed with 300 ml of water and than with a linear gradient of ammonium bicarbonate buffer (0 - 0.15 M, total volume 600 ml). The product was eluted at 0.08 M NH_4HCO_3. The aqueous solvent was evaporated and the residue was repeatedly (5 x) dissolved in water (10 ml) and concentrated to dryness to give the ethyl phosphonate **5c**. A solution of **5c** in DMF (5 ml) was treated with bromotrimethylsilane (0.5 ml) and the mixture was kept at room temperature overnight. The solution was concentrated to dryness and title compound **5d** was isolated by ion-exchange chromatography using a gradient of NH_4HCO_3 (0 - 0.3 M) as eluent. The phosphonate **5d** was eluted at 0.15 M NH_4HCO_3. The solvent was evaporated and the residue repeatedly (5 x) dissolved in water (10 ml) and concentrated to dryness. The residue was dissolved in water (2 ml) and purified on a LiChroprep RP-8 column (2.5 x 25 cm). Elution with water followed by freeze-drying gave the nucleotide **5d** (42 mg, 30 %) as a free acid. UV (H_2O, pH 7) λ_{max}: 262.5 nm; FAB MS: 312 ($M^+ + 1$). ^1H NMR (D_2O): 8.14 (s, 1H, H-2), 8.02 (s, 1H, H-8), 6.49 (dt, 1H, H-3', $J_{3',2'} = 6$, $J_{3',1'} = J_{3',4'} = 2$), 6.28 (dq, 1H, H-2', $J_{2',1'} = 2$, $J_{3',4'} = 1$), 5.75 (m, 1H, H-1'), 5.04 (m, 1H, 4'-OH), 3.70 (m, 2H, CH_2P, $^2J = 13$, $J_{H,P} = 9$), 2.57 (m, 1H, H-5'a, $J = 4$, $J = 8$, $^2J = 15$), 2.29 (m, 1H, H-5'b, $J = 8$, $J = 4$).

The same procedure was used to prepare (1'R,4'R)-9-[4'-(phosphonomethoxy)cyclopent-2'-enyl]adenine *ent*-**5d** (40 mg, 29 %) from *ent*-**5b** (110 mg, 0.5 mmol). The spectral data of *ent*-**5d** were identical with those of **5d**.

(1'S,4'R)-9-[4'-(Phosphonomethoxy)cyclopent-2'-enyl]adenine (**4d**) and *(1R,4S)-4-[1-Ethoxyhydroxyphosphorylmethyl)-6-imino-1,6-dihydropurin)-9-yl]-cyclopent-2-enyloxymethylphosphonic acid ethyl ester* (**12**).

The nucleoside analogue **4d** was obtained from **4c** (110 mg, 0.5 mmol) by phosphonylation as described above for **5b**. The ethyl phosphonate **4c** was isolated by chromatography on DEAE using a linear gradient of NH_4HCO_3 (0 - 0.25 M). Ethyl phosphonate **4c** was eluted at 0.08 M NH_4HCO_3. The by-product **12** was eluted at 0.16 M NH_4HCO_3. The phosphonate **4c** was deprotected and purified as descibed for **5c** to yield the title compound **4d** (42 mg (30%). UV (H_2O, pH 7): λ_{max}: 262.5 nm, FAB MS: 312 ($M^+ + 1$). ^1H NMR (D_2O): 8.18 (s, 1H, H-2), 8.16 (s, 1H, H-8), 6.47 (dt, 1H, H-3', $J_{3',2'} = 6$, $J_{3',1'} = J_{3',4'} = 2$), 6.23 (dq, 1H,

H-2', $J_{2',1'} = 2$, $J_{2',4'} = 1$), 5.50 (m, 1H, H-1'), 4.80 (4'-OH and H_2O overlapping), 3.72 (d, 2H. CH_2P, $J_{H,P} = 9$), 3.03 (m, 1H, H-5'a), 1.97 (m, 1H, H-5'b, $J = 8$, $J = 4$, $^2J = 15$).

The solution of the ethyl phosphonate **12** was concentrated to dryness, repeatedly (5 x) dissolved in water (10 ml) and concentrated. The residue was purified by reversed phase chromatography as mentioned above to afford **12** (30 mg, 13%). UV (H_2O, pH 7): λ_{max}: 270.7 nm, FAB MS: 461 ($M^+ + 1$). ^1H NMR (D_2O): 8.33 (s, 1H, H-2), 8.18 (s, 1H, H-8), 6.50 (m, 1H, H-3'), 6.30 (m, 1H, H-2'), 5.58 (m, 1H, H-1'), 4.82 (4'-OH and H_2O overlapping), 3.93 (m, 6H, N-CH_2P + CH_2CH_3), 3.75 (d, 2H, CH_2P, $J_{H,P} = 9$), 3.09 (m, 1H, H-5'a), 1.99 (m, 1H, H-5'b, $J = 8$, $J = 4$, $^2J = 15$), 1.03 (m, 6H, CH_3). ^{31}P NMR (D_2O): 18.94 (s) and 18.59 (s).

Using the same procedure *ent*-**4d** and *ent*-**12** were obtained in similar yields (30 and 15 %). The spectral data were identical with those of **4d** and **12**, respectively.

(1R,4S)-4-(2-Hydroxy-2-oxo-2,3-dihydro-1,3a,5,6,8-pentaaza-2-phospa-as-indacen-6-yl)-cyclopent-2-enyloxymethylphosphonic acid (**13**).

A solution of the ethyl phosphonate **12** (30 mg, 0.065 mmol) in DMF (5 ml) was treated with bromotrimethylsilane (0.3 ml). After 12 h the reaction mixture was concentrated under reduced pressure. The title compound **13** (3 mg, 13 %) was isolated by ion-exchange chromatography as described for **4c**. UV (H_2O, pH 7): λ_{max}: 270.7 nm, FAB MS: 386 ($M^+ + 1$). ^1H NMR (D_2O): 8.29 (s, 1H, H-2), 8.13 (s, 1H, H-8), 6.47 (dt, 1H, H-3', $J_{3',2'} = 6$, $J_{3',1'} = J_{3',4'} = 2$), 6.24 (dq, 1H, H-2', $J_{2',1'} = 2$, $J_{2',4'} = 1$), 5.54 (m, 1H, H-1'), 4.84 (4'-OH and H_2O overlapping), 3.85 (br. d, 2H, N-CH_2P, $J_{H,P} = 12$), 3.75 (d, 2H, CH_2P, $J_{H,P} = 9$), 3.14 (m, 1H, H-5'a), 1.98 (dt, 1H, H-5'b, $J_{5'b,1'} = J_{5'a,4'} = 4$, $^2J = 15$). ^{31}P NMR (D_2O): 16.76 (s) and 16.51 (s).

General procedure for the preparation of diphosphorylphosphonates.

A solution of the phosphonate **4d** (and *ent*-**4d**, **5d**, or *ent*-**5d**, respectively) (16 mg, 0.05 mmol) in DMF (5 ml) was treated with dimethylformamide dimethyl acetal (0.3 ml) and kept at room temperature overnight. After evaporation of the solvent, the residue was dissolved in DMF (3 ml) and treated with N,N'-carbonyldiimidazole (24 mg, 0.15 mmol). After 12 h a 1 M solution of dibutylammonium pyrophosphate in DMF (1 ml) was added and the mixture was kept at room temperature for 2 h. Then this mixture was treated with NH_4OH (1 ml) and subsequently concentrated under reduced pressure for 1 h. The residue was purified by chromatography on DEAE with a linear gradient of NH_4HCO_3 (0 - 0.4 M) as eluent. The triphosphate analogues **4e**, *ent*-**4e**, **5e**, and *ent*-**5e**, were eluted at 0.3 M NH_4HCO_3. Repurification was carried out by reversed phase chromatography on a LiChroprep C-18 column (2 x 30 cm). The average yield was 12% (rel. to the corresponding phosphonates). FAB MS: 490 ($M^+ + 3$), 506 ($M^+ + 2 + NH_3$), 522 ($M^+ + 1 + 2\ NH_3$). The spectra for all stereoisomers were identical.

(1'S,4'R)-9-[4'-(Diphosphorylphosphonomethoxy)cyclopent-2'-enyl]adenine (**4e**): Retention time: 11.7 min. The ^1H NMR spectrum was identical with that of **4d**. ^{31}P NMR (D_2O): 10.01 (d, P-α, $J_{\alpha,\beta} = 26.23$), –9.56 (d, P-γ, $J_{\gamma,\beta} = 20.50$), –21.97 (br.s, P-β).

(1'R,4'S)-9-[4'-(Diphosphorylphosphonomethoxy)cyclopent-2'-enyl]adenine (*ent*-**4e**): Retention time: 12.0 min. The ^1H NMR spectrum was identical with that of **4d**. ^{31}P NMR (D_2O): 10.01 (d, P-α, $J_{\alpha,\beta} = 26.23$), –9.56 (d, P-γ, $J_{\gamma,\beta} = 20.50$), –21.97 (br.s, P-β).

(1'S,4'S)-9-[4'-(Diphosphorylphosphonomethoxy)cyclopent-2'-enyl]adenine (**5e**): Retention time: 10.4 min. The ^{1}H NMR spectrum was identical with that of **5d**. ^{31}P NMR (D_2O): 10.12 (d, P-α, $J_{\alpha,\beta}$ = 24.67), –9.21 (d, P-γ, $J_{\gamma,\beta}$ = 21.29), –21.45 (br.s, P-β).

(1'R,4'R)-9-[4'-(Diphosphorylphosphonomethoxy)cyclopent-2'-enyl]adenine (*ent*-**5e**): Retention time: 10.8 min. The ^{1}H NMR spectrum was identical with that of **5d**. ^{31}P NMR (D_2O): 10.02 (d, P-α, $J_{\alpha,\beta}$ = 26.72), –9.33 (d, P-γ, $J_{\gamma,\beta}$ = 18.04), –21.90 (br.s., P-β).

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