five *exo*. In Fig. 23.3.2.4 (top), pushing the C3′ atom of the C3′-*endo* conformation into the plane of the ring would tend to push C2′ below the ring, passing through a T state and creating a C2′-*exo* conformation. C2′ can, in turn, be returned to the ring plane if C1′ is pushed above the ring, forming C1′-*endo*, and so on, around the ring. In this way, a contiguous series of alternating *endo/exo* conformations is produced, as listed in Table 23.3.2.2.

This ten-conformation *endo/exo* cycle can be generalized to a continuous distribution of intermediate conformations, characterized by a pseudorotation angle, *P* (Altona *et al.*, 1968; Altona & Sundaralingam, 1972), with the ten *endo/exo* conformations spaced 36° apart (Table 23.3.2.2). Fig. 23.3.2.5 shows the calculated potential energy of conformations around the pseudorotation cycle (Levitt & Warshel, 1978). Note that C2′-*endo* and C3′-*endo* are most stable, that the pathway between them along the right half of the circle remains one of low energy, but that a large 6 kcal mol^{-1} potential energy barrier (1 kcal mol^{-1} = 4.184 kJ mol^{-1}) effectively forbids conformations around the left half of the circle.

As Fig. 23.3.2.4 indicates, the main-chain torsion angle, δ, is sensitive to ring conformation, because the C5′—C4′ and C3′—O3′ bonds that define the angle shift as ring puckering changes. The idealized relationship between torsion angle, δ, and pseudorotation angle, *P* (Saenger, 1984), is

$$\delta = 40^\circ \cos(P + 144^\circ) + 120^\circ.$$

Fig. 23.3.2.6 shows the observed torsion angles, δ, and pseudorotation angles, *P*, from X-ray crystal structure analyses of synthetic DNA oligonucleotides: 296 examples from A-DNA and 280 from B-DNA. The most striking aspect of this plot is the radically different behaviour of A- and B-DNA. The prototypical sugar conformation for A-DNA obtained from fibre diffraction modelling, C3′-*endo*, is, in fact, adhered to quite closely in A-DNA crystal structures.

However, B-DNA shows a quite different behaviour. Although earlier fibre diffraction led one to expect C2′-*endo* sugars, the actual experimental distribution is quite broad, extending up the right-hand side of the pseudorotation circle of Fig. 23.3.2.5, through C1′-*exo*, O1′-*endo* and C4′-*exo*, in some cases all the way to C3′-*endo* itself. Indeed, the mean value of δ observed in B-DNA oligomer crystal structures is 128° rather than 144° (Table 23.3.2.1), making C1′-*exo* a better description of sugar conformation in B-DNA than C2′-*endo*. Old habits die hard, however, and the B-DNA sugar conformation is still colloquially termed C2′-*endo*, a designation of historical significance but of little practical value. The apparent greater malleability of the B helix compared to A may indeed be one feature that makes B-DNA particularly suitable for expressing its base sequence to drugs and control proteins *via* local helix structure changes.

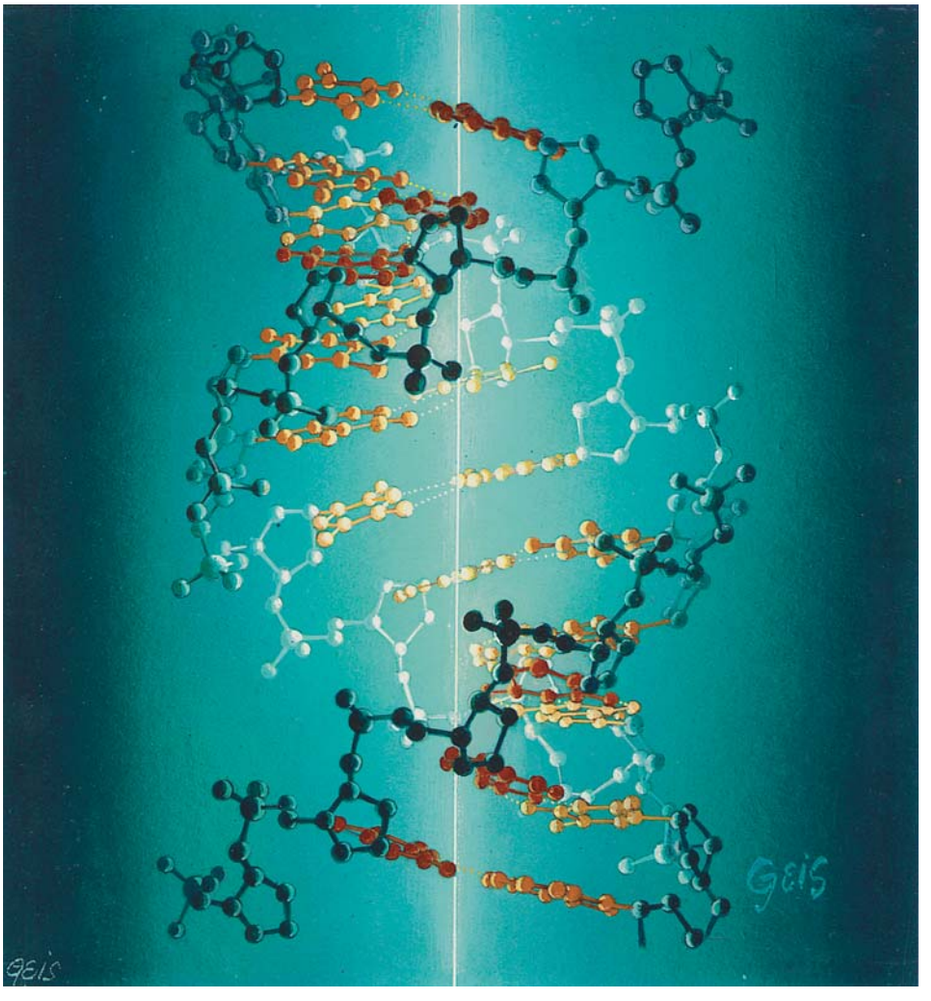

Fig. 23.3.1.1. 'Hot wire' painting of A-DNA by Irving Geis. Geis produced two dramatic paintings of horse-heart cytochrome *c*, in which the sole light source was the central iron atom within the haem, producing a glowing 'molecular lantern' effect. One painting showed this central luminous haem surrounded by hydrophobic side chains; the other featured the polar side chains extending out from the surface. These are to be seen today on the front and back covers of Voet & Voet's *Biochemistry* (Voet & Voet, 1990, 1995). In the present A-DNA painting, Geis chose the imaginary central axis of the helix as a monofilament light source, thereby reversing the conventional illumination: atoms lining the deep major groove glow brightly, whereas the outer surface of the helix is in dark silhouette. Geis struggled with the B helix as an artistic subject, but was never satisfied with the results. Hence, this glowing A-DNA helix represents his nucleic acid artistic legacy. Reprinted courtesy of the estate of Irving Geis. Rights owned by Howard Hughes Medical Institute.

23.3.2.3. *Base pairing*

The key to the biological role of DNA is that one of the two purines can pair with only one of the pyrimidines: A with T, and G with C. Hence, genetic information present in one strand is passed on to the complementary strand. The standard two-base pairs are shown in Fig. 23.3.2.7 along with the conventional numbering of the atoms. Backbone sugar and phosphate atoms are primed while base atoms are unprimed, as, for example, C1′ and N9 at opposite ends of a purine glycosydic bond. The G·C base pair is held together by three hydrogen bonds, whereas an A·T pair has only two. This means that A·T pairs show less resistance to propeller twisting (counter-rotation of the two bases about their common long axis), and this will have an effect on minor groove width, as seen later. The patterns of hydrogen-bond acceptors (*A*) and donors (*D*) on the major and minor groove edges of base pairs are important elements in recognition of base sequence by drugs and control proteins.

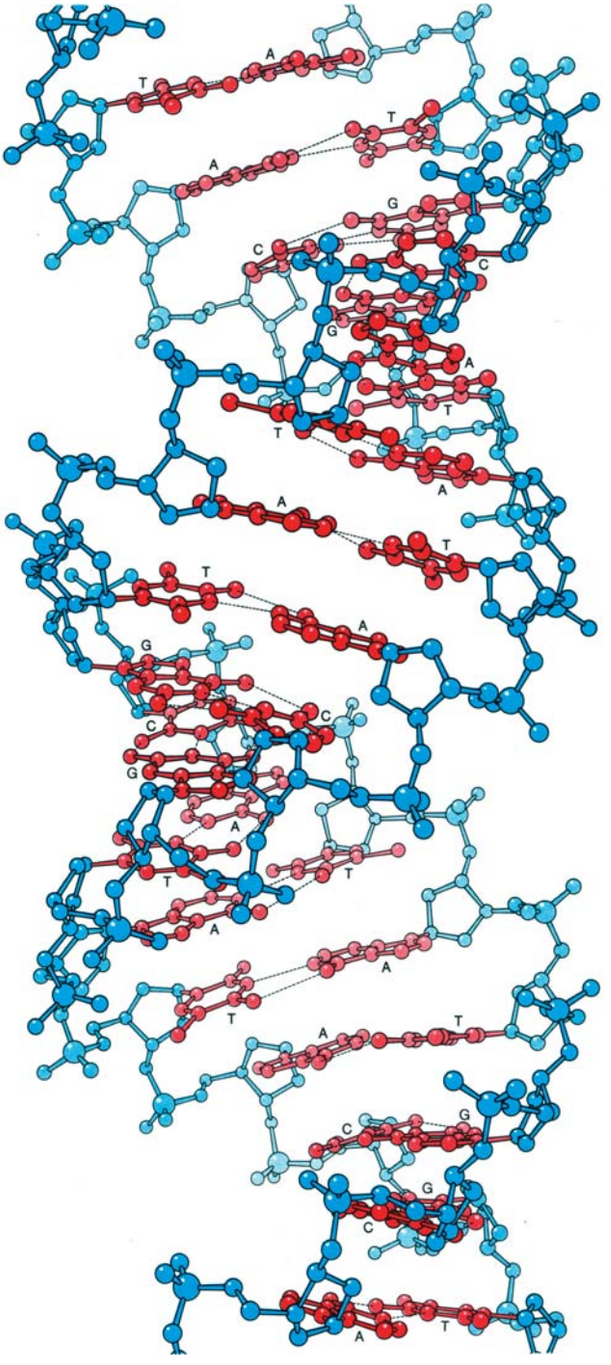

Fig. 23.3.1.2. Infinite A-DNA helix, generated from the X-ray crystal structure of the hexamer G-G-T-A-T-A-C-C (references A2 and A7 in Table A23.3.1.1) by deleting the outer base pair from each end and stacking images of the resulting truncated hexamer so their outer phosphate groups overlapped. This generates an endless helix that exhibits the local structural features of the X-ray crystal structure. Note the degree to which the A helix resembles an antiparallel double-stranded ribbon wound around an invisible helical core (the 'hot wire' axis of Fig. 23.3.1.1). (From Dickerson, 1983.) Reprinted courtesy of the estate of Irving Geis. Rights owned by Howard Hughes Medical Institute.

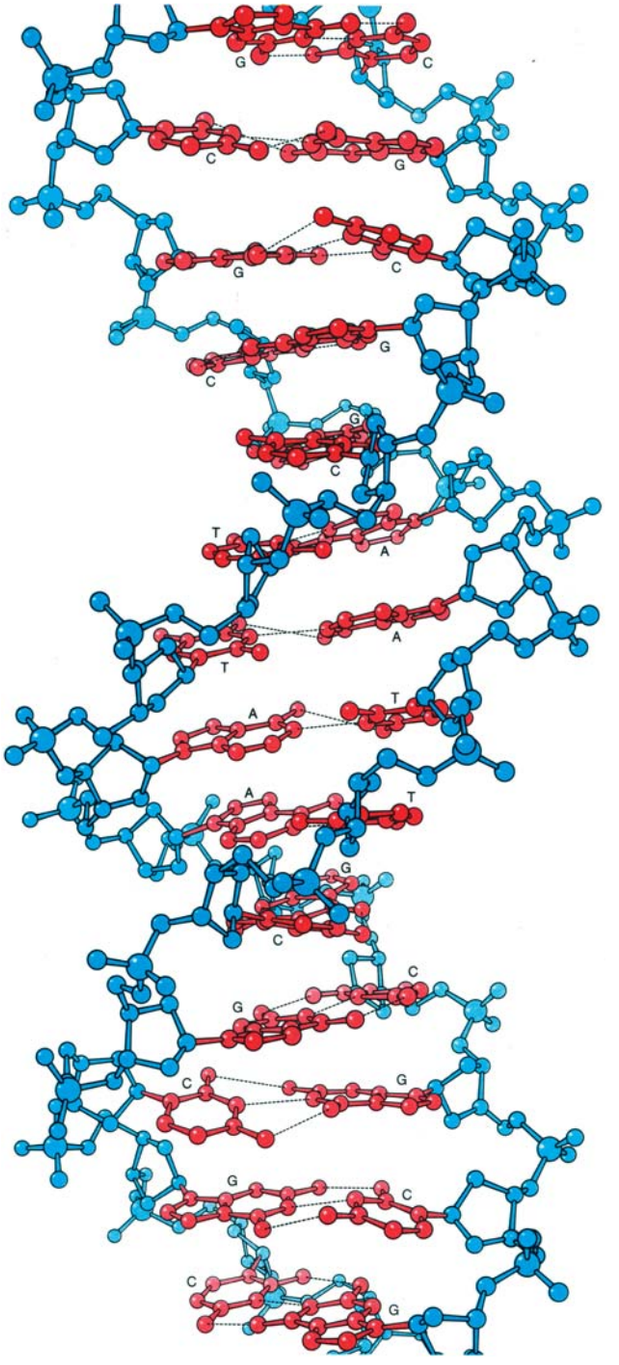

Fig. 23.3.1.3. Infinite B-DNA helix, generated in a similar manner to Fig. 23.3.1.2 from the central ten base pairs of the dodecamer C-G-C-G-A-A-T-T-C-G-C-G (B1–B5). Note that the minor groove is narrow in the AT region facing the viewer at the centre, but appreciably wider in the GC regions on the back side of the helix at top and bottom. Propeller twisting, or deviations of bases from coplanarity within one pair, is one sequence-dependent aspect of DNA that was not suspected from the averaged structures obtained from fibres. (From Dickerson, 1983.) Reprinted courtesy of the estate of Irving Geis. Rights owned by Howard Hughes Medical Institute.

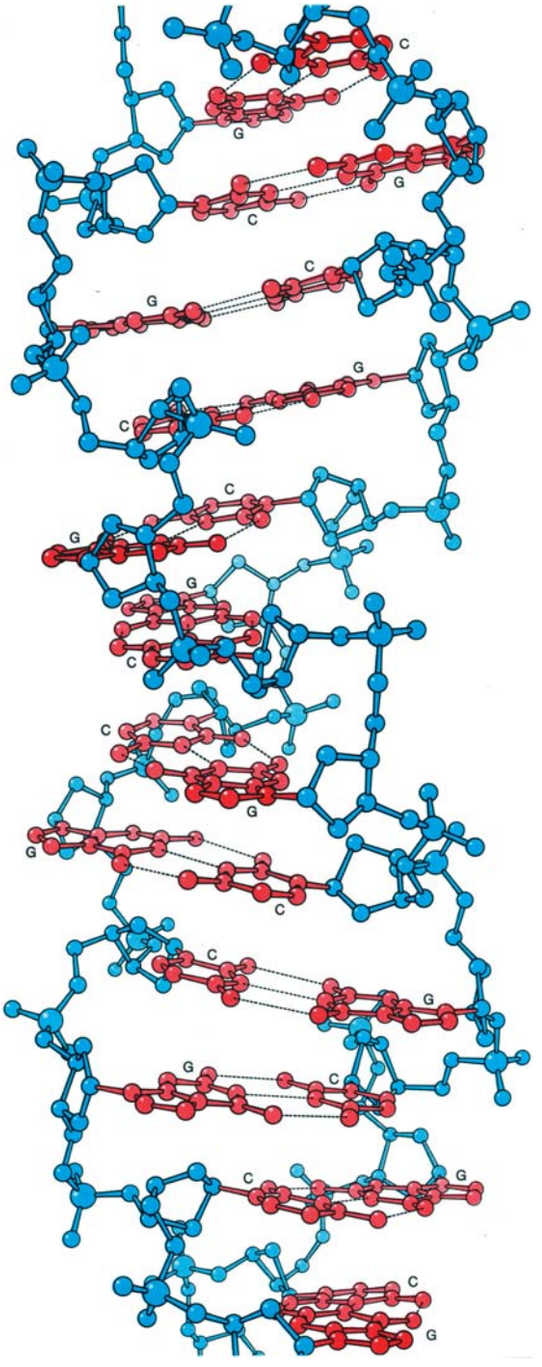

Fig. 23.3.1.4. Infinite Z-DNA helix, generated as before from the central four base pairs of the hexamer C-G-C-G-C-G (Z1). G and C bases alternate along each chain. The sugar–phosphate backbone adopts a pronounced zigzag pathway, rising vertically past each guanine, but travelling horizontally across the helix at cytosines. Hence, the formal helix repeat is two base pairs, G followed by C, rather than a single base pair, as in the A and B helices. Note that the structures of Z-DNA and A-DNA are in many ways the inverse of one another. The Z helix is left-handed, tall and slim, with a deep minor groove, a flattened major groove and small propeller twist. The A helix is right handed, short and broad, with a deep major groove, a shallow minor groove and large propeller twist. (From Dickerson, 1983.) Reprinted courtesy of the estate of Irving Geis. Rights owned by Howard Hughes Medical Institute.

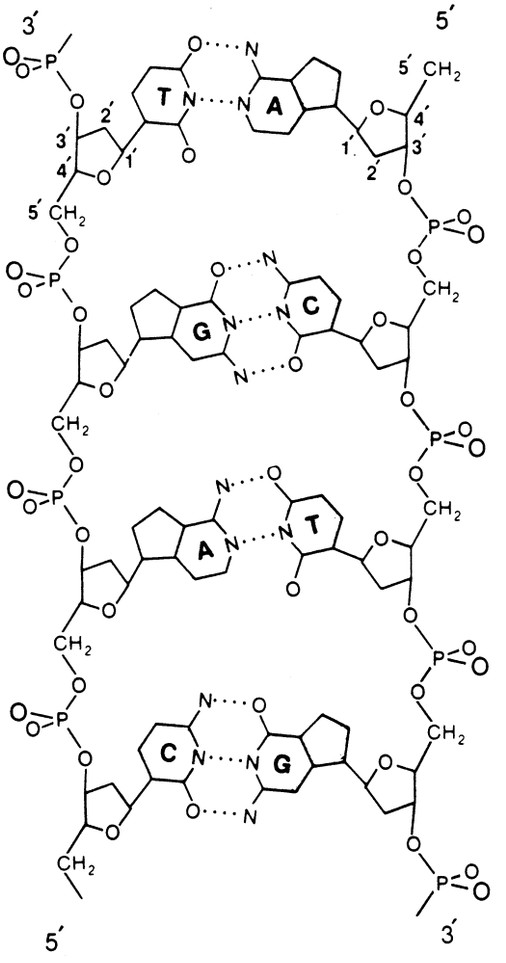

Fig. 23.3.2.1. Unrolled schematic of A- or B-DNA, viewed into the minor groove. Paired bases are attached to backbone chains that run in opposite directions: downward on the right and upward on the left. Z-DNA differs from A- and B-DNA in that the two backbone chains run in opposite directions from those shown here. Hence, Z-DNA cannot be obtained from A- or B-DNA by simple twisting around the helix axis.

Other related but nonstandard base pairs are compared in Fig. 23.3.2.8. Inosine (I) is useful in studying properties of DNA in that, when paired with cytosine (C), it creates a G·C-family base pair having overall similarity to A·T. Similarly, diaminopurine (DAP) [also known as 2-aminoadenine (2aA)], when paired with thymine (T), creates a G·C-like pair from A·T-family bases. Hence, in a given experimental situation, one can unscramble the relative significance of number of hydrogen bonds *versus* identity and location of exocyclic groups.

The conventional Watson–Crick base pairing of Fig. 23.3.2.7 uses the hexamer 'end' of the purine base. A different type of base pairing was proposed many years ago by Hoogsteen (1963), in which the upper edge of the purine was used: N7 and N6/O6. Hoogsteen base pairing is shown between the left-hand two bases in each part of Fig. 23.3.2.9. Note that in Hoogsteen base pairing of A and T, each ring provides both a hydrogen-bond donor and an acceptor. Guanine cannot do this, since both its N7 and O6 positions are acceptors. As a consequence, in a G·C pair, C must supply both of the hydrogen-bond donors. It can only form a Hoogsteen base pair with G when the cytosine ring is protonated. This would lead one to expect triplex formation only at low pH. However, the stability of a triplex can, to a certain extent, alter the pK_a of the N—H proton itself. (Recall the shift in pK_a of buried Asp and His

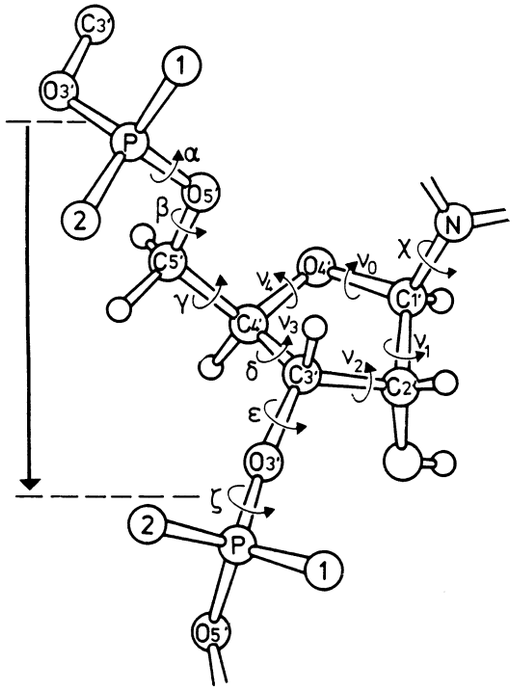

Fig. 23.3.2.2. Sugar–phosphate backbone of RNA and DNA polynucleotides. One nucleotide begins at a phosphorus atom and extends just short of the phosphorus atom of the following nucleotide, with the conventional positive direction being P→O5′—C5′—C4′—C3′—O3′→P, as indicated by the arrows. Main-chain torsion angles are designated α through ζ, and torsion angles about the five bonds of the ribose or deoxyribose ring are ν_0 through ν_4, as shown. If one imagines atoms O3′—P—O5′ as a hump-backed bridge, as one crosses the bridge in a positive chain direction, oxygen atom O1 is to the left and O2 is to the right. These oxygens, accordingly, are sometimes designated O_L and O_R. The —OH group attached to the C2′ atom of the ribose ring in RNA shown here is replaced by —H in the deoxyribose ring of DNA. Atom N to the right is part of the base attached to the sugar ring: N1 in pyrimidines and N9 in purines. Torsion angle χ is defined by O4′—C1′—N1—C2 in pyrimidines and O4′—C1′—N9—C4 in purines.

groups in the active sites of enzymes.) Hence, with a single-chain DNA, G-A-G-A-G-A-A-C-C-C-C-T-T-C-T-C-T-T-C-T-T-T-C-T-C-T-C-T-T, that folds back upon itself twice to build a triplex, NMR experiments indicate a significant amount of triplex remaining even at pH 8.0 (Sklenár & Feigon, 1990; Feigon, 1996).

23.3.2.4. *Helix parameters*

An important advantage of single-crystal oligonucleotide structures over fibre-based models is that one can actually observe local sequence-based departures from ideal helix geometry. B-DNA fibre models indicated a mean twist of *ca* 36° per step, or ten base pairs per turn, whereas A-DNA fibre patterns indicated less winding: *ca* 33° per step or 11 base pairs per turn. Twist, rise per base pair along the helix axis, horizontal displacement of base pairs off that axis, and inclination of base pairs away from perpendicularity to the axis are all intuitively obvious parameters. But when single-crystal structures began appearing in great numbers in the mid-1980s, it became imperative that uniform names and definitions be used for these and for less obvious, but increasingly significant, local helix parameters.

An EMBO workshop on DNA curvature and bending, held at Churchill College, Cambridge, in September 1988, led to an agreement on definitions and conventions that was published

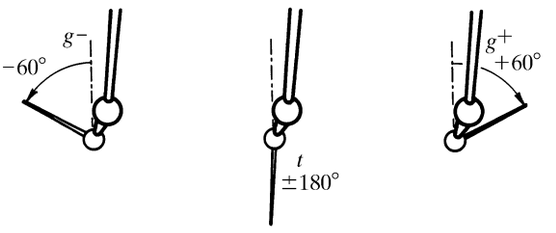

Fig. 23.3.2.3. Definition of torsion angles. A positive angle results from clockwise rotation of the farther bond, holding the nearer bond fixed. Torsion angle +60° is designated as *gauche*$^+$ or g^+, angle 180° is *trans* or *t* and angle −60° is *gauche*$^-$ or g^-.

simultaneously in four journals (Dickerson *et al.*, 1989). Fig. 23.3.2.10 shows the reference frames for two successive base pairs, and Figs. 23.3.2.11 and 23.3.2.12 illustrate local helix parameters involving rotation and translation, respectively. Subsequent experience has shown the most useful parameters to be inclination, propeller, twist and roll among the rotations, and *x* displacement, rise and slide among the translations. As mentioned at the beginning of this chapter, inclination and *x* displacement are the two properties that best differentiate A- from B-DNA. The four most widely used computer programs for calculation of local helix parameters are

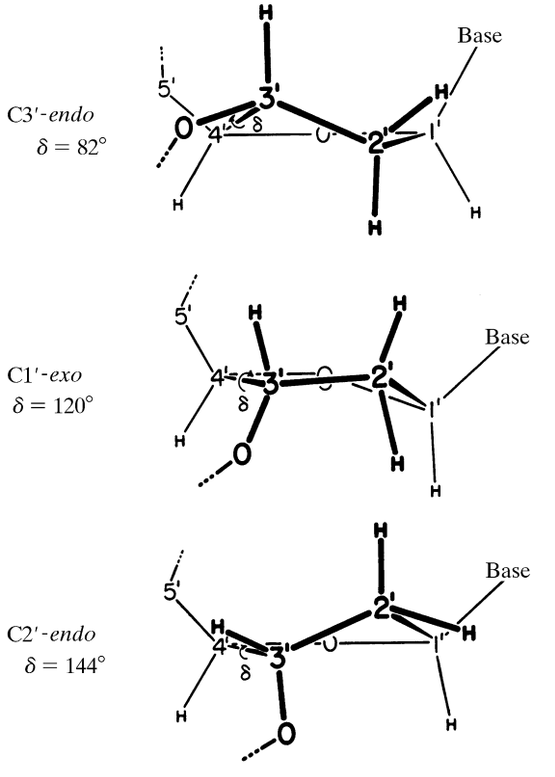

Fig. 23.3.2.4. The three most common furanose ring geometries. The planar form of the five-membered ribose or deoxyribose ring is unstable because of steric hindrance from side groups; one of the five atoms prefers to pucker out-of-plane on one side of the ring or the other. Puckering toward the same side of the ring as the C5′ atom is termed *endo*, and puckering toward the opposite 'outside' surface is termed *exo*. The main-chain torsion angle δ is related to sugar ring conformation because of the motion undergone by the C3′—O3′ bond during changes in puckering.

references