Preparation of Uniformly Isotope-labeled DNA Oligonucleotides for NMR Spectroscopy*

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Two methods for the large scale preparation of uniformly isotope-labeled DNA for NMR studies have been developed. The first method comprises the growth of a suitable plasmid harboring multiple copies of the desired oligonucleotide in a medium based on $^{15}$N and $^{13}$C nutrients. The second method uses a polymerase chain reaction (PCR)-based approach with $^{15}$N- and/or $^{13}$C-labeled deoxyribonucleoside triphosphates. The novelty of our PCR strategy over existing ones is that the primer and template are identical molecules, resulting in an exponential growth in the length of the double strand that contains tandem repeats of the target DNA sequence. This novel PCR approach, which we have termed ESRA for endonuclease-sensitive repeat amplification, is easy to use, results in high yields, and can be accomplished at low costs. The utility of both methods is demonstrated for the preparation of a double-stranded 21-mer uniformly labeled with $^{15}$N and a double-stranded 17-mer DNA uniformly labeled with $^{15}$N and $^{13}$C.

Recent advances in NMR spectroscopy have had a major impact on macromolecular structure determination largely due to the advent of double and triple resonance three- and four-dimensional heteronuclear experiments (1–3). Similarly, structural characterization of folded RNA molecules and their complexes by NMR has been greatly aided by the application of multidimensional heteronuclear techniques (for reviews, see Refs. 4 and 5). The marked success of these modern NMR techniques for proteins and RNA is in stark contrast to their rare application in DNA investigations. This is mainly due to the difficulties in obtaining labeled oligodeoxynucleotides. Labeling proteins with NMR observable isotopes ($^{13}$C and $^{15}$N) is commonly achieved by expressing the protein of interest in a suitable host, commonly in Escherichia coli, using a growth medium containing $^{15}$N and $^{13}$C as sole nitrogen and carbon sources, respectively. Labeled RNA is generally obtained by in vitro transcription using T7 RNA polymerase (6) and $^{13}$C- and/or $^{15}$N-labeled ribonucleoside triphosphates prepared from RNA of organisms grown with $^{15}$N- and $^{13}$C-enriched nutrients (7–11). Isotope labeling of DNA has been difficult and most heteronuclear NMR studies on DNA oligonucleotides have been carried out using either $^{13}$C at natural abundance (12–14) or site specific $^{15}$N- and/or $^{13}$C-labeled chemically synthesized oligonucleotides. Isotopes have been incorporated either at particular atomic positions (15), uniformly in single nucleotides (16), or uniformly in all nucleotides of a particular type (17). The fact that structural studies using uniformly isotopically labeled DNA are lagging behind is almost exclusively due to the fact that the labeled phosphoamidites necessary for chemical synthesis are not commercially available and that their preparation is both extremely labor intensive and costly. More recently, a procedure for the enzymatic synthesis of uniformly $^{13}$C- and $^{15}$N-labeled DNA oligonucleotides has been reported (18). In this approach, labeled deoxynucleoside triphosphates are prepared from DNA of organisms grown with $^{15}$N- and $^{13}$C-enriched nutrients in a similar manner to that described for the ribonucleoside triphosphates. DNA synthesis is carried out with DNA polymerase I and isotope-labeled triphosphates using a primer-template with a ribonucleotide at the 3’ terminus that allows for alkaline hydrolysis. Cleavage yields labeled primer-template and the labeled, newly synthesized single-stranded oligonucleotide. In this manner, uniformly labeled DNA can be prepared and structurally characterized by multidimensional heteronuclear NMR (18–20).

In this paper we present two additional methods for the large scale preparation of uniformly isotope-labeled DNA. The first method is akin in spirit to expressing the protein of interest in E. coli, using a growth medium based on $^{15}$N and $^{13}$C nutrients. The second method, which we have termed ESRA for Endonuclease Sensitive Repeat Amplification, is PCR based and makes use of labeled deoxynucleoside triphosphates similar in spirit to the above mentioned enzymatic approach. The novelty of our PCR strategy over existing ones consists of primer and template being the identical molecule, resulting in an exponential growth in the length of the double strand, which contains tandem repeats of the target DNA sequence. Both methods have several advantages compared with chemical synthesis and the Zimmer and Crothers (18) procedure. In particular, ESRA is superior in terms of ease of use, yields, and costs. We demonstrate the utility of both methods for the preparation of two double-stranded oligonucleotides: a 21-mer uniformly labeled with $^{15}$N and a 17-mer uniformly labeled with $^{15}$N and $^{13}$C.

EXPERIMENTAL PROCEDURES

Construction of the Oligonucleotide-containing Plasmid—Two complementary SRY oligonucleotides, 5’-AATTCTCCGTTGTTGTCAG and 5’-AATTCTGCAAACACCCGGG were annealed and ligated overnight. The products of this ligation reaction, exhibiting a distribution of lengths around ~200 bp, were cloned into the EcoRI site of pUC8 (21). Novablue cells (Novagen) were transformed with pUC8-SRY DNA and transformants were screened by restriction enzyme analysis. Several candidates containing multiple copies of the 21-bp oligonucleotide

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¶ The abbreviations used are: ESRA, endonuclease sensitive repeat amplification; PCR, polymerase chain reaction; bp, base pair(s); HSQC, heteronuclear single quantum coherence; SRY, male sex-determining factor Y.
were further analyzed and propagated. Insert instability was observed and resulted in multiple shorter insert lengths after prolonged growth. One candidate plasmid pUC8-SRY#27 was transferred to E. coli Sure® (Stratagene) for storage and subsequently into E. coli GM1674 (kindly provided by M. G. Marinus, University of Massachusetts Medical School, Worcester) for plasmid production.

Preparation of 15N-labeled Plasmid DNA and 21-mer Oligonucleotide—Cells bearing pUC8-SRY#27 were grown in 30 liters of minimal M9 medium (22) supplemented with 15NH4Cl and in the presence of 0.5% glucose, 2 µg/ml thiamine, 0.1 µg/ml biotin, and trace elements in a 50-liter fermentor until the glucose was depleted from the medium. An optical density of 7.0 at 600 nm roughly corresponds to a yield of 300 g wet weight of cells. Plasmid DNA was isolated using the Giga plasmid isolation kit (Qiagen Inc, Chatsworth, CA). Typically 10–12 mg of plasmid DNA was purified from 40 g of cells and stored at 2 mg/ml in 6 mM Tris-HCl at pH 7.4, 6 mM NaCl, and 0.2 mM EDTA at 4 °C. Restriction endonuclease digestion was performed in batches of 25 mg of plasmid at 0.4 mg of plasmid/ml of buffer and 0.25 units SmaI of plasmid for 15–17 h at 25 °C according to the specifications of the provider (New England Biolabs, Beverly, MA). The digested DNA was dialyzed against a 20-fold excess of 50 mM Tris-HCl, pH 8.0, 1 mM EDTA using 2 kDa molecular mass cut-off membranes (Pierce) for 4 h at ambient temperature, diluted 2-fold in the same buffer, and subjected to anion-exchange column chromatography (Poros 50 HQ 1 × 10 cm, Perceptive Biosystems, Framington, MA). The double-stranded SRY/Sma oligonucleotide (SRY-21) was fractionated with a gradient of 0.6–1 M NaCl in 20 column volumes at a flow-rate of 7.5 ml/min. Peak fractions were concentrated to ~1.5 ml in a Centriprep 3 (Amicon, Beverly, MA) and ethanol precipitated. The precipitate was recovered by centrifugation, washed with 95% cold ethanol, dried, and dissolved in DNA buffer (10 mM sodium phosphate, pH 6.8, 25 mM NaCl, 0.2 mM EDTA). A yield of ~1.1 mg of pure SRY-21 was obtained from 75 mg of plasmid and used for NMR studies.

**PCR Synthesis of 15N,13C-labeled 17-mer Oligonucleotide**—The oligonucleotide 5′-GGGTGTTTGTGCAGCCCGGTTCCTTAAAGGG-3′, which represents a repeat of the sequence 5′-GGGTGTTTGTGCAGCCCGGTTCCTTAAAGGG-3′, in tandem, and its complement were synthesized and purified on 15% acrylamide, 7 M urea gels (Novex, San Diego, CA). 60 ng of each oligonucleotide were used in an amplification reaction using the Expand high fidelity PCR kit (Boehringer Mannheim) in the presence of 0.2 mM uniformly labeled 13C,15N dNTPs (Martek Bioscience Corp., Gaithersburg, MD) and cycled 8 rounds at 95 °C for 1 min, 55 °C for 2 min, and 72 °C for 3 min in a GeneAmp 9600 cycler (Perkin Elmer). This amplified mixture (700 µl) was used to further amplify 12.5 ml of reaction mixture cycled 40 times under the same conditions in 100-µl aliquots. Aliquots were pooled and digested with SmaI and subjected to column (0.5 × 5 cm) chromatography under conditions as described above for the 21-mer. The double-stranded SRY-17 oligonucleotide (SRY-17) was purified using a NaCl gradient of 0.5–0.9 M in 50 column volumes at a flow rate of 5 ml/min. Peak fractions were concentrated, dialyzed extensively against DNA buffer, and concentrated further to yield a 0.3 mM solution. The concentrations of oligonucleotides were estimated both spectrophotometrically and by densitometry following gel electrophoresis with ethidium bromide staining.

**NMR Spectroscopy**—NMR experiments were carried out on a Bruker DMAX600 spectrometer equipped with x,y,z-shielded gradient triple resonance probes. The 1H,13N HSQC spectrum with a water flip back pulse and the 1H,15N constant time HSQC spectrum were recorded as described in Refs. 23 and 24, respectively.

**RESULTS AND DISCUSSION**

The application of multidimensional heteronuclear NMR techniques, which revolutionized solution structure determination of proteins, was intimately linked to advances in molecular biology that allowed for easy cloning of genes and the expression and purification of their isotopically labeled products. Access to labeled nucleic acids, on the other hand, has been difficult and up until now only isotopically enriched RNA oligonucleotides could be prepared with relative ease. Although chemical synthesis of DNA oligomers is a simple and automated procedure, its use for preparing labeled DNA is limited to those laboratories that have the manpower and chemical expertise to synthesize uniformly labeled phosphoramidites (16, 25, 26). Apart from chemical synthesis, only one alternative approach has been reported in the literature for the preparation of labeled single-stranded DNA oligonucleotides (18). We therefore set out to develop efficient and simple methods to prepare labeled double-stranded DNA that would bring structural studies of DNA by NMR to the same level of sophistication as that of proteins.

The first procedure exploits our knowledge of biosynthetic labeling in E. coli. At first sight, production of the desired DNA sequence in bacteria grown with 15N and 13C nutrients should be a simple and easily accomplished task. Indeed, 13N-labeled DNA has had an illustrious history in molecular biology (27). Most DNA within the cell, however, constitutes chromosomal DNA, and therefore one has to devise an efficient way to increase the amount of a desired short sequence such that it can be detected and purified from the vast excess of undesired sequences. Clearly, introduction of a defined sequence can be detected and purified from the vast excess of undesired sequences. Clearly, introduction of a defined sequence via a plasmid or plasmid, rather than introduction into the E. coli genome is the method of choice. We chose plasmid-mediated introduction since, in general, plasmids are smaller than phage genomes and higher numbers of extrachromosomal DNA copies of plasmid can be achieved. Based on size (2664 bp) and copy numbers (700) we chose pUC8 (21) as our cloning vector. The oligonucleotide for insertion into the plasmid was designed in the following fashion. One end has an EcoRI restriction site, whereas the other end has the Smal recognition sites, respectively. DS, double-stranded.
any recombinant vector originating from pUC8 containing single or multiple copies with the blunt-end creating enzyme SmaI. The reason for this is that the multiple cloning site in pUC8 contains a SmaI site adjacent to the EcoRI site. (Note, this is different for other members of the pUC series.) Multiple repeats of the original 21-mer were created by ligation and successfully inserted into the EcoRI site of pUC8. Stable clon- ing of repeat sequences is not easily achieved, and indeed we observed shortening of inserts for a large number of transfor- mants. For production purposes we chose a particular pUC8 derivative designated pUC8-SRY#27. It contains seven tandem copies of the original 21-mer. The fact that no 34-mer SmaI fragments were observed for this candidate indicates that inverted repeats are extremely unstable and are lost from the plasmid rapidly.

E. coli strain GM1674 (kindly provided by M. G. Marinus) was transformed with pUC8-SRY#27 and used for plasmid DNA preparation. This particular strain was chosen to avoid the introduction of any heterogeneity caused by selective methylation. It lacks both the Dam and Dcm methylases that modify the N6 position of adenine residues and the C5 position of cytosine residues with methyl groups in GATC or CC(A/T)GG sequences, respectively. On average, Dam sites occur at a frequency of ~1 site per 256 bp and Dcm sites at ~1 site per 512 bp in DNA of random sequence. Sites for the third E. coli DNA methylase, EcoKI, are much less common (~1 site per 8 kilobase pairs) such that it is not necessary to worry about this modification. Although heterogeneity within the 21-mer oligonucleotide due to Dam and Dcm methylases would be too small to be of concern, preparation of labeled nucleotides from chromosomal DNA or the vector portion of the plasmid DNA (see second method below) may not be advisable.

We grew pUC8-SRY#27-transformed GM1674 in minimal medium using 15NH4Cl as the sole nitrogen source, isolated the plasmid DNA, and carried out endonucleolytic cleavage with the enzyme SmaI. The 21-bp oligonucleotide, designated SRY-21, with the sequence

\[
5'\text{GGGATTCCTGGCACAAAAACCCC} 3'
\]

1.1 mg of purified oligonucleotide was obtained from 75 mg of plasmid DNA, which corresponds to an overall final yield of ~30%. Although the final amount of 15N-labeled 21-mer seems to be rather small compared with the amount of labeled DNA produced in E. coli, the latter constitutes an excellent source for the preparation of labeled deoxynucleoside triphosphates by previously established methods (18, 20).

Fig. 2 presents the cytosine amino (A) and imino (B and C) regions of the 1H-15N HSQC spectrum recorded at 30 °C on a 0.3 mM sample of 15N-labeled SRY-21 in phosphate buffer containing 90% H2O, 10% D2O. A, the cytosine amino; B, the guanine imino; and C, the thymine imino regions. The spectrum shown in panel A was recorded in 2 h of measurement time with 64* (t1) × 1024* (t2) complex points, acquisition times of 51.2 and 68.25 ms in the 15N and 1H dimensions, respectively, and the 15N carrier at 97 ppm. The spectrum shown in panels B and C was recorded in 1.5 h of measurement time with 100* (t1) × 1024* (t2) complex points, acquisition times of 80 and 68.25 ms in the 15N and 1H dimensions, respectively, and the 15N carrier at 155 ppm.

Fig. 3 illustrates the PCR-based ESRA approach for the production of the SRY-17 duplex oligodeoxynucleotide. The sequences of the two starting single-stranded oligonucleotides are given at the top. Bases that constitute the SmaI recognition sequence are shown in italics. During the first PCR cycle, extension to a triplet repeat occurs; the second cycle generates triple, quadruple, and pentuple repeats with subsequent cycles leading to exponential growth in chain length.

FIG. 2. Selected regions of the 1H-15N HSQC spectrum recorded at 30 °C on a 0.3 mM sample of 15N-labeled SRY-21 in phosphate buffer containing 90% H2O, 10% D2O. A, the cytosine amino; B, the guanine imino; and C, the thymine imino regions. The spectrum shown in panel A was recorded in 2 h of measurement time with 64* (t1) × 1024* (t2) complex points, acquisition times of 51.2 and 68.25 ms in the 15N and 1H dimensions, respectively, and the 15N carrier at 97 ppm. The spectrum shown in panels B and C was recorded in 1.5 h of measurement time with 100* (t1) × 1024* (t2) complex points, acquisition times of 80 and 68.25 ms in the 15N and 1H dimensions, respectively, and the 15N carrier at 155 ppm.

FIG. 3. Illustration of the PCR-based ESRA approach for the production of the SRY-17 duplex oligodeoxynucleotide.
shift dispersion for these cross peaks, it is possible to identify the imino proton and nitrogen resonances for all but the two terminal base pairs. The latter are not visible due to fraying of the strands at the ends of the duplex. We were able to assign these resonances using a conventional two-dimensional $^1$H-$^1$H nuclear Overhauser effect spectrum (data not shown), and cross peaks are labeled with the base pair number. The isotopic enrichment of SRY-21 was ascertained from a one-dimensional proton spectrum recorded without nitrogen decoupling (data not shown) and by mass spectrometry. By both methods, the oligonucleotide is $>$95% labeled with $^{15}$N.

The second procedure, ESRA, exploits the power of PCR technology for DNA amplification in a novel way. Relying on an enzymatic synthesis step, this approach is similar in spirit to the methodology developed for the preparation of labeled RNA (29, 30) and subsequently DNA (18) oligonucleotides. The major difference and clear advantage of our method is based on the fact that a large number of repeated cycling steps can be performed, thus resulting in the production of copious amounts of DNA.

An outline of the PCR-ESRA approach is illustrated in Fig. 3. The starting material consists of two oligonucleotides prepared by chemical synthesis using standard methodologies. Their sequences comprise the desired DNA sequence repeated twice and embodied between two restriction sites. For the present purposes we have chosen Smal sites since we had already developed efficient cleavage conditions for large amounts of DNA for our first methodology (described above). Any other restriction site can also be employed, and indeed HaeIII works equally well.2 Annealing of the two oligonucleotides will, in addition to the complete double-stranded 34-mer, yield partial double-stranded species that are amenable to filling in by polymerases. In this manner each single strand functions simultaneously as a template and as a primer. Using natural abundant deoxynucleoside triphosphates will result in unlabeled DNA, whereas $^{15}$N- and/or $^{13}$C-labeled deoxynucleoside triphosphates allow for the preparation of uniformly $^{15}$N- and/or $^{15}$N-$^{13}$C labeled DNA. In the first extension reaction (see Fig. 3) the product is a 3-fold repeat of the target sequence. Multiple cycles of annealing and extension will result in hundreds of tandem copies of the target double-stranded oligonucleotide. Although initially only a small percentage of the annealed sequences will have single-stranded ends, these are the only species competent in the extension reaction, and multiple rounds of melting and annealing will completely consume these initial and intermediate blunt-end species. Restriction endonuclease treatment

FIG. 4. Electrophoretic analysis of DNA products generated using ESRA. A, amplification of the repeat DNA as a function of cycle number 0 through 25. 5 μl of each reaction mixture was electrophoresed on a 0.8% agarose gel in Tris-Borate-EDTA (TBE) buffer. M denotes the marker DNA in kilobases (lambda DNA digested with HindIII). B, 5 μl of the reaction mixture after 25 cycles of PCR, undigested (−) and digested (+) with Smal separated on a 10% acrylamide gel in TBE buffer. C, purified oligonucleotides: lane 1, SRY-17 prepared by chemical synthesis; lane 2, SRY-17 prepared by ESRA; lane 3, SRY-21 prepared by the plasmid approach. DS, double-stranded.

FIG. 5. Selected regions of the constant time $^1$H-$^1$C HSQC experiment recorded at 30 °C on a 0.3 mM sample of $^{15}$N-$^{13}$C-labeled SRY-17 in phosphate buffer containing 99.99% D$_2$O. A, C1’-H1’; B, C3’-H3’; C, C4’-H4’; and D, T(CH3) regions of the spectrum. The spectrum was recorded in 3 h of measurement time with a constant time period of 26.6 ms, 282* (t1) x 512* (t2) complex points, acquisition times of 26.5 and 85.2 ms in the $^{13}$C and $^1$H dimensions, respectively, and the $^{13}$C carrier at 70 ppm.

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of the several kilobase pairs-long product results in the desired double-stranded oligonucleotide in large amounts. We prepared a uniformly $^{15}$N-$^{13}$C-labeled 17-bp double-stranded oligonucleotide, designated SRY-17, with the following sequence.

$$
\begin{align*}
3' & \text{CCCGACCCG} \\
5' & \text{GGGGTCACAAACCC}
\end{align*}
$$

The DNA fragment was purified by ion exchange chromatography in the same manner as SRY-21. Fig. 4 illustrates the results of the annealing/extension protocol. A timecourse for the PCR reaction is shown in the left panel, the product before and after endonuclease cleavage is shown in the middle panel, and the final purified product is shown in the right-hand side panel. As is easily appreciated, cleavage with Smal yields predominantly the 17-mer with only a small amount of undigested material migrating to the position of a dimer. The latter most likely occurs as a result of a single base 3' overhang in the extension products that is not efficiently removed by the 3'-5' exonuclease activity (31). This, in turn, may oblate the Smal recognition site resulting in some oligonucleotides that cannot be cleaved to monomers. The amount of dimer is less than 10% and is easily removed in the chromatography step. The overall yield of purified labeled double-stranded 17-mer is ~50% calculated with respect to the amount of input labeled deoxynucleoside triphosphates, illustrating the cost effectiveness of this methodology (i.e. ~50% of the labeled deoxynucleoside triphosphates are incorporated into the final product). Most of the loss could be due to the instability of deoxynucleoside triphosphates over extended cycles of heating and cooling used in PCR. The final product (~1 mg) is >95% labeled with $^{15}$N and $^{13}$C, as evidenced from an undecoupled one-dimensional $^1$H NMR spectrum as well as mass spectrometry.

Fig. 5 shows four regions of the constant time $^1$H-$^13$C HSQC spectrum recorded at 30 °C on a 0.3 mM sample of $^{15}$N,$^{13}$C-labeled SRY-17 in phosphate buffer containing 99.99% D$_2$O. The C1'-H1' region is surprisingly well resolved, and 29 of the 34 cross peaks can be identified. The remaining ones are hidden in the severely overlapped portions of this region of the spectrum. Naturally the C3'-H3' and, more markedly, the C4'-H4' regions exhibit extensive overlap. In panel D all cross peaks for the thymine methyl groups can be identified. As can be appreciated, all expected resonances are observed, indicating complete $^{13}$C-labeling of the oligonucleotide. A $^1$H-$^13$C HSQC spectrum, analogous to that presented in Fig. 2, demonstrates complete $^{15}$N-labeling as well (data not shown).

In summary, we have developed two efficient and economical methods for the large scale production of isotopically labeled double-stranded DNA oligonucleotides. Although the plasmid-based approach is currently limited to $^{15}$N-labeled DNA for reasons of cost, the yields may easily be improved using fed-batch fermentation protocols developed for large scale plasmid production (32). Improvements in the cloning of multiple repeats using a variation of the ESRA approach in conjunction with specialized strains may also be possible. The ESRA approach, on the other hand, is cost effective for any type of uniform isotopic labeling.

As has been eluded to above, the PCR-based ESRA methodology presented here is not only applicable to the preparation of labeled oligodeoxynucleotides for NMR structural studies. Indeed, it may well be the method of choice for the large scale production of double-stranded oligonucleotides per se, particularly in cases that require a minimum length where chemical synthesis and purification of large amounts becomes difficult due to contamination with n-1 products (i.e. 60 bp and longer). Thus DNA fragments required for structural studies by x-ray crystallography may present another system that would benefit from the ESRA approach. No doubt, the availability of uniformly isotopically labeled DNA will make NMR studies of DNA oligonucleotides, either free or complexed, amenable to the same level of sophistication that can be applied to proteins.

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REFERENCES

1. Clore, G. M., and Gronenborn, A. M. (1991a) Annu. Rev. Biophys. Biophys. Chem. 20, 29–63
2. Clore, G. M., and Gronenborn, A. M. (1991b) Science 253, 1390–1399
3. Baz A., and Grzesiek, S. (1995) Acc. Chem. Res. 26, 131–138
4. Pandit, A. (1995) Methods Enzymol. 261, 350–380
5. Varani, G., Aboul-el, F., and Allain, F. H.-T. (1996) Proc. Nucl. Mag. Reson. Spect. 29, 51–127
6. Milligan, J. F., Groebe, D. R., Witherell, G. W., and Uhlenbeck, O. C. (1987) Nucleic Acids Res. 15, 8783–8788
7. Batey, R. T., Inada, M., Kujawański, E., Puglisi, J. D., and Williamson, J. R. (1992) Nucleic Acids Res. 20, 4515–4523
8. Nkonowicz, E. P., and Pardi, A. (1992) Nature 355, 184–186
9. Nkonowicz, E. P., and Pardi, A. (1992) J. Am. Chem. Soc. 114, 1082–1083
10. Nkonowicz, E. P., Sirr, A., Legalow, J., Pucker, F. M., Baer, L. M., and Pardi, A. (1992) Nucleic Acids Res. 20, 4507–4513
11. Michnicka, M. J., Harper, J. W., and King, G. C. (1993) Biochemistry 32, 395–400
12. Ashcroft, J., Live, D. H., Patel, D. J., and Cowburn, D. (1991) Biopolymers 31, 45–55
13. Schmieder, P., Ippel, J. H., van den Elst, H. H. van der Marel, G. A., and van Boom, J. H. Altona, C., and Kessler, H. (1992) Nucleic Acids Res. 20, 4747–4751
14. Radha, P. K. (1996) Magn. Reson. Chem. 34, 518–522
15. Michalezyk, R., Silks, L. A. L., and Russu, I. M. (1996) Magn. Reson. Chem. 34, 897–8104
16. Ono, A., Tate, S., Ishido, Y., and Kainosho, M. (1994) J. Biomol. NMR. 4, 381–386
17. Tate, S., Ono, A., and Kainosho, M. (1994) J. Am. Chem. Soc. 116, 5977–5978
18. Zinner, D. P., and Crothers, D. M. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 1091–1095
19. Zinner, D. P., Marino, J. P., and Griesing, C. (1996) Magn. Reson. Chem. 34, S177–S186
20. Batey, R. T., Battiste, J. L., and Williamson, J. R. (1995) Methods Enzymol. 228, 252–268