Journal of Biomolecular NMR, 5 (1995) 87–92
ESCOM

J-Bio NMR 237

Sequential correlation of anomeric ribose protons and intervening phosphorus in RNA oligonucleotides by a $^{1}H,^{13}C,^{31}P$ triple resonance experiment: HCP-CCH-TOCSY

John P. Marino[a], Harald Schwalbe[b,*], Clemens Anklin[c], Wolfgang Bermel[d], Donald M. Crothers[a] and Christian Griesinger[b,**]

[a]*Department of Chemistry, Yale University, P.O. Box 6666, New Haven, CT 06511, U.S.A.*
[b]*Institut für Organische Chemie, Universität Frankfurt, Marie Curie Strasse 11, D-60439 Frankfurt, Germany*
[c]*Bruker Analytische Meßtechnik GmbH, Silberstreifen, D-76287 Rheinstetten, Germany*
[d]*Bruker Instruments, Inc., Manning Park, 19 Fortune Drive, Billerica, MA 018212-3991, U.S.A.*

Received 23 September 1994
Accepted 12 October 1994

Keywords: Triple resonance; 3D NMR; ^{13}C-labeled RNA; HCP; C,C-TOCSY; *ColE1*

Summary

A three-dimensional $^{1}H,^{13}C,^{31}P$ triple resonance experiment, HCP-CCH-TOCSY, is presented which provides unambiguous through-bond correlation of all ^{1}H ribose protons on the 5' and 3' sides of the intervening phosphorus along the backbone bonding network in ^{13}C-labeled RNA oligonucleotides. The correlation of the complete ribose spin system to the intervening phosphorus is obtained by adding a C,C-TOCSY coherence transfer step to the triple resonance HCP experiment. The C,C-TOCSY transfer step, which utilizes the large and relatively uniform $^{1}J(C,C)$ coupling constant (~40 Hz for ribose carbons), efficiently correlates the phosphorus-coupled carbons observed in the HCP correlation experiment (i.e., C4' and C5' in the 5' direction and C4' and C3' in the 3' direction) to all other carbons in the ribose spin system. Of the additional correlations observed in the HCP-CCH-TOCSY, that to the relatively well-resolved anomeric H1',C1' resonance pairs provides the greatest gain in terms of facilitating assignment. The gain in spectral resolution afforded by chemical shift labeling with the anomeric resonances should provide a more robust pathway for sequential assignment over the intervening phosphorus in larger RNA oligonucleotides. The HCP-CCH-TOCSY experiment is demonstrated on a uniformly $^{13}C,^{15}N$-labeled 19-nucleotide RNA stem-loop, derived from the antisense RNA I molecule found in the *ColE1* plasmid replication control system.

^{1}H NMR spectroscopy has been proven to be a powerful tool in determining the conformation and dynamics of small oligonucleotides in solution (Van de Ven and Hilbers, 1988; Varani and Tinoco, 1991). The first step in any high-resolution ^{1}H NMR structural study of nucleic acids involves the complete assignment of ^{1}H resonances. The standard strategy for nucleic acid resonance assignment depends on the observation of sequential NOESY cross peaks predicted from the structural models of double-helical RNA or DNA (Feigon et al., 1983; Hare et al., 1983; Scheek et al., 1984). Many structurally interesting RNA oligonucleotides, however, often adopt noncanonical secondary and tertiary conformations, such as bulges, loops and triplets (Chastain and Tinoco, 1991; Wyatt and Tinoco, 1993). It is usually in these regions of the molecules where structural characterization provides the most interesting information on the biological questions being raised, but for which assignment by the standard NOESY strategy becomes most precarious. Methods for sequential unambiguous through-bond assignment in these noncanonical regions, as well as in helical regions of RNA oligonucleotides, would obviously be quite advantageous in structural studies. We have therefore pursued triple resonance $^{1}H,^{13}C,^{31}P$ NMR methods for sequential through-bond assignment of backbone ribose protons and intervening phosphorus atoms along the phosphodiester backbone in RNA.

Sequential through-bond assignment of the ribose protons across the phosphate backbone of oligonucleo-

*Present address: New Chemistry Laboratory, University of Oxford, South Park Road, Oxford OX1 3QT, U.K.
**To whom correspondence should be addressed.

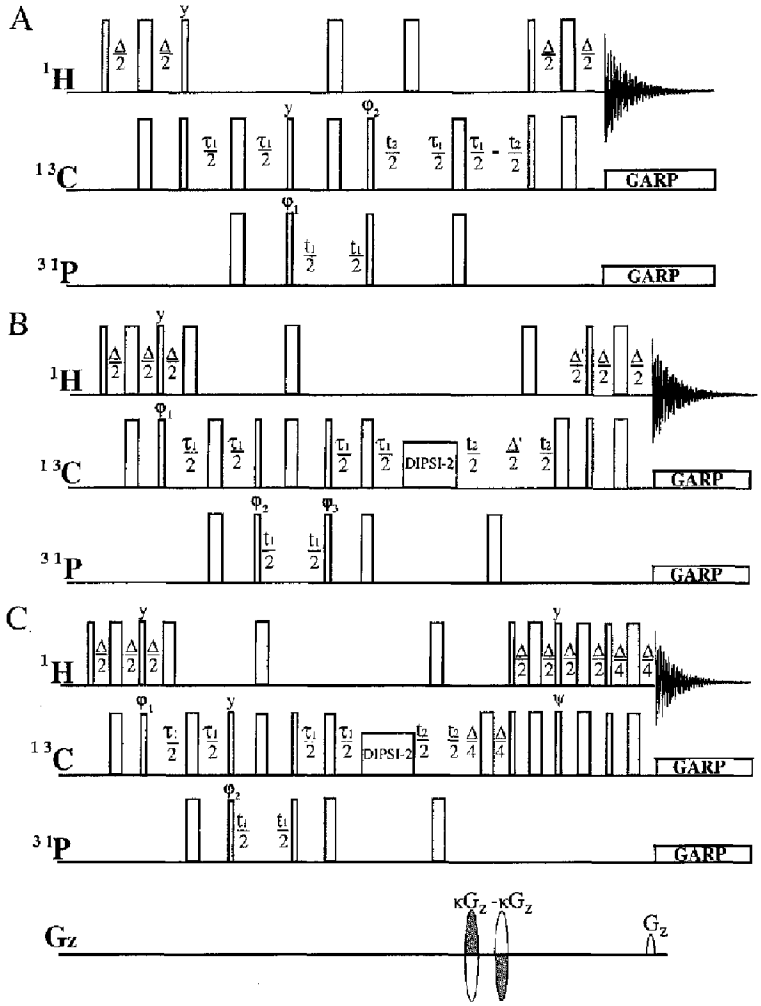


Fig. 1. The pulse schemes of the 3D (A) HCP; (B) HCP-CCH-TOCSY; and (C) sensitivity-enhanced gradient HCP-CCH-TOCSY triple resonance experiments used to correlate ribose ^{1}H,^{13}C spin systems with their respective intervening phosphorus. Narrow bars represent $\pi/2$ pulses and wide bars represent π pulses. All pulses without phase indication were applied along the x-axis. For the 3D HCP experiment (A), the four-step phase cycle used was as follows: $\phi_1 = x,-x$; $\phi_2 = 2(y),2(-y)$; Acq = (x,−x,−x,x). For the 3D HCP-CCH-TOCSY experiment (B), the eight-step phase cycle used was as follows: $\phi_1 = 4(x),4(-x)$; $\phi_2 = x,-x$; $\phi_3 = 2(x),2(-x)$; Acq = (x,−x,−x,x),(−x,x,x,−x). The delays were $\Delta/2 = 1.5$ ms (1/4J(CH), where J(CH) = 165 Hz), $\tau_1 = 26$ ms. In the HCP-CCH-TOCSY experiment, the refocusing delay was optimized for CH groups, $\Delta'/2 = 1.5$ ms, and so the H5'/H5" methylene protons were not observed. In the HCP experiment (A), the constant-time evolution of ^{13}C ($\tau_1 = 1/J(CC) = 26$ ms) is achieved simultaneously with the C,H-INEPT delay. In the HCP-CCH-TOCSY experiment, a 15 ms carbon spin-lock period along the x-axis is achieved by using a DIPSI-2 pulse sequence with an rf field strength of 5 kHz centered on the ribose carbons (~80 ppm). For both experiments, the ^{13}C carrier was centered in the middle of the ribose area (~80 ppm) and the ^{31}P carrier was centered in the middle of the phosphorus resonances (−4.10 ppm). GARP decoupling (Shaka et al., 1985) was used to decouple ^{13}C during acquisition. Quadrature in the ω_1 and ω_2 dimensions was obtained with the TPPI-States method (Marion et al., 1989). For the sensitivity-enhanced gradient HCP-CCH-TOCSY (C), the gradient acting on carbon was inverted in concert with the phase ψ (y,−y) to select the echo and anti-echo in alternate scans. Gradients were applied along the z-axis with κ = 2. Spectra with pure phase in ω_2 were obtained from the original data set according to the method described by Cavanagh et al. (1991). This sequence could not be used because a probe equipped with gradients was not available at the time the experiments were performed.

tides can in principle be obtained from 2D ^{1}H-^{31}P-correlated experiments when the heteronuclear correlation information is coupled with connectivity information obtained from 2D ^{1}H-^{1}H COSY experiments (Pardi et al., 1983). In practice, however, this correlation scheme is quite difficult to accomplish, even in moderately sized oligonucleotides, due to the severe overlap of backbone ^{1}H and ^{31}P resonances (which is especially true in RNA). Additionally, small J(H5',P), J(H5",P) coupling constants and overlapped geminal H5',H5" pairs can result in a breakdown of the sequential backbone assignment. More recently, 2D and 3D ^{1}H-^{31}P correlations based on heteronuclear TOCSY (Bearden and Brown, 1989; Artemov, 1991; Morris and Gibbs, 1991; Kellogg, 1992) have been used to make sequential correlations along the DNA backbone by correlation to the H3' proton on both the 5' and 3' sides of the intervening phosphorus (Kellogg and Schweitzer, 1993). The resolution of H3' protons, together with favorable J(H,P) coupling constants, make this a more efficient method for sequential assignment in DNA. Unfortunately, in RNA molecules the ^{1}H-^{31}P magnetization transfer by H,P-TOCSY occurs almost exclusively

to the ribose on the 5' side of the intervening phosphorus (Kellogg and Schweitzer, 1993). In addition, the transfer usually terminates at the H2' proton due to the vanishingly small ^{3}J(H1',H2') coupling constant that is characteristic of the C3'-endo sugar conformation adopted by riboses found in an A-form helical geometry. Therefore, as a result of the more severe spectral overlap of backbone ^{1}H resonances and unfavorable J(H,P) coupling constants in RNA, sequential through-bond correlations to resolvable resonances are not obtained with the H,P-TOCSY. An additional NOESY transfer step after the H,P-TOCSY provides H1',P correlations in RNA, however, with much reduced sensitivity (Kellogg et al., 1992). Using uniformly ^{13}C-labeled RNA oligonucleotides, unambiguous through-bond correlations of the H4',C4' and H5'/H5",C5' resonances on the 5' side and the H3',C3' and H4',C4' resonances on the 3' side of the intervening phosphorus in the HCP experiment (Heus et al., 1994; Marino et al., 1994) can provide sequential assignment. The experiment has sufficient sensitivity due to the favorable ^{2}J(C,P) couplings of 3–5 Hz and ^{3}J(C4',P) couplings of 8–10 Hz (Schwalbe et al., 1993,1994). However, due to severe overlap in the C3', C4' and C5' regions of carbon spectra of larger RNA oligonucleotides, additional correlations to more resolved resonances are required.

In this communication, we describe a 3D triple resonance $^{1}H,^{13}C,^{31}P$ experiment, HCP-CCH-TOCSY, a concatenation of HCP (Heus et al., 1994; Marino et al., 1994) and HCCH-TOCSY (Bax et al., 1990; Fesik et al., 1990; Pardi and Nikonowicz, 1992) experiments, providing unambiguous through-bond correlation of all ribose protons, ribose carbons and intervening phosphorus resonances via the coupling network along the backbone in ^{13}C-labeled RNA oligonucleotides. The goal of the experiment is to label the phosphorus-coupled carbons (C3' and C4' in the 5' direction; C4' and C5' in the 3' direction) observed by the P, C and H correlations of the HCP experiment with the chemical shift of the anomeric C1' and H1' resonances found in the same ribose. In RNA, the anomeric H1',C1' resonances usually show the highest chemical shift dispersion and do not overlap with any other ribose resonances. These anomeric resonances are consequently the most attractive candidates for chemical shift labeling to enhance the spectral resolution of any experiment involving the backbone resonances in RNA.

The 3D triple resonance HCP-CCH-TOCSY experiment (B) and the HCP experiment (A), from which it is derived, are shown in Fig. 1. The HCP-CCH-TOCSY experiment correlates backbone proton, carbon and phosphorus resonances in an analogous manner to the HCP experiment. The HCP part of the sequence resembles the HNCO experiment in its out and back version and consists of two sequential ^{1}H-^{13}C and ^{13}C-^{31}P INEPT and reverse INEPT coherence transfer steps (Morris and Freeman, 1979). Proton excitation provides the optimal sensitivity, due to the higher gyromagnetic ratio relative to carbon and phosphorus. Moreover, since ^{31}P has the smallest T_2, this sequence profits from the minimal delay during which ^{31}P is transverse. The delay τ_1 is set to 26 ms, which is approximately $1/(4\,^{3}J(C4',P))$ and also $1/^{1}J(C,C)$. Thus, coherence is transferred optimally from C4' to P and sensitivity losses due to the inevitable evolution of the homonuclear ^{1}J(C,C) coupling are minimized. For the RNA molecule under investigation here, 26 ms was the optimal delay despite the rather short T_2 of the carbons. For larger RNA oligonucleotides, a shorter τ_1 delay may turn out to be optimal when carbon T_2 relaxation is considered.

The C,C-TOCSY correlation of all ribose carbons, particularly the C1' carbons, to the phosphorus-correlated carbons is accomplished with a DIPSI-2 isotropic mixing sequence (Shaka et al., 1988) of 15 ms, covering a 5 kHz field spectral width. The evolution of carbon chemical shift during t_2 after the C,C-TOCSY and finally the C,H transfer optimized for CH groups ($\Delta = 1/(2\,^{1}J(C,H))$ results in the correlation of the intervening phosphorus resonances with all H,C pairs of the riboses in both the 5' and 3' directions along the RNA backbone. In particular, the well-resolved H1',C1' resonances of each ribose are correlated with the adjacent phosphorus resonances. The magnetization pathway followed in the HCP and HCP-CCH-TOCSY experiments is shown schematically in Fig. 2.

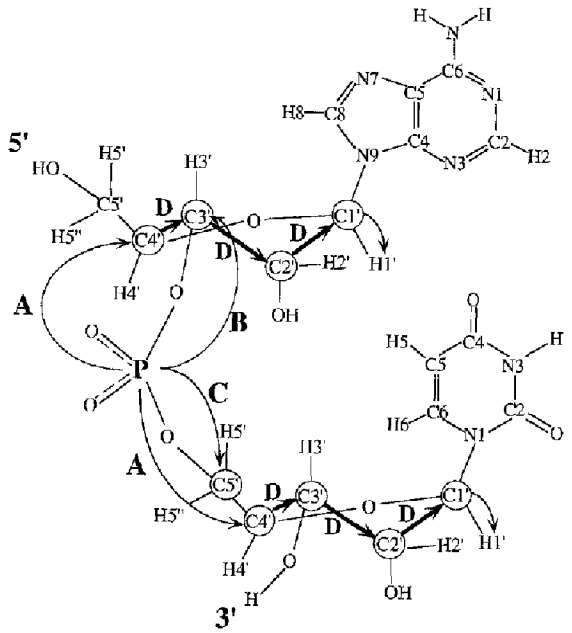
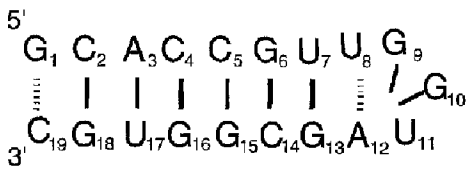


Fig. 2. A schematic showing the through-bond correlations observed in the HCP and HCP-CCH-TOCSY experiments. The three-bond ^{13}C, ^{31}P correlation pathway (A), ^{3}J(C4',P) ~ 8–10 Hz, and the two-bond $^{13}C,^{31}P$ correlation pathway (B and C), ^{2}J(C,P) ~ 3–5 Hz, observed in the HCP 'out and back' transfers are indicated. In the HCP-CCH-TOCSY experiment pathway C is suppressed due to methin selective excitation. The ribose carbons correlated by the C,C-TOCSY are shadowed and the correlation between the phosphorus-correlated carbons (C3',C4' in the 3' direction and C5',C4' in the 5' direction of the intervening phosphorus) of the HCP experiment and the C1' carbons is shown by pathway (C).

In our hands, the chemical shift evolution of ^{13}C after the C,C-TOCSY was found to be superior to the alternative experiment that accomplished ^{13}C evolution before the C,C-TOCSY part. The alternate approach would then give a 3D experiment in which the chemical shifts of the backbone phosphorus-correlated carbons, namely C3' and C4' (C5' is suppressed due to the refocussing delay set to 1/2J), are evolved and then further labeled in the third dimension with the chemical shift of the anomeric ^1H in the ribose spin system. This implementation has the advantage, as in the HCP experiment, that the information about the direction of the sequence is not lost. However, the chemical shift dispersion afforded by evolution of the C3' and C4' carbon resonances is usually worse when compared to the C1' resonances. In addition, the evolution of carbon chemical shift after the TOCSY mixing leads to an increased sensitivity when compared to evolution of carbon chemical shift before the TOCSY. For the proposed approach, the detected proton derives its magnetization from all carbon coherences filtered in the HCP step, giving rise to four signals per ribose and phosphorus. In the alternative implementation the detected proton derives its magnetization individually from the selected carbon coherence filtered in the HCP step, giving rise to eight signals per ribose and phosphorus.

The sequences A and B could be further improved by implementing sensitivity enhancement (Palmer et al., 1991) in connection with the formation of a heteronuclear gradient echo (Kay et al., 1992; Schleucher et al., 1993). This would allow the experiments to be measured in H_2O. The respective pulse sequence with carbon evolution after the TOCSY mixing period is given in Fig. 1C. The experiment in Fig. 1C uses a pulsed in-phase coherence order-selective coherence transfer (ICOS-CT), resulting in a sensitivity enhancement of $\sqrt{2}$ compared to the non-gradient version (Sattler et al., 1995).



Scheme 1. Numbering of the residues in the RNA stem-loop 19-mer under investigation.

The 3D HCP-CCH-TOCSY experiment can also be easily converted into a 2D version by removing the ^{13}C chemical shift labeling. The resulting 2D ^{31}P-^1H experiment could be used to correlate H1' protons on the 5' and 3' sides of the intervening phosphorus and provide complete sequential assignments if the spectral resolution of the H1' protons and phosphorus resonances is sufficient. A 2D version of the HCP-CCH-TOCSY experiment, applied to the 19-mer RNA stem-loop (Scheme 1) derived from the RNA I antisense repressor molecule of the *ColE1* replication control system, is shown in Fig. 3. The solid line traces the H1',P correlations for the 'loop' residues U8 through A12.

The ^{13}C-^{31}P correlations of the C1' regions at the anomeric ^1H slices of the HCP-CCH-TOCSY experiment, applied to the 19-mer RNA stem-loop, are shown in Fig. 4. Each ^1H plane shows a given C1' carbon correlated to the phosphorus in both the 5' and 3' directions of the intervening ribose ring. Sequential connectivities along the entire backbone of the 19-mer stem-loop, as shown by solid lines in Fig. 3, are made by connecting two cross peaks in a given C1',P plane and then connecting these C1',P cross peaks from one ^1H anomeric plane to the next. An analogous assignment pathway can be used by looking at the ^1H,P planes in the H1' region of the slices at the C1'

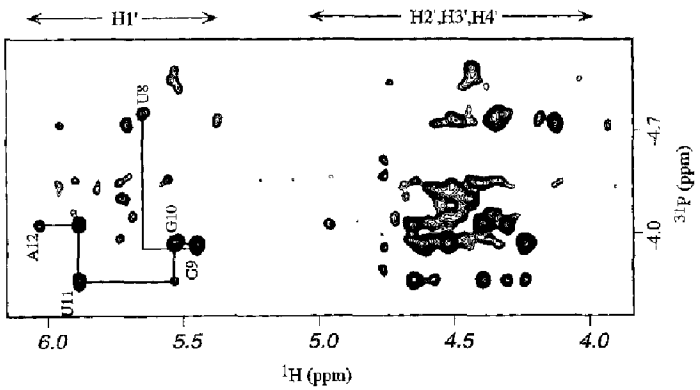


Fig. 3. A ^1H,^{31}P plot of the 2D HCP-CCH-TOCSY experiment applied to the RNA I stem-loop in 25 mM NaCl, 1 mM cacodylate (pH = 6.5) dissolved in 99.996% D_2O. The RNA oligonucleotide was enzymatically synthesized from a DNA template using T7 polymerase run off transcription (Milligan et al., 1987). The ^{13}C,^{15}N-labeled NTPs were prepared from RNA, isolated from *Methylophilus methylotrophus* grown in minimal media with ^{13}C-methanol and ^{15}N-NH_4Cl as the sole carbon and nitrogen sources (Batey et al., 1992). The sample concentration was ~1.5 mM. The experiment was collected on a Bruker four-channel AMX600 spectrometer equipped with a triple resonance ^1H,^{13}C,^{31}P probe at 25 °C. Correlations are observed between the ^{31}P resonances and all ^1H resonances in the riboses at the 5' and 3' sides of the phosphorus. A sequential trace of the anomeric H1',P cross peaks, with the ribose H1' chemical shifts labeled, of the 'loop' residues A12 through U8 is indicated by the solid line. The experiment was collected with 512 and 32 complex points in t_2 and t_1, respectively, 256 scans per t_1, t_2 increment and spectral widths of 6000 and 425 Hz for the respective dimensions. Total experiment time was 8 h. The spectrum was processed with a 60°-shifted sine bell in t_2 (using 256 points) and t_1 and was zero-filled to a final matrix size of 1024 × 256.

carbon chemical shifts and correlating H1',P cross peaks from one C1' carbon plane to the next. Additionally, sequential phosphorus correlations to H4',C4', H3',C3' and H2',C2' resonances are observed in the experiment (methylene H5',H5'' are not observed, since $\Delta' = 1/2J(CH)$). In the event that overlapped resonances are observed in the anomeric region of the spectrum, these other correlations may be resolved and provide the missing assignments. The ^{31}P plane at the G10 phosphorus resonance is shown in Fig. 5, with the correlations to the C1',H1', C2',H2', C3',H3' and C4',H4' resonances of the G10 and U11 riboses labeled. In general, however, the dispersion afforded by the H1' and C1' resonances is in most cases better than any other resonances correlated to the intervening phosphorus by either the direct HCP or indirect C,C-TOCSY transfers.

In short, the 3D HCP-CCH-TOCSY experiment de-

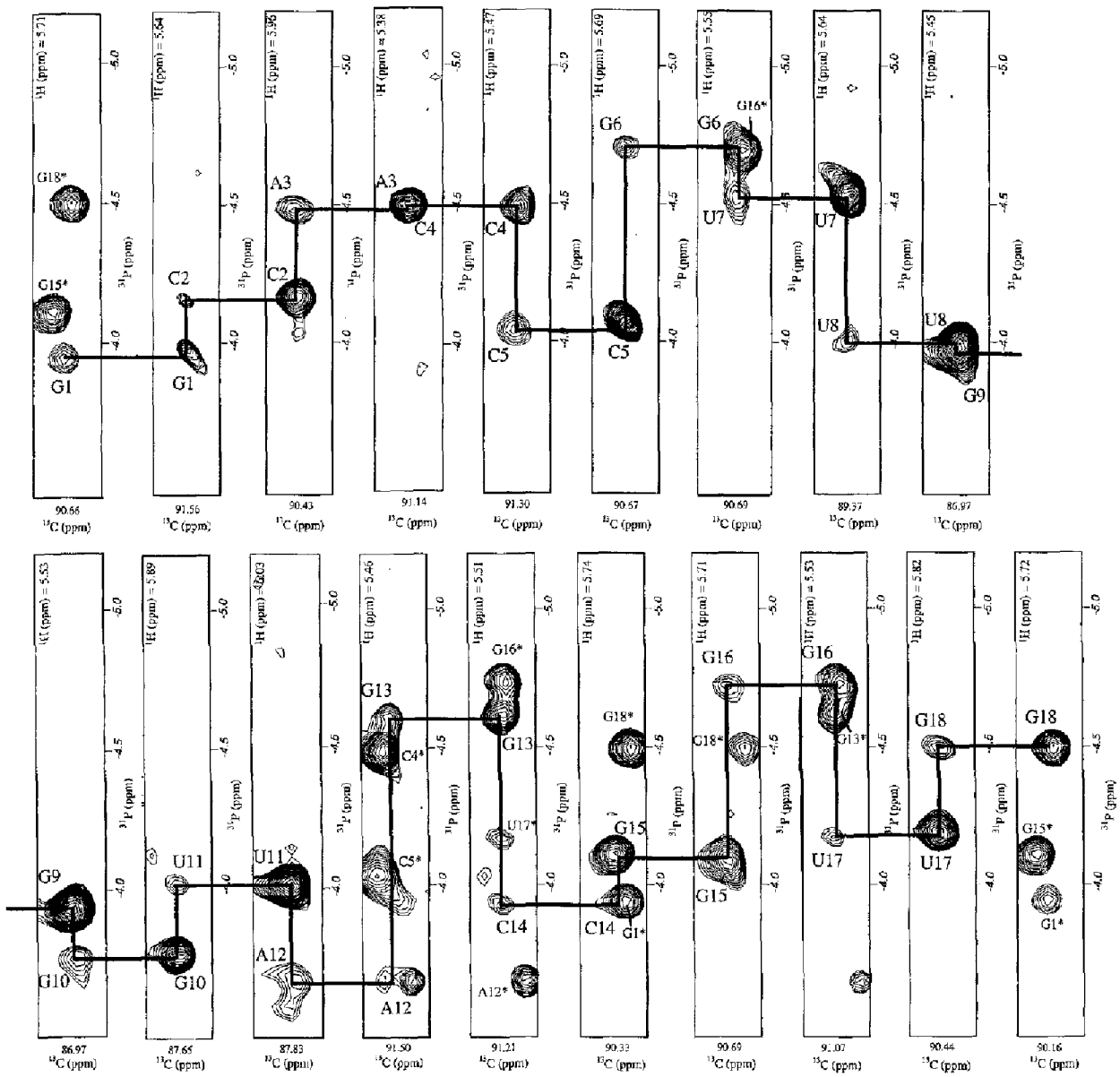


Fig. 4. Expansion of the C1',P regions of the $^{13}C,^{31}P$ planes at the ^1H chemical shift of anomeric H1' protons in the 3D HCP-CCH-TOCSY experiments applied to the RNA I stem-loop. The experiment was collected on a Bruker four-channel DMX600 spectrometer (Bruker Instruments, Billerica), equipped with a quadruple resonance $^1H,^{13}C,^{15}N,^{31}P$ probe at 25 °C. Each $^{13}C,^{31}P$ plane shows two correlations in the C1',P region to the phosphorus on both the 5' and 3' sides of an intervening ribose H1',C1' resonance. Sequential assignment along the entire oligonucleotide sequence of the stem-loop is shown by the solid lines connecting the labeled C1',P cross peaks from one ^1H plane to the next. The cross peaks are labeled by the nucleotide assignment for a particular H1',C1' pair. The additional peaks labeled with asterisks in the selected C1',P regions result from overlap in the $^1H1$ dimension. The experiment was collected with 512, 70 and 19 complex points in t_3, t_2 and t_1, respectively, 24 scans per t_1, t_2 increment and spectral widths of 6000, 8400 and 425 Hz for the respective dimensions. Total experiment time was 48 h. The spectrum was processed with a 60°-shifted sine bell in t_3 (using 256 points), t_2 and t_1 and was zero-filled to a final matrix size of 1024 × 256 × 64. Note that the C1',P planes have been plotted at different levels, as a reflection of the differing cross-peak intensities observed, and that the splitting observed for the A12 resonance is not understood at this time.

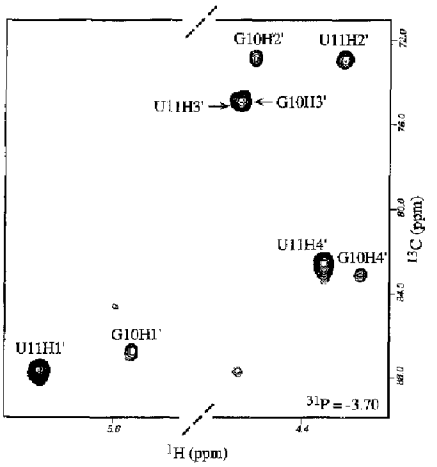


Fig. 5. Plane at the ^{31}P resonance of G10 in the 3D HCP-CCH-TOCSY experiment, applied to the RNA I stem-loop. The correlations of the G10 phosphorus resonance to the C1',H1', C2',H2', C3',H3' and C4',H4' resonances of the G10 and U11 riboses are indicated. The C3',H3' resonances of G10 and U11 are overlapped in this plane and are assigned based on a 3D HCCH-TOCSY experiment that has been recorded with the RNA I sample. Note that the ^{31}P resonances are numbered by the preceding 5' nucleotide to which they are bound, which is consistent with the convention of previous nucleic acid NMR studies, but does not conform to IUPAC standards.

scribed here contains three favorable components that make it a powerful tool for sequential backbone assignment in RNA: (i) the favorable J(C,P) allows efficient transfer to the ribose rings on both the 5' and 3' sides of the intervening phosphorus; (ii) the C,C-TOCSY provides efficient correlation of all ring carbons that is independent of ring geometry; and (iii) the evolution of the ^{13}C chemical shift provides additional spectral dispersion. The HCP sequential correlations discussed here can be taken together with other through-bond correlations obtained in double ^{1}H,^{13}C (Pardi and Nikonowicz, 1992) and triple ^{1}H,^{13}C,^{15}N resonance (Farmer et al., 1993,1994; Sklenář et al., 1993a,b) experiments developed for RNA assignment to provide unambiguous assignment of all backbone ^{1}H, ^{13}C, ^{15}N and ^{31}P resonances. The ability to completely assign these backbone resonances now provides an opportunity to extract additional structural information contained in the chemical shifts, relaxation behavior, NOEs and coupling constants associated with these resonances. This additional information on the backbone structure and dynamics should enhance the ability to obtain high-resolution solution NMR structures of RNA molecules.

Acknowledgements

This work was supported by the NIH (U.S.A.), Grant GM21966, to D.M.C. and by the Fonds der Chemischen Industrie to C.G. J.P.M. thanks Prof. J.H. Prestegard for many enlightening discussions. H.S. is supported by an EC Fellowship ('Human Capital and Mobility') and thanks Dr. C. Dobson for encouragement.

References

Artemov, D.Y. (1991) *J. Magn. Reson.*, **91**, 405–407.
Batey, R.T., Inada, M., Kujawinski, E., Puglisi, J.D. and Williamson, J.R. (1992) *Nucleic Acids Res.*, **20**, 4515–4523.
Bax, A., Clore, G.M. and Gronenborn, A.M. (1990) *J. Magn. Reson.*, **88**, 425–431.
Bearden, D.W. and Brown, L.R. (1989) *Chem. Phys. Lett.*, **163**, 432–436.
Cavanagh, J., Palmer III, A.G., Wright, P.E. and Rance, M. (1991) *J. Magn. Reson.*, **91**, 429–436.
Chastain, M. and Tinoco Jr., I.J. (1991) *Prog. Nucleic Acid Res. Mol. Biol.*, **41**, 131–177.
Farmer II, B.T., Müller, L., Nikonowicz, E.P. and Pardi, A. (1993) *J. Am. Chem. Soc.*, **115**, 11040–11041.
Farmer II, B.T., Müller, L., Nikonowicz, E.P. and Pardi, A. (1994) *J. Biomol. NMR*, **4**, 129–133.
Feigon, J., Leupin, W., Denny, W.A. and Kearns, D.R. (1983) *Biochemistry*, **22**, 5943–5951.
Fesik, S.W., Eaton, H.L., Olejniczak, E.T., Zuiderweg, E.R.P., McIntosh, L.P. and Dahlquist, F.W. (1990) *J. Am. Chem. Soc.*, **112**, 886–888.
Hare, D.R., Wemmer, D.E., Chou, S., Drobny, G. and Reid, B.R. (1983) *J. Mol. Biol.*, **171**, 319–336.
Heus, H.A., Wijmenga, S.S., Van de Ven, F.J.M. and Hilbers, C.W. (1994) *J. Am. Chem. Soc.*, **116**, 4983–4984.
Kay, L.E., Keifer, P. and Saarinen, T. (1992) *J. Am. Chem. Soc.*, **114**, 10663–10664.
Kellogg, G.W. (1992) *J. Magn. Reson.*, **98**, 176–182.
Kellogg, G.W., Szewczak, A.A. and Moore, P.B. (1992) *J. Am. Chem. Soc.*, **114**, 2727–2728.
Kellogg, G.W. and Schweitzer, B.I. (1993) *J. Biomol. NMR*, **3**, 577–595.
Marino, J.P., Schwalbe, H., Anklin, C., Bermel, W., Crothers, D.M. and Griesinger, C. (1994) *J. Am. Chem. Soc.*, **116**, 6472-6473.
Marion, D., Ikura, R., Tschudin, R. and Bax, A. (1989) *J. Magn. Reson.*, **85**, 393.
Milligan, J.F., Groebe, D.R., Witherell, G.W. and Uhlenbeck, O.C. (1987) *Nucleic Acids Res.*, **15**, 8783–8798.
Morris, G.A. and Freeman, R.J. (1979) *J. Am. Chem. Soc.*, **101**, 760–762.
Morris, G.A. and Gibbs, A. (1991) *J. Magn. Reson.*, **91**, 444–449.
Pardi, A. and Nikonowicz, E.P. (1992) *J. Am. Chem. Soc.*, **114**, 9202–9203.
Pardi, A., Walker, R., Rappoport, H., Wider, G. and Wüthrich, K. (1983) *J. Am. Chem. Soc.*, **105**, 1652–1653.
Palmer III, A.G., Cavanagh, J., Wright, P.E. and Rance, M. (1991) *J. Magn. Reson.*, **93**, 151–170.
Sattler, M., Schmidt, P., Schleucher, J., Schedletzky, O., Glaser, S.J. and Griesinger, C. (1995), *J. Magn. Reson.*, in press.
Scheek, R.M., Boelens, R., Russo, N., Van Boom, J.H. and Kaptein, R. (1984) *Biochemistry*, **23**, 1371–1376.
Schleucher, J., Sattler, M. and Griesinger, C. (1993) *Angew. Chem., Int. Ed. Engl.*, **32**, 1489–1491.
Schwalbe, H., Marino, J.P., King, G.C., Wechselberger, R., Bermel, W. and Griesinger, C. (1994) *J. Biomol. NMR*, **4**, 631–644.
Schwalbe, H., Samstag, W., Engels, J.W., Bermel, W. and Griesinger, C. (1993) *J. Biomol. NMR*, **3**, 479–486.
Shaka, A.J., Barker, P. and Freeman, R. (1985) *J. Magn. Reson.*, **64**, 547–552.
Shaka, A.J., Lee, C.J. and Pines, A. (1988) *J. Magn. Reson.*, **77**, 274–293.
Sklenář, V., Peterson, R.D., Rejante, M. and Feigon, J. (1993a) *J. Biomol. NMR*, **3**, 721–727.
Sklenář, V., Peterson, R.D., Rejante, M.R., Wang, E. and Feigon, J. (1993b) *J. Am. Chem. Soc.*, **115**, 12181–12182.
Van de Ven, F.J.M. and Hilbers, C.W. (1988) *Eur. J. Biochem.*, **178**, 1–38.
Varani, G. and Tinoco Jr., I.J. (1991) *Q. Rev. Biophys.*, **24**, 479–532.
Wyatt, J.R. and Tinoco Jr., I.J. (1993) *The RNA World*, Cold Spring Harbor Laboratory Press, Plainview, NY.