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Supplementary Material for Microfilm Edition

Sequential Assignments.

The sequential assignment procedure started with the analysis of the characteristic thymine $5CH_3$ and cytosine 5H resonances. Of particular value were the sequential $d_S(6,8;5H,5CH_3)$ NOEs, providing 22 (out of 30 possible) well-resolved connectivities. In addition, all $d_S(5H,5CH_3;5H,5CH_3)$ NOEs for cytidines next to a thymidine were readily observed (T_6/C_7, C_{21}/T_{22}, T_{22}/C_{23}, C_{42}/T_{43}, C_{56}/T_{57}), as were the sole $d_S(5H;5H)$ NOE (C_{50}/C_{51}) and $d_S(5CH_3;5CH_3)$ NOEs (T_{36}/T_{37}, T_{60}/T_{61}).

Careful processing of the JR-NOESY spectrum acquired at 305K, optimizing for resolution, allowed for identification of numerous of the standard B-type DNA sequential NOEs $d_S(1'H,2'H,2''H;6H,8H)$ (Figure S1 and S2). At least one such NOE was resolved for 88% of the residues (13 in strand 1, 13 in strand 2, 13 in strand 3, 14 in strand 4). Another important sequential NOE was the $d_S(6H,8H;6H,8H)$, which provided the critical connectivity for certain residues.

In the following, a rather detailed description of the sequential resonance assignment of strand 1 is given, illustrating the manner in which critical NOE connectivities are pieced together.

A good starting point for the assignment of strand 1 is the unique d-(AATC) tract. Only two pairs of adjacent adenine residues, A_4/A_5 and A_{28}/A_{29}, are found in J2P1. Both pairs were linked by readily identified $d_S(1'H;8H)$ NOEs. $d_S(8H;5CH_3)$ and $d_S(1'H,2'H,2''H;6H)$ NOEs extended the connectivity for both, to T_6 and T_{30}, respectively. Observation of a $d_S(6H;5H)$ NOE to C_7 positively identified which of the two d-(AAT) segments corresponded to strand 1 ($A_{28}A_{29}T_{30}$ is followed by G_{31} in strand 3). Further evidence for the sequential connectivity to C_7 was obtained from the $d_S(1'H,2'H,2''H;6H)$ NOEs observed in the 2D plane at $\omega_1 = \delta(C_7\ 5H)$ of the 3D TOCSY/NOESY experiment. Connectivity to A_8 could be established despite the problem with the assignment of the 2"H

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resonance of C_7, through $d_S(6H;8H)$ and $d_S(2'H,3'H;8H)$ NOEs together with a $d_S(1'H;8H)$ NOE identified in the NOESY spectrum recorded in D_2O. The $d_S(1'H;8H)$ NOE could not be unambiguously identified in the JR-NOESY spectra due to overlap with one of the $d_i(5H;4NH_2)$ NOEs for C_7.

T_9 was difficult to assign because the 6H resonance of T_9 is in a very crowded region, broader lineshapes were observed for the resonances of this residue, and there is distorted conformation at the junction point for this crossover strand (see main text). T_9 could only be connected to A_8 by weaker $d_S(1'H,2'H,2''H,3'H;5CH_3)$ NOEs and a very weak $d_S(8H;5CH_3)$ NOE to the $5CH_3$ protons. There is a gap between T_9 and G_{10} because sequential NOEs between these two residues were ambiguous due to chemical shift degeneracy of the 8H resonance of G_{10} with several other guanosine 8H resonances. A_{11} showed well resolved resonances and extension backwards to G_{10} was possible from identification of $d_S(1'H,2'H,2''H,3'H;8H)$ and $d_S(8H,8H)$ NOEs. Connectivities on to G_{12}, C_{13}, A_{14} and C_{15} were obtained by identification of one or several of $d_S(1'H,2'H,2''H;6H,8H)$ NOEs and, where possible, $d_S(8H;5H)$ NOEs. These connectivities uniquely identified this tract as belonging to strand 1. Extension to G_{16} was obtained through resolved $d_S(1'H,2'H,2''H;8H)$, even though the G_{16} 8H resonace has the same chemical shift as five other guanosine 8H resonances. The uniquely downfield shift of the 1'H of a guanosine residue at the 3'-end of the DNA strand give further evidence for the 8H chemical shift of G_{16}.

Returning back to the 5'-end, C_1 is readily identified through it's uniquely down-field shifted 6H resonace. Sequential connectivity to G_2 was established through $d_S(1'H,2'H,2''H;8H)$ NOEs, and on to C_3 through $ds(1'H,2'H,2''H;6H)$ and $d_S(8H;5H)$ NOEs, which uniquely identifies this stretch to strand 1, and not strand 2. Observation of $d_S(1'H,2'H,2''H;8H)$ to A_4, finally completes the sequential assignment of strand 1.

In summary, the segments on either side of T_9/G_{10} were sequentially assigned on the basis of at least one unambiguous NOE, together with observation of several other sequential

and intra-residue cross peaks. No evidence for connectivity between the two fragments was obtained, but the sequential assignments are still unambiguous as the sequences uniquely fit to strand 1.

The sequential assignment of the labile protons relies primarily on observation of connectivities between adjacent imino protons, together with NOEs corresponding to short inter-base-pair distances between the imino proton and the neighboring adenine 2H or cytosine $4NH_2$ protons. More indirect pathways exists, involving various NOEs to the adenine 2H, cytosine 5H and thymine $5CH_3$. Figure S3 shows a summary of all of the sequential and intra-base-pair connectivities identified for J2P1 in the various JR-NOESY spectra recorded at different temperatures.

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Figure S1A

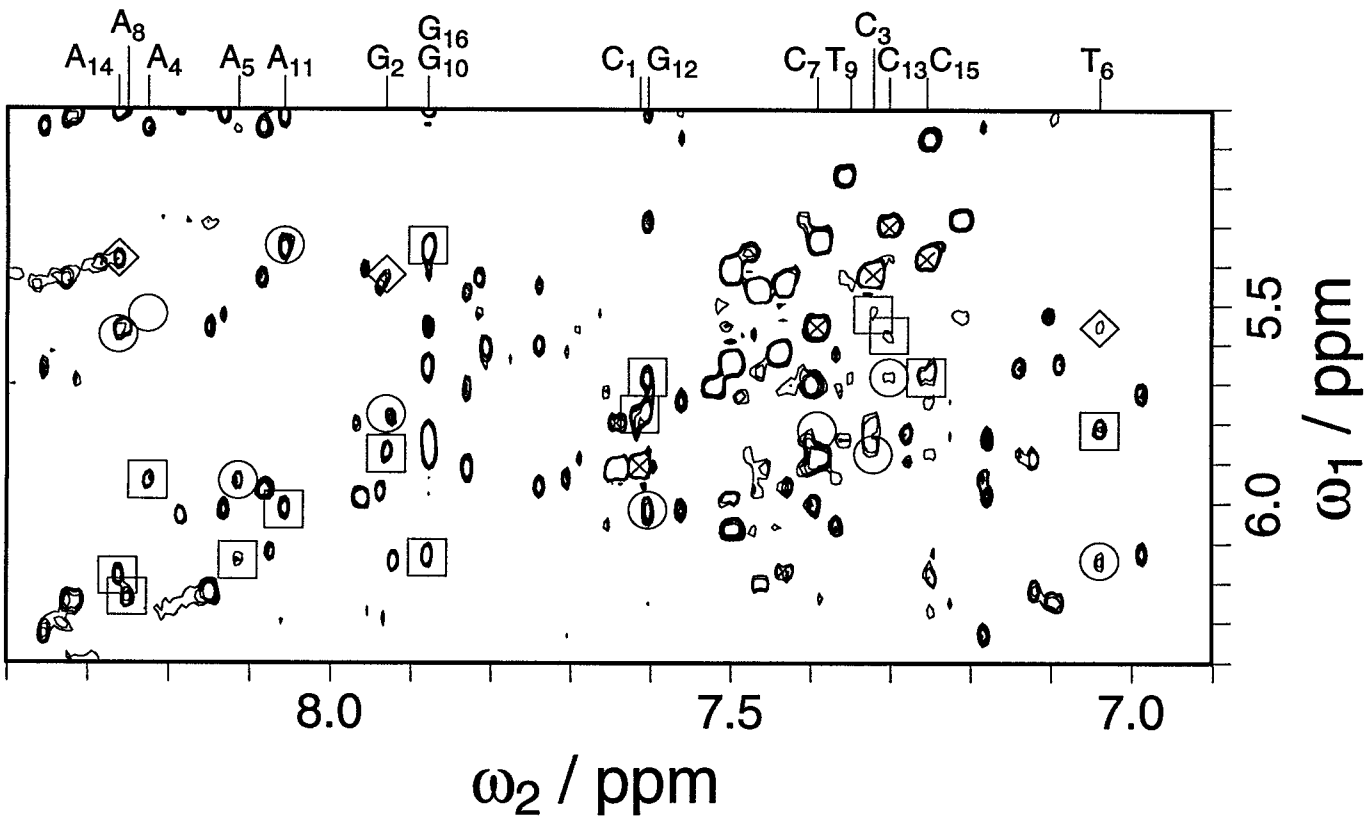

Figure S1. Region of the 600 MHz JR-NOESY spectrum of ~1 mM J2P1 containing cross peaks from 1'H and 5H resonances to base proton resonances. Separate panels are shown for each of the four strands: A) 1-16; B) 17-32; C) 33-48; D) 49-64. The spectrum was acquired at 305K with a mixing time of 200 ms from a H_2O solution. The base proton frequencies are indicated at the top of each panel with unambiguously identified intraresidue and sequential NOEs identified by boxes and circles, respectively. The strong intraresidue cytosine $d_i(5H;6H)$ NOEs are marked with an X within the crosspeak, and the unambiguously identified sequential $d_s(6H,8H;5H)$ NOEs are identified by diamonds.

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Figure S1B

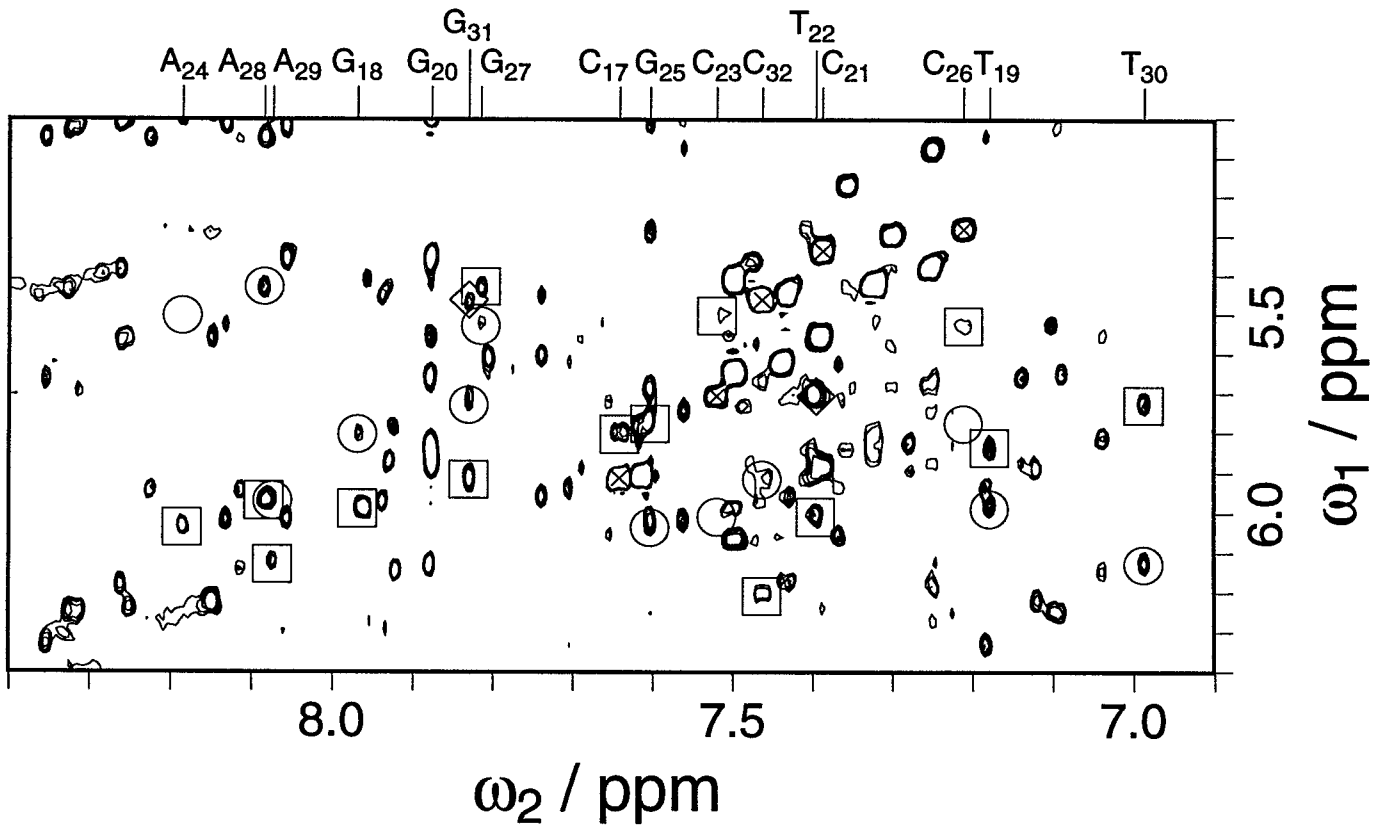

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Figure S1C

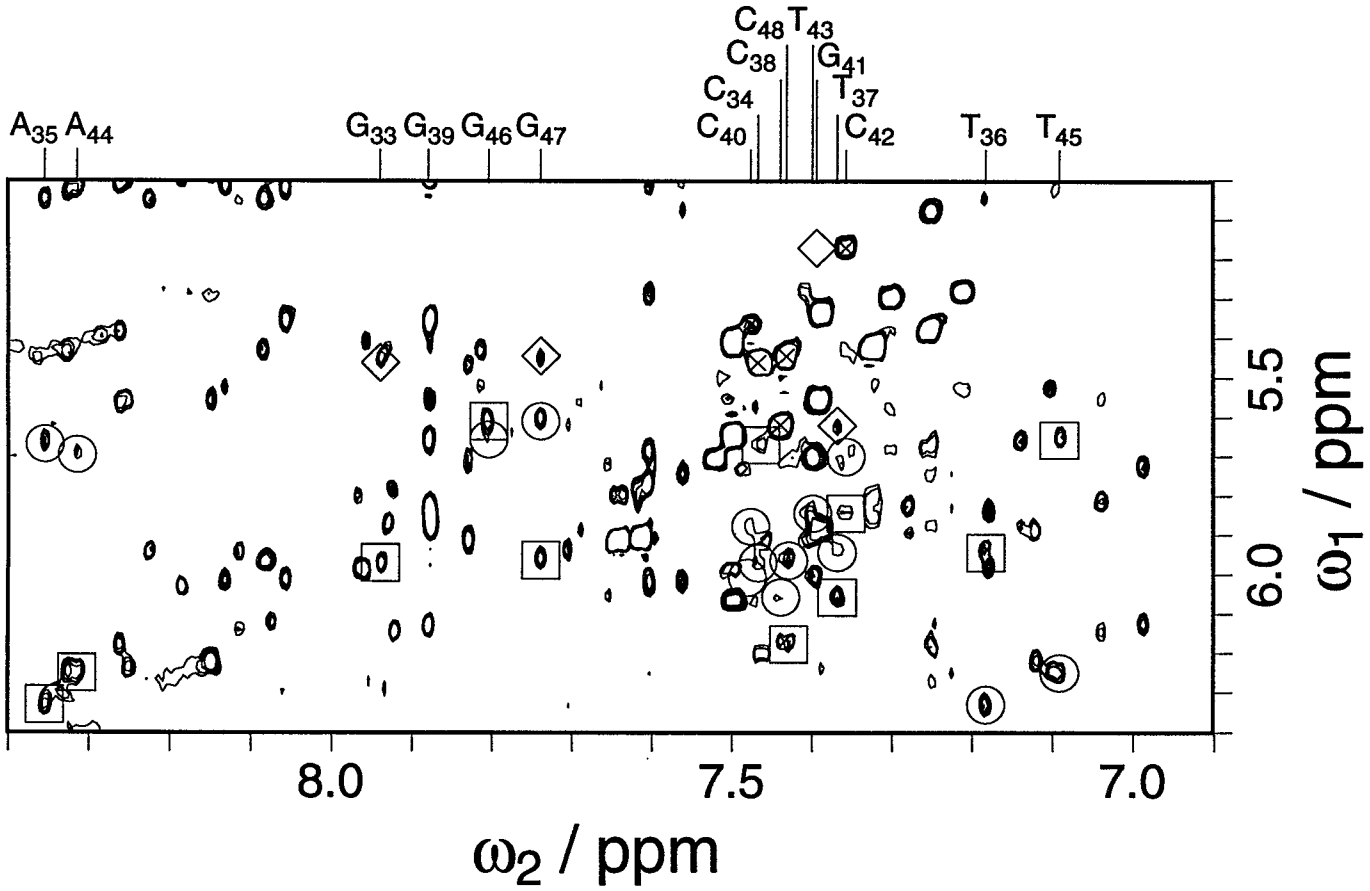

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Figure S1D

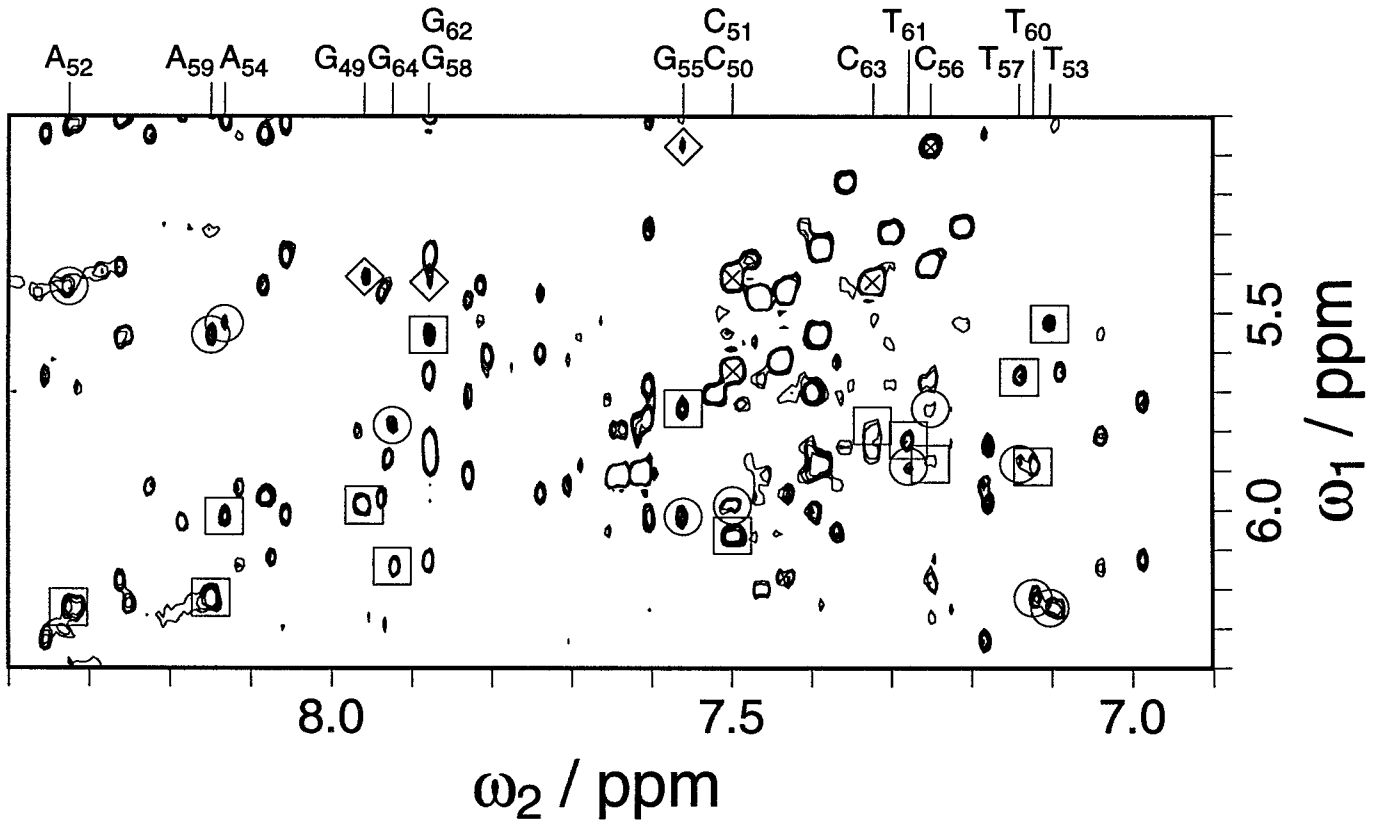

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Figure S2A

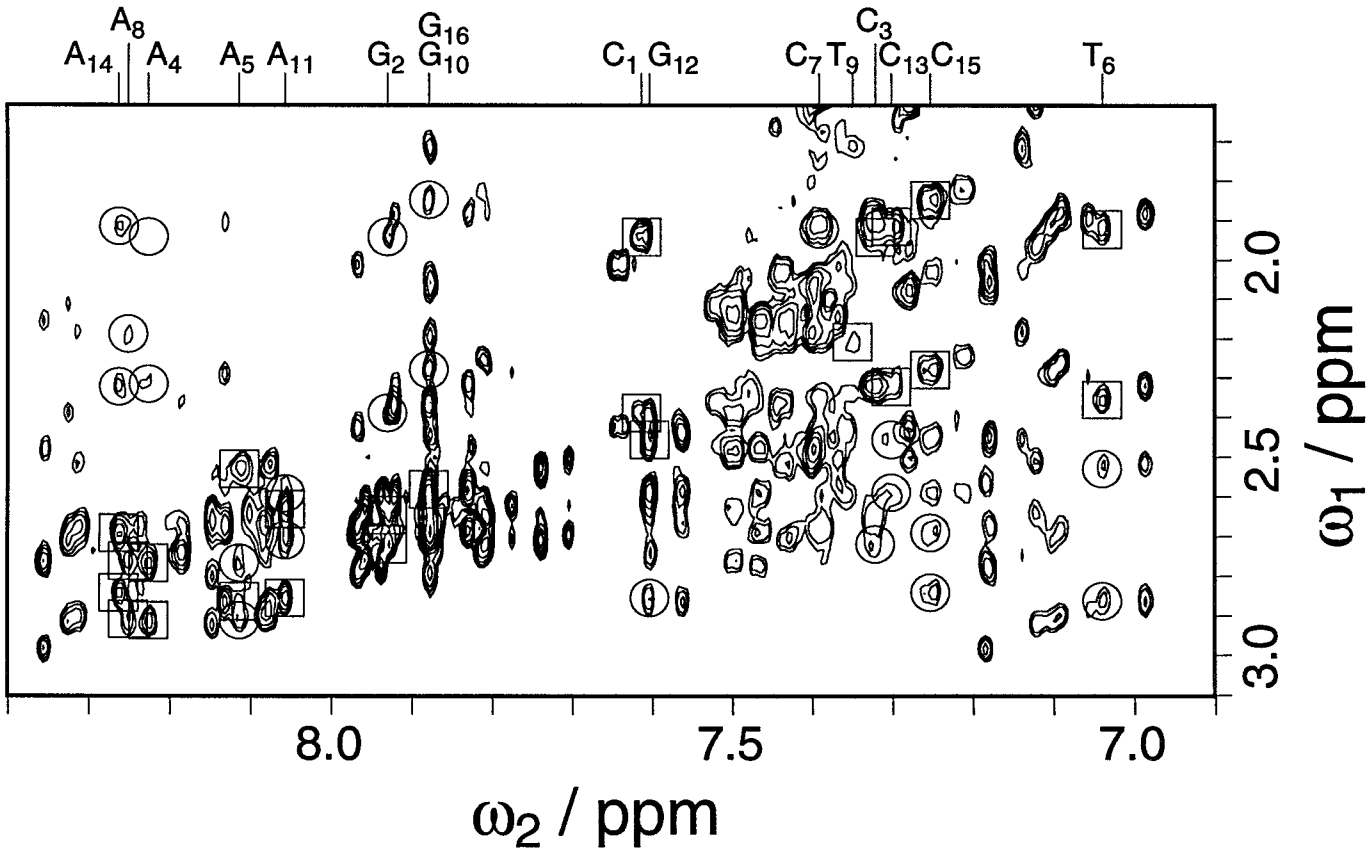

Figure S2. Region of the 600 MHz JR-NOESY spectrum of ~1 mM J2P1 containing cross peaks from 2'H and 2"H resonances to base proton resonances. Separate panels are shown for each of the four strands: A) 1-16; B) 17-32; C) 33-48; D) 49-64. The spectrum was acquired at 305 K with a mixing time of 200 ms from a H_2O solution. The base proton frequencies are indicated at the top of each panel with unambiguously identified intraresidue and sequential NOEs identified by boxes and circles, respectively.

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Figure S2B

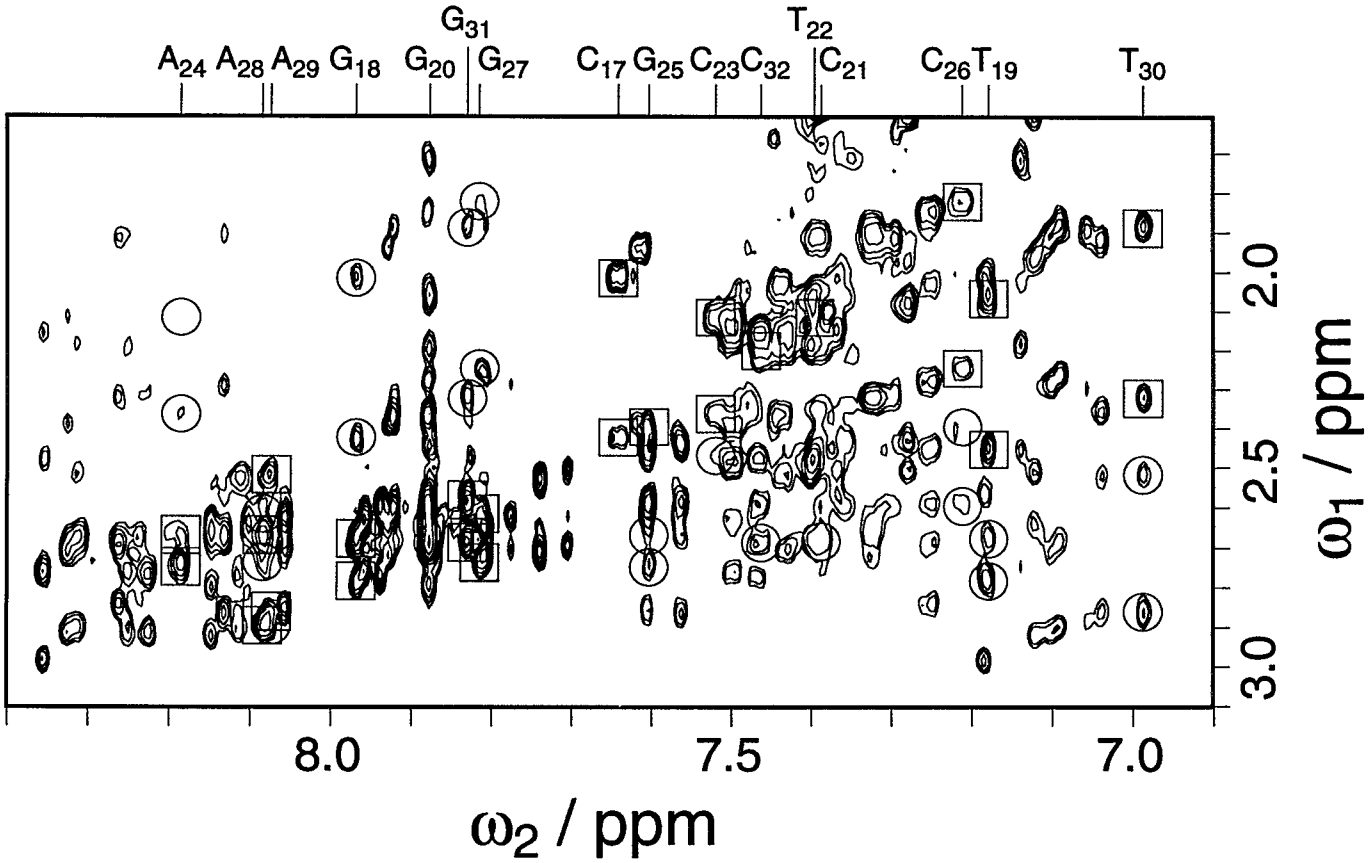

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Figure S2C

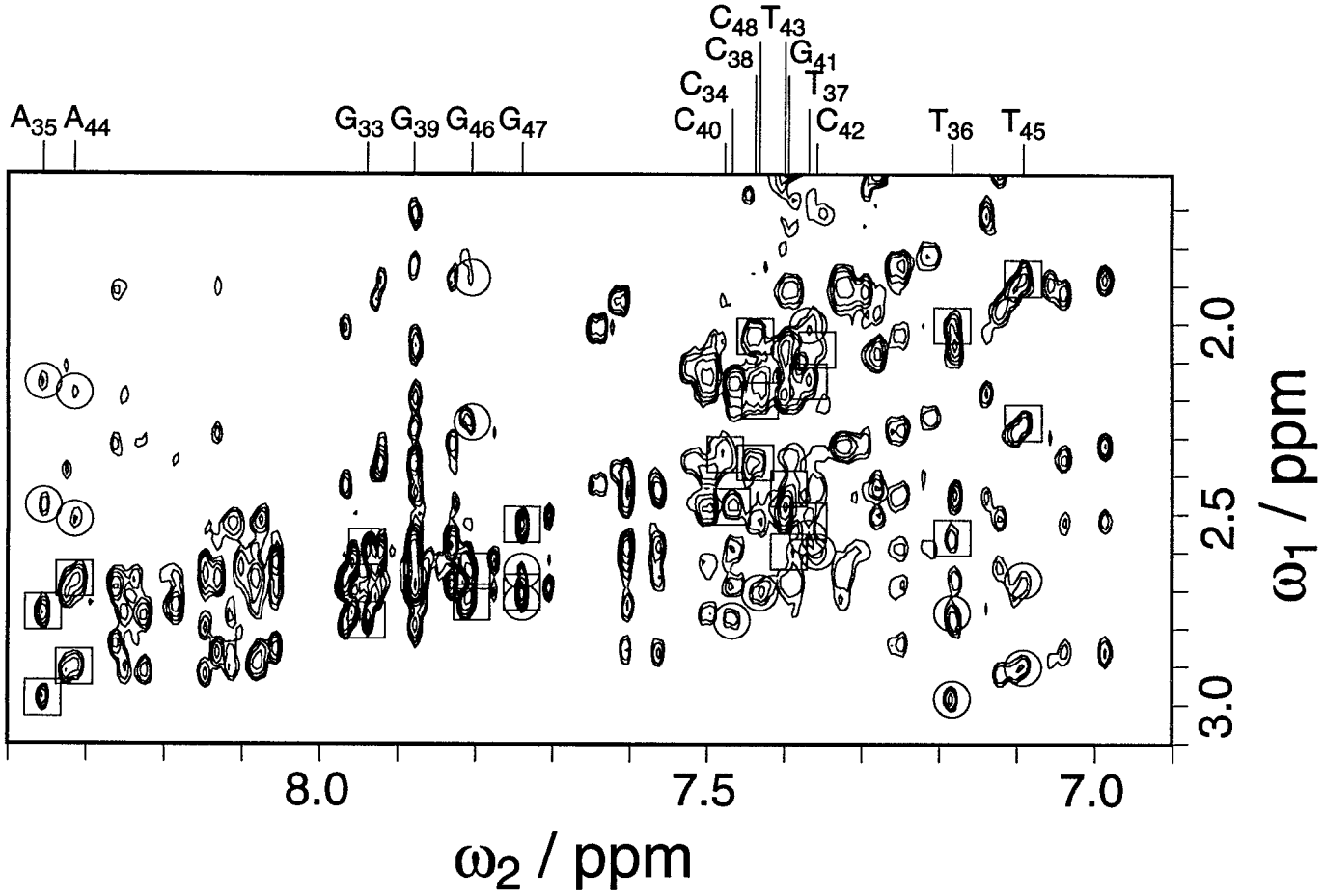

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Figure S2D

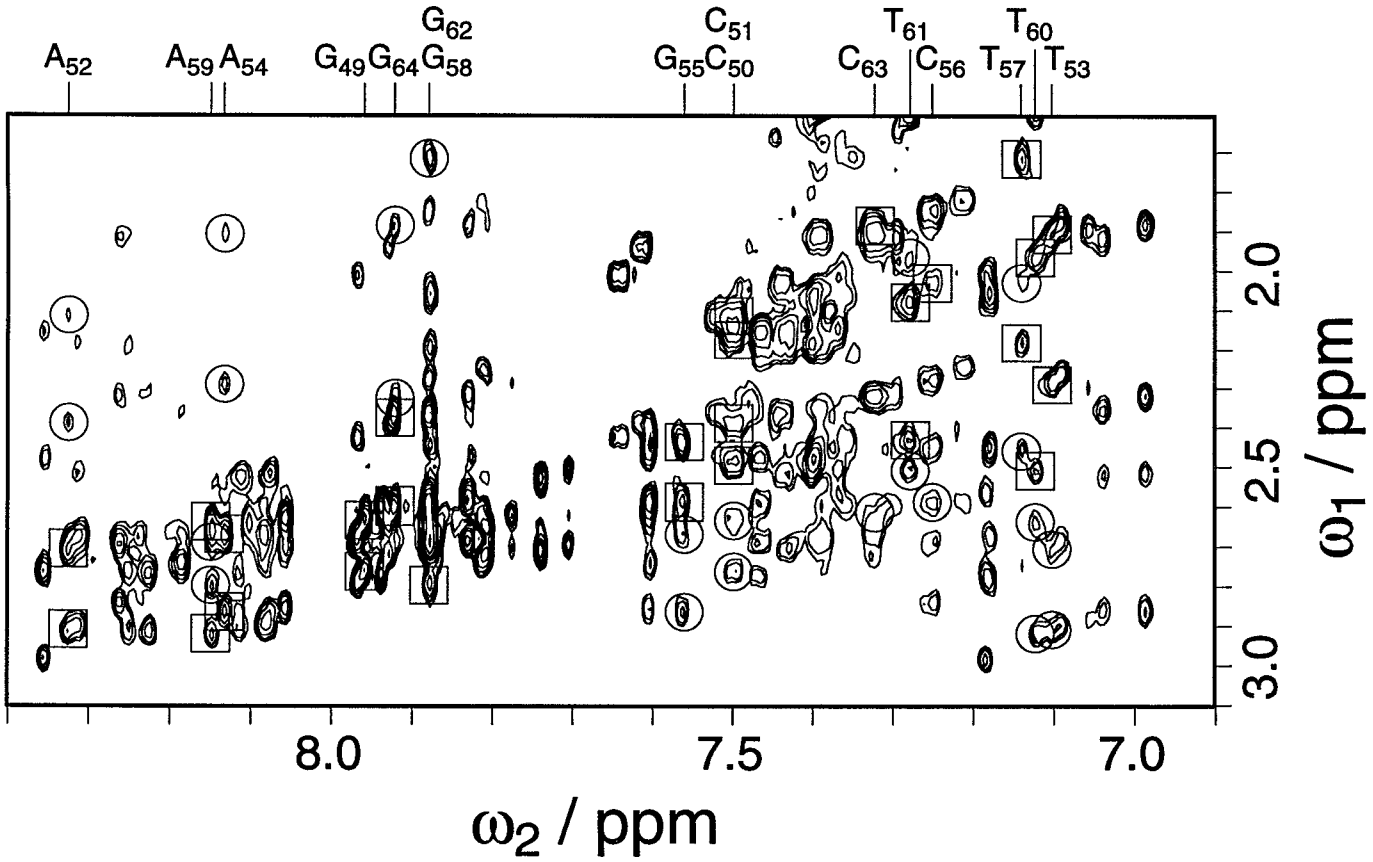

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Figure S3

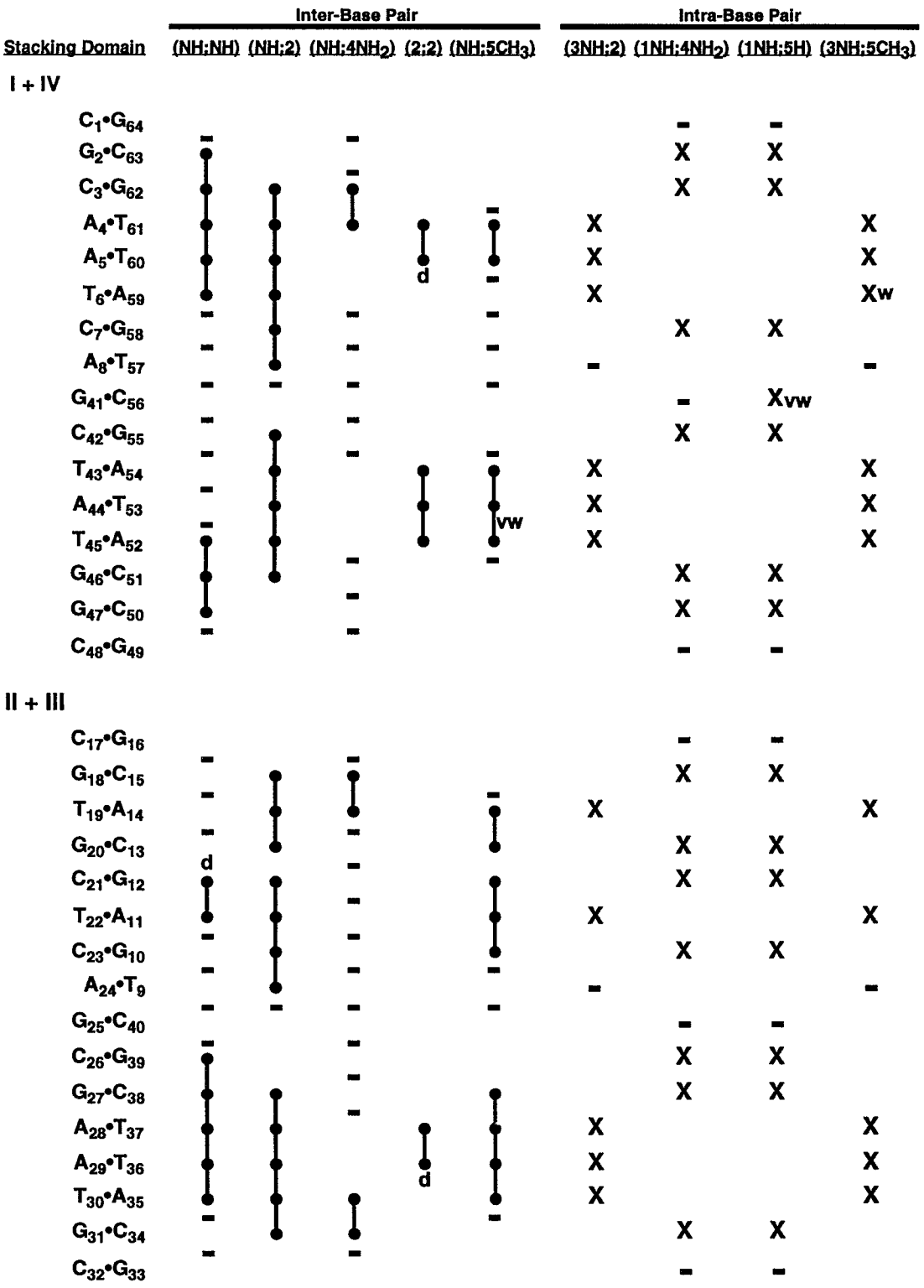

Figure S3. Summary of NOE cross peaks involving the labile protons of J2P1 observed in 2D JR-NOESY spectra obtained at 285, 293, 296, 300, and 305 K. A 'd' indicates that this NOE could not be observed due to degeneracy, and a 'w' and a 'vw' indicates that the observed crosspeak was 'weak' and 'very weak', respectively.