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Synthesis and Conformational Analysis of a Ribo-Type Cyclohexenyl Nucleoside

Sara Vijgen, Koen Nauwelaerts, Jing Wang, Arthur Van Aerschot, Irene Lagoja, and Piet Herdewijn*

Laboratory of Medicinal Chemistry, Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000, Leuven, Belgium

piet.herdewijn@rega.kuleuven.ac.be

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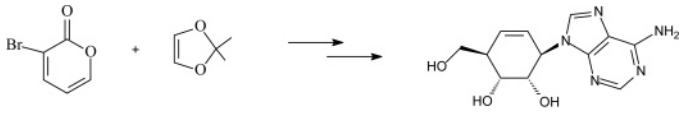

"S-type" conformation

A straightforward approach to a novel class of ribo-type cyclohexenyl nucleosides is described. An electron-demand Diels−Alder reaction forms the key-step of the chosen synthetic pathway. Although the difference is small, conformational analysis using NMR shows that this nucleoside analogue adopts preferentially an $^2H_3$ conformation (S-type), while the "deoxy" cyclohexenyl analogue has a preference for a C3′ *endo* conformation (N-type). Analyses of the conformational equilibrium reveal that, in the given experimental conditions, the difference between adenosine and its cyclohexenyl congener resides in their different ΔG values; furthermore, in adenosine, the conformational preference is of enthalpic origin, whereas in the cyclohexenyl congener, the conformational preference is of entropic origin.

Introduction

The discovery of the multiple functions of RNA (mRNA, ribozymes, ^{t}RNA, ribosomes, in replication and splicing, riboswitches, siRNA, and microRNA) has stimulated the search for synthetic RNA mimics. This is not an easy undertaking, as RNA has extensive structural (folding in a variety of tertiary structures) and functional versatility (catalysis of different chemical reactions). The way we approached the problem is by substituting the ring oxygen atom of the furanose sugar of a natural nucleoside by a double bond.[1−7] The presence of the double bond induces flexibility in the six-membered ring, similar to the flexibility of a furanose ring. We first synthesized a "2′-deoxy analogue" and demonstrated that such nucleosides can be potent antivirals,[1,6,8] and, when incorporated in oligonucleotides, they increase the thermal stability of DNA−RNA duplexes as well as the nuclease stability.[5] This motivates us to test cyclohexenyl nucleic acids (CeNA) in antisense experiments and in siRNA experiments. The conformational adaptability of a cyclohexenyl nucleoside to external agents was demonstrated by the observation that its conformation is different ($^3H_2$ or $^2H_3$) when incorporated in different double-stranded DNA sequences.[9] Moreover, CeNA hybridizes both with DNA and with RNA, although this effect is sequence-dependent.[5,10] Constitutionally, a "deoxy" cyclohexenyl nucleoside is a mimic of a natural deoxynucleoside. With respect to conformational preference and hybridization (a deoxycyclohexenyl nucleoside prefers the $^3H_2$ conformation), it is a mimic of a natural ribonucleoside. This difference in conformation of a deoxynucleoside and a deoxycyclohexenyl nucleoside can be ascribed to differences in stereo-

* Corresponding author. Phone: +3216337387. Fax: +3216337341.

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(8) Racemic cyclohexenyl-C shows an EC_{50} of 0.05 μg/mL against varicella-zoster virus in human embryonic lung cells, which is 10 times lower than the EC_{50} value of acyclovir (0.5 μg/mL) in the same test system.

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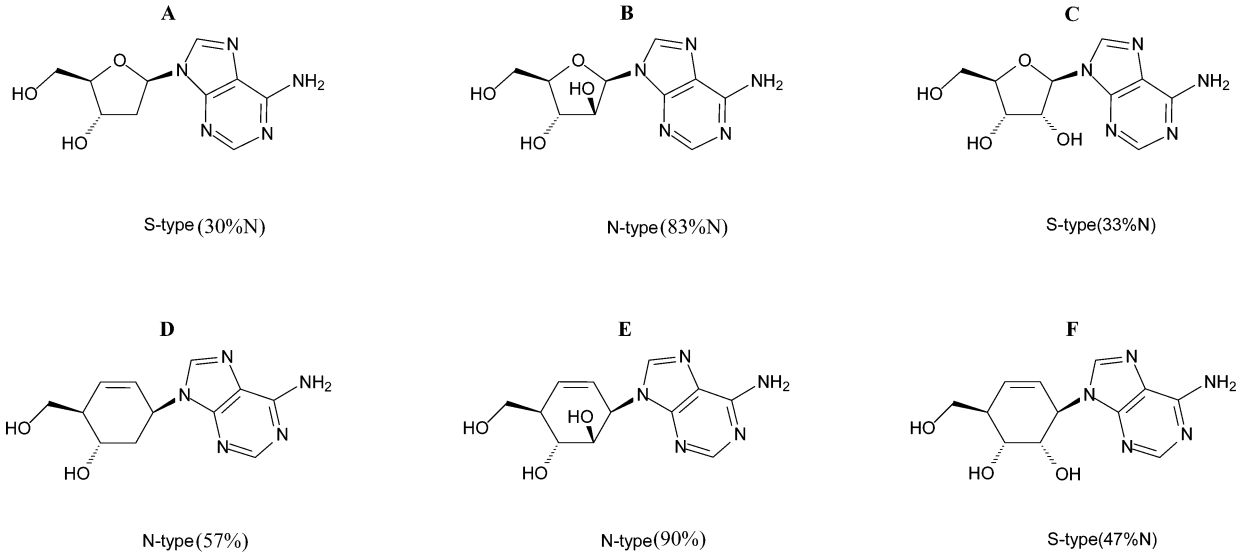
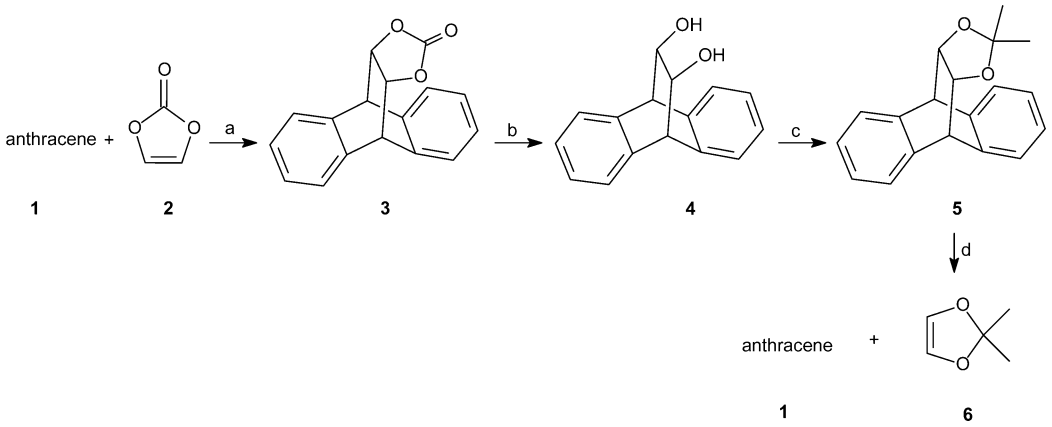


FIGURE 1. Structure of natural deoxy- (A), arabino- (B), and ribonucleosides (C) and their cyclohexenyl congeners (D−F). The % N-conformation (C3′ *endo*; $^3H_2$) of the "sugar" moiety (298 K) is indicated. The preferred conformations of the furanose nucleosides (A and C) in the solution state are described in the literature.[11] The % $^3H_2$-conformation of the cyclohexenyl nucleosides is derived from NMR coupling constants as given in Table 1. The % C3′ *endo* of compound B was calculated using the pseurot program[12] based on scalar coupling constants measured in a one-dimensional ^{1}H NMR experiment of compound B.

SCHEME 1. Synthesis of Dienophile 6 Starting from Anthracene 1[a]



[a] Conditions: (a) 1,2-dichlorobenzene, reflux, 1.5 days; (b) NaOH, MeOH/H_2O, reflux overnight; (c) $Me_2C(OMe)_2$, PTSA, rt, 24 h; (d) BHT, Δ.

electronic effects (anomeric effect, gauche effect, allylic effect) and steric effects. In continuation of the search for more "perfect" RNA mimics, we synthesized a "ribo"-type cyclohexenyl nucleoside with an adenine base moiety and studied its solution-state conformation using NMR. This modified nucleoside, having also a 2′- and a 3′-hydroxyl group, can be considered as a potential, functional, and constitutional analogue of natural adenosine. Here we describe its synthesis and conclude that introduction of an OH in the ribo position shifts the conformation (toward an $^2H_3$ conformation) in a way that is opposite to what has been observed in natural ribonucleosides.

Results and Discussion

The key step of the synthesis of ribocyclohexenyl adenosine **18** is an inverse-electron-demand Diels−Alder cycloaddition reaction[13,14] of 2,2-dimethyl-1,3-dioxole[15] (dienophile) **6** with 3-bromo-2*H*-pyran-2-one (diene) **10a** to construct a bicyclic intermediate **11**. 2,2-Dimethyl-1,3-dioxole **6** can be obtained via the cascade of Diels−Alder (DA) and retro-Diels−Alder (RDA) reactions[15,16] outlined in Scheme 1.

Diels−Alder reaction of anthracene **1** and vinylene carbonate **2** provided **3** in high yield (94%). Hydrolysis of **3** with NaOH in MeOH gave rise to the diol **4** (76%). To obtain **6**, diol **4** was first converted into the acetal **5** (96%) using 2,2-dimethoxypropane/*p*-toluenesulfonic acid at room temperature. Thermal cracking of **5** led to 55%

(11) Thibaudeau, C.; Chattopadhyaya, J. *Stereoelectronic Effects in Nucleosides and Nucleotides and Their Structural Implications*; Uppsala University Press: Uppsala, Sweden, 1999; ISBN 91-506-1351-0.
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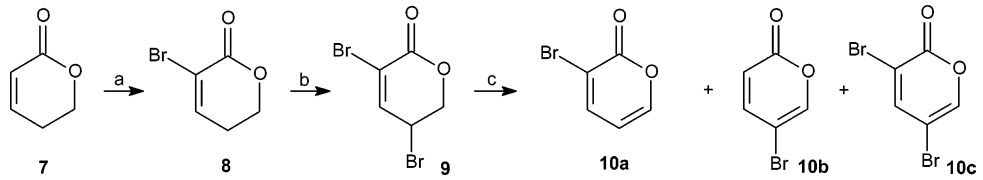
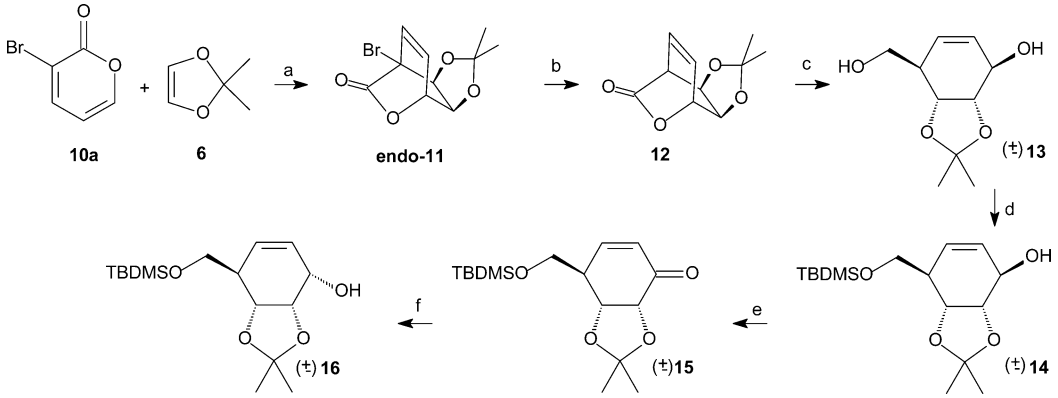
(16) Field, N. D. *J. Am. Chem. Soc.* **1961**, *83*, 3504−5307.

SCHEME 2. Synthesis of Diene 10a Starting from 5,6-Dihydro-2*H*-pyran-2-one 7[a]



[a] Conditions: (a) (1) Br_2/CH_2Cl_2; (2) Et_3N. (b) NBS, CCl_4, benzoylperoxide, 100 °C, 5.5 h. (c) Et_3N, CH_2Cl_2, rt.

SCHEME 3. Synthesis of the Nucleoside Precursor 16[a]



[a] Conditions: (a) CH_2Cl_2, 90 °C, 4 days; (b) *n*-Bu_3SnH, AIBN, toluene; (c) $LiAlH_4$, THF, 0 °C; (d) TBDMS−Cl, imidazole, DMF, 0 °C; (e) MnO_2, CH_2Cl_2, rt, overnight; (f) $NaBH_4$, $CeCl_3 \cdot 7\ H_2O$, MeOH, rt.

yield of **6** by retro-Diels−Alder reaction (RDA). Diene **10a** was obtained by a sequence of selective bromination reactions, followed by elimination as outlined in Scheme 2.

Selective bromination in position 3 of 5,6-dihydro-2*H*-pyran-2-one **7** in CH_2Cl_2 gave 3-bromo-5,6-dihydro-2*H*-pyran-2-one **8** (82%). A second bromination of **8** at the allylic position was carried out with *N*-bromosuccinimide (NBS) to obtain **9** (89%). Subsequent elimination with Et_3N yielded 3-bromo-2*H*-pyran-2-one[13,17] **10a** (43%). The formation of the major byproduct, 5-bromo-2*H*-pyran-2-one **10b**, resulted from prototropic migration in a basic medium followed by elimination of HBr.[17]

The key step Diels−Alder reaction[13,14] was carried out by heating **10a** and **6** together with a small amount of ethyldiisopropylamine in a sealed pressure tube at 90 °C for 4 days. Replacement of the bridgehead bromine by hydrogen using tributyltin hydride and AIBN[13] (radical mechanism) provided the halogen-free bicyclic lactone **12**. Reduction and ring opening of the lactone **12** with lithium aluminum hydride[18] gave diol **13** in good yield (86%). Treating **13** with 1.2 equiv of *tert*-butyldimethylsilyl chloride in DMF in the presence of 1.5 equiv of imidazole at 0 °C allowed protection of the primary hydroxyl group. Monosilylated **14** was obtained in 59% yield, while 17% of the starting material **13** was recovered.

Cycloadduct **11** was formed in a 4:1 mixture of *endo*:*exo* isomers. Structural proof for the *endo* isomer could be achieved with the help of NOE difference spectroscopy. Irradiation of H5/6 caused positive NOE enhancement

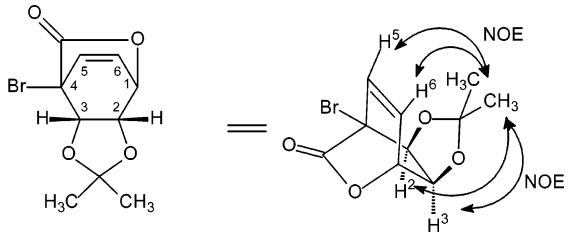


FIGURE 2. Spectroscopic proof of the *endo*-isomer

(2.53%) of CH_3 a/b, whereas irradiation of CH_3 a/b led to positive NOE of 1.6% (H2/3) and 0.5% (H5/6), respectively (Figure 2).

To obtain rCe-A (**18**), the configuration of the allylic hydroxyl group of **14** needs to be inverted. Therefore, the intermediate **14** was oxidized to the corresponding enone **15** using manganese dioxide in CH_2Cl_2 (84%). Reduction of the enone **15** with $NaBH_4$ in the presence of $CeCl_3 \cdot 7H_2O$ provided the α-alcohol **16** (72%; Scheme 3). A small amount of the *β*-alcohol **14** (9%) was likewise obtained. Introduction of the base moiety onto the cyclohexenyl ring was effected by a S_N2 reaction, following the Mitsunobu protocol (Scheme 4).

Treatment of **16** with adenine in the presence of PPh_3 and DIAD in dry dioxane at room temperature gave **17** (62%). No N-7 isomer was found. Complete deprotection of **17** with TFA/H_2O (3:1) at room-temperature overnight afforded the adenine congener (±)-**18** as a racemic mixture. The potential of this nucleoside analogue to mimic adenosine in biological systems was tested by its substrate specificity for an adenosine metabolic enzyme, i.e., adenosine deaminase.[19] Likewise, this enzyme may be used to resolve the obtained racemic mixture of ribocyclohexenyl-A ((±)-**18**).[20] We may conclude that resolution of both enantiomers of (±)-rCe-A **18**, with

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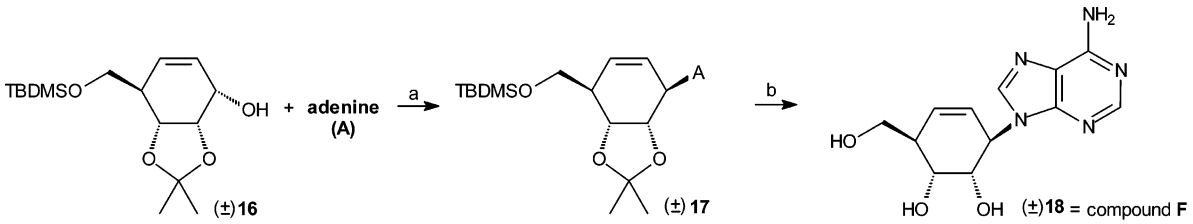
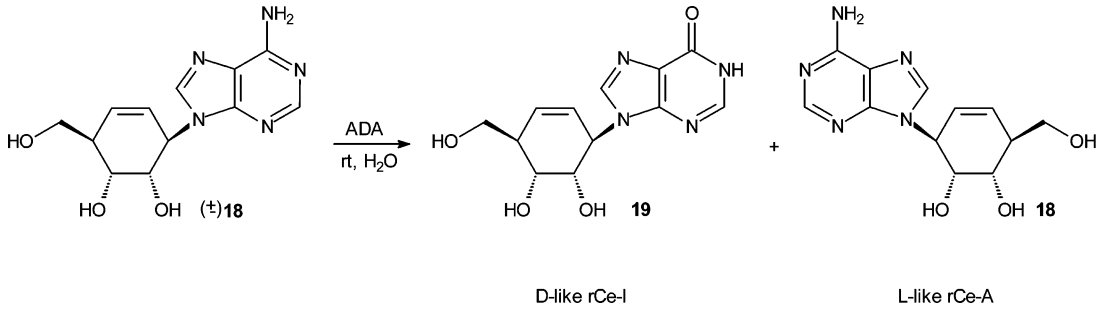
(18) Roberts, S. M.; Sutton, P. W. *J. Chem. Soc., Perkin. Trans. 1* **1995**, *12*, 1499−1504.

SCHEME 4. Introduction of Adenine in Allylic Position[a]



[a] Conditions: (a) DIAD, PPh_3, dry dioxane; (b) TFA/H_2O (3:1), rt.

SCHEME 5. Enzymatic Deamination



concomitant conversion of the D-like enantiomer into an inosine analogue, is possible by selective enzymatic deamination reaction of (±)-**18** using adenosine deaminase (ADA)[19] (Scheme 5).

Racemic **18** dissolved in ethanol is well resolved using a Chiralpak column and hexane/EtOH 85:15 as an eluent (Figure 3a). After treatment of **18** with ADA, a progressive disappearance of the first peak is observed (Figure 3B or 3C) after 12 and 24 h of incubation, whereas the second peak remains unaffected. Therefore, the HPLC analyses indicate the more mobile enantiomer (retention time ~55 min) to be a substrate for ADA, indicating that this isomer corresponds to a D-like nucleoside, with the less mobile enantiomer (retention time ~66 min) resembling a L-nucleoside. The formation of the polar inosine analogue **19** could be demonstrated by TLC separation and mass spectrometry, indicating that ADA transferred one enantiomer of (±)-**18** into the inosine-analogue **19** (FAB^+ *m/z* $C_{12}H_{14}N_4O_4$ = 278.1015).

Like furanose nucleosides, cyclohexenyl nucleosides occur as an equilibrium between different conformations in which one conformer is generally predominant. When the "sugar" moiety is considered, this equilibrium is described as a function of the percentage of C3′ *endo* (northern) versus the percentage of C2′ *endo* (southern).[21] Conformational analysis of cyclohexenyl nucleosides shows that at the molecular level, they exist in an equilibrium between two half-chair conformations ($^3H_2$ and $^2H_3$).[9] The $^3H_2$ and $^2H_3$ conformations of the cyclohexenyl ring mimic the 3′-*endo* and 2′-*endo* sugar conformations, respectively, of a natural nucleoside.[2] Potential energy calculations could show that the equilibrium between the $^3H_2$ and the $^2H_3$ conformation can be tuned by changing the substituents of the cyclohexene ring. Whereas a deoxycyclohexenyl nucleoside (Figure 1D) was shown to occur predominantly in a $^3H_2$ conformation, introduction of a hydroxyl group in the ribo position (Figure 1F) on C2′ shifted the conformation toward $^2H_3$ with the base moiety occupying a pseudoequatorial position. The introduction of a hydroxyl group in the "arabino" position (Figure 1E) on C2′, however, shifted the conformation further toward the $^3H_2$ conformation.[9] The results as discussed in the modeling experiments[9] were confirmed by NMR spectroscopy. The conformational equilibrium of the rCe-A compound was calculated by the Hexrot program on the basis of the observed scalar coupling constants.[9] Comparison of these results with the results of similar calculations done on the deoxy- and arabino-cyclohexenyl congeners with an adenine base moiety (Figure 1D,E and Table 1) shows that introduction of a 2′-OH substituent in the down position shifts the conformation toward $^2H_3$ (S-type), whereas introduction of a 2′-OH in the up position shifts the conformation toward $^3H_2$ (N-type). The synthesis and NMR analysis of the arabino-type cyclohexenyl compound have been previously described.[22]

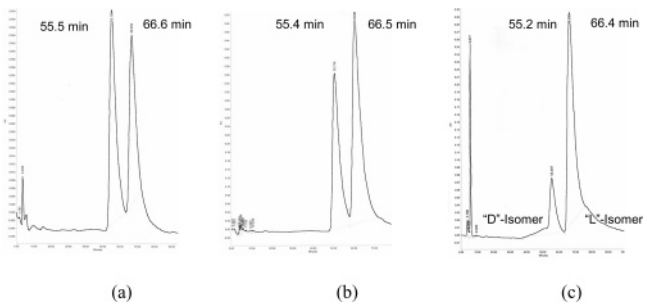


FIGURE 3. Deamination reaction was followed with chiral HPLC using Chiralpak AD column (250 × 4.6 mm): racemic (±)-rCe-A 18 (a) and the progress of the deamination process (b and c).

(19) Adenosine deaminase (EC 3.5.4.4) from calf intestinal mucosa, purchased from Sigma-Aldrich; product no. A-1030, type VIII. One unit of this enzyme preparation will deaminate 1.0 μmol of adenosine to inosine per minute at pH 7.5 at 25 °C.

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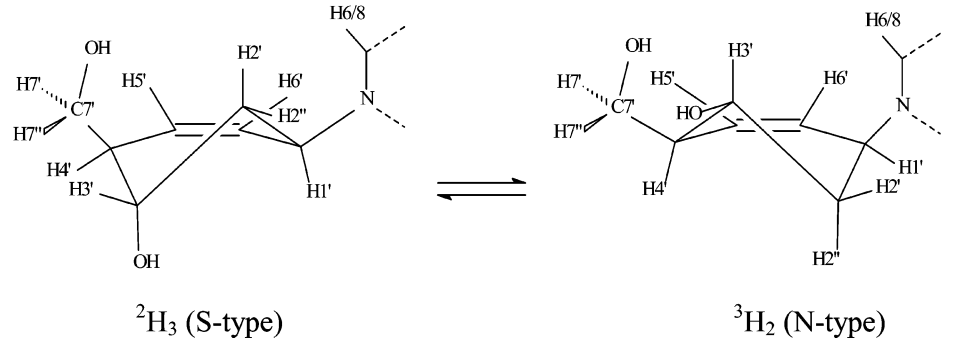
TABLE 1. Temperature Dependence of the Scalar Coupling Constants from the Cyclohexenyl Moiety of Compounds D and F from Figure 1 and Results of Hexrot[9] and van't Hof Calculations[a]



$^2H_3$ (S-type) $^3H_2$ (N-type)

	rCe-A						ara-Ce-A						dCe-A					
$^3J_{H-H}$ (Hz)	10 °C	20 °C	30 °C	40 °C	50 °C	60 °C	10 °C	20 °C	30 °C	40 °C	50 °C	60 °C	10 °C	20 °C	30 °C	40 °C	50 °C	60 °C
H1′ H2′	6.1	6.1	6.2	6.3	6.3	6.3							5.7	5.7	5.6	5.6	5.5	5.4
H1′ H2″							5.4	5.4	5.5	5.5	5.5	5.5	5.7	5.8	5.8	5.9	5.9	5.9
H2′ H3′	2.9	2.8	2.9	2.9	2.9	2.9							3.7	3.6	3.6	3.6	3.5	3.4
H2″ H3′							10.3	10.3	10.1	10.1	10.1	9.9	8.4	8.2	8.1	7.9	7.8	7.7
H3′ H4′	5.0	4.9	4.9	4.9	4.8	4.8	8.8	8.4	8.4	8.3	8.2	8.2	5.7	5.7	5.6	5.5	5.4	5.4
% N	48	47	47	46	46	46	92	90	89	88	87	87	58	57	56	55	55	54
ΔH (kJ)			1.32						8.66						2.55			
$T^*\Delta S$ (kJ)			1.59						3.10						1.85			
ΔG (kJ)			0.27						5.56						0.70			

[a] NMR spectra were recorded in D_2O at pH = 7. dCe-A, deoxycyclohexenyl-A (Figure 1D); rCe-A, ribocyclohexenyl-A (Figure 1F); ara-Ce-A, arabino-cyclohexenyl-A (Figure 1E).

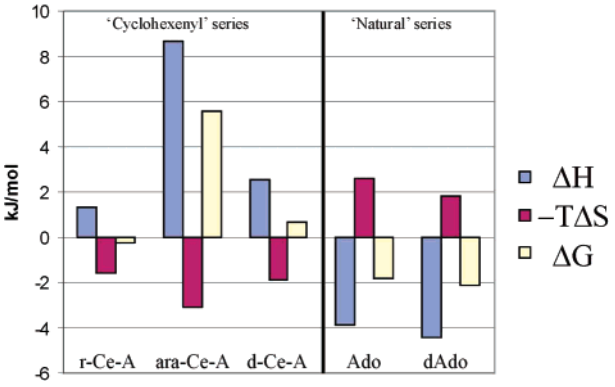


FIGURE 4. Comparison of ΔH, $-T\Delta S$, and ΔG of the N↔S equilibrium of natural (C3′ *endo*↔C2′ *endo*′) and cyclohexenyl ($^3H_2$↔$^2H_3$) nucleosides. Thermodynamic values were obtained using standard van 't Hof plots. Ado, Adenosine; dAdo, 2′-deoxyadenosine.

During a more profound NMR analysis, ΔS, ΔH, and ΔG values (Figure 4) were obtained for the equilibrium between the $^3H_2$ and $^2H_3$ conformers of the deoxy-, ribo-, and arabino-cyclohexenyl nucleosides. First, the scalar coupling constants in these compounds were determined at various temperatures, and the corresponding % $^3H_2$ conformer (N-conformer) was calculated using the Hexrot program (Table 5). Second, ΔS and ΔH values were derived using standard van't Hof plots.

Comparison of the thermodynamic parameters of the cyclohexenyl compounds with their natural congeners shows that in the "natural" series, enthalpy favors the "C2′ *endo*" (S-type) and entropy favors "C3′ *endo*" (N-type), while the "cyclohexenyl" series, "$^2H_3$" (S-type), is favored by entropy and "$^3H_2$" (N-type) by enthalpy.

Conclusion

In search for a mimic of a ribonucleoside, we have synthesized a ribo-type cyclohexenyl nucleoside with an adenine base moiety. The synthetic scheme is based on a Diels−Alder reaction between 2,2-dimethyl-1,3-dioxole **6** and 3-bromo-2*H*-pyran-2-one **10** to obtain the cyclohexene scaffold and a Mitsunobu-type reaction for introduction of the base moiety. Adenosine deaminase can be used to resolve the obtained racemic mixture. NMR analysis shows the conformational shift of cyclohexenyl nucleosides to be opposite to what has been observed for natural nucleosides in the deoxy and ribo series. The arabino compound (Figure 1E) as well as the ribo compound (Figure 1F) will be evaluated as mimics of a natural ribonucleoside in functional biological assays.

Experimental Section

The starting products for the key step of this synthesis, the RDA reaction, anthracene-2,2-dimethyl-1,3-dioxole adduct (**5**)[15,16] and 3-bromo-2*H*-pyran-2-one (**10**),[13,17] were prepared according to the literature.

2,2-Dimethyl-1,3-dioxole (6).[15,16] Before starting the reaction, all glasswork was dried overnight in an oven at 80 °C. The starting material (21.0 g, 0.075 mol of **5** and a few crystals of BHT) was placed in a 100 mL flask and lyophilized for 24 h to remove all water. The whole apparatus (see picture below) was flushed three times with nitrogen. The RDA reaction was carried out under N_2 protection. The temperature of the collecting tube has been adjusted to −50 °C with acetone/dry ice. (Dioxole **6** is a volatile liquid, solidifying at −70 °C.) After the solid was melted with a heat gun and the temperature was increased to about 600 °C, the RDA reaction started, indicated by vigorous boiling. The formed dioxole **6** was collected in the precooled tube; heating was continued until no more product could be distilled (1.5 h). Dioxole **6** (4.01 g, 55%) was collected as a colorless liquid and stored at −20 °C. The identity of **6** was proven by means of NMR spectroscopy:

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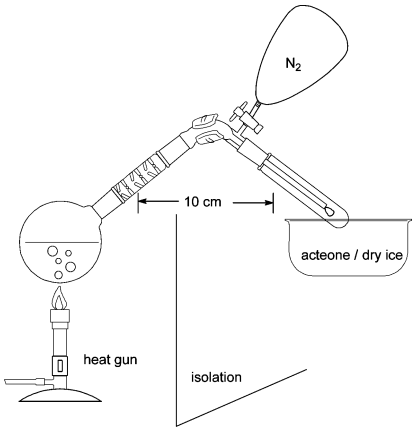
^{1}H NMR ($CDCl_3$) δ 1.52 (s, 6H), 6.17 (s, 2H) ppm; ^{13}C NMR ($CDCl_3$) δ 24.8, 114.1, 126.6.



1-Bromo-4,4-dimethyl-3,5,8-trioxa-tricyclo[5.2.2.0]undec-10-en-9-one (11).[13,14] A 15 mL pressure tube (Aldrich) was charged with **10a** (1.28 g, 7.31 mmol), **6** (3.56 g, 35.59 mmol, 4.87 equiv), and Hünig's base (EtN(*i*-Pr)$_2$, 85.3 mg 0.66 mmol, 0.09 equiv). After CH_2Cl_2 (5.6 mL) was added, the tube was sealed and placed in an oven (90 °C) for 4 days. After cooling to room temperature, the resulting brown-yellow solution was concentrated. The residue was purified by flash chromatography on silica gel (50 g of SiO_2, the column was packed with hexanes−EtOAc (10:1 + 1% Et_3N) and eluted with hexanes−EtOAc (1:1 + 1% Et_3N) in less than 3 min due to the instability of **11** on the silica gel). The resulting yellow solution was concentrated to give an orange-yellow oil as a mixture of the *endo* and *exo* isomers. The spectroscopic data of the *endo* isomer are given: R_f 0.68 (hexanes−EtOAc 2:1); ^{1}H NMR ($CDCl_3$) δ 1.38 (s, 3H), 1.42 (s, 3H), 4.63 (dd, 1H, J = 6.9, 1.2 Hz), 4.77 (dd, ^{1}H, J = 7.0, 4.4 Hz), 5.27 (td, 1H, J = 4.5, 2.2 Hz), 6.34−6.48 (m, 2H) ppm; ^{13}C NMR ($CDCl_3$) δ 25.3, 25.4, 60.4, 73.2, 76.7, 79.4, 114.5, 129.1, 135.8, 166.0 ppm.

4,4-Dimethyl-3,5,8-trioxa-tricyclo[5.2.2.0]undec-10-en-9-one (12).[13] A solution of **11** (1.34 g, 4.87 mmol), tributyltin hydride (*n*-Bu_3SnH, 1.94 mL, 7.31 mmol, 1.5 equiv), and AIBN (0.28 g, 0.49 mol, 0.1 equiv) in dry toluene (32 mL) was degassed under nitrogen. The solution was immersed in a preheated bath (130 °C) and refluxed for 1 h. The reaction mixture was cooled and concentrated. The residue was purified on a silica column. The column was eluted with hexane (500 mL) (to remove *n*-Bu_3SnH), hexanes−Et_2O 1:1 (500 mL), and hexanes−Et_2O 1:2 (600 mL) to afford **12** (690 mg, 72.25%): R_f 0.62 (hexanes−EtOAc 2:1) ^{1}H NMR ($CDCl_3$) δ 1.33 (s, 3H), 1.34 (s, 3H), 3.87 (ddd, 1H, J = 1.8, 4.0, 7.4 Hz), 4.60 (dd, 1H, J = 6.9, 3.8 Hz), 4.70 (dd, 1H, J = 6.8, 4.0 Hz), 5.26 (td, 1H, J = 4.4, 2.2 Hz), 6.40−6.51 (m, 2H) ppm; ^{13}C NMR ($CDCl_3$) δ 25.3, 25.4, 46.4, 72.5, 75.1, 75.8, 113.6, 129.6, 130.3, 170.4 ppm.

(±)-(3a*S*,4*R*,7*S*,7a*R*)-7-(Hydroxymethyl)-2,2-dimethyl-3a,4,7,7a-tetrahydro-1,3-benzodioxo-4-ol (13).[18] To a mixture of $LiAlH_4$ (119 mg, 3.13 mmol, 1.5 equiv) in dry THF (20 mL) at 0 °C was added a solution of **12** (410 mg, 2.09 mmol) in THF (8 mL) slowly. The reaction mixture was stirred at 0 °C for an additional 15 min and at room temperature overnight. A saturated sodium bisulfite solution was added dropwise to the reaction mixture until a precipitate was formed. EtOAc (3 mL) was added, and the mixture was stirred for an additional 0.5 h. The precipitate was filtered, and the filtrate was concentrated. The residue was purified with a silica column (the column was eluted with hexanes−EtOAc 1:1) to give **13** (360 mg, 86%): R_f 0.15 (hexanes−EtOAc 1:1); ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 3H), 1.47 (s, 3H), 2.40 (m, 2H), 3.05 (br-s, 1H), 3.78 (m, 2H), 4.10−4.52 (m, 3H), 5.68 (ddd, 1H, J = 9.85, 3.7, 2.2 Hz), 5.97 (dt, 1H, J = 9.6, 2.7 Hz) ppm; ^{13}C NMR ($CDCl_3$) δ 24.7, 27.1, 42.3, 64.0, 69.7, 75.3, 80.5, 108.7, 127.2, 131.8 ppm; FAB$^+$ 223.1 (M + Na)$^+$; HRMS calcd for $C_{10}H_{16}O_4$ (M + H)$^+$ 223.0946, found 223.0949. Anal. Calcd ($C_{10}H_{16}O_6$): C, 59.98; H, 8.05. Found: C, 59.48; H, 7.93.

(±)-(3a*S*,4*R*,7*S*,7a*R*)-7-({[*tert*-Butyl(dimethyl)silyl]oxy}-methyl)-2,2-dimethyl-3a,4,7,7a-tetrahydro-1,3-benzodioxo-4-ol (14). To a solution of **13** (620 mg, 3.09 mmol) in dry DMF (13 mL) at 0 °C was added imidazole (316 mg, 4.64 mmol, 1.5 equiv), followed by TBDMSCl (560 mg, 3.72 mmol, 1.2 equiv) in three portions (after 0.5 h). The reaction was stirred at 0 °C for 10 min and at room temperature overnight and quenched with water. The resulting mixture was evaporated to remove DMF. The residue was absorbed on silica and chromatographed (hexanes−EtOAc 10:1, 5:1, 1:1, 1:2) to yield **14** (580 mg, 59.6%) and unreacted **13** (110 mg, 17.7%) both as an oil. Spectroscopic data of **14** are given: R_f 0.57 (hexanes−EtOAc 2:1); ^{1}H NMR ($CDCl_3$) δ 0.084 (s, 6H), 0.91 (s, 9H), 1.36 (s, 3H), 1.45 (s, 3H), 2.37 (m, 1H), 2.60 (br-s, 1H), 3.78 (d, 2H, J = 1.8 Hz), 4.05−4.21 (m, 3H), 5.73 (ddd, 1H, J = 9.8, 3.5, 2.0 Hz), 5.93 (dt, 1H, J = 10.4, 2.6 Hz) ppm; ^{13}C NMR ($CDCl_3$) δ −5.55, 18.3, 24.7, 25.9, 27.2, 42.9, 64.1, 69.5, 74.1, 80.3, 108.4, 128.3, 130.8 ppm; FAB$^+$ 337.2 (M + Na)$^+$; HRMS calcd for $C_{16}H_{30}O_4Si$ (M + Na)$^+$ 337.1811, found 337.1807. Anal. Calcd ($C_{16}H_{30}O_4Si$): C, 61.11; H, 9.61. Found: C, 60.69; H, 9.19.

(±)-(3a*R*,7*S*,7a*R*)-7-({[*tert*-Butyl(dimethyl)silyl]oxy}-methyl)-2,2-dimethyl-7,7a-dihydro-1,3-benzodioxo-4(3a*H*)-one (15). A mixture of **14** (120 mg, 0.38 mmol) and activated MnO_2 (332 mg, 3.82 mmol, 10 equiv) in dry CH_2Cl_2 (6 mL) was stirred vigorously. After 12 h, MnO_2 (332 mg, 3.82 mmol, 10 equiv) was added to the mixture. After another 12 h, the completion of the reaction was checked by TLC. Again, MnO_2 (66 mg, 0.76 mmol, 2 equiv) was added, and stirring was continued overnight. The reaction mixture was diluted with CH_2Cl_2, filtered through Celite, and concentrated. The residue was chromatographed on silica gel (hexanes−EtOAc 6:1) to give **15** (100 mg, 84%) as a white solid: R_f 0.68 (hexanes−EtOAc 2:1); ^{1}H NMR ($CDCl_3$) δ 0.00 (s, 3H), 0.03 (s, 3H), 0.83 (s, 9H), 1.35 (s, 3H), 1.40 (s, 3H), 2.99 (m, 1H), 3.74 (dd, 1H, J = 10.1, 3.0 Hz), 3.92 (dd, 1H, J = 10.0, 4.0 Hz), 4.35 (d, 1H, J = 5.0 Hz), 4.51 (dt, 1H, J = 3.6, 1.65 Hz), 6.19 (d, 1H, J = 10.2 Hz), 6.74 (dddd, 1H, J = 10.3, 5.2, 1.8 Hz) ppm; ^{13}C NMR ($CDCl_3$) δ −5.7, 18.1, 25.7, 25.8, 27.4, 41.6, 63.3, 75.7, 77.0, 108.5, 129.8, 147.2, 196.1 ppm; FAB$^+$ 335.1 (M + Na)$^+$; HRMS calcd for $C_{16}H_{28}O_4Si$ (M + Na)$^+$ 335.1654, found 335.1645. Anal. Calcd ($C_{16}H_{28}O_4Si$): C, 61.50; H, 9.03. Found: C, 61.52; H, 8.57.

(±)-(3a*S*,4*S*,7*S*,7a*R*)-7-({[*tert*-Butyl(dimethyl)silyl]oxy}-methyl)-2,2-dimethyl-3a,4,7,7a-tetrahydro-1,3-benzodioxo-4-ol (16). To a solution of **15** (110 mg, 0.35 mmol) in MeOH (6 mL) at room temperature was added $CeCl_3 \cdot 7H_2O$ (197 mg, 0.53 mmol, 1.5 equiv). The mixture was stirred for 1 h, and a clear solution was obtained. $NaBH_4$ (16 mg, 0.42 mmol, 1.2 equiv) was added in portions, and H_2 evolved. The reaction mixture was stirred for 2 h and quenched with crushed ice. The mixture was stirred for 0.5 h and concentrated. The residue was diluted with EtOAc (15 mL), washed with H_2O and brine, dried over Na_2SO_4, and concentrated. The residue was chromatographed on silica gel (hexanes−EtOAc 5:1) to give **16** (80 mg, 72.3%) as a colorless oil and **14** (10 mg, 9.1%) as a side product: R_f 0.63 (hexanes−EtOAc 2:1); ^{1}H NMR ($CDCl_3$) δ 0.05 (s, 6H), 0.89 (t, 9H, J = 3.0 Hz), 1.39 (s, 3H), 1.45 (s, 3H), 2.60 (m, 2H, H4), 3.61 (dd, 1H, J = 10.3, 4.7 Hz), 3.69 (dd, 1H, J = 10.2, 5.0 Hz), 4.33−4.44 (m, 3H), 5.82 (dd, 1H, J = 10.9, 4.5 Hz), 5.92 (dd, 1H, J = 11.5, 2.6 Hz) ppm; ^{13}C NMR ($CDCl_3$) δ −5.6, 18.3, 24.5, 25.8, 26.4, 42.0, 64.0, 64.6, 74.3, 75.7, 108.5, 129.4, 131.1 ppm; FAB$^+$ 337.2 (M + Na)$^+$; HRMS calcd for $C_{16}H_{30}O_4Si$ (M + Na)$^+$ 337.1811, found 337.1807.

(±)-9-[(3a*S*,4*R*,7*S*,7a*R*)-7-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)-2,2-dimethyl-3a,4,7,7a-tetrahydro-1,3-benzodioxol-4-yl]-9*H*-purin-6-amine (17). To a mixture of **16** (220 mg, 0.70 mmol), adenine (189 mg, 1.40 mmol, 2 equiv), and PPh_3 (367 mg, 1.40 mmol, 2 equiv) in dry dioxane (11 mL) under N_2 at room temperature was added DIAD (278 μL, 1.40 mmol) very slowly. The reaction mixture was stirred at room-

temperature overnight and concentrated. The resulting residue was chromatographed on silica gel (CH_2Cl_2−MeOH, 98:2) to yield **17** (170 mg, 62.28%) as a white solid: R_f 0.12 (CH_2Cl_2−MeOH 98:2); ^{1}H NMR ($CDCl_3$) δ 0.10 (s, 6H), 0.93 (s, 9H), 1.32 (s, 3H), 1.55 (s, 3H), 2.55 (m, 1H), 3.78−3.97 (m, 2H), 4.25 (t, 1H, J = 7.0 Hz), 4.48 (t, 1H, J = 7.0 Hz), 4.98−5.02 (m, 1H), 5.80(s, 2H), 5.90−6.07 (m, 2H), 7.87 (s, 1H), 8.37 (s, 1H) ppm; ^{13}C NMR ($CDCl_3$) δ −5.8, −5.6, 18.1, 25.4, 25.7, 27.4, 42.9, 56.7, 63.7, 73.7, 76.2, 109.2, 119.0, 126.4, 130.9, 139.9, 152.9, 155.5 ppm; FAB$^+$ 432.2 $(M + H)^+$; HRMS calcd for $C_{21}H_{34}N_5O_3Si$ $(M + H)^+$ 432.2431, found 432.2428.

(±)-(1*R*,2*S*,3*R*,4*S*)-9-(2,3-Dihydroxy-4-hydroxymethyl-5-cyclohexen-1-yl)adenine (18) (Ribo-Type Cyclohexenyl Adenine, rCe-A). Compound **17** (150 mg, 0.35 mmol) was treated with TFA−H_2O (3:1, 7 mL) at room-temperature overnight. The reaction mixture was concentrated and co-evaporated with toluene (three times). The residue was chromatographed on silicagel (CH_2Cl_2−MeOH, 9:1, 8:1, 7:1, 7:3) to afford **18** (77 mg, 80.2%) as a yellow-white solid: R_f 0.16 (CH_2Cl_2−MeOH 6:1); ^{1}H NMR (DMSO) (500 MHz) δ 2.41 (m, 1H), 3.65 (m, 2H), 3.94 (m, 1H), 4.74 (m, 1H), 4.76 (m, 2H), 5.07 (m, 1H), 5.60 (dt, 1H, j= 4.2, 1.0 Hz), 5.82 (dt, 1H, J = 3.8, 1.2 Hz), 7.14 (br-s, 2H), 8.02 (s, 1H), 8.12 (s, 1H) ppm; ^{13}C NMR (DMSO) (500 MHz) δ 45.7, 55.3, 61.8, 67.9, 69.4, 119.2, 124.5, 131.2, 140.0, 149.7, 152.2, 156.0 ppm; FAB$^+$ 278.1 $(M + H)^+$; HRMS calcd for $C_{12}H_{15}N_5O_3$ $(M + H)^+$ 277.1175, found 278.1250.

(±)-(1*R*,2*S*,3*R*,4*S*)-9-(2,3-Dihydroxy-4-hydroxymethyl-5-cyclohexen-1-yl)hypoxanthine (19). A portion of 5 mg (0.018 mmol) of racemic **18** was dissolved in 1 mL of hot water. The solution was cooled to room temperature; ADA (10 μL of a suspension containing 50 units of adenosine deaminase) was added in one portion, and the reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated and dissolved in 1 mL of EtOH, and 20 μL of this solution was examined by TLC (CH_2Cl_2−MeOH 7:1) and HPLC, showing deamination of only one of the enantiomers. The newly formed product was identified as the hypoxanthine congener by MS.

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Supporting Information Available: General methods and instrumentation; NMR spectral data of **6** and **11**−**18**. This material is available free of charge via the Internet at http://pubs.acs.org.

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