

A possible conformation for double-stranded polynucleotides

(DNA structure/x-ray calculations/optical rotation)

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ABSTRACT A model is presented for double-stranded polynucleotides which involves side-by-side meshing of the two strands rather than double helical intertwining. The sugar-phosphate backbone has a twisted strip-like character, yet base-pairing of the Watson-Crick type is still possible. Structural features of the basic model are described and coordinates are presented for a representative example. The structure has, on the whole, reasonable stereochemical contacts, and can be shown to produce a fiber diffraction pattern with x-rays not unlike that of the B form of DNA.

The Watson-Crick model (1) has been outstandingly successful in providing a framework for understanding a wide range of observations of duplex structures. Initial concern about its intertwined nature (2) has now diminished. Nonetheless, as this feature remains something of a puzzle, we recently attempted to construct a base-paired model where the strands do not intertwine. We have found that such a model, having a side-by-side association of single strands, can be built without gross stereochemical difficulty. While this structure may not be generally applicable to duplexes it does have sufficiently attractive features for us to consider it appropriate to present the model at this time for appraisal as an additional or alternative conformation to the double helix. We outline broad structural details of the initial model together with a preliminary analysis of it in relation to various physical properties of duplexes. One key feature of the side-by-side (SBS) model is that it can be mathematically shown to be capable of producing the characteristic x-ray diffraction pattern of the B form of DNA.

Structural basis of the model

Using the Watson-Crick mode of base pairing and antiparallel strands we have constructed a model that does not involve gross intertwining of the phosphate backbones. This was achieved by constructing approximately half of the repeat unit in a basically Watson-Crick manner for a right-handed double helix with acceptable structural parameters (3), and then continuing the model in the form of a left-handed double helix. The model retains the essential features of base stacking and repeat distance of the Watson-Crick model that are required by x-ray data. However, by alternating the helical sense every half-repeat distance a net winding of the phosphate strands is minimized and the structure becomes a side-by-side intermeshing of strands rather than an intertwining of them. Various views of the side-by-side (SBS) structure are shown in Figs. 1-4. One view (Figs. 2b and 3a) resembles that of the Watson-Crick model. Other views (Figs. 1-4) however reveal marked differences. The SBS structure has a twisted strip-like character that contrasts with the rod-like appearance of a double helix. The structure approximates to that of a strip of corrugated sheet cut on the diagonal with the cut edges representing the phosphate strands.

Abbreviation: SBS, side-by-side.

The major difference between the SBS and Watson-Crick structures, apart from the difference in helical sense of one half of the repeat unit, is the existence of bends in phosphate SBS backbone structure. These bends occur in pairs approximately every five base pairs. They not only enable the changes in handedness along the molecule to be accomplished but they also have the effect of destroying the helical character of the backbone. While a relatively high proportion of the phosphate structure approximates to sections or mirror images of sections of a right-handed helix, it does not have a smooth helical form. Rather, the phosphate backbone has more the appearance of hinged rods.

In constructing the model, two kinds of bending of the backbone (p and q) were employed. The p bend involves a change from the *gauche-gauche* (gg) nucleotide conformation [that is used in the right-handed section, as for refined models of double helical DNA and RNA (4)] to the *gauche-trans* (gt) conformation (5) (that is necessary for building left-handed portions). The p bend change from gg to gt involves rotation about the C₄'-C₅' bond to the sugar, as used in the formation of a Crick-Klug "kink" (6). For q bends, an additional nucleotide conformation is required to achieve the gg to gt change. The recently observed *trans-gauche* (tg) conformation (7), or one close to it, appears to be the most favorable one to employ. The nucleotide sequence for q bends is therefore . . . gg-tg-gt The p bends are distinguished from the q type by being somewhat smoother and more open, and by their disposition along the chains. The pattern along the two strands is

p q p
q p q

with bends of each pair occurring at about the same level.

When viewed down the molecular axis, the SBS model has a heart-shaped rather than circular outline (Fig. 4). The p bends lie along the 1 and 2 directions while the q bends lie adjacent to 3 when viewed in projection. The asymmetry in projection arises from the orientation of the linkages to the bases. They are always disposed towards the 3 side. This produces an open face or broad groove on the 1,2 side and a narrower one on the 3 side of the molecule. The particular disposition of the linkages from the backbone to the bases also accounts for the q bends being somewhat tighter than those of the p type. Further, the SBS configuration has a unique geometrical association of the complementary strands with the 5'-end of necessity being on the 2,3 side and the 3'-end on the 1,3 side for the Fig. 4 view. The model has 2-fold rotation axes (dyads) perpendicular to the molecular axis every ½ repeat distance. These are contained in the vertical plane passing through 3 and the mid-point of a line joining 1 and 2 (Fig. 4) and lie midway between pairs of bends as shown in Fig. 1b.

The (C3 endo) sugar conformation appears to be the most favorable one for left-handed sections of the structure. The C3 endo conformation or alternative conformations [(C2 endo) and

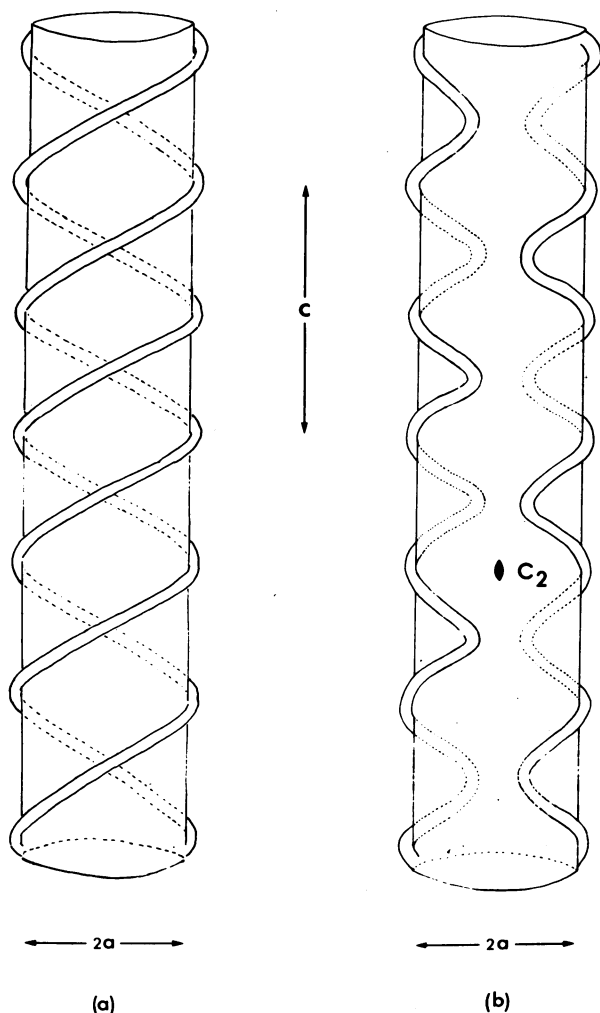


FIG. 1. Idealized drawings of (a) an elementary Watson-Crick model and (b) an elementary side-by-side (SBS) structure; c = axial length of the repeat unit; a = approximate radius of phosphate strands; C_2 = 2-fold rotation (dyad) axis (perpendicular to paper) which relates the p and q bends in the following manner



(C3 exo)] may be used for the right-handed sections. The use of more than one sugar conformation is probably necessary for the construction of SBS structures particularly in q bend regions (see *Appendix*).

Because the sugars in the two halves of opposite helical sense in the SBS model are necessarily of the same (D) optical type, one half is not the exact stereochemical inverse or mirror image of the other. In addition, the phosphate groups in the left-handed portion are splayed out to a slightly greater extent than in the right-handed section. Thus, the diameters of the two sections differ by perhaps one or two Angstrom units. By comparison with the Watson-Crick model where each successive phosphate group must bend towards the center of the duplex in order to retain the helical character of the backbone, the phosphate groups in the SBS model are generally freer to adopt positions at larger radial distances.

There are many more degrees of freedom in constructing an SBS model (because of the different types of juxtapositioning of base-pair units) than for a Watson-Crick type, where the whole structure can be built by successive 36° rotation of a basic nucleotide base-pair unit. Thus, it is difficult to ensure that our model is the best representation of a side-by-side structure and

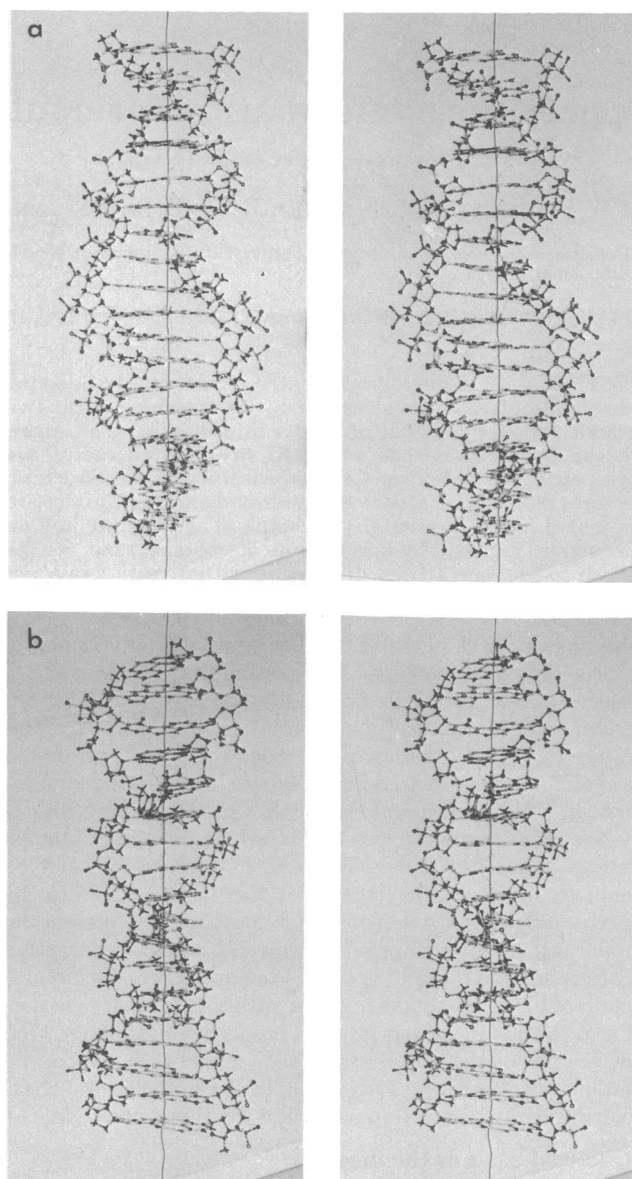


FIG. 2. Stereo views of a side-by-side (SBS) model (a) viewed from the narrow groove side and (b) viewed at a position 90° from (a).

details presented here must consequently be treated with caution. Also, while we have used established conformations for the components of the structure and ensured that all close contacts are minimized, we cannot guarantee that our model satisfies all stereochemical constraints. Further studies, with computer model building in particular, will be necessary to more rigorously check the stereochemical viability of the basic model.

We stress that our model is only a preliminary one and that various modifications are possible, such as (i) bending the two backbones at different levels, (ii) varying the lengths of the left-handed and right-handed portions, and (iii) allowing the sections of opposite helical sense to zig-zag to some extent along the axis.

Models with even right-handed, left-handed arrangements have a net long range right-handed twist (as indicated in the stereo diagrams and described in the *Appendix*). This arises from the greater ease of rotation for right-handed helices. A set

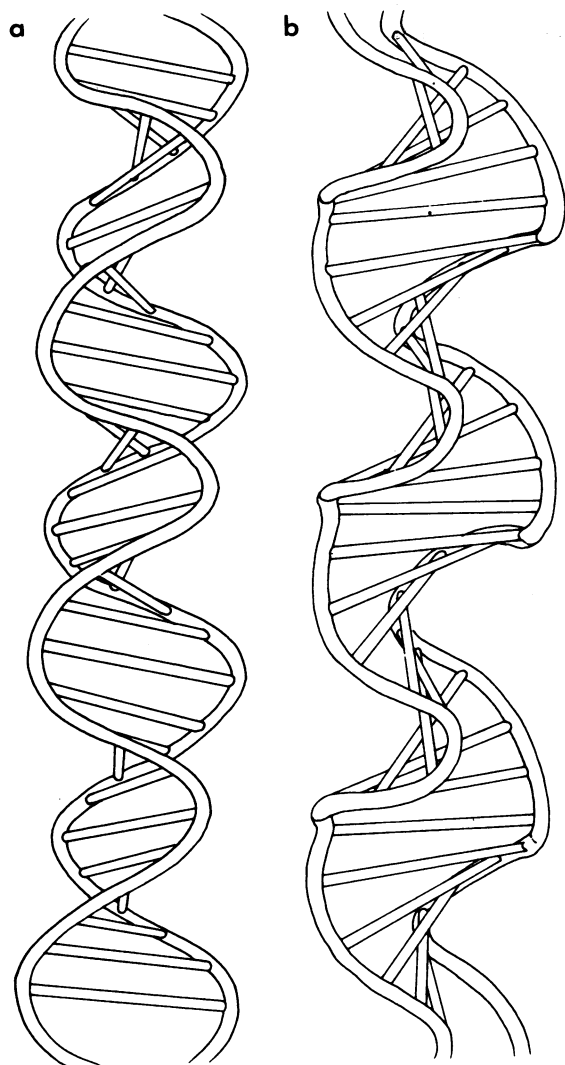


FIG. 3. Sketches of a simplified model of the side-by-side (SBS) structure, (a) side-view corresponding to Fig. 2b, (b) an angular side-on view.

of approximate coordinates for a representative SBS structure is presented in the *Appendix*. This has an even (5 right, 5 left) arrangement that produces a net right-handed rotation of about 35° .

Assessment of data for duplexes in terms of the SBS model

We consider that a crucial feature of the SBS structure is its ability to produce the characteristic x-ray pattern of the fiber form of DNA. This is discussed in part (a) below. In part (b) of this section we refer to a possible case where the SBS structure plays a role in a synthetic system and in part (c) we comment briefly on other physical properties of duplexes.

Mathematical Calculation of Diffraction Patterns. A fiber specimen of DNA consists of a large number of individual fibers bundled together. All the fibers are roughly parallel and the molecules in a single fiber are quite well ordered. However, the relative angular orientation of the fibers is essentially random, and they are in thermal motion. So, the intensity of the observed diffraction pattern is roughly proportional to the intensity of the diffraction pattern of a single molecule averaged by rotation. The most striking feature of the observed diffraction pattern is the absence of intensity in the double wedge region

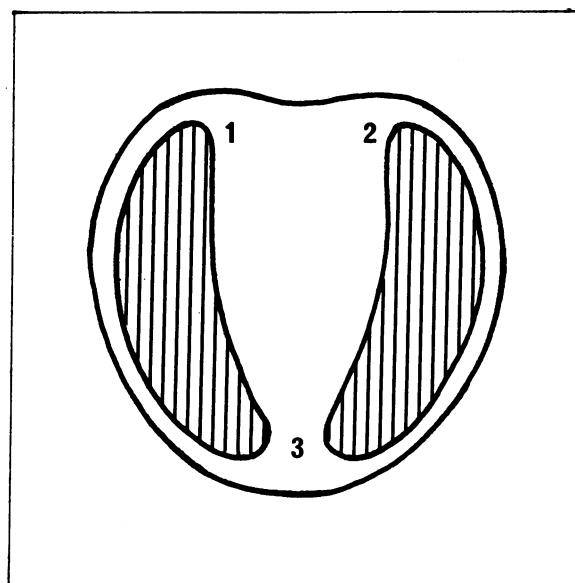


FIG. 4. Idealized view along axis of side-by-side (SBS) structure (shaded regions indicate the phosphate strands in projection).

which has the layer line axis (meridional axis) for its axis of symmetry (this gives the famous "cross pattern" of the B-fiber form of DNA).

The simplest useful representations of the Watson-Crick and SBS models are pairs of identical filamentary strands of electron density. In the Watson-Crick model, the strands are helices lying on the surface of a circular cylinder of radius roughly 9 Å, as in Fig. 1a. In the SBS model, the strands are sinusoidal curves lying on the surface of a similar cylinder, as shown in Fig. 1b. We find that the sinusoids comprising the elementary SBS model can be adjusted to produce a diffraction pattern which is in as close agreement with observation as is the corresponding pattern from the elementary Watson-Crick model. There is only one parameter (the relative phase of the two helices, i.e., their relative displacement parallel to the fiber axis) which is available for gross adjustment in the Watson-Crick model. In the SBS model, there are three adjustable parameters (the relative phase and angular displacement of the sinusoids, and the amplitude of the sinusoids). However, these three parameters are not completely independent because of stereochemical constraints.

It is worth remarking here that our first representation of the SBS model was a double helix of one hand, spanning half a repeat unit, then a similar double helix of opposite hand, also spanning half a repeat unit. This gives rise to the characteristic cross-pattern, but it is virtually impossible to adjust the details of the representation (to improve agreement with observation) without a contamination of the double wedge region with significant diffracted intensity.

We later realized that the pair of sinusoidal curves depicted in Fig. 1b is a superior representation of the essential features of the SBS model. The characteristic cross-pattern is obtained for a wide range of adjustments and deformations of this representation.

Optical Rotation of a Synthetic Polynucleotide. Evidence for the possible existence of an SBS structure appears to come from an observation of the optical isomerization of the double-stranded synthetic polynucleotide [poly(dG-dC)] (8-10). In 0.2 M NaCl, this polynucleotide has a circular dichroism spectrum characteristic of a right-handed helical structure but at 3.9 M NaCl the spectrum inverts to give features which could

Table 1. Numbering scheme and sugar/phosphate conformations for nucleotide residues of 10 base-pair repeat unit*

A strand		B strand	
3'		5'	
gg C3 endo	A1	B1	C3 endo ^{gg}
■ gg C3 endo	A2	B2	C3 exo ^{gg●}
■ gt C3 endo	A3 †	B3	C3 endo ^{tg●}
gt C3 endo	A4	B4	C3 endo ^{gt●}
gt C3 endo	A5	B5	C3 endo ^{gt}
gt C3 endo	A6	B6	C3 endo ^{gt}
● gt C3 endo	A7	B7	C3 endo ^{gt}
● tg C3 endo	A8 †	B8	C3 endo ^{gt■}
● gg C3 exo	A9	B9	C3 endo ^{gg■}
gg C3 endo	A10	B10	C3 endo ^{gg}

■, p bend region. ●, q bend region. ◊ = 2-fold axis.

* The asymmetric unit is boxed.

† Base pairs at which change in helical sense occurs. The region between these positions is left-handed. (The diagram represents the view from the narrow groove side.)

be interpreted in terms of a left-handed form (11). This process is reversible, and kinetic and equilibrium measurements indicate an intramolecular process of zero enthalpy change. Such a facile interconversion could arise for an SBS structure where relatively small structural changes could alter the net rotation from one hand to the other. Alternative explanations have been presented for this phenomenon in terms of double helix structures (11, 12).

Other Physical Properties of Duplexes. The SBS arrangement appears to allow double-stranded structures to bend more readily than a double helix. In addition, molecules having the SBS structure can themselves intermesh to a considerable extent. These two properties could allow regions of double-stranded DNA to pack in a reasonably tight manner.

The SBS structure could have a number of other implications for interpreting the function of properties of duplexes. To take one example, among topological properties of duplexes the sideways association of the strands must be one of its most important features.

Energy considerations

The SBS structure would appear to be significantly less stable than a right-handed double helix because of the presence of left-handed sections and bending of the phosphate backbone. However, association of protein molecules with DNA could at least for limited sections stabilize the SBS arrangement. Another

Table 2. Atomic coordinates for nucleotide residues of asymmetric unit

A6			B6			
N	-4.55,	0.00,	0.00	4.55,	0.00,	0.00
O5	-6.05,	-1.25,	-1.15	6.15,	-1.20,	1.35
C1	-5.45,	-1.20,	0.10	5.50,	-1.05,	0.00
C2	-6.35,	-0.90,	1.15	6.60,	-0.80,	-0.80
C3	-7.35,	-0.10,	0.45	7.50,	0.10,	-0.15
C4	-7.50,	-0.75,	-0.80	7.55,	-0.75,	1.10
C5	-7.90,	0.00,	-2.00	7.90,	0.00,	2.00
O4	-7.85,	-0.90,	-3.15	7.85,	-0.90,	3.15
P	-8.50,	-2.35,	-2.90	8.50,	-2.35,	2.90
O1	-7.25,	-3.40,	-2.50	7.25,	-3.40,	2.50
A7			B7			
N	-3.55,	-2.25,	-3.50	4.55,	1.85,	-3.55
O5	-4.45,	-3.95,	-4.60	6.50,	1.65,	-2.35
C1	-3.85,	-3.65,	-3.35	5.90,	1.20,	-3.55
C2	-4.85,	-3.80,	-2.35	6.70,	1.85,	-4.65
C3	-6.00,	-3.35,	-3.15	6.95,	3.05,	-3.80
C4	-5.80,	-4.20,	-4.25	7.45,	2.60,	-2.55
C5	-6.50,	-3.70,	-5.60	7.75,	3.60,	-1.40
O4	-6.15,	-4.80,	-6.60	8.05,	2.80,	-0.20
P	-6.15,	-6.20,	-6.00	9.25,	1.80,	-0.20
O1	-4.50,	-6.60,	-5.90	8.65,	0.40,	-0.80
A8			B8			
N	-2.15,	-3.50,	-6.80	3.60,	3.70,	-7.10
O5	-2.05,	-5.25,	-8.15	5.70,	4.05,	-6.10
C1	-1.80,	-4.80,	-6.75	4.95,	3.35,	-7.15
C2	-2.65,	-5.40,	-5.75	5.60,	3.80,	-8.35
C3	-3.70,	-5.75,	-6.70	6.25,	5.00,	-8.00
C4	-3.00,	-6.15,	-7.90	6.55,	4.90,	-6.65
C5	-3.95,	-6.00,	-9.20	6.65,	6.30,	-5.95
O4	-5.25,	-6.55,	-9.15	6.95,	6.00,	-4.55
P	-6.45,	-5.90,	-10.05	8.25,	5.25,	-4.20
O1	-6.90,	-4.80,	-9.05	7.95,	3.75,	-4.45
A9			B9			
N	-4.00,	-1.60,	-10.30	4.55,	1.40,	-10.50
O5	-5.55,	-2.95,	-11.40	6.65,	1.10,	-9.70
C1	-4.50,	-2.95,	-10.40	5.55,	0.50,	-10.35
C2	-5.35,	-3.00,	-9.15	6.05,	0.35,	-11.65
C3	-6.75,	-3.35,	-9.45	7.45,	1.00,	-11.85
C4	-6.80,	-3.00,	-10.90	7.75,	1.25,	-10.50
C5	-7.75,	-1.95,	-11.20	8.15,	2.80,	-10.40
O4	-7.50,	-0.80,	-10.30	6.95,	3.55,	-10.90
P	-8.15,	0.65,	-10.55	7.00,	5.05,	-10.45
O1	-8.70,	0.60,	-12.15	7.30,	5.15,	-8.80
A10			B10			
N	-4.80,	1.10,	-13.80	4.00,	-1.45,	-13.85
O5	-6.65,	1.05,	-15.00	5.90,	-2.90,	-13.15
C1	-5.90,	0.20,	-14.10	4.70,	-2.85,	-13.90
C2	-6.60,	-0.20,	-12.95	4.80,	-3.25,	-15.30
C3	-7.60,	0.85,	-12.95	6.15,	-2.90,	-15.40
C4	-7.80,	1.25,	-14.35	6.85,	-3.20,	-14.10
C5	-8.00,	2.65,	-14.35	7.75,	-2.10,	-13.90
O4	-7.35,	3.50,	-13.40	7.40,	-0.85,	-14.45
P	-6.85,	4.90,	-14.05	8.40,	0.35,	-14.15
O1	-7.00,	4.90,	-15.70	8.30,	0.15,	-12.50

Distances (x,y,z) in Å from origin (0,0,0), located midway between the nitrogen atoms of the A6 and B6, for right-handed coordinate system.

factor which may be important for an SBS region is the stabilizing effect of base-pair stacking. Unlike a double helix which has base pairs displaced from each other by about 36°, SBS base pairs oscillate from one extreme to another within a narrower

range and without giving full 360° rotation. This could have a stabilizing effect because it allows base pairs to locate more precisely one above the other.

Conclusion

We have constructed a model which demonstrates the possibility of side-by-side association of two intermeshing, anti-parallel polynucleotide strands with the Watson-Crick mode of base pairing. This model has a number of attractive features. We present it for consideration as a polynucleotide conformation which may exist at least under some conditions.

Appendix

Approximate Coordinates for a Representative SBS Model.

The following coordinates were derived from a 1 Å = 2 cm wire model of a SBS structure with the assistance of R. H. Nokes and B. H. Thompson. This particular model was built with base-pair planes perpendicular to the molecular axis and spaced at the 3.45 Å separation of B DNA. It has a five left-handed and five right-handed arrangement which gives a basic ten base-pair repeat unit, and a net rotation of about 35° per repeat unit. (SBS models with an even right-left arrangement give a net right-handed twist because of the greater magnitude of rotation of right-handed sections.) The coordinates are presented for the phosphate-sugar backbone atoms (except for the terminal phosphate oxygen atoms, O2 and O3). A constant distance of 9.2 Å was maintained between corresponding nitrogen atoms of the two strands. The centers of lines joining adjacent pairs of nitrogen atoms were constrained to lie above each other as closely as possible. Coordinates for only half the repeat unit are presented because the remainder can be generated by 2-fold rotation about a dyad axis which lies along the line joining the points (0, 0, 1.70) and (-1.00, -4.20, 1.70) of the cartesian coordinate system. This system has its origin midway between the nitrogen atoms of the A6 and B6 residues; z is the vertical, molecular axis. The dyad axis is positioned as shown in Fig. 1b although it should be noted that Fig. 1b does not take account of the long range twist mentioned above. The sugar and sugar/phosphate conformations employed are given in Table 1.

Bond distances and bond angles, as calculated from the coordinates in Table 2, are within 0.15 Å and 15° of recognized standard values. The present coordinates reveal four nonbonded contacts less than 2.7 Å [A704-A801 (2.55), A704-A8C3 (2.65), A705-A8C2 (2.60), B1004-B10C3 (2.60)] and indicate contacts of the same order of magnitude for one of the terminal oxygen atoms linked to A8P and for certain (base) carbon atoms (for the zero twist positions of the base planes). While some individual atomic positions have been adjusted there has been no

refinement of group positions or torsional angles. Considerably more variable parameters are available for the refinement of a SBS model than have been used for helical structures. We therefore consider this illustrative model is sufficiently flexible to allow adjustments to be made to relieve any unacceptably short contact distances. Also, in attempting to match as closely as possible the regularity of double helix models, unnecessarily rigid constraints may have been imposed on this SBS structural example. For example there is no evidence to suggest that base planes may not be able to stack at slightly larger distances than 3.45 Å in bend regions.

This work arose from the concern expressed by Clive H. Rowe, of the University of Canterbury about the intertwined nature of the Watson-Crick model.

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