

Article

New WC-Type DNA/RNA Microhelices Determined by a DFT Study with the Comparison of Their Structural Geometric Parameters and Their Intra-/Intermolecular H-Bonds

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Abstract

Background: The computation of DNA is a field of growing interest, primarily focused on studying its individual constituents, but a few authors have gone further by analyzing standard microhelices with DFT methods. **Objective:** Because these studies did not consider that, at the atomic level, other possible DNA and RNA helices with the WC-type arrangement are also stable, this is the main objective here. **Methods:** Therefore, forty microhelices were optimized in a simple model with three WC base pairs composed of nucleosides using the M06-2X DFT method. **Results:** Based on the spatial arrangement of the intramolecular H-bond with the 2'-OH group, five types of RNA:RNA double microhelices were obtained; by this arrangement, they were related to the corresponding DNA:DNA ones. In addition to A-type and B-type microhelices, two new types were found, conveniently named B1-type and C-type. The structural geometric parameters of all these optimized microhelices in an isolated state were compared and analyzed in detail. The total counterpoise-corrected interaction energies were determined in these microhelices. RNA microhelices with different types of intramolecular H-bond arrangements involving the 2'-OH group in each single strand were also optimized, as well as their corresponding DNA helices having the same spatial arrangement. Different DNA:RNA hybrid microhelices were also considered, and their structural geometric parameters were compared to those of other microhelices. **Conclusions:** the identification and detailed characterization, for the first time, of ten main stable spatial geometric shapes with different exocyclic and endocyclic torsional angles. Several relationships among the structural geometric parameters of these microhelices were also established. Although the new DNA/RNA helix types containing theoretically obtained WC pairs have not been found in biological helices, they could be synthesized and they may open the possibility of being used for other purposes.

Keywords: DNA; RNA; microhelices; helical geometric parameters; WC pairs; DFT methods

Academic Editor: Michael Fried

Received: 26 April 2026

Revised: 21 June 2026

Accepted: 2 July 2026

Published: 21 July 2026

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1. Introduction

The discovery of DNA/RNA structure is one of the most significant achievements in science as these biomolecules represent the molecular basis for life [1]. Thus, understanding their molecular spatial structure and functions, or those of their fragments, is of great importance and has been the subject of numerous research studies [2].

Although modern and cryo-EM techniques [3] can provide high-resolution structural information on biological macromolecules [4,5], unfortunately, most of this information is about the secondary DNA helix structure and it is commonly performed at a resolution quite far from the atomic level. Therefore, it is not very useful for describing in detail their geometrical parameters, such as the whole set of bond lengths, bonds and torsion angles, and interatomic distances and angles [6].

Therefore, the computational study of DNA helices is a research area of increasing interest today. The molecular and electronic structure of nucleobases, as well as their stability, flexibility, tautomerism, vibrational frequencies and interactions with metals and with other molecules, has been theoretically studied by many researchers using accurate *ab initio* and DFT quantum chemical methods [7–9]. Their possible WC and non-WC base pair combinations have also been studied as possible factors in DNA replication errors [10,11]. However, nucleosides, nucleotides and their pairing combinations have been studied less extensively using full optimization with these *ab initio* and DFT methods [12–15]. Some of these studies have focused on the design of new antiviral or anticancer drugs [16,17]. The transferability of these results from individual DNA constituents to large DNA structures using these accurate quantum chemical methods has been analyzed by only a few authors. This may be due to the complexity of the system and the high computational requirements, especially for system convergence. Thus, a few studies involving two nucleotide base pairs [1,18–22], three nucleotide base pairs [16,23–28] and five nucleotide pairs [29] with these DFT methods can be highlighted. Most of these studies are focused on calculating the different energies that appear between the strands. Previous publications by us using three nucleotide pairs [16–18,23] were focused on how different anticancer/antiviral drugs can interact with and distort DNA/RNA growth.

However, in the present manuscript, our aim was to study the helix geometry at the atomic level, especially the arrangement of the sugar ring atoms and the intramolecular H-bond types that can be established through the 2'-OH hydroxyl group. We have related these H-bond types in double-stranded RNA helices to the corresponding DNA helices and to the experimental ones, describing new helix types as well as new DNA/RNA double helices with different combination types on each strand.

Because the DNA molecule has a strong ability to undergo many structural changes depending on a wide variety of factors such as nucleotide sequence, environment, and interactions with ions and other molecules (e.g., inorganic compounds or drugs), numerous helix types can be observed experimentally. These transitions are due to the positional polymorphism of the base pair, which can adopt multiple conformations, as well as to the phosphate groups. Therefore, the DNA constituents have the capacity to form different conventional and non-conventional H-bonds; some of them with small differences appear labeled as B', C, and D to Z [6,30]. Among all these possibilities, we considered it of interest to first study, using accurate DFT methods, those DNA/RNA helices with the standard WC base pair and without the influence of external ions or other molecules. Based on experimental structural information on these standard WC pair helices, only three types labeled as A-type, B-type and Z-types have been reported [31]. Thus, the present study focuses only on them and on two other new structures that have been calculated theoretically. However, due to the significant difference in all geometrical

parameters and energies of the Z-type helix compared to the A- and B-types, we consider it appropriate to include many of the Z-type results in a new future publication.

In these double-stranded helices, both strands are of the same type. However, our interest here also lies in explaining that other arrangements are possible, where stable helices can exist with strands of different types, as well as other combinations in which different types exist within a single strand. This could explain some anomalies in the DNA/RNA growth. Therefore, one of the aims in the present manuscript was not only to study those experimentally found helices (A-, B- and Z-types), but also those that may be present (although less stable), and those that could be synthesized for other purposes [32–34]. We will also study how helices are structurally possible with different strand types. The study of the DNA/RNA helices at the atomic level can improve our understanding of these important biomolecules and their role in life.

Because DFT optimization of helices containing more than three nucleoside pairs exceeds the available computational time and memory, and also presents large convergence problems, only helices with three nucleoside base pairs were optimized, called microhelices or mini-helices [24].

Thus, a summary of the main objectives of the present manuscript with optimized DNA/RNA microhelices of different strands and types was as follows: (i) To describe the inter- and intramolecular H-bonds that occur in the DNA/RNA microhelices, in particular those formed by rotation of the 2'-OH hydroxyl group. (ii) To characterize new double-stranded RNA microhelices stabilized by this 2'-OH group and their corresponding DNA helices, which were also computationally stable, and which for convenience were labeled as B1- and C-types. (iii) To analyze new double DNA and RNA microhelices with different combinations between A-, B-, and Z-types (and others labeled for convenience as B1- and C-types) in each strand. (iv) To describe the special characteristics of the intermolecular H-bonds in the different microhelix types by means of the interaction energy calculation and its effect on the helix stability. (v) To determine the helical parameters and NBO charges of all combination types and microhelix strands and find relationships between them.

2. Computational Methodology

Full geometry optimization of all microhelices was carried out using Density Functional (DFT) computations [35] mainly through the Minnesota exchange-correlation functional M06-2X [36] with the 6-31G(d,p) and 6-311+G(d,p) basis sets. These basis sets represent one of the best ratios between the accuracy obtained in the calculations and the time and computer memory consumed. They are highly standardized and have been used for a long time in many molecular structures by many authors, and they were also used successfully in previous publications with microhelices [17,18,23,27]. Only DNA/RNA combinations with nucleobases in the standard WC orientation were studied here. All calculations were performed using the Gaussian16 software package [37] running on the Brigit supercomputer of the Computing Center in the Complutense University at Madrid. Standard parameters were utilized for the calculations in the program UNIX version.

The M06-2X DFT method was selected because it appears to be one of the best choices for considering dispersion-bound systems [38,39] from a geometric point of view, in particular nucleobase stacking [40–42], compared to other commonly used meta-generalized gradient functionals. Therefore, in previous publications [16,17,23,24,27], it provided the best geometric result among several DFT methods, including M06HF [43], wB97XD [44] and B97D [45]. Furthermore, it was significantly better than the M05-2X, LC-wPBE, B3LYP and MPWB1K methods, which failed to optimize double-stranded DNA/RNA microhelices [16]. Also, the M06-2X method has demonstrated reasonable

accuracy in mimicking the DNA/RNA backbone geometries [18,28,46] and predicting possible antiviral modified nucleosides [47,48]. Especially, the calculated geometric parameters in DNA microhelices at the M06-2X/6-31G(d,p) level were reported to be quantitatively similar to those at the B97-D3/def2-SV(P) level [49] with a few exceptions [26,28]. Furthermore, the interaction energies calculated for the stacking of DNA base dimers with thymine/thymine and adenine/adenine and RNA dimers with uracil/uracil [50–52] using the M06-2X method showed a good accordance with those obtained by CCSD(T)/CBS. Although these London dispersion corrections (for example with D3/D4 functionals) could further refine our calculated energies, they are not altered qualitatively. As an example and for comparative purposes, the geometrical values obtained in a few selected microhelices using the dispersion correction D3 [53,54] with the B3LYP and M06-2X methods were included in Tables and Figures of the present manuscript. Because the geometrical parameters and relative energies were similar to those obtained without this dispersion correction, and the calculations required a longer computational time and memory, no further optimizations were performed with them.

Because our focus was on the geometry and spatial arrangement of the helices, and for comparison purposes with other works, in all optimization processes the Berny algorithm was utilized under standard convergence criteria and with the default fine integration grid. Frequency calculations were only performed on the most important optimized structures due to the huge amount of computer memory that this required.

The microhelices were optimized in a simple model with three nucleoside base pairs. The large amount of computer memory, the time required and the convergence criterion pose a significant problem for increasing the system. However, these small microhelices appear to be large enough to realistically mimic and calculate many parameters of DNA structure [24,26]. Only bicatenary forms were optimized in both DNA and RNA helices for obtaining all geometric parameters, although a few monocatenary forms were also optimized for the calculation of the deformation energy values in the double-stranded formation from a single strand. To simplify and facilitate the interpretation of the different changes in the helices, most of the microhelices studied have one strand formed only by pyrimidine nucleosides and another strand formed only by purine nucleosides, that is, as WC triribonucleoside diphosphate homopolymer forms [24] and in the 5'- to -3' sequence. The microhelices with uridine (RNA) or thymidine (DNA) nucleosides were notably easier to optimize than those with cytidine. Therefore, microhelices with uridine/thymidine in one strand and adenosine in another strand were mainly optimized, with the discussion and analysis of the different types of spatial arrangements focusing mainly on them. The difficulty in optimizing microhelices with cytidine are due to the large interplanar H-bonds/interactions that significantly deform the helix and result in unrealistic forms.

The DNA:DNA microhelices were obtained from the prior optimization of the corresponding RNA:RNA microhelices, after removing the 2'-OH group and including the methyl group at the 5th position of the uracil ring. The ends of the backbone chains at H5'1 ($n-1$ plane) and H3'1 ($n+1$ plane) were conveniently oriented to avoid false intramolecular H-bond formation that could alter the geometry and helix energy. Therefore, with the exception of the microhelices containing cytidine, these ends were not replaced by methyl groups, as has been described in similar calculations by other authors [24]. The microhelices were optimized with a global negative charge of -4 .

In an initial study, the role of the hydration shell and Na⁺ cations was analyzed by us using different DNA microhelices. This study was conducted with four Na⁺ cations, one around each phosphate group, and with ten explicit water molecules surrounding the phosphates and Na⁺ cations [16]. Subsequently, this influence was studied in other helices [17,23]. Although small changes in the geometric parameters were observed, the

trends remained consistent compared to other microhelices. Therefore, due to the significant computational time required, these calculations were not included in the present manuscript. Moreover, the aim of this manuscript was to theoretically study those microhelices that, under the WC arrangement, can be stable and can present different intramolecular H-bond types in each strand and which could be synthesized for other purposes.

2.1. Interaction Energy Calculation in the Microhelices

This E^{int} energy corresponds to that existing between both helix strands, and it was determined only in the main microhelices described here, with the sugar ring as either β -D-2'-deoxyribose (DNA) or β -D-ribose (RNA). Their values between strand-A (pyrimidine strand) and strand-B (purine strand) were determined using the following relationship [27,28]:

$$E^{\text{int}}(\text{AB}) = E_{\text{AB}}^{\text{AB}}(\text{AB}) - E_{\text{A}}^{\text{AB}}(\text{AB}) - E_{\text{B}}^{\text{AB}}(\text{AB}) \quad (1)$$

where $E_{\text{AB}}^{\text{AB}}(\text{AB})$ corresponds to the computed electronic energy over the whole microhelix (AB), while $E_{\text{A}}^{\text{AB}}(\text{AB})$ (or $E_{\text{B}}^{\text{AB}}(\text{AB})$) represents the electronic energy of each of the isolated strands A (or B) within the entire system (AB).

The deformation energy $E^{\text{def}}(\text{AB})$ (also called relaxation energy ΔE_r by other authors [28]) produced in the double-stranded DNA/RNA helix formation from the optimized monocationary form can be described as:

$$E^{\text{def}}(\text{AB}) = E_{\text{A}}^{\text{def}}(\text{AB}) + E_{\text{B}}^{\text{def}}(\text{AB}) \quad (2)$$

where $E_{\text{A}}^{\text{def}}$ or $E_{\text{B}}^{\text{def}}$ of each monocationary helix ($X \equiv \text{A}$ or B) can be calculated by:

$$E_{\text{X}}^{\text{def}}(\text{AB}) = E_{\text{X}}(\text{AB}) - E_{\text{X}}(\text{X}) \quad (3)$$

In this equation, the parentheses indicate whether the computations were carried out at the optimized molecular geometry of the single-stranded helix (A or B) or to the whole system with the double-stranded helix (AB).

The calculated interaction energies were corrected by these deformation energies according to the Boys and Bernardi procedure for basis set superposition error [50,51], leading to the total counterpoise (CP)-corrected interaction energies $\Delta E_{\text{AB}}^{\text{CP}}$. These energies were determined using the following relationship:

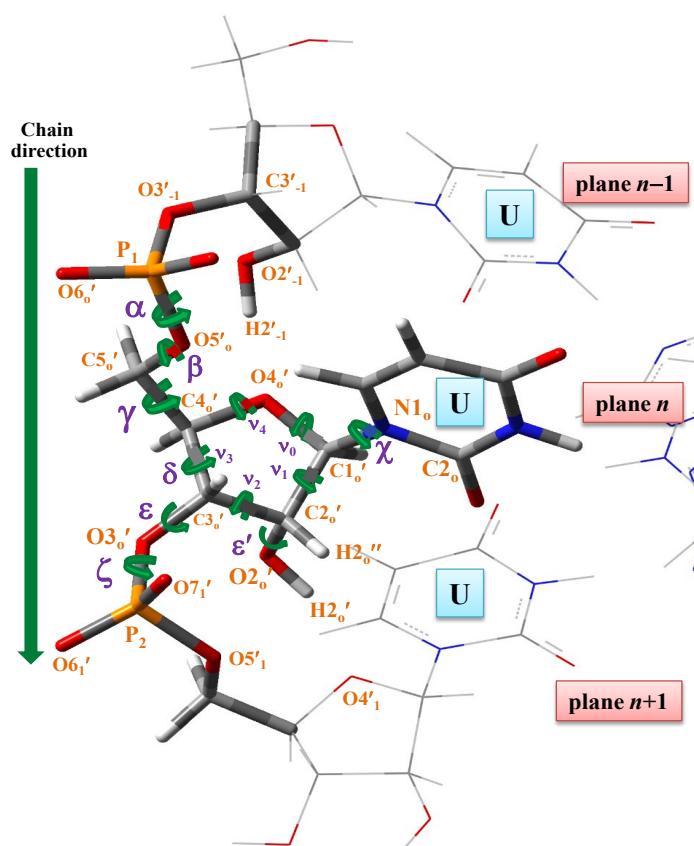
$$\Delta E_{\text{AB}}^{\text{CP}} = E^{\text{int}}(\text{AB}) + E^{\text{def}}(\text{AB}) \quad (4)$$

These energies were calculated under two main models, according to that followed by other authors [24]: In Model 1 (vacuum), with a negative charge on the helix, our values were also characterized as a true local minimum on the endothermic energy scale [24]. In Model 2 (vacuum), where the negative charge is compensated by counterions, either H^+ or Na^+ cations [25], the calculations were also carried out by us using Na^+ . Since we considered the results obtained with Model 2 to be of greater interest in the E^{int} calculation, their values were the only ones collected in the figures of the present manuscript.

2.2. Characteristic Conformational Parameters in Nucleotides

In the optimization with three nucleoside base pairs, the geometric parameters obtained in the nucleoside of plane- n (Scheme 1) were mainly considered, and only these are collected in the tables and figures of this manuscript. This is because they appear to be the closest to those that would be present in a longer helix. The labeling of atoms in these microhelices is also included in Scheme 1, where their numbering follows the Saenger standard notation [55] used in many publications [16,23]. This conventional notation has

also been utilized for the exocyclic (χ , ζ , α , β , γ , δ , ϵ , and ϵ') and endocyclic (ν_0 to ν_4) torsional angles of the nucleotides in the helix. The subscripts (0, 1 or -1) correspond to the pair plane (n , $n+1$ or $n-1$, respectively) in the microhelix. The colored atom notation is as follows: red (O), blue (N), orange (P), gray (H) and white (H).



Scheme 1. Numbering of the main atoms and definition of the exocyclic and endocyclic torsion angles in the 5'-UUU-3' pyrimidine strand of A-type in the UUU:AAA microhelix.

Among the exocyclic torsional angles, the parameter χ (O4'-C1'-N1-C2), also known as the glycosidic angle, appears to be the most important for defining the relative position of the nucleobase with respect to the sugar ring, with three main forms: *syn*, *anti*, and *high-anti*. The remaining exocyclic angles are mainly related to the helix chain flexibility. Their description can be summarized as follows:

(i) The skeletal ζ (C3'-O3'-P1-O5') angle [16] defines the relative orientation of the furanose-phosphate backbone with respect to the helical twist. (ii) The torsion α (O3'-P1-O5'-C5') angle describes the O3' oxygen atom position [16] within the phosphate group of the chain. (iii) The torsion β (P1-O5'-C5'-C4') angle indicates the phosphate moiety orientation relative to the sugar ring, which is found rotated to reduce non-bonding interactions. (iv) The torsion γ (O5'-C5'-C4'-C3') angle defines the O5' oxygen relative orientation and the sugar ring, which is found rotate to ease the formation of the weak/very weak intramolecular O5'...H6 (pyrimidine) or O5'...H8 (purine) H-bonds. (v) The torsion δ (C5'-C4'-C3'-O3') angle is the principal that describes the furanose ring symmetry. (vi) The torsion ϵ (C4'-C3'-O3'-P2) angle defines the orientation of the phosphate moiety of plane $n-1$ relative to the furanose ring and contributes to the helical twist. This angle, along with β , is normally present in the helix in *trans* form. (vii) The torsion angle ϵ' (H2'-O2'-C2'-C3') of RNA strands indicates the hydroxyl group O2'-H orientation in the furanose ring, and it is responsible for the helix type. (viii) The pucker **P**

(pseudorotation phase angle) establishes the sugar ring symmetry, and thus the helix type, and is defined as:

$$\text{tg } P = \frac{(v_4 + v_1) - (v_3 + v_0)}{2 v_2 (\sin(36) + \sin(72))} \quad (5)$$

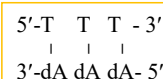
where v_0 to v_4 correspond to the endocyclic angles [55] of the furanose ring.

The maximum torsion angle (degree of pucker), $v_{\max}$, has been correlated with the sugar ring flexibility, where a high value indicates a greater flexibility. It is described as:

$$v_{\max} = \frac{v_2}{\cos P} \quad (6)$$

2.3. Notation Used for Microhelix

The nucleotides in the microhelices were countered in the standard way from top (plane $n-1$) to bottom (plane $n+1$), following the $O5' \rightarrow O3'$ direction. The two helix strands were labeled as *I* and *II* for convenience, where strand *I* corresponds to that with a pyrimidine nucleobase in plane n , and only this strand was represented in the simplified microhelix notation, i.e., the microhelix:



was only denoted by 5'-TTT-3' or by TTT:dAdAdA, focusing only on the pyrimidine nucleosides. This notation facilitated the discussion and analysis of the different microhelices. Only the nucleotide values in this plane n were considered and collected in Tables.

2.4. Characteristic Parameters in Helices

Numerous parameters that explain the geometric relationships between the nucleobases of the helix are described in the bibliography [56–58]. For simplicity, only a few of these parameters were considered and discussed in the present manuscript. The definition of those considered as the most important is as follows:

- (i) *Translational parameters for base stacking:*
 - The **rise** parameter (Dz) corresponds to the distance between nucleobases in a stacked form [16]. It was determined for both strands, using the notation Dz_{-1} for the calculated distance between the geometric center of the six-atom ring in the nucleobase of the $(n-1)$ plane with that of the n plane, while Dz_0 corresponded to the calculated distance between the n and $n+1$ planes.
 - The **slide** parameter (Dy) (in Å) describes the relative lateral displacement ($\bar{Y}$ axis) of one WC base pair with respect to the next pair. The notation (Dy_{-1}) corresponds to the displacement of the nucleobase ring between plane $(n-1)$ and plane n .
 - The **shift** parameter (Dx) (in Å) describes the displacement, similarly to the Dy parameter, but in this case, it was determined along the $\bar{X}$ axis. The notation (Dx_{-1}) corresponds to the displacement of the nucleobase ring between plane $(n-1)$ and plane n .
- (ii) *Rotational parameters for base stacking:*
 - The **helical twist** ω (also labeled as Ω) corresponds to the angle between the $\bar{Y}$ axes of the stacked nucleobases of the planes $n-1$ and n . It is calculated in the pyrimidine and purine strands.
- (iii) *Rotational base-pair parameters:*
 - The **opening** σ angle (in degrees) was measured in the plane- n and it corresponds to the in-plane angle between the nucleobases of the pair.

- The **propeller twist** θ_p (also labeled as ω) was calculated as the torsion angle between individual planes of the purine and pyrimidine rings and was only measured in the plane- n . It can be approximately determined if it is observed along the longitudinal axis of the WC base pair and, therefore, by way of the torsion angle φ^{II} [16] defined as $\text{C}4_0^{T/dC}-\text{N}3_0^{T/dC}\dots\text{N}1_0^{dA/dG}-\text{C}6_0^{dA/dG}$.
 - The **buckle** κ angle (in degrees) corresponds to the complementary dihedral angle between the nucleobase rings along their short axis, after θ_p was rotated to 0° ($\theta_p = 0$).
- (iv) *Another base-pair parameter:*
- The **inclination** angle INC (also labeled as η) of the base pair in plane- n was calculated with respect to the helix axis O_0 passing through $\text{C}8_0^{dA/dG}$ [16].

3. Results and Discussion

The formation of different DNA/RNA helices and their stability result from the distinct H-bonds that can be established. Therefore, the first point to study was focused on a detailed description of these H-bonds. Fourteen distinct H-bonds were identified in the theoretical calculated RNA:RNA microhelices of previous publications [16,23], and they were labeled from ① to ⑭. Four correspond to intermolecular H-bonds between both helix strands (①, ②, ⑨ and ⑫), and ten to intramolecular H-bonds (③ to ⑧, ⑩–⑪, ⑬–⑭) within the same strand. Among them, only a few of those that we consider as the most important will now be described and analyzed in detail in the present manuscript.

3.1. Intramolecular H-Bonds Determined in RNA Microhelices Compared to Those in DNA

H2' hydroxyl hydrogen presents a high reactivity, and because of that four types of intramolecular H-bonds were found through the 2'-OH hydroxyl group in the optimized RNA:RNA microhelices, Figures 1–3. These figures mainly show the pyrimidine strand of the UUU:AAA RNA microhelices. These four types of H-bonds were labeled as A, B, C and Z, and they were compared to a similar geometric arrangement of the furanose and purine/pyrimidine rings in the TTT:dAdAdA DNA:DNA microhelices, which lack the 2'-OH group. For simplicity, these figures only include the furanose ring of the central fragment (plane- n) (all have the same arrangement) with the phosphate backbone. For a better characterization of them, these H-bonds were marked in a bright yellow color circle with the labeling of the atoms involved. The H-bond length values, along with several characteristic exocyclic torsional angles, the pseudorotation phase angle (P) and the total microhelix energy, were included in these figures and in Tables 1 and 2. The detailed description of these H-bonds through the H2' hydroxyl hydrogen of plane- n was as follows:

- (i) **H-bond to O4'1** in the plane $n+1$, Figure 1a: This H-bond was initially labeled by us as ④ [12] and now labeled as **A-type**, and it leads to A-type RNA helices, in analogy to A-type DNA helices, Figure 1b with the TTT:dAdAdA helix. The furanose ring appears in RNA with 3E symmetry, although it is 3T ($P = 7.8$) in the DNA microhelix with the 6-31G(d,p) basis set. Although DNA helices do not have the 2'-OH hydroxyl group, the geometric arrangement of these DNA and RNA microhelices appear similar, with closely exocyclic and endocyclic torsional angles. E.g., $\chi = -154.9^\circ$ in RNA vs. -151.7° in DNA, and $\zeta = -70.9^\circ$ in RNA vs. -64.2° in DNA. The remaining exocyclic angles are also similar, Table 1. The intramolecular H-bond distance $\text{H}2'_0 \cdots \text{O}4'_1$ in RNA is also somewhat close to that of $\text{H}2''_0 \cdots \text{O}4'_1$ in DNA, 2.286 Å. These results indicate that the $\text{O}2'-\text{H}2'_n \cdots \text{O}4'_{n+1}$ H-bond has little effect on the geometric arrangement of the helix.

Table 1. Calculated backbone parameters at the M06-2X/6-31G(d,p) level in the nucleoside base pair of central plane n according to Scheme 1. Exocyclic torsional angles and pseudorotational angle P are in degrees. The calculated values using the M06-2X/6-311+G(d,p) level are shown in italic form, while those underlined correspond to the B3LYP-D3/6-31G(d,p) level. Microhelices are defined by the pyrimidine strand.

	Microhelix	Type	Pyrimidine Strand										Purine Strand									
			χ	α	β	γ	δ	ϵ	ζ	P	S	$v_{\max}$	χ	α	β	γ	δ	ϵ	ζ	P	S	$v_{\max}$
RNA:RNA	5'-U U U-3'	A	-154.9	-69.4	168.2	65.6	83.8	-160.6	-70.9	9.0	³ E	37.6	-130.5	-73.6	170.7	63.4	89.5	-162.2	-68.5	2.6	³ <i>T</i>	33.1
			-155.3	-71.3	167.6	66.6	82.8	-160.5	-70.6	9.3		37.9	-128.2	-74.5	170.2	63.4	89.3	-163.8	-70.8	3.5		32.6
		B	-111.6	-64.4	169.8	53.4	143.8	-175.9	-113.9	157.4	² E	43.1	-110.1	-59.2	167.1	52.5	137.7	153.2	-84.3	150.7	² <i>T</i>	48.1
		B1	-107.0	-64.3	175.1	51.0	148.7	-175.5	176.3	158.9	² E	46.5	-116.7	12.0	98.9	50.3	136.0	-166.5	-143.1	142.9	² <i>T</i>	49.6
		C	-112.1	-72.0	167.4	54.3	124.6	-165.3	-100.8	129.9	¹ E	48.3	-112.3	-70.5	166.6	53.9	124.7	-162.1	-103.3	129.8	¹ E	47.1
		A-C ^a	-155.8	-70.7	169.2	65.3	84.0	-159.8	-70.2	9.7	³ E	37.3	-113.1	-68.7	166.8	55.7	124.4	-161.5	-103.9	129.5	¹ E	47.0
	5'-C C C-3'	B-C ^b	-111.8	-63.8	170.0	52.7	142.4	-174.8	-115.5	153.8	² E	43.9	-113.3	-69.1	167.1	55.5	124.5	-162.5	-103.3	129.5	¹ E	47.1
		A	-154.9	-64.9	164.9	63.5	84.8	-160.8	-75.1	5.2	³ <i>T</i>	38.2	-152.0	-73.7	169.6	69.1	87.2	-167.1	-72.3	10.1	³ E	32.8
	5'-A U A-3' ^f	B	-99.0	-57.8	165.9	66.0	148.3	172.4	-105.6	167.0	² E	43.8	-110.0	-64.1	-175.7	55.3	147.7	150.4	-86.2	177.8	² <i>T</i>	42.7
		A	-159.6	-74.1	168.8	65.9	82.6	-159.2	-68.9	9.1	³ <i>T</i>	38.9	-153.9	-72.2	173.8	64.6	80.5	-166.9	-74.8	17.3	³ E	38.2
	5'-TTT-3'	B	-109.3	-64.8	173.8	51.4	142.4	-176.3	-109.2	153.2	² E	44.9	-122.3	-66.7	170.2	49.1	138.0	156.7	-85.6	151.6	² <i>T</i>	46.7
		A ^c	-152.1	-72.9	169.3	65.0	85.6	-154.4	-65.1	8.8	³ <i>T</i>	35.7	-129.2	-73.7	171.6	62.7	89.9	-161.6	-67.7	3.0	³ <i>T</i>	33.0
DNA:RNA		B ^d	-115.6	-64.7	-175.9	56.2	136.1	168.7	-96.5	157.5	² E	32.5	-109.8	-60.0	167.3	54.8	139.4	154.3	-85.2	153.1	² E	47.3
DNA:DNA	5'-T T T-3'	A	-152.1	-74.5	168.5	65.5	85.8	-152.6	-63.3	7.8	³ <i>T</i>	36.0	-140.3	-66.0	159.2	67.0	82.6	-159.5	-60.6	17.1	³ E	36.8
			-151.7	-74.0	168.0	64.1	85.5	-156.7	-64.2	9.1	³ E	35.9	-149.0	-67.0	155.9	70.2	85.8	-160.5	-61.7	25.1		40.4
		B	-116.3	-59.4	-175.1	58.1	134.8	165.9	-97.3	157.7	² E	31.3	-107.1	-62.8	164.6	56.8	130.7	140.3	-76.1	142.9	² <i>T</i>	42.8
			-117.1	-58.7	-177.8	58.0	133.9	168.7	-97.8	156.7		31.1	-104.1	-61.8	167.0	55.6	130.8	140.2	-75.8	145.8		41.7
		B1	-109.5	-63.4	-177.1	58.6	148.9	-175.1	-178.5	163.4	² E	40.5	-112.7	6.7	97.1	47.0	136.5	-173.6	-161.0	144.4	² <i>T</i>	46.6
		C	-148.5	-71.8	161.8	56.9	81.9	171.0	-88.8	72.5	⁰ ₄ <i>T</i>	43.1	-139.4	-68.7	161.6	61.1	87.3	172.9	-87.3	77.8	⁰ ₄ <i>T</i>	40.0
	5'-dCdCdC-3'	A-B ^e	-154.7	-73.9	166.0	65.0	86.0	-155.6	-63.4	7.4	³ <i>T</i>	36.0	-109.7	-77.9	166.8	47.8	102.0	98.3	-49.9	110.4	⁰ ₁ <i>T</i>	40.7
		B-C ^b	-114.9	-64.2	-175.0	55.8	132.5	167.4	-97.9	150.9	² <i>T</i>	32.2	-120.7	-67.5	160.8	60.3	100.6	171.9	-86.6	98.2	⁰ E	37.9
	5'-dATdA-3' ^f	A	-160.8	-56.1	130.4	66.7	86.3	-139.3	-67.1	7.4	³ <i>T</i>	39.3	-161.5	-76.8	169.4	69.6	86.9	-161.3	-58.5	191.6	³ E	35.0
		B	-117.9	-47.2	127.8	71.6	147.7	169.8	-92.0	166.9	² E	40.2	-113.0	-64.1	-171.6	60.5	150.1	147.4	-78.4	185.1	² <i>T</i>	38.8
	5'-dATdA-3' ^f	A	-158.8	-72.1	171.4	67.0	82.2	-157.6	-66.1	6.9	³ <i>T</i>	38.8	-157.5	-70.3	171.6	61.5	78.9	-170.9	-77.2	34.5	³ <i>T</i>	37.7
		B	-123.6	-62.4	169.9	58.0	127.0	153.7	-81.2	135.4	² <i>T</i>	47.4	-137.6	-65.2	172.4	52.8	123.0	149.1	-79.5	131.7	¹ E	47.7

Notation used from Ref. [55]: χ (O4'-C1'-N1-C2), α (O3'-P-O5'-C5'), β (P-O5'-C5'-C4'), γ (O5'-C5'-C4'-C3'), δ (C5'-C4'-C3'-O3'), ϵ (C4'-C3'-O3'-P₂), ζ (C3'-O3'-P-O5'), P (pseudorotation phase angle), S(furanose puckering mode), $v_{\max} = v_2/\cos P$. ^aPyrimidine strand is of A-type and purine strand is of C-type. ^bPyrimidine strand is of B-type and purine strand is of C-type. ^cWith DNA pyrimidine strand and RNA purine strand, TTT:AAA microhelix. ^dWith DNA pyrimidine strand and RNA purine strand, TTT:AAA microhelix. ^ePyrimidine strand is of A-type and purine strand is of B-type. ^fFor convenience, the pyrimidine strand in this microhelix is that with a pyrimidine nucleobase in plane n .

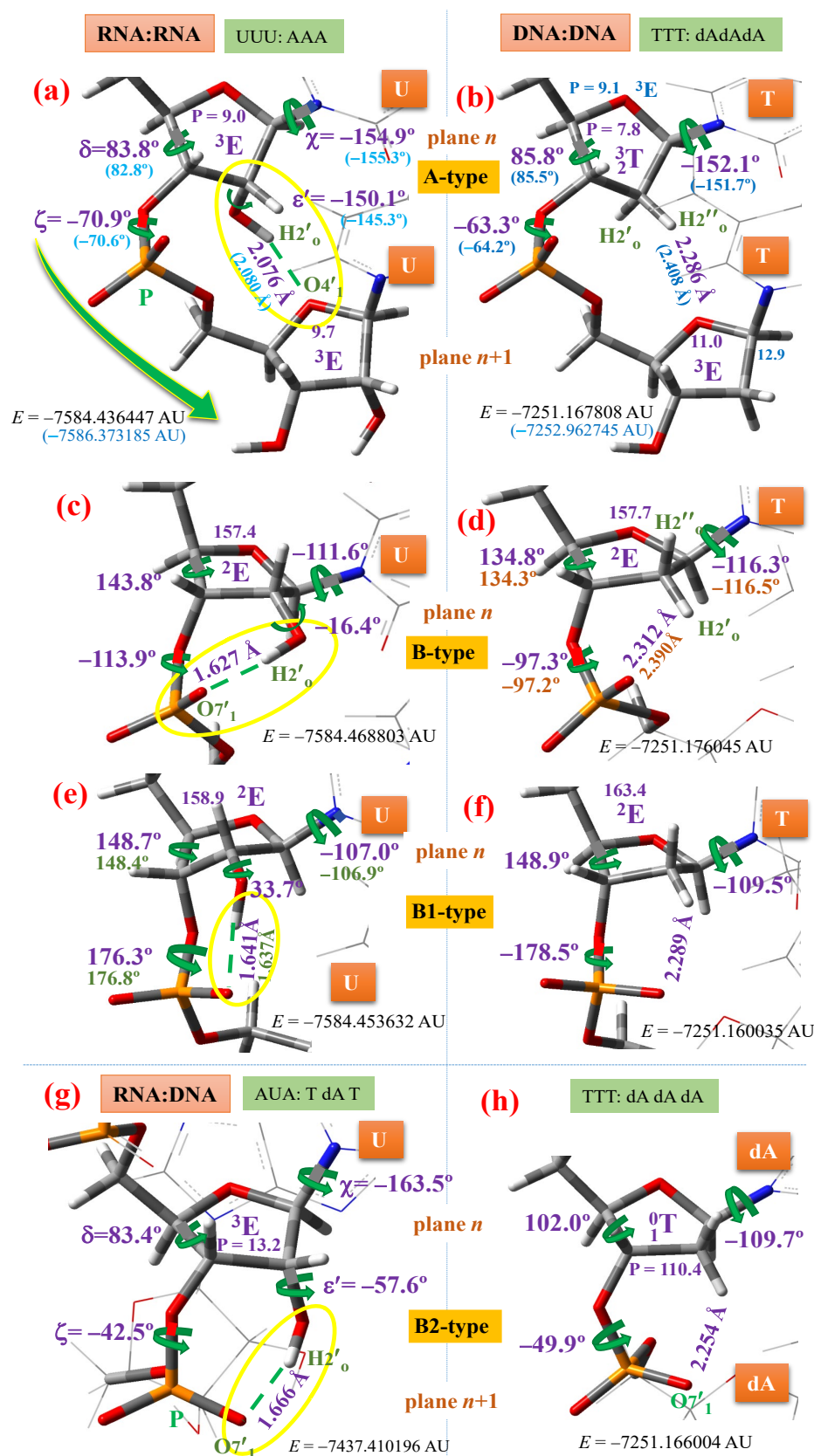


Figure 1. (a–f) Main types of intramolecular H-bonds through the 2'-OH hydroxyl group calculated in the 5'-UUU-3' pyrimidine strand of the UUU:AAA RNA microhelices and compared with a similar geometric arrangement in TTT:dAdAdA DNA ones. (g,h) Geometrical values in the B2-type molecular arrangement. The values of the most characteristic parameters calculated at the M06-2X/6-31G(d,p) level are shown in violet color, while those in blue correspond to

M06-2X/6-311+G(d,p). The values in dark orange are obtained at the B3LYP-D3/6-31G(d,p) level and those in green at the M06-2X-D3/6-31G(d,p) level.

- (ii) **H-bond to O7'₁** oxygen of the phosphate group in the plane $n+1$, Figure 1c: This H-bond was labeled as ⑧ [16] and now renamed as **B-type**, and it leads to B-type RNA helices, so labeled in analogy to B-type DNA helices, Figure 1d. In RNA, this H-bond is stronger than A-type, with a H2'_o...O7'₁ distance of 1.627 vs. 2.076 Å in A-type. It is noted in the DNA helix that the intramolecular distance (or very weak H-bond) H2'_o...O7'₁ in B-type is with H2'_o (Figure 1d), whereas it is with H2'_o in A-type, Figure 1b. This B-type is characterized by the furanose ring with ²E symmetry, and by the exocyclic torsional angles δ , ζ and ϵ' with values of 143.8°, −113.9° and −16.4°, respectively, in the UUU:AAA RNA helix. However, these values differ by ca. 10° in the DNA helix, which means that the O2'-H2'_n...O7'_{n+1} H-bond has some influence on the helix geometric arrangement. Significant differences also appear in the purine strand where the furanose ring symmetry changes to ²₁T instead of ²E. Two new stable B-subtypes, H-bonded to the phosphate oxygen O7'₁, have been identified with different values for the optimized exocyclic torsional angles, which are described as follows:
- Figure 1e: There are noticeably different values of the exocyclic torsional angles ϵ' (which is positive) and ζ (which is large), 33.7° and 176.3°, respectively, compared to −16.4° and −113.9° in UUU:AAA of B-type. The remaining exocyclic angle values are collected in Table 1. Since these new torsion angle values differ markedly from those obtained in B-type, for convenience, they were labeled as **B1-type**. The corresponding B1-type in DNA was also optimized, Figure 1f. The intramolecular distance/H-bond H2'_o...O7'₁ in this B1-type was slightly longer than in B-type and, therefore, slightly less stable.
 - Figure 1g: There is ³E symmetry in the furanose ring, as in A-type microhelices, instead of the ²E orientation in B-type, and also with clear and remarkable differences in most of the torsional angles. Due to these features and for convenience, it was labeled as **B2-type**. It was only found in the AUA:TdAT hybrid microhelix. The corresponding B2-type in DNA, with the closest geometric arrangement, was found in the TTT:dAdAdA microhelix of Figure 1h, although with very large differences in the furanose ring symmetry and in several exocyclic torsional angles.
 - TTT:dAdAdA DNA: In the purine strand of the this microhelix was observed significant differences in several exocyclic torsional angles and in the furanose ring symmetry, compared to B-type. For this reason, it was labeled as **B3-type** and plotted in a later figure. This geometric arrangement resulted in the least stable microhelix.
- (iii) **H-bond to O5'₁** of the plane $n+1$, Figure 2a: This corresponds to the H-bond ⑭ and now renamed as **C-type**. This H-bond between H2'_o hydroxyl hydrogen and O5'₁ is slightly weaker than B-type but stronger than A-type and it leads to a new helix type that for convenience, we have labeled as **C-type**. This notation does not correspond to the C-form of DNA used by some authors [6,24] for a structure that differs in the base orientation from the standard WC. Moreover, the notation previously used for B1 and B2 does not correspond to B1 and B11 used for non-standard WC pairs [6,24]. The present C-type is characterized by a furanose ring with ¹E symmetry, totally different from that of A-type and B-type, and by several exocyclic torsion angles. Although it may have some similarities to B-type, it differs markedly in the δ and ϵ' values, which are 124.6° (143.8° in B-type) and 40.7° (−16.4° in B-type), respectively.

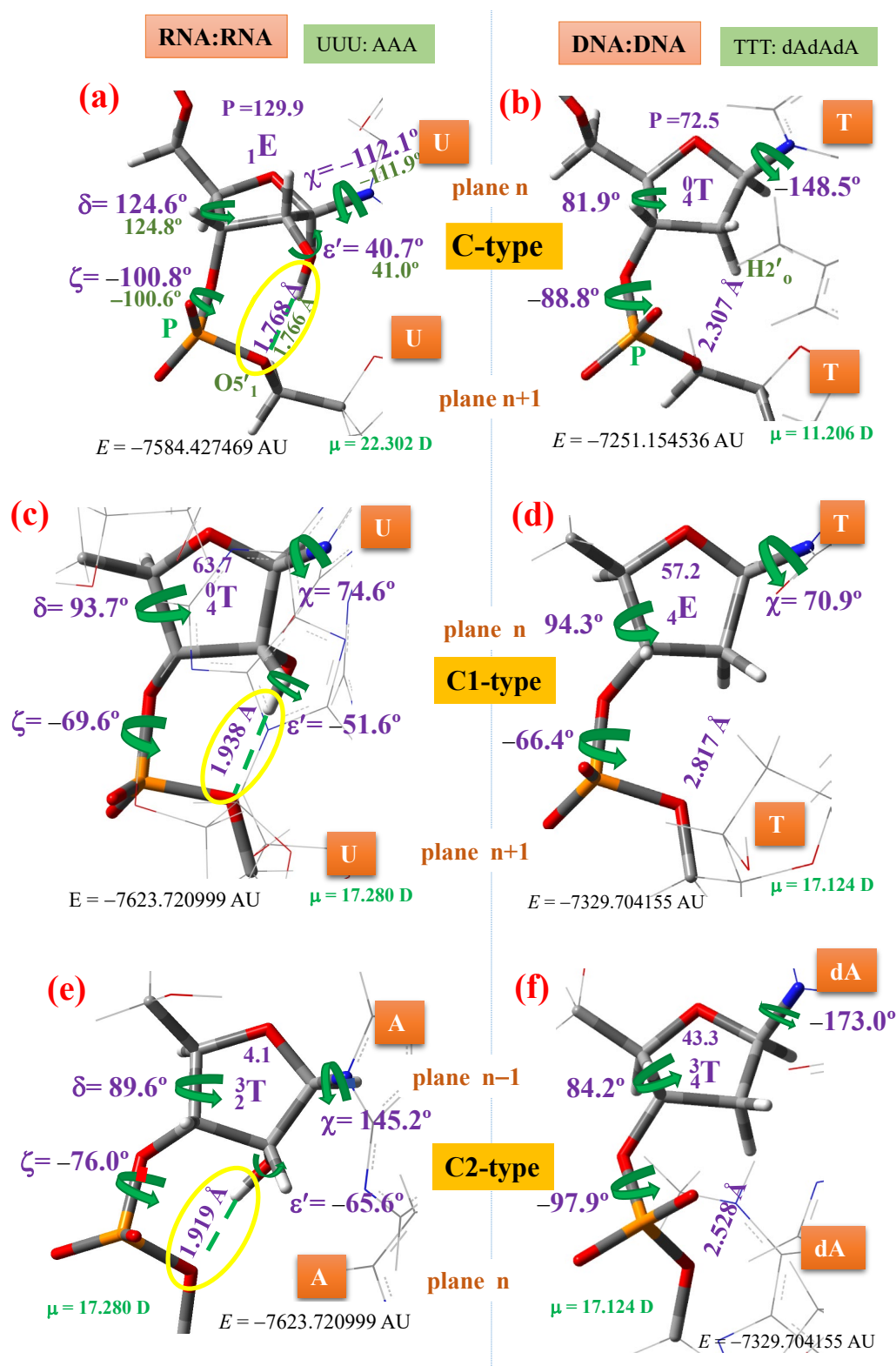


Figure 2. Different intramolecular H-bond types were calculated with the 2'-OH hydroxyl group in the plane n and the O5'₁ oxygen in the plane $n+1$: (a) C-type in the pyrimidine strand of the UUU:AAA RNA:RNA microhelix. (b) C-type in the pyrimidine strand of TTT:dAdAdA DNA:DNA microhelix. (c,d) C1-type in the pyrimidine strand of UUU:AAA and TTT:dAdAdA microhelices, respectively. (e,f) C2-type in the purine strand of UUU:AAA and TTT:dAdAdA microhelices, respectively. The geometrical values in green correspond to the M06-2X-D3/6-31G(d,p) level.

The corresponding C-type in DNA was also theoretically optimized, Figure 2b, but showed large differences compared to that of RNA. E.g., the furanose ring symmetry 0_4T ($P = 72.5$) fully differs from all other optimized microhelices. The χ torsion angle value of -148.5° also differs from that calculated in RNA (-112.1°), although it shows some similarity to A-type of DNA (-152.1°). The δ value of 81.9° differs from that with RNA (124.6°), although it is also similar to that of A-type in DNA (85.8°). It is noted that the intramolecular distance $H2'\cdots O5'$ of $2.307 \text{ \AA}$ (and represented in Figure 2b) is with $H2'$, in analogy to B-type and with a similar value, $2.312 \text{ \AA}$.

Two new stable C-subtypes H-bonded to $O5'_{n+1}$ oxygen were identified with both different furanose ring symmetry and optimized exocyclic torsion angles, which are as follows:

- a. Figure 2c: With the furanose ring symmetry 0_4T , which remarkably differs from C-type, as well as the χ angle (74.6°), ζ angle (-69.6°), δ angle and ϵ' . Therefore, for convenience, it was labeled as **C1-type**. This arrangement has been identified in one of the nucleosides of the pyrimidine strand in the UUU:AAA microhelix. The corresponding DNA microhelix (Figure 2d) was also optimized with a similar geometric arrangement, although with a slight difference in the furanose ring symmetry with 4E ($P = 57.2$). Similar exocyclic torsional angle values were obtained in the dATdA:UAU mixed hybrid DNA:RNA microhelix shown in a later figure.
- b. Figure 2e: With different values of all exocyclic torsional angles compared to C-type and C1-type, and also with different furanose ring symmetry (3_2T). Because of that, for convenience, it was labeled as **C2-type**. It was obtained in the purine strand of the UUU:AAA microhelix. Its pseudorotation phase angle value of $P = 4.1$ appears similar to that of A-type (Figure 1a), and this similarity can also be observed in the χ , δ and ζ angles, with values of 145.2° (-154.9° in A-type), 89.6° (83.8° in A-type) and -76.0° (-70.9° in A-type), respectively. However, its ϵ' angle differs remarkably, -65.6° vs. -150.1° in A-type.

The corresponding DNA microhelix was also optimized, Figure 2f, but it shows remarkable differences in the furanose ring symmetry with 3_4T ($P = 43.3$), as well as in the χ angle of -173.0° vs. 145.2° in RNA, and in the ζ angle of -97.9° vs. -76.0° in RNA. However, the dATdA:TdAT mixed DNA, and plotted in a latter figure, was found with similar exocyclic torsional angles, although with very different furanose ring symmetry.

- (iv) **H-bond to $O3'$** , but both in the plane $n-1$, Figure 3a: This $H2'\cdots O3'$ H-bond between the hydroxyl hydrogen $H2'$ and the neighboring $O3'$ oxygen is weak, especially because the $O2'-H2'\cdots O3'$ angle value of 118.4° in the UUU:AAA microhelix differs from 180° , where the maximum stretching occurs. This new type of H-bond, labeled as **Z-type**, leads to the Z-type helices, with a very different arrangement from the previous A-, B- and C-types. It appears with the furanose ring in the 3E orientation ($P = 26.6$ in RNA), as in A-type, and also with similar χ and δ angles. However, the ζ angle differs remarkably, with a value of 77.5° vs. -70.9° in A-type, and especially the ϵ' angle, with a value of -35.4° vs. -150.1° in A-type. The corresponding DNA helix included in Figure 3b has similar exocyclic torsional angles, which indicates the very small effect of the $H2'\cdots O3'$ H-bond on the geometric arrangement and, consequently, on the helix stability.

Several new stable Z-subtypes H-bonded to $O3'$ oxygen have been identified with different furanose ring symmetry and exocyclic torsion angles, Figure 3. A short description of some of these spatial geometric arrangements was as follows:

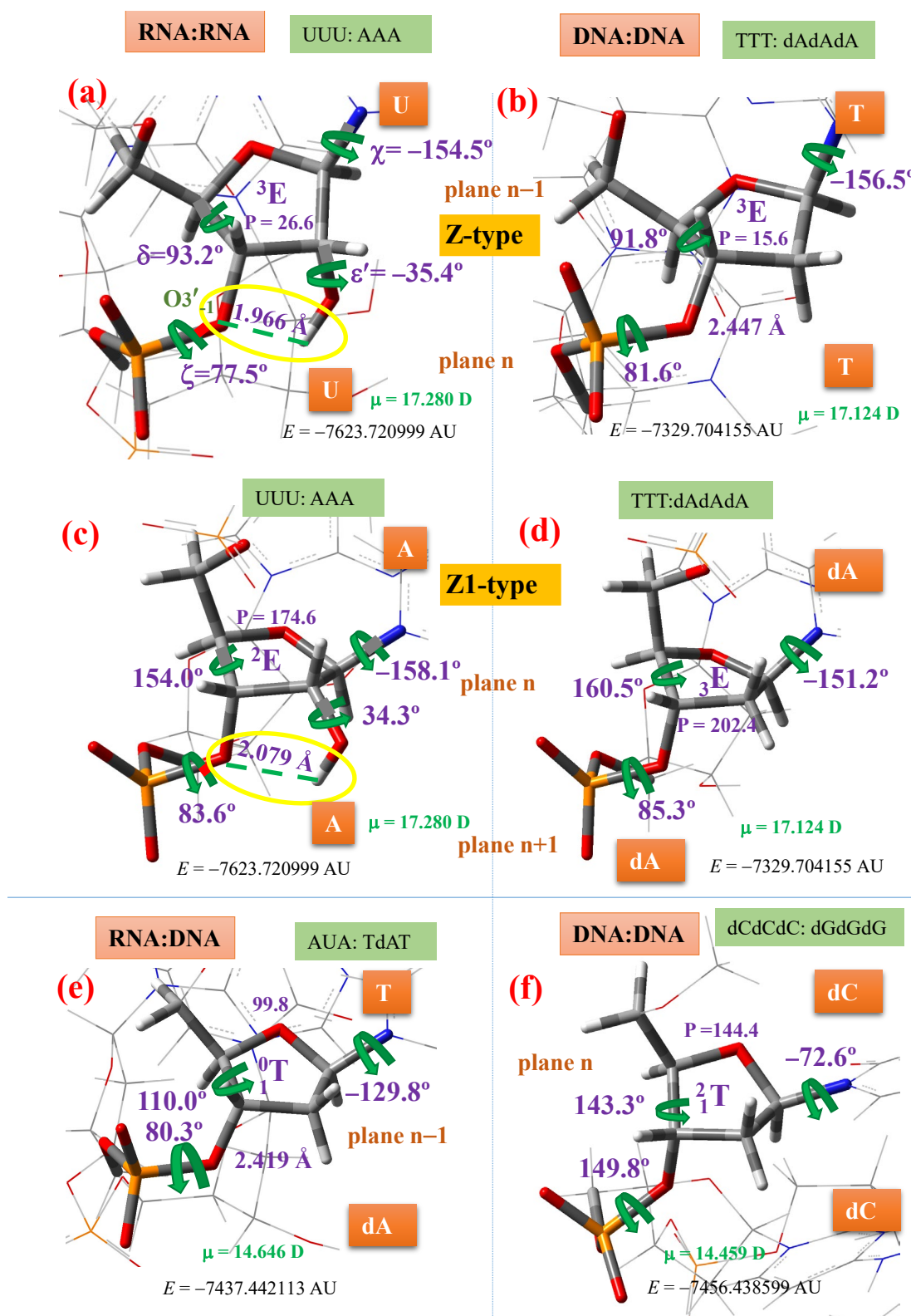


Figure 3. Intramolecular H-bond types through the 2'-OH hydroxyl group and the O3' oxygen, compared to similar geometric arrangement in the corresponding DNA one, of the following microhelices: (a,b) Pyrimidine strand of UUU:AAA and TTT:dAdAdA Z-type microhelices, respectively. (c,d) Purine strand in the UUU:AAA and TTT:dAdAdA Z1-type microhelices, respectively. (e) TdAT strand in the mixed hybrid AUA:TdAT RNA:DNA microhelix. (f) Pyrimidine strand in the dCdCdC:dGdGdG DNA microhelix.

- Figure 3c: With a furanose ring of 2E symmetry ($P = 174.6$), which is very different from that of Z-type, and with a δ angle of 154.0° compared to 93.2° in Z-type. For convenience, this geometric arrangement was called **Z1-type**. It was obtained in the purine strand of the UUU:AAA microhelix. The corresponding DNA one is shown in Figure 3d, although it differs in the furanose ring symmetry with 3E ($P = 202.4$).
- Figure 3e: With the furanose ring of 0_1T symmetry ($P = 99.8$), which is very different from that of Z-type, as well as in the δ angle, 110.0° vs. 93.2° in Z-type, and in the χ angle. It was obtained in the DNA strand of the AUA:TdAT hybrid mixed microhelix.
- Figure 3f: With the furanose ring of 2_1T symmetry ($P = 144.4$), which is very different from Z-type of DNA, as well as in the δ angle, 143.3° vs. 91.8° in Z-type, and in the χ and ζ angles. It was obtained in the dCdCdC:dGdGdG DNA microhelix.

Figure 4 includes several spatial arrangements of the furanose ring symmetry observed in specific microhelices, in particular those corresponding to B3-, C1, and C2-types and described in previous paragraphs.

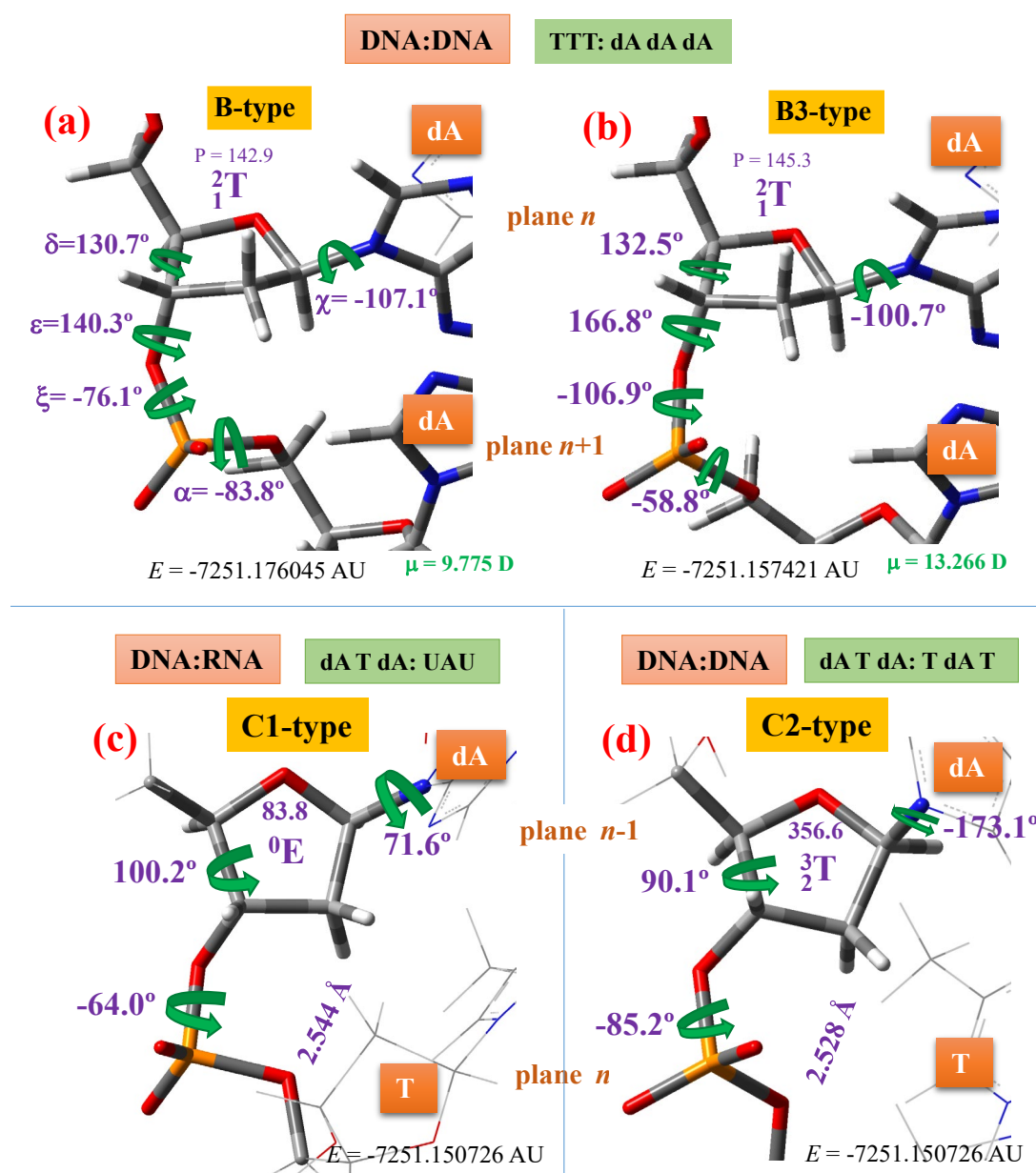


Figure 4. Other calculated spatial arrangements of the furanose ring symmetry in the following microhelices: (a,b) In the purine strand of TTT:dAdAdA of B-type compared to that found in a

new, less stable microhelix called B3-type. (c) C1-type in the 5'-dATdA-3' strand of dATdA:UAU DNA:RNA mixed hybrid microhelix. (d) C2-type in the 5'-dATdA-3' strand of the mixed dATdA:TdAT DNA:DNA microhelix.

Other intramolecular H-bonds/contacts have been observed in the calculated RNA:RNA microhelices, providing additional stability to the helix structure, Figure 5. These are briefly described below:

- (i) Figure 5a shows a very weak intramolecular H-bond/contact between the O5'H hydroxyl oxygen of plane $n-1$ and the H6 hydrogen of the nucleobase, which stabilizes this hydroxyl group position in the microhelices. This H-bond/contact appears only at the first nucleoside (plane $n-1$) of all our microhelices. The O5'...H6 distance is less than 2.4 Å in the pyrimidine nucleoside of the RNA/DNA helices of the A- and C-types. The arrangement in purines is similar, but the O5'...H6 distance is generally much longer than 2.6 Å, and in B-types this distance is even larger, indicating a negligible effect on them.
- (ii) Figure 5b: In B-type microhelices, the hydroxyl group position on the first nucleoside (plane $n-1$) is stabilized by an intramolecular H-bond between H5'1 and O4'1. This H-bond is remarkably stronger than that shown in Figure 5a, which facilitates the OH group fixing for subsequent calculations. The value included in this figure corresponds to the 5'-UUU-3' microhelix.
- (iii) Figure 5c: In RNA microhelices of B-type, a weak intramolecular H-bond/contact between the O3' oxygen of plane n and the H6 of the nucleobase in the plane $n+1$ appears to fix this nucleobase inside the helix.
- (iv) Figure 5d shows two intramolecular H-bonds: One of them is between O3'1 and H2'1, which appears only in the primer (plane $n+1$) of our RNA microhelices, stabilizing both hydroxyl group positions. The example corresponds to the pyrimidine strand of the 5'-UUU-3' microhelix calculated at the M06-2X/6-311+G(d,p) level. Another H-bond appears between O5'1 and H6'1, which stabilizes the nucleobase position. Although the C-H...O bond is weaker than the N-H...O, the O5'...H6 distance of 1.995 Å is shorter than that shown in Figure 5a and it appears to better fix the nucleobase inside the helix. This H-bond only appears in RNA/DNA helices of A-type, with the distance being slightly longer in DNA, 2.050 Å, and even longer in the purine strand, 2.165 Å in RNA vs. 2.229 Å in DNA.
- (v) Figure 5e: The H-bonds arise due to the two amino hydrogens of the NH₂ group in A/dA. One of the hydrogens is intermolecularly H-bonded to the carbonyl oxygen O4 of the U/T nucleobase, while another hydrogen appears to be available to form a weak intramolecular H-bond with O4 of U/T in a different (interplanar) plane of the same up/down-positioned strand. The example with dATdA:TdAT DNA shows these H-bonds, but only the interplanar ones are highlighted in yellow color. These interplanar H-bonds were observed in both RNA/DNA microhelices and they increase the helix roll (ρ) parameter. With cytidine nucleosides, the effect of these interplanar H-bonds is much greater.
- (vi) Figure 5f: Other H-bonds are observed in Z-helices, but for simplicity and as an example, only the H-bond between the O7' phosphate oxygen and the NH₂ hydrogen of guanine nucleobase was included. This H-bond type stabilizes the special Z-helix structure.

Diagonal interactions (cross-term interactions [25]) were not observed in the studied microhelices, as they usually appear in CCG, GCG or similar sequences. These interactions seem to have a strong influence on the duplex stability, stabilizing or destabilizing it depending on the net electrostatic interaction of the functional groups involved in the diagonal.

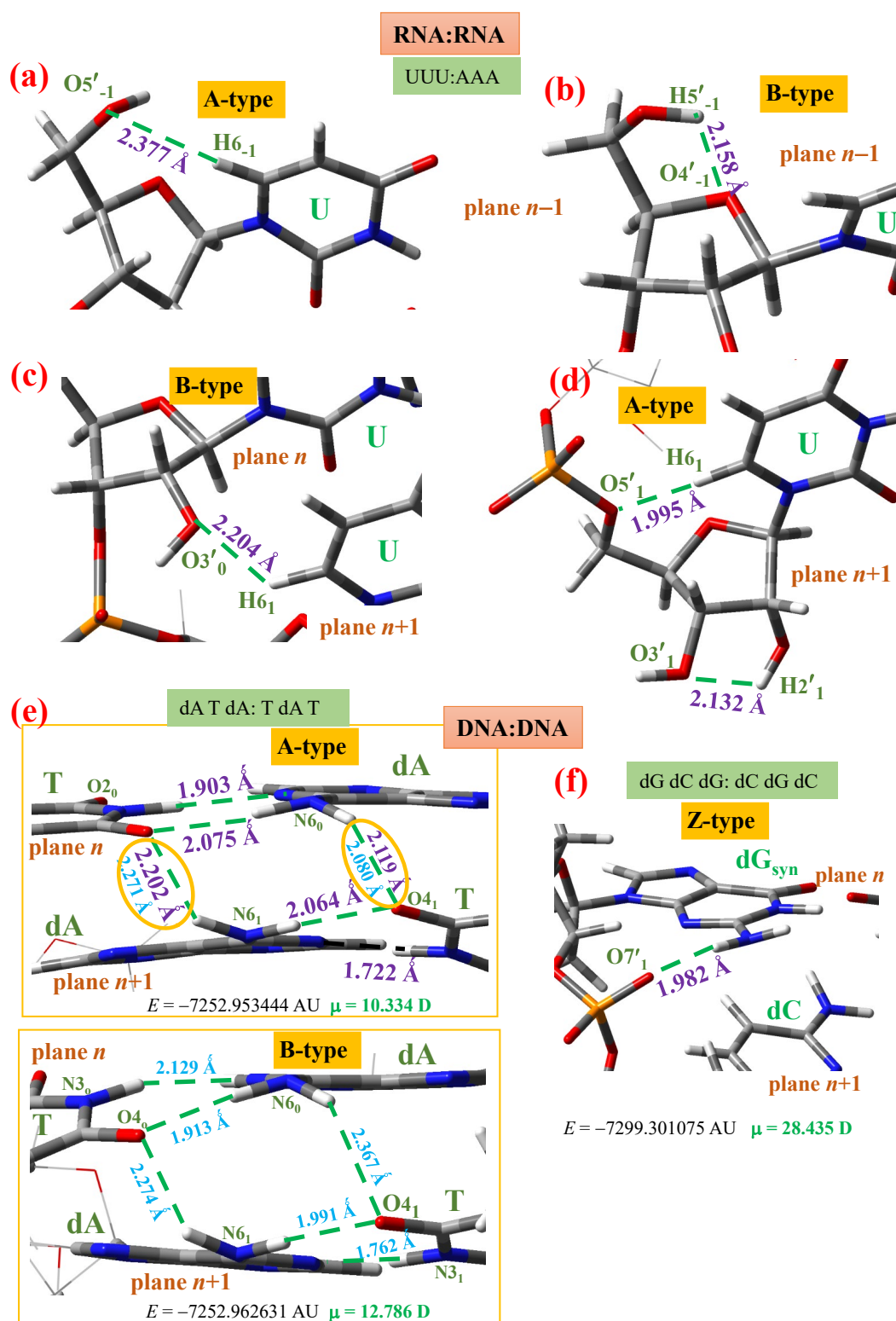


Figure 5. Other intramolecular H-bonds/contacts that provide stability to the helix structure. (a) Weak intramolecular H-bond/contact between the O5'H of plane *n*−1 and H6 hydrogen of the nucleobase for A-type microhelices. The calculated value was at the M06-2X/6-311+G(d,p) level. (b) Intramolecular H-bond of O5'H in plane *n*−1 with O4' in 5'-UUU-3' microhelix of B-type. (c) Weak intramolecular H-bonds/contacts of the O3' oxygen of plane *n* with H6₁ of uracil in the plane *n*+1, which stabilizes it. (d) Intramolecular H-bonds in the plane *n*+1 that stabilize both the hydroxyl group positions and nucleobase position in A-type helices. (e) Weak interplanar H-bonds between one of the amino hydrogens of the NH₂ group of dA and the carbonyl oxygen O4 of T. (f) Intramolecular H-bond between the amino hydrogen NH₂ of dG and O7' of the phosphate group in the DNA:DNA microhelix of Z-type.

3.2. Types of Polynucleotide Double Helices

Based on the four possible intramolecular H-bonds through the 2'-OH hydroxyl hydrogen in the RNA helices described in Figures 1–3, four main types of RNA:RNA microhelices were optimized, along with the corresponding four DNA:DNA ones. For a better analysis and comparison of the obtained values, the UUU:AAA and TTT:dAdAdA microhelices were mainly considered. A general description of them is as follows:

- (i) **A-type:** When this intramolecular H-bond of H2' is with O4' of the next plane on the same strand, the O2'-H2'_(n)...O4'_(n+1) A-type H-bond. It was labeled by analogy to A-type DNA polynucleotide double helices (or A-DNA [55]). It is mainly characterized by the nucleobase ring in *high-anti* orientation (χ ca. -150° in pyrimidine strand and ca. -130° in purine) with respect to the furanose ring, and by a 3'-*endo* orientation 3E of this ring (3T in the purine strand). As an example, Figure 6a shows only the pyrimidine strand of the optimized UUU:AAA microhelix, with the values of the main exocyclic torsion angles, the furanose ring symmetry and the total energy of the system. These values, for both strands, were listed in Table 1 and included in Figure S1a,b (Supplementary Material Section). Due to this A-type intramolecular H-bond, the helix flexibility measured by the $v_{\max}$ parameter is remarkably lower (37.6° in the pyrimidine strand) than that found in the other microhelix types. This $v_{\max}$ value is also lower in the purine strand, 33.1° in Table 1. The influence of the 2'-OH group conformation on the stability of A-form RNA helices has been reported [59].

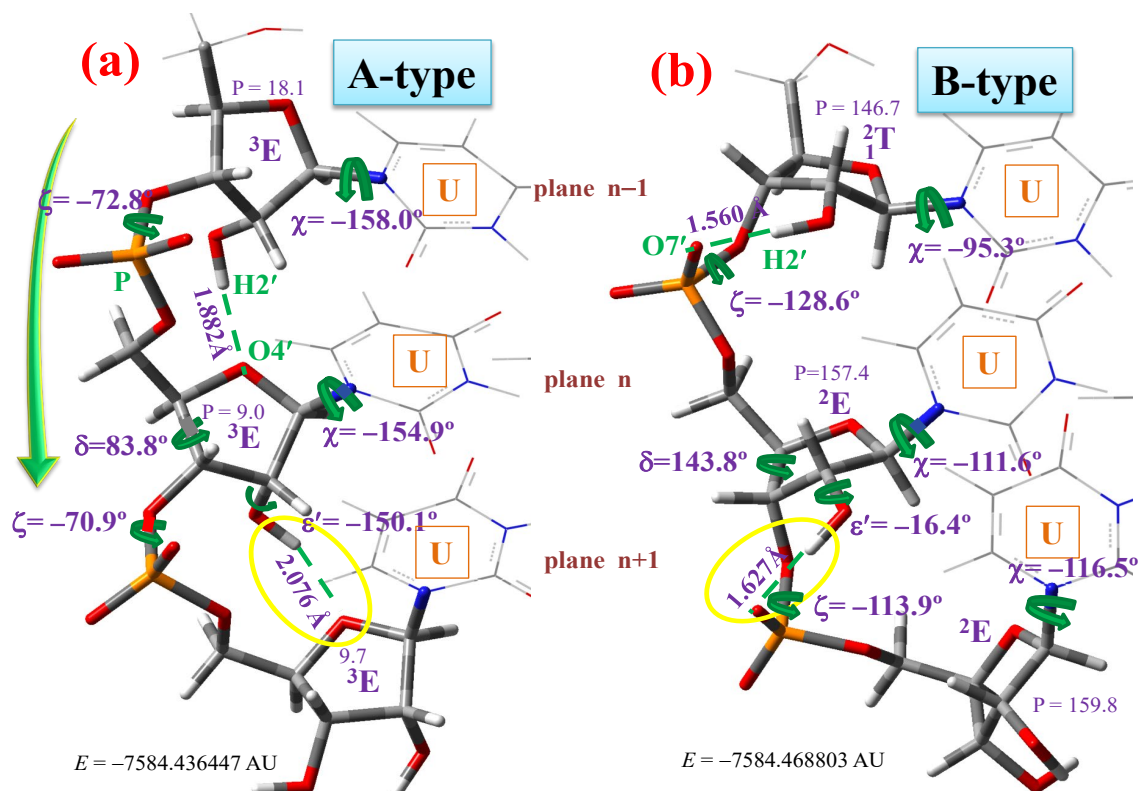
Although DNA helices lack the 2'-OH hydroxyl group, their geometric arrangement (exocyclic and endocyclic torsion angles) in A-type appears similar to that of A-type RNA. As an example, the main geometric parameters in the TTT:dAdAdA DNA microhelix are listed in Table 1 and plotted in Figure S2a,b. As expected, the most affected angles (ca. $5\text{--}8^\circ$) corresponds to α , ϵ and ξ , with an increase in α and a decrease in ϵ and ξ compared to the RNA ones. All calculated exocyclic torsion angle values agree well with the experimental ranges reported by X-ray for this A-type [60,61].

- (ii) **B-type:** When this intramolecular H-bond is with O7' of the phosphate group, the B-type H-bond corresponding to O2'-H2'_(n)...O7'_(n+1). It was labeled by analogy with B-type DNA helices (or B-DNA [55]). This notation was also used in related works [16,23,62]. This B-type is mainly characterized by both a nucleobase ring in the *anti* orientation (χ ca. -110°) related to the furanose ring and by a 2'-*endo* orientation 2E of this ring. In purine nucleobases, this ring orientation slightly differs from 2T (P around $140\text{--}150$). This *anti* orientation is more stable than the *high-anti* (in A-type). Because of that, and mainly due to this B-type H-bond being remarkably stronger than A-type, the B-type helix is the most common form, and all RNA/DNA helices will attempt to raise this form. Thus, and due to its extensive structural heterogeneity [63], B-type is present under physiological conditions [13,58], takes part in most biochemical processes and, hence, has been studied by many authors [13,64,65]. This B-type, as well as A-type, is the biological form of DNA:DNA helices that has been considered the most significant.

Figure 6b shows as an example, only the pyrimidine strand of the UUU:AAA microhelix, which can be easily compared to A-type, Figure 6a. The values in both strands are listed in Table 1 and included in Figure S1c,d. A larger exocyclic angle ζ in absolute value in B-type than in A-type leads to a higher helical twist (ω) rotation than in A-type. This microhelix type is notably more stable (84.9 kJ/mol) than A-type. It is worth noting that the optimized uridine nucleoside in isolated state with 3'-*endo* (A-type) orientation of the furanose ring is 2.58 kJ/mol more stable than with 2'-*endo* (B-type). This small difference has also been found in NMR experi-

ments, where both forms in solution appeared in rapid equilibrium [55]. Under water hydration, ${}^2\text{E}$ orientation is slightly more stable than ${}^3\text{E}$.

The geometric arrangement of B-type DNA closely resembles that of RNA, with the highest difference (ca. 10°) in the δ , ϵ and ζ torsional angles. The differences, especially in the ζ angle, can be attributed to a slightly lower NBO charge on the phosphorus atom of DNA (Table 2) than on the corresponding RNA one, due to the lack in DNA helices of the intramolecular H-bond between H2' with O7'. In general, in A- and B-DNA microhelices, H2' hydrogen perfectly replaces the 2'-OH group of RNA, preserving the same orientation of the nucleobase ring (*anti* or *high-anti*), with respect to the furanose ring (${}^2\text{E}$ or ${}^3\text{E}$). Analogously, in DNA microhelices the furanose ring orientation of the purine strand (${}^2\text{E}$) differs from the pyrimidine one (${}^2_1\text{T}$), Table 1 and Figure S2c,d. The TTT:dAdAdA microhelix is also more stable (21.6 kJ/mol) in B-type than in A-type, although the difference is four times smaller than in RNA helices. This higher difference between RNA and DNA can be explained by two features: (i) Only in RNAs does an intramolecular H-bond appear through H2', which is notably stronger in B- than that in A-type. (ii) The *anti* orientation in B-type DNA is more stable than the *high-anti* form of A-type [16]. Therefore, this difference is smaller in DNAs because only this second feature contributes.



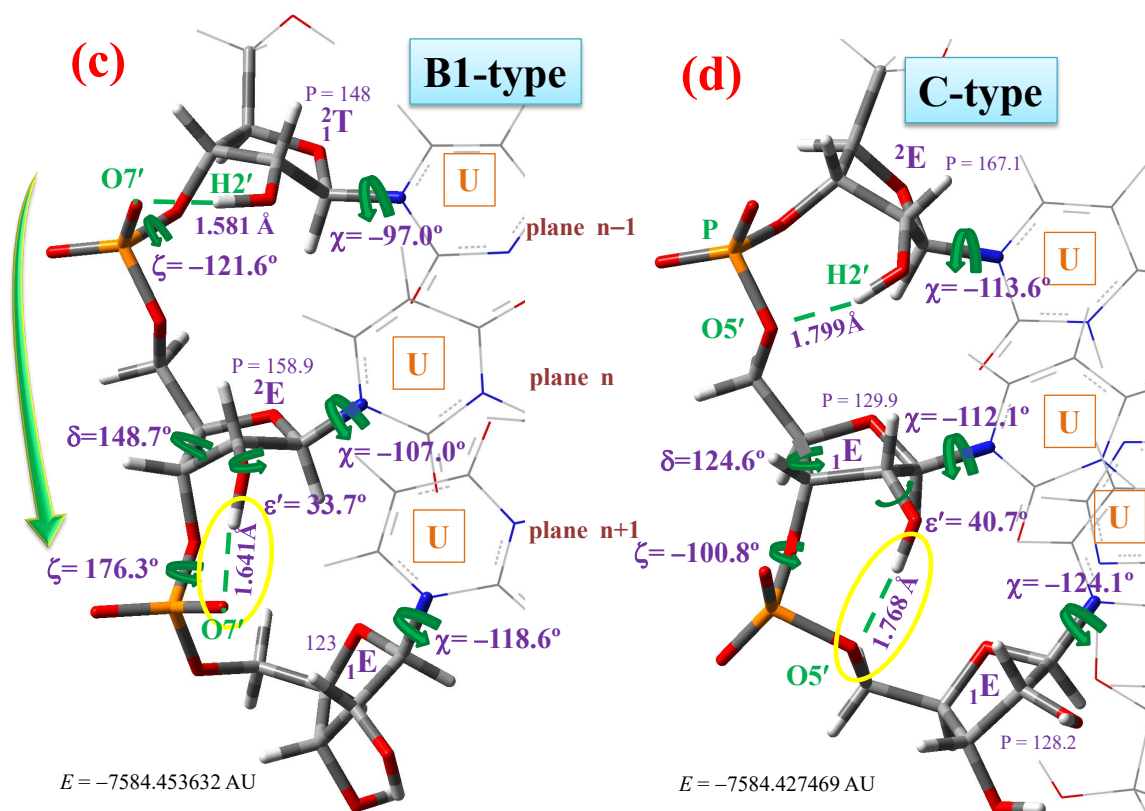


Figure 6. Comparison of the main optimized geometric parameters in the pyrimidine strand of the UUU:AAA RNA:RNA microhelices with intramolecular H-bond through H2' of: (a) A-type, (b) B-type, (c) B1-type, and (d) C-type.

This energy difference between A- and B-types in the TTT:dAdAdA microhelix is relatively small and could be in agreement with X-ray studies [6,66] where A- and B-types DNA can easily interconvert by lengthening the C1'–C2' and C2'–C3' bonds and shortening the corresponding C3'–C4' [67]. A crystallographic map for the transition from B-DNA to A-DNA has also been reported [68]. This is possible due to the large flexibility of the sugar ring and the glycosyl N–C1' bond, which is more facilitated in purines than in pyrimidines [27].

In the torsional angles, γ is ca. 10° smaller in B- than A-type, while the δ angle is around 50 – 60° larger than in A-type. Only the β torsional angle values appear similar in A- and B-types. In all microhelices studied, as well as in other microhelices [16,27], B-type was always more stable than A-type, in contrast to reports by other authors [26,69]. The comparison of the calculated exocyclic torsional angle values with those obtained experimentally by X-ray in B-DNA (by B1 conformation) [60,61] was as follows: (i) The experimental ϵ angle values in the 120° and 210 (-150°) range, with an average value of 184° (-176°), were in good accordance with our calculations in the pyrimidine strand of 5'-TTT-3' with 165.9° (140.3° in purine strand) and in the 5'-dCdCdC-3' with 169.8° (147.4° purine strand). (ii) The experimental ζ values in the 230° to 300° (-130° to -60°) range, with an average of 265° (-105°), were also in agreement with our calculated values in the 5'-TTT-3' pyrimidine strand at -97.3° (-76.1° in purine) and in 5'-dCdCdC-3' at -92.0° (-78.4° in purine). Schneider et al. [60] have reported that when these ϵ and ζ ranges for BI match to a residue, then this residue was labeled as belonging to the B1 conformation. According to this definition, as well as the values range of the remaining exocyclic angles, the BI form corresponds to our B-type.

Based on the **B1-type** intramolecular H-bond described in Figure 1e with its exocyclic torsion angles ϵ' and ζ , microhelices with B1-type in both strands were optimized. As an example, Figure 6c shows the pyrimidine strand of the UUU:AAA microhelix, while the remaining values for both strands are shown in Table 1 and Figure S3a,b. This B1-type is slightly less stable (39.8 kJ/mol) than B-type, especially due to its weaker intramolecular H-bond $H2'_n \cdots O7'_{n+1}$. Its different exocyclic torsion angles compared to B-type, however, do not affect the NBO charges of the phosphate atoms in the microhelices.

The corresponding B1-type values with DNA are shown in Figure S4a,b. Most of the exocyclic torsion angle values are similar to those of RNA helices, and all them were also included in Table 1. This DNA microhelix is 42.0 kJ/mol less stable than B-type. This lower stability (also found in RNA) may be due to the large deformation in the α (6.7°) and β (97.1°) torsion angle values of the purine strand, which are fully different from those of any other microhelix. One possible explanation for this feature is considering the furanose ring flexibility measured from $v_{\max}$, which is noticeably greater than in B-type, in both pyrimidine and purine strands, and in both RNA and DNA microhelices. Its value in the purine strand is significantly higher than in the pyrimidine strand and, therefore, the purine strand is the one that is mainly deformed to build the B1-type microhelix. Due to this deformation in the purine strand, none of its furanose rings have 2E symmetry, as in B-type, nor do two of its rings in the pyrimidine strand. This feature could contribute to the large deformation in the α and β torsion angles of the purine strand. The NBO charges on the phosphorus atoms are also slightly lower than those of the related RNA microhelices.

Comparing the calculated exocyclic torsion angles in 5'-TTT-3' of B-type with those experimentally reported in B-DNA (BII conformation [60]), the following was observed: (i) The experimental ϵ values in the 210° to 300° (-150° to -60°) range, with an average value of 246° (-114°), were in good agreement with our calculations with -175.1° in the pyrimidine strand and -173.6° in the purine strand. (ii) The experimental ζ values in the 150° to 210° (-150°) range, with an average of 174° , were also in accordance with our calculated values in the pyrimidine strand with -178.5° and in purine with -161.0° . (iii) The experimental χ range from 200° to 300° (-160° to -60°) and average value of 271° (-89°) appears in good agreement with the calculated ones of -109.5° and -112.7° in pyrimidine and purine strands, respectively. (iv) The δ angle in the 70 to 180° range with average value of 144° is in good accordance with 148.9° and 136.5° in pyrimidine and purine strands, respectively. (v) The experimental β angle with a 130° to 200 (-160°) range and an average of 146° is also in agreement with our calculated value of -177.1° in the pyrimidine strand, but remarkably differs in the purine strand with 97.1° . Therefore, it is concluded that our B1-type could be the BII conformation found experimentally [60].

- (iii) **C-type:** When the intramolecular H-bond is to $O5'$ of the next plane, $O2'-H2'_{(n)} \cdots O5'_{(n+1)}$. It differs from A-type in the χ , δ and ζ angles. To our knowledge, helices with this H-bond type have not been reported experimentally. This may be because they resemble B-type helices and because the X-ray experimental data cannot accurately determine the hydrogen positions.

These optimized microhelices were slightly less stable than both A- and B-types. For these reasons, and for convenience, they were labeled as C-type. As an example, Figure 6d shows the pyrimidine strand of the UUU:AAA microhelix with some values, while the remaining values for both strands appear in Table 1 and in Figure S3c,d. In this microhelix, the phosphorus atom charges have the lowest values and ca. $0.01e$ lower than in A-type. The dipole moment obtained is the largest for the UUU:AAA microhelix, 22.302 D, which suggests that it may be the most stable in water solution. This large increase in

the dipole moment was also observed in the mixed microhelices such as AUA:UAU and hybrid ones with C-type.

The corresponding C-type DNA is also less stable than A- and B-types, in analogy to that found in the RNA helix, but it shows noticeable geometric changes compared to RNA ones, especially in the χ and δ angles, as can be seen in Table 1 and is plotted in Figure S4c,d. Unlike RNA, this C-type DNA appears more similar to A-type than to B-type. The main differences with A-type are found in the ϵ and ζ angles, but when comparing these values, as well as those of the other exocyclic angles, with experimental X-ray values [60], a good agreement was observed. This feature means that, experimentally (as well as theoretically by other authors), a differentiation in A-type microhelices has not been carried out between A- and C-types, as has been done in the present manuscript. The differences in the ϵ and ζ angles, and especially in the furanose ring symmetry with 0_4T and P around 70, are clearly different from the values obtained in the other microhelix types.

The charges on the phosphorus atoms have similar values in DNA helices, which means that the exocyclic angle values mainly depend on the intermolecular H-bonds and the π - π stacking interactions within the helix. The furanose ring flexibility [70] is the largest in the 5'-TTT-3' pyrimidine strand ($v_{\max} = 43.1^\circ$), and it is also large in the purine strand. In general, the flexibility of the furanose ring, measured by the $v_{\max}$ parameter, is significantly lower in DNA helices than in RNA ones. The dipole moment is remarkably reduced compared to RNA, but close to that of A-type.

- (iv) **Z-type:** When this intramolecular H-bond is to O2', the O2'-H2'_(n)...O3'_(n) and labeled as Z-type, Figure 3. It was not possible to optimize an RNA (or DNA) microhelix with only this H-bond type in both strands, nor a single strand with only this Z-type H-bond. These H-bonds must necessarily be mixed with C-type H-bonds, and in an alternative way, in both strands. Therefore, microhelices with Z-type (Z- plus C-type H-bonds in an alternative way) were optimized, appearing as the only possible combination in one strand. As an example of these possible combinations, the RNA:DNA hybrid microhelix AUA:TdAT in Figure 7a,b shows the combination with C1-type and B2-type in the RNA strand and with Z-type and C2-type in the DNA strand. Figure 7c,d shows the example with C1- and Z1-types in the RNA strand and with Z- and C2-types in the DNA strand. These Z-type helices are noticeably deformed compared to the A-, B- and C-types described above and are left-handed. For these reasons, these microhelices are remarkably the least stable. The dipole moment is very large and close to that obtained in C-type. Due to the significant difference in all geometrical parameters and energies of this Z-type helix compared to A-, B- and C-types, we consider it appropriate to include its study in other future publications.

For a better comparison of these A-, B-, C- and Z-type forms between UUU:AAA (RNA) and TTT:dAdAdA (DNA) microhelices, Figure S5 shows the main differences observed in the optimized geometric parameters in the plane-*n* of the pyrimidine strand. In addition, Figure 8 shows a summary in graphical form of the main differences observed utilizing two or three exocyclic (δ , ζ and χ) torsional angles, as well as in the furanose ring symmetry, in dark violet, in the RNA types of the 5'-UUU-3' pyrimidine strand. The four possible intramolecular H-bonds of H2'₁ with O4'₁ (A-type), O7'₁ (B-type), O3'₁ (C-type) and O3'₀ (Z-type) are indicated with a blue arrow.

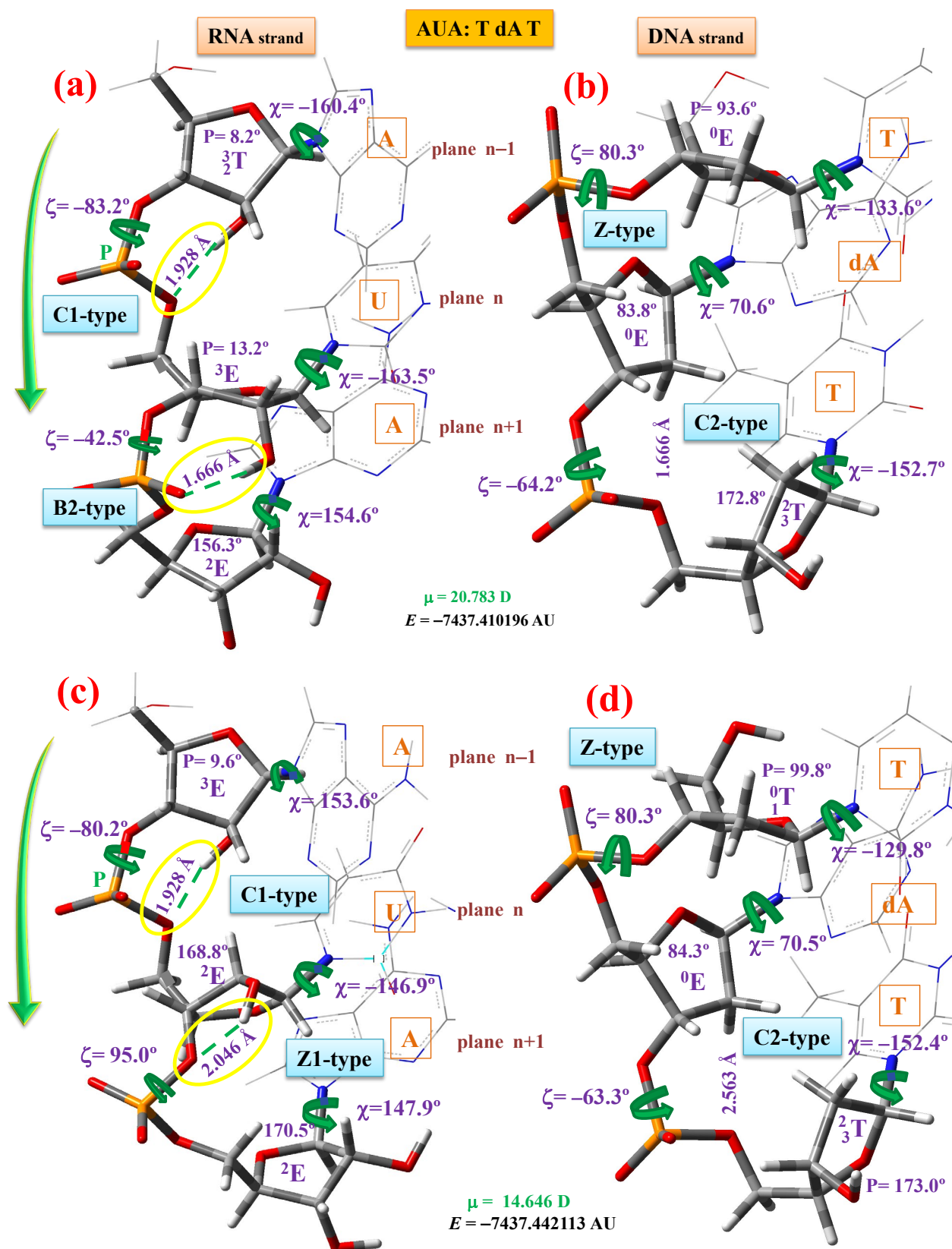


Figure 7. Different combinations of the intramolecular H-bonds types through H2' that can be found in the optimized AUA:TdAT RNA:DNA hybrid microhelices: (a,b) With C1-type and B2-type in the RNA strand and with Z-type and C2-type in the DNA strand. (c,d) With C1-type and Z1-type in the RNA strand and with Z-type and C2-type in the DNA strand.

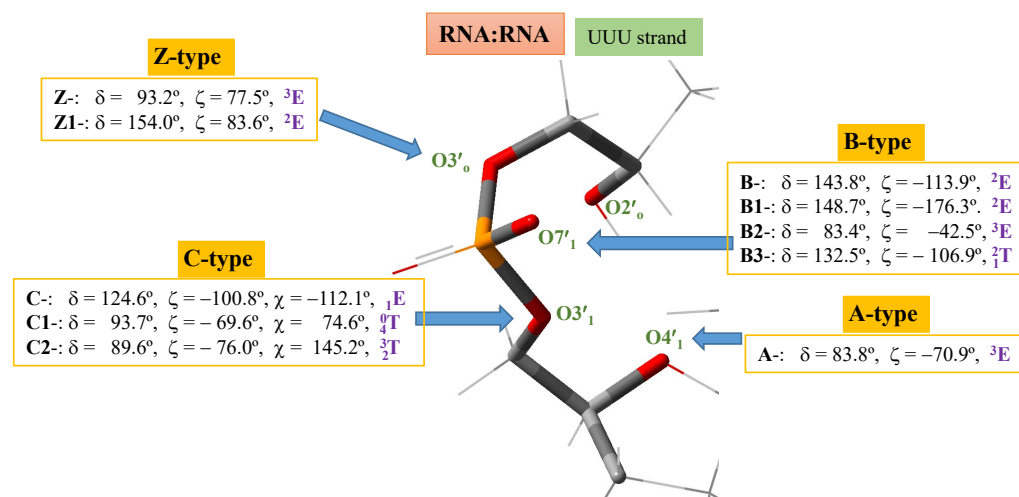


Figure 8. Summary with the main differences in two/three exocyclic angles among the RNA types found in the 5'-UUU-3' pyrimidine strand, as well as in the furanose ring symmetry, in dark violet. Blue arrow indicates the four possible intramolecular H-bonds of H2'1 with O4'1 (A-type), O7'1 (B-type), O3'1 (C-type) and O3'0 (Z-type). The exceptions are: B2-type with calculated values in the 5'-AUA-3' strand, and C2- and Z1-types with values in the 5'-AAA-3' strand.

3.3. Intermolecular H-Bonds Determined in RNA:RNA and DNA:DNA Microhelices

Intermolecular H-bonds between the two helix strands, which form the canonical WC pairs, appear to be the most important characteristic responsible for maintaining the double helix. An analysis of these H-bonds was carried out in each microhelix type and the RNA:RNA, DNA:DNA and DNA:RNA microhelices. Furthermore, it was computationally determined that microhelices with different types of intramolecular H-bonds in each strand were possible and stable, and these were also optimized and analyzed in the present manuscript. Therefore, Figures 9–11 graphically show the H-bond lengths corresponding to the plane n of these microhelices, as well as the two main torsion angles involved and the total interaction energy. For simplicity, only microhelices with pyrimidines on one strand and purines on another strand were included, i.e., the UUU:AAA and CCC:GGG RNA microhelices and their corresponding TTT:dAdAdA and dCdCdC:dGdGdG DNA ones. Due to the special characteristics of the Z-type microhelices, they were not included nor analyzed in the present manuscript. The description of the intermolecular H-bonds in several selected optimized microhelices is as follows:

3.3.1. Microhelices with the Same Type of Intramolecular H-Bond via H2' in Both Strands

A first comparison of the intermolecular H-bond lengths in microhelices with the same type of intramolecular H-bonds through H2' in both strands was performed, and their variation in the different helix types is graphically indicated in Figure 9 and in Table 2. The interaction energy E^{int} between both helix strands was calculated, as well as the counterpoise (CP)-corrected interaction energy ΔE^{CP} . The monocatenary DNA microhelix with the C-type purine strand was not stable, because one of its nucleosides was converted to B-type. A description of the main features observed in these microhelices was as follows:

- (i) With UUU:AAA RNA microhelices, the $N3^U\cdots N1^A$ intermolecular H-bond (labeled as ① in Ref. [16]) is always longer than $C=O4^U\cdots H6^A$ (labeled as ②). The difference (①–②) is remarkably larger in C-type (0.141 Å in Figure 9g) and B-type (Figure 9c) than in A-type, 0.04 Å (0.076 Å with the 6-311+G(d,p) basis set) in Figure 9a. This feature is also observed in DNA:DNA microhelices (Figures 9b,d,f), with the

exception of C-type (Figure 9h), which is reversed, due to the helix deformation caused by the exocyclic torsion angles of this C-type.

With the exception of C-type microhelices, H-bond ① always appears shorter in RNA than in DNA helices. It was also noted that the intermolecular H-bond ② appears more flexible than ①, with a larger variation range. This range is also notably greater in RNA microhelices than in DNA ones. In C-type, the H-bond lengths appear different, which can be interpreted as a consequence of the $H2'_n \cdots O5'_{n+1}$ H-bond forcing the helix into a deformation of the exocyclic torsion angles, as well as of the intermolecular H-bonds between both strands.

- (ii) Although in RNA microhelices the intramolecular H-bonds through $H2'$ appear to reduce its helix flexibility compared to those of DNA, the large flexibility of the intermolecular H-bonds between both strands provides enough flexibility to the RNA/DNA helices for their double-stranded formation.
- (iii) The H-bonds in B-type RNA are shorter than their corresponding TTT:dAdAdA DNA ones, in accordance with a higher ΔE^{CP} value in RNA (−238.6 kJ/mol) than in DNA (−185.7 kJ/mol), Figure 9. This feature is contributed to by the significantly greater E^{def} (ca. ten times) of the DNA pyrimidine strand than that of RNA. A similar feature was observed in B1-type (Figure 9e,f) with higher ΔE^{CP} in RNA than in DNA. In general, ΔE^{CP} values are higher in RNA helices than in their corresponding DNA ones, mainly due to the greater E^{def} in DNA than in RNA.
- (iv) Both intermolecular H-bonds in the B-type RNA microhelices are shorter than those in A-type, in accordance with their higher stability. However, in B1-type both H-bonds have almost the same length in accordance with a lower stability and with a ΔE^{CP} value, −233.6 vs. 238.6 kJ/mol in B-type, especially due to the E^{def} of the pyrimidine strand being twice higher in B1- than in B-type.
By contrast, DNA microhelices of A- and C-types generally appear to have shorter intermolecular H-bonds than those of RNA and higher $E^{int.}$ and ΔE^{CP} values. However, H-bond ① is longer in A-type DNA, and H-bond ② is longer in C-type than in the corresponding RNA one. The highest $E^{int.}$ value occurs in A-type RNA, −264.6 kJ/mol, Figure 9a, as well as in its ΔE^{CP} value.
- (v) Figure 9i–l: The intermolecular H-bonds in the CCC:GGG microhelices are in general weaker than those in UUU:AAA, although this is compensated by three H-bonds. B-type microhelices are also more stable than A-type and with stronger intermolecular H-bonds in both RNA and DNA helices.
- (vi) φ^I and φ^{II} torsion angle values (Figure 9), which appears to be directly related to the propeller twist θ_P parameter, are notably greater (due to a more rotated nucleobase in the pair) in DNA microhelices than in RNA ones. This can be explained by the fact that the lack of the 2'-OH group in DNA helices facilitates their rotation compared to RNA, where their structure appears more fixed. The exception is the C-type microhelix, which exhibits the opposite behavior. The values in A-type are larger than in B-type to facilitate the weak $H2'_n \cdots O4'_{n+1}$ H-bond.
- (vii) The $N3^U \cdots H \cdots N1^A$ intermolecular angle, which appears related to the opening σ angle, shows a large linearity in the optimized microhelices, being larger in the B-type helices, Table 2.

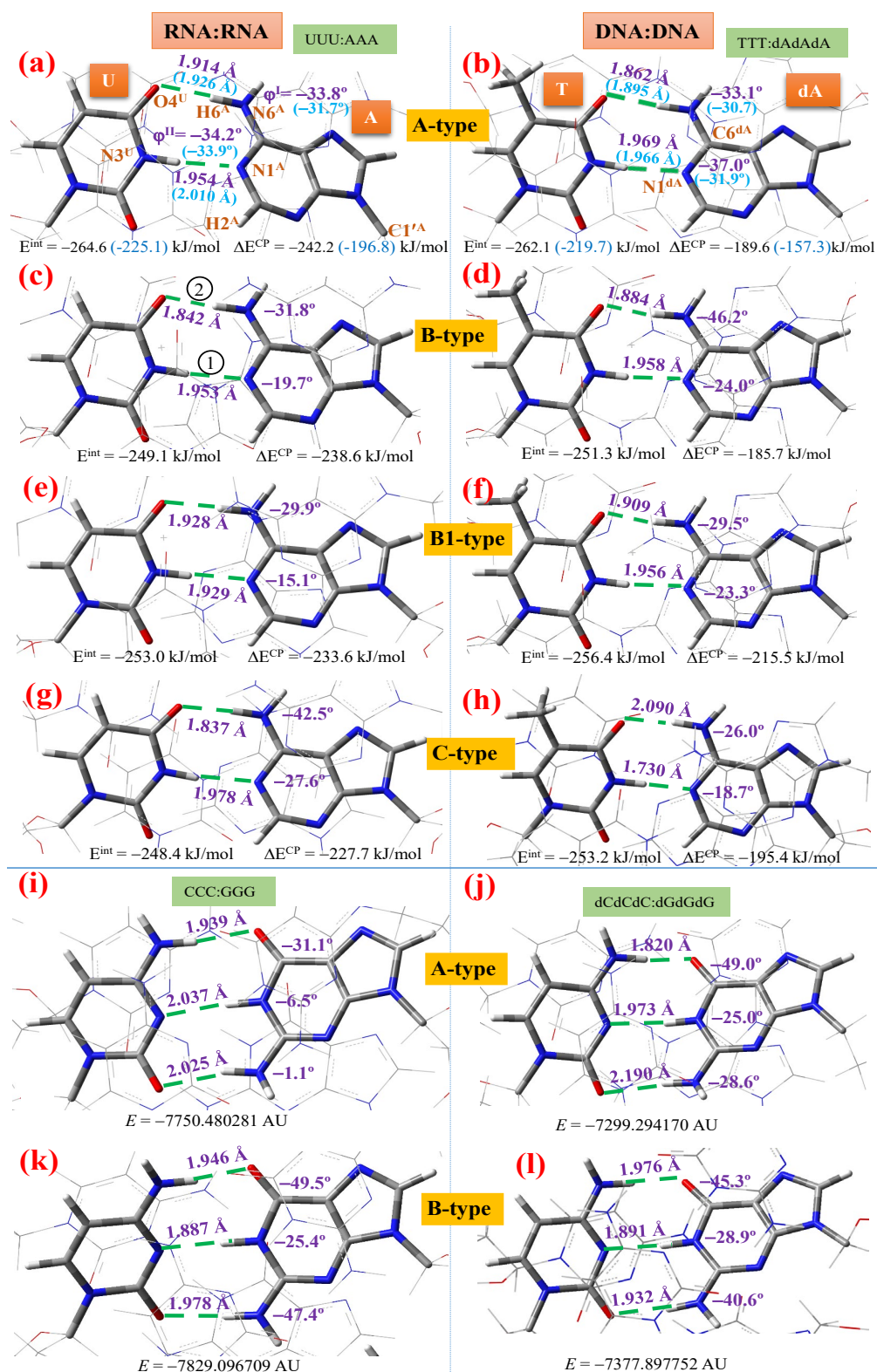


Figure 9. Comparison of the different intermolecular H-bond types between the two strands of the following microhelices: (a,b) A-type in UUU:AAA RNA vs. similar arrangement in TTT:dAdAdA DNA. (c,d) B-type in UUU:AAA RNA vs. TTT:dAdAdA DNA. (e,f) B1-type in UUU:AAA RNA vs. TTT:dAdAdA DNA. (g,h) C-type in UUU:AAA RNA vs. TTT:dAdAdA DNA. (i,j) A-type in CCC:GGG RNA vs. dCdCdC:dGdGdG DNA. (k,l) B-type in CCC:GGG RNA vs. dCdCdC:dGdGdG DNA and in both microhelices with a $-\text{CH}_3$ group instead of $\text{H3}'$ in the primer nucleoside (plane $n+1$). The notation for the torsional angles is: $\varphi^{\text{I}} = \text{C4}^{\text{U/T}} = \text{O}^{\text{U/T}} \dots \text{N6}^{\text{A/dA}} \text{C6}^{\text{A/dA}}$, $\varphi^{\text{II}} = \text{C4}^{\text{U/T}} \text{N3}^{\text{U/T}} \dots \text{N1}^{\text{A/dA}} \text{C6}^{\text{A/dA}}$. The values of the most characteristic parameters calculated at the M06-2X/6-31G(d,p) level are shown in violet color, while those in blue correspond to M06-2X/6-311+G(d,p).

Table 2. Main structural parameters optimized corresponding to the nucleoside base pair of central plane n according to Scheme 1. Intermolecular H-bonds (in Å) and their angles (in degrees) were included, as well as the corresponding NBO charges and deformation energies involved in the microhelices. In italic form appears the calculated values with the 6-311+G(d,p) basis set. Microhelices appear defined by the pyrimidine strand.

	Microhelix	Type	Pyrimidine Strand	Purine Strand	H-Bond					NBO Charges								
			C1'-N1 ^{U/T}	C1'-N1 ^{A/dA}	① H3 ₀ ^{U/T} ...N1 ₀ ^{A/dA}	② O4 ₀ ^{U/T} ...H6 ₀ ^{A/dA}	N3-H ^{U/T} ...N1 ^{A/dA}	φ ^I	φ ^{II}	O ₂	N3	O4	N1 ^{A/dA}	N6 ^{A/dA}	P ₁	P ₂	P ₃	P ₄
RNA:RNA	5'-U U U-3'	A	1.478	1.457	1.954	1.914	174.3	-33.8	-34.2	-0.674	-0.706	-0.680	-0.629	-0.847	2.585	2.588	2.586	2.584
			1.480	1.457	2.010	1.926	173.0	-31.7	-33.9	-0.664	-0.706	-0.682	-0.636	-0.801	2.510	2.514	2.513	2.508
		B	1.451	1.435	1.953	1.842	176.2	-31.8	-19.7	-0.639	-0.710	-0.683	-0.624	-0.854	2.606	2.603	2.608	2.603
		B1	1.450	1.438	1.929	1.928	176.5	-29.9	-15.1	-0.646	-0.708	-0.686	-0.624	-0.859	2.606	2.604	2.610	2.610
		C	1.451	1.441	1.978	1.837	171.9	-42.5	-27.6	-0.628	-0.716	-0.673	-0.626	-0.858	2.578	2.576	2.577	2.577
		A-C ^a	1.477	1.442	1.885	1.903	177.0	-35.7	-23.8	-0.674	-0.709	-0.676	-0.628	-0.856	2.585	2.586	2.577	2.577
		B-C ^b	1.452	1.443	1.935	1.897	176.0	-39.1	-25.9	-0.643	-0.711	-0.683	-0.625	-0.857	2.606	2.604	2.577	2.578
	5'-C C C-3' ^g	A	1.480	1.458	2.037	1.939	164.7	-31.1	-6.5	-0.722	-0.664	-0.830	-0.685	-0.688	2.589	2.590	2.585	2.583
		B	1.453	1.437	1.887	2.019	165.0	-51.0	-26.6	-0.686	-0.670	-0.827	-0.679	-0.733	2.609	2.608	2.606	2.604
		5'-A U A-3' ^h	A	1.482	1.460	2.026	2.013	176.2	-12.9	-0.8	-0.669	-0.699	-0.700	-0.628	-0.857	2.584	2.584	2.588
B	1.451		1.438	2.024	1.920	174.4	-6.5	-4.7	-0.630	-0.699	-0.710	-0.628	-0.846	2.606	2.601	2.610	2.606	
DNA:RNA	5'-TTT-3'	A ^c	1.485	1.457	1.988	1.926	175.4	-31.8	-42.1	-0.669	-0.701	-0.691	-0.626	-0.843	2.590	2.591	2.586	2.584
		B ^d	1.464	1.435	1.908	1.877	177.8	-31.6	-19.4	-0.642	-0.702	-0.695	-0.625	-0.857	2.584	2.586	2.608	2.604
DNA:DNA	5'-T T T-3'	A	1.485	1.465	1.969	1.862	174.6	-33.1	-37.0	-0.667	-0.700	-0.690	-0.621	-0.844	2.591	2.591	2.584	2.582
			1.484	1.466	1.966	1.895	173.2	-30.7	-31.9	-0.659	-0.698	-0.696	-0.634	-0.796	2.516	2.518	2.509	2.505
		B	1.465	1.440	1.958	1.884	175.8	-46.3	-24.0	-0.642	-0.700	-0.702	-0.622	-0.851	2.585	2.589	2.598	2.583
		B1	1.459	1.440	1.956	1.909	178.8	-29.5	-23.3	-0.641	-0.701	-0.696	-0.626	-0.855	2.584	2.591	2.591	2.589
		C	1.464	1.448	1.730	2.090	176.4	-26.0	-18.7	-0.658	-0.704	-0.686	-0.623	-0.851	2.584	2.592	2.586	2.583
		A-B ^e	1.485	1.446	2.014	1.812	174.2	-30.4	-28.3	-0.662	-0.700	-0.687	-0.622	-0.842	2.591	2.590	2.590	2.586
		B-C ^f	1.465	1.446	1.888	1.894	175.5	-42.8	-26.2	-0.649	-0.701	-0.700	-0.627	-0.848	2.583	2.587	2.583	2.583
	5'-dCdCdC-3'	A	1.491	1.465	1.973	1.820	163.8	-49.0	-25.0	-0.725	-0.669	-0.824	-0.682	-0.705	2.583	2.583	2.582	2.581
		B	1.463	1.443	1.892	1.976	169.3	-45.3	-28.9	-0.693	-0.670	-0.827	-0.682	-0.728	2.587	2.586	2.598	2.584
		5'-dATdA-3' ^h	A	1.487	1.462	1.903	2.075	178.3	-19.1	-0.7	-0.671	-0.692	-0.720	-0.624	-0.860	2.587	2.583	2.592
B	1.453		1.447	1.882	1.940	165.1	-33.8	-28.7	-0.631	-0.689	-0.714	-0.621	-0.862	2.583	2.590	2.590	2.582	

Notation used for H-bonds [16]: ① corresponds to H3^{U/T}...N1^{A/dA} (or H3^{C/dC}...N1^{G/dG}), ② corresponds to O4^{U/T}...H6^{A/dA} (or N4^{H4^{C/dC}}...O6^{G/dG}). $\varphi_o^I = C4_0O4_0^{U/T} \dots N6_0^{A/dA} - C6_0$. $\varphi_o^{II} = C4_0 - N3_0^{U/T} \dots N1_0^{A/dA} - C6_0$. ^a Figure 9a with pyrimidine strand of A-type and purine strand of C-type. ^b With pyrimidine strand of B-type and purine strand of C-type. ^c With pyrimidine strand DNA and purine strand RNA, TTT:AAA microhelix. ^d With pyrimidine strand DNA and purine strand RNA, TTT:AAA microhelix. ^e With pyrimidine strand of A-type and purine strand of B-type. ^f With pyrimidine strand of B-type and purine strand of C-type. ^g With N4^C instead of O4 and with O6^G instead of N6^{A/dA}. ^h For convenience, the pyrimidine strand in this microhelix is that with a pyrimidine nucleobase in plane n .

- (viii) The helix optimization with the 6-311+G(d,p) basis set hardly modifies the torsional angles compared to those obtained with 6-31G(d,p). However, the differences appear significant in the intermolecular H-bond values, and especially in the interaction energies, which are remarkably lower (in absolute value). E.g., in the TTT:dAdAdA microhelix of A-type, the value is -262.1 kJ/mol vs. -219.7 kJ/mol by 6-311+G(d,p) basis set, being $\Delta E = 42.4$ kJ/mol. Thus, using this approximate difference, the calculated ΔE^{CP} value of -185.7 kJ/mol for the B-type DNA microhelix with the 6-31G(d,p) basis set would give us a value of -143.3 kJ/mol, which is very close to the formation energy ΔE value of ca. -33 kcal/mol (-138.1 kJ/mol) reported [25] using a higher BLYP-D3(BJ)/TZ2P level. That is, using of the 6-311+G(d,p) basis set instead of 6-31G(d,p) does not significantly change the geometric parameters, although it has an influence on the absolute energy values, but it is not on their relative ones.

3.3.2. Microhelices with Different Type of Intramolecular H-Bonds in Both Strands

Microhelices with different types of intramolecular H-bonds in the strands were optimized. For simplicity, Figure 10 only includes combinations with A- or B-type in the pyrimidine strand and mainly C-type in the purine strand, which are more interesting. Attention was focused on the combinations with this new C-type helix, in its stability and geometric characteristics. The NBO charge values on the phosphorus atoms of each helix type are conserved in their combinations, as it is shown in Table 2. The values in Figure 10 correspond to plane n of the microhelix. A description of the features observed was as follows:

- (i) Figure 10a: The RNA helix combination of the A-type with the C-type is notably less stable than the A-type (Figure 9a) or C-type (Figure 9e) double-stranded helices. It can be interpreted by the large deformation required to form this helix combination with $E^{\text{int.}}$ of -261.7 kJ/mol, which is slightly lower than that of A-type. The intermolecular H-bond φ is the strongest in all RNA microhelices, while H-bond ② is stronger than that of A-type (Figure 9a) but weaker than that of C-type (Figure 9g). The dipole moment value is smaller than A- or C-types.
- (ii) Figure 10b: The combination of A- with B-types in the DNA helix results in a slightly less stable microhelix (only 4.7 kJ/mol) than A-type, and with an interaction energy (-253.2 kJ/mol) slightly higher than B-type helices. However, due to the large E^{def} (68.8 kJ/mol) the ΔE^{CP} energy is smaller than A- and B-types. The H-bond φ is weaker than those of A- and B-types, but H-bond ② is the strongest. These values indicate that this combination appears possible to occur in biological media. The dipole moment value (11.409 D) is intermediate between that of A-type (14.695 D) and B-type (9.775 D).
- (iii) Figure 10c: The microhelix with RNA combination of B- and C-types appears slightly less stable (34.0 kJ/mol) than that of B-type, but more stable than those of C- or A-types. The $E^{\text{int.}}$ value is slightly higher than that of B- and C-types. The H-bond φ is stronger and ② weaker than those of B- and C-types. The dipole moment is very high (17.599 D), which is almost intermediate between that of B-type (6.258 D) and C-type (22.302 D). These values indicate that this combination appears possible to occur in biological media, providing the C-type RNA helix with a high dipole moment.
- (iv) Figure 10d: It corresponds to the DNA microhelix plotted in Figure 10c. It appears less stable than B- and C-types, and with $E^{\text{int.}}$ very close to that of B-type, but due to the large E^{def} energy, ΔE^{CP} is lower than B- and C-types. The H-bond φ is stronger and ② weaker than that of B-type. The φ^{I} and φ^{II} torsional angle values are very similar to those of B-type. Although this microhelix is less stable than B-type, its high dipole moment of 16.442 D could facilitate its presence in biological media.

- (v) Figure 10e: The combination of A- and B-types in the CCC:GGG RNA microhelix results in noticeably stronger intermolecular H-bonds than those of A- or B-types (Figure 9i,k). The torsional angles are very similar to those of B-type. It is noted that with this combination, it was not necessary to introduce the methyl $-\text{CH}_3$ group into the microhelix primer (plane $n+1$) for optimization. Due to this feature and the considerable difficulty in optimizing a realistic (without adding water molecules) CCC:GGG microhelix, we think that computationally, this combination could be present in biological media.
- (vi) Figure 10f: The intermolecular H-bonds in this combination are also stronger than those of A- and B-type DNA. The dipole moment value is remarkably lower than that of the corresponding TTT:dAdAdA. It was not necessary to introduce the $-\text{CH}_3$ group and, therefore, we think that this combination could also be present in biological environments.

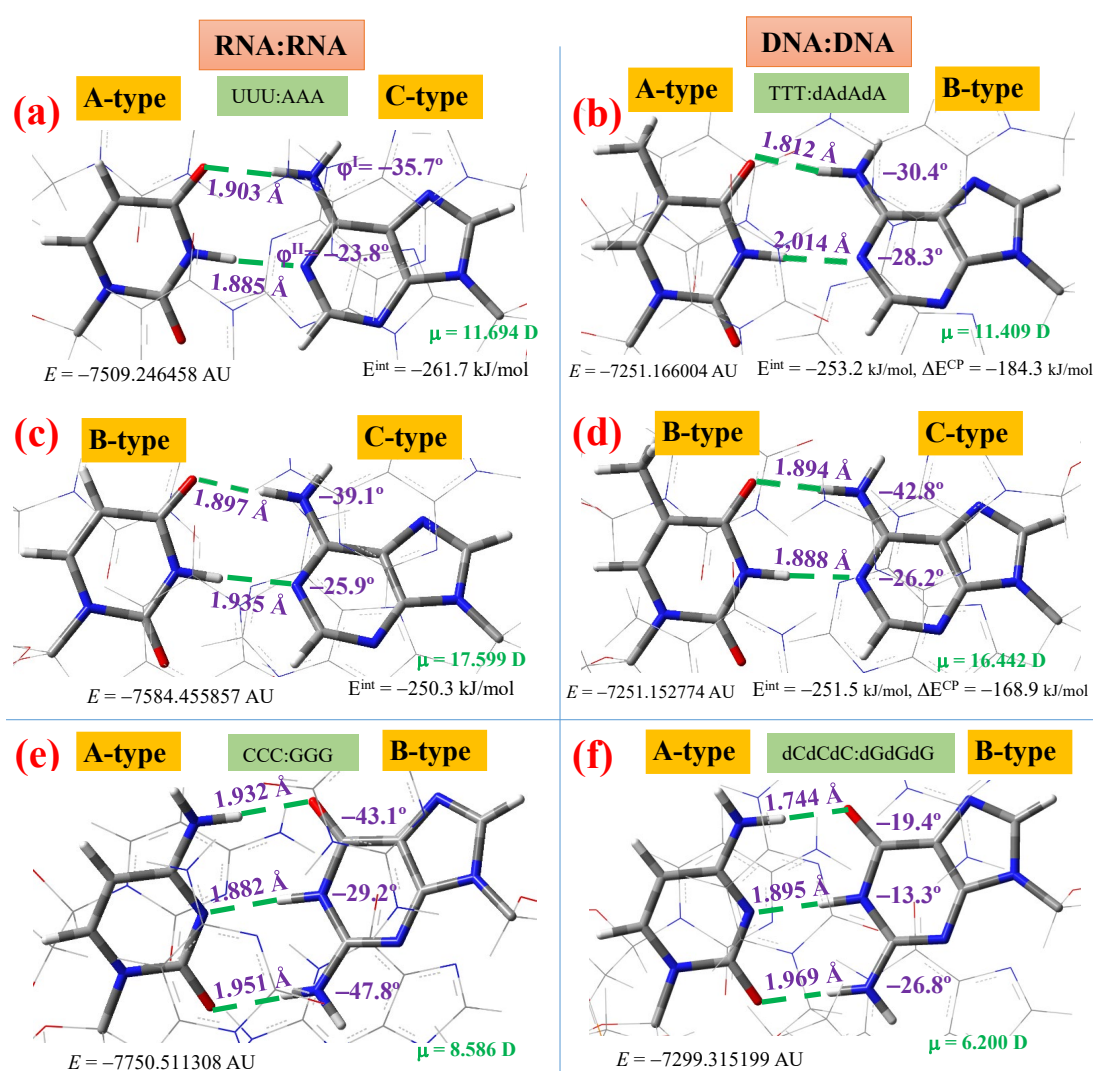


Figure 10. Comparison of the intermolecular H-bonds in microhelices with different types of strands: (a) With A-type pyrimidine strand and C-type purine strand in the UUU:AAA RNA microhelix. (b) DNA microhelix with 5'-TTT-3' strand of A-type and 5'-dA dA dA-3' strand of B-type. (c) RNA microhelix with 5'-UUU-3' of B-type and 5'-AAA-3' of C-type. (d) DNA microhelix with 5'-TTT-3' strand of B-type and 5'-dA dA dA-3' strand of C-type. (e) RNA microhelix with 5'-CCC-3' of A-type and 5'-GGG-3' of B-type. (f) DNA microhelix with 5'-dCdCdC-3' strand of A-type and 5'-dG dG dG-3' strand of B-type.

3.4. Intermolecular H-Bonds Determined in DNA:RNA and RNA:DNA Hybrid Microhelices

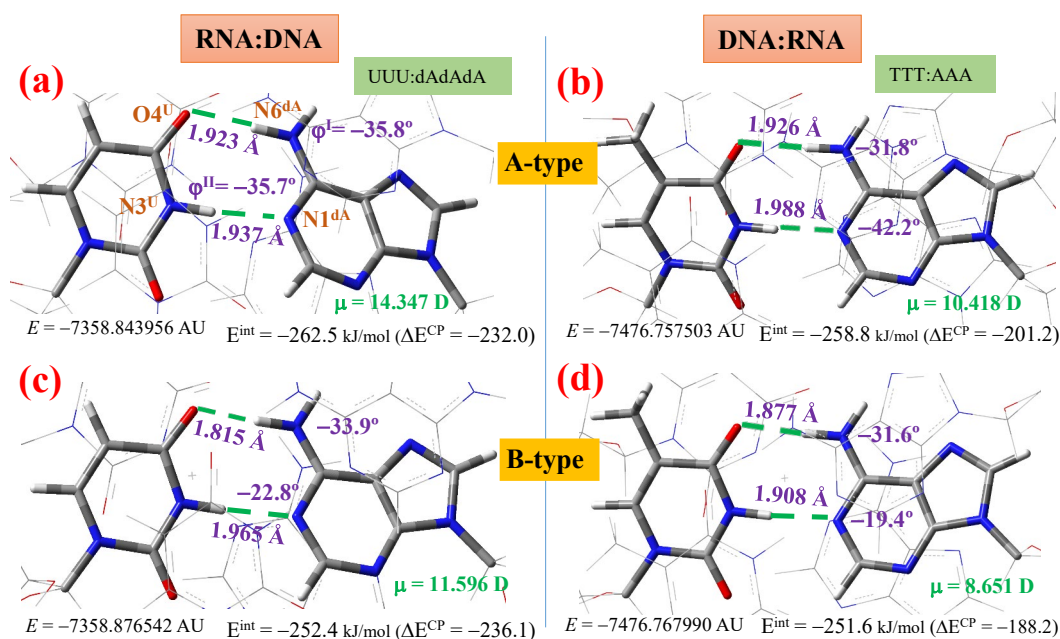
Comparing intermolecular H-bonds in hybrid microhelices (one nucleotide with ribose ring, and another with 2'-deoxyribose) is of great interest due to these being essential biological intermediates that appear to be involved in the reverse transcription process and in DNA synthesis [71]. Comparisons using MD simulations with these hybrid helices have been reported [72], but now the attention is focused on how the H-bonds and the interaction energies change depending on the helix type compared to RNA:RNA and DNA:DNA helices.

3.4.1. Microhelices with the Same Type of Intramolecular H-Bonds in Both Strands

Figure 11a–d graphically compare the intermolecular H-bonds in microhelices with the same type of intramolecular H-bonds in both strands. The observed features are described as follows:

- (i) Figure 11a: In the UUU:dAdAdA microhelix of A-type, the intermolecular H-bond ① is stronger than that calculated in the corresponding UUU:AAA (RNA:RNA, Figure 9a) and TTT:dAdAdA (DNA:DNA, Figure 9b) optimized microhelices, while H-bond ② is weaker than in both RNA:RNA and DNA:DNA. This feature facilitates the nucleobase rotation in the pair, leading to a slight increase in the φ^I and φ^{II} torsional angles and a small decrease in the interaction energy (-262.5 kJ/mol) and ΔE^{CP} (-232.0 kJ/mol) compared to the RNA:RNA microhelix, -262.6 and -242.2 kJ/mol, respectively.

However, in the TTT:AAA microhelix (Figure 11b), both ① and ② H-bonds are weaker than in the RNA:RNA and DNA:DNA microhelices, further facilitating nucleobase rotation, with a large φ^{II} value of -42.2° . E^{int} and ΔE^{CP} have shorter values than those in Figure 11a, but with higher ΔE^{CP} than in DNA:DNA microhelices due to a small E^{def} value in the purine strand. The dipole moment is significantly reduced to 10.418 D vs. 14.347 D in Figure 11a.



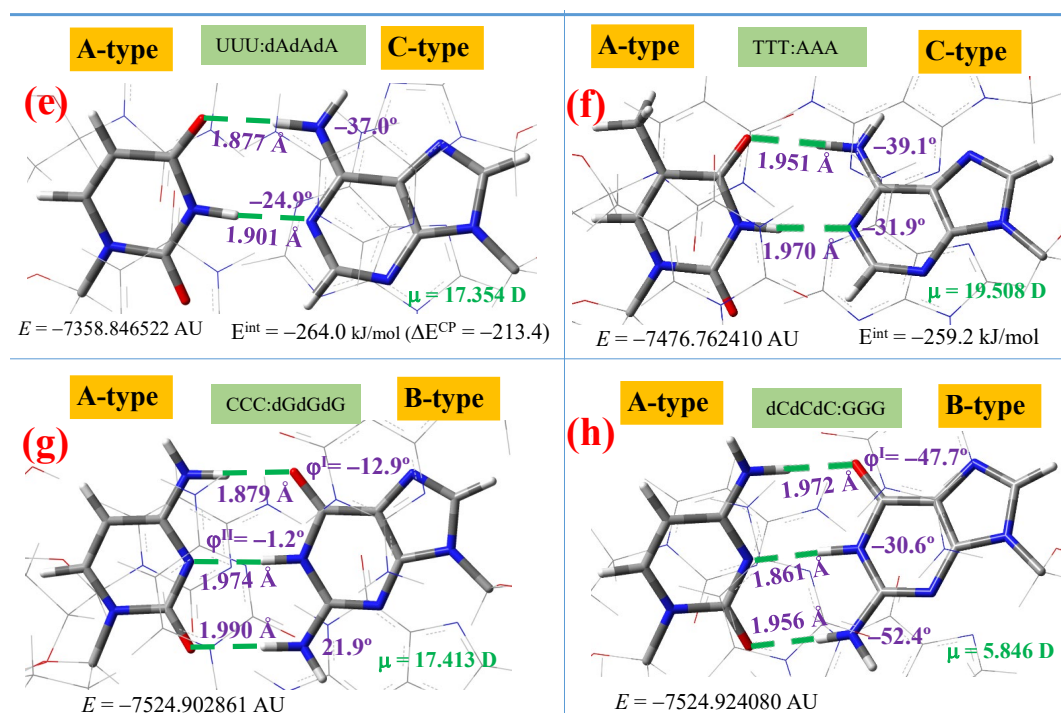


Figure 11. Comparison of the intermolecular H-bonds in hybrid microhelices with one strand of RNA and another of DNA: (a) In the UUU:dAdAdA (RNA:DNA) microhelix of A-type. (b) In the TTT:AAA (DNA:RNA) microhelix of A-type. (c) In the UUU:dAdAdA (RNA:DNA) microhelix of B-type. (d) In the TTT:AAA (DNA:RNA) microhelix of B-type. (e) Pyrimidine strand is A-type RNA, while purine strand is C-type DNA. (f) Pyrimidine strand is A-type DNA, while purine strand is C-type RNA. (g) Pyrimidine strand is CCC (RNA) of A-type, while purine strand is dGdGdG (DNA) of B-type. (h) Pyrimidine strand is dCdCdC (DNA) of A-type, while purine is GGG (RNA) of B-type.

- (ii) Figure 11c: In B-type microhelices, the effect was the opposite to that in A-type. Therefore, H-bond ① is weaker and ② stronger than in both RNA:RNA and DNA:DNA, while in TTT:AAA (Figure 11d) H-bond ① is stronger and ② weaker than in DNA:DNA. These B-type hybrid microhelices appear less rotated than A-type, with lower interaction energies and dipole moments. In B-type DNA, both rotation and dipole moment are reduced compared to those in RNA. The interaction energies have similar values in Figure 10c as in Figure 11d.

The combination of DNA with pyrimidine and RNA with purine appears to provide slightly higher helix flexibility, with a greater rotation in A-type and a lower dipole moment than when the DNA is in the purine strand.

3.4.2. Hybrid Microhelices with Different Intramolecular H-Bond Types in Both Strands

Hybrid microhelices with different types of intramolecular H-bonds in the strands were optimized. The intermolecular H-bonds in the plane n of them are shown graphically in Figure 11e–h. The features observed were described as follows:

- (i) Figure 11e: The combination of RNA pyrimidine strand of A-type with DNA purine strand of C-type appears more stable (6.7 kJ/mol) than the combination of both RNA and DNA strands of A-type (Figure 11a). In this microhelix with C-type DNA, a notable increase in the helical parameters and a considerably larger dipole moment of 17.354 D were observed. It can be explained by the fact that the helix diameter d of 17.717 Å is noticeably increased compared to 17.098 Å in A-type DNA, but slightly smaller than that of C-type DNA. It is also explained by the helical radius R , which

falls between the 8.558 Å in A-type RNA and the 10.152 Å in C-type DNA, while the propeller twist θ_p appears markedly decreased. This leads to stronger H-bonds ① and ② than in A-type RNA, while the H-bond ② is remarkably stronger than in C-type DNA, leading to a higher stability. Compared to RNA:RNA of Figure 10a, the φ^I and φ^{II} torsion angles appear with similar values, but the H-bond φ is slightly weaker and ② stronger, with E^{int} being slightly higher.

- (ii) Figure 11f: When the pyrimidine strand is DNA and the purine strand is RNA, this is the opposite compared to Figure 11e. However, with this combination, both φ and ② H-bonds are remarkably weakened, resulting in a significant nucleobase torsion in the pair (greater φ^I and φ^{II} torsion angles) with an increase in the dipole moment and decrease in E^{int} .
- (iii) Figure 11g: This combination appears with two weaker H-bonds and one stronger than the RNA:RNA of Figure 10e, and with a dipole moment twice higher.
- (iv) Figure 11h: With this combination, two H-bonds are stronger and one is weaker than in Figures 10f and 11h, which significantly increases the nucleobase rotation and considerably reduces the dipole moment.

3.5. Differences in the Helical Parameters Among Different Microhelices

Figure 12 graphically compares the spatial orientation of the three nucleobase pairs in the microhelices of A-, B-, B1- and C-types. For comparison purposes, only the UUU:AAA RNA and TTT:dAdAdA DNA microhelices, with the same type of intramolecular H-bonds in both strands, were considered. The dipole moment values as well as the helix diameter d [16] were also included. Due to the nearly equal NBO charge values on the phosphorus atoms in each strand, as well as the similarity between the nucleobase values in the plane- n and those in the planes $n-1$ and $n+1$, an almost identical stacking effect of the atoms in planes $n-1$ and $n+1$ on the atoms in plane n is expected. The comparison in the calculated helical parameters is as follows:

$P_1 \cdot P_2$ (in the pyrimidine strand) and $P_3 \cdot P_4$ (purine strand) interatomic distances between the phosphorus atoms of each strand: Their values were initially used to analyze the skeletal backbone in the different helix types. In the UUU:AAA RNA microhelices of A-type, both distances are similar, 6.401 and 6.404 Å, respectively (Table 3). However, in the corresponding DNA microhelices, it is larger in the purine strand than in the pyrimidine one (6.561 Å vs. 6.340 Å, respectively), which means that in this DNA microhelix, the purine strand contributes more to the helix rotation. This feature leads to a twist angle value $\vartheta_{pu}(R)$ of the purine strand remarkably larger than $\vartheta_{py}(R)$ of the pyrimidine one.

Similarly, in B-type these distances are also noticeably larger in the purine strand than in the pyrimidine one by both RNA (7.315 and 7.158 Å, respectively) and DNA (7.352 and 7.088 Å, respectively) helices. By contrast, it is the opposite in B1-type helices, while in C-type, it is larger in the pyrimidine strand of the RNA helix but smaller in the corresponding DNA helix. In 5'-CCC-3' microhelices and in mixed and hybrid ones, the value in the purine strand is in general larger than in the pyrimidine one, especially in A-type. This feature also leads to higher $\vartheta_{pu}(R)$ than $\vartheta_{py}(R)$. These $P_1 \cdot P_2$ and $P_3 \cdot P_4$ distances are noticeably shorter in A-type than in B- and C-types, in accordance with the experimentally average value of 5.9 Å in A-type and 7.0 Å in B-type [30].

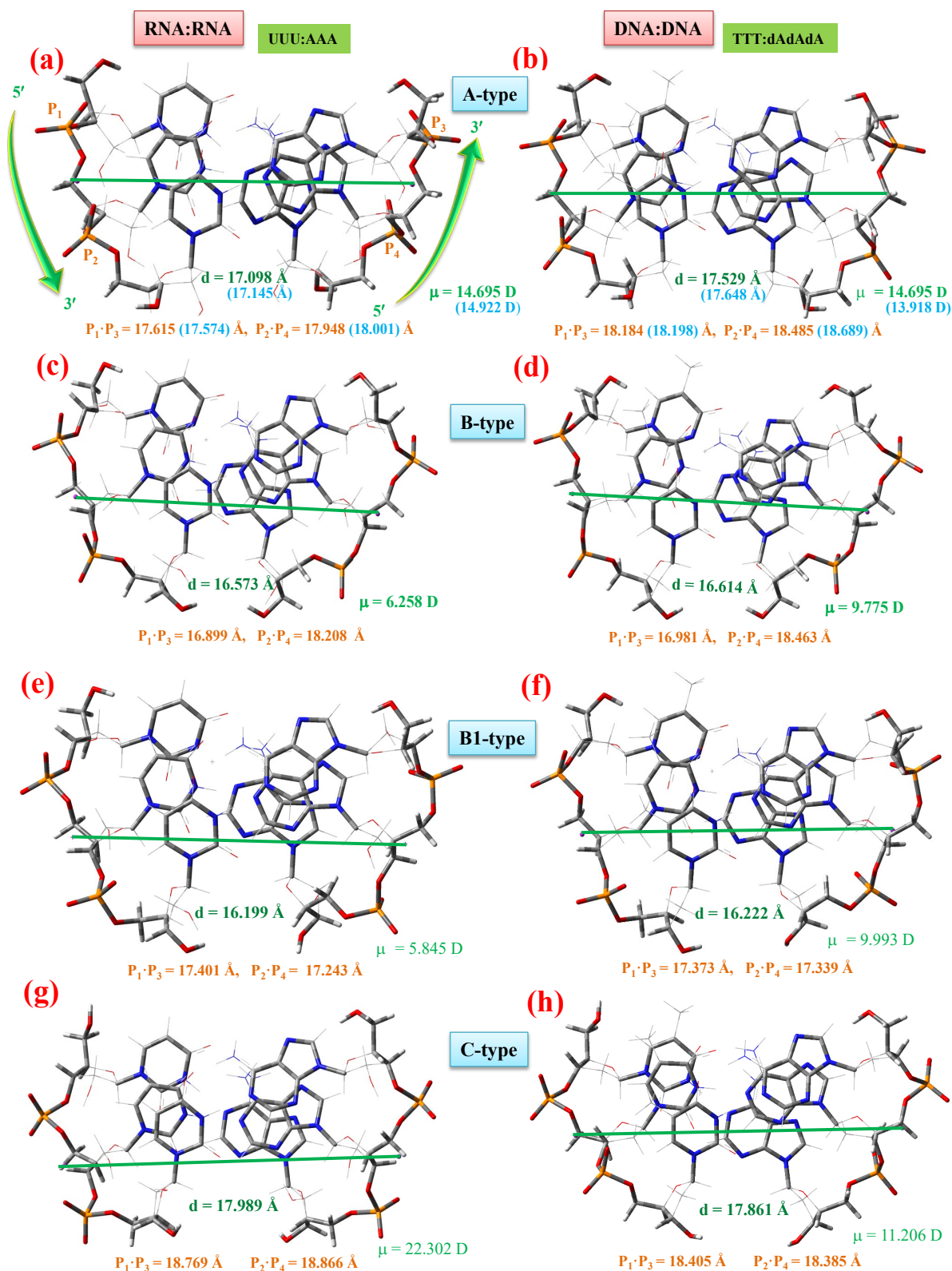


Figure 12. Comparison of the spatial nucleobase arrangement in the helix from the perspective of the $\bar{Z}$ axis, along with the skeletal backbone in the RNA:RNA (UUU:AAA) and DNA:DNA (TTT-dAdAdA) microhelices of: (a,b) A-type, (c,d) B-type, (e,f) B1-type, and (g,h) C-type. The values of the helix diameter d (in green), the phosphorus distances $P_1 \cdot P_3$ and $P_2 \cdot P_4$ (in orange) and dipole moment (μ) were also included. Values with the 6-311+G(d,p) basis set are shown in blue.

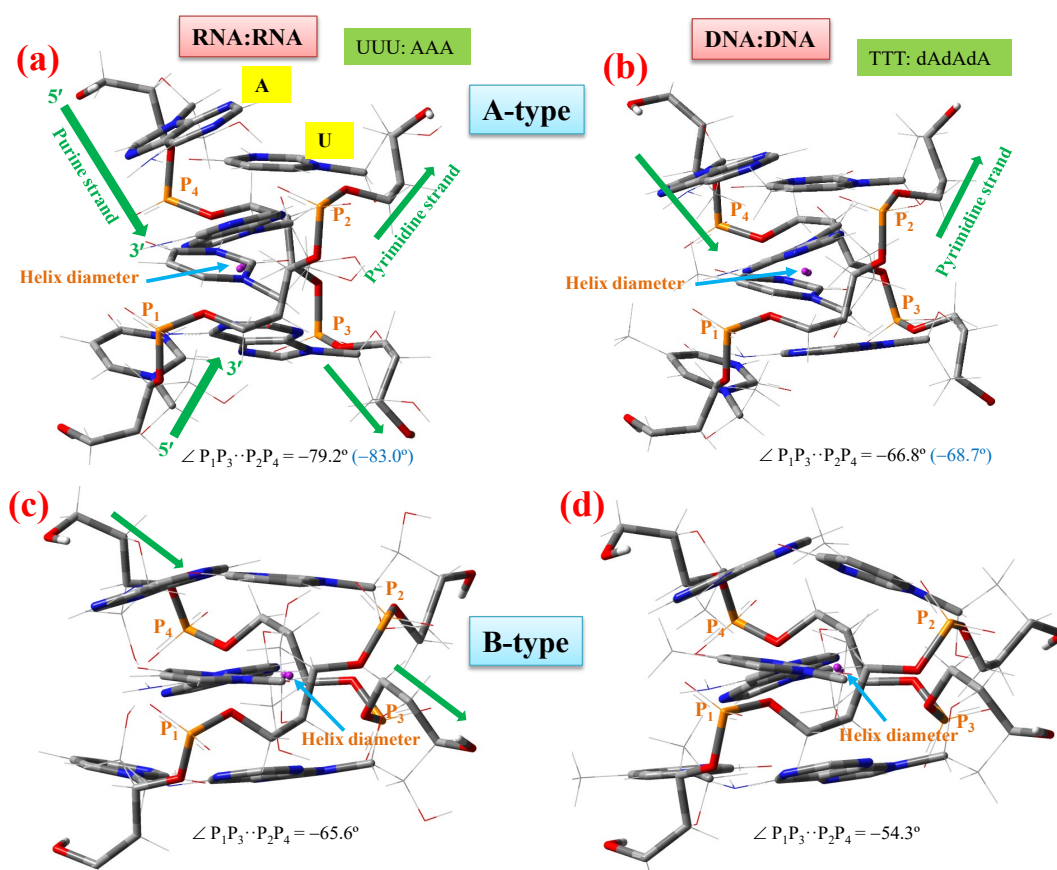
Table 3. The calculated helical parameters in the microhelices are in degrees, while those corresponding to intermolecular distances and rise parameters are in Å. In italic form appears the calculated values with the 6-311+G(d,p) basis set. Microhelices appear defined by the pyrimidine strand.

	Microhelix	Type	Helix		Pyrimidine Strand					Purine Strand					Helix		S(R) §			
			P ₁ P ₂	P ₃ P ₄	d	Dz ₋₁	Dz ₀	ω	Dy ₋₁	Dx ₋₁	Dz ₀	Dz ₋₁	ω	σ	κ	θ _p	R	INC	Pyrimidine	Purine
RNA:RNA	5'-U U U-3'	A	6.401	6.404	17.098	3.353	3.317	29.3	0.058	1.797	3.442	3.352	27.0	8.1	19.4	-28.7	8.558	8.3	-12.4	-22.7
			6.411	6.432	17.145	3.358	3.363	28.8	0.003	1.836	3.526	3.381	27.5	9.1	21.1	-26.2	8.584	12.8	-10.3	-22.4
		B	7.158	7.315	16.573	2.965	3.037	36.0	0.005	2.371	3.155	3.156	32.6	2.5	4.0	-19.0	9.564	16.2	17.9	-27.1
		B1	7.142	6.964	16.199	2.930	2.977	31.3	0.231	2.325	3.146	3.196	36.3	2.8	5.9	-17.7	8.826	19.3	-20.1	0.0
		C	7.043	7.003	17.989	3.178	3.145	27.3	0.394	1.916	3.087	3.188	28.5	3.3	3.2	-24.3	10.060	12.2	-9.4	-15.3
		A-C ^a	6.371	7.018	17.422	3.329	3.330	28.4	0.082	1.796	3.061	3.175	29.0	0.7	2.0	-25.6	9.100	22.7	-15.4	-7.4
	5'-C C C-3'	B-C ^b	7.108	7.022	17.413	2.944	3.128	33.0	0.180	2.284	3.083	3.203	35.0	0.4	1.8	-23.9	9.579	15.5	-9.5	-12.2
		A	6.407	6.586	17.117	3.359	3.375	33.3	0.223	1.399	3.173	3.288	34.6	5.3	7.9	-17.3	8.599	15.2	-7.4	-7.1
	5'-A UA-3' ^h	B	7.445	7.407	15.469	3.220	3.605	49.0	1.219	0.295	3.225	3.176	48.1	3.1	22.6	-30.6	8.894	17.0	-19.7	-9.3
		A	6.379	6.316	17.285	3.224	3.066	9.0	1.093	0.530	3.398	2.915	39.7	3.6	7.5	-4.9	8.932	13.4	-11.1	19.0
		B	7.053	7.242	16.598	3.402	3.546	5.6	0.966	0.953	3.483	1.983	37.7	4.0	11.4	2.1	10.102	5.4	3.1	7.7
		5'-TTT-3' (Figure 11)	A ^c	6.333	6.372	17.276	3.282	3.465	31.6	0.258	1.649	3.497	3.364	11.2	9.0	12.9	-33.0	8.639	12.7	-13.7
B ^d	7.044		7.352	16.555	3.273	3.154	34.8	0.357	1.974	3.121	3.167	34.6	0.5	1.7	-21.5	9.160	18.1	11.8	-26.6	
DNA:DNA	5'-T T T-3'	A	6.340	6.561	17.529	3.236	3.405	30.7	0.331	1.672	3.327	3.241	27.1	0.4	1.3	-32.9	8.799	11.0	13.6	-43.9
			6.381	6.605	17.648	3.365	3.508	29.9	0.437	1.669	3.240	3.247	30.6	2.0	1.6	-32.3	8.920	11.1	9.7	-33.9
		B	7.088	7.352	16.614	3.467	3.009	34.6	0.392	1.978	3.168	3.253	33.6	3.6	9.6	-28.6	9.307	21.4	29.0	-53.3
		B1	7.100	6.838	16.222	3.277	3.109	36.3	0.387	1.912	3.220	3.210	26.4	4.7	4.8	-22.0	8.529	19.8	-11.8	-10.3
		C	6.774	6.851	17.861	3.211	3.345	36.8	0.178	0.453	3.312	3.151	31.7	6.9	12.4	-26.2	10.152	5.2	-10.5	-6.3
		A-B ^e	6.427	7.070	17.329	3.314	3.375	31.8	0.365	1.511	3.122	3.183	23.2	4.1	2.5	-24.4	9.148	21.1	9.2	-25.1
	5'-dCdCdC-3'	B-C ^f	7.011	7.005	17.496	3.452	3.027	32.7	0.207	1.979	3.179	3.221	32.4	3.0	8.5	-28.4	9.202	16.4	5.7	-28.8
		A	6.405	6.613	16.739	3.675	3.611	32.5	0.642	1.731	3.099	3.275	40.7	10.0	22.2	-32.8	8.461	16.4	32.2	-73.3
	5'-dATdA-3' ^h	B	7.376	7.331	14.913	3.030	3.522	52.7	2.003	0.130	3.305	3.165	49.6	1.3	20.4	-30.7	9.755	21.5	13.9	-51.3
		A	6.352	6.416	17.560	3.501	3.240	13.0	0.827	0.525	3.574	3.016	41.7	7.3	3.5	-7.3	9.291	8.7	2.8	10.6
		B	7.289	7.232	15.879	3.109	4.423	13.8	0.953	0.658	3.236	2.660	47.4	0.5	38.9	-24.6	11.776	4.0	-17.4	6.3

Notation used according to Refs. [16,55]: d (microhelix diameter), Dz₋₁ (rise between planes *n*-1 and *n*), Dz₀ (between planes *n* and *n*+1), ω (helical twist) between *n* and *n*-1 planes, Dy₋₁ (slide), Dx₋₁ (shift), σ (opening), κ (buckle), θ_p (propeller twist), R (helical radius), INC (inclination), ^aPyrimidine strand is of A-type and purine strand is of C-type, Figure 10a. ^bPyrimidine strand is of B-type and purine strand is of C-type, Figure 10c. ^cWith DNA pyrimidine strand and RNA purine strand, TTT:AAA microhelix, Figure 11b. ^dWith DNA pyrimidine strand and RNA purine strand, TTT:AAA microhelix, Figure 11d. ^ePyrimidine strand is of A-type and purine strand is of B-type, Figure 10b. ^fPyrimidine strand is of B-type and purine strand is of C-type, Figure 10d. § $\Theta_{py}(R)$ twist of pyrimidine strand related to helix axis O_o, $\Theta_{pu}(R)$ twist of purine strand related to helix axis O_o. ^hFor convenience, the pyrimidine strand in this microhelix is that with a pyrimidine nucleobase in plane *n*, while purine strand is 5'-UAU-3'.

The calculated microhelix diameter d is larger (wider helix) in the TTT:dAdAdA DNA microhelix of A-, B- and B1-types than in the corresponding RNA ones (Figure 12). This is due to both by a distance between the phosphorus atoms of different strands ($P_1 \cdot P_3$ and $P_2 \cdot P_4$) larger in DNA than in RNA ones, and by a rotation angle $P_1P_3 \cdots P_2P_4$ between the phosphorus atoms remarkably smaller in DNA than in RNA. The only exception is C-type, where d is shorter in DNA than in RNA. Moreover, this C-type has the largest d value in accordance with the highest rotation angle of -98.5° in DNA vs. -70.3° in RNA, Figure 13. The different backbone arrangements in these microhelix types can be clearly observed from the helix axis point of view, indicated in Figure 13.

The d value is larger in A-type than B-type in both DNA and RNA microhelices, but the difference increases ca. 1 Å in DNA (17.529 Å in A-type vs. 16.614 Å in B-type), while it is ca. 0.5 Å in RNA. This lengthening in A-type appears to be a consequence of longer glycosidic C1'-N1 bonds in A-type than in B-type in both pyrimidine and purine strands. E.g., in the pyrimidine strand of A-type DNA is 1.485 Å vs. 1.465 Å in B-type, while it is 1.478 Å in A-type RNA vs. 1.451 Å in B-type. In the dATdA:TdAT mixed microhelix, the d value increases in both RNA and DNA, and it is larger in A-type than in B-type. A slight increase in the glycosidic C1'-N1 bonds is also generally observed with the mixing. The low calculated value of 15.879 Å in the mixed microhelix of B-type is due to inter-planar H-bonds between the amino hydrogens of the adenine NH_2 group and the carbonyl oxygen O4 of thymine (Figure 5e)



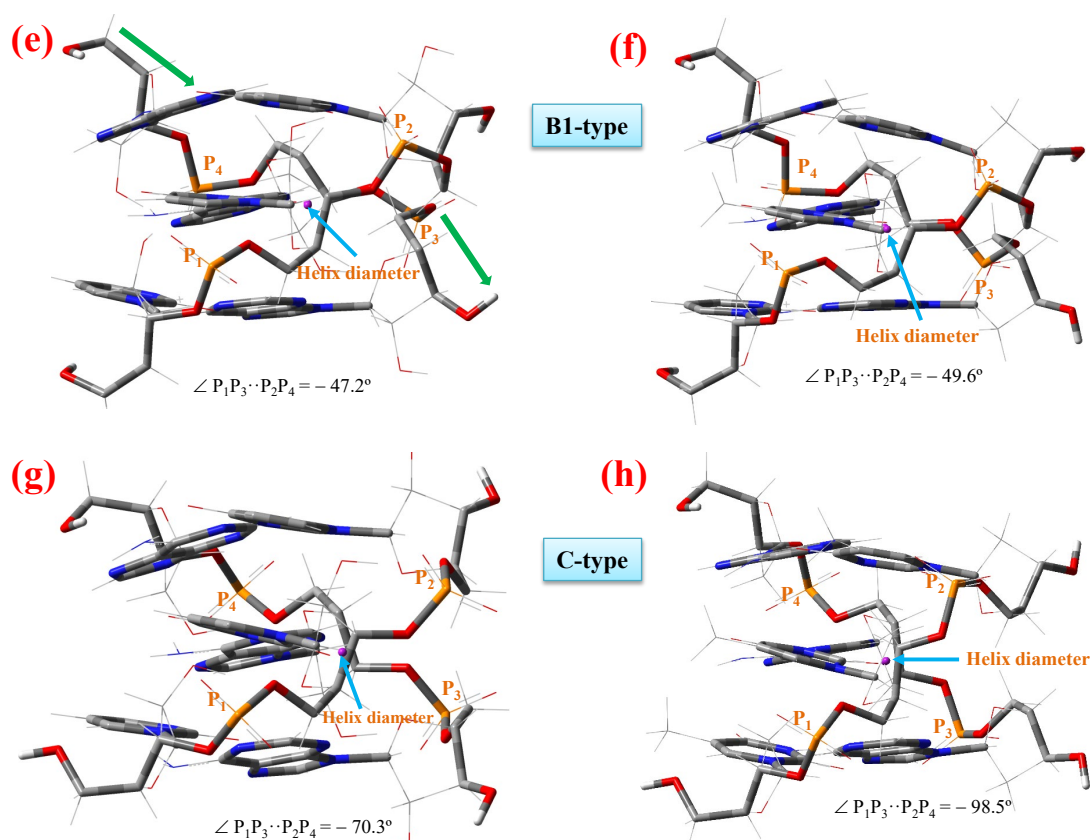


Figure 13. Comparison of the spatial backbone arrangement in the different optimized microhelices from the perspective of helix diameter (small red ball indicated by a blue arrow)) direction in the RNA:RNA (UUU:AAA) and DNA:DNA (TTT:dAdAdA) microhelices of: (a,b) A-type, (c,d) B-type, (e,f) B1-type, and (g,h) C-type. The $P_1P_3 \cdots P_2P_4$ torsional angle is also included, which is greater in A-type than in B- and B1-types. The values in blue correspond to the 6-311+G(d,p) basis set. Green arrows indicate the purine and pyrimidine strand directions.

A lengthening in the C1'-N1 bond leads to a shortening in the neighboring bond lengths of the furanose ring, reducing its flexibility. That is, the maximum torsion angle $v_{\max}$ decreases. A linear relationship/trend is observed between both parameters, Figure 14a. Another notable difference between A- and B-types appears in the furanose ring flexibility measured by $v_{\max}$, which is remarkably higher in B- than A-types in both pyrimidine and purine strands, Table 1, with its value in the purine strand being noticeably higher than in the pyrimidine strand. An explanation of this behavior can be found in the observed correlation in most of the values between $v_{\max}$ and the glycosyl C1'-N bond length (Table 2). Although different factors affect the $v_{\max}$ values, a shortening of the C1'-N bond length generally appears to contribute to a high $v_{\max}$ value. Therefore, purine strands with shorter C1'-N^A bond lengths than pyrimidine strands have higher $v_{\max}$ values. In RNA microhelices, the NBO atomic charges on the phosphorus atoms in B-type are higher than in A-type. This feature may be influenced by the intramolecular H2'...O7' H-bond with the phosphate oxygen. It is also observed in this microhelix, as in A-type RNA, that the charges of these phosphorus atoms have almost the same value in both strands, indicating that their value is independent of whether the nucleobase is uracil or adenine.

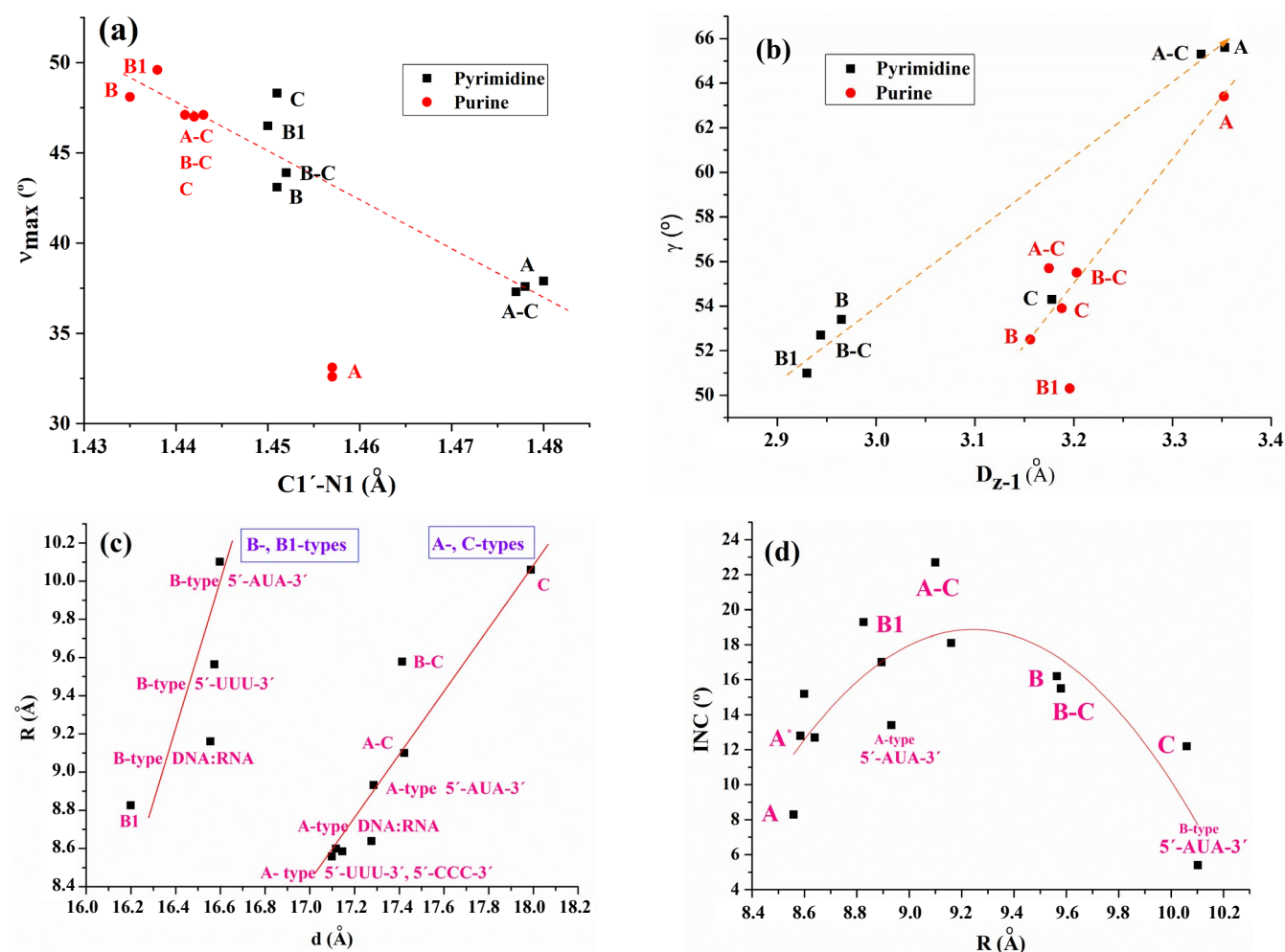


Figure 14. The relationships established in the purine strand (in red) and pyrimidine strand (in black) in the A-, B-, C- B1- A-C and B-C microhelices between: (a) the maximum torsional angle $v_{\max}$ and the glycosidic C1'-N1 bond in RNA helices, and (b) the torsional angle γ and the rise D_{z-1} parameter in RNA helices. The relationships established with the helical parameters in these microhelices are in pick corresponding to: (c) the helical radius R and microhelix diameter d , and (d) the inclination INC angle and the helical radius R in RNA helices. The letters inside the plots correspond to the microhelix types shown in Table 3.

In our calculations for the 5'-UUU-3' and 5'-AUA-3' RNA microhelices, the A-type form differed among many parameters, by having both a longer d and a remarkably higher dipole moment than the corresponding B-type. Because A-type is the form that appears in the RNA duplex [73,74], our calculations in the isolated and hydration states suggest that this could be interpreted as follows: the A-type form (3E) of uridine nucleoside appears to be slightly more stable than the corresponding B-type (2E). In other words, the calculated most stable sugar ring form in the primer was A-type, and in this form, the 3'-OH group appears with the best orientation for the nucleophilic attack line by the subsequent nucleotide to form the chain [74]. Therefore, the helix is expected to grow in this A-type. Because B-type appears mainly when the 2'-OH moiety is H-bonded to O7' of phosphate, this cannot happen in the primer since it is preferentially in A-type. Therefore, B-type helix formation appears to be mainly possible via an internal reorientation to get the minimum energy.

The rise parameter values (in Å) between the planes $n-1$ and n (D_{z-1}), and between the planes n and $n+1$ (D_{z0}), were included in Table 3 for both pyrimidine and purine strands. A detailed analysis of these data yielded the following results:

- (i) In the 5'-UUU-3' RNA:RNA microhelices, the average value of the D_z parameter (D_{z-1} and D_{z0}) appears slightly higher in A-type than in B-type, as is expected and in accordance with that found experimentally [55]. It can be interpreted by a helical twist ω and shift D_x values smaller in A- than in B-types, as well as by the intramolecular H-bond $H2'_n \cdots O4'_{n+1}$ with the 2'-OH group in A-type being weaker than $H2'_n \cdots O7'_{n+1}$ in B-type. This fact may compress the interplanar D_z distance less in A-type. In the purine strand, with a larger nucleobase ring size than that of pyrimidine and therefore greater π - π interactions, the effect of this intramolecular H-bond on the D_z value is slightly smaller (i.e., it compresses less). This results in a slightly higher D_z value than in the pyrimidine strand. This feature is also confirmed in the DNA-DNA helices, where this H-bond through $H2'$ is absent (i.e., the helix is not compressed), and thus a large change appears in B-type with an increase in its D_z value.
- (ii) Base stacking interactions in the helix lead to changes in the exocyclic torsion angles. Among them, an almost linear relationship was obtained between the D_{z-1} values in the RNA:RNA microhelices and their $\gamma(O5'-C5'-C4'-C3')$ values, Figure 14b. The plotted values correspond to those listed in Tables 1 and 3. An increase in D_{z-1} values leads to an increment in γ values. In the pyrimidine strand, microhelices with A- and A-C types have the largest D_{z-1} and γ values, while those with B-, B1 and B-C types appear grouped at lower values. The only exception to this linear relationship is C-type.
Due to a weaker effect of the intramolecular H-bond by the 2'-OH hydroxyl group in the purine strand, its D_{z-1} value range is narrower than that of the pyrimidine strand and is found at higher values. However, in C-type microhelices, both purine and pyrimidine strands have almost the same D_{z-1} and γ values. As expected, this relationship was not clearly observed in DNA:DNA helices due to the lack of the 2'-OH group.
- (iii) In A-C and B-C microhelices with different types of intramolecular H-bond in both strands (Figure 10), a small effect is observed in the pyrimidine strand of C-type with a slight decrease in the D_{z-1} and γ values. By contrast, in the purine strand the effect is notably greater (Figure 14b) and the D_{z-1} and γ values in the A-C and B-C microhelices are very similar to those of C-type in both purine and pyrimidine strands.
- (iv) In the 5'-CCC-3' microhelix of A-type, an increment in the average D_z value is observed in the pyrimidine strand, while its value is reduced in the purine one compared to 5'-UUU-3'. This effect is also observed in its DNA microhelix, although the differences are larger. This feature leads to a higher inclination (INC) value in 5'-CCC-3' and 5'-dCdCdC-3' than in the corresponding 5'-UUU-3' and 5'-TTT-3', respectively.
- (v) In mixed microhelices, because weak interplanar H-bonds are established with one of the amino hydrogens of the NH_2 group (Figure 5e), a noticeable lengthening in the D_z value is observed in the pyrimidine strand compared to the corresponding 5'-TTT-3' microhelix. Because these interplanar H-bonds appear stronger in B-type, and especially in the purine strand, a very low D_{z-1} value of 1.983 Å was obtained in the 5'-AUA-3' microhelix. In DNA microhelices, the D_z values in pyrimidine and purine strands appear higher in both A- and B-type than those in RNA ones, especially in B-type, which can be explained by the lack of the 2'-OH hydroxyl group that lengthened the D_z values.
- (vi) In the hybrid 5'-TTT-3' microhelix, the D_z values are closer to those of DNA than to RNA ones, and with slightly greater differences between the pyrimidine and purine strand values than those corresponding to DNA:DNA microhelices.

The slide parameter values (in Å) between the planes $n-1$ and n (Dy_{-1}) were calculated for simplicity only in the pyrimidine strand, Table 3. In a detailed analysis of these data, the main results obtained were the following:

- (i) In the 5'-UUU-3' and 5'-AUA-3' RNA microhelices, the A-type values are larger than B-type, which can be explained by the greater flexibility of A- than B-type because the intramolecular H-bond of A-type is weaker than that of B-type. In 5'-TTT-3' DNA microhelices, the values are significantly larger than in RNA, and in this case, B-type values are larger than A-type due to the absence of the 2'-OH hydroxyl group, which facilitates helical flexibility.
- (ii) In the 5'-AUA-3' RNA mixed microhelix, the Dy_{-1} value is significantly larger than in 5'-UUU-3'. It can be explained by the fact that in mixed helices the nucleobases are more widely separated, with a larger stretch Sy parameter and longer intermolecular H-bonds ① and ②. It is worth noting that these intermolecular H-bonds in the helix are slightly larger than those calculated in a single WC nucleoside pair alone.
- (iii) The Dy_{-1} value is remarkably larger in B1-type RNA than in B-type due to the large change in the torsional angle ξ . The value increases in DNA microhelix and approaches that of B-type, which can be explained by the lack of the 2'-OH hydroxyl group.
- (iv) A large value (0.394 Å) of Dy_{-1} appears in the C-type RNA microhelix, although a noticeable reduction is observed in its combinations with another strand in A-type (0.082 Å) and B-type (0.180 Å), as well as in the DNA helix.
- (v) The largest values appear in 5'-CCC-3' and 5'-dCdCdC-3' microhelices of B-type due to the great helix deformation.

The shift parameter values (in Å) between the planes $n-1$ and n (Dx_{-1}) were also calculated but, for simplicity, only those of the pyrimidine strand are included in Table 3. These Dx_{-1} values in the $\bar{X}$ -axis are remarkably larger (ca. 2 Å in B-type) than those previously calculated for the $\bar{Y}$ -axis, the slide parameter Dy_{-1} . However, in the 5'-AUA-3' and 5'-dATdA-3' mixed microhelices, the lengthening is less than 1 Å and slightly shorter for Dx_{-1} than for Dy_{-1} . In B-type, the values are remarkably larger than in A-type, for both RNA and DNA helices. A large value in Dx_{-1} generally corresponds to high helical twist ω values, as expected.

The helical twist ω values in the pyrimidine and purine strands were included in Table 3. In a detailed analysis of these data, the main results obtained were the following:

- (i) The values in the microhelices of A- and B-types are slightly higher in the pyrimidine strand than in the purine one, as well as in the DNA microhelices of B1- and C-types, and in their combinations with A- and B-types in another strand. By contrast, they are lower in the pyrimidine strand of B1- and C-types of RNA, as well as in their A-C and B-C combinations with another strand. This behavior can be explained by considering the Dz , Dy and Dx values.
- (ii) The values in B-type are larger than in A-type in both strands and in RNA and DNA helices, in accordance with those observed experimentally and calculated by other authors [28,75].
- (iii) The lowest values appear in the pyrimidine strand of mixed microhelices. E.g. in B-type it is 5.6° in RNA and 13.8° in DNA, although these low values are countered by large values in the purine strand. In these mixed microhelices, the ω values in DNA are larger than in RNA. E.g. they appear more rotated due to the absence of the 2'-OH group that fixes the structure.

The opening σ angle in the plane n was also included in Table 3. In the 5'-UUU-3' microhelix, its value is more than three times higher in A- than in B-types in accordance with a closer $N3-H^U \cdots N1^A$ angle in A-type (174.3°) than in B-type (176.2°). This feature

was also observed in the hybrid DNA:RNA microhelix, with a σ value of 9.0° ($N3-H^T \cdots N1^A = 175.4^\circ$) in A-type, while it is 0.5° ($N3-H^T \cdots N1^A = 177.8^\circ$) in B-type, as well as in the mixed 5'-dATdA-3' microhelix with a large value of 7.3° in A-type vs. 0.5° in B-type.

The buckle κ angle in the plane n differs from the planar form in the 5'-UUU-3' microhelix of A-type and especially in the mixed DNA helix of B-type (38.9°), which may be due to interplanar H-bonds between nucleobases (Figure 5e). Its value in the B-type RNA helix appears closer to the linear form than its homolog DNA, which may be because the intermolecular H-bonds ① and ② are slightly stronger in RNA than in DNA.

The propeller twist angle θp is larger in A- than in B-types in both RNA and DNA helices, and in both types it is larger in DNA than in RNA, due to the lack of the 2'-OH hydroxyl group. In mixed microhelices, the values are remarkably lower in accordance with lower ω values, and to the $20\text{--}30^\circ$ ranges for homopolymer forms and to $10\text{--}20^\circ$ for mixed forms [24].

The helical radius R generally appears geometrically related to microhelix diameter d . A good relationship was established for the RNA:RNA and DNA:RNA values, Figure 14c. An increase in R is almost linearly related to larger d values, although different trends appear for B- and B1 types than for A- and C-types, because B- and B1-types have significantly shorter d values than A- and C-types. R values appear remarkably larger in B-type than A-type. E.g., in the 5'-UUU-3' microhelix, the value is $8.558 \text{ \AA}$ in A-type vs. $9.564 \text{ \AA}$ in B-type, and in the mixed 5'-AUA-3' microhelix, it is $8.932 \text{ \AA}$ in A-type vs. $10.102 \text{ \AA}$ in B-type. This feature is also observed in the hybrid DNA:RNA microhelix. B1-type exhibits low R and d values, while in C-type the opposite occurs, with very large R and d values. The A-C and B-C types also have large R and d values, although lower than those of C-type. Due to the strong deformation of the 5'-CCC-3' microhelix of B-type, its values were omitted in the figure.

In DNA helices, no clear relationship was observed between d and R values, possibly due to the absence of the 2'-OH hydroxyl group that fixes the structural values. The difference in R values between A- and B-types is ca. $1.1 \text{ \AA}$ in RNA helices, while it is $0.508 \text{ \AA}$ in DNA and $2.485 \text{ \AA}$ in the mixed 5'-AUA-3'. It should be noted that the R value was calculated using only three nucleoside pairs and, therefore, may have some margin of error.

The inclination angle INC considerably differs between helices, although some relationship with the radius R was found and plotted in Figure 14d for RNA helices. In microhelices with large R ($>9.3 \text{ \AA}$), an increase in its value leads to a decrease in INC values. However, with lower R values, the trend appears to reverse, and a shortening of R seems to decrease INC . Microhelices of A-type have low R and INC values, while B-type has large R and INC values. The lowest INC value obtained in the mixed 5'-AUA-3' microhelix of B-type appears to be due to interplanar H-bonds, Figure 5e. Low values are also observed in C-type, perhaps due to the largest R values. In DNA helices, and for large R values, an increase in R also leads to a decrease in INC , although the relationship is less clear than in RNA helices.

The calculated twist $\vartheta(R)$ values in the pyrimidine and purine strands, and related to the helix axis, are listed in the last two columns of Table 3. Pyrimidine strands are generally less twisted than purine ones. In 5'-UUU-3' microhelices, slightly larger values are observed in B- than in A-types in accordance with higher R and INC values in B-type. The lowest twist values observed in mixed 5'-AUA-3' microhelices may be due to their interplanar H-bonds. Based on their geometric arrangement, B1- and C-types have the lowest values. Because the $\vartheta(R)$ values were calculated using only three nucleoside pairs, some error is expected in them, especially in the purine strand of A- and B-type DNA.

3.6. Comparison of 5'-UUU-3' and 5'-TTT-3' Values vs. Those in Their Mixed Forms

A summary of the main differences observed between microhelices with only pyrimidine nucleobases on one strand and purine on another strand (non-mixed forms) vs. mixed microhelices in both RNA and DNA is as follows:

- (i) Mixed 5'-AUA-3' RNA microhelix appears to be slightly more stable than 5'-UUU-3', both in A-type (10.5 kJ/mol) and B-type (3.1 kJ/mol). This can be explained by the presence in mixed helices of additional interplanar H-bonds between one of the amino hydrogens of the NH₂ group of adenosine and the carbonyl oxygen O4 of uridine, as well as by additional uridine–adenosine stacking interactions between different planes.
The mixed 5'-dATdA-3' DNA microhelix is remarkably more stable than 5'-TTT-3' in both A-type and B-type. In other words, helices in which purine/pyrimidine nucleosides are significantly mixed within in each strand appear to be more stable than those with a smaller mixture. Due to this noticeably higher stability of mixed DNA helices than RNA ones, it is expected that in bicatenary DNA helices, the purine–pyrimidine mixture within each strand will be significantly more preferred than in bicatenary RNA ones.
- (ii) Mixed microhelices with a purine nucleobase in plane-*n* (5'-UAU-3' form) appear markedly more twisted (high ω) than those with a pyrimidine nucleobase (5'-AUA-3'). By contrast, similar twisting is observed in both strands of 5'-UUU-3' and 5'-TTT-3' non-mixed microhelices, with pyrimidine nucleobases in one strand and purine in another.
- (iii) The dipole moment is noticeably higher in mixed B-type microhelices than in non-mixed forms, in both RNA and DNA. E.g., in RNA it is 14.695 D vs. 6.258 D in 5'-UUU-3', and in DNA is 15.704 D vs. 9.775 D in 5'-TTT-3'. By contrast, in A-type DNA, the value is remarkably lower in the mixed microhelix (9.901 D) than in non-mixed 5'-TTT-3' (14.695 D). These features indicate that mixed RNA/DNA microhelices of B-type appear to be favored in water environments, while mixed DNA forms of A-type appear to be favored in low or poor water environments.
- (iv) With mixing, the exocyclic torsional angles in DNA helices are more affected than their corresponding RNA ones, due to the absence of the 2'-OH group, which hinders changes in the torsional angle. These changes are also noticeably greater in the purine strand than in the pyrimidine one. The main geometrical changes in the DNA purine strand appear in the χ angle, with 17° in A-type and 30° in B-type, in both cases increasing the *anti* form with mixing. An increment is also observed in both the β angle (12° in A-type and 8° in B-type), as well as in the negative value of ζ (17° and 3°, A- and B-types, respectively), and in ϵ (11° and 9°, respectively).
- (v) The pseudorotation phase angle *P* appears remarkably increased with mixing in the purine strand of A-type, from 2.6° to 17.3° in RNA, leading to a change in the furanose ring symmetry, from 3_2T to 3E . A similar increase in *P* is also observed in the corresponding A-type DNA, from 17.1° to 34.5°, changing the symmetry from 3E to 3_4T . Different behavior appears in B-type microhelices, especially in the DNA ones, with a marked decrease in *P* from 157.7° (2E) to 135.4° (2_1T) in the pyrimidine strand of the mixed form, and from 142.9° (2_1T) to 131.7° (1E) in the purine strand.
- (vi) With mixing, the rise parameter *Dz* is shortened in the pyrimidine strand of A-type RNA and lengthened in B-type, while in the purine strand it is shortened in both cases due to strong interplanar H-bonds. Due to the high flexibility of DNA helices, large differences between *Dz*₋₁ and *Dz*₀ are observed with mixing. The propeller twist θ_p decreases markedly with mixing, while the helical radius *R* is increased.

- (vii) The intermolecular H-bonds ① and ② in A- and B-types RNA helices are significantly weakened by mixing, while ① is strengthened and ② weakened in DNA helices.

4. Conclusions

A comprehensive DFT study of the different spatial geometric shapes and energies of double-stranded DNA, RNA and hybrid DNA:RNA microhelices with the standard WC base pair was carried out. The main findings obtained were the following:

1. Because four possible intramolecular H-bonds appear in double-stranded RNA helices through the 2'-OH group, 10 main stable spatial geometric shapes with different exocyclic and endocyclic torsional angles were identified and characterized in detail. Although DNA helices lack this 2'-OH group and therefore cannot form these intramolecular H-bonds, they exhibit a high degree of geometric similarity to RNA helices. Consequently, ten DNA helices with geometric shapes closely resembling those of RNA were also optimized and, analogously to some of those already reported in the literature, they were labeled as A-, B-, B1-, B2-, B3-, C-, C1-, C2-, Z-, and Z1-type.

Although a hydration shell with 10 explicit water molecules surrounding the phosphates and 4 Na⁺ cations produce small changes in the geometric parameters of the helices, the trends remained consistent compared to other microhelices.

2. A new relationship was established between the different DNA types and their corresponding RNA double helices. Of the 10 geometric shapes, only 4 helix types with the same form in all nucleoside pairs were stable in both DNA and RNA helices. In addition to the A- and B-types reported in the bibliography, two new ones were determined and described in detail: B1-type and C-type. The experimentally observed Z-type helices are a combination of the H-bonds of Z- and C-types.

The B1-type is similar to a B-type helix but is slightly less stable and mainly characterized by its larger ζ torsional angle, which is 176.3° in the pyrimidine strand of the 5'-UUU-3' RNA microhelix vs. -113.9° in its corresponding B-type. It is further characterized by its ϵ' angle of 33.7° vs. -16.4° in the B-type and by its shortest R and d values.

The C-type structure arises in RNA helices through the intramolecular O2'-H2'_(n)...O5'_(n+1) H-bond, which is weaker than the B-type but stronger than the A-type. Due to its lower stability and its potential to resemble the B-type to some extent, helices with this C-type have not been identified experimentally by X-ray. Geometrically, it is characterized by a furanose ring with 1E symmetry and by δ and ϵ' values different from those of the A- and B-types. In DNA helices, the furanose ring symmetry changes significantly to $\text{0}_4\text{T}$, and the pyrimidine strand has the greatest v_{max} . This C-type has the largest R and d values in both RNA and DNA helices.

This finding suggests a new RNA double helix classification based on the intramolecular H-bond type through the 2'-OH group, compared to the corresponding classifications in DNA.

3. The two strands of the RNA:RNA microhelix exhibit stronger intermolecular H-bonding than those of the DNA:DNA. The calculated interaction energy E^{int} between both helix strands is higher in RNA helices than in DNA helices, in both A- and B-types.
4. B-type RNA helices are more stable than A-type because of both an intramolecular H-bond H2'_n...O7_n, which is stronger than H2'_(n)...O4'_(n+1), and intermolecular H-bonds which are stronger in B- than in A-type. B-type helices also exhibit a noticeably shorter helix diameter d in both DNA and RNA than A-type. B-type DNA helices are also more stable than A-type, but the difference is around four times

smaller than with the corresponding RNA one. Compared to A-type, B-type helices have a shorter C1'-N1 glycosidic bond in both purine and pyrimidine strands, slightly lower D_z and markedly lower opening σ values in RNA helices, as well as lower θ_p values and higher ω , D_x and R values.

5. The calculated values of the helical twist ω , helix diameter d , inclination angle INC and rise parameter D_z are generally greater in DNA than in RNA. The helical radius R is also larger in A-type DNA than in RNA, but it is opposite in the B-type. In DNA helices and in B-type RNA, the maximum torsional angle $v_{\max}$ and the twist angle $\vartheta_{pu}(R)$ in the purine strand are significantly higher than $\vartheta_{py}(R)$ in the pyrimidine strand. The lowest $v_{\max}$ occurs in A-type microhelices.
6. Microhelices with different types of intramolecular H-bonds through the 2'-OH group in each strand appear possible, and several combinations were optimized. Because the combination of A-type DNA in one strand and B-type DNA in another is almost as stable as double-stranded A-type DNA, it appears possible for it to occur in biological media. Another possible combination with RNA B- and C-types was optimized and it is more stable than C- or A-types, with a high dipole moment μ and large R and d values. The corresponding DNA helix is also possible due to its high μ value.
7. In both A- and B-types, mixed microhelices with purine–pyrimidine nucleosides in each strand are remarkably more stable than those with only purine in one strand and pyrimidine in another. This stability is notably higher in mixed DNA helices than in RNA ones, i.e., the mixing in DNA helices is expected to be larger than in RNA. Due to their high μ value, B-type mixed microhelices are favored in water environments, while A-type mixed forms are more stable in low or poor water environments. With mixing, the helix expands more (higher R), compresses more (shorter D_z), and exhibits a lower θ_p angle.
8. Several relationships/tendencies between the geometric structural parameters of RNA helices were established for the first time. An increase in the glycosidic C1'-N1 bond leads to a reduction in $v_{\max}$, while an increase in the torsional angle γ appears to increment the rise D_{z-1} value. Likewise, with the lengthening of the helical radius R , an increment in diameter d is observed and, for low values, larger INC angles.

The theoretical results presented in this manuscript aim to provide new insights into the geometric parameters, stability, NBO charges in different helix types, and especially the geometric relationship between the DNA helix types and their corresponding bi-catenary RNA helices. Although the conclusions were obtained from the optimization of 40 microhelices with three nucleoside pairs, it is expected that they can be applied to other combination types.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/dna6030034/s1>, Figure S1: Comparison of the optimized geometric parameters in the purine and pyrimidine strands of the UUU:AAA RNA microhelix with (a,b) A-type form, (b,c) B-type form; Figure S2: Comparison of the optimized geometric parameters in the purine and pyrimidine strands of the TTT:dA dA dA DNA microhelix with (a,b) A-type form, (b,c) B-type form; Figure S3: Comparison of the optimized geometric parameters in the purine and pyrimidine strands of the UUU:AAA RNA microhelix with (a,b) B1-type form, (b,c) C-type form; Figure S4: Comparison of the optimized geometric parameters in the purine and pyrimidine strands of the TTT:dA dA dA DNA microhelix with (a,b) B1-type form, (b,c) C-type form; Figure S5: Comparison of the optimized geometric parameters in the plane- n of the pyrimidine strands of the UUU:AAA (RNA) vs. TTT:dA dA dA (DNA) microhelices of A-, B-, C- and Z-type forms.

Author Contributions: Conceptualization, M.A.P.; methodology, M.A.P.; software, M.A.P.; validation, M.A.P. and J.I.; formal analysis, M.A.P.; investigation, M.A.P. and J.I.; resources, M.A.P.; data curation, M.A.P.; writing—original draft preparation, M.A.P.; writing—review and editing, M.A.P. and J.I.; visualization, M.A.P. and J.I.; supervision, M.A.P. and J.I. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

WC	Watson–Crick
DFT	Density Functional Theory
NBO	Natural Bond Orbital

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