NMR spectrum of the parent metallabutadiyne, **1**, exhibits 0.8 Hz splittings of the *H*—C≡W—C≡C—*H* resonances, arising from the coupling of these two nuclei over five bonds; by comparison, $^5J_{HH}$ = 2.2 Hz for butadiyne.[15] This result is indicative of π-electron delocalization within the C≡W—C≡C backbone of **1**.[16]

The molecular structure of **2** was determined by a single-crystal X-ray diffraction study,[17] which revealed methylidyne and (trimethylsilyl)ethynyl ligands arranged trans to each other about a pseudooctahedral tungsten center (Figure 1). Along the backbone, the C—W—C—C—Si assemblage is nearly linear, with only the methylidyne hydrogen lying off this axis; the reasons for the bending of the methylidyne ligand have been discussed previously for W(CH)(dmpe)$_2$(*n*-Bu).[13] The bond distances within the backbone indicate that the bond order alternates along the chain according to H—C≡W—C≡C—Si: the W≡C (1.801 (7) Å) and C≡C (1.228 (9) Å) distances are within the normal ranges for those of triply bonded W(CH)(dmpe)$_2$X (X = *n*-Bu, 1.826 (5) Å;[13] X = Cl, 1.797 (10) Å[18]) and metal-alkynyl complexes,[5c,19,20] respectively, while the HCW—CCR bond is significantly longer (2.246 (6) Å) than either of the bonds flanking it. This latter distance is less than that expected for a W—C single bond in this environment, however, as evidenced by the fact that it is 0.16 Å shorter than that for the nonconjugated compound W(CH)(dmpe)$_2$(*n*-Bu) (2.402 (7) Å);[13] based on the single-bond covalent radii of sp- and sp^3-hybridized carbons, a difference of only 0.08 Å between the W—C bond distances is expected. This contraction of the W—C bond for **2** suggests the presence of a π-bonding interaction between the C≡W and C≡C fragments.

The π(C≡W—C≡C) conjugation suggested by the NMR data for **1** and the structural data for **2** is strongly manifested by the electronic spectra of metallabutadiynes **1-4**. These compounds, and other derivatives of the type W(CH)(dmpe)$_2$X (X = Cl, I, *n*-Bu),[21] display a weak ($\epsilon \cong 400$) band as the lowest energy feature. It has been suggested previously that the orbital character of this electronic transition in molecules of the type W(CPh)L$_4$X (X = halide) is $d_{xy} \rightarrow \pi^*$(W≡CPh), the terminating orbital of which possesses metal d_{xz} or d_{yz} parentage (these levels are nondegenerate under C_{2v} symmetry);[22,23] by analogy, we suggest that the $d_{xy} \rightarrow \pi^*$(W≡C) assignment is also appropriate here. For nonconjugated W(CH)(dmpe)$_2$X complexes, the energy of this transition is largely insensitive to the nature of the trans ligand (X = Cl, $\bar{\nu}_{max}$ 24 810 cm^{-1}; X = I, $\bar{\nu}_{max}$ 24 210; X = *n*-Bu, $\bar{\nu}_{max}$ 23 470).[21] In contrast, this absorption band lies to distinctly lower energy for **1-4** (**1**, $\bar{\nu}_{max}$ 22 270 cm^{-1}; **2**, 21 830; **3**, 21 280; **4**, 20 240) and, impressively, spans a broader energy range as a function of the alkynyl R group (2000 cm^{-1}) than do the corresponding bands of the halide and alkyl derivatives (1300 cm^{-1}).

The structural and spectroscopic data for **1-4** indicate that the C≡W—C≡C backbones of these metallabutadiynes are π-conjugated. We advance the following molecular orbital interpretation for these results. The energy and orbital interactions of the tungsten d_{xy} orbital are independent of the nature of the axial ligand, to first order, since this orbital is nonbonding (δ-symmetry) with respect to the HC≡W—C≡CR axis. In contrast, mixings among the π*(C≡CR) and π*(W≡C) orbitals are symmetry allowed; these should stabilize π*(W≡C) and destabilize π*(C≡CR), resulting in a red shift of the $d_{xy} \rightarrow \pi^*$(W≡C) transition of metallabutadiynes **1-4** relative to W(CH)(dmpe)$_2$X derivatives with σ or, more so, σ/π-donor X ligands. The overall trend in spectroscopic energies (halide > alkyl > alkynyl) is consistent with this, as is the red shift within the metallabutadiyne series (**1** > **2** > **3** > **4**) with increasing π-conjugation of the alkynyl R group. Since the π*(C≡CR)–π*(W≡C) interactions occur among virtual orbitals, however, they cannot be responsible for the shortened HCW—CCR bond. This must arise from interactions between occupied and virtual orbitals, of which π(C≡CR)–π*(W≡C) and π(W≡C)–π*(C≡CR) are the most likely candidates. We are not able to distinguish between these possibilities with our current data, although we note that the latter is consistent with the observation of a relatively long C≡C bond in **2**.

In conclusion, we have discovered a class of π(C≡W—C≡C)-conjugated metallabutadiynes. The synthesis of these internally substituted butadiynes opens a wide range of possibilities as regards the perturbation of the properties and electronic structures of polydiacetylenes and related oligomers; such compounds, and long-chain metallabutadiynes, are currently under investigation.

Acknowledgment. We thank the National Science Foundation for the support of this research.

Registry No. **1**, 143493-89-4; **2**, 143493-90-7; **3**, 143493-91-8; **4**, 143493-92-9; W(CH)(dmpe)$_2$(O$_3$SCF$_3$), 143493-93-0.

(15) Snyder, E. I.; Roberts, J. D. *J. Am. Chem. Soc.* **1962**, *84*, 1582-1586.
(16) (a) McConnell, H. M. *J. Mol. Spectrosc.* **1957**, *1*, 11-17. (b) Karplus, M. *J. Chem. Phys.* **1960**, *33*, 1842-1849. (c) Cunliffe, A. V.; Grinter, R.; Harris, R. K. *J. Magn. Reson.* **1970**, *3*, 299-318.
(17) X-ray crystallographic data for **2**: $P2_1/n$; a = 9.840 (2) Å, b = 16.569 (3) Å, c = 17.391 (3) Å, β = 100.21 (1)°, V = 2790.5 (10) Å^3, Z = 4, D_c = 1.415 g cm^{-3}, μ(Mo Kα) = 44.13 cm^{-1}, T = 225 K. Of 4387 reflections collected (4° ≤ 2θ ≤ 48°), 3339 with $F_o > 4.0\sigma(F_o)$ were used in the refinement of the structure. Data were corrected for absorption (XABS) and extinction effects. The structure was solved by direct methods. All non-hydrogen atoms were located and refined anisotropically, except for the C-C backbone of one of the dmpe ligands, which is disordered and was refined isotropically into two configurations. The methylidyne hydrogen was located, and refined isotropically (U = 0.089 (29) Å^2). All other hydrogen atom positions were calculated (d(C-H) = 0.96 Å). The structure was refined to R_F = 2.68%, R_{wF} = 3.25%, GOF = 0.99; Δ/ρ = 0.94 e Å^{-3} (near W).
(18) Manna, J.; Geib, S. J.; Hopkins, M. D. Unpublished results.
(19) (a) Nast, R. *Coord. Chem. Rev.* **1982**, *47*, 89-124. (b) Erker, G.; Frömberg, W.; Benn, R.; Nynott, R.; Angermund, K.; Krüger, C. *Organometallics* **1989**, *8*, 911-920.
(20) The C≡C distance for **2** appears to be slightly long, although data for η^1-CCSiMe$_3$ complexes are scarce: C≡C distances of 1.193 (6) and 1.202 (9) Å, respectively, are found for Pd(N(CH$_2$CH$_2$PPh$_2$)$_3$)(CCSiMe$_3$)Br (de Graaf, W.; Harder, S.; Boersma, J.; van Koten, G.; Kanters, J. A. *J. Organomet. Chem.* **1988**, *358*, 545-562) and [Rh(dmpe)$_2$H(CCSiMe$_3$)]$_2$(μ-dmpe) (Chow, P.; Zargarian, D.; Taylor, N. J.; Marder, T. *J. Chem. Soc., Chem. Commun.* **1989**, 1545-1547).
(21) Mlinar, L. A.; Hopkins, M. D. Unpublished results.
(22) Manna, J.; Gilbert, T. M.; Dallinger, R. F.; Geib, S. J.; Hopkins, M. D. *J. Am. Chem. Soc.* **1992**, *114*, 5870-5872.
(23) Bocarsly, A. B.; Cameron, R. E.; Rubin, H.-D.; McDermott, G. A.; Wolff, C. R.; Mayr, A. *Inorg. Chem.* **1985**, *24*, 3976-3978.

Specific Abstraction of the 5′(*S*)- and 4′-Deoxyribosyl Hydrogen Atoms from DNA by Calicheamicin γ$_1^I$

Jon J. Hangeland, James J. De Voss, Julie A. Heath, and Craig A. Townsend*

Department of Chemistry, The Johns Hopkins University Baltimore, Maryland 21218

Wei-dong Ding, Joseph S. Ashcroft, and George A. Ellestad*

American Cyanamid Company, Medical Research Division Lederle Laboratories, Pearl River, New York 10965

Received July 2, 1992

The 1,4-diyl (**2**) generated from calicheamicin γ$_1^I$ (**1**, CLM) by reductive activation[1,2] and rearrangement[3,4] is believed to initiate DNA cleavage by hydrogen atom abstraction from both strands of the helix to give the reduced form of the drug, CLM ϵ (**3**).

(1) Lee, M. D.; Dunne, T. S.; Siegel, M. M.; Chang, C. C.; Morton, G. O.; Borders, D. B. *J. Am. Chem. Soc.* **1987**, *109*, 3464-3466. Lee, M. D.; Dunne, T. S.; Chang, C. C.; Ellestad, G. A.; Siegel, M. M.; Morton, G. O.; McGahren, W. J.; Borders, D. B. *J. Am. Chem. Soc.* **1987**, *109*, 3466-3468. Ellestad, G. A.; Hamann, P. R.; Morton, G. O.; Siegel, M. M.; Pastel, M.; Borders, D. B.; McGahren, W. J. *Tetrahedron Lett.* **1989**, *30*, 3033-3036.
(2) (a) Zein, N.; Sinha, A. M.; McGahren, W. J.; Ellestad, G. A. *Science* **1988**, *240*, 1198-1201. (b) Zein, N.; Poncin, M.; Nilakantan, R.; Ellestad, G. A. *Science* **1989**, *244*, 697-699.
(3) Bergman, R. G. *Acc. Chem. Res.* **1973**, *6*, 25-31. Darby, N.; Kim, C. U.; Salaün, J. A.; Shelton, K. W.; Takada, S.; Masamune, S. *J. Chem. Soc., Chem. Commun.* **1971**, 1516-1517. Wong, H. N. C.; Sondheimer, F. *Tetrahedron Lett.* **1980**, *21*, 217-220 and references cited.
(4) De Voss, J. J.; Hangeland, J. J.; Townsend, C. A. *J. Am. Chem. Soc.* **1990**, *112*, 4554-4556.

0002-7863/92/1514-9200$03.00/0

Scheme I

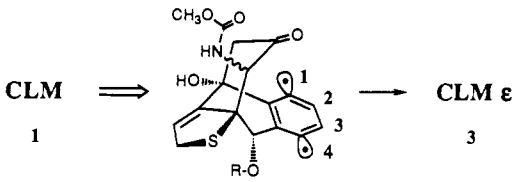
5'-d(G G G T C[5]C T A A A T T)-3'
3'-d(C C[23]C A G G A T T T A A)-5'

4

5'-d(C C C G G T C[7]C T A A G)-3'
3'-d(G G G C[21]C A G G A T T C)-5'

5

Atom-transfer experiments with synthetic dodecamers containing a TCCT recognition sequence have shown that deuterium label is selectively removed from the 5'-carbon of the penultimate 5'-pyrimidine (dC) during cleavage by the drug.[5] In this paper we demonstrate the principal site of hydrogen abstraction on the complementary strand and establish for the first time in a small molecule·DNA interaction that one of the two diastereotopic 5'-hydrogens is specifically removed from the TCCT strand. The remarkable specificity of these transfers with respect to both the origins and termini of atom migrations defines structural and kinetic elements of the drug/DNA reaction.

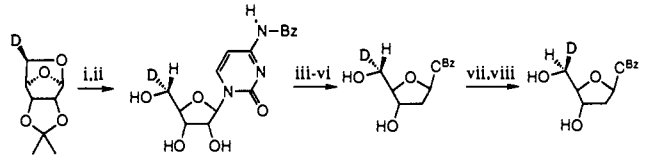


2

To determine the site of hydrogen atom abstraction from the strand complementary to the TCCT recognition sequence, it was necessary to redesign the dodecamer used in previous experiments[5] (**4**, Scheme I) to remove end effects that greatly reduced the efficiency of hydrogen atom transfer from dC[23].[6] To ensure stable helical structure at both sites, the TCCT sequence was moved toward the 3'-end of the strand bearing the TCCT sequence thereby placing the opposing strand cleavage site four base pairs from its 5'-end (**5**, Scheme I). High-resolution polyacrylamide gel electrophoresis (PAGE) analysis coupled with phosphorimager quantification of DNA fragments of **5** produced by the action of CLM showed dC[7] and dC[21] to be cleaved to the extent of 98 ± 1% and 94 ± 5%, respectively.[7]

We first reexamined the atom-transfer process from [5'-$^2H_2$]dC[7] in dodecamer **5** under conditions identical to those reported previously with the hope of confirming our earlier findings with labeled **4**.[5] We were pleased that the efficiency of atom transfer (81 ± 1%)[8] was identical within experimental error and occurred exclusively to C-4 of **3**. A control experiment carried out with unlabeled dodecamer **5** in a deuteriated medium revealed that, in contrast to our previously reported observation with dodecamer **4**,[5] solvent-derived deuterium accounted for 10 ± 1% d_1 and 2 ± 1% d_2 content in the derived CLM ε (**3**).[8] The distribution of heavy isotope between the two aryl sites in **3** was estimated by careful integration of the corresponding ^1H NMR spectrum

Scheme II[a]



[a] (i) N^4,O^2-bis(trimethylsilyl)-N^4-benzoylcytosine, $SnCl_4$, ACN;[13] (ii) 95% aqueous TFA;[14] (iii) 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, pyridine;[15] (iv) phenyl thionochloroformate, DMAP, ACN;[15] (v) Bu_3SnH, AIBN, benzene;[15] (vi) TBAF, THF;[15] (vii) DEAD, PPh_3, 4-nitrobenzoic acid (5 equiv), HMPA;[16] (viii) NaOMe, MeOH/THF.[17]

at C-4 (ca. 2%) and C-1 (ca. 12%). In contrast to the high efficiency of deuterium transfer from [5'-$^2H_2$]dC[7], no isotope was detected from [4'-^{2}H]- or [1'-^{2}H]dC[7] **5**.[10] The principal site(s) of hydrogen abstraction from the AGGA strand of dodecamer **5** was established in like fashion. Thus when CLM was incubated with [4'-^{2}H]dC[21] **5**, the CLM ε (**3**) isolated showed complementary deuterium incorporation[8] at only C-1 to the extent of 63 ± 2%, entirely consistent with a single drug orientation[5] and the observation of 3'-phosphoglycolate oligonucleotide fragments by high-resolution gel electrophoresis.[2b] No isotopic enrichment was seen when [1'-^{2}H]dC[21] **5** was employed whereas a small amount, ca. 2%, was detected from [5'-$^2H_2$]dC[21] **5**.

Atom-transfer events from the TCCT strand were further resolved using (5'*R*)- and (5'*S*)-[5'-^{2}H]dC[7] **5** synthesized from the corresponding stereospecifically labeled monomers. (5'*R*)- and (5'*S*)-[5'-^{2}H]-N^4-benzoyl-2'-deoxycytidines (**8** and **9**) were available in 11% and 5% overall yields, respectively, from the (5'*R*)-[5-^{2}H]-1,5-anhydroribofuranose **6**[11] of Ohrui (Scheme II).[12] As anticipated for stereospecific diyl abstraction, only the 5'*S*-diastereomer transferred deuterium (89 ± 1% exclusively to C-4).[8]

The behavior of radiolabeled CLM-induced DNA fragments on high-resolution PAGE shows a 3-bp stagger disposed toward the 3'-end of each opposing strand consistent with CLM binding and cleavage in the minor groove.[2] This structural view is secured here by independent means to define virtually exclusive abstraction of DNA hydrogens from the 5'*S*- and 4'-loci of dC[7] and dC[21] of dodecamer **5**. These two hydrogens point into the minor groove at comparatively exposed sites near the exterior of the helix with proper alignment for reaction with CLM.[18] The specific transfer of heavy isotope from these sites to C-4 and C-1, respectively, of the proposed diyl **2** defines precisely the location and orientation of the cleavage portion of the drug within the minor groove,

(5) De Voss, J. J.; Townsend, C. A.; Ding, W.-d.; Morton, G. O.; Ellestad, G. A.; Zein, N.; Tabor, A. B.; Schreiber, S. L. *J. Am. Chem. Soc.* **1990**, *112*, 9669–9670.

(6) De Voss, J. J.; Townsend, C. A.; Ding, W.-d.; Ashcroft, J. S.; Ellestad, G. A. Unpublished results.

(7) More complex fragmentation patterns were observed on the AGGA strand, leading to larger errors. Percent of total cleavage at dC[21] of oligonucleotide **5** was estimated after piperidine treatment and observed to be remarkably reproducible for extents of reaction of <10–90%.

(8) Analytical methods improved over those used earlier[5] were applied in the atom-transfer experiments. Resonances for H-1 (δ 7.57) and H-4 (δ 7.18) in CLM ε (**3**) were well-separated at 500 MHz and were integrated instrumentally and by the cut-and-weigh method (±3%). CLM ε was then degraded[1] to the crystalline naphtho[2,3-*b*]thiophene-4,10-dione[9] and its deuterium content accurately determined by mass spectrometry (±1%).

(9) Doadt, E. G.; Iwao, M.; Reed, J. N.; Sniekus, V. In *Polynuclear Aromatic Hydrocarbons: Formation, Metabolism and Measurement*; Cooke, M., Dennis, A. J., Eds.; Batelle Press: Columbus, 1983.

(10) These deuterium incorporations are corrected for the actual deuterium content of each labeled form of dodecamer **5** accurately determined by mass spectrometry of stable intermediates in the respective syntheses: [5'-$^2H_2$]dC, 97 ± 1%; [4'-^{2}H]dC, 93 ± 1%; [1'-^{2}H]dC, 98 ± 1%; (5'*R*)- and (5'*S*)-[5'-^{2}H]dC, 97 ± 1%. A full accounting of the synthesis of each labeled 2'-deoxynucleoside will be reported elsewhere.

(11) Ohrui, H.; Misawa, T.; Meguro, H. *Agric. Biol. Chem.* **1984**, *48*, 1825–1829. Ohrui, H.; Misawa, T.; Meguro, H. *Agric. Biol. Chem.* **1985**, *49*, 239–240. For the syntheses of (5'*R*)- and (5'*S*)-[5'-^{2}H]adenosine, see: Ohrui, H.; Meguro, H. *Nucleic Acids Res.* **1986**, *17*, 29–32. For an example of a nonstereospecific synthesis of [5'-^{2}H]thymidine, see: Orban, J.; Reid, B. R. *J. Labelled Compd. Radiopharm.* **1989**, *27*, 195–198.

(12) Labeled ribonucleosides and 2'-deoxyribonucleosides containing any base should be accessible by this method. General applications of the diastereospecifically labeled 5'-^{2}H materials in NMR studies of DNA and RNA can be readily identified (e.g., see: Kline, P. C.; Serianni, A. S. *Magn. Reson. Chem.* **1988**, *26*, 120–123).

(13) Schneider, K. C.; Benner, S. A. *Tetrahedron Lett.* **1990**, *30*, 335–338. Vorbruggen, H.; Krolikiewicz, K.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1235–1255.

(14) Espie, J. C.; Lhomme, M. F.; Morat, C.; Lhomme, J. *Tetrahedron Lett.* **1990**, *31*, 1423–1426.

(15) Robbins, M. J.; Wilson, J. S.; Hansske, F. *J. Am. Chem. Soc.* **1983**, *105*, 4059–4064.

(16) Mitsunobu, O.; Kimura, J.; Fujisawa, Y. *Bull. Chem. Soc. Jpn.* **1972**, *45*, 245–247. Mitsunobu, O. *Synthesis* **1981**, *1*, 1–28.

(17) Rammler, D. H.; Khorana, H. G. *J. Am. Chem. Soc.* **1962**, *84*, 3112–3118.

(18) These experiments make possible revision of an earlier view of this process based on molecular modeling exercises: Hawley, R. C.; Kiessling, L. L.; Schreiber, S. L. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 1105–1109.

although greater solvent participation is seen on the AGGA side.[19] On the basis of the limited data and methods available earlier, no kinetic isotope effect appeared associated with removal of substrate deuterium by the activated drug.[5] However, careful phosphorimager evaluation of the several labeled oligonucleotides available in these and other experiments[20] have shown that small but reproducible isotope effects can be measured for the TCCT ($k_H/k_D = 1.2 \pm 0.2$) and the AGGA strands ($k_H/k_D = 1.4 \pm 0.1$). A full description of these experiments will be reported in due course.[21]

Acknowledgment. We are grateful to the National Institutes of Health (CA 54421) for partial financial support of this research. J.J.H. is partially supported by an A. H. Corwin fellowship. We are pleased to acknowledge Professor T. D. Tullius for use of a Molecular Dynamics phosphorimager obtained with funds from a NSF Biological Research Centers award (DIR 87-21059) and the W. M. Keck Foundation.

(19) Hydroxyl radical footprinting experiments have defined in greater detail the nature of the interactions of the entire drug with DNA at several binding and cleavage sites: Mah, S. C.; Townsend, C. A.; Tullius, T. D. Unpublished.
(20) Hangeland, J. J.; Townsend, C. A. Unpublished.
(21) See for comparison: Frank, B. L.; Worth, L., Jr.; Christner, D. F.; Kozarich, J. W.; Stubbe, J.; Kappen, L. S.; Goldberg, I. H. *J. Am. Chem. Soc.* **1991**, *113*, 2271-2275. Kappen, L. S.; Goldberg, I. H.; Frank, B. L.; Worth, I., Jr.; Christner, D. F.; Kozarich, J. W.; Stubbe, J. *Biochemistry* **1991**, *30*, 2034-2042.

Simple Procedure for Resonance Assignment of the Sugar Protons in ^{13}C-Labeled RNAs

Arthur Pardi* and Edward P. Nikonowicz

Department of Chemistry and Biochemistry
Campus Box 215, University of Colorado, Boulder
Boulder, Colorado 80309-0215
Received July 2, 1992

Scalar homonuclear 2D NMR experiments (such as DQF-COSY, RELAY, or TOCSY) are generally used to make resonance assignments of the sugar protons in nucleic acids.[1,2] However, the 2′ through 5′/5″ sugar protons in RNAs resonate over a very narrow chemical shift range (<1.0 ppm), making it difficult to resolve and assign these protons.[3] The utility of these homonuclear experiments is further limited in RNAs because the very small (≈1 Hz) H1′ to H2′ J coupling constant in A-form RNA makes it difficult to transfer magnetization from the crowded 2′ through 5′/5″ protons, to the well-resolved 1′ protons. To overcome this problem, we have designed a novel strategy for the unambiguous assignment of all protons in a ribose spin system involving application of 2D HCCH-COSY, HCCH-RELAY, and HCCH-TOCSY experiments.[4-6] This method is easily applied to any ^{13}C-labeled RNA and is illustrated here on the uniformly ^{13}C labeled RNA duplex, r(GGCGCUUGCGUC)$_2$.

In an HCCH experiment, connectivities between two protons in an individual ribose ring are made by transferring magnetization through their intervening carbon atoms.[4-6] For example, an HCCH-TOCSY experiment on an RNA duplex identifies all protons in the same ribose spin system (Figure S1, supplementary material). However, the proton type cannot be assigned from the TOCSY experiment alone; therefore, a series of HCCH experiments (Figure 1) are employed to assign the 2′, 3′, 4′, and 5′/5″

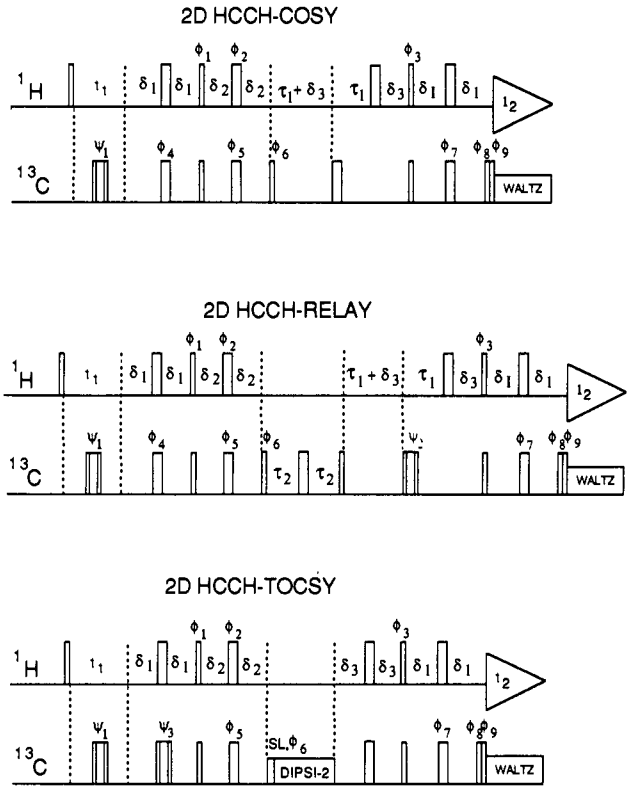


Figure 1. Pulse sequences used to collect the 2D HCCH experiments.[4,5] Narrow bars represent $\pi/2$ pulses, and wide bars represent π pulses. ψ denotes a composite π pulse of the form x–y–x. Unless otherwise stated, all pulses were of phase x. The phases of the pulses, are $\phi_1 = y, -y$; $\phi_2 = 4(x), 4(-x), 4(y), 4(-y)$; $\phi_3 = 4(x), 4(-x)$; $\phi_4 = 2(y), 2(-y)$; $\phi_5 = 2(x), 2(y), 2(-x), 2(-y)$; $\phi_6 = 4(x), 4(-x)$; $\phi_7 = 4(x), 4(-x)$, $\phi_8 = 16(x)$; $\phi_9 = 4(x), 4(-x)$; $\psi_1 = 8(x), 8(-x)$; $\psi_2 = 16(x)$; $\psi_3 = 2(x), 2(-x)$; and receiver = $(x, -x, -x, x, -x, x, x, -x)$. In the HCCH-TOCSY experiments, SL is a trim pulse of 1.2-ms duration. The delays in each of the experiments were $\delta_1 = 1.7$ ms, $\delta_2 = 1.1$ ms, $\delta_3 = 1.1$ ms, $\tau_1 = 1.6$ ms, and $\tau_2 = 4.6$ ms, except for one of the HCCH-TOCSY experiments, where $\delta_3 = 2.3$ ms, so that the 5′/5″ proton resonances are opposite in sign to the other ribose proton resonances. The HCCH-TOCSY experiments employed a 23-ms spin lock period using the DIPSI-2 sequence. In all the experiments the ^{13}C carrier was positioned in the center of the ribose region (~80 ppm). Sweep widths of 1600 and 3200 Hz were used in t_1 and t_2, respectively, 80 scans were collected for each FID, 120 complex points were collected in t_1, and 1024 complex points were collected in t_2. The NMR data were processed using FELIX (Hare, Inc.). The data were zero filled in t_1 and t_2 before Fourier transformation to give final real matrix sizes of 1024 × 2048 points.

protons as illustrated in Figure 2. Figure 2a shows part of the H1′ (ω_1) to the H2′ through H5′/H5″ (ω_2) region of a 2D HCCH-COSY experiment on the RNA duplex. The HCCH-COSY experiment only transfers magnetization through a single carbon–carbon bond,[4,5] and therefore the H1′ to H2′ is the only cross peak in this region. Figure 2b shows the same region of an HCCH-RELAY spectrum where magnetization is transferred up to two carbon–carbon bonds. Thus the new cross peak in this spectrum can only arise from a H1′ to H3′ connectivity. To assign the 4′ and 5′/5″ protons we employ refocused INEPT/reverse INEPT proton–carbon polarization transfers[7,8] in the HCCH-TOCSY experiment to differentiate between methylene and methyne protons (Figure 1). The HCCH-TOCSY experiment is used to filter out, or to select for, 5′/5″ methylene protons as illustrated in parts c and d, respectively, of Figure 2. In the first experiment (F_1), the reverse INEPT delay was set so that the 5′/5″ (methylene) protons are opposite in sign of all the other ribose protons. In the second experiment (F_2), the delay was set so that all the ribose protons have the same sign and similar intensities.

* Author to whom correspondence should be addressed.
(1) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; John Wiley & Sons: New York, 1986.
(2) van de Ven, F. J. M.; Hilbers, C. W. *Eur. J. Biochem.* **1988**, *178*, 1.
(3) Varani, G.; Tinoco, I. *Q. Rev. Biophys.* **1991**, *24*, 479.
(4) Kay, L. E.; Ikura, M.; Bax, A. *J. Am. Chem. Soc.* **1990**, *112*, 888.
(5) Clore, G. M.; Bax, A.; Driscoll, P. C.; Wingfield, P. T.; Gronenborn, A. M. *Biochemistry* **1990**, *29*, 8172.
(6) Fesik, S. W.; Eaton, H. L.; Olejniczak, E. T.; Zuiderweg, E.; Mcintosh, L. P.; Dahlquist, F. W. *J. Am. Chem. Soc.* **1990**, *112*, 886.
(7) Burum, D. P.; Ernst, R. R. *J. Magn. Reson.* **1980**, *39*, 163.
(8) Morris, G. A.; Freeman, R. *J. Am. Chem. Soc.* **1979**, *101*, 760.

0002-7863/92/1514-9202$03.00/0