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Spatial Configuration of Ordered Polynucleotide Chains. 3. Polycyclonucleotides[1]

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Abstract: Approximate details of the spatial configuration of the ordered 8-2′-purine polycyclonucleotide chain in dilute solution are reported from a combined theoretical analysis of chain flexibility and base stacking. The limited experimental evidence detailing the rotational preferences in this molecule is here supplemented by semiempirical energy estimates of conformation. Only a fraction of the wide variety of regular polycyclonucleotide helices generated on this basis is found to accommodate the array of stacked bases that characterizes the ordered form of the molecule. The bases comprising these stacked helices are arranged, as has been observed previously, almost exclusively in left-handed stacking patterns. In contrast to earlier suggestions, however, the backbone structures to which the stacked polycyclonucleotide bases attach are observed to be right-handed helices. In fact, the sugar–phosphate units of these polycyclonucleotide helices are identical with backbone conformations deduced in x-ray fiber diffraction analyses of ordered double-stranded polynucleotides. Unlike the bases aligned in planes approximately perpendicular to the long axes of ordered polynucleotide chains, the polycyclonucleotide bases attached to the same backbone frameworks are found to stack in planes that approximately parallel the helix axis. This unusual parallel alignment permits the bases of the polycyclonucleotide simultaneously both to exhibit left-handed stacking and to conform to right-handed helical organization.

It has been well established that the introduction of covalent chemical linkages between the sugar and base moieties in a so-called polycyclonucleotide chain will alter the physical and biological properties of the nucleic acid system.[2-10] This effect is especially dramatic in the case of 8-2′-purine and 6-2′-pyrimidine polycyclonucleotide chains where the covalent attachment between the base and 2′-carbon fixes the glycosyl rotation χ (describing the mutual orientation of the two moieties) in the unusual so-called[11] "high anti" conformation. This conformation is an approximately intermediate arrangement between the commonly occurring anti and syn glycosyl conformations. As evident from Figure 1 [where a unit of the 8-2′-anhydro-8-X-9-(β-D-arabinofuranosyl)adenyl or poly(cyclo-A) chain is depicted], the base plane in the high anti orientation approximately parallels the sense of direction of the sugar–phosphate backbone repeating unit. The rotation angle defined by atoms C(2′)–C(1′)–N(9)–C(4) of this unit is found in a trans arrangement. This unusual conformation also characterizes the crystal structures of a number of naturally occurring azanucleosides including formycin,[11] 8-azaadenosine,[12] and 6-azacytidine.[13,14] In the unmodified polynucleotide chain, on the other hand, the anti or syn bases are oriented approximately perpendicular to the direction of the chain repeat unit. In the former conformation the rotation

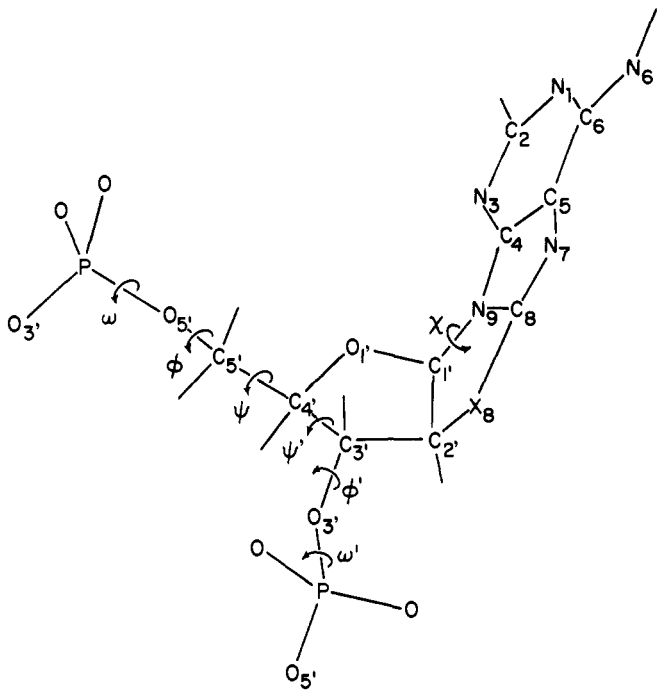

Figure 1. Section of the poly(cyclo-A) chain backbone showing chain atoms and rotation angles. The rotation angle convention adopted here (trans = 0°) differs from that used by crystallographers (cis = 0°).

angle H(1′)-C(1′)-N(9)-C(4) is approximately trans and in the latter conformation this angle is approximately cis.

The unusual optical and nuclear magnetic resonance (NMR) properties of model poly(cyclo-A) chains in solution[2-6,9] suggest that this compound assumes a molecular arrangement quite different from the usual spatial configuration of the unmodified anti adenine polyribonucleotides. The poly(rA) chain is well known to exhibit an ordered arrangement in dilute solution in which the bases are positioned roughly perpendicular to the long axis of the chain and such that each "stacks" one above the other in a parallel fashion. Furthermore, according to both circular dichroism (CD)[15,16] and NMR analyses,[17-21] the stacking of adjacent adenine bases in such helices may be described in terms of a right-handed cylindrical transformation of coordinates. Atoms of each base in the chain may be related to the corresponding atoms in the neighboring parallel base through a right-handed rotation $\pm\Theta$ (of magnitude less than 180°) about a vertical axis perpendicular to the planes of the bases followed by a displacement Z (of the same sign) along this base stacking axis.[22] These base stacking parameters are only slightly different from the helical parameters z and θ (see below) which describe the regularly repeating right-handed RNA-A type poly(rA) structure[23] which accommodates the right-handed stacks. With increasing temperature or upon alteration of solvent conditions this relatively compact helix undergoes a noncooperative structural transition to a less ordered (randomly coiling) form characterized (according to theoretical analysis)[24] by a sizable proportion of extended unstacked conformations.

According to both the ultraviolet (uv) absorption and the CD spectra determined for model dimers and oligomers, adjacent bases in the poly(cyclo-A) chains exhibit strong base stacking interactions. All accumulated evidence, however, points to the occurrence of left-handed stacking in the cyclo-A systems.[2-6,9] The CD spectra of both the model S-cyclo-A dimer (A^SpA^S) and the O-cyclo-A dimer (A^OpA^O) are approximate mirror images of that of ApA.[2-5,9] This splitting pattern plus observed ring current shielding effects[3] from the π electrons in the bases of the A^SpA^S model supports the occurrence of the left-handed base arrangements. In contrast to poly(rA) where the quotient of the base stacking parameters Z/Θ is a positive quantity, the ratio of these parameters is thus negative in the polycyclonucleotides. In comparison to the poly(rA) right-handed bases, the left-handed stacks are relatively stable against thermal perturbations.[3-6] Furthermore, the cyclo-A dimers are resistant to hydrolysis by spleen and venom phosphodiesterases as opposed to ApA which is readily degraded.[3,9]

Since the unusual spectral properties of the polycyclonucleotides and their low-molecular-weight analogues provide no details of the conformation of the chain backbone, it is impossible to draw any immediate conclusions regarding the nature of this unusual helix. In fact, at present, aside from the x-ray diffraction of polynucleotide fibers and the infrared dichroism of polynucleotide films, there is no physicochemical method available to elucidate the handedness of the nucleic acid sugar–phosphate backbone. Furthermore, until experimental techniques are developed to analyze the phosphodiester conformation, there is no experimental means to describe the handedness of any ordered polynucleotide system in solution.

The cylindrical parameters which describe the polynucleotide helix[22] (z = the vertical displacement of adjacent residues along a rectilinear or helix axis, r = the radial distance of equivalent atoms from the helix axis, and θ = the cylindrical rotation angle about the helix axis between corresponding atoms of neighboring units) depend only upon the values of the six backbone rotation angles of the sugar–phosphate repeating unit (see Figure 1), the six chemical bond lengths of the skeletal backbone, and the six valence bond angles along the unit. These cylindrical parameters and, hence, the handedness of the helix (defined as right-handed by positive values of the ratio z/θ and left-handed by negative values) depend in no way upon either the glycosyl orientation or the consequent stacking arrangement (see below) of the heterocyclic bases attached to the furanoses. In the helical structure it is necessary only that each base be positioned in the same manner with respect to the polymer backbone. In the polycyclonucleotide this requirement is assured by the covalent linkage between the base and the 2′-carbon.

Recent work from this laboratory has demonstrated how a single right-handed helical backbone can accommodate several modes of both right-handed and left-handed base stacking depending upon the orientation of the bases.[22] The handedness of the base stack correlates with the handedness of the helix only in the rare instances when the base stacking axis Z and the helix axis z coincide approximately. In this situation the bases are oriented not only perpendicular to the stacking axis but also at 90° angles to the helix axis. It appears fortuitous then that the right-handed poly(rA) helix with bases approximately perpendicular to the helix axis also accommodates right-handed stacking.

The previously reported assignments of helical backbone sense from the NMR and CD of stacked cyclonucleotides, however, were made under the false assumption that the turn or handedness of the polynucleotide backbone follows the handedness of the base stacking.[3,21] When, as sometimes happens, the glycosyl rotation places the base planes in directions which approximately parallel the helix axis, the handedness of the base stacks and the helix may be of the opposite sense. In fact, the right-handed helix treated in the above-mentioned study will accommodate left-handed stacking of high anti bases.[22]

In this paper we present a detailed theoretical reinvestigation of the helical structure of the polycyclonucleotides. We utilize the spectroscopic information detailing the left-handed base stacking in cyclonucleotide analogues in conjunction with the known conformational flexibility of the chain in order to estimate the more probable spatial arrangements of a polycyclo-

Table I. Structural Data of Bridgehead Ether Ring of Poly(O-cyclo-A)

Bond lengths, Å		Valence bond angles, deg	
C(1′)-C(2′)	1.52	C(1′)-C(2′)-O(8)	101.1
C(2′)-O(8)	1.52	C(1′)-N(9)-C(8)	103.0
C(1′)-N(9)	1.47	N(9)-C(8)-O(8)	118.0
C(8)-N(9)	1.37	N(9)-C(1′)-C(2′)	110.8
C(8)-O(8)	1.36	C(8)-O(8)-C(2′)	107.1

nucleotide helix in dilute solution. We calculate the helical and base stacking parameters for a broad spectrum of regularly repeating cyclonucleotide structures which are consistent with both available experimental evidence and theoretical energy estimates of the conformational preferences of low-molecular-weight model cyclonucleotides. The analysis is restricted to the structure of the purine cyclonucleotides [such as poly(cyclo-A)] since the bases of pyrimidine systems [such as poly(cyclo-U)] exhibit little tendency for base stacking.[7,8] The structure of pyrimidine polycyclonucleotides at low temperature, however, may be related to the structures deduced for the purine systems since it has been found that model cyclo-A and cyclo-U oligomers can form stable double- and triple-stranded complexes.[7]

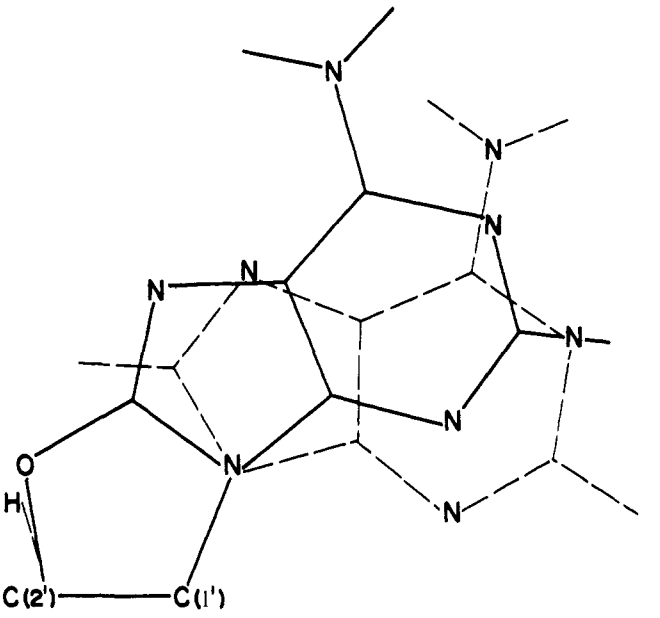

Figure 2. Comparative structural orientation of the base moieties of poly(O-cyclo-A) (solid figure) and poly(rA) (dashed figure) with respect to the pentose atoms. Comparative structural parameters are listed in Table I.

Polycyclonucleotide Structure

The spatial configuration of the polycyclonucleotide chain is determined by the bond rotations χ, ψ', ϕ', ω', ω, ϕ, and ψ (see Figure 1) describing each repeating unit. The rotation about a given bond is taken here to be zero in the planar trans conformation and is assigned positive values for right-handed rotations. The rotation χ about the glycosyl bond is measured relative to the trans orientation of bond vectors O(1′)-C(1′), C(1′)-N(9), and N(9)-C(4).

The fixed structural parameters (consisting of bond lengths and valence bond angles) required for the present analysis of the poly(cyclo-A) system have been approximated as outlined below in view of the absence in the x-ray crystallographic literature of geometric details of model 8-2′-purine cyclonucleotides. All structural modifications associated with the cyclization at atom X(8) are postulated for simplicity to manifest themselves only in changes to the normal exocyclic valence bond angles of the sugar and base moieties directly involved in the formation of the new planar ring [C(2′)-C(1′)-N(9)-C(8)-X(8)]. Similar structural modifications have been observed in the geometry of 2-2′-cyclo-U derivatives compared to that of normal uridine.[25,26] The remaining valence bond angles and bond lengths used to describe the repeating unit (with the exception of those of the 2′-hydroxyl group which is replaced by hydrogen in the cyclonucleotide) are those that have been detailed previously for the poly(rA) system.[27]

The structural parameters thus determined for the ether rings linking the bases and pentoses of the poly(O-cyclo-A) chain are listed in Table I. Aside from the chemical replacement of the H(2′) and H(8) atoms of adenosine by the O(8) bridgehead atom in cyclo-A, the principal difference between the present cyclo-A structures and poly(rA) (as evident from the visual comparison in Figure 2) lies in the relative orientations of the base and pentose. The two valence angles [C(1′)-N(9)-C(8) and N(9)-C(8)-X(8)] are significantly altered from their normal values in the theoretical cyclo structure. According to Table I the former angle is drastically bent from its usual value of 125.5° to 103° in order to permit the formation of the cyclic ether. Although this change is undoubtedly overexaggerated in the model, we note that the corresponding exocyclic angle of the formycin crystal structure[11] (where the base is in a high anti conformation similar to the cyclonucleotide) exhibits a similar decrease of 5° in value from that of adenosine. The valence angle at C(8) in the present cyclo-A structure is also about 6° smaller than the analogous H(8)-C(8)-N(9) angle of the adenosine structure. The two C-O bonds generated in this hypothetical structure are significantly different in length (1.36 and 1.52 Å). One bond is shorter than the mean C-O single bond distance (1.46 Å) and the other bond is even longer than the average. A similar but less exaggerated trend has been reported in the C-O distances of the five-membered ether rings of the 2-2′-cyclo-U crystal structures.[25,26] The valence bond angle formed at the bridgehead oxygen of the hypothetical structure differs by only 2° from the mean angle (109.2°) observed in the cyclo-U structures.[25,26]

It is important to emphasize at this point that the geometric uncertainties associated with the cyclic ether moieties in the present hypothetical structure have only minor influence on the conformational preferences of the poly(cyclo-A) chain backbone as described in the section that follows. The deductions of base stacking and helical structure associated with this particular model thus apply equally well to any feasible cyclo-A system as well as to a number of high anti deazapurine polynucleotides such as polyformycin and polylaurusin.[28,29]

Preferred Conformation Ranges

Glycosyl Rotation. The immediate conformational consequence imposed by the covalent attachment between the base and sugar of the cyclonucleotide is the severe restriction to internal rotation about the glycosyl bond. The flexibility (i.e., puckering) of the five-membered ether bridgehead ring revealed upon inspection of molecular models suggests that the χ rotation is not rigidly confined to a unique angle by the structural requirements of cyclization but may adopt a variety of values centered in the high anti rotation range about χ = 120°. Because of the narrow size of this interval, however, we may represent χ in good approximation by the single rotational isomeric state associated with the planar arrangement of the ether ring. The exact value of χ associated with this conformation depends upon the puckering of the pentose ring. The variations in the internal valence bond angles at C(1′) with different ring puckerings affect the relative positions of the two planes [O(1′)-C(1′)-N(9) and C(2′)-C(1′)-N(9)] describing the dihedral angle of interest. For C(2′)-endo ring puckering

Table II. Comparative Distributions of Partial Electronic Charges in Poly(O-cyclo-A) and Poly(rA)

Atom	Charge (O-cyclo-A)	Charge (rA)
N(1)	−0.391	−0.391
C(2)	0.254	0.254
N(3)	−0.401	−0.401
C(4)	0.205	0.205
C(5)	0.055	0.055
C(6)	0.334	0.334
N(7)	−0.606	−0.406
C(8)	0.450	0.164
N(9)	0.127	0.169
H(2)	0.043	0.043
N(6)	−0.409	−0.409
Amino H	0.224	0.224
H(8)		0.043
O(8)	−0.154	
C(1′)	0.131	0.127
H(1′)	0.055	0.054
C(2′)	0.098	0.107
H(2′-ribose)		0.052
OH(2′-ribose)		−0.157
H(2′-arabinose)	0.051	

(see below) we adopt the value $\chi = 118.3°$ and for C(3′)-endo puckering $\chi = 118.5°$. We note also that these angles place the base and ether rings of the cyclonucleotide in a coplanar arrangement. A rotation of $\chi = 122°$ was observed in the crystal structure of an S-cyclo-A analogue.[30]

Pentose Puckering. The only available experimental evidence detailing the conformation of the pentose moieties of purine polycyclonucleotides in dilute solution and consequently the approximate value of the ψ' rotation angle is proton–proton coupling constants of model nucleoside analogues. The large values of $J_{1'-2'}$ (5–7 Hz) observed in the model S-cyclo-A dimer in aqueous solution[3,6] suggest that a sizable proportion of pentose units of the polymer may be in a C(2′)-endo like conformation. The reported values of $J_{1'-2'}$, $J_{2'-3'}$, and $J_{3'-4'}$ for *S-cyclo*-guanosine in dimethyl sulfoxide[31] also support this conclusion. The conformational flexibility we observe within the pentose moieties of molecular models of poly(cyclo-A), however, leads us to believe that C(3′)-endo type puckering cannot be ignored in our calculations. We have assigned to the ψ' rotation the isomeric states 317 and 266° corresponding to N(2′)-endo and C(3′)-endo units, respectively. We have ignored the unusual C(4′)-endo puckering reported in the crystal structure of the S-cyclo-A 3′,5′-cyclic monophosphate.[30] We recognize that C(4′)-endo puckering is closely related to C(2′)-endo puckering on the pseudorotation pathway of internal motion in the pentose.

Allowed Rotations of ϕ'. The ϕ' rotation about the C(3′)–O(3′) bond has been observed in experimental[32–37] and theoretical[38–40] studies on a wide variety of model compounds to assume a trans arrangement of the chain backbone atoms C(4′)–C(3′)–O(3′)-P. Furthermore, all crystallographic[32] and theoretical[27,39,40] evidence indicates that the conformation of this angle exhibits little if any dependence upon either the nature or the conformation of the attached base. We thus approximate this rotation in our polycyclonucleotide model with the rotational isomeric state of 35° used in previous treatments of polyribonucleotides.[40]

$\phi\psi$ Rotation Pair. The only acyclic rotation along the sugar-phosphate backbone known (from NMR,[41] x-ray,[32] and theoretical[27,42–44] studies) to be influenced by the nature and conformation of the attached base is the angle ψ about the C(5′)–C(4′) bond. No experimental evidence, however, has been reported to date detailing the conformation of this angle in purine polycyclonucleotide analogues in either the crystalline state or in dilute aqueous solution. For this reason we have resorted to a semiempirical energy estimate of the preferred ψ conformations. In view of the absence of any experimental information related to the conformation of the ϕ rotation about bond C(5′)–O(5′) and also in light of the known rotational interdependence[40] between the ϕ and ψ angles, the energy computations have been carried out simultaneously for the pair. The potential energy function utilized was partitioned into bond torsional strain terms, van der Waals repulsions, London attractions, coulombic interactions, and charge-induced dipole interactions as detailed elsewhere.[40] All parameters, with the exceptions of the partial charges listed in Table II for the O-cyclo-A base, were those also described previously.[40] These charges were obtained, as previously, from the combined σ and π charges calculated using the method of Del Re[45] and Hückel[46] theory, respectively. The two angles (ϕ and ψ) were varied from 0 to 330° in increments of 30°. Both C(2′)-endo and C(3′)-endo chains were examined. The χ rotation was fixed in the high anti conformation at the values listed above and the ψ' rotation at 35°. The contributions to the nonbonded energy terms dependent upon the $\phi\psi$ pair comprised all significant interactions between the atoms of the base and the atoms of the chain backbone located between and including the phosphate moieties on either side of the pentose–base unit of interest (i.e., the 3′,5′-O-cyclo-A mononucleoside diphosphate residue).

Table III. Comparative Statistical Weights of the $\phi\psi$ Conformations in Poly(O-cyclo-A) and Poly(rA)

	C(2′)-endo		C(3′)-endo	
Minimum	w_M (O-cyclo-A)	w_M (rA)	w_M (O-cyclo-A)	w_M (rA)
tt	0.06	0.24	0.66	0.24
tg^+	0	0	0	0
tg^-	0.92	0.76	0.31	0.02
g^+t	0	0	0.02	0.53
g^+g^+	0	0	0	0
g^+g^-	0	0	0	0
g^-t	0	0	0.01	0.20
g^-g^+	0	0	0	0
g^-g^-	0.02	0	0	0.01

The relative importance of each of the low-energy regions on the $\phi\psi$ energy map is readily determined from the relative statistical weights associated with each of these domains. The statistical weight w_M is the fractional contribution from minimum M on the $V(\phi, \psi)$ energy surface to the configurational partition function $z_{\phi,\psi}$ and, as outlined previously,[24] may be expressed with sufficient accuracy as

$$w_M = \frac{\sum\sum_M \exp[-V(\phi,\psi)/RT]}{\sum\sum_S \exp[-V(\phi,\psi)/RT]}$$

The summation in the numerator is evaluated at 30° intervals over all ϕ and ψ within minimum M while the summation in the denominator representing $z_{\phi,\psi}$ is evaluated at 30° intervals over all ϕ and ψ on the entire energy surface S.

As evident from the statistical weights listed in Table III, two distinct energy minima are found to be favored overwhelmingly from the semiempirical estimates of the $\phi\psi$ internal rotations in poly(cyclo-A). Both domains are located within the trans (t) range of ϕ centered ±60° about $\phi = 0°$. The ψ rotation exhibits either a t or a g^- (gauche$^-$) orientation. The latter range defines the area centered ±60° about $\psi = 240°$. The theoretical approximations find the tg^- minimum significantly favored in C(2′)-endo units where $w_{tg^-} = 0.92$ and

Table IV. Statistical Weights of the $\omega'\omega$ Conformations in Poly(O-cyclo-A)

	$\phi\psi$ = tt		$\phi\psi$ = tg$^-$	
Minimum	w_M C(2′)-endo	w_M C(3′)-endo	w_M C(2′)-endo	w_M C(3′)-endo
tt	0.46	0.42	0.26	0.33
tg$^+$	0	0	0.08	0.12
tg$^-$	0.01	0.02	0.01	0
g$^+$t	0.28	0.34	0.05	0.23
g$^+$g$^+$	0.06	0.08	0.59	0.32
g$^+$g$^-$	0	0	0	0
g$^-$t	0.19	0.14	0.01	0
g$^-$g$^+$	0	0	0	0
g$^-$g$^-$	0	0	0	0

the tt the major domain in C(3′)-endo units where w_{tt} = 0.66.

For comparison, the corresponding statistical weights determined previously[24] for the poly(rA) unit are also listed in Table III. According to these data there are only minor differences between the conformational preferences of C(2′)-endo poly(rA) and C(2′)-endo poly(cyclo-A) units. The tg$^-$ domain is somewhat more favored in the latter chain. The conformational preferences associated with the C(3′)-endo chains, however, change dramatically when the poly(rA) residue is replaced by the poly(cyclo-A) residue. Electrostatic interactions between the phosphate moieties of the poly(rA) unit favor the t range of ψ and permit the ϕ rotation to assume the g$^+$ (gauche$^+$ where $\phi \sim 120 \pm 60°$) and the g$^-$ as well as the t conformation. In the C(3′)-endo cyclonucleotides, steric interactions between the 5′-phosphate and the high anti base disfavor the g$^+$t domain. Coulombic interactions between the 5′-phosphate and the cyclonucleotide base are responsible for the observed stabilization of the tt region over the g$^-$t minimum in this unit.

In the analysis of base stacking and chain helicity that will follow we thus choose to restrict the poly(cyclo-A) ϕ rotation to the t conformation and the ψ rotation to the t and g$^-$ ranges. We assign the rotational isomeric states $\phi = 0°$ and $\psi = 0°$ or $\psi = 240°$ in the calculations. Small deviations in ϕ and ψ from these values have negligible influence on the base stacking patterns outlined below.

$\omega'\omega$ Rotation Pair. The conformational preferences of the $\omega'\omega$ pair of rotations about the phosphodiester backbone have also been estimated using semiempirical energy functions. The computations were carried out on the dinucleoside triphosphate unit (P-cyclo-A-P-cyclo-A-P) in accordance with the suggestions of Sundaralingam.[47] The ϕ' rotation was fixed at 35°, the ϕ rotation at 0°, and the ψ rotation at either 0 or 240° in the calculations. Both C(2′)-endo and C(3′)-endo units were treated with the χ rotation fixed at the appropriate value (see above). The contributions to the energy from nonbonded interactions between the two bases (i.e., a stacking energy) were ignored in the calculations. Examination of molecular models reveals that the additional interactions between the high anti bases do not exclude any of the conformationally feasible minima described in the following analysis of the energy.

The statistical weights of the four $\omega'\omega$ energy surfaces thus examined are presented in Table IV. These results indicate the $\omega'\omega$ rotations to be a much more flexible angle pair than the $\phi\psi$ rotations examined above. Six distinct minima located at the familiar tt, tg$^+$, tg$^-$, g$^+$t, g$^+$g$^+$, and g$^-$t positions appear on the energy surfaces. The relative magnitude of the statistical weights associated with these regions reflects principally the different extent of coulombic interaction between the negative terminal phosphate moieties of the dinucleoside triphosphate unit in each conformation. Interactions between atoms of the high anti base and the chain backbone have negligible influence on the observed energy surfaces. Any favorable base-base stacking energy not taken into account in the present calculations may affect the relative importance of the various conformational domains as estimated by the statistical weights in Table IV. Any stacking, however, would not alter the zero statistical weights assigned the g$^+$g$^-$, g$^-$g$^+$, and g$^-$g$^-$ conformations. These three regions are exluded by severe steric interactions between nonbonded atom pairs of the sugar-phosphate backbone. Finally, comparison of the statistical weights determined for the C(3′)-endo and C(2′)-endo units reveals the phosphodiester conformation to depend only to a slight extent upon the pucker of the sugar. The $\omega'\omega$ energy surfaces calculated previously[40] for a smaller unit of the polynucleotide chain [extending from atom O(5′) of one pentose to C(4′) of the next] also show only a slight dependence upon ring conformation.

Table V. Allowed Conformations of the Polycyclonucleotide Repeating Unit

χ	ψ'	ϕ'	$\omega'\omega$	$\phi\psi$
High anti	C(2′)-endo or C(3′)-endo	t	tt, tg$^+$, tg$^-$, g$^+$t, g$^+$g$^+$, and g$^-$t	tt or tg$^-$

The apparent flexibility of the phosphodiester rotations in the above energy estimates suggests treatment of the ω' and ω angles as threefold. With the exception of the sterically infeasible g$^+$g$^-$, g$^-$g$^+$, and g$^-$g$^-$ domains cited above, we thus examine all combinations of the t, g$^+$, and g$^-$ conformations of these two angles in the stacking analysis that follows. We vary both ω' and ω at 10° intervals over each of the six remaining minima. The t range is chosen to extend between −20 and 20°, the g$^+$ range between 90 and 120°, and the g$^-$ range between 220 and 260°. These boundaries correspond to the approximate outer limits of conformational feasibility in the P-O rotations based upon the energy estimates (i.e., these boundaries contain those conformations for which the relative energy is less than or equal to 4 kcal/mol and hence for which the statistical weight at 25 °C is greater than 0.001).

Helical Conformations. In retrospect, we find that a single unit of the polycyclonucleotide chain can assume 24 different conformational categories which correspond to all combinations of the allowed ranges of the seven rotatable bonds per residue. These possibilities are listed in outline form in Table V. In order to avoid any conformational bias associated with either the approximate nature of the semiempirical energy functions or the exclusion of base stacking contributions in the above calculations, all 24 sterically feasible conformations (i.e., those conformations which entail no close van der Waals contacts) are included in the following analysis of base stacking. The energy calculations have served merely to differentiate sterically allowed from sterically disallowed conformations of the polycyclonucleotide backbone rather than to predict the relative energies of various helical structures. Since the observed flexibility arises from backbone rotations only, 24 categories of regularly repeating or helical polycyclonucleotide structures can thus be generated. In the section that follows we search to identify those regular structures which can accommodate the left-handed base stacks known to occur in the ordered cyclonucleotide chain.

Treatment of Base Stacking

The mutual orientation of two neighboring bases i and $i+1$ in the polynucleotide chain is determined by the two glycosyl rotations (χ_i and χ_{i+1}) as well as by the values of the seven

Table VI. Mean Helix and Base Stacking Parameters of Ordered Poly(cyclo-A) Chains

Category	Conformation: ψ (range)	Conformation: $\omega'\omega$ (domain)	Conformation: ψ' (pucker)	Helix parameters: $\langle z \rangle$, Å	Helix parameters: $\langle r \rangle$, Å	Helix parameters: $\langle \theta \rangle$, deg	Base stacking parameters: $\langle Z \rangle$, Å	Base stacking parameters: $\langle \Lambda \rangle$, deg	Base stacking parameters: $\langle \Theta \rangle$, deg	Base stacking parameters: $\langle \eta \rangle$, deg
I	t	g^+t	C(2′)-endo	5.74	3.75	74.2	0.25	65.3	42.2	35.9
	t	g^+t	C(3′)-endo	4.96	7.40	32.2	1.05	31.5	0.2	85.9
I	g^-	tg^+	C(2′)-endo	2.60	13.43	−8.7	5.67	28.8	0.6	71.2
	g^-	tg^+	C(3′)-endo	3.00	3.98	−75.6	3.05	74.6	−21.5	83.1
III	g^-	g^+t	C(2′)-endo	1.26	4.13	−104.9	5.60	68.4	−101.2	46.2
	g^-	g^+t	C(3′)-endo	2.94	2.84	−117.2	3.84	18.9	−126.7	11.2
IV	g^-	g^+g^+	C(2′)-endo	4.87	3.42	83.7	0.62	71.5	59.1	62.5
	g^-	g^+g^+	C(3′)-endo	3.06	5.97	46.5	1.15	42.0	−8.9	69.7

intervening rotations (ψ'_i, ϕ'_i, ω'_i, ω_i, ϕ_i, ψ_i, and ψ'_{i+1}) along the sugar–phosphate backbone. In regular structures where the rotation angles are identically conformed, the number of parameters detailing the stacking is reduced to seven. In the present model of the regular polycyclonucleotide where all χ, all ϕ', and all ϕ may be assigned fixed values, the arrangement of any pair of adjacent bases is determined by only four parameters (ψ', ω', ω, and ψ).

As outlined above, base stacking can be described in terms of the mean distance Z between the planes of the two bases and by the angle Θ relating these two moieties about a base stacking axis. This axis will exist only if the two base planes are precisely parallel.[22] Because of the congruency of two parallel bases, all angles formed by corresponding vectors in the two moieties are identical with the angle Θ. The values of Θ reported below for nonparallel base pairs (i and $i + 1$) are the angles formed by unit vectors along the principal axes of each base [i.e., the vectors extending from O(8) to the midpoint of bond N(1)–C(2)] in the plane of base i. We have also determined Λ, the angle between the two base planes, and η, the angle formed between the base stacking and helix axes. These stacking parameters as well as the parameters z, r, and θ describing the helix backbone were determined using the single virtual bond scheme reported recently.[22]

We consider a pair of adjacent bases to be stacked if the following approximate criteria are fulfilled: (1) the two bases partially overlap one above the other (i.e., no intervening moieties, such as the chain backbone, fall between the two bases); (2) the bases are approximately parallel ($\Lambda < 45°$); (3) the bases are at an appropriate separation in space (3.0 Å $\leq Z \leq$ 4.0 Å). If the planes approach any closer than 3.0 Å, excessive van der Waals repulsions will occur between the carbon and nitrogen atoms of the two bases. At distances greater than 4.0 Å theoretical considerations[18,48] indicate that the optical rotation and hypochromicity, although observable, should be substantially weaker than that observed in solution studies of polycyclonucleotides. In addition, the left-handed stacking associated with the polycyclonucleotide chain requires that the ratio Z/Θ be a negative quantity. Finally, the experimental observations of complex formation between *cyclo*-purine and *cyclo*-pyrimidine oligomers further suggest that the bases of the polycyclonucleotide helix are located inside the ordered framework. Those polycyclonucleotide structures which position bases outside the helical framework can form double-stranded complexes only with complementary strands of much wider diameter (and hence of a very different backbone conformation) and with bases directed inside the (second) helical framework. In view of the observed independence of preferred helical backbone conformation upon base sequence in x-ray,[32] NMR,[21] and Raman[49] studies of ordered naturally occurring polynucleotides, it is highly unlikely that complementary strands of polycyclonucleotides would adopt entirely different helical conformations.

Results

Listed in Table VI are the helical and base stacking parameters associated only with those ordered polycyclonucleotide structures which entail partial base–base overlaps. The helices are described in terms of the rotation ranges assumed by the variable angles ψ', ω', ω, and ψ of the polycyclonucleotide model. As noted above, the computations were performed at 10° intervals within the specified ranges of ω' and ω. For simplicity, only the mean values of the parameters associated with each of the regions of $\omega'\omega$ conformation space are reported in Table VI. In order to differentiate these values which do not refer to a real structure from the values of z, r, θ, Z, Λ, Θ, and η associated with a given helix, the parameters are enclosed by angle brackets. In the case of the g^+t helices, for example, the bracketed values in the table represent the mean parameters of the 20 g^+t helices which fall at 10° increments within the area bounded by the 90 and 120° ω' axes and the ±20° ω axes. These mean data are usually representative of the ordered structures of the entire population of helices within a particular region of $\omega'\omega$ conformation space. Those regions containing helices of sizes which diverge significantly from the listed average dimensions are noted in the text.

Cursory inspection of the data in Table VI reveals that only four of the twelve sterically feasible combinations of the ω', ω, and ψ rotations permit the high anti bases of the polycyclonucleotide to adopt an overlapping spatial configuration. The categories I–IV appearing in the table refer to these conformational domains. Both the C(2′)-endo and the C(3′)-endo helices associated with each of these four regions can accommodate overlapping arrangements of neighboring base moieties; hence, the list in Table VI includes a total of eight ordered structures corresponding to the four combinations of each puckered form. The mode of ring puckering associated with a particular combination of the ω', ω, and ψ angles, however, has a significant effect on certain mean structural parameters detailing the base stacking. According to the values of Λ in Table VI only one of the two helices associated with each conformational category (I–IV) meets the loose classification of "stacked" as defined by a simple parallel overlapping arrangement of bases. Examination of molecular models indicates that minor adjustments of approximately ±20° in the rotations ϕ', ϕ, ψ, and χ from the rotational isomeric states chosen in these calculations will transform the four nonparallel base arrangements of Table VI into stacked structures of the same handedness. Such adjustments, however, do not increase the number of helices which can accommodate overlapping base arrangements beyond the eight already cited.

In this paper we do not search to identify precise combinations of the chain rotation angles which produce the best aligned base stacking arrangements. Conformational details of polycyclonucleotide helices which can accommodate both the experimentally observed left-handed base stacking and the

formation of double- and triple-stranded helices will be presented in the following paper of this series.[50] Our interest here is to identify approximately those regular conformations of the polycyclonucleotide chain which can accommodate the left-handed base stacks found in experimental work.

Inspection of molecular models as well as examination of the data in Table VI reveals that variation in the sugar pucker between the C(2′)-endo and C(3′)-endo forms does not usually affect the handedness of stacking associated with each of the conformational categories of the ω', ω, and ψ angles nor significantly alter the mean distance between the planes of neighboring bases. Thus, we can limit the following discussion of geometric details of stacking to the four helices (I-3′, II-2′, III-3′, and IV-3′ of Table VI) in which the bases are approximately in parallel alignment. Minor conformational adjustments of the ϕ', ϕ, ψ, and χ angles will transform the corresponding four helices of opposite pucker (I-2′, II-3′, III-2′, and IV-2′) into stacked configurations very similar to the four categories described below.

Helix Category I. According to the data listed in Table VI, the average polycyclonucleotide type I-3′ structure is a right-handed helix which accommodates bases oriented in a right-handed pattern but separated by distances which violate normal van der Waals contacts. In at least 60% of the I-3′ helices, however, the bases are ordered in left-handed arrangements. The base stacking angles of the entire population of I-3′ helices are restricted to a narrow range centered approximately ±15° about $\Theta = 0°$. In view of the relatively small magnitude of Θ over the entire helix I-3′ conformational domain, the mean base alignment is more aptly termed a straight stack. Although the mean stacking distance of type I-3′ helices is too small, the Z value of a few of the helices approaches the ideal 3.0-Å separation. These few structures located in the conformational domain where the $\omega'\omega$ angles are approximately 90°,−20° thus meet the criteria of left-handed base stacking.

In contrast to the close separation of the high anti bases of the polycyclonucleotide chain, the mean distance between anti bases attached to a helix I-3′ backbone such as in poly(rA) greatly exceeds the boundary of a normal stacking interaction. The average Z determined for type I-3′ poly(rA) chains with the attached bases at orientations of $\chi = 30°$ is 5.7 Å.[23] This distance is possibly large enough to permit intercalation of a planar molecule between the bases. In the polycyclonucleotide chain, however, such a phenomenon is clearly impossible. A visual comparison of the unstacked anti bases and the stacked high anti bases of a I-3′ helix is afforded from the computer generated diagrams in Figure 3. In both structures the bases are located inside the same loosely wound helical framework permitting the formation of multistranded complexes. The bases of the poly(rA) model, however, are oriented approximately perpendicular to the helix axis while those of the poly(cyclo-A) schematic are approximately parallel to this axis.

Helix Category II. The polycyclonucleotide chain ordered in the II-2′ arrangement (of Table VI) is a loosely wound left-handed helix containing bases attached in a right-handed pattern on the outside of the molecular framework. About 40% of the base stacks could be defined as left-handed. The best description of the average base alignment, however, is a straight stack in view of the limited deviations of Θ about the 0° rotation (−10 to +15°) in the type II-2′ helices. The occurrence in dilute solution of a regular polycyclonucleotide structure fashioned from long sequences of II-2′ helical units appears to be very improbable. In the first place, any formation of double-stranded complexes between II-2′ chains is highly unlikely for reasons cited above. Furthermore, the high anti bases along the chain are too widely separated in space (Z = 4.0–6.6 Å) to account for the observed solution stacking properties. The neighboring anti bases of the poly(rA) II-2′ chain are also separated by large distances ($\langle Z \rangle$ = 6.2 Å).[23] The extent of partial overlap of adjacent anti bases in poly(rA), however, is considerably less than that between the high anti bases along the II-2′ polycyclonucleotide chain.

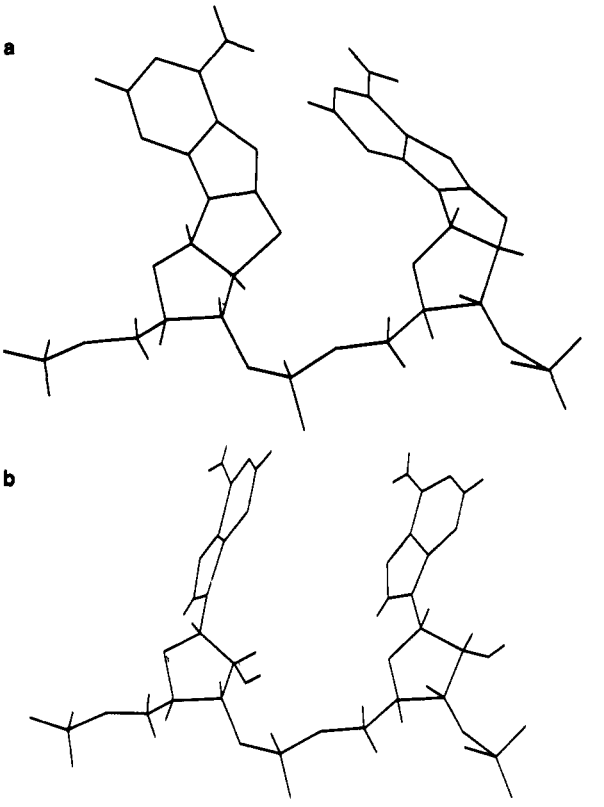

Figure 3. Computer-generated representations of the trinucleotide diphosphate units of (a) poly(cyclo-A) and (b) poly(rA) in the helix I-3′ (see Table VI) conformation. The backbone rotations of both units are fixed at the following values: $\psi' = 266°$, $\phi' = 35°$, $\omega' = 110°$, $\omega = 350°$, $\phi = 0°$, $\psi = 0°$. The high anti polycyclonucleotide base is oriented at the angle $\chi = 118.5°$ and the anti polynucleotide base at the value $\chi = 30°$.

Helix Category III. The regularly repeating III-3′ polycyclonucleotide chain is a very tightly wound left-handed helix according to the structural data in Table VI. In contrast to the large diameter, loosely wound helices I-3′, II-2′, and IV-3′ containing eight or more repeating residues per turn of the helix, a single turn of the III-3′ helix is comprised of only 3–5 chain units. Neighboring bases along the chain are not only ordered in a left-handed arrangement but also separated by the appropriate base stacking distance. Although the high anti bases fulfill all the criteria of normal left-handed stacking interactions, the regular III-3′ configuration remains an unlikely possibility available to the ordered polycyclonucleotide chain in solution. As evident from Figure 4, the bases are laterally attached to the outside of the helix and, consequently, formation of complementary structures is prevented. The stability of such a structure in aqueous solution is also doubtful in view of the strong electrostatic repulsions that would arise between the closely spaced negative phosphate moieties along the inner chain backbone and also in view of the unfavorable interactions that would be associated between the polar solvent and the hydrophobic base moieties on the outside of the helix. Although adjacent high anti bases attached to the III-3′ helix are aligned in a parallel and overlapping fashion, the anti bases along the III-3′ poly(rA) chain fulfill neither criterion. The mean value of Λ is found to be 64.2° for a III-3′ poly(rA) helix with attached bases fixed by an anti glycosyl angle of 30°[23] and only 18.9° when the bases are in a high anti conformation in poly(cyclo-A).

Helix Category IV. The high anti bases attached to the IV-3′ polycyclonucleotide helix appear to fulfill all the geometric

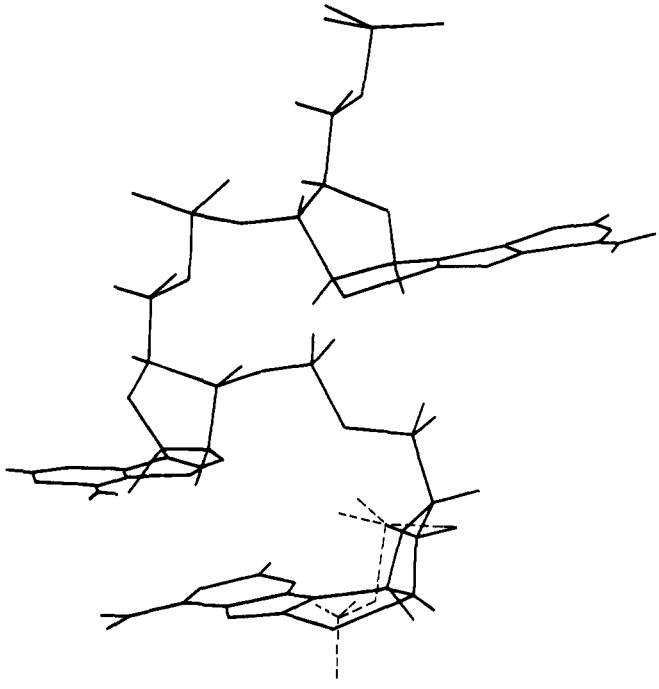

Figure 4. Computer-generated representation of one helical turn of the helix III-2′ (cf. Table VI) polycyclonucleotide chain. Rotation angles of the structure are fixed at the following values: $\psi' = 317°$, $\phi' = 35°$, $\omega' = 90°$, $\omega = 0°$, $\phi = 0°$, $\psi = 240°$, $\chi = 118.3°$. Bonds comprising the next helical turn of the chain are represented by dashed lines.

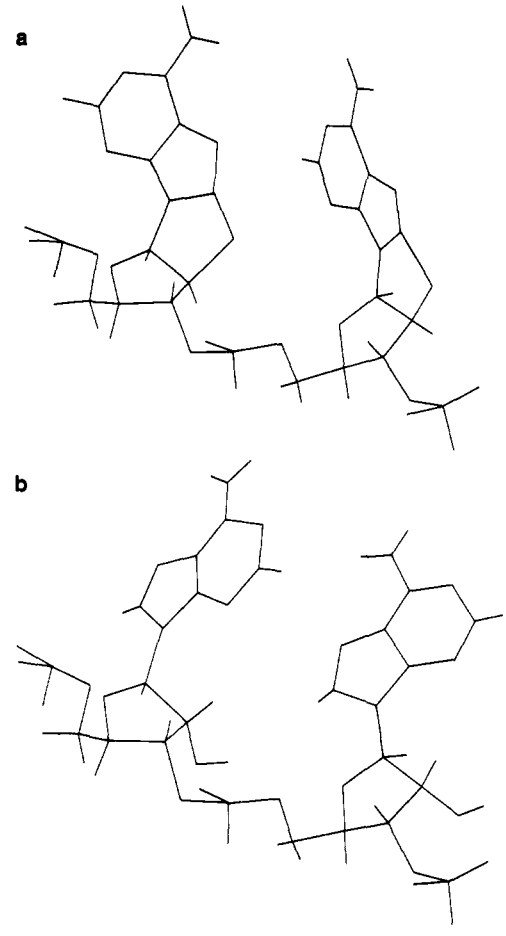

Figure 5. Computer-generated representations of the trinucleotide diphosphate units of (a) poly(cyclo-A) and (b) poly(rA) in the helix IV-3′ (see Table VI) conformation. The backbone rotations of both units are fixed at the following values: $\psi' = 266°$, $\phi' = 35°$, $\omega' = 90°$, $\omega = 110°$, $\phi = 0°$, $\psi = 240°$. The glycosyl angle is fixed at $\chi = 118.5°$ in poly(cyclo-A) and $\chi = 30°$ in poly(rA).

criteria of left-handed stacking (cf. Table VI) with the exception of vertical separation by an appropriate $\langle Z \rangle$ distance. The mean base plane spacing for all polycyclonucleotide helices in the IV-3′ category is 1.15 Å. This distance, however, increases beyond 3 Å in certain IV-3′ helices located near the 90°,90° conformational domain of the $\omega'\omega$ rotation pair. The IV-3′ helix thus also appears to be a probable conformation of the polycyclonucleotide in solution.

These IV-3′ structures are loosely wound right-handed helices. The backbone geometry is very similar to the sequence of rotations that describe the familiar RNA-A type double helices deduced from x-ray fiber diffraction studies[32] and the single-stranded poly(rA) helix in dilute solution.[23] The bases are located inside the helix framework permitting the formation of complex multistranded structures. In contrast to the RNA-A and poly(rA) structures where the anti bases are oriented approximately perpendicular to the helix axis, the bases along the IV-3′ polycyclonucleotide chain are directed parallel to the helix axis. As evident from the schematic diagrams in Figure 5, not only the handedness of the base stacks but also the areas of base–base overlap depend upon the orientation of the base. The base–base overlaps in a sequence (3′- to 5′-phosphate linkages) of anti right-handed stacks position the six-membered pyrimidine ring of the ith purine base (e.g., 3′-end) opposite the five-membered imidazole ring of base $i + 1$ (e.g., 5′-end). In contrast, along a sequence of overlapping high anti bases, the five-membered ring of the ith base covers the pyrimidine ring of the $(i + 1)$th purine.

Discussion

According to the analysis presented above, the only feasible structures which can accommodate the array of base stacks that characterize the ordered polycyclonucleotide in dilute solution are the two right-handed helices I and IV of Table VI. Both structures are large diameter, compact helices potentially capable of accommodating similar intertwined ordered chains in a multistranded polycyclonucleotide complex. As we emphasize elsewhere,[50] these multistranded helices are rather different from the helical model one usually visualizes. Whereas normal anti (or syn) base pairs form horizontally across the double-stranded structure, between complementary residues located at the same z distance or vertical displacement along the helix axis, the base pairing of cyclonucleotide bases occurs vertically between residues at different z positions. One polycyclonucleotide chain positions the hydrogen bonding atoms of its bases upward and the self-complementary chain (running in the opposite direction) positions the hydrogen bonding atoms of its bases downward.

It is also interesting to note from the statistical weights listed in Tables III and IV that the repeating units comprising the two helices are among the lowest energy conformers of the polycyclonucleotide chain. The spatial configuration of the sugar–phosphate unit of helix I resembles that deduced in the original Watson–Crick fiber diffraction analysis of DNA,[51] while the rotational parameters of helix IV describe a backbone arrangement similar to those associated with the "accurate" RNA and DNA double helices of more refined x-ray studies.[32] Unlike the anti bases aligned one above the other in planes approximately perpendicular to the long axes of these two familiar polynucleotide structures, the high anti bases attached to the same backbone networks in a polycyclonucleotide stack in planes that parallel the helix axis. This unique parallel alignment allows the bases of the polycyclonucleotide simultaneously to exhibit a left-handed base stacking pattern and to conform to right-handed helical organization. Despite the

different spatial configurations of the two right-handed polycyclonucleotide helices, the base pairs comprising the two systems exhibit very similar stacking patterns. The calculated mean stacking parameters in Table VI suggest that pairs of neighboring bases along the chains exhibit only slight rotations ($|\langle\Theta\rangle| < 10°$) about their base stacking axes and possibly constitute a system of straight stacks. Theoretical considerations of the exciton interactions determining optical spectra predict the optical rotation to be very weak in such a system of straight stacks.[52] Corresponding bond vectors in adjacent bases attached to specific regularly repeating polycyclonucleotide chains of these helical categories, however, describe left-handed stacking angles as great as $-19°$ in the case of helix I and $-27°$ for helix IV. Base pairs oriented at angles of Θ of this magnitude can account not only for the optical properties of model cyclonucleotides[2-6,9] but also for the reported NMR ring current and shielding effects.[3]

It is also important to emphasize that vertically stacked polycyclonucleotide bases provide approximately the same amount of conformational stabilization to the single-stranded helical backbone as the conventional horizontal base stacking arrangement. One must recall that the bases of a familiar single-stranded helix such as poly(rA) are not completely stacked one on top of the other but are only partially overlapping. As sketched in Figure 6 a, partial overlap of the adenine bases leads to a staggered arrangement of the base stacks within the helix. Under the assumption that base stacking interactions involve only bases which mutually overlap at close distances (i.e., less than 10Å), the stacking enthalpy associated with a given adenine base in a single-stranded helix arises from its interactions with its two nearest neighbors. One sees very similar nearest neighbor overlaps in the vertically stacked polycyclonucleotide model sketched in Figure 6 b and, hence, expects stacking energies between the cyclonucleotide base pairs roughly comparable to those of the same anti bases.

Two other arrangements of the polycyclonucleotide backbone can accommodate high anti bases ordered in parallel fashion as was noted in Table VI. Both configurations (II and III) describe a system of left-handed base stacks joined to a left-handed helical framework. The fact that the bases are located on the outside of the helices, however, prevents the formation of the double- and triple-stranded polycyclonucleotide complexes that have been observed at low temperatures.[7,8] The left-handed chains also violate certain structural criteria of helix stability as cited above. Thus, we do not predict the occurrence of long sequences of the repeating units of helix II or of helix III in polycyclonucleotides at low temperature. The estimates of conformational energy in Tables III and IV, however, point to the occurrence of these conformations in the polycyclonucleotide at higher temperature.

For comparison, it is noteworthy that only one arrangement of the polynucleotide backbone can accommodate the stacking of anti bases at low temperatures. These bases are arranged in the well-known right-handed pattern of planes spaced at 3.5-Å intervals in an array perpendicular to the helix axis. Among the numerous conformations accessible to the polynucleotide at higher temperature are three additional arrangements which also position the attached anti bases in parallel alignment.[23] The stacking in two of these structures is left-handed while that in the third is right-handed. As the temperature increases and both the chain backbone and glycosyl rotations assume a wide variety of conformations, the organization of identical right-handed base stacks within the polynucleotide helix is disrupted. Although each of the four "stacked" arrangements of the polynucleotide bases exhibits optical activity, the net optical rotation associated with equal populations of two types of right-handed stacks and two types of left-handed stacks should be very low. Furthermore, the majority of conformations accessible to the melted polynucleotide unit may be described as unstacked structures which exhibit no optical rotation.[23]

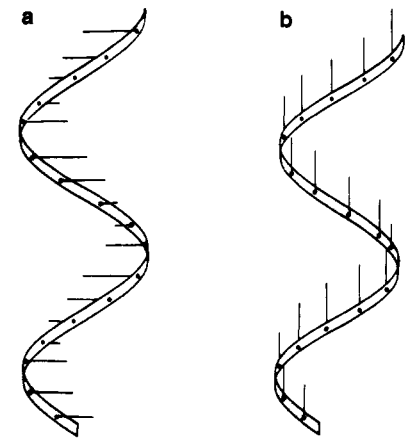

Figure 6. Schematic representations of the base stacking in single-stranded polynucleotide helices. The chain backbone is designated by the ribbon-like structure and is fixed in the helix IV-3′ (see Table VI) conformation. The bases are denoted by the heavy solid lines: (a) the conventional horizontal base stacking of anti and syn bases; (b) the proposed vertical base stacking of high anti polycyclonucleotide bases.

As the polycyclonucleotide melts, however, conformational changes are induced only by the rotations comprising the sugar–phosphate backbone. Of the 24 conformations theoretically accessible to the randomly coiling polycyclonucleotide, eight arrangements [corresponding to helices I–IV of Table VI in both C(2′)-endo and C(3′)-endo chains] may be classified loosely as stacked structures. In addition, these stacks are ordered in either left-handed or straight stacking patterns which contribute in an additive fashion to the optical activity. It is possible then that the increased thermal stability of cyclonucleotide dimers compared with dimers bearing anti bases in CD studies[3,5,9] arises from the large proportion of left-handed stacked conformers that populate the melted cyclonucleotide unit.

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Biosynthesis of Griseofulvin

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Abstract: The antifungal antibiotic griseofulvin (**1**) is a polyketide metabolite of *Penicillium griseofulvum* for which the present study has revealed that at least two and probably all three of the *O*-methyl groups are introduced after both carbocyclic rings have been formed. Benzophenone **11**, the monomethylated precursor predicted by earlier workers, could not be detected in cultures by carrier dilution experiments. Instead benzophenone **14** was shown to be a precursor of **1** by a feeding experiment in which **14** containing a tritium label in the *O*-methyl group was incorporated (14%) into **1**. Demethylation of labeled **1** first to **16** and then to **17** showed that less than 10% randomization of the label had occurred during biotransformation of **14** into **1**. The possibility that unmethylated benzophenone **18** was the precursor of **14** was considered, but synthetic **18** was found to be too unstable for use in carrier dilution or incorporation experiments, undergoing facile cyclization to xanthone **19**. The latter compound was, however, found to be a metabolite of *P. griseofulvum*, which lends support to the hypothesis that both **19** and **14** arise in the fungal culture from **18**. Earlier workers had postulated that the grisan ring was formed by oxidative cyclization of benzophenone **2** to give dehydrogriseofulvin but in vivo confirmation of this process had not been obtained. Another possible precursor to dehydrogriseofulvin, normethyldehydrogriseofulvin (**20**), has been synthesized and shown to be incorporated (44%) into **1**. These findings, in conjunction with those of previous studies, support the biosynthetic sequence: acetate → heptaacetic acid (**8**) → benzophenone **18** → benzophenone **14** → benzophenone **4** → benzophenone **3** → grisan **20** → dehydrogriseofulvin (**7**) → griseofulvin (**1**).

Griseofulvin (**1**), a chlorine-containing, antifungal antibiotic elaborated by *Penicillium griseofulvum* and related strains of *Penicillia*, has been the subject of numerous chemical and biological studies.[1] The polyketide origin of this compound was demonstrated in 1958 by Birch[2] using [1-^{14}C]acetate and later by Tanabe[3] with [2-^{13}C]acetate as the metabolic precursor. Cometabolites of **1** have provid considerable insight into its biosynthesis; compounds bearing a clear structural relationship include benzophenones **2–4** which have been given the trivial names griseophenones A, B, and C, respectively, xanthones **5** and **6**, known as griseoxanthones B and C, and dehydrogriseofulvin (**7**).[4] As presently understood, the biosynthesis of **1** involves cyclization of a polyketo acid to a benzophenone; the heterocyclic ring of the grisan structure is then formed by an intramolecular oxidative coupling.[5] The timing of methylations and chlorination is important. Sequential involvement of benzophenones **4** and **3** in the biosynthesis of **1** has been demonstrated.[6] Similar attempts to incorporate **2** have met with failure[6] but dehydrogriseofulvin (**7**) is efficiently transformed into **1**.[5b,7] The xanthones are apparently by-products rather than intermediates in the pathway. These relationships are summarized in Scheme I.

Benzophenone **4** is the most primitive compound in the pathway, other than acetate, thus far to be identified; in the present study a search has been made for aromatic precursors of **1**. In addition, the unresolved question of the stage at which formation of the grisan ring system occurs has been investigated and the paradoxical failure of **2** to be transformed into **7** and **1** can now be rationalized.

Aromatic Precursors of Griseophenone C (4). Heptaacetic acid (**8**) is a putative intermediate in the biosynthesis of griseofulvin and other metabolites derived from 7 acetate molecules.[5] The transformation of **8** into **4** requires (a) a Claisen cyclization, (b) an aldol cyclization, (c) dehydration,