Transfer RNAs play a key role in decoding genetic information and protein synthesis. Although all tRNAs have similar shapes in order to fit properly to the ribosome, they each have distinct structural features that allow accurate recognition by their cognate aminoacyl-tRNA synthetases to ensure faithful translation in accordance to the genetic code (1). To understand the detailed mechanisms of the biochemical steps involved requires knowledge of the structures of tRNAs and their complexes at atomic resolution. Efforts in the past 20 years have resulted in obtaining crystal structures of several tRNAs (2–7) and complexes of tRNAs with their cognate synthetases (8–9). Unfortunately, due to its large molecular weight, there has been no single solution structure of tRNA established until now. As a powerful tool for structural determination of biomolecules in solution, NMR has long been used to study the structural features, we uniformly labeled the tRNA Trp with 15N. Wild type and mutant tRNAs were studied by proton and 15N multidimensional NMR spectroscopy. Imino protons in hydrogen-bonded base pairs, including several tertiary base pairs, were assigned. The thermal stability of tRNA Trp was also analyzed. Two components with different base modifications endowed with different thermal stability can be distinguished.

MATERIALS AND METHODS

Preparation of tRNA Trp—B. subtilis tRNA Trp was hyperexpressed in E. coli and purified as described by Xue et al. (19). Briefly, E. coli JM109 cells, transformed by recombinant pGEM-9Zf (−)–derived plasmid containing synthetic B. subtilis tRNA Trp gene between the Sfi I and HindIII sites, were grown in M9-glycerol medium supplemented with 100 μg/ml ampicillin. When the cells reached an absorbance of about 0.15 at 600 nm, isopropyl-1-thio-β-D-galactopyranoside was added to a final concentration of 0.2 mM. About 4 h later, the cells were harvested by centrifugation. Total tRNAs were prepared by phenol extraction and further purified using a DEAE-Sepharose CL-6B column. Purification of tRNA Trp was achieved by HPLC using a 250 × 10 mm Vydac C4-derivatized silica column. Peaks I and II were pooled separately and precipitated by ethanol. 15N-Labeled tRNA Trp samples were prepared as above except that NH4Cl was replaced by 15NH4Cl (Isotec Inc.) in the M9-glycerol minimal medium.

The abbreviations used are: NOE, nuclear Overhauser effect; NOESY, nuclear Overhauser effect spectroscopy; HSQC, heteronuclear single-quantum correlation; HPLC, high pressure liquid chromatography.

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For the NMR experiments, the samples were dissolved in a buffer containing 10 mM sodium phosphate, 100 mM sodium chloride, and 10 mM MgCl₂, pH 6.5, and then concentrated and washed three times using a Centracon-10 concentrator (Amicon). The final volumes of the samples were about 0.5 ml with 25 μl of D₂O added. Sample concentrations were 1–2 mM in tRNA. A small amount of 2,2-dimethyl-2-silapentane-5-sulfonic acid was added as an internal reference for proton chemical shift.

**NMR Spectroscopy**—All the NMR experiments were performed on Varian INOVA 500 and 750 spectrometers. One-dimensional NMR spectra were recorded using jump-and-return sequence (23) to suppress water signal, with the carrier on the water frequency and the delay set to yield the maximum intensity at the middle of the imino proton region. Phase-sensitive two-dimensional NOESY spectra were recorded at 30 °C with the hypercomplex method (24) for quadrature detection in the F₁ dimension. 256 t₁ increments were recorded over a spectral width of 12,000 Hz, each with 4096 data points and a mixing time of 120 ms. Solvent suppression was achieved by replacing the third pulse by the jump-and-return sequence, with the carrier and delay set as in the one-dimensional experiments.

Sensitivity-enhanced gradient two-dimensional ¹H-¹⁵N HSQC spectra (25) were recorded at 30 °C. Spectral widths at the proton and nitrogen dimensions were 12,000 and 6000 Hz, respectively. A total of 128 t₁ increments were acquired, each with 2048 points. Three-dimensional NOESY-HSQC spectra were also recorded using a gradient sensitivity-enhanced version (26) with NOESY mixing time of 70 ms. The size of the data set was 512 × 128 × 32 complex points. The spectral widths were 3041 Hz for the nitrogen dimension and 12,000 Hz for both proton dimensions. Data were processed using NMR pipe (27). Linear prediction method (28) was used in both non-acquisition dimensions to improve resolution. Variable temperature experiments were carried out on Varian INOVA 750 spectrometer equipped with a triple resonance probe.

**RESULTS**

**Assignment of Imino Protons**—In tRNA, the imino protons in each base pair can be observed in the proton spectrum in the low field region. Two-dimensional NOESY, two-dimensional ¹H-¹⁵N HSQC, and three-dimensional NOESY-HSQC experiments were employed to assign these imino protons. There is little difference between wild type and A73 mutant spectra (Fig. 1, B and C) except for the relative intensities of some peaks owing to the different percentages of a minor form in the sample, as discussed below. The peaks in the spectrum were labeled with their positions in sequence. Some imino groups with degenerate proton chemical shifts, but different nitrogen chemical shifts, were clearly observable in the HSQC spectrum of A73 mutant (Fig. 2).

**D Stem**—The GU wobble pairs in this tRNA provided a suitable starting point for the chemical shift assignments of the imino protons, because the two imino protons in GU pairs appear at the up-field part of the imino proton region and give rise to strong mutual NOE cross-peaks (29). In the two-dimensional NOESY spectrum of tRNA<sup>Trp</sup> (Fig. 3), there were strong mutual NOE cross-peaks between peaks at 12.15 and 9.75 ppm and 11.33 and 10.00 ppm, respectively. These were assigned to the GU pairs, G₂₂-U₁₃ and G₅₁-U₆₃ as shown in the cloverleaf structure of tRNA<sup>Trp</sup> (Fig. 1A). The peak at 14.69 ppm was assigned to s<sup>4</sup>U₈, which comes from one of the two conserved reversed Hoogsteen pairs in the tRNA tertiary structure (10), namely T₅₄-A₅₈ and s<sup>4</sup>U₈-A₁₄ in tRNA<sup>Trp</sup>, according to its special nitrogen chemical shift at 181.0 ppm (30). It gave rise to a rather strong NOE at 12.15 ppm and a weaker one at 9.75 ppm, to which the U₁₃ and G₂₂ were assigned, respectively. According to the crystal structure...
of other tRNAs (2–7), the s^U8-A14 is stacked above the 13–22 base pair in the D arm. Both s^U8 and U13 gave rise to NOEs at 11.90 ppm, which was assigned as G46, the base forming a tertiary interaction with U13 and G22. U12, U11, and G10 were assigned to the peak at 12.76, 14.69, and 12.35 ppm, respectively, according to the NOE connectivity between them and between U12 and U13, by using two-dimensional NOESY, HSQC, and three-dimensional NOESY-HSQC spectra. U11, U12, and U13 all gave rise to NOEs at 9.32 ppm, the high field shoulder of a broad peak, consisting of two components. This peak displayed a nitrogen chemical shift of 94.0 ppm (data not shown), typical for an amino nitrogen. It also gave NOEs to a broad peak at 8.65 ppm and a sharp peak at 8.18 ppm, which should be an amino and an aromatic proton, respectively. These signals were therefore assigned to the two amino protons of A23 (9.32 and 8.65 ppm) and the H8 (8.18 ppm) of A9, which forms a reversed Hoogsteen base pair with A23 and is a component of the U12-A23-A9 base triplet (31).

T'C Stem—Having assigned U13-G22, another wobble pair, G51U63, was therefore assigned to the peaks at 10.00 and 11.90 ppm, respectively. T54, a ribothymidine, is a special base forming another reversed Hoogsteen pair with A58. The protons of the 5-methyl group of T54 had several NOE cross-peaks of 11.90 ppm, respectively. T54, a ribothymidine, is a special base.

G27 was assigned unambiguously by utilizing the HSQC spectrum of the G1C72 mutant, where G27 and G52 were well separated from G2 and G3 due to the shift of the G2 and G3 peaks.

Acceptor Stem—The remaining unassigned peaks in the spectra were from GC pairs and should be from the acceptor stem. There were five unassigned GC imino protons distributed in several overlapped peaks, and only one NOE cross-peak between peaks at 13.18 and 12.76 ppm in the two-dimensional NOESY spectra. Several 15N-labeled or non-labeled mutants were constructed to facilitate the assignment. In the two-dimensional NOESY spectra of A3U70, A4U69, and A5U68 mutants, the resonant peak of the guanine 5' to the adenine was shifted upfield, and the one 3' to the adenine was shifted downfield. By combining the information from the two-dimensional HSQC and two-dimensional NOESY spectra of various mutants, the G-C pairs in the acceptor stem were completely assigned as shown in Table I.

So far, all the signals in the two-dimensional HSQC spectrum were assigned, except one very weak peak at 13.42 ppm with nitrogen chemical shift of 162.2 ppm. This must be contributed by one of the three unassigned AU pairs, namely U31-A39 in the anticodon stem and A1-U72 and A7-U66 in the acceptor stem, all of which are at the end of helical regions. The disappearance of these imino protons suggests that they are in fast exchange with water or are open under the measurement conditions.

Tertiary Base Pairs—There are several conserved tertiary base pairs in the tRNA^Trp structure. Both reversed Hoogsteen pairs, s^U8-A14 and T54-A58, have been previously assigned. As mentioned before, the imino proton and methyl group proton of T54 could give NOEs to the two imino protons of s^55, as was observed in the two-dimensional NOESY spectrum. Thus the s^55N3 proton, which is hydrogen-bonded to phosphate group of A58 (31), and s^55N1 proton were assigned to peaks at 11.54 and 10.46 ppm, respectively. Their characteristic nitrogen chemical shift confirmed the assignment (32, 33). G18, hydrogen-bonded to s^55 and stacked together with T54-A58 (31), was assigned to the peak at 9.40 ppm based on its NOE connections to T54 and s^55. Based on an NOE connection to s^U8 and HSQC spectrum, U48, which forms a reversed Watson-Crick base pair with A15 and is stacked with s^U8-A14, was assigned to the peak at 13.14 ppm with a nitrogen chemical shift of 162.2 ppm. A broad but rather strong peak observed in the HMQC spectra acquired at 10 and 20 °C, with the nitrogen chemical shift of 150.0 ppm and proton chemical

| Table I |
| Proton and nitrogen chemical shifts of imino groups of tRNA^Trp in 10 mM MgCl2 at 30 °C |

| H | N | Assignment | H | N | Assignment |
|---|---|------------|---|---|------------|
| 14.69 | 163.7 | U11 | 12.69 | 147.9 | G27 |
| 14.50 | 181.0 | s^U8 | 12.68 | 147.5 | G52 |
| 13.98 | 163.5 | U50 | 12.67 | 148.1 | G3 |
| 13.98 | 162.8 | U42 | 12.66 | 147.7 | G2 |
| 13.91 | 163.4 | U50 | 12.35 | 148.2 | G10 |
| 13.82 | 160.2 | T54 | 12.15 | 159.5 | U13 |
| 13.77 | 160.1 | T54' | 12.02 | 158.8 | U13 |
| 13.79 | 162.5 | U8 | 11.90 | 147.6 | G46 |
| 13.31 | 148.7 | G30 | 11.54 | 160.5 | ¥55N3 |
| 12.20 | 148.2 | G49 | 11.54 | 160.1 | ¥55N3' |
| 13.18 | 149.0 | G5 | 11.40 | 157.8 | U53 |
| 13.14 | 160.4 | U48 | 11.33 | 157.8 | U63 |
| 13.14 | 149.0 | G67 | 10.46 | 135.0 | ¥55N1 |
| 13.05 | 148.3 | G53 | 10.33 | 135.2 | ¥55N1 |
| 13.00 | 148.4 | G53' | 10.08 | 143.2 | G5' |
| 12.93 | 161.9 | U12' | 10.00 | 143.1 | G51 |
| 12.86 | 147.7 | G29 | 9.81 | 139.4 | G22 |
| 12.76 | 162.0 | U12 | 9.75 | 139.2 | G22 |
| 12.76 | 148.2 | G4 | 9.47 | 146.3 | G18' |
| 9.40 | 146.6 | G18 |

FIG. 3. Imino proton region of NOESY spectrum of B. subtilis tRNA^Trp A73 mutant at 30 °C in H2O with 10 mM MgCl2, pH 6.5.
shifts of 10.09 ppm, was assigned to D20, a nucleotide in the D loop region. It has characteristic proton and nitrogen chemical shifts due to its hydrogen bonding with oxygen (32).

**Sample Components**—There were many peaks, mainly from the D stem and the TψC stem, in the HSQC spectrum (Fig. 2), each having an associate peak with lower intensity. These peaks also displayed NOE patterns in the two-dimensional NOESY spectrum similar to those of their associated peaks, except that the U13 peak (the associate peak of U13) had an NOE to a peak with a proton chemical shift of 13.79 ppm and a nitrogen chemical shift of 162.5 ppm, which are typical chemical shifts for a uridine. This peak was therefore assigned to U8. The only difference between the two sets of peaks is that there was an s^4U8 in one set and a U8 in the other set, demonstrating that there were two species of tRNA^{TTP} in the sample. The molar ratio of s^4U8 to U8 in the hyperexpressed tRNA^{TTP} was reported to be only about 0.2, but the actual percentage might be higher because of its instability (20). The peak intensity of s^4U8 was almost as high as that of U11 in the spectrum of the A73 mutant, whereas it was much lower than that of U11 in the wild type spectrum. The percentage of thiolation ranged from about 40% to about 95% as revealed by HSQC spectra. We therefore suggest that the other set of peaks was derived from the species in the tRNA^{TTP} sample containing U8 instead of s^4U8. The results of the various assignments are summarized in Table I.

**Effect of Temperature on tRNA Structure**—Fig. 4 shows the imino proton spectra of the tRNA^{TTP} wild type at different temperatures. As has been mentioned before, D20 could only be observed in the HSQC spectra acquired at 10 and 20 °C and disappeared at 30 °C on account of the increased exchange rate. In contrast, U12, G18, and G46 were not clearly observed in both one-dimensional and two-dimensional spectra at a temperature lower than 30 °C. U50 and G52 were weak at 10 and 20 °C. G67 became stronger and was upfield-shifted extensively with the increase of temperature. These results indicate that there might be a minor conformational change when temperature was raised from below room temperature. Some other peaks were also shifted slightly. No significant changes were observed when the temperature was raised from 30 to 50 °C except that the δ55 N1 proton signal, which is not hydrogen-bonded, became broadened at 40 °C and vanished at 60 °C, because of an increased exchange rate of this proton with water. When the temperature was increased to 60 °C, the intensities of all the peaks decreased slightly. The peak intensities of U42, G29, and G30 from the anticodon stem were dramatically decreased, indicating that the exchange rates of these nucleotides were increased, with the possible existence of partial melting of the anticodon stem. Also, the peaks of U50 and G18 were decreased. At 70 °C, the anticodon stem melted extensively as indicated by the disappearance of U42, G30, and further decrease of G29. G18 disappeared too, indicating the disrupted linkage between D loop and TψC loop. The tertiary base pair T54-A58 in TψC loop melted at 75 °C, along with the G51U63 base pair in T stem. When the temperature was raised to 80 °C, all the peaks disappeared except that the very small residual peak for s^4U8 remained visible but broadened, indicating the complete melting of the whole structure of this tRNA molecule.

The methyl group signals are shown in the right column of Fig. 4. The peaks at 1.06 and 1.00 ppm at 30 °C were assigned to the methyl groups of T54 of the tRNA^{TTP} containing s^4U8 or U8, respectively, based on their chemical shifts and NOEs observed in the two-dimensional NOESY spectrum (35). The two well resolved sets of resonant peaks for the s^4U8-containing and U8-containing tRNA^{TTP} species made possible the separate monitoring of the thermal stability of these two tRNA^{TTP} species. From 10 to 70 °C, the signal of T54 of the species containing s^4U8 was only shifted slightly with no loss in intensities. This suggests that there was no conformational change around the D loop and TψC loop at below 70 °C. The peaks from the tRNA^{TTP} species containing U8, however, behaved differently. The T54 methyl signal of this species started to decrease at 50 °C and almost disappeared at 70 °C. When the temperature was increased further to 75 °C, the methyl group signal of the native state of the species containing s^4U8 also decreased. Finally, at 80 °C, a strong peak at 1.73 ppm appeared, corresponding to the methyl group of T in the random coil state (34). In summary, the whole structure of the tRNA^{TTP} species containing s^4U8 is not altered even at 50 °C. Its anticodon stem becomes partially melted at 60 °C, whereas the TψC stem and the tertiary base pair between δ55 and G18 are partially melted at 70 °C. At 80 °C, the structure of the molecule is disrupted and becomes a random coil. In comparison, the U8-containing tRNA^{TTP} species was less stable. Its conformation started to change at about 50 °C, as indicated by the diminished intensity of its T methyl signal, in contrast to the s^4U8-containing species which started to change only above 60 °C. These findings suggest that the thiolation of U8 stabilizes the tRNA^{TTP} structure.

**DISCUSSION**

**Uridine Thiolation**—The base modification pattern of B. subtilis tRNA^{TTP} hyperexpressed in E. coli is more similar to that of E. coli tRNA^{TTP} than to that of native B. subtilis tRNA^{TTP} (20, 21). Two molecular species were found in the present study, one with s^4U at position 8 and the other without such thiolation. Due to their similar chromatographic properties, these two species could not be separated by HPLC (19). This was evident in the 15N-H HSQC spectra, and proton NOESY spectra in which two distinct signals were observed for the same tertiary base pair between positions 8 and 14. The ratio between these components varied for different batches of tRNA^{TTP}, ranging from about 40 to 95%. Incomplete thiolation at position 8 was fa-
vored by overproduction of the cloned heterologous tRNA_Tp in minimal medium. Even for the native tRNAs of E. coli, the degree of thiolation in individual tRNA species is known to depend on bacterial growth rates (35). The differences in the chemical shifts of the two molecular species mainly reside in the T stem and D stem and in the tertiary interactions between T and D loops, clearly indicating that the base modification at position 8 causes a conformational change in this tertiary region.

Assignment of Imino Protons—The imino protons of 15N-labeled B. subtilis tRNA_Tp were completely assigned with the use of multidimensional NMR techniques and comparison of wild type and mutant tRNA_Tp. In the two-dimensional NOESY spectrum, most of the imino peaks were correlated by NOE cross-peaks, permitting sequential assignment by NOEs. The commonly used starting points in assignment, such as sU and GU pairs, were also utilized in the present study. For NOE cross-peaks from overlapped peaks, three-dimensional NOESY-HSQC was used to render the assignments unambiguous.

Several tertiary base pairs were observed in the present study. Two reverse Hoogsteen pairs, sU8-A14 and T54-A58, were assigned according to their characteristic chemical shifts and their NOEs to other imino protons as reported for other tRNAs (14, 30, 31). A15-U48, a reverse Watson-Crick base pair connecting the D loop and variable loop, was assigned according to its NOE to sU8. Also, the pairing of G18 and 89 55 was observed and assigned. However, the G19-C56 base pair connecting the D loop and TjC loop was not observed. This might not be entirely surprising in the light of differences among the D loop sequences of tRNA_Tp and those of yeast tRNA_Sp and tRNA_Phe. The G18G19 doulet in the D loop of tRNA_Tp is in the same sequence position as that in tRNA_Sp but different from that in tRNA_Phe, even though both of the latter two molecules have an 8-membered D loop, whereas tRNA_Tp has only a 7-membered D loop. The crystal structure of yeast tRNA_Sp also lacks any interaction between G19 and C56 (36), and the same applies to Bombyx mori tRNA_Glu (37), which has a similar D stem and loop sequence. This has been attributed to the occurrence of anticodon-anticodon interaction (36). In tRNA_Tp, which has a CCA anticodon, apparently no anticodon-anticodon interaction can be expected. However, it is noteworthy that all these three tRNAs lack bases at positions 17 and 47, with a wobble pair instead at the 13-22 position in tRNA_Tp and tRNA_Sp to compensate for the lack of base at 47 (38), and another wobble pair at 10-25 in tRNA_Sp and tRNA_Phe. This implies that it is the characteristic structural feature of these tRNAs, rather than the anticodon-anticodon interactions, that results in the lack of interactions between G19 and C56 in solution.

We have noticed that U12 resonates at 12.76 ppm, which is in a rather high field region compared with the chemical shifts of normal Watson-Crick A-U pairs. Similar situations were reported for U27 (12.70 ppm) in E. coli initiator tRNA_Met (39), U7 (12.6 ppm) in E. coli tRNA_Al (15), and U7 in Thermus thermophilus tRNA_Ac (40). In comparison, the U12 in tRNA_Sp and tRNA_Ac resonates in a far more downfield region, although they all have similar sequences in the D stem. A possible explanation is that there exists a rather special local conformation in tRNA_Tp different from that in tRNA_Sp and tRNA_Ac. In the present study, three AU pairs, two from the acceptor stem and one from the anticodon stem, were not assigned in NMR spectra. All of these AU pairs are located at the end of the stem and thus may exchange very rapidly with water. In the HSQC spectrum, a very weak cross-peak from uridine was not assigned. If the acceptor stem of the tRNA_Tp is also stacked on the TjC stem as in the crystal structure of yeast tRNA_Phe or E. coli tRNA_Met in solution (41), the A7-U66 base pair should be protected from fast exchange with water, as was observed in the studies of tRNA_Sp (42). We therefore tentatively assign this peak to A7U66.

Thermal Melting—Thermal melting experiments showed that in the presence of high magnesium ion concentration the melting of the structure was cooperative in character, compared with a more sequential behavior in the absence of magnesium (43). The D stem and sU8-A14 base pair were stabilized with Mg2+ binding. The tRNA_Tp species containing sU8 is more stable thermally by about 20 °C than that containing U8 with regard to the initiation of thermal unfolding, thus providing striking evidence that the thiolation of U8 stabilizes the tertiary structure of tRNA_Tp.

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REFERENCES
1. Schimmel, P. (1989) Biochemistry 28, 2747–2759
2. Kim, S. H., Quigley, G. J., Suddath, F. L., McPherson, A., Snedden, D., Kim J. J., Weinzierl, J., and Rich, A. (1973) Science 179, 285–288
3. Robertus, J. D., Lower J. N. F., Finch, J. J., Rhodes, D., Brown, R. B. C., and Klug, A. (1974) Nature 250, 546–551
4. Griebe, R., Moras, D., and Thierry, J. C. (1977) J. Mol. Biol. 115, 91–96
5. Moras, D., Comarranond, M. B., Fischer, J., Weiss, R., Thierry, J. C., Ebel, J. P., and Griebe, R. (1986) Nature 328, 669–674
6. Schevitz, R. W., Podjarny, A. D., Krishnamachari, N., Hughes, J. J., Sigler, P. B., and Sussman, J. L. (1979) Nature 287, 188–190
7. Wou, N. H., Roe, B. A., and Rich, A. (1980) Nature 286, 346–351
8. Roald, M. A., Perona, J. S., Soll, D., and Steitz, T. A. (1989) Science 246, 1135–1143
9. Ruff, M., Krishnaswamy, S., Bleggin, M., Peterszam, A., Mitschler, A., Podjarny, A., Rees, B., Thierry, J. C., Moras, D. (1991) Science 252, 1682–1689
10. Schimmel, P. R., and Redfield A. G. (1986) Annu. Rev. Biophys. Bioeng. 9, 181–221
11. Reid, B. R. (1981) Annu. Rev. Biochem. 50, 969–996
12. Holley, R. W., Appar, J., Everett, G. A., Madison, J. T., Marquise, M., Merill, S. H., Penwick, J. R., and Zamir, A. (1965) Science 1462–1465
13. Sanchez, V., Redfield, A. G., Johnston, P. D., and Trapp, J. O. (1986) Proc. Natl. Acad. Sci. U. S. A. 77, 5659–5662
14. Roy, S., and Reddick, A. G. (1981) Nucleic Acids Res. 9, 7073–7083
15. Harre, D. R., Ribeiro, N. S., Wemmer, D. E., and Reid, B. R. (1985) Biochemistry 24, 4300–4306
16. Niimi, T., Kawai, G., Takayanagi, M., Noguchi, T., Hayashi, N., Kohn, T., Muto, Y., Watanabe, K., Miyazawa, T., and Yokoyama, S. (1993) Biochimie Paris 75, 1109–1115
17. Wallis, N. G., Dardel, F., and Blanquet, S. (1995) Biochemistry 34, 7668–7677
18. Ikenura, T. (1985) Mol. Biol. Evol. 2, 13–34
19. Xue, H., Shen, W., and Wong, J. T. (1993) J. Chromatogr. 613, 247–255
20. Xue, H., Glasser, A., Desgres, J., and Grosjean, H. (1993) Nucleic Acids Res. 21, 2479–2486
21. Matsugi, J., Murao, K., and Ishikura, H. (1992) Nucleic Acids Res. 20, 3514
22. Xue, H., Shen, W., Giege, R., and Weng, J. T. (1993) J. Biol. Chem. 268, 9316–9322
23. Plateau, P., and Gueron, M. (1983) J. Am. Chem. Soc. 105, 7310–7311
24. States, D., Harberkon, R. A., and Ruben, D. J. (1982) J. Magn. Reson. 48, 456–462
25. Kay, L. E., Keifer, P., and Saarinen, T. (1992) J. Am. Chem. Soc. 114, 10663–10665
26. Zhang, O. Kay, L. E., Olivier, J. P., and Forman-Kay, J. D. (1994) J. Biol. Chem. 269, 845–858