

Efficient and Accurate Potential Energy Surfaces of Puckering in Sugar-Modified Nucleosides

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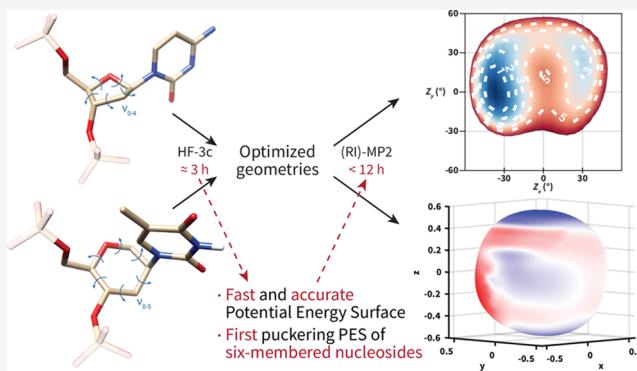
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ABSTRACT: Puckering of the sugar unit in nucleosides and nucleotides is an important structural aspect that directly influences the helical structure of nucleic acids. The preference for specific puckering modes in nucleic acids can be analyzed via *in silico* conformational analysis, but the large amount of conformations and the accuracy of the analysis leads to an extensive amount of computational time. In this paper, we show that the combination of geometry optimizations with the HF-3c method with single point energies at the RI-MP2 level results in accurate results for the puckering potential energy surface (PES) of DNA and RNA nucleosides while significantly reducing the necessary computational time. Applying this method to a series of known xeno nucleic acids (XNAs) allowed us to rapidly explore the puckering PES of each of the respective nucleosides and to explore the puckering PES of six-membered modified XNA (HNA and β -homo-DNA) for the first time.



1. INTRODUCTION

For nucleic acids, the concept of puckering is used to describe the structural behavior of the sugar moiety, which constitutes one of the three fundamental elements of nucleotides. This out-of-plane deviation naturally occurs to reduce electrostatic repulsion and steric strain that a planar conformation induces in a nonaromatic cyclic compound having more than four atoms in the ring. The energetic favorable conformers are actively influenced by substituents attached to the cyclic moiety. Puckering of nonaromatic cyclic compounds is typically studied by reducing the dimensionality of the out-of-plane deviation by the concept of pseudo-rotation.^{1–3} The five-membered ring of (deoxy)ribofuranose in DNA and RNA typically adopts one of two low-energy conformations, namely, C3'-*endo* and C2'-*endo* conformation (Figure S1 in the Supporting Information), which directly influences the helical structure of the oligomer.^{4,5} Investigating and understanding the influence of substituents on the puckering behavior in sugar-modified nucleosides is of high importance in drug discovery⁶ and structural biology.⁷ In light of therapeutic strategies, modified nucleic acids have been developed for anticancer strategies,⁸ antiviral compounds,^{8,9} antisense technology,¹⁰ and aptamers.¹¹ Modifying the carrier of genetic information in light of xenomicobiology¹² has led to the rise of xeno nucleic acids (XNA), where alternative moieties replacing the (deoxy)ribose ring show several benefits for XNAs over their natural counterparts, including increased

thermal stability, increased nuclease resistance, and orthogonal hybridization (i.e., disfavoring interactions with natural nucleic acids). With recent advances being made in the rational design of XNA processing enzymes,¹³ the employed models should be able to correctly interpret puckering preferences for these XNA species to correctly interpret and predict protein and substrate interactions. Traditional force fields, used in the analysis of biomolecular structures and interactions, are typically parametrized based on quantum mechanics (QM) studies on building blocks of those biomolecules.^{14–17} Although computational studies on single molecules with extensive QM methods have become commonplace, the amount of optimizations required for elaborate conformational analyses do form a bottleneck, especially when computational resources are scarce.

While seeking an *in silico* tool to perform structural characterization of potential XNA candidates, we evaluated the use of a lightweight method (HF-3c)¹⁸ for the geometry optimizations combined with a re-evaluation of the electronic energy of each conformer at the Moller–Plesset second-order

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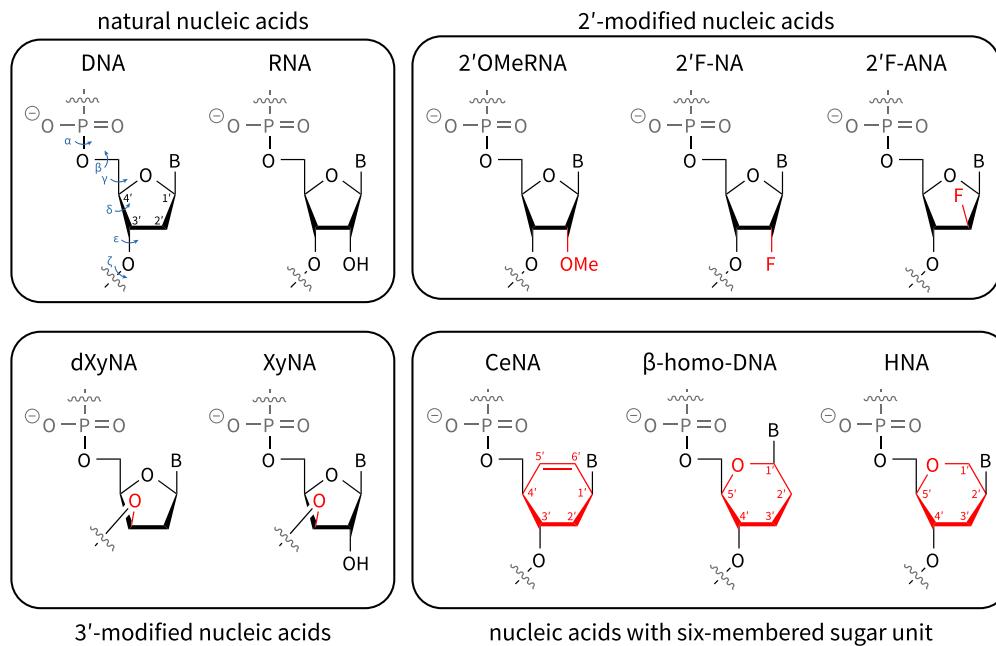


Figure 1. Overview of nucleic acids (gray) subject of this study. All calculations were performed on nucleosides (black). Modifications compared to natural nucleic acids are highlighted in red.

perturbation (MP2) level of theory for a set of nucleic acids (Figure 1) that ranges from natural DNA and RNA over XNA with minor modifications in the five-membered ribose ring, e.g., 2'-O-methylribonucleic acid (2'OMeRNA) and 2'-deoxy-2'-fluororibonucleic acid (2'F-RNA), to more significant changes in five-membered ring, e.g., 2'-deoxy-2'-fluoroarabinofuranosyl nucleic acid (2'F-ANA), 2'-deoxxylylucleic acid (dXyNA), and xylonucleic acid (XyNA), to XNA with six-membered sugar moieties, i.e., hexitol nucleic acids (HNA) and β -2',3'-dideoxyglucose nucleic acids (further referred to as homo-DNA). To our knowledge, the only modified, six-membered nucleoside for which a conformational analysis has been previously performed at semiempirical level (PM3) is the cyclohexene nucleic acid (CeNA).¹⁹ The unsaturated bond in the sugar moiety allowed for a sampling approach similar to five-membered rings where the two unsaturated carbons are considered as a single pseudo-atom and is therefore termed as a pseudo-five-membered nucleoside. Here, we present an evaluation of the CeNA puckering potential energy surface (PES) at the HF-3c/RI-MP2 level, together with a first conformational analysis of HNA and homo-DNA as true six-membered nucleosides using Pickett angles^{20,21} to sample their conformational globe (Figure S2 in the Supporting Information).

2. METHODS

2.1. Sampling of Five-Membered Nucleosides. For five-membered nucleosides, conformational sampling of the PES for ring puckering was performed according to methodology reported by Huang et al.²² In brief, this method expresses the typical polar coordinates (A_ν , P_θ)³ in Cartesian coordinates (Z_x , Z_y) (Figure S1). The Cartesian coordinates Z_x and Z_y are empirically related to two endocyclic torsions, ν_1 and ν_3 , by the following expressions:

$$Z_x = \frac{\nu_1 + \nu_3}{2 \cos(4\pi/5)} \quad (1)$$

$$Z_y = \frac{\nu_1 - \nu_3}{2 \sin(4\pi/5)} \quad (2)$$

Equations 1 and 2 can simply be rearranged to obtain expressions for ν_1 and ν_3 , as functions of Z_x and Z_y . Both Z_x and Z_y were sampled from -60° to $+60^\circ$ in intervals of 6° for a total of 441 (21×21) conformations for each five-membered nucleoside (see Figure S3 in the Supporting Information). PES contours were plotted as contour plots using numpy²³ and matplotlib²⁴ after interpolating the data at a 1° interval for both Z_x and Z_y ordinates.

For each (modified) sugar, nucleosides were considered with adenine, guanine, cytosine, and uracyl (or thymine in similarity with DNA if a CH_2 group in the sugar moiety is flanking the nucleobase substituent at the 2'-position).

The influence of the 3'-substituent was analyzed for dxC and dC with NBO7.^{25,26} Deletion analysis of donor–acceptor interactions between Lewis (filled) and non-Lewis (empty) orbitals was employed to address the effects of the 3'-epimerization on stereoelectronic interactions with the 3'-OH substituent.

2.2. Sampling of Six-Membered Nucleosides. Pseudo-rotation in six-membered puckered rings follow the same principle as pseudo-rotation in five-membered rings. With a well-chosen mean plane, one can quantify the deviation of the atoms with respect to this reference plane. As such, an N -membered ring can be represented by an $N - 3$ hypersurface. For the particular case of a six-membered ring, this implies that all conformers can be represented on a sphere using the polar coordinates (Q , θ , ϕ) (see Figure S2A in the Supporting Information). Whereas Q indicates the total puckering amplitude, the distortion of each conformer is indicated by θ and ϕ .³ Based on the value of these latter parameters, IUPAC has determined 38 distinct conformers describing the different geometries attainable by six-membered puckered rings (Figure S2B in the Supporting Information).²⁷

Since atomic positions are not easily inferred from Cremer–Pople (CP) polar coordinates, the conformational sampling of

Table 1. Backbone Restraints Used for Scanning PES of Puckering in Specified XNA Species^a

	DNA ^b	RNA ^b	2'OMeRNA ^c	2'F-NA ^c	2'F-ANA ^c	dXyNA ^d	XyNA ^d	CeNA ^e	homo-DNA ^e	HNA ^e
β (°)	208.5	180.1	180.1	180.1	180.1	180.1	180.1	180.1	180.1	180.1
γ (°)	30.9	47.4	47.4	47.4	47.4	240.0	240.0	60.0	60.0	60.0
ϵ (°)	159.1	208.3	208.3	208.3	208.3	151.7	151.7	180.0	180.0	180.0
χ (°)	260.6	193.9	193.9	193.9	193.9	193.9	193.9	—	—	—
$2'$ (°)	—	190.3	190.3	—	—	—	190.3	—	—	—

^aThe 2' torsion indicates C3'-C2'-O2'-H(O2') in the case of RNA and XyNA or C3'-C2'-O2'-C(O2') in the case of 2'OMeRNA. ^bValues were taken from ref 22 for comparison purposes. ^cTypical A-type helical values were chosen due to their chemical similarity with RNA. ^dValues were chosen based on observed dihedral values in NMR structures.^{29,30} ^eValues were chosen to avoid intramolecular interactions while keeping conformational strain to a minimum.

the puckering PES was accomplished by transforming the polar CP coordinates (Q, θ, ϕ) to a set of Pickett–Strauss (PS) dihedral values (script was available online at <http://www.science.unitn.it/~sega/sugars.html><http://www.science.unitn.it/~sega/sugars.html>)²⁸ for HNA and homo-DNA. The CP coordinates were uniformly sampled on the conformational globe for each nucleoside by dividing the surface area of the globe over each point (pseudocode was retrieved from https://www.cmu.edu/biophys/deserno/pdf/sphere_equi.pdf) for a given Q , resulting in 630 conformations per conformational globe. For each of the obtained data points (Q, θ, ϕ), a respective set of PS angles was then derived and used as optimization restraints to obtain the conformational sampling. The spherical PES plots were generated using the gridfit function available in Matlab (r2020a) instead of the python libraries.

2.3. Computational Details. To reduce influence of backbone dihedral variation (e.g., intramolecular hydrogen bonding between 5'-OH and O' or nucleobase in nucleosides, which does not occur in nucleotides), restraints were applied to β , γ , ϵ , and χ dihedrals (Table 1). For XNA with six-membered sugar units (HNA and homo-DNA), backbone torsion angles outside the sugar ring were restrained to reduce steric strain: β and ϵ were restrained in the *antiperiplanar* (*ap*) range, whereas γ was restrained in the *+synclinal* (*+sc*) range (see Table 1).

Unless stated otherwise, all conformations required for a puckering PES of all included nucleic acids in this study were optimized using the HF-3c¹⁸ method, followed by a single point energy calculation using RI-MP2³¹ level of theory with the 6-311++G(2df,2p) basis, further referred to as the “HF-3c/RI-MP2” method. For geometry optimization of local minima at MP2 level, we compared RI-MP2 optimizations for DNA and RNA to assess whether a triple- ζ (tz) basis (6-311++G(2df,2p)) set is necessary when applying RI approximations to both MP2 density and HF step, i.e., Coulomb and exchange integrals. For all RI-MP2 calculations, the def2-qzvpp/C basis was employed as an auxiliary basis set for the RI approximation of the MP2 density and the def2/JK auxiliary basis set for the RI approximation of Coulomb and exchange integrals in the HF step. All calculations were performed using ORCA³² (v4.2.1). RI-MP2 optimizations of local minima were performed using strict convergence settings (keyword VerTightOpt).

All discussed computational times are based on our workstation, which is equipped with an Intel Xeon E5-2640 v4 (10 cores/20 threads) and 64 GB RAM (2333 MHz). The actual required time will differ for various systems. However, the relative computational cost will remain similar, according to the investigated molecule.

3. RESULTS AND DISCUSSION

3.1. Benchmark: DNA and RNA. Huang and co-workers²² provided benchmark PESs regarding the puckering of DNA and RNA nucleosides. However, their methodology included the geometry optimization of each selected point on the PES at the MP2 level. On our workstations, a geometry optimization at the RI-MP2 level with a double- ζ (dz) basis set for these systems using 8 CPU threads required between 45 min and 4 h for our test optimizations, depending on the starting conformation. Performing all 441 calculations in series would then at least require ~13.7 days to fully scan the puckering in a single nucleoside on our system (see Figure S4.A in the Supporting Information). On the other hand, an RI-MP2 optimization using only a single CPU thread required ~4 h. In the theoretical case where we could run 16 optimizations independently, this would result in a wall-clock time of ~4.6 days to run all optimizations for only a single nucleoside, reducing the calculation runtime by a factor of 3 (Figure S4.B in the Supporting Information). Running all 441 optimizations at the MP2 level at the same time would be an ideal situation. Unfortunately, this well exceeds the specifications of commonplace workstations since most *ab initio* methods are very demanding at the hardware level.

The HF-3c method¹⁸ is a very lightweight method that aims to correct the errors in an HF calculation based on a small basis set using three corrections: geometric counterpoise, dispersion correction, and correction for incompleteness of the basis set in short ranges. An optimization using the HF-3c required ~2–4 min to complete using only a single CPU thread. Parallelization of the geometry optimizations and resources in mind, a full set of optimizations could be achieved within less than 3 h if 16 optimizations can run simultaneously, which is a significant improvement over optimizing at the (RI)-MP2 level (Figure S4.C in the Supporting Information). However, running 16 optimizations independently requires each of these to have a starting conformation. The further the starting conformation from the applied restraints during the optimization, the greater the possibility of the geometry optimization engine falling in the trap of broken geometries. To circumvent this obstacle, we scripted the workflow so that each optimization started from a neighboring conformation to reduce the possibility of the geometry optimization engine falling into this trap and lowering the required time for the respective optimization (Figure S4.D). While this does introduce a hierarchy in the order wherein optimizations are to be executed, the relative speed to perform a full puckering PES scan for each nucleoside at HF-3c level well surpasses that of (RI)-MP2, whereafter single-point (SP) calculations can be performed. Ultimately, the respective minima are still

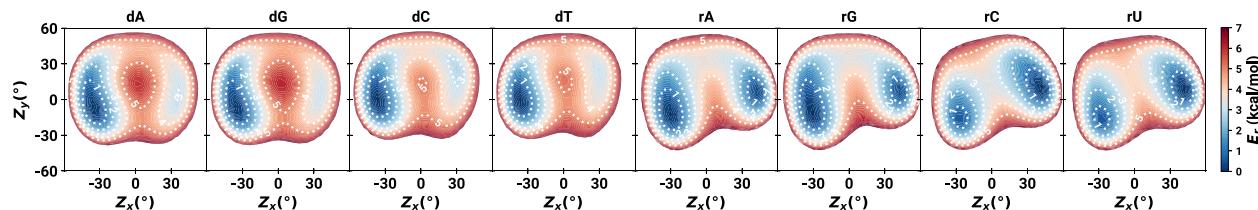


Figure 2. PES of puckering coordinates in DNA (dA, dG, dC, dT) and RNA (rA, rG, rC, rU) nucleosides with the HF-3c/RI-MP2 method. The PES plots for DNA nucleosides show a clear preference for the C2'-endo conformation, whereas the relative energy for the C3'-endo conformation is considerably lower for RNA nucleosides.

Table 2. Comparison of Relative Energy Difference and Structural Parameters for Localized Minima at the RI-MP2 Level of Theory for DNA and RNA Nucleosides^a

		C3'-endo (N)			C2'-endo (S)		
		P_θ (°)	A_r (°)	ΔE (kcal mol ⁻¹)	P_θ (°)	A_r (°)	ΔE (kcal mol ⁻¹)
dA	MP2 ²²	358.7	34.3	2.9	189.8	34.7	0.0
	RI-MP2(dz)	358.9	33.9	3.1	190.3	35.4	0.0
	RI-MP2(tz)	3.0	35.1	3.0	190.2	35.4	0.0
dG	MP2 ²²	357.8	33.9	3.1	190.6	34.8	0.0
	RI-MP2(dz)	358.0	33.5	3.4	191.2	34.6	0.0
	RI-MP2(tz)	5.0	35.4	3.1	190.8	35.5	0.0
dC	MP2 ²²	36.8	37.5	2.8	180.2	35.4	0.0
	RI-MP2(dz)	38.8	35.0	3.1	180.3	38.8	0.0
	RI-MP2(tz)	38.7	39.4	2.8	180.9	36.0	0.0
dT	MP2 ²²	26.5	33.5	3.2	180.8	35.4	0.0
	RI-MP2(dz)	30.7	34.5	3.4	180.8	35.1	0.0
	RI-MP2(tz)	36.8	38.1	3.2	181.6	36.0	0.0
rA	MP2 ²²	8.5	42.5	0.0	200.8	33.1	0.0
	RI-MP2(dz)	8.6	42.0	0.7	201.1	32.8	0.0
	RI-MP2(tz)	8.3	42.3	0.1	202.0	32.8	0.0
rG	MP2 ²²	9.1	42.5	0.1	197.9	33.3	0.0
	RI-MP2(dz)	9.4	42.0	0.6	198.3	33.1	0.0
	RI-MP2(tz)	8.9	42.4	0.2	198.3	33.1	0.0
rC	MP2 ²²	13.5	41.4	0.0	207.5	31.1	0.9
	RI-MP2(dz)	14.0	41.0	0.0	208.2	31.0	0.5
	RI-MP2(tz)	13.5	41.5	0.0	210.1	31.2	0.7
rU	MP2 ²²	12.7	42.0	0.0	207.8	31.9	0.9
	RI-MP2(dz)	13.2	41.5	0.0	208.5	31.9	0.3
	RI-MP2(tz)	12.5	41.9	0.0	209.9	32.0	0.6

^aValues for geometries optimized using the 6-31++G(d,p) basis set are indicated with RI-MP2(dz), and values for geometries optimized using the 6-311++G(2df,2p) are indicated with RI-MP2(tz).

examined at the RI-MP2 level to give more accurate energy differences.

This approach moved the computational bottleneck from the optimizations to the SP calculations with the extended basis set, which typically required 30 min or less per calculation on a single CPU thread. Since all geometries were available for the SP calculations, we could parallelize as many calculations as our CPU allows, keeping in mind some overhead to not oversubscribe the CPU too extensively and creating an additional bottleneck. Parallelizing the SP calculations over 16 CPU threads allowed us to complete all SP calculations in usually less than 12 h, depending on the molecule.

The puckering PESs of DNA and RNA nucleosides (Figure 2) obtained by the HF-3c/RI-MP2 method are qualitatively almost identical to the plots obtained at the MP2/6-311+ +G(3df,2p)//MP2/6-31++G(d,p) level of theory.²² Only two observable differences appear in the presented PESs. First, an additional 5 kcal mol⁻¹ contour appears at (0, 15) for dC, indicating the relative energy for conformations within this contour to be higher than 5 kcal mol⁻¹. Second, the puckering PES for rU shows a lower transitional barrier, depicted by the broader 4 kcal mol⁻¹ contour. Since these differences are only minor to the PESs from Huang and co-workers, our HF-3c/RI-MP2 approach can be considered as an efficient yet accurate

method to describe the sugar puckering in (deoxy)ribose nucleosides. In addition, the geometries of optimized minima were compared to evaluate the necessity of the triple- ζ basis set (Table 2). Both RI-MP2 with a double and triple- ζ basis set deviated several degrees for both P_θ and A_r . Overall, the geometries obtained with the double- ζ basis set were closer to the geometries obtained by Huang and co-workers. The deviation in structural parameters was slightly larger for the geometries obtained with the triple- ζ basis set (e.g., C3'-*endo* of dA and dG) with a maximum ΔP_θ of 10.3° and ΔA_r of 4.6° for dT. The relative energy for DNA nucleosides corresponded well for both basis sets. However, the double basis set was less accurate at determining the relative energies between both minima for RNA nucleosides. The offset varied between 0.4 kcal mol⁻¹ to 0.6 kcal mol⁻¹ whereas the maximum offset for relative energy obtained from triple- ζ basis set optimizations was 0.2 kcal mol⁻¹ for RNA nucleosides (Table 2). These results indicate that the use of triple- ζ basis set is recommended to obtain more accurate relative energies.

3.2. Five-Membered Modified Nucleosides. 3.2.1. 2'-Modified Nucleosides: 2'OMeRNA, 2'F-NA, 2'F-ANA. Modifications at C2', e.g., 2'F-NA and 2'OMeRNA, constitute some of the XNA more closely resembling their natural counterparts, because the sugar moiety differs little in chemical structure. In RNA, the 2'-OH substituent acts both as acceptor and donor in a variety of intramolecular hydrogen bonds and plays an important role in enzymatic and nonenzymatic catalysis. The substitution of the reactive hydroxyl group with a chemically more stable methoxy substituent in 2'OMeRNA results in stable oligonucleotide structures with reduced flexibility.³³

In experimental structures of 2'OMeRNA duplexes,^{33,34} the C3'-C2'-O2'-CH₃ dihedral is typically found in the *ap* region. The PES plots (Figure 3) of 2'OMeRNA were

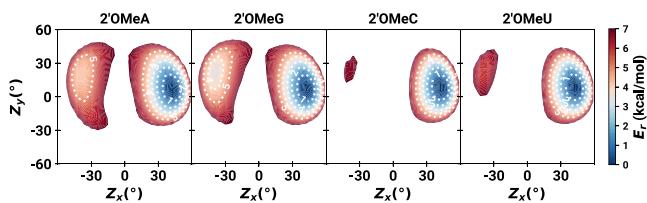


Figure 3. PES of puckering coordinates in 2'OMeRNA nucleosides with the HF-3c/RI-MP2 method.

generated at the HF-3c/RI-MP2 level (see the Methods section). For all four nucleosides, the 2'-methoxy substituent shifts the equilibrium toward C3'-*endo* conformations in comparison with natural (deoxy)riboses, since the relative energy difference between C2'-*endo* and C3'-*endo* minima is enlarged. This preference in puckering is identical to the puckering of 2'OMeRNA residues in crystal structures.^{33,34} In

contrast to DNA and RNA, this difference is more pronounced for pyrimidines (2'OMeU = 5.5 kcal mol⁻¹, 2'OMeC = 6.4 kcal mol⁻¹) than for purines (2'OMeG = 2.9 kcal mol⁻¹, 2'OMeA = 3.6 kcal mol⁻¹) for optimized geometries at the RI-MP2(tz) level.

In 2'F-NA, the 2'-hydroxyl is replaced by a single fluorine substituent. The PES plots for 2'F-NA nucleosides show the same pattern as seen for 2'OMeRNA, albeit less pronounced, i.e., a shift toward C3'-*endo* conformation (Figure 4). Also, a similar trend is observed in the difference in relative energy between C3'-*endo* and C2'-*endo* conformation, which ascends from purine to pyrimidine nucleosides: fG (2.8 kcal mol⁻¹) < fA (3.3 kcal mol⁻¹) < fU (3.9 kcal mol⁻¹) < fC (5.5 kcal mol⁻¹). These results agree with experimental crystal structures where all 2'F-NA residues adopt a C3'-*endo* conformation.^{35,36} Additionally, these results confirm the general consensus that electronegative 2'-substituents influence the puckering in preference of the C3'-*endo* conformation.³⁷

Experimental 2'F-ANA structures show a more diverse profile in puckering of the fluoroarabinose sugar moiety, compared to five-membered sugars mentioned above. Incorporation of 2'F-ANA thymine residues in B-DNA results in more stable duplexes while retaining the overall B-helical shape. The 2'F-ANA residues were identified to adopt a O4'-*endo* conformation, which was believed to be a result of predominantly steric interactions between 2'F and C6 of the thymine base.³⁸ Although the χ angle was restrained in the *ap* range (A-type helix) instead of *-anticlinal (-ac)* range (B-type helix), the PESs of all 2'F-ANA nucleosides show a reduced relative electronic energy for the O4'-*endo* conformation (Figure 4) instead of the C2'-*endo* conformation. Except for the cytosine analogue, all nucleosides favor the C3'-*endo* conformation over the O4'-*endo* conformation at the RI-MP2 level, albeit with a small margin (fG = 2.1 kcal mol⁻¹, fA = 1.4 kcal mol⁻¹, fU = 0.8 kcal mol⁻¹, fC = 0.3 kcal mol⁻¹ in favor of O4'-*endo*). This preference for the sterically less strained C3'-*endo* conformation corresponds well with the puckering observed in A-type helices in 2'F-ANA crystal structures.³⁹

3.2.2. 3'-Modified Nucleosides: dXyNA and XyNA. When reviewing literature regarding elucidated (d)XyNA structures,^{29,30,40–42} the major difference with the employed backbone restraints is found in the γ -dihedral, which is mostly found in the *-ac* range. Therefore, we decided to evaluate the puckering PESs of (d)XyNA nucleosides with the γ -dihedral restrained in an *-ac* fashion. The subsequent orientation of O5' did not result in any overlap with O3', and, hence, the PES showed a shift in favor of C3'-*endo* conformations (Figure 5), in agreement with the predominant conformation found in NMR solution structures of (d)XyNA.^{30,41,42}

The only difference between DNA and dXyNA is the 3'-epimerization. This simple, yet drastic, change completely

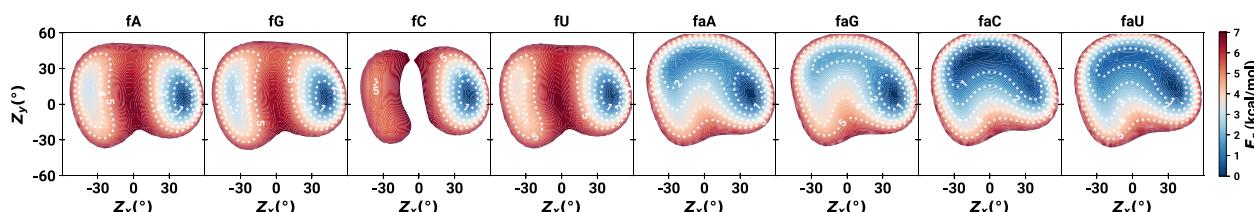


Figure 4. PES of puckering coordinates in 2'F-NA (fA, fG, fC, fU) and 2'F-ANA (faA, faG, faC, faU) nucleosides with the HF-3c/RI-MP2 method.

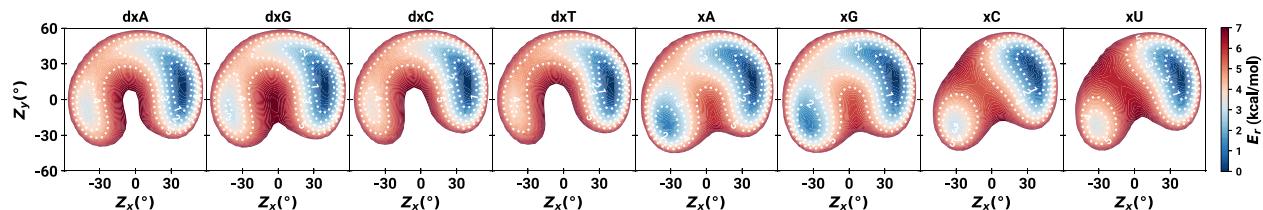


Figure 5. PES of puckering coordinates in dXyNA (dxA, dxG, dxC, dxT) and XyNA (xA, xG, xC, xU) nucleosides with the HF-3c/RI-MP2 method.

inverts the conformational preference of the 2'-deoxy nucleosides in favor of the C3'-*endo* conformation. Previous reports already observed this preference in solution NMR, but never rationalized why the epimerization would have such an effect.^{30,41–45} In the current work, deletion analysis of donor–acceptor interactions at the HF level with NBO7 indicated that interactions with O3' for dC stabilize the C2'-*endo* conformation ~1 kcal mol⁻¹ more than the C3'-*endo* conformation, whereas the inverse is observed for dxC (Table 3). As such, the epimerization shifts the balance by ~2 kcal

Table 3. Donor–Acceptor Interactions with O3' in dxC and dC Calculated with Deletion Analysis in NBO7^a

donor	acceptor	E_{del} (kcal mol) ⁻¹			
		dC		dxC	
		C2'- <i>endo</i>	C3'- <i>endo</i>	C2'- <i>endo</i>	C3'- <i>endo</i>
O3' (LP1)	C3'-C4'*	2.3	2.7	2.0	1.7
O3' (LP1)	C3'-H3'*	0.3	0.3	5.2	4.8
O3' (LP1)	C3'-C2'*	2.9	3.5	0.0	0.0
O3' (LP2)	C3'-C4'*	0.8	0.9	3.6	3.4
O3' (LP2)	C3'-H3'*	14.9	14.1	3.2	3.0
O3' (LP2)	C3'-C2'*	5.1	5.1	12.3	10.3
C2'-H2'	O3'-C3'*	4.7	0.0	1.3	1.6
C2'-H2''	O3'-C3'*	1.8	2.1	0.0	5.5
C2'-C1'	O3'-C3'*	0.0	3.5	4.7	0.0
C4'-H4'	O3'-C3'*	2.5	1.3	0.0	5.2
C4'-C5'	O3'-C3'*	1.7	0.0	0.9	1.1
C4'-O4'	O3'-C3'*	0.0	2.3	2.4	0.1
total		37.1	36.0	35.5	36.6

^aA higher E_{del} value indicates a stabilizing effect of the interaction.

mol⁻¹ in favor of C3'-*endo* conformation. Notably, interactions with O3'-C3'* as acceptor seem to be influenced the most by this epimerization (Table 3). In contrast to natural nucleic acids, the presence of a 2'-hydroxy substituent actually lowers the relative electronic energy of the C2'-*endo* conformation, instead of favoring the C3'-*endo* conformation. This can be attributed to the diaxial orientation of 2'-hydroxy and 3'-hydroxy substituents in the C3'-*endo* conformation. Such diaxial orientation of vicinal hydroxides is known to be sterically less favorable.^{46,47}

3.3. Pseudo-Five-Membered Nucleosides: CeNA. Previously reported analysis of the puckering in CeNA¹⁹ exploited the rigid endocyclic torsion over the double bond (ν_0) to consider both unsaturated carbons and the interconnecting double bond as a single pseudo-atom. In this approach, the puckering of CeNA was described on a conformational wheel, similar to nucleosides with a five-membered sugar moiety. To re-evaluate the PES of CeNA puckering at the HF-3c/RI-MP2 level, we transformed the (Z_x ,

Z_y) coordinates to the polar puckering parameters (P_θ , A_r) to follow the Fourier transform procedure as previously described.¹⁹

The evaluation of CeNA puckering at the RI-MP2 level shows qualitatively the same preference for C3'-*endo* conformations (Figure 6), as previously reported at the PM3

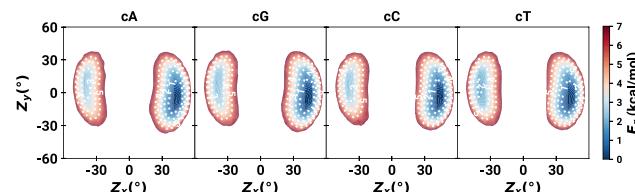


Figure 6. PES of puckering coordinates in CeNA nucleosides with the HF-3c/RI-MP2 method.

level.¹⁹ However, the energy difference between both conformers ($cG = 2.3$ kcal mol⁻¹, $cA = 2.5$ kcal mol⁻¹, $cT = 2.0$ kcal mol⁻¹, $cC = 2.5$ kcal mol⁻¹) is larger than the difference previously reported at the PM3 level (~0.5 kcal mol⁻¹).¹⁹ It is important to note that backbone dihedrals for CeNA nucleosides have been restrained in this study for the consistency of the methodology and to prevent influence of backbone dihedrals. As the previous PM3 study did not restrain backbone dihedrals and did not report on conformational changes in backbone dihedrals, it is difficult to compare the results. However, it is unlikely that the dihedrals remained constant over the PES without restraints, possibly reducing the energy difference between the C3'-*endo* and C2'-*endo* conformation due to favorable reorientation of backbone dihedrals in different nucleoside conformations. In either case, the C3'-*endo* remains the preferred conformation on the puckering PES of CeNA. The unrestrained χ dihedral remained in the high *anti* region for all optimized CeNA geometries at the RI-MP2(tz) level of theory (see Table S1 in the Supporting Information).

3.4. Six-Membered Modified Nucleosides: HNA and homo-DNA. HNA and homo-DNA are both XNA with six-membered sugar moieties that have been examined from a therapeutic and xenobiological perspective. Experimental duplex structures for both XNA have been described at the atomic resolution,^{48–51} but no single-stranded structure with different secondary motifs, e.g., loops, have been experimentally elucidated to date. Although both XNA show a structural preference for a particular chair conformation of the six-membered sugar moiety, a duplex structure for homo-DNA has been identified where a single residue adopted a boat conformation.^{48,51}

Obtained conformational globes of hA and ddA are quite similar when compared from different angles (Figure 7). However, rather low transition energies between different ddA

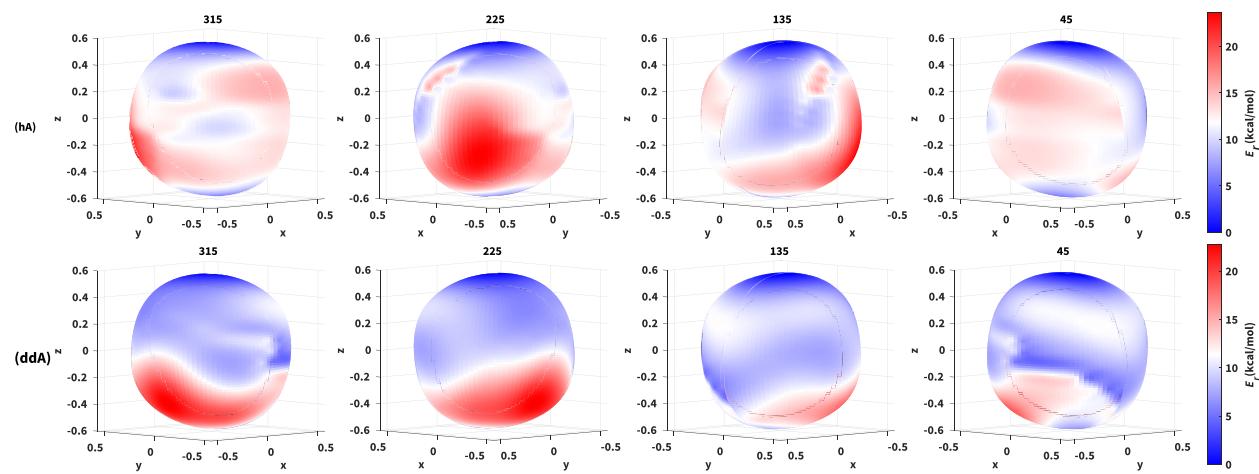


Figure 7. Comparison of corresponding views on the conformational globe of hA (HNA) and ddA (homo-DNA). Erratic changes in E_r are due to unrestrained changes in the χ -angle.

Table 4. Low-Energy Conformers for homo-DNA Nucleosides

	conformation	θ (deg)	ϕ (deg)	Q (deg)	χ (deg)	E_r (kcal mol ⁻¹)
ddA	${}^4C_{1'}$	1.2	92.8	32.9	201.5	0.0
	${}^1C_{4'}$	167.2	340.3	28.5	312.5	4.5
	${}^{1'A'}B$	85.4	220.6	38.8	296.2	6.2
	${}^{1'A'}B$	85.3	71.6	38.4	221.0	5.9
	${}^1S_{5'}$	91.4	278.3	41.2	178.9	2.5
	${}^0S_{2'}$	93.8	326.8	43.7	193.7	3.2
ddG	${}^4C_{1'}$	1.4	88.4	32.9	206.4	0.0
	${}^1C_{4'}$	167.5	343.9	28.3	310.2	3.6
	${}^{1'A'}B$	86.2	224.3	38.5	296.5	5.5
	${}^{1'A'}B$	85.2	71.5	38.3	232.2	5.6
	${}^1S_{5'}$	92.2	281.9	40.9	184.2	2.9
	${}^0S_{2'}$	93.9	327.8	43.8	195.8	3.2
ddC	${}^4C_{1'}$	1.5	72.9	33.0	203.1	0.0
	${}^1C_{4'}$	168.8	336.3	28.3	128.2	8.3
	${}^{1'A'}B$	89.6	230.4	38.3	294.6	9.9
	${}^{1'A'}B$	84.8	70.3	38.4	206.4	6.8
	${}^1S_{5'}$	92.5	285.6	40.5	193.1	3.7
	${}^0S_{2'}$	93.2	327.8	43.7	198.2	3.7
ddT	${}^4C_{1'}$	1.3	91.8	32.9	211.8	0.0
	${}^1C_{4'}$	169.4	339.9	28.3	127.7	6.0
	${}^{1'A'}B$	89.5	231.2	38.1	295.8	7.7
	${}^{1'A'}B$	86.2	72.3	38.0	226.3	6.0
	${}^1S_{5'}$	93.0	287.1	40.3	194.2	3.8
	${}^0S_{2'}$	94.0	326.1	43.1	200.1	3.8

conformations are visible, in contrast to hA. From the analyzed low-energy conformers of homo-DNA (Table 4), the ${}^1S_{5'}$ and ${}^0S_{2'}$ conformations are conformations with similar relative energy, compared to five-membered nucleosides (~ 3 kcal mol⁻¹). The boat conformation identified by Egli and co-workers⁴⁸ seems to be of the ${}^0S_{2'}$ type, which is closely related to the ${}^0S_{2'}$ conformation. Although the relative energy of the

${}^1C_{4'}$ conformation for homo-DNA purines remains below 5 kcal mol⁻¹, the relative energy of these conformations for the pyrimidine counterparts well exceeds 5 kcal mol⁻¹. The necessary change in χ to the -sc range causes a short distance between O2 of pyrimidine nucleobases and H3', which probably introduces strain on the molecule. For HNA (see Table 5), the search for low-energy conformers seems even

Table 5. Low-Energy Conformers for HNA Nucleosides

	conformation	θ (deg)	ϕ (deg)	Q (deg)	χ (deg)	E_r (kcal mol ⁻¹)
hA	${}^4C_{1'}$	0.8	38.5	31.9	210.6	0.0
	${}^1C_{4'}$	173.1	292.2	30.6	245.2	4.6
	${}^{1'A'}B$	91.5	222.9	44.0	71.9	5.4
	${}^1S_{3'}$	86.8	200.8	42.8	215.7	6.0
	${}^3S_{1'}$	87.4	29.9	43.3	243.5	3.9
	${}^4C_{1'}$	0.6	0.1	31.9	210.5	0.0
hG	${}^1C_{4'}$	172.7	290.4	30.7	249.0	3.7
	${}^{1'A'}B$	91.7	222.3	43.7	64.5	4.8
	${}^1S_{3'}$	87.2	202.2	43.1	216.4	5.4
	${}^3S_{1'}$	87.9	28.2	43.5	240.8	5.4
	${}^4C_{1'}$	2.6	245.5	31.5	204.4	0.0
	${}^1C_{4'}$	175.2	290.2	30.2	241.8	5.5
hC	${}^{1'A'}B$	90.8	219.3	43.0	269.6	7.8
	${}^1S_{3'}$	86.3	199.5	42.2	213.2	7.3
	${}^3S_{1'}$	88.8	25.2	43.6	220.3	3.5
	${}^4C_{1'}$	2.5	233.3	31.3	207.5	0.0
	${}^1C_{4'}$	175.8	289.3	30.2	243.3	4.5
	${}^{1'A'}B$	91.5	219.6	42.7	253.0	7.0
hT	${}^1S_{5'}$	87.4	200.3	41.8	219.8	6.8
	${}^3S_{1'}$	88.8	26.0	43.4	228.4	2.8

more difficult. Except for hG, the ${}^3S_{1'}$ conformations returned the lowest relative energy, compared to the respective ${}^4C_{1'}$ conformation (hA = 3.9 kcal mol⁻¹, hT = 2.8 kcal mol⁻¹, hC = 3.5 kcal mol⁻¹ mol). The relatively low energy of the ${}^3S_{1'}$ conformations are possibly attributed to favorable electrostatic interactions between O' and H8/H6 atoms. The position of the nucleobase on C2' requires a less drastic change in χ -angle

for the ${}^4C_{1'}$ conformation, resulting in a relatively low energy of this conformation, especially for purines ($hA = 4.6 \text{ kcal mol}^{-1}$, $hG = 3.7 \text{ kcal mol}^{-1}$, $hC = 5.5 \text{ kcal mol}^{-1}$, $hT = 4.5 \text{ kcal mol}^{-1}$). The complete set of conformational globes for homo-DNA (Figure S5) and HNA (Figure S6) are available in the Supporting Information.

Especially for HNA, where the energy minima seem to be isolated by higher transition states on the conformational globe, overall flexibility of the XNA could be significantly hampered. To fold in a stable tertiary structure, an XNA oligonucleotide should be able to form loops, which are one of the simplest secondary structural elements in oligonucleotides.^{52,53} The formation of such loops typically requires the extension of the P–P distance between nucleotides, usually arising from an increased flexibility in sugar moieties or a change in sugar conformation of loop residues, compared to the helical residues.^{4,52} As mentioned before, such an alternating puckering motif has only been experimentally observed for homo-DNA and not HNA.

4. CONCLUSION

A combined approach of the lightweight HF-3c method with the well-known (RI-)MP2 method was evaluated for the efficient and accurate calculation of the PES of puckering in natural nucleic acids and several XNA. Although the method cannot attain the accuracy of MP2 geometries and electronic energies (without RI approximations), the error is almost negligible and, as such, the method can be employed for general conformational sampling approaches when computational resources are limited or access to high-performance computing is unavailable.

The puckering PES of selected five-membered XNA species and CeNA showed great resemblance with available data on these XNAs. The effect of electronegative 2'-substituents was confirmed by exploring the PES of 2'OMeRNA and 2'F-NAs. A first attempt at describing the influence of the 3'-epimerization in (d)XyNA on the stereoelectronics was performed, indicating that predominantly donor–acceptor-type interactions with O3'-C3'* are influenced by this epimerization. Although the relative energy for the C2'-*endo* conformation seems enlarged at the MP2 level, the re-evaluation of CeNA at the MP2 level did not drastically alter the previous perspective.

Folded structures of single-stranded XNA with six-membered “sugars” in the backbone are not experimentally determined so far. The formation of stable tertiary structures requires secondary structural elements (e.g., loops) that contain a variation in puckering modes in available structures of folded nucleic acids with five-membered sugars. The relatively high electronic energy of conformers, other than the most stable ${}^4C_{1'}$, observed here for HNA and homo-DNA could indicate that such required flexibility might be hampered and dependent on external influences.

The efficient method presented in this work to determine the complete puckering PES of potential new XNA candidates can provide interesting structural information at the start of projects. Calculating the PES of the “sugar” in XNA can be a helpful tool in the selection of most suitable XNA candidates in applications for which stable folding is required, e.g., aptamer development. Moreover, it can complement and facilitate the experimental structure determination of novel XNA structures.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jctc.1c00270>.

Graphical illustrations of the pseudorotation in both five- and six-membered rings, as well as illustrations of the sampling approach and HNA/homo-DNA PES plots (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

- (1) Kilpatrick, J. E.; Pitzer, K. S.; Spitzer, R. The Thermodynamics and Molecular Structure of Cyclopentane. *J. Am. Chem. Soc.* **1947**, *69*, 2483–2488.
- (2) Altona, C.; Sundaralingam, M. Conformational analysis of the sugar ring in nucleosides and nucleotides. New description using the concept of pseudorotation. *J. Am. Chem. Soc.* **1972**, *94*, 8205–8212.
- (3) Cremer, D.; Pople, J. A. A General Definition of Ring Puckering Coordinates. *J. Am. Chem. Soc.* **1975**, *97*, 1354–1358.
- (4) Saenger, W. *Principles of Nucleic Acid Structure*; 1984; pp 1–8.
- (5) Vargason, J. M.; Henderson, K.; Ho, P. S. A crystallographic map of the transition from B-DNA to A-DNA. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 7265–7270.
- (6) Yamamoto, T.; Nakatani, M.; Narukawa, K.; Obika, S. Antisense drug discovery and development. *Future Med. Chem.* **2011**, *3*, 339–365.
- (7) Anosova, I.; Kowal, E. A.; Dunn, M. R.; Chaput, J. C.; Van Horn, W. D.; Egli, M. The structural diversity of artificial genetic polymers. *Nucleic Acids Res.* **2016**, *44*, 1007–1021.
- (8) Jordheim, L. P.; Durantel, D.; Zoulim, F.; Dumontet, C. Advances in the development of nucleoside and nucleotide analogues for cancer and viral diseases. *Nat. Rev. Drug Discovery* **2013**, *12*, 447–464.

- (9) Yates, M. K.; Selye-Radtke, K. L. The evolution of antiviral nucleoside analogues: A review for chemists and non-chemists. Part II: Complex modifications to the nucleoside scaffold. *Antiviral Res.* **2019**, *162*, 5–21.
- (10) Khvorova, A.; Watts, J. K. The chemical evolution of oligonucleotide therapies of clinical utility. *Nat. Biotechnol.* **2017**, *35*, 238–248.
- (11) Keefe, A. D.; Pai, S.; Ellington, A. Aptamers as therapeutics. *Nat. Rev. Drug Discovery* **2010**, *9*, 537–550.
- (12) Pinheiro, V. B.; Taylor, A. I.; Cozens, C.; Abramov, M.; Renders, M.; Zhang, S.; Chaput, J. C.; Wengel, J.; Peak-Chew, S.-Y.; McLaughlin, S. H.; Herdewijn, P.; Holliger, P. Synthetic Genetic Polymers Capable of Heredity and Evolution. *Science* **2012**, *336*, 341–344.
- (13) Vanmeert, M.; Razzokov, J.; Mirza, M. U.; Weeks, S. D.; Schepers, G.; Bogaerts, A.; Rozenski, J.; Froeyen, M.; Herdewijn, P.; Pinheiro, V. B.; Lescrinier, E. Rational design of an XNA ligase through docking of unbound nucleic acids to toroidal proteins. *Nucleic Acids Res.* **2019**, *47*, 7130–7142.
- (14) Zgarbová, M.; Otyepka, M.; Šponer, J.; Mládek, A.; Banáš, P.; Cheatham, T. E.; Jurečka, P. Refinement of the Cornell et al. Nucleic Acids Force Field Based on Reference Quantum Chemical Calculations of Glycosidic Torsion Profiles. *J. Chem. Theory Comput.* **2011**, *7*, 2886–2902.
- (15) Zgarbová, M.; Luque, F. J.; Šponer, J.; Cheatham, T. E., III; Otyepka, M.; Jurečka, P. Toward Improved Description of DNA Backbone: Revisiting Epsilon and Zeta Torsion Force Field Parameters. *J. Chem. Theory Comput.* **2013**, *9*, 2339–2354.
- (16) Zgarbová, M.; Šponer, J.; Otyepka, M.; Cheatham, T. E.; Galindo-Murillo, R.; Jurečka, P. Refinement of the Sugar-Phosphate Backbone Torsion Beta for AMBER Force Fields Improves the Description of Z- and B-DNA. *J. Chem. Theory Comput.* **2015**, *11*, 5723–5736.
- (17) Wang, L. P.; McKiernan, K. A.; Gomes, J.; Beauchamp, K. A.; Head-Gordon, T.; Rice, J. E.; Swope, W. C.; Martínez, T. J.; Pande, V. S. Building a More Predictive Protein Force Field: A Systematic and Reproducible Route to AMBER-FB15. *J. Phys. Chem. B* **2017**, *121*, 4023–4039.
- (18) Sure, R.; Grimme, S. Corrected small basis set Hartree-Fock method for large systems. *J. Comput. Chem.* **2013**, *34*, 1672–1685.
- (19) Nauwelaerts, K.; Lescrinier, E.; Sclep, G.; Herdewijn, P. Cyclohexenyl nucleic acids: Conformationally flexible oligonucleotides. *Nucleic Acids Res.* **2005**, *33*, 2452–2463.
- (20) Pickett, H. M.; Strauss, H. L. Conformational Structure, Energy, and Inversion Rates of Cyclohexane and Some Related Oxanes. *J. Am. Chem. Soc.* **1970**, *92*, 7281–7290.
- (21) Pickett, H. M.; Strauss, H. L. Symmetry and Conformation of the Cycloalkanes. *J. Chem. Phys.* **1971**, *55*, 324–334.
- (22) Huang, M.; Giese, T. J.; Lee, T. S.; York, D. M. Improvement of DNA and RNA sugar pucker profiles from semiempirical quantum methods. *J. Chem. Theory Comput.* **2014**, *10*, 1538–1545.
- (23) Harris, C. R.; Millman, K. J.; van der Walt, S. J.; Gommers, R.; Virtanen, P.; Cournapeau, D.; Wieser, E.; Taylor, J.; Berg, S.; Smith, N. J.; Kern, R.; Picus, M.; Hoyer, S.; van Kerkwijk, M. H.; Brett, M.; Haldane, A.; del Río, J. F.; Wiebe, M.; Peterson, P.; Gérard-Marchant, P.; Sheppard, K.; Reddy, T.; Weckesser, W.; Abbasi, H.; Gohlke, C.; Oliphant, T. E. Array programming with NumPy. *Nature* **2020**, *585*, 357–362.
- (24) Hunter, J. D. Matplotlib: A 2D Graphics Environment. *Comput. Sci. Eng.* **2007**, *9*, 90–95.
- (25) Glendening, E. D.; Landis, C. R.; Weinhold, F. NBO 7.0: New vistas in localized and delocalized chemical bonding theory. *J. Comput. Chem.* **2019**, *40*, 2234–2241.
- (26) Burai Patrascu, M.; Malek-Adamian, E.; Damha, M. J.; Moitessier, N. Accurately modeling the conformational preferences of nucleosides. *J. Am. Chem. Soc.* **2017**, *139*, 13620–13623.
- (27) IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN). Conformational Nomenclature for Five and Six-Membered Ring Forms of Monosaccharides and Their Derivatives. Recommendations 1980. *Eur. J. Biochem.* **1980**, *111*, 295–298.
- (28) Segà, M.; Autieri, E.; Pederiva, F. Pickett angles and Cremer-Pople coordinates as collective variables for the enhanced sampling of six-membered ring conformations. *Mol. Phys.* **2011**, *109*, 141–148.
- (29) Ramaswamy, A.; Smyrnova, D.; Froeyen, M.; Maiti, M.; Herdewijn, P.; Ceulemans, A. Molecular Dynamics of Double Stranded Xylo-Nucleic Acid. *J. Chem. Theory Comput.* **2017**, *13*, 5028–5038.
- (30) Mattelaer, C.; Maiti, M.; Smets, L.; Maiti, M.; Schepers, G.; Mattelaer, H.-P.; Rosemeyer, H.; Herdewijn, P.; Lescrinier, E. Stable Hairpin Structures Formed by Xylose-Based Nucleic Acids. *ChemBioChem* **2021**, *22*, 1638.
- (31) Feyereisen, M.; Fitzgerald, G.; Komornicki, A. Use of approximate integrals in ab initio theory. An application in MP2 energy calculations. *Chem. Phys. Lett.* **1993**, *208*, 359–363.
- (32) Neese, F. The ORCA program system. *Wiley Interdiscip. Rev.: Comput. Mol. Sci.* **2012**, *2*, 73–78.
- (33) Lubini, P.; Zürcher, W.; Egli, M. Stabilizing effects of the RNA 2'-substituent: crystal structure of an oligodeoxynucleotide duplex containing 2'-O-methylated adenosines. *Chem. Biol.* **1994**, *1*, 39–45.
- (34) Adamiak, D. A.; Milecki, J.; Popenda, M.; Adamiak, R. W.; Dauter, Z.; Rypniewski, W. R. Crystal structure of 2'-O-Me-(CGCGCG)₂, an RNA duplex at 1.30 Å resolution. Hydration pattern of 2'-O-methylated RNA. *Nucleic Acids Res.* **1997**, *25*, 4599–4607.
- (35) Manoharan, M.; Akinc, A.; Pandey, R. K.; Qin, J.; Hadwiger, P.; John, M.; Mills, K.; Charisse, K.; Maier, M. A.; Nechev, L.; Greene, E. M.; Pallan, P. S.; Rozners, E.; Rajeev, K. G.; Egli, M. Unique Gene-Silencing and Structural Properties of 2'Fluoro-Modified siRNAs. *Angew. Chem., Int. Ed.* **2011**, *50*, 2284–2288.
- (36) Pallan, P. S.; Greene, E. M.; Jicman, P. A.; Pandey, R. K.; Manoharan, M.; Rozners, E.; Egli, M. Unexpected origins of the enhanced pairing affinity of 2'-fluoro-modified RNA. *Nucleic Acids Res.* **2011**, *39*, 3482–3495.
- (37) Deleavey, G. F.; Damha, M. J. Designing chemically modified oligonucleotides for targeted gene silencing. *Chem. Biol.* **2012**, *19*, 937–954.
- (38) Berger, I.; Tereshko, V.; Ikeda, H.; Marquez, V. E.; Egli, M. Crystal structures of B-DNA with incorporated implications of conformational preorganization for duplex stability. *Nucleic Acids Res.* **1998**, *26*, 2473–2480.
- (39) Li, F.; Sarkhel, S.; Wilds, C. J.; Wawrzak, Z.; Prakash, T. P.; Manoharan, M.; Egli, M. 2'-Fluoroarabino- and Arabinonucleic Acid Show Different Conformations, Resulting in Deviating RNA Affinities and Processing of Their Heteroduplexes with RNA by RNase H †, ‡. *Biochemistry* **2006**, *45*, 4141–4152.
- (40) Ramaswamy, A.; Froeyen, M.; Herdewijn, P.; Ceulemans, A. Helical structure of xylose-DNA. *J. Am. Chem. Soc.* **2010**, *132*, 587–595.
- (41) Maiti, M.; Siegmund, V.; Abramov, M.; Lescrinier, E.; Rosemeyer, H.; Froeyen, M.; Ramaswamy, A.; Ceulemans, A.; Marx, A.; Herdewijn, P. Solution structure and conformational dynamics of deoxyxylonucleic acids (dxNA): An orthogonal nucleic acid candidate. *Chem. - Eur. J.* **2012**, *18*, 869–879.
- (42) Maiti, M.; Maiti, M.; Knies, C.; Dumbre, S.; Lescrinier, E.; Rosemeyer, H.; Ceulemans, A.; Herdewijn, P. Xylonucleic acid: Synthesis, structure, and orthogonal pairing properties. *Nucleic Acids Res.* **2015**, *43*, 7189–7200.
- (43) Rosemeyer, H.; Seela, F. 1-(2'-Deoxy- β -D-xylofuranosyl)-thymine Building Blocks for Solid-Phase Synthesis and Properties of Oligo(2'-Deoxyxylonucleotides). *Helv. Chim. Acta* **1991**, *74*, 748–760.
- (44) Roscmeycr, H.; Krecmerova, M.; Seela, F. 9-(2'-Deoxy- β -D-xylofuranosyl)adenine Building Blocks for Solid-Phase Synthesis and Properties of Oligo(2'-deoxy-xylonucleotides). *Helv. Chim. Acta* **1991**, *74*, 2054–2067.
- (45) Rosemeyer, H.; Strothholz, L.; Seela, F. 2'-Deoxy- β -D-xylothymidinyl-(3', 5')-2'-deoxy- β -D-xylothymidylate: stereochemical

course of dinucleoside phosphonate formation and conformational properties. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1201–1206.

(46) Abraham, R. J.; Koniotou, R.; Sancassan, F. Conformational analysis. Part 39. A theoretical and lanthanide induced shift (LIS) investigation of the conformations of cyclopentanol and cis- and trans-cyclopentane-1,2-diol. *J. Chem. Soc., Perkin Trans.* **2002**, *2*, 2025–2030.

(47) Chen, X.; Walthall, D. A.; Brauman, J. I. Acidities in cyclohexanediols enhanced by intramolecular hydrogen bonds. *J. Am. Chem. Soc.* **2004**, *126*, 12614–12620.

(48) Egli, M.; Pallan, P. S.; Pattanayek, R.; Wilds, C. J.; Lubini, P.; Minasov, G.; Dobler, M.; Leumann, C. J.; Eschenmoser, A. Crystal structure of homo-DNA and nature's choice of pentose over hexose in the genetic system. *J. Am. Chem. Soc.* **2006**, *128*, 10847–10856.

(49) Lescrinier, E.; Esnouf, R.; Schraml, J.; Busson, R.; Heus, H.; Hilbers, C.; Herdewijn, P. Solution structure of a HNA–RNA hybrid. *Chem. Biol.* **2000**, *7*, 719–731.

(50) Lescrinier, E.; Esnouf, R. M.; Schraml, J.; Busson, R.; Herdewijn, P. Solution Structure of a Hexitol Nucleic Acid Duplex with Four Consecutive T·T Base Pairs. *Helv. Chim. Acta* **2000**, *83*, 1291–1310.

(51) Sheehan, J. H.; Smith, J. A.; Pallan, P. S.; Lybrand, T. P.; Egli, M. Molecular dynamics simulation of homo-DNA: The role of crystal packing in duplex conformation. *Crystals* **2019**, *9*, 532.

(52) Varani, G. Exceptionally stable nucleic acid hairpins. *Annu. Rev. Biophys. Biomol. Struct.* **1995**, *24*, 379–404.

(53) Zacharias, M. Conformational analysis of DNA-trinucleotide-hairpin-loop structures using a continuum solvent model. *Biophys. J.* **2001**, *80*, 2350–2363.