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Influence of the glycosidic torsion angle on ^{13}C and ^{15}N shifts in guanosine nucleotides: Investigations of G-tetrad models with alternating *syn* and *anti* bases

Karen L. Greene[c,*], Yong Wang[b] and David Live[a,**]

[a]*Cellular Biochemistry and Biophysics Program, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, U.S.A.*
[b]*Department of Biochemistry and Molecular Biophysics, College of Physicians and Surgeons, Columbia University, New York, NY 10032, U.S.A.*
[c]*Analytical Services, 110 Technology Parkway, Norcross, GA 30092, U.S.A.*

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Summary

The effect of the glycosidic torsion angle on ^{13}C and ^{15}N shifts of the sugar and base moieties of guanosine nucleotides was investigated by comparing the sites in two model G-tetrad oligodeoxynucleotides that contain guanosine residues alternately with *syn* and *anti* bases. The sugar puckering has been shown to be C2'-*endo* for both cases. It was observed that, for the instances with *syn* bases, the C1' through C4' carbons showed shifts that may be distinguished from those normally found in B-DNA-like structures. C1', C3' and C4' moved to lower field, while C2' moved to higher field. Effects of the change in glycosidic torsion angle were also seen in the shifts of base carbons and nitrogens in the five-membered ring portion of the base. Characterization of the shift variation associated with this conformational change may be useful in developing the use of ^{13}C shifts as a tool in conformational analysis of oligonucleotides.

Introduction

The advent of indirect-detection proton experiments has made observation of ^{13}C signals in oligonucleotides and their assignments readily accessible, even at natural abundance (Leupin et al., 1987; Sklenář et al., 1987; LaPlante et al., 1988a; Ashcroft et al., 1989,1991; Varani and Tinoco, 1991). The enhanced shift dispersion of the carbons and the discrete regions in which specific kinds of carbons resonate can, in concert with heteronuclear correlation experiments, be a significant aid to proton assignments as well. For RNA, these methods in conjunction with labeling strategies have proven almost indispensable for NMR analysis (Varani and Tinoco, 1991; Michnicka et al., 1993; Nikonowicz and Pardi, 1993). Additional information may be extracted from the ^{13}C chemical shifts if conformational contributions to these shifts are understood. The dependance of ^{13}C shifts on sugar conformation has been recognized for some time and has been demonstrated by shifts that are associated with melting of duplex structures (Lankhorst et al., 1983; LaPlante et al., 1994). These shifts have been related to the fractional populations of N- and S-type sugar conformation. Similar trends have been observed in the isotropic ^{13}C shifts of solid crystalline nucleotides of known crystal structures as well (Santos et al., 1989). The impact of base orientation on the shifts of the sugar carbons is less well established than the effects of sugar pucker, perhaps due to the absence of suitable conformationally defined examples. Regarding the base component, limited data are available for shifts of base carbons in DNA (LaPlante et al., 1988b) for cases with *anti* bases, and some data for protonated base carbons in RNA are available from a small hairpin (Varani and Tinoco, 1991). Calculations on a model system provide insight into the variation of the C8 carbon shift anticipated for a change of the glycosidic

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**To whom correspondence should be addressed.

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torsion angle to a *syn* orientation (Ghose et al., 1994), with supporting evidence for the predicted trend provided from the case of a *syn* guanosine residue in an RNA compound (Varani and Tinoco, 1991). Little is known about the conformational dependence of shifts for the nonprotonated ^{15}N sites in the bases. As interest increases in examining DNA and RNA molecules with unusual structures, where the glycosidic bond angle is less predictable, considerations of the impact of this angle on chemical shift become more relevant for providing supporting evidence for structural conclusions. For NMR solution studies of structures that deviate from the canonical duplex organization, ^{13}C chemical shift information may make up for the absence of or ambiguities in NOE information. Furthermore, for adducts to the C8 position of purines, shift information may compensate for the information otherwise available from NOE interactions to the H8 proton.

The molecules investigated here, based on the G-rich regions of DNA found in telomeres at the end of chromosomes (Blackburn, 1991), are an example of the expanding repertoire of structural motifs that have been shown to display particularly stable structures (e.g., Kang et al., 1992; Smith and Feigon, 1992; Wang and Patel, 1993; Schultze et al., 1994). They provide some of the first examples of categorizing the effects of *syn* base orientation on chemical shifts in oligonucleotides. The particular sequences used were d(GGTTTTCGG) (**I**) and d(GGTCGG) (**II**), with sodium counterions. Both sequences adopt G-tetrad structures (Wang et al., 1991) that display guanosine bases alternately with *syn* or *anti* orientation (Fig. 1), while the sugars in both cases are found to be in a C2'-*endo* configuration.

Methods

Preparation of the samples and NMR spectroscopy were carried out as described by Wang et al. (1991). ^{1}H-^{13}C HMQC spectra of **I** were recorded on a GN-500 spectrometer with the dephasing delay set for a $^{1}J_{HC}$ of 150 Hz. For each of the 128 t_1 values, 800 scans were collected. The final spectra were obtained by Fourier transforming the data, 1024 points in the ^{1}H dimension, and zero-filling to 512 points in the ^{13}C dimension. The ^{1}H carrier was positioned at 4.00 ppm, with a sweep width of 8.26 ppm, and the ^{13}C carrier was placed at 80.0 ppm, with a sweep width of 65.6 ppm. Spectra of **II** were collected as for **I**, except that 220 scans were used for each increment. Additional ^{1}H-^{13}C HMQC spectra were recorded for **II** on a Varian Unity plus 500 MHz spectrometer, where improvements in sensitivity of the instrument allowed us to limit the number of transients to 64 for each increment. ^{1}H-^{13}C and ^{1}H-^{15}N HMBC experiments (Bax and Summers, 1986) were carried out on **II** using a Varian Unity plus 500 MHz spectrometer. For long-range carbon detection, 48 increments of 512 transients each for a ^{13}C sweep width of 64.0 ppm were collected. The ^{1}H transmitter was positioned at 4.95 ppm, with a sweep width of 8.5 ppm. The ^{13}C transmitter was centered at 132.5 ppm. In order to minimize relaxation losses, the delay for the long-range coupling was set to 40 ms, even though the optimum value for this coupling is expected to be somewhat longer (Ashcroft et al., 1989). For long-range detection of ^{15}N, 64 increments of 800 transients each, for a sweep width of 120.0 ppm, were collected with the same ^{1}H conditions and dephasing delay as for ^{13}C. The ^{15}N transmitter was set at 190.5 ppm. ^{13}C chemical shifts are referenced to TSP (Ashcroft et al., 1991) and ^{15}N shifts to NH_3 (Live et al., 1984). Sample conditions were as follows. The concentration of **I** was 11.6 mM of monomer units in a 0.5 ml D_2O solution (pH 6.8) with 100 mM NaCl and 10 mM phosphate. The data were acquired at 2 °C. The concentration of **II** was 15 mM or 19 mM of monomer units in a 0.5 ml D_2O solution (pH 6.8) with 100 mM NaCl and 10 mM phosphate. Spectra were run at 3 °C.

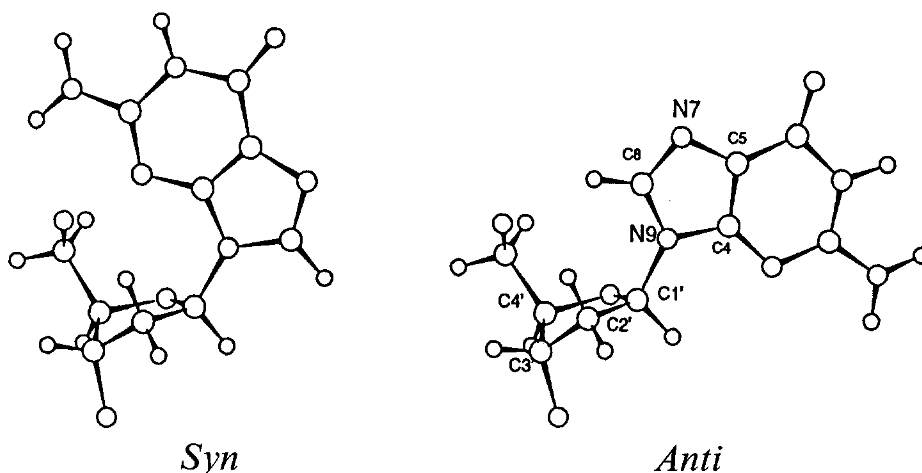

Fig. 1. Illustration of the *anti* and *syn* conformation of a deoxyguanosine residue.

TABLE 1
^{13}C CHEMICAL SHIFTS (ppm) OF PROTONATED CARBONS OF d(GGTTTTCGG)[a]

Residue	C1'	C3'	C4'	C6/C8	CH_3/C5
G1	89.70	81.83	89.54	140.55	
G2	83.65	78.11	87.13	138.45	
T3	86.10	78.33	86.55	138.49	14.54
T4	85.12	79.75	85.89	139.11	14.47
T5	88.39	79.17	86.56	139.73	14.73
T6	88.39	79.21	88.69	139.47	15.73
C7	89.70	80.57	86.87	[b]	98.24
G8	89.31	80.52	89.04	141.44	
G9	84.11	72.70	87.49	138.46	

[a] From Wang et al. (1991). Shifts are relative to TSP. C5' carbons were not assigned due to absence of H5' and H5'' assignments, and C2' carbons were not assigned due to complications arising from overlap in the cross peaks between ^{13}C and H2'/H2'', as well as the inability to observe some of them.
[b] Resonance not observed.

Results and Discussion

The proton and protonated carbon assignments of **I** have been reported previously (Wang et al., 1991). The ^{13}C shifts of **I** are presented in Table 1. NOE experiments (Wang et al., 1991) show that as far as the guanosine bases are concerned, both sequences adopt similar arrangements, having two G-tetrads each. The first and penultimate guanosine residues display a *syn* orientation, while the second and terminal guanosines have *anti* arrangements. The ^{13}C shifts for **II** are given in Table 2. (^1H assignments are available from the authors as Supplementary Material.) The base orientation of **II** has been further confirmed by heteronuclear couplings between H1' and base C8 and C4 atoms (Zhu et al., 1994). Examination of homonuclear and heteronuclear coupling constants shows that the sugars are in C2'-*endo* conformation for **II** (Zhu et al., 1994), and similar conclusions can be drawn for the sugar residues in **I** based on proton experiments.

A section of the contour plot for the region of the C1'-H1' correlations of sequence **II** is shown in Fig. 2. A striking feature of the data in Tables 1 and 2 is the clear discrimination between the ^{13}C shifts of C1', C2', C3' and C4' in the sugars associated with *syn* guanosines (G1 and G5 or G8), and those for *anti* guanosine residues (G2 and G6 or G9). Carbon shifts for C1', C3' and C4' of residues in the *syn* configuration are downfield with respect to the cases where bases are *anti*, while the C2' for *syn* orientations is found distinctly upfield for **II**, which was the only molecule for which C2' shifts could be fully analyzed. Relatively little effect is seen for the C5' shifts. As indicated above, in all cases the sugar conformation is of the C2'-*endo* type, eliminating variation in sugar pucker as a factor. The average effect on carbon shifts in going to *syn* is +5.02 ppm for C1', −7.00 ppm for C2', +4.06 ppm for C3' (shift values for the 3'-terminal C3' carbons were not included in this average) and +1.46 ppm for C4'. Comparison of the shifts of C1' and C2' of the *syn* bases with those reported for a number of DNAs assumed to be of B-type (i.e., with C2'-*endo* sugar pucker and *anti* base orientation) (Borer et al., 1984; Leupin et al., 1987; LaPlante et al., 1988b,1994; Ashcroft et al., 1991; Wang et al., 1992) shows that these shifts fall outside the range of analogous sites of B-type internal residues. Shifts of the C3' and C4' are to lower field or are very near the low-field limit of those found for the B-DNA cases. The guanosines with *anti* orientation fall in the normal range for B-DNA. In sequence **II**, with only a dinucleotide connection between pairs of guanosines, the C1' resonances of the thymidine and cytidine residues are at rather low field, presumably due to effects of the unusual conformation of this tight loop, that has not been as well defined. Even in regular DNA structures, the C1' of pyrimidine nucleotides are found on average to lower field than those of purines (LaPlante et al., 1994), thus the additional displacement from the conformational effect needed to

TABLE 2
^{13}C CHEMICAL SHIFTS (ppm) OF PROTONATED CARBONS FOR d(GGTCGG)[a]

Residue	C1'	C2'	C3'	C4'	C5'	C6/C8	CH_3/C5
G1	90.11	37.94	81.62	89.51	65.33	140.31	
G2	85.45	43.89	77.80	88.75	69.99	140.18	
T3	89.10	43.46	78.82	89.51	69.27	138.01	15.17
C4	90.02	44.04	80.73	87.22	68.38	142.22	98.17
G5	89.35	35.84	80.09	89.00	69.78	142.98	
G6	85.20	43.89	72.83	87.86	67.62	138.65	

[a] Shifts relative to TSP.

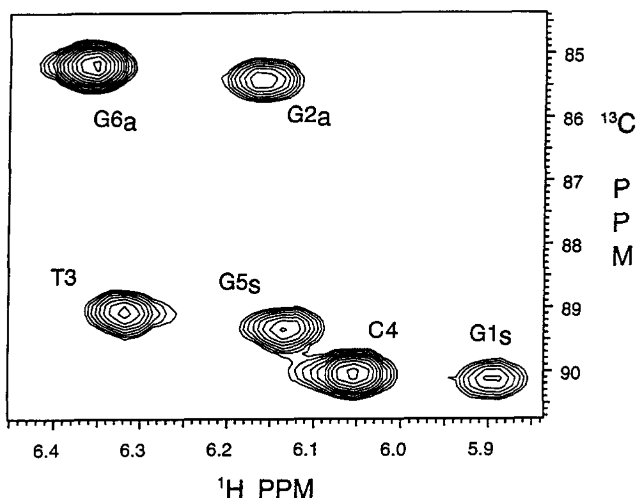

Fig. 2. Expanded region of an HMQC spectrum of d(GGTCGG), showing the H1'-C1' correlations. Cross peaks are marked by residue assignment and subscripts a and s refer to *anti* and *syn* base orientations, respectively.

shift the resonances of loop pyrimidine residues to the position of the *syn* guanosine bases is smaller than that caused by the glycosidic bond effect on the guanosine residues. In the base portion, a trend of average value +2.38 ppm is observed for the shifts of the C8 in guanosine bases with *syn* orientation compared to those in *anti* configuration.

While it is more difficult to observe unprotonated nuclei in the bases through multiple-bond HMBC experiments, this is generally feasible in labeled samples and, as has been previously shown, may also be accessible at natural abundance (Ashcroft et al., 1989). We have used the HMBC experiment (Bax and Summers, 1986) to observe the carbon and nitrogen nuclei of **II** in the vicinity of the glycosidic bond (Table 3), and have analyzed effects of conformational change on their shifts. The C4 and C5 carbons are shifted to slightly lower field for the *syn* bases relative to the *anti* bases, with the C5, more remote from the glycosidic bond, showing the larger effect. The average effects are 0.65 ppm for C4 and 1.15 ppm for C5. While the shifts of the C5 for guanosines in the *anti* arrangement are consistent with shifts previously found for this position in B-DNA-type systems (LaPlante et al., 1988b), the shifts of the *syn* bases are to lower field compared to more typical duplex samples. In the case of the N7 nitrogen, participating in the glycosidic bond, and the more remote N9, the trend is for those in *syn* bases to be shifted upfield relative to the *anti* bases. Average differences are 5.92 ppm for N7 and about 3.95 ppm for N9. Thus, the nonprotonated sites may also be useful as markers of base orientation.

For sugar carbons, possible explanations for the shifts could derive from variation in the ring current effect caused by the change in base orientation, or some conformational changes related to the changes in glycosidic torsion angle. Modulation in the ring current effect does not provide an adequate rationalization of the shift trends noted here, since an expected quantitative correlation with the chemical shifts of the protons attached to the respective carbon nuclei is not present. Thus, the bond structure and/or angle changes are the likely cause of this phenomenon. Support for the latter explanation comes from crystal structures available for guanosine (Thewalt et al., 1970) in the *anti* conformation and *N,N*-dimethylguanosine (Brennan et al., 1972) in the *syn* conformation. Bond angle and length variations can be observed between the structures. A notable example is the C1'-H1' bond length of 0.93 Å for the *syn* case and 0.99 Å for the *anti* case. The C1'-N distance is about 0.01 Å longer for *syn* compared to *anti*. Based on calculations that have been done for peptide residues, a lengthening of the C^{α}-N bond would result in a downfield shift of the carbon, although of smaller magnitude than observed here (De Dios et al., 1993).

Recently, efforts have been made to predict the effect of glycosidic torsion angles on the shift of the C8 carbon using molecular orbital calculations (Ghose et al., 1994), and it was predicted on theoretical grounds that guanosine bases in the *syn* configuration should have C8 shifts downfield from those of *anti* bases. The experimental results presented here provide qualitative experimental confirmation for this prediction. The experimental shifts, on average 2 ppm downfield for *syn* bases in the G-tetrad models (compared to *anti* bases), are also consistent with the shift seen for a *syn* guanosine in an RNA tetraloop (Varani and Tinoco, 1991; Varani et al., 1991). Compari-

TABLE 3
^{13}C AND ^{15}N CHEMICAL SHIFTS (ppm) OF SOME NONPROTONATED BASE ATOMS OF d(GGTCGG)[a]

Residue	C4	C5	C2	N7	N9
G1	155.37	119.71		234.48	168.50
G2	155.28	117.95		240.21	169.32
T3	167.30	114.53	154.13		
C4	168.42		160.60		
G5	155.96	118.71		232.25	165.27
G6	154.76	118.13		238.30	172.35

[a] Shifts are relative to TSP for ^{13}C and to NH_3 for ^{15}N. Shifts were observed by HMBC experiments using H8 of guanosine residues, H6 and CH_3 of thymidine, and H6 of cytidine. Long-range correlations to nitrogens were not observed for the pyrimidine bases.

son of the attached proton shifts indicates that ring current effects are not a major factor for this variation. Relating experimental shifts for the nonprotonated nuclei with possible future calculations may be useful in providing guidance for assessing the overall accuracy of theoretical chemical shift predictions in these systems.

Results for Z-DNA (Sklenář et al., 1987) provide an opportunity to experimentally evaluate the predicted combined effects of changes in both glycosidic torsion angle and sugar pucker on the ^{13}C shifts, using the already described effects of sugar conformation and the results for *syn* and *anti* base orientation reported here. An *anti* to *syn* change would induce downfield shifts at C1', C3' and C4', in order of decreasing magnitude. A transition of sugar pucker from S-type to N-type, best quantified by Lankhorst et al. (1983), would result in downfield shifts of C1' and C2', while C3' and C4' would shift upfield. The reported (Sklenář et al., 1987) downfield shift for the C1' of the guanosine residues in $d(Gm^5C)_{20}$ on going from B- to Z-DNA (in the former the guanosine is *anti*, with a C2'-*endo* sugar, and in the latter it is *syn*, with a C3'-*endo* sugar) is consistent with the downfield changes expected for both aspects of the conformational change, although the magnitude is weaker than predicted in both cases. The guanosine C3' shift is almost unaffected by the transition, while the C4' resonance moves upfield 1.26 ppm. The latter results are consistent with the opposite shift influences of the glycosidic and pucker contributions, respectively, that would tend to cancel, with the size of the effect due to the change in glycosidic torsion angle being less for C4' than for C3'. For C2' it is expected that the change in sugar pucker would induce a downfield shift for this carbon, while the glycosidic torsion angle change would induce an upfield shift of apparently larger magnitude. Experimentally, an upfield shift of about 0.4 ppm is observed.

It would be rather surprising if the results were simply additive in detail based on model system data, since the combined change of glycosidic angle and sugar pucker would very likely induce additional steric interactions, and cause minor additional structural adjustments affecting the chemical shift. Furthermore, the data were obtained with a sample that had been 85% converted to the Z-form (Sklenář et al., 1987), which leaves open the question whether the Z species in the sample would display the full aspects of a canonical Z-DNA structure under these conditions. Nonetheless, the overall qualitative consistency suggests that the analysis of ^{13}C shift trends as a function of conformation is meaningful.

The extension of the trends identified here to RNA can be assessed by comparing results from several guanosine residues in a stable tetraloop hairpin (Varani and Tinoco, 1991; Varani et al., 1991). In this instance all sugar moieties of the guanosine residues are C3'-*endo*, with three of the guanosines *anti*, and one *syn*. The C1' and C3' of the *syn* residues resonate downfield compared to their *anti* counterparts. In fact, the C1' of the *syn* residue displays the most extreme downfield shift of this group in the molecule. These sites show a general consistency with the effects in the DNA case. The C2', however, is downfield in the *syn* relative to the *anti* case, opposite to what is seen in the DNA case. It is possible that the difference in the chemical and steric environments at the 2' position is responsible for the deviation at this site.

Conclusions

The data reported here show that a change in the base orientation of guanosine residues from *anti* to *syn* causes significant effects on the shifts of carbon and nitrogen atoms in sugar and base moieties. While these studies involve residues with C2'-*endo* conformation, comparison with the limited data available (Sklenář et al., 1987; Varani and Tinoco, 1991) indicates that similar trends are present where the sugar is C3'-*endo*. It is likely that the same trends will hold for adenine and perhaps pyrimidine bases, but experimental confirmation for this suggestion is as yet not available. Theoretical prediction of the shift trends of the C8 carbon as a function of glycosidic torsion angle shows general consistency with the values from experiment and additional calculations may provide support for the extension of these trends to other nucleotide residues. Knowledge of this conformational effect on carbon shifts will be of help in trying to use these parameters in conformational analysis, however, this must be done in the context of the interplay of other factors, most notably sugar pucker, with these shifts as well. While there has been success in using theory to correlate shift effects and conformation in nucleotides (e.g. Ghose et al., 1994) and in protein residues (e.g. De Dios et al., 1993), obtaining the same degree of success for oligonucleotides relative to proteins may be elusive, since the rigidity of structures adopted by oligonucleotides is likely to be less than that of the packed interior of a protein, and hence a greater effort to account for the presence of some conformational averaging may be needed.

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Note added in proof

Two guanosine residues in an RNA hairpin loop have recently been observed (N. Greenbaum, I. Radakrishnan, D. Hirsh and D. Patel, unpublished results) displaying

C2'-*endo* sugar pucker with the base *syn* in one case and *anti* in the other. The C1' shifts are 88.0 and 85.9 ppm, respectively. For the C2' site, the shifts are 71.8 and 74.5 ppm for the *syn* and *anti* residues, respectively. The trends for these sites agree with the DNA data from the G-tetrad model. While the absolute values of the C2' shifts in the RNA hairpin compared to DNA reflect the change in carbon substituents at this site, the C1' values are quite consistent with those for the G-tetrad model. The C4' chemical shift of the residue with the *syn* base is downfield with respect to that of the *anti* residue, while the C3' shift of the *syn* residue is slightly upfield compared to that of the *anti* residue. The base C8 behavior is consistent with that observed previously for both DNA and RNA. These data allow the additional comparison of the effects of glycosidic torsion angle between DNA and RNA with C2'-*endo* sugar pucker.

References

Ashcroft, J., LaPlante, S.R., Borer, P.N. and Cowburn, D.C. (1989) *J. Am. Chem. Soc.*, **111**, 363–365.

Ashcroft, J., Live, D., Patel, D.J. and Cowburn, D. (1991) *Biopolymers*, **31**, 45–55.

Bax, A. and Summers, M.F. (1986) *J. Am. Chem. Soc.*, **108**, 2093–2094.

Borer, P.N., Zanatta, N., Holak, T.A., Levy, G.C., Van Boom, J. and Wang, A.-H.J. (1984) *J. Biomol. Struct. Dyn.*, **1**, 1373–1386.

Brennan, T., Weeks, C., Shefter, E., Rao, S.T. and Sundaralingam, M. (1972) *J. Am. Chem. Soc.*, **94**, 8548–8553.

De Dios, A.C., Pearson, J.G. and Oldfield, E. (1993) *J. Am. Chem. Soc.*, **115**, 9968–9773.

Ghose, R., Marino, J.P., Wiberg, K.B. and Prestegard, J.H. (1994) *J. Am. Chem. Soc.*, **116**, 8827–8828.

Kang, C.H., Zhang, X., Ratliff, R., Mpyzis, R. and Rich, A. (1992) *Nature*, **356**, 126–131.

Lankhorst, P.P., Erkelens, C., Haasnoot, C.A.G. and Altona, C. (1983) *Nucleic Acids Res.*, **11**, 7215–7230.

LaPlante, S.R., Ashcroft, J., Cowburn, D., Levy, G.C. and Borer, P.N. (1988a) *J. Biomol. Struct. Dyn.*, **5**, 1089–1099.

LaPlante, S.R., Bourdreau, E.A., Zanatta, N., Levy, G.C., Borer, P.N., Ashcroft, J. and Cowburn, D. (1988b) *Biochemistry*, **27**, 7902–7909.

LaPlante, S.R., Zanatta, N., Hakkinen, A., Wang, A.H.-J. and Borer, P.N. (1994) *Biochemistry*, **33**, 2430–2440.

Leupin, W., Wagner, G., Denny, W. and Wüthrich, K. (1987) *Nucleic Acids Res.*, **15**, 269–275.

Live, D., David, D.G., Agosta, W. and Cowburn, D.C. (1984) *J. Am. Chem. Soc.*, **106**, 1939–1943.

Michnicka, M.J., Harper, J.W. and King, G.C. (1993) *Biochemistry*, **32**, 395–400.

Nikonowicz, E.P. and Pardi, A. (1993) *J. Mol. Biol.*, **232**, 1141–1156.

Santos, R.A., Tang, P. and Harbison, G.S. (1989) *Biochemistry*, **28**, 9372–9378.

Schultze, P., Macaya, R.F. and Feigon, J. (1994) *J. Mol. Biol.*, **235**, 1532–1547.

Sklenář, V., Bax, A. and Zon, G. (1987) *J. Am. Chem. Soc.*, **109**, 2221–2222.

Smith, F.W. and Feigon, J. (1992) *Nature*, **356**, 164–168.

Thewalt, U., Bugg, C.E. and Marsh, R.E. (1970) *Acta Crystallogr.*, **B26**, 1089–1101.

Varani, G., Cheong, C. and Tinoco Jr., I. (1991) *Biochemistry*, **30**, 3280–3289.

Varani, G. and Tinoco Jr., I. (1991) *J. Am. Chem. Soc.*, **113**, 9349–9354.

Wang, Y., De los Santos, C., Gao, X., Greene, K., Live, D. and Patel, D.J. (1991) *J. Mol. Biol.*, **222**, 819–832.

Wang, K.Y., Heffron, G.J., Bishop, K.D., Levy, G.C., Garbesi, A.M., Tondelli, L., Medley, J.H. and Borer, P.N. (1992) *Magn. Reson. Chem.*, **30**, 377–380.

Wang, Y. and Patel, D.J. (1993) *Structure*, **1**, 263–282.

Zhu, G., Live, D. and Bax, A. (1994) *J. Am. Chem. Soc.*, **116**, 8370–8371.