Journal of Biomolecular NMR, 4 (1994) 595–601
ESCOM

J-Bio NMR 218

Determination of the backbone torsion angle ε in nucleic acids

M.J.J. Blommers[a,*], D. Nanz[b,**] and O. Zerbe[b,***]

[a]*Department of Physics, Ciba-Geigy AG, P.O. Box, CH-4002 Basel, Switzerland*
[b]*Organisch-chemisches Institut der Universität Zürich, CH-8057 Zürich, Switzerland*

Received 13 June 1994
Accepted 15 July 1994

Keywords: Nucleic acids; Conformation; Coupling constants; E.COSY

SUMMARY

The multiplet structure of cross peaks in double-quantum-filtered COSY NMR spectra is analysed for those resonances that include passive heteronuclear couplings. Interestingly, the cross peak involving the sugar-ring protons H2' and H3' in nucleic acids display an E.COSY-type appearance exclusively when the backbone torsion angle ε (C4'-C3'-O3'-P) adopts a gauche(−) conformation. This observation allows an unambiguous analysis of the conformation around ε, without the knowledge of $^3J_{CP}$ coupling constants.

INTRODUCTION

Efficient methods for studying structures of nucleic acids using NMR methods are well established (Van de Ven and Hilbers, 1988; Wijmenga et al., 1993). They generally require the measurement of NOEs, which are interpreted using physical relaxation models to account for spin diffusion. Furthermore, coupling constants defining the sugar-ring conformation (Altona, 1982) are evaluated as additional restraints. However, homo- and heteronuclear coupling constants reflecting the conformation of the phosphate backbone have been largely neglected, although they are of particular interest for structures different from A- or B-type helices, such as hairpins, bulges, mismatches or certain nucleic acid analogues designed for antisense DNA application.

In this paper, we report a simple method to distinguish between the gauche(+), trans and gauche(−) conformations around the backbone torsion angle ε (C4'-C3'-O3'-P). It will be demonstrated below that this information is easily extracted from a conventional double-quantum-

*To whom correspondence should be addressed.
**Present address: Department of Chemistry, University of California, Berkeley, CA, U.S.A.
***Present address: Institut für Molekularbiologie und Biophysik, ETH-Hönggerberg, CH-8093 Zürich, Switzerland.

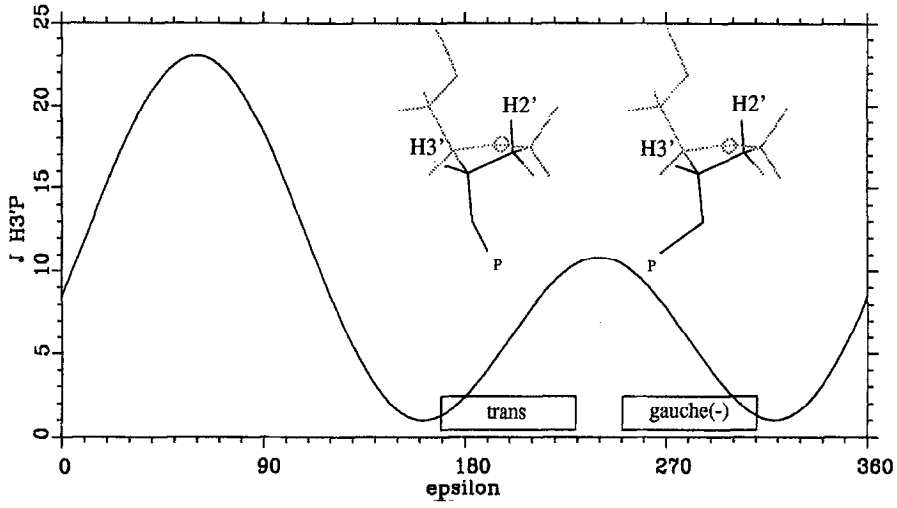


Fig. 1. Karplus curve representing the relationship between the $^3J_{H3'P}$ coupling constant and the backbone torsion angle ε (C4'-C3'-O3'-P) parameterised by Lankhorst et al. (1984): $^3J_{H3'P} = 15.3 \cos^2(\varepsilon + 120) - 6.2 \cos(\varepsilon + 120) + 1.5$. The sterically allowed regions for this torsion angle, trans and gauche(−), are indicated, as well as the corresponding conformations. The bonds which connect the H2', H3' and P spins are indicated in bold.

filtered COSY experiment (2QF-COSY) (Piantini et al., 1982) by considering the appearance of the H2'-H3' cross peak.

METHODS

Unambiguous determination of the torsion angle ε requires the knowledge of three coupling constants, i.e., $^3J_{H3'P}$, $^3J_{C2'P}$ and $^3J_{C4'P}$ (Blommers et al., 1988). Due to the degeneracies in the Karplus curve (Lankhorst et al., 1984) presented in Fig. 1, ε may not be restricted to a single value when only the $^3J_{H3'P}$ value is known. In the gauche(+) conformation the phosphate lies below the sugar ring, leading to severe van der Waals repulsion, and hence this conformation has never been observed so far. However, in modified nucleoside analogues the gauche(+) conformation may well be sterically allowed and would be conveniently recognised from the large $^3J_{H3'P}$ coupling around 23 Hz. Unfortunately, for the trans and gauche(−) conformations this coupling is of similar magnitude.

The backbone of nucleic acids is not necessarily rigid and conformational transitions may be expected between the trans and gauche(−) conformations. The observed coupling constants then display a time-averaged value, which may be described as:

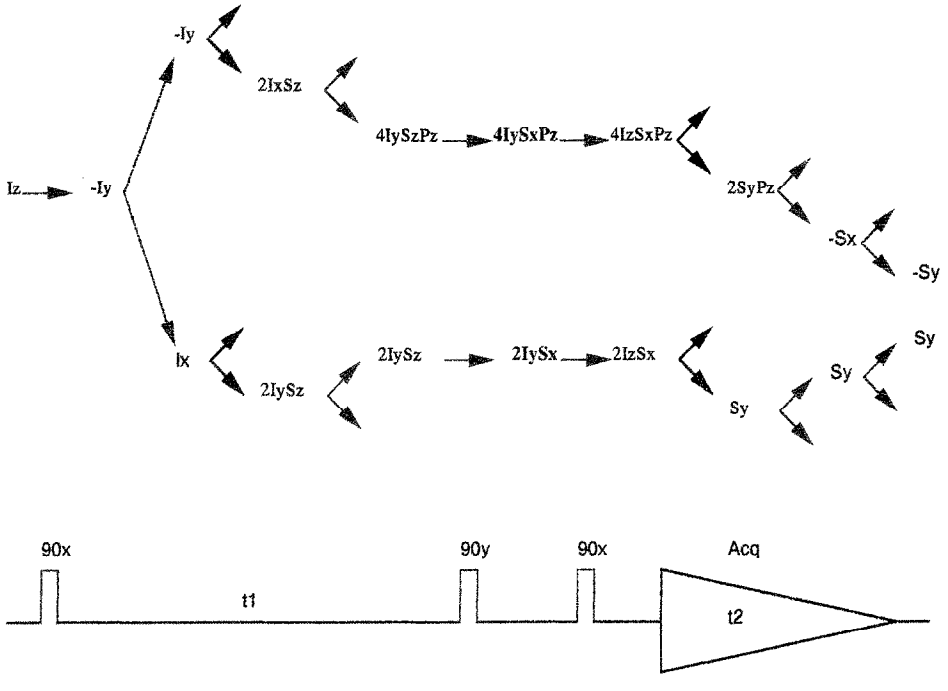
$$J_{obs} = x_{trans} J_{trans} + (1 - x_{trans}) J_{-}$$

where x_{trans} is the molar fraction of the trans conformation and J_{trans} and J_{-} the $^3J_{H3'P}$ coupling constants for the trans and gauche(−) conformations, respectively. Since J_{trans} and J_{-} have similar values, neither the molar fraction nor the torsion angle value of the rotamers can be obtained from the $^3J_{H3'P}$ coupling constant solely.

For those cases where coupling constants involving ^{13}C nuclei are not easily available, the four-bond coupling constant $^4J_{H2'P}$ may be of particular use. This coupling is only resolved for gauche(−) conformations, where the four bonds involved are found to be coplanar and in zig-zag (W) formation (cf. Fig. 1). This was previously shown by ^{31}P-^1H heteronuclear correlation experiments (Blommers et al., 1991). When ε changes from gauche(−) to trans, the P-O bond turns out of this plane, and as a consequence the four-bond coupling is not observable anymore. It is interesting to note that, when ε adopts a gauche(−) conformation, the sugar conformation is restricted to the S-type (Altona, 1982). This follows from Ramachandran plots calculated for nucleic acids (Mooren, 1993) and is confirmed when the crystal structure of $tRNA^{Phe}$ (Westhof and Sundaralingam, 1986) is analysed: when ε is observed in the gauche(−) rotamer, the ribose sugar is consistently found in an S-type conformation. Therefore, the $^4J_{H2'P}$ coupling is a unique feature of the ε gauche(−) conformation.

Even when the $^4J_{H2'P}$ coupling is too small to be detected in a ^{31}P-^1H correlation spectrum, it may well be manifest in homonuclear correlated spectra; in fact, the H2'-H3' cross peaks display E.COSY-type multiplet patterns only for gauche(−) conformations. By using the product operator formalism for weakly coupled spins (Sørensen et al., 1983; Van de Ven and Hilbers, 1983) it can be shown that this unique feature is due to the (passive) phosphorus coupling.

The scheme below shows a product operator description of the coherence pathways responsible for the H2'-H3' cross peak, where the transfer functions have been omitted for clarity. I, S and P denote the H2', H3' and ^{31}P spins, respectively.



Upwards and downwards pointed arrows denote cosine and sine modulation (Van de Ven and Hilbers, 1983). Right-handed rotations are used (Ernst et al., 1987). Evolution due to chemical

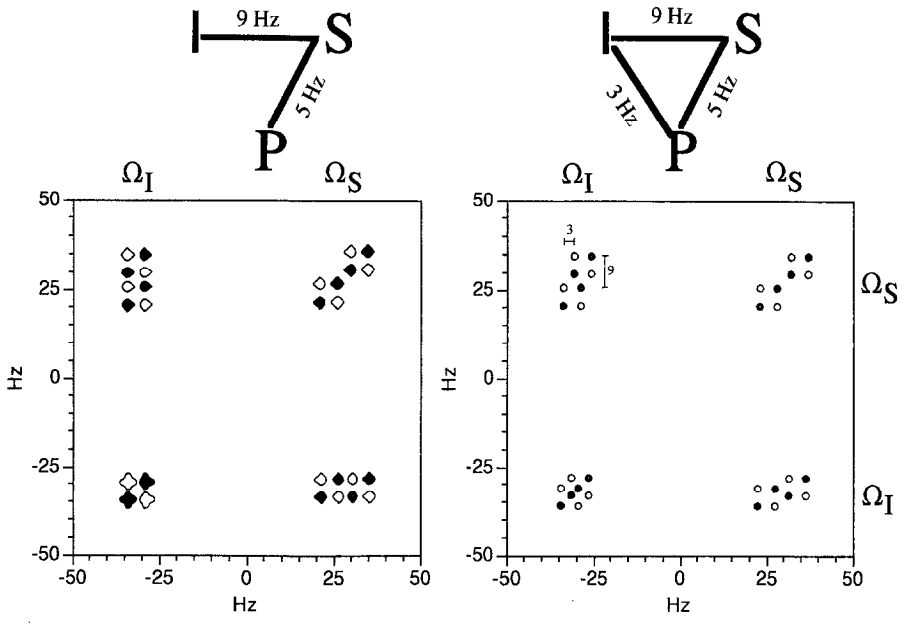


Fig. 2. Simulations of the 2QF-COSY experiment obtained using the program SMART. I and S denote ^{1}H spins, whereas P denotes ^{31}P. The following coupling constants are used: J_{IS} = 9 Hz; J_{SP} = 5 Hz, J_{IP} = 0 Hz (left panel) or 3 Hz (right panel). The J-connectivities of the two spin systems are shown on top. E.COSY-type cross peaks are observed only when both spins I and S are coupled to the heteronucleus P.

shift, homonuclear and heteronuclear J-coupling is indicated by green, black and red arrows, respectively. Only those pathways which lead to observable cross peaks are indicated. Two pathways are responsible for detection of the cross peaks that correlate the spins I and S. The uppermost pathway leads via IS double-quantum coherence, which is in anti-phase with respect to the heteronuclear coupling J_{IP}. This coherence is converted into detectable single-quantum coherence during t_2, provided that $J_{PS} \neq 0$. It is evident that this pathway only leads to detectable cross peaks when both protons I and S are coupled to the same phosphorus. The bottom pathway does not involve the heteronuclear coupling actively and corresponds to the normal pathway in a double-quantum-filtered experiment. The transfer function for the cross peak I $\rightarrow$ S is given by:

$$-S_y \cos(\Omega_I T_1) \sin(\pi J_{IS} t_1) \sin(\pi J_{IP} t_1) \sin(\pi J_{IS} t_2) \sin(\pi J_{SP} t_2) \sin(\Omega_S t_2)$$
$$+ S_y \sin(\Omega_I t_1) \sin(\pi J_{IS} t_1) \cos(\pi J_{IP} t_1) \sin(\pi J_{IS} t_2) \cos(\pi J_{SP} t_2) \cos(\Omega_S t_2)$$

and that for S $\rightarrow$ I is given by:

$$-I_y \cos(\Omega_S t_1) \sin(\pi J_{IS} t_1) \sin(\pi J_{SP} t_1) \sin(\pi J_{IS} t_2) \sin(\pi J_{IP} t_2) \sin(\Omega_I t_2)$$
$$+ I_y \sin(\Omega_S t_1) \sin(\pi J_{IS} t_1) \cos(\pi J_{SP} t_1) \sin(\pi J_{IS} t_2) \cos(\pi J_{IP} t_2) \cos(\Omega_I t_2)$$

The combination of the two terms that give rise to the cross peak results in cancellation of half of the multiplet components present in the cross peak, leading to an appearance as a so-called E.COSY-type cross peak (Griesinger et al., 1985).

In order to verify the effect, 2QF-COSY spectra of an I-S-P spin system have been simulated using the program SMART (Studer, 1988), implemented on an X32 computer. When only one of the coupled protons has a resolved phosphorus coupling, the E.COSY-type multiplet pattern can be seen in its diagonal peak only; otherwise it can be observed in both the diagonal and the cross peak (cf. Fig. 2). The phosphorus, in contrast to the protons, is not affected by the mixing pulses, and hence no higher nonobservable heteronuclear coherence orders are excited. Additional protons in the spin system, such as H1', H2" and H4', will lead to additional in-phase splittings of the multiplets (Blommers et al., 1988).

Since such a simultaneous phosphorus coupling to both H2' and H3' only exists for gauche(−) conformations, as discussed above, the evaluation of the cross-peak patterns may be used to discriminate between the two cases in nucleic acids.

RESULTS AND DISCUSSION

An application of the described method is demonstrated for the dodecamer of oligonucleotide d(C_1-C_2-T_3-A_4-T_5-T_6-T_7-A_8-T_9-A_{10}-G_{11}-G_{12}), which forms a hairpin in solution. The H2'-H3' cross peaks, as observed in the 2QF-COSY spectrum, are presented in Fig. 3. The enlarged cross peaks correspond to residue C_2 in the double-stranded part (Fig. 3A) and residue T_7 in the loop (Fig. 3B). From the E.COSY-type multiplet structure of the H2'-H3' cross peaks it is obvious that only T_7 is in a gauche(−) conformation around ε. The heteronuclear couplings $^4J_{H2'P}$ and $^3J_{H3'P}$ can readily be estimated from the displacements of the multiplet components, preferably in the F2 dimension. It is a special advantage of the method that, for cases where one of the two protons displays a relatively large phosphorus coupling, the other one is still measurable, even if the coupling is smaller than the natural line width of the involved proton. This is due to the displacement in the other frequency dimension, and has been exploited recently to determine ^{13}C-^1H or ^{15}N-^1H coupling constants in labelled proteins (Montelione et al., 1989; Schmieder et al., 1991). It is expected that the E.COSY-type cross peaks can be recognised in much larger systems than the one presented here, provided that the individual cross peaks display no severe overlap.

In the case of the investigated oligonucleotide the residues T_7 and A_8 are involved in a sharp π-turn: the backbone inverts its direction after the third loop residue. The gauche(−) conformation around ε_7 is clearly established, based on the $^4J_{H2'P}$ coupling detected in a ^{31}P-^1H correlation experiment (not shown). It is obvious, however, that the 2QF-COSY experiment is the experiment of choice, since it is much more sensitive, especially when $^4J_{H2'P}$ is small.

Since the four-bond coupling only exists for the gauche(−) conformation, the population of this rotamer can be calculated by:

$$X_{\varepsilon(-)} = {}^4J_{H2'P\,(obs)} / {}^4J_{H2'P\,(-)}$$

where $^4J_{H2'P(-)}$ denotes the coupling constant observed for a pure gauche(−) conformation of ε. For residue T_7 of the dodecamer, $^4J_{H2'P}$ = 2.3 Hz is observed. In the NMR study of a cyclic trinucleotide, i.e., r < pGpGpG > (Mooren et al., 1994), the torsion angle ε is found in equilibrium between gauche(−) (35%) and trans (65%) conformations. This follows from the $J_{C4'P}$ and $J_{C2'P}$ coupling constants. The $^4J_{H2'P}$ coupling constant for this case amounts to 0.8 Hz. When these data are combined, it appears that a $^4J_{H2'P}$ of 2.3 Hz, as found for residue T_7, corresponds to a pure gauche(−) conformation.

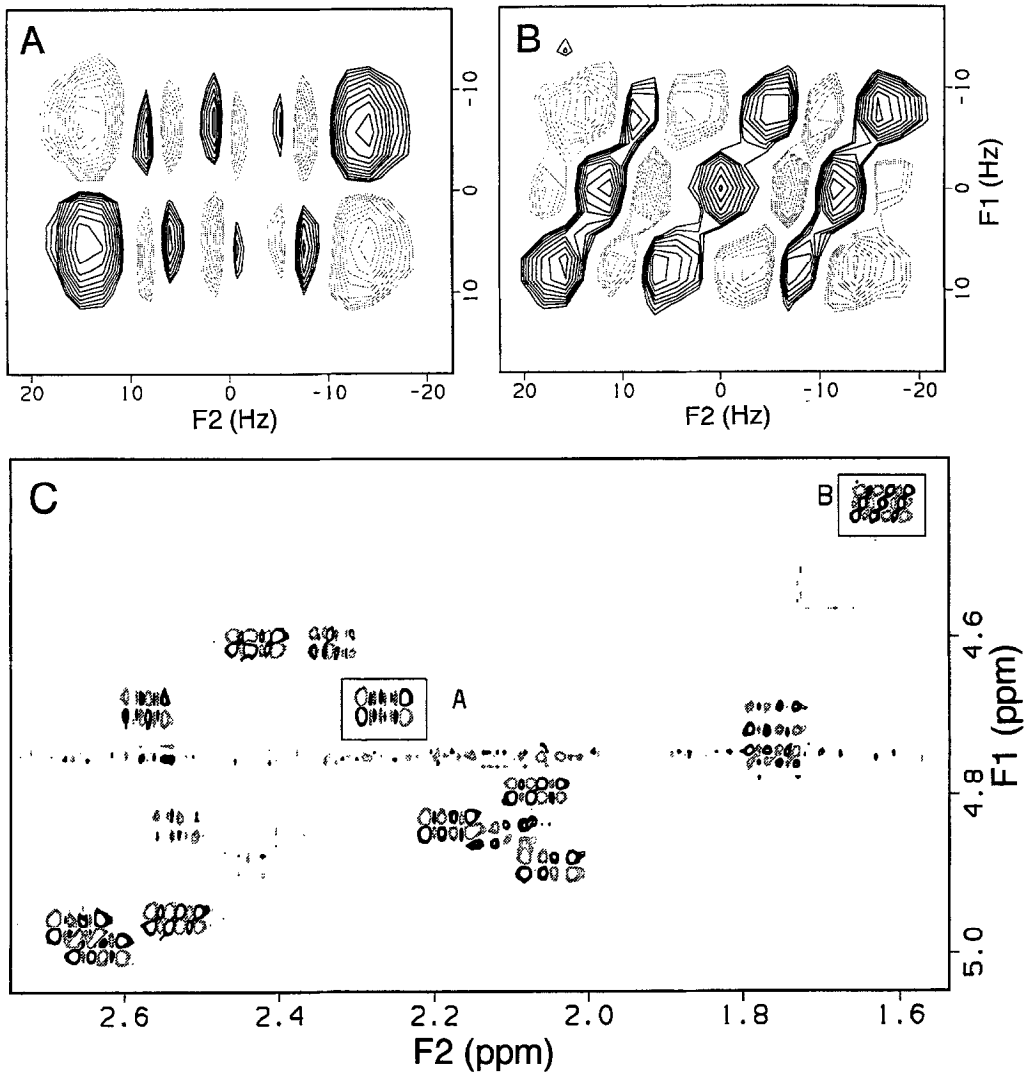


Fig. 3. Part of the 2QF-COSY spectrum of d(C_1-C_2-T_3-A_4-T_5-T_6-T_7-A_8-T_9-A_{10}-G_{11}-G_{12}), recorded at 25 °C, pH 7.0. The spectrum was recorded with 4098 and 600 complex points in t_2 and t_1, respectively, and processed using shifted sine-bell apodisation. The region encompassing most of the H2',H2" resonances (F2) and H3' resonances (F1) is shown in part C. Cross peaks A and B are enlarged in the two panels at the top. These cross peaks correlate the H2' and H3' of residues C_2 and T_7, respectively.

The analysis of the torsion angles ε can now be completed. For example, ε_2 is found to be in a trans conformation for more than 90%. In combination with the $^3J_{H3'P}$ value of 7.0 Hz, the Karplus curve depicted in Fig. 1 can be used to determine the value of torsion angle ε to be 207°. In contrast, ε_7 is found exclusively in a gauche(−) conformation. Here, $^3J_{H3'P}$ = 7.7 Hz, which corresponds to ε_7 = 269°. It should be noted that such an analysis for nucleic acids is only allowed when ε adopts only one conformation, which can be checked by means of an inspection of the 2QF-COSY spectrum. Because, in general, it is difficult to exclude minor (< 10%) contributions

of a second conformation, it would be realistic to assume an error of about ± 5° in the obtained value of ε. A more qualitative interpretation, e.g., all torsion angles ε in our duplex adopt a trans conformation (Blommers et al., 1994), is useful as well.

Similar effects relate to H4'-H5' and H4'-H5" cross peaks, although for the investigated dodecamer this is only observed for a few cases, due to resonance overlap in that particular area of the spectrum. For these cases, the E.COSY-type cross peaks account for the torsion angle γ (O5'-C5'-C4'-C3') to be in a gauche(+) and the torsion angle β (P-O5'-C5'-C4') to be in a trans conformation. Only for this combination, the bonds involving P-O5'-C5'-C4'-H4' are in a zig-zag (W) conformation, where the $^4J_{H4'P}$ coupling is resolved. Then, the H4'-H5' and H4'-H5" are both coupled to the same phosphorus. Although we believe that this method finds only limited application for the determination of the backbone torsion angles β and γ, in case these cross peaks do not exhibit resonance overlap, the interpretation can in principle be done along the same lines as outlined for the torsion angle ε.

CONCLUSIONS

We are convinced that the knowledge of the conformation around ε which can be readily obtained as outlined in this paper provides useful additional restraints for structure calculations and will therefore improve the quality of nucleic acid structures derived from NMR measurements.

REFERENCES

Altona, C. (1982) *Recl. Trav. Chim. Pays-Bas*, **101**, 413–433.

Blommers, M.J.J., Haasnoot, C.A.G., Walters, J.A.L.I., Van der Marel, G.A., Van Boom, J.H. and Hilbers, C.W. (1988) *Biochemistry*, **27**, 8361–8369.

Blommers, M.J.J., Van de Ven, F.J.M., Van der Marel, G.A., Van Boom, J.H. and Hilbers, C.W. (1991) *Eur. J. Biochem.*, **201**, 33–51.

Blommers, M.J.J., Tondelli, L. and Garbesi, A. (1994) *Biochemistry*, **33**, 7886–7896.

Ernst, R.R., Bodenhausen, G. and Wokaun, A. (1987) *Principles of Nuclear Magnetic Resonance in One and Two Dimensions*, 1st ed., Clarendon Press, New York, NY.

Griesinger, C., Sørensen, O.W. and Ernst, R.R. (1985) *J. Am. Chem. Soc.*, **107**, 6394–6396.

Lankhorst, P.P., Haasnoot, C.A.G., Erkelens, C. and Altona, C. (1984) *J. Biomol. Struct. Dyn.*, **1**, 1387–1405.

Montelione, G.T., Winkler, M.E., Rauenbuchler, P. and Wagner, G. (1989) *J. Magn. Reson.*, **82**, 198–204.

Mooren, M.M.W. (1993) Ph.D. Thesis, University of Nijmegen, Nijmegen.

Mooren, M.M.W., Wijmenga, S.S., Van der Marel, G.A., Van Boom, J.H. and Hilbers, C.W. (1994) *Nucleic Acids Res.*, **22**, 2658–2666.

Piantini, U., Sørensen, O.W. and Ernst, R.R. (1982) *J. Am. Chem. Soc.*, **104**, 6800–6801.

Schmieder, P., Kurtz, M. and Kessler, H. (1991) *J. Biomol. NMR*, **1**, 403–420.

Sørensen, O.W., Eich, G.W., Levitt, M.H., Bodenhausen, G. and Ernst, R.R. (1983) *Prog. NMR Spectrosc.*, **16**, 163–192.

Studer, W. (1988) *J. Magn. Reson.*, **77**, 424–438.

Van de Ven, F.J.M. and Hilbers, C.W. (1983) *J. Magn. Reson.*, **54**, 512–520.

Van de Ven, F.J.M. and Hilbers, C.W. (1988) *Eur. J. Biochem.*, **178**, 1 38.

Westhof, E. and Sundaralingam, M. (1986) *Biochemistry*, **25**, 4868–4878.

Wijmenga, S.S., Mooren, M.M.W. and Hilbers, C.W. (1993) In *NMR of Biological Macromolecules: A Practical Approach* (Ed, Roberts, G.C.K.) The Practical Approach Series, Oxford University Press, Oxford, pp. 217–288.