Application of the Mitsunobu-Type Condensation Reaction to the Synthesis of Phosphonate Derivatives of Cyclohexenyl and Cyclohexanyl Nucleosides

María-Jesús Pérez-Pérez, Jef Rozenski, Roger Busson, and Piet Herdewijn*

Laboratory of Medicinal Chemistry, Rega Institute of Medical Research, K. U. Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

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1,4-*trans*-cyclohexanediol has been used as starting material for the synthesis of cyclohexenyl and cyclohexanyl nucleosides functionalized with a $OCH_2P(O)(OH)_2$ moiety in a 1,4-*cis* relationship with the heterocyclic base. The methodology used is based on the Mitsunobu-type condensation of a conveniently functionalized alcohol with both purines and pyrimidines bases. In all cases (except for cytosine) the natural substituted derivative (N-9 for purines and N-1 for pyrimidines) were obtained as the major isomers. The N-1 cytosine derivative was thus obtained from its uracil analogue. Yields were higher when an allylic alcohol was used in the condensation. For this reason, cyclohexanyl nucleosides were obtained from their cyclohexenyl analogues by reduction of the 2′,3′-double bond. The phosphonomethoxy moiety was introduced prior to the coupling with the heterocyclic base to avoid protection or undesired alkylations of the bases during the derivatization of the 4′-alcohol function.

Introduction

Carbocyclic nucleosides, both natural and synthetic, have been the subject of considerable antitumoral and antiviral studies.[1] The replacement of the oxygen atom in the sugar moeity of nucleosides by a methylene group confers chemical and enzymatic stability to the sugar–base linkage compared to their natural counterparts. By removal of the oxygen function, however, the anomeric center disappears and the electronic properties of the five-membered ring is altered. As a consequence, carbocyclic nucleosides show a different structure–activity relationship than do natural nucleosides. As with their natural congeners, in most of the cases, carbocyclic nucleosides need to be transformed to their triphosphates to exert their antiviral activity. In this activation, the generation of the monophosphate is normally the limiting step. One extensively used and successful strategy to overcome this first phosphorylation is the preparation of the phosphonate derivatives of the nucleoside. It has been demonstrated that, for the generation of a biological active compound, the replacement of the monophosphate moeity should be as well isosteric as isoelectronic, and the $OCH_2P(O)(OH)_2$ moeity fulfills both requirements.[2-4] Despite the fact that carbocyclic nucleosides have been extensively studied, very few efforts have been directed toward the synthesis of six-membered carbocyclic nucleosides,[5-7] and only one phosphonate derivative of a six-membered carbocyclic nucleoside has recently been reported.[8]

As a continuation of our work on biologically active 1,4-substituted six-membered nucleosides,[9-11] we considered of interest the synthesis and antiviral evaluation of 1,4-*cis*-substituted cyclohexenyl nucleosides of formula **1**, and their cyclohexanyl counterparts **2**, both series functionalized with a phosphonomethoxy moiety at their C-4′ position (Figure 1). These molecules can be considered isosteres of monophosphates of 1,4-substituted carbocyclic six-membered nucleosides.

Following a convergent approach for the synthesis of the cyclohexenyl nucleosides **1**, we envisioned two possible strategies (Scheme 1): (a) Pd(O)-catalyzed alkylation of heterocyclic bases by the convenient allylic acetate or epoxide,[12] followed by introduction of the phosphonomethyl moiety (pathway A); (b) Mitsunobu-type condensations[13] of the bases on the corresponding carbocyclic alcohol (pathway B). Attempts to follow the first approach by introducing the heterocyclic base on 3,4-epoxycyclohexene mediated by Pd(O) complexes [pathway A(a)] were disappointing due to the low yield of this reaction (15–20%) and the difficult purification of the intermediates. These results contrast with some recent reports where this strategy has been successfully used to obtain adenine[6] or thymine[7] cyclohexenyl nucleosides.

Therefore we turned our attention to the Mitsunobu-type condensation for introduction of the base moiety. Although this strategy has been used recently in acyclic[14] and cyclopentyl[15] systems, no use of this reaction in

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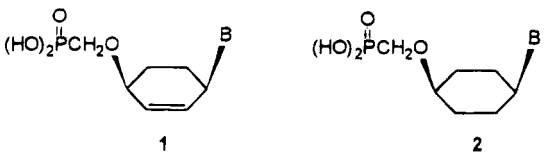
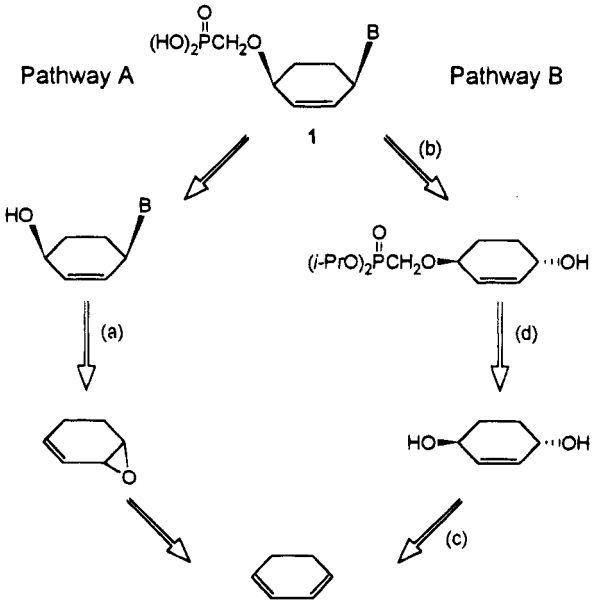


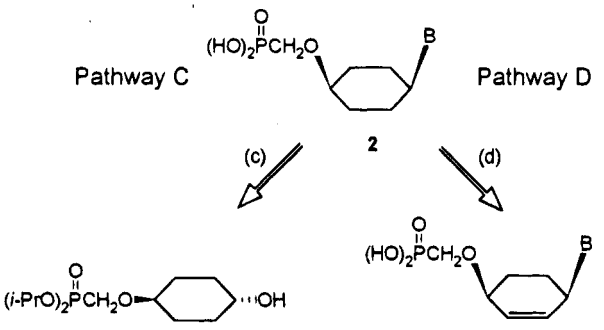
a: thymin-1-yl; b: cytosin-1-yl; c: guanin-9-yl; d: adenin-9-yl

Figure 1.

Scheme 1



Scheme 2



cyclohexyl systems has been reported. As Mitsunobu-type condensations normally proceed with inversion of configuration [pathway B(b)], a 1,4-*trans*-diol is required as starting material [pathway B(c)]. In order to be able to synthesize the different carbocyclic nucleosides from a common synthon, we preferred to introduce the phosphonomethyl moiety onto the diol prior to the Mitsunobu coupling [pathway B(d)]. In a similar way, the cyclohexanyl series **2** could be obtained by carrying out the Mitsunobu-type condensation on the corresponding alcohol (Scheme 2, pathway C). However, when the Mitsunobu coupling was performed on this saturated alcohol, yields were lower than when the reaction was carried out on the unsaturated alcohol (52% with thymine on cyclohexanyl alcohol versus 88% on the cyclohexenyl alcohol). This may be explained by the higher reactivity of allylic alcohols in nucleophilic substitution reactions. Therefore, it seems to be more convenient to synthesize the cyclohexanyl nucleosides **2** by reduction of the 2′,3′-double bond of their cyclohexenyl congeners (pathway D).

Results and Discussion

The starting material used for the synthesis of the carbocyclic nucleoside phosphonates, 1,4-*trans*-cyclohexenediol (**3**), was obtained from 1,3-cyclohexadiene following a described procedure.[16] Attempts to introduce directly the phosphonomethyl moiety on this symmetric alcohol, by generation of the alkoxide and treatment with 1.1 equiv of diisopropyl[(*p*-toluenesulfonyl)oxy]methanephosphonate,[17] gave **4** in very poor yields (Scheme 3). Therefore, the diol **3** was monoprotected with an acid labile protecting group prior to alkylation. Reaction of **3** with trityl chloride, triethylamine (Et_3N), and catalytic DMAP in CH_2Cl_2 provided the monotritylated compound, which was then treated with NaH and the above mentioned tosylate in DMF. The trityl protecting group was removed by heating at 60 °C in 80% AcOH. This three-step procedure afforded the alcohol **4** in 22% overall yield.

Condensation of **4** (1 equiv) with N^3-benzoylthymine[18] (2 equiv) in the presence of triphenylphosphine (Ph_3P, 2 equiv) and DEAD (2 equiv) in dioxane at room temperature, followed by debenzoylation with methanolic ammonia, afforded the N-1-alkylated derivative **5** in 54% yield, together with 23% of the less polar O-2-isomer **6**. The structure determination of both isomers was based on their NMR spectra (^1H and ^{13}C). For the O-2-isomer **6**, the signals corresponding to H-1′ and C-1′ (cyclohexene moiety)[19] and the signals corresponding to C-2, C-5, and C-6 (pyrimidine base moiety) are shifted downfield relative to the same signals in the N-1 isomer. These data agree with those reported in the literature.[20]

When the condensation was performed with N^4-benzoylcytosine,[21] using the same reaction circumstances, the desired N-1 alkylated compound was obtained in very low yield (8%), the O-2 isomer being the major product. This observation sustained our strategy to synthesize the cytosine derivative through transformation of the uracil analogue. Reaction of **4** with N^3-benzoyluracil,[18] Ph_3P, and DEAD, followed by debenzoylation, afforded the N-1 isomer **7** (53% yield) as the major compound and the O-2

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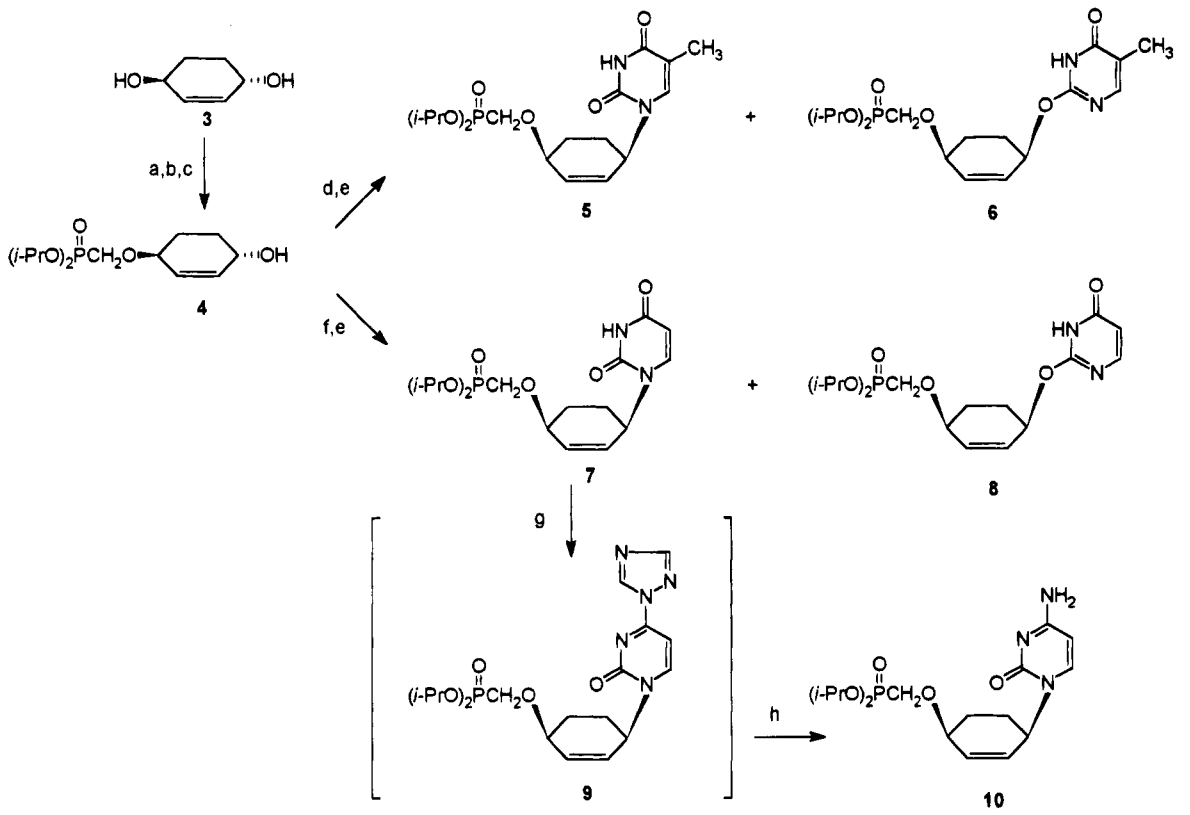
Scheme 3[a]



[a] (a) Ph_3CCl, Et_3N, DMAP (cat.), CH_2Cl_2, rt; (b) $(i\text{-PrO})_2POCH_2OTs$, NaH, DMF, −20 °C to rt; (c) 80% AcOH 60 °C; (d) N^3-benzoylthymine, Ph_3P, DEAD, dioxane, rt; (e) $MeOH/NH_3$; (f) N^3-benzoyluracil, Ph_3P, DEAD, dioxane, rt; (g) $POCl_3$, 1,2,4-triazole, Et_3N, MeCN, 0 °C to rt; (h) NH_4OH, dioxane, rt.

isomer **8** as a minor compound (15%). The assignment of both structures was done analogously to that described for the thymine derivatives. Reaction of **7** with $POCl_3$ and 1,2,4-triazole in Et_3N and acetonitrile[22] afforded the intermediate 1,2,4-triazol-1-yl derivative **9** which was treated with NH_4OH in dioxane. The cytosine derivative **10** was obtained in 22% yield from the alcohol **4**.

For the synthesis of the guanine analogue by Mitsunobu reaction (Scheme 4), the use of the commercially available 2-amino-6-chloropurine instead of N^2-isobutyryl-O^6-[(*p*-nitrophenyl)ethyl]guanine seems to be more attractive.[14,23] Reaction of **4** with 2-amino-6-chloropurine under the above mentioned conditions afforded **11** in 35% yield. The UV spectroscopic λ_{max} value and the chemical shifts in the ^1H and ^{13}C NMR spectra clearly indicate N-9 substitution for **11**.[23] The transformation of **11** into the guanine analogue **12** was accomplished by treatment with trifluoroacetic acid:H_2O (3:1)[24] for two days at room temperature (73%).

Adenine derivatives can be generally obtained from the 6-chloropurine analogues. Reaction of **4** with 6-chloropurine yielded the N-9 isomer **13** in 40% yield together with the N-7 (10%) and a third compound, probably the N-3 isomer (5%). Similar results have been reported for the coupling reaction of 6-chloropurine with *cis*-2-cyclopentene-1,4-diol mono-*tert*-butyldimethylsilyl ether.[15b] As alternative, the condensation reaction using N^6-benzoyladenine,[25] that to our knowledge has not been used under Mitsunobu conditions, was carried out. This reaction, followed by deprotection with $MeOH/NH_3$ afforded the N-9 isomer of adenine **14** in 22% yield. Although the yield is low, the advantage of obtaining directly the adenine nucleoside convinced us to select this strategy for the synthesis of the cyclohexenyl nucleoside. The chloropurine nucleoside **13** was used to synthesize the cyclohexanyl congener. Thus, the 6-chloro atom of **13** was substituted by an azido group on treatment with NaN_3 in DMF at 70 °C, giving **15**, which was hydrogenated to yield the saturated adenine derivative **16** in 45% yield from **13**.

The diisopropyl ester functions of the phosphonates **5**, **10**, **12**, and **14** were hydrolyzed by reaction with trimethylsilyl bromide (Me_3SiBr) in DMF followed by treatment with aqueous NH_4OH (Scheme 5). The unsaturated carbocyclic nucleosides **1a–d** were isolated as monoammonium salts after purification on an XAD column and a DEAE Sephadex column. The corresponding cyclohexanyl derivatives **2a–c** were obtained by hydrogenation of the cyclohexenyl congeners (**5**, **10**, **12**) on 10% Pd/C, followed by deprotection of the phosphonate group and purification. The adenine derivative **2c** was directly obtained by deprotection of **16**.

The 1,4-*cis* substitution pattern could be confirmed from the coupling constants observed in the ^1H NMR spectra of the cyclohexanyl derivatives **2a–d**. Selective decoupling experiments carried out in **2a** indicate that

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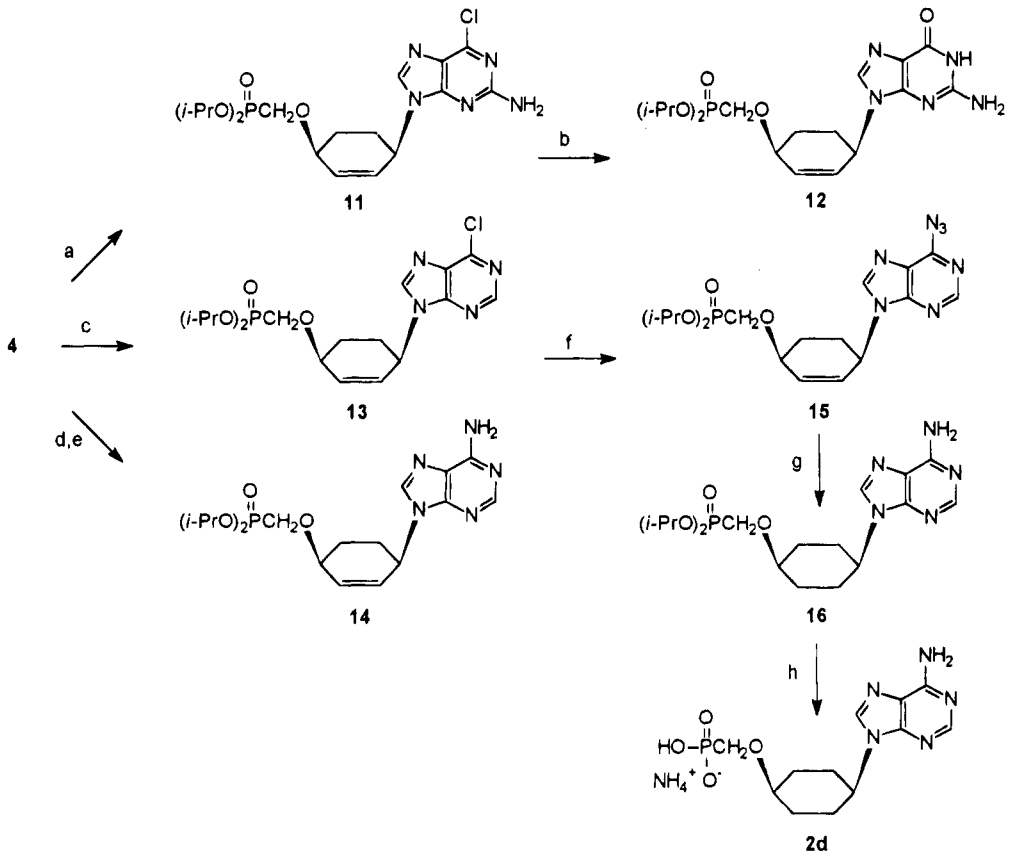
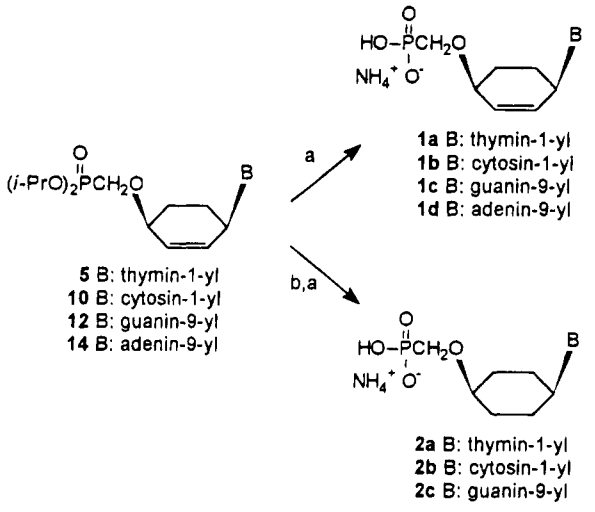
Scheme 4[a]



[a] (a) 6-Chloro-2-aminopurine, Ph_3P, DEAD, dioxane, rt; (b) CF_3COOH:H_2O (3:1), rt; (c) 6-chloropurine, Ph_3P, DEAD, dioxane, rt; (d) N^6-benzoyladenine, PH_3P, DEAD, dioxane, rt; (e) NH_3/MeOH; (f) NaN_3, DMF, 70 °C; (g) 10% Pd/C, H_2, EtOH, rt; (h) Me_3SiBr, DMF.

Scheme 5[a]



[a] (a) Me_3SiBr, DMF; (b) 10% Pd/C, H_2, EtOH.

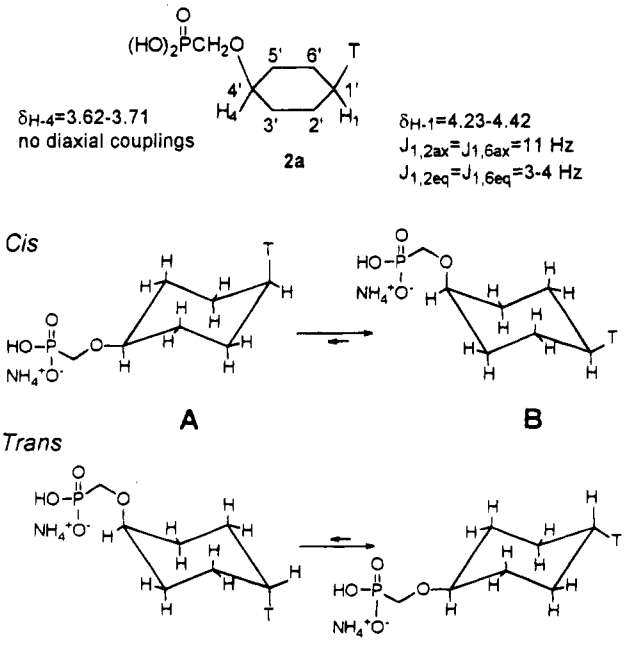


Figure 2.

the signal at 4.32 ppm in the ^1H NMR spectrum corresponds to the proton attached to the carbon that resonates at 57.4 ppm in ^{13}C NMR, that is C-1′ (C–N). So, this signal at 4.32 ppm is assigned to H-1′ and appears as a double triplet with coupling constants of 11.0 and 3.4 Hz, while H-4′ appears as a narrow multiplet at 3.67 ppm. These coupling constants suggest an axial orientation of H-1′ with large diaxial coupling constants ($J_{1',2'ax} \simeq J_{1',6'ax} \simeq 11$ Hz, $J_{1',2'eq} \simeq J_{1',6'eq} \simeq 3-4$ Hz) and an equatorial orientation of H-4′ with small coupling constants ($J_{3',4'}$ and $J_{4',5'}$). These values fit well with the more favorable B conformer of **2a** (Figure 2), having the heterocyclic moiety in an equatorial orientation. In the case of a 1,4-*trans* relationship, large diaxial coupling constants for both H-1′ and H-4′ should be expected. A similar pattern for both protons is observed in the other structures of this series (**2b–d**). As these cyclohexanyl nucleosides were obtained from their cyclohexenyl analogues, the 1,4-*cis* substitution is also confirmed for the

laters (**5**, **10**, **12** and **14**) and for their deprotected analogues **1a–d**.

The equatorial orientation of the base moiety in the cyclohexanyl nucleosides **2a–d** as deduced from their ^{1}H NMR coupling constants contrast with the axial orientation of the heterocyclic base observed in a related series of compounds, namely the 4-hydroxylated 1,5-anhydrohexitol nucleosides.[10] The results obtained here seem to indicate the importance of 1,3-dipolar interactions in the previously described 1,5-anhydronucleosides to force the base moiety into an axial orientation. This interaction is not present in the cyclohexanyl nucleosides reported here. Further experiments including NMR dynamics and molecular modeling are being carried out to clarify this issue.

Conclusion

Mitsunobu-type condensations have recently been reported as a useful tool in the synthesis of five-membered carbocyclic nucleosides.[15] We have now successfully applied this procedure for the synthesis of 1,4-*cis* substituted six-membered carbocyclic nucleosides, both purine and pyrimidines derivatives. In all cases, except for cytosine, the natural substituted derivatives (N-9 for purines and N-1 for pyrimidines) were obtained as the major compounds. Due to the higher reactivity of allylic alcohols in SN reactions, cyclohexanyl nucleosides, as exemplified by **2a–d**, are best obtained from their cyclohexenyl counterparts (**1a–d**).[26]

Experimental Section

Analytical instruments used were described previously.[10] Liquid secondary ion mass spectra (LSIMS) were obtained using glycerol (GLY) or thioglycerol (THGLY) as matrix. Column chromatography was performed on silica gel (0.060–0.200 nm and 0.030–0.750 nm). Sephadex A-25 (HCO_3^--form) was used for ion-exchange chromatography. Organic extracts were dried on $MgSO_4$.

(±)-*trans*-4-[(Diisopropoxyphosphinyl)methoxy]-2-cyclohexenol (4). To a suspension of *trans*-2-cyclohexene-1,4-diol[16] (**3**) (2.28 g, 20 mmol) in dry CH_2Cl_2 (80 mL) placed in an ice-bath were added Et_3N (3.09 mL, 22 mol), 4-dimethylaminopyridine (97 mg, 0.8 mmol) and trityl chloride (6.13 g, 22 mmol). The resulting mixture was stirred at room temperature for 2 days. Then CH_2Cl_2 (100 mL) and H_2O (100 mL) were added. The aqueous phase was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic extract was washed with brine and dried. After filtration and evaporation the residue was purified by column chromatography [(1) hexane–EtOAc (8:1), (2) hexane–EtOAc (5:1)] to yield 4 g (11.4 mol) (51%) of the monoprotected diol. A solution of this and diisopropyl[(*p*-toluenesulfonyl)oxy]methanephosphonate (6.1 g, 17 mmol) in dry DMF (60 mL) was cooled to −20 °C. Then, NaH (60% suspension, 1.0 g, 25 mol) was added and the reaction was allowed to reach room temperature and stirred overnight. The reaction mixture was neutralized with acetic acid, evaporated, coevaporated with xylene, and treated with 80% acetic acid at 60 °C for 30 min. The reaction was quenched with ethanol and evaporated. The residue was taken up into brine (50 mL) and extracted with EtOAc (200 mL). The organic phase was dried, filtered, and evaporated. Purification by column chromatography [CH_2Cl_2:MeOH (100:1.5)] afforded 1.4 g (42%) of **4** as a syrup. LSIMS (GLY) 293 $(M + H)^-$. HRMS calcd for $C_{13}H_{26}O_5P$ $(M + H)^-$ 293.1516, found 293.1532. ^{1}H NMR ($CDCl_3$) δ 3.74 (2H, $J_{H,P}$ = 9.3 Hz), 4.07 (1H), 4.24 (1H), 4.75 (2H), 5.85 (2H). ^{13}C NMR ($CDCl_3$) δ 62.5 ($J_{C,P}$ = 170 Hz), 65.9, 71.0 ($J_{C,P}$ = 7 Hz), 75.4 ($J_{C,P}$ = 12 Hz), 129.2, 133.9.

General Procedure for the Condensation of 4 with Heterocyclic Bases. To a suspension containing the alcohol **4** (292 mg, 1 mmol), Ph_3P (525 mg, 2 mmol), and the heterocyclic base (2 mmol) in dry dioxane (10 mL) was slowly added a solution of DEAD (0.31 mL, 2 mmol) in dioxane (3 mL). The mixture was stirred at room temperature for 2–16 h. Volatiles were removed, and the residue was either directly purified by column chromatography or purified after deprotection.

(±)-1-{*cis*-4′-[(Diisopropyloxyphosphonyl)methoxy]-2′-cyclohexenyl}thymine (5) and 2-O-isomer 6. Reaction of the alcohol **4** with N^3-benzoylthymine (460 mg, 2 mmol) following the above described procedure gave a residue that was directly debenzoylated by treatment with methanolic ammonia (24 h). Evaporation of the volatiles and purification by flash column chromatography [(1)CH_2Cl_2:MeOH (99:1), (2) CH_2Cl_2:MeOH (98:2)] yielded 216 g (54%) of the N-1 isomer **5** and 92 g of the O-2 isomer **6** (23%). For **5**:LSIMS (THGLY) 401 $(M + H^+)$, 205. HRMS calcd for $C_{18}H_{30}N_2O_6P$ $(M + H^+)$ 401.1838, found 401.1848. UV λ_{max} (MeOH) = 272 nm (ϵ = 15 300). ^{1}H NMR ($CDCl_3$) δ 1.84 (3H, s), 3.75 (2H, $J_{C,P}$ = 9.1 Hz), 3.93 (1H), 4.70 (2H), 5.11 (1H), 5.65 (1H, dd, $J_{2',3'}$ = 10 Hz, $J_{1',2'}$ = 2.3 Hz), 6.18 (1H), 7.01 (1H, s), 9.90 (1H, br s). ^{13}C NMR ($CDCl_3$) δ 12.8, 51.6, 64.1 ($J_{C,P}$ = 170 Hz), 71.5 ($J_{C,P}$ = 6.3 Hz), 72.7 ($J_{C,P}$ = 11 Hz), 111.4, 130.4, 133.0, 137.5, 151.7, 164.7. For **6**: LSIMS (THGLY) 401 $(M + H^+)$, 275, 191. UV λ_{max} (MeOH) = 272 nm. ^{1}H NMR ($CDCl_3$) δ 1.97 (3H, d, J = 1.0 Hz), 3.77 (2H), 3.99 (1H), 4.75 (2H), 5.42 (1H), 5.97–6.07 (2H), 7.53 (1H, d). ^{13}C NMR ($CDCl_3$) δ 12.8, 63.5 ($J_{C,P}$ = 169 Hz), 71.4, 71.6 ($J_{C,P}$ = 6.7 Hz), 74.7 ($J_{C,P}$ = 11.6 Hz), 118.1, 128.7, 132.9, 151.4, 155.4, 164.9.

(±)-1-{*cis*-4′-[(Diisopropyloxyphosphonyl)methoxy]-2′-cyclohexenyl}uracil (7) and 2-*O*-Isomer 8. Reaction of **4** with N^3-benzoyluracil (433 mg, 2 mmol) followed by treatment with methanolic ammonia afforded a residue that was dissolved in CH_2Cl_2, filtrated through Celite, and purified by flash column chromatography [(1) CH_2Cl_2:MeOH (99:1), (2) CH_2Cl_2:MeOH (98:2), (3) CH_2Cl_2:MeOH (97:3)]. A 204 mg (53%) amount of **7** was obtained as a syrup, followed by 57 mg (15%) of the O-2-isomer **8**. For **7**: LSIMS (THGLY) 387 $(M + H^+)$, 191, 113. UV λ_{max} (MeOH) = 266 nm. ^{1}H NMR ($CDCl_3$) δ 3.77 (2H, $J_{H,P}$ = 9.2 Hz), 3.98 (1H), 4.76 (2H), 5.15 (1H), 5.69 (2H), 6.26 (1H, $J_{2',3'}$ = 10 Hz), 7.27 (1H, $J_{5,6}$ = 8 Hz), 9.29 (1H, br s). ^{13}C NMR ($CDCl_3$) δ 51.8, 64.1 ($J_{C,P}$ = 169 Hz), 71.6 ($J_{C,P}$ = 6 Hz), 72.8 ($J_{C,P}$ = 11 Hz), 102.8, 129.6, 133.8, 141.8, 151.4, 163.5. For **8**: LSIMS (THGLY) 387 $(M + H^-)$, 275, 191, 113. UV λ_{max} (MeOH) = 269 nm. ^{1}H NMR ($CDCl_3$) δ 3.72 (2H, $J_{H,P}$ = 9.3 Hz), 3.97 (1H), 4.73 (2H), 5.44 (1H), 6.08–5.96 (3H), 7.67 (1H, $J_{5,6}$ = 6.6 Hz). ^{13}C NMR ($CDCl_3$) δ 63.4 ($J_{C,P}$ = 170 Hz), 71.7 ($J_{C,P}$ = 6 Hz), 74.8 ($J_{C,P}$ = 12 Hz), 109.4, 128.6, 132.9, 155.4, 157.4, 165.7.

(±)-1-{*cis*-4′-[(Diisopropoxyphosphinyl)methoxy]-2′-cyclohexenyl}cytosine (10). A suspension of 1,2,4-triazole (660 mg, 9.5 mol) and $POCl_3$ (0.29 mL, 2.0 mmol) in dry acetonitrile (4 mL) was stirred at 0 °C for 5 min. Then Et_3N (1.5 mL) was slowly added. The resulting mixture was stirred at 0 °C for 1 h and then a solution of **7** (250 mg, 0.65 mmol) in CH_3CN (2 mL) was added. The mixture was stirred at room temperature for 5 h and then filtered. The filtrate was diluted with EtOAc (50 mL), washed with aqueous $NaHCO_3$ (25 mL), brine (25 mL), dried, filtered, and evaporated and coevaporated with dioxane. The residual oil was dissolved in dioxane (4 mL) and treated with NH_4OH (4 mL). After 6 h, solvents were removed and the residue was purified by flash column chromatography [(1) CH_2Cl_2:MeOH (98:2), (2) CH_2Cl_2:MeOH (92:8)] to afford 100 mg (40%) of **10** as a foam. LSIMS (GLY) 386 $(M + H^+)$, 190, 112. HRMS calcd for $C_{17}H_{29}N_3O_5P$ $(M + H)^+$ 386.1841, found 386.1841. UV λ_{max} (MeOH) = 276 nm (ϵ = 13 600). ^{1}H NMR ($CDCl_3$) δ 3.73 (2H, $J_{H,P}$ = 9.4 Hz), 3.96 (1H), 4.72 (2H), 5.19 (1H), 5.67 (1H, dd, $J_{1',2'}$ = 2.5, $J_{2',3'}$ = 10 Hz), 5.80 (1H, d, $J_{5,6}$ = 7.3 Hz), 6.16 (1H), 7.26 (1H, d). ^{13}C NMR ($CDCl_3$) δ 52.1, 64.0 ($J_{C,P}$ = 170 Hz), 71.6 ($J_{C,P}$ = 6 Hz), 73.6 ($J_{C,P}$ = 12 Hz), 95.2, 130.7, 132.8, 142.8, 157.0, 166.2.

(26) When tested for inhibitory activity against herpes viruses (HSV-1, HSV-2, VZV, CMV) and HIV replication, compounds **1a–d** and **2a–d** were found inactive at concentrations up to 50 μg/mL.

(±)-2-Amino-6-chloro-9-{*cis*-4′-[(diisopropoxyphosphinyl)methoxy]-2′-cyclohexenyl}purine (11). Following the general procedure, **4** reacted with 2-amino-6-chloropurine (340 mg, 2 mmol). Solvents were removed, and the residue was taken up into EtOAc, and filtered through Celite, and then purified by flash column chromatography [(1) CH_2Cl_2:MeOH (99:1), (2) CH_2Cl_2:MeOH (98:2)] to afford 155 mg (35%) of **11** as a syrup. LSIMS (THGLY) 444 ($M + H^+$), 191, 170. UV (MeOH) λ_{max} = 249 and 311 nm. ^1H NMR ($CDCl_3$) δ 3.83 (2H, $J_{P,H}$ = 9.4 Hz), 4.08 (1H), 4.75 (2H), 4.98 (1H), 5.37 (2H, br s), 5.86 (1H, dd, $J_{1',2'}$ = 2.2, $J_{2',3'}$ = 10 Hz), 6.23 (1H), 7.78 (1H s). ^{13}C NMR ($CDCl_3$) δ 49.8, 63.3 ($J_{C,P}$ = 170 Hz), 71.2 ($J_{C,P}$ = 5 Hz), 72.9 ($J_{C,P}$ = 13 Hz), 125.4, 128.1, 132.7, 140.9, 151.3, 153.3, 159.0.

(±)-9-{*cis*-4′-[(Diisopropylphosphinyl)methoxy]-2′-cyclohexenyl}guanine (12). A solution of **11** (200 mg, 0.45 mmol) in CF_3COOH:H_2O (3:1) (4 mL) was stirred at room temperature for 2 days. Solvents were evaporated and coevaporated with H_2O. The residue was cooled and treated with MeOH:NH_4OH (10:1) (15 mL) and evaporated and purified by flash column chromatography [(1) CH_2Cl_2:MeOH (95:5), (2) CH_2Cl_2:MeOH (90:10)] to yield 140 mg (73%) of **12** as an amorphous solid. LSIMS (THGLY) 448 ($M + Na)^+$. HRMS calcd for $C_{18}H_{29}N_5O_5P$ ($M + H)^+$ 426.1901, found 426.1923. UV (MeOH) λ_{max} = 255 nm (ϵ = 17 000). ^1H NMR (DMSO-d_6) δ 3.86 (2H, d, $J_{C,P}$ = 9 Hz), 4.02 (2H), 4.62 (2H), 4.82 (1H), 5.94 (1H, dd, $J_{2',3'}$ = 10 Hz), 6.13 (1H), 6.51 (2H, br s), 7.48 (1H, s). ^{13}C NMR (DMSO-d_6) δ 49.0, 62.5 ($J_{C,P}$ = 165 Hz), 70.4 ($J_{C,P}$ = 6 Hz), 72.8 ($J_{C,P}$ = 12 Hz), 116.8, 128.9, 131.9, 135.1, 150.9, 153.8, 157.0.

(±)-9-{*cis*-4′-[(Diisopropyloxyphosphinyl)methoxy]-2′-cyclohexenyl}-6-chloropurine (13). According to the general procedure, **4** was reacted with 6-chloropurine (309 mg, 3 mmol) for 2 h at room temperature. Volatiles were removed, and the residue was purified by flash column chromatography [(1) CH_2Cl_2:MeOH (99:1), (2) CH_2Cl_2:MeOH (98:2)] to afford 340 mg (40%) of **13**. LSIMS (THGLY) 429 ($M + M)^+$ 233, 191, 155. HRMS calcd for $C_{18}H_{27}N_4O_4PCl$ ($M + H)^+$ 429.1454, found 429.1457. UV (MeOH) λ_{max} = 266 nm. ^1H NMR ($CDCl_3$) δ 3.80 (2H), 4.10 (1H), 4.75 (2H), 5.27 (1H), 5.93 (1H, dd, $J_{1',2'}$ = 2.3, $J_{2',3'}$ = 9.9 Hz), 6.33 (1H), 8.16, 8.75 (each 1H, 2 × s). ^{13}C NMR ($CDCl_3$) δ 50.6, 64.1 ($J_{C,P}$ = 169 Hz), 71.6 ($J_{C,P}$ = 6.4 Hz), 73.7 ($J_{C,P}$ = 11 Hz), 127.5, 132.3, 134.4, 144.3, 151.5, 151.8, 152.3.

(±)-9-{*cis*-4′-[(Diisopropylphosphinyl)methoxy]-2′-cyclohexenyl}adenine (14). Reaction of the alcohol **4** (1 mmol) with N^6-benzoyladenine (478 mg, 2 mmol) followed by deprotection with methanolic ammonia afforded a residue that was suspended in CH_2Cl_2, filtered through Celite, and purified by flash column chromatography [(1) CH_2Cl_2:MeOH (98:2), (2) CH_2Cl_2:MeOH (95:5)] giving 90 mg (22%) of **14** as an oil. LSIMS (THGLY) 410 ($M + H)^+$. HRMS calcd for $C_{18}H_{29}N_5O_4P$ ($M + H^+$) 410.1953, found 410.1963. UV (MeOH) λ_{max} = 262 nm. ^1H NMR ($CDCl_3$) δ 3.82 (2H), 4.08 (1H), 4.77 (2H), 5.20 (1H), 5.87 (2H, br s), 5.94 (1H, dd, $J_{1',2'}$ = 2.5, $J_{2',3'}$ = 9.8 Hz), 6.27 (1H), 7.86, 8.35 (each 1H, 2 × s). ^{13}C NMR ($CDCl_3$) δ 49.7, 64.0 ($J_{C,P}$ = 169 Hz), 71.7 ($J_{C,P}$ = 6 Hz), 74.1 ($J_{C,P}$ = 11 Hz), 120.2, 128.4, 133.5, 139.5, 150.0, 153.4, 156.1.

(±)-9-{*cis*-4′-[(Diisopropoxyphosphinyl)methoxy]cyclohexanyl}adenine (16). To a solution of **13** (300 mg, 0.7 mmol) in dry DMF (6 mL) was added NaN_3 (46 mg, 0.7 mmol), and the mixture was heated at 70 °C for 3 h. Solvent was removed and the residue was coevaporated with xylene and passed through a silica gel column and eluted with CH_2Cl_2:MeOH (98:2), to give 270 mg of crude **15** [LSIMS (THGLY) 436 ($M + H^+$), 375, 291, 249, 207]. This oil was dissolved in EtOH (15 mL) and hydrogenated in the presence of 10% Pd/C (100 mg) at room temperature for 5 h. The mixture was filtrated and evaporated. Purification by flash column chromatography [(1) CH_2Cl_2:MeOH (95:5), (2) CH_2Cl_2:MeOH (90:10)] afforded 130 mg (45%) of **16** as an oil. LSIMS (THGLY) 412 ($M + H)^+$, 328, 216. HRMS calcd for $C_{18}H_{31}N_5O_4P$ ($M + H)^+$ 412.2109, found 412.2124. UV (MeOH) λ_{max} = 262 nm. ^1H NMR ($CDCl_3$) δ 3.71 (3H, $J_{H,P}$ = 9.4 Hz), 4.48 (1H, $J_{1',2'ax} \simeq J_{1',6'ax} \simeq$ 12 Hz, $J_{1',2'eq} \simeq J_{1',6'eq} \simeq$ 4 Hz), 4.77 (2H), 6.14 (2H, br s), 7.86 (1H, s), 8.31 (1H, s). ^{13}C NMR ($CDCl_3$) δ 53.4, 63.5 ($J_{C,P}$ = 170 Hz), 71.5 ($J_{C,P}$ = 6.3 Hz), 74.5 ($J_{C,P}$ = 12 Hz), 120.0, 138.4, 150.0, 153.2, 156.1.

General Procedure for the Deprotection of Phosphonate Ester. The protected phosphonate (0.3–0.5 mmol) was dissolved in DMF (3 mL) and treated with Me_3SiBr bromide (0.3 mL) for 20–48 h. The reaction was cooled and treated with NH_4OH (4 mL) for 6 h. Solvents were removed, and the residue was applied on a XAD-column and eluted with H_2O and H_2O:MeOH (70:30). UV positive fractions were collected, evaporated and purified by DEAD Sephadex A25 (HCO_3^- form) eluting with a gradient H_2O–0.1 M NH_4HCO_3. Appropriate fractions were evaporated, coevaporated with water, and lyophilized.

(±)-1-[*cis*-4′-(Phosphonomethoxy)-2′-cyclohexenyl]thymine Ammonium Salt (1a). Compound **5** (100 mg, 0.25 mmol) was deprotected and isolated as the ammonium salt following the general procedure (57 mg, 69%). LSIMS (THGLY) 315 ($M - H)^-$, HRMS calcd for $C_{12}H_{16}N_2O_6P$ 315.0743, found 315.0758. UV (H_2O) λ_{max} = 274 nm (ϵ = 11 600). ^1H NMR (D_2O) 1.64–1.80 (7H), 3.56 (2H, $J_{H,P}$ = 8.5 Hz), 3.92 (1H), 4.86 (1H), 5.65 (1H, dd, $J_{1',2'}$ = 10 Hz), 6.13 (1H), 7.40 (1H, s). ^{13}C NMR (D_2O) δ 14.0, 26.3, 26.6, 54.8, 67.5 ($J_{C,P}$ = 158 Hz), 75.5 ($J_{C,P}$ = 11 Hz), 113.5, 131.7, 135.5, 142.9, 155.0, 169.5.

(±)-1-[*cis*-4′-(Phosphonomethoxy)-2′-cyclohexenyl]cytosine Ammonium Salt (1b). Compound **10** (90 mg, 0.23 mmol) after deprotection and purification yielded **18** (30 mg, 42%) as a white lyophilate. LSIMS (GLY) 300 $(M-H)^-$. HRMS calcd for $C_{11}H_{15}N_3O_5P$ $(M-H)^-$ 300.0746, found 300.0740. UV (H_2O) λ_{max} = 279 nm (ϵ = 13 060). ^1H NMR (D_2O) 1.72–1.85 (4H), 3.60 (2H, d, $J_{H,P}$ = 9.2 Hz), 3.98 (1H), 4.97 (1H), 5.68 (1H, dd, $J_{2',3'}$ = 10, $J_{1',2'}$ = 3.3 Hz), 6.01 (1H, d, $J_{5,6}$ = 7.7 Hz), 6.22 (1H), 7.74 (1H, d). ^{13}C NMR (D_2O) δ 26.1, 26.8, 55.7, 67.3 ($J_{C,P}$ = 157 Hz), 75.8 ($J_{C,P}$ = 11 Hz), 97.6, 130.6, 136.7, 148.9, 154.6, 163.9.

(±)-9-[*cis*-4′-(Phosphonomethoxy)-2′-cyclohexenyl]guanine Ammonium Salt (1c). Compound **12** (120 mg, 0.28 mmol) was deprotected and purified following the general procedure to yield 46 mg (46%) of **1c**. LSIMS (THGLY) 340 $(M - H)^-$. HRMS calcd for $C_{12}H_{15}N_5O_5P$ $(M - H)^-$ 340.0806, found 340.0800. UV (H_2O) λ_{max} = 253 nm (ϵ = 13 200). ^1H NMR (D_2O) 1.70–1.96 (4H), 3.69 (2H, J = 9.1, 9.7 Hz), 4.09 (1H), 4.85 (1H), 5.90 (1H, dd, $J_{1',2'}$ = 2.5 Hz, $J_{2',3'}$ = 10.6 Hz), 6.23 (1H), 7.98 (1H, br s). ^{13}C NMR (D_2O) δ 26.3, 28.0, 52.7, 67.3 ($J_{C,P}$ = 158 Hz), 76.5 ($J_{C,P}$ = 11 Hz), 118.2, 130.5, 135.7, 141.2 (br s), C-4 could not be detected, 156.5, 161.3.

(±)-9-[*cis*-4′-(Phosphonomethoxy)-2′-cyclohexenyl]adenine Ammonium Salt (1d). Compound **14** (90 mg, 0.22 mmol) was deprotected and purified, as described in the general procedure to yield 52 mg (49%) of **1d** as a white lyophilate. HRMS calcd for $C_{12}H_{15}N_5O_4P$ $(M - H)^-$ 324.0857, found 324.0867. UV (H_2O) λ_{max} = 263 nm (ϵ = 15 100). ^1H NMR (D_2O) 1.5–2.0 (4H), 3.65 (2H), 4.08 (1H), 4.90 (1H), 5.89 (1H, dd, $J_{1',2'}$ = 2.7 Hz, $J_{2',3'}$ = 9.7 Hz), 6.23 (1H), 8.01, 8.03 (each 1H, 2 × s). ^{13}C NMR (D_2O) δ 26.1, 28.2, 52.8, 67.3 ($J_{C,P}$ = 157 Hz), 76.7 ($J_{C,P}$ = 10.4 Hz), 121.3, 121.3, 136.1, 143.9, 150.9, 154.2, 157.7.

(±)-[*cis*-4′-(Phosphonomethoxy)-2′-cyclohexanyl]thymine Ammonium Salt (2a). A solution of **5** (200 mg, 0.50 mol) in EtOH (15 mL) was hydrogenated in the presence of 10% Pd/C (100 mg) at 30 psi for 5 h. The mixture was filtered and passed through a short silica gel column eluting with EtOAc:MeOH (100:3). The residue obtained after evaporation of the appropriate fractions (110 mg) was dissolved in DMF (3 mL) and deprotected following the general procedure. After purification, 87 mg (52%) of **2a** were obtained as a white powder. LSIMS (THGLY) 317 $(M - H)^-$. HRMS calcd for $C_{12}H_{18}N_2O_6P$ $(M - H)^-$ 317.0899, found 317.0898. UV (H_2O) λ_{max} = 275 nm (ϵ = 11 330). ^1H NMR (D_2O) 1.60–2.18 (11H), 3.54 (2H, d, J = 9.3 Hz), 3.62–3.71 (1H), 4.32 (1H, $J_{1',2'ax} \simeq J_{1',2'ax} \simeq$ 12, $J_{1',2'eq} \simeq J_{1',6'eq} \simeq$ 4 Hz), 7.62 (1H, s). ^{13}C NMR (D_2O) δ 14.2, 27.7, 30.8, 57.4, 66.1 ($J_{C,P}$ = 159 Hz), 76.7 ($J_{C,P}$ = 11 Hz), 113.6, 142.5, 155.1, 169.4.

(±)-1-[*cis*-4′-(Phosphonomethoxy)cyclohexanyl]cytosine Ammonium Salt (2b). A solution of **10** (200 mg, 0.52 mmol) in EtOH (15 mL) was hydrogenated in the presence of 10% Pd/C (60 mg) at 30 psi for 4 h. The mixture was filtrated

and passed through a short silica gel column, eluting with EtOAc:MeOH (100:15). The residue obtained after coevaporation (115 mg) was dissolved in DMF (3 mL), deprotected with Me_3SiBr and purified following the general procedure, giving 62 mg (37% from **10**) of **2b** as a white lyophilate. LSIMS (THGLY) 302 $(M - H)^-$ HRMS calcd for $C_{11}H_{17}N_3O_5P$ $(M - H)^-$ 302.0902, found 302.0880. UV (H_2O) λ_{max} = 280 nm (ϵ = 12 100). ^1H NMR (D_2O) δ 1.4–2.2 (8H), 3.55 (2H, d, $J_{H,P}$ = 9.3 Hz), 3.63–3.72 (1H), 4.39 (1H, $J_{1',2'ax} \simeq J_{1',6'ax} \simeq 12$, $J_{1',2'eq} \simeq J_{1',6'eq} \simeq 3.7$ Hz), 6.08 (1H, d; $J_{5,6}$ = 7.7 Hz), 7.91 (1H, d). ^{13}C NMR (D_2O) δ 27.7, 30.7, 58.5, 66.4 ($J_{C,P}$ = 158 Hz), 76.5 ($J_{C,P}$ = 11 Hz), 97.7, 148.4, 154.6, 163.5.

(±)-9-[*cis*-4′-(Phosphonomethoxy)cyclohexanyl]guanine Ammonium Salt (2c). A solution of **12** (200 mg, 0.47 mmol) in EtOH (10 mL) was hydrogenated in the presence of 10% Pd/C (100 mg) at 30 psi for 4 h. The mixture was filtered, evaporated, and coevaporated with toluene. The residue was dissolved in DMF (3 mL) and treated with Me_3SiBr, following the general procedure for deprotection of phosphonate esters. After purification 88 mg (52%) of **2c** were obtained. LSIMS (THGLY) 342 $(M - H)^-$. HRMS calcd, for $C_{12}H_{17}N_5O_5P$ $(M - H)^-$ 342.0962, found 342.0957. UV (MeOH) λ_{max} = 253 nm (ϵ = 12 440). ^1H NMR (D_2O) δ 1.42–2.12 (8H), 3.57 (2H, d, $J_{H,P}$ = 9 Hz), 3.65–3.74 (1H), 4.05 (1H, $J_{1',2'ax} \simeq J_{1',6'ax} \simeq 11$, $J_{1',2'eq} \simeq J_{1'6'eq} \simeq 4$ Hz), 7.88 (1H, br s). ^{13}C NMR (D_2O) δ 29.1, 30.6, 56.2, 66.5 ($J_{C,P}$ = 158 Hz), 76.8 ($J_{C,P}$ = 11 Hz), 117.9, 140.1 (br s), C-4 could not be detected, 156.3, 161.2.

(±)-9-[*cis*-4′-(Phosphonomethoxy)cyclohexanyl]adenine Ammonium Salt (2d). Deprotection of **16** (80 mg, 0.20 mol) and purification following the general procedure afforded **2d** (47 mg, 68%) as a white lyophiliate. HRMS calcd for $C_{12}H_{17}N_5O_4P$ $(M - H)^-$ 326.1013, found 326.0987. UV (H_2O) λ_{max} = 263 nm (ϵ = 14 800). ^1H NMR (D_2O) δ 1.43–2.20 (8H), 3.39 (2H, d, $J_{H,P}$ = 9.2 Hz), 3.67–3.76 (1H), 4.21–4.39 (1H), 8.06, 8.20 (each 1H, 2 × s). ^{13}C NMR (D_2O) δ 29.1–30.5 (3 × s), 56.4, 68.1 ($J_{C,P}$ = 151 Hz), 76.1 ($J_{C,P}$ = 9.8 Hz), 120.8, 142.7, 150.6, 154.4, 157.7.

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Supplementary Material Available: ^{13}C NMR spectra for compounds **1a–d**, **2a–d**, **4–8**, **10–14**, and **16** and HRMS data for compounds **1a–d**, **2a–d**, **4**, **5**, **10**, **12–14**, and **16** (19 pages). This materials is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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