Supercoiling-Induced DNA Bending†

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ABSTRACT: Local DNA bending is a critical factor for numerous DNA functions including recognition of DNA by sequence-specific regulatory binding proteins. Negative DNA supercoiling increases both local and global DNA dynamics, and this dynamic flexibility can facilitate the formation of DNA−protein complexes. We have recently shown that apexes of supercoiled DNA molecules are sites that can promote the formation of an alternative DNA structure, a cruciform, suggesting that these positions in supercoiled DNA are under additional stress and perhaps have a distorted DNA geometry. To test this hypothesis, we used atomic force microscopy to directly measure the curvature of apical positions in supercoiled DNA. The measurements were performed for an inherently curved sequence formed by phased A tracts and a region of mixed sequence DNA. For this, we used plasmids in which an inverted repeat and A tract were placed at precise locations relative to each other. Under specific conditions, the inverted repeat formed a cruciform that was used as a marker for the unambiguous identification of the A tract location. When the A tract and cruciform were placed diametrically opposite, this yielded predominantly nonbranched plectonemic molecules with an extruded cruciform and A tract localized in the terminal loops. For both the curved A tract and mixed sequence nonbent DNA, their localization to an apex increased the angle of bending compared to that expected for DNA unconstrained in solution. This is consistent with increased helical distortion at an apical bend.

DNA bending is a common feature required for the interaction of DNA with many site-specific binding proteins (*1*−*3*). The axial deformation of DNA can be a mechanism for regulation of site-specific DNA recognition, thereby facilitating or preventing protein binding. In addition to sequence-specific DNA flexibility (*4*), negative DNA supercoiling can change the angle of the bend (*5*) and thus modulate protein−DNA interaction. Importantly, there is another interesting feature of DNA supercoiling that can be involved with local sequence-specific DNA curvature. Experimental evidence, including atomic force microscope (AFM)[1] imaging, has shown that negatively supercoiled DNA is a plectonemic superhelix and that an important feature of a plectonemic superhelix is the terminal loops. These sites in supercoiled DNA are predominantly occupied by intrinsically curved DNA regions such as A tracts (*6*) and cruciforms when they adopt folded X-type conformation (*7*, *8*). It is intuitively obvious that DNA at the apex of a superhelix is necessarily bent. Earlier studies suggested that A tracts in terminal loops of supercoiled DNA are distorted (*6*, *9*, *10*). The major objective of this paper was to test the hypothesis that the curvature of DNA when localized to the apex of the superhelix is greater than expected based on random dynamic fluctuation. For these experiments, we utilized an X-type cruciform, which becomes localized at the apex of a superhelix (*11*), to place the DNA sequence located at a 6 o'clock position to the opposite apex of the plectonemic superhelix. In different plasmids, a mixed sequence, noncurved DNA fragment or a sequence containing a 180° bend was introduced opposite the cruciform-forming sequence. Recently, we have shown that the relative positioning of these structural features is critical for the overall geometry of the plasmid (*11*). When these structural features were positioned in plasmids at 90° relative to each other, an increase in the fraction of branched molecules was observed. In the same study, when the A tract and the cruciform were positioned diametrically opposite, unbranched plectonemic molecules were observed with a high frequency. Moreover, in these 6 o'clock constructs, the cruciform transition point shifts to lower DNA supercoiling, supporting the hypothesis that DNA supercoiling leads to the distortion of the double helix at the apical loops of the superhelix. In this paper, we analyzed the curvature of the terminal loop in supercoiled DNA and showed that DNA localized to the apex of a superhelix has a greater degree of bending than expected for DNA not localized at an apex.

EXPERIMENTAL PROCEDURES

Plasmid DNA. Plasmid constructs (Figure 1) have been recently described (*11*). Briefly, plasmids are based on

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[1] Abbreviations: AFM, atomic force microscope; APS, 1-(3-aminopropyl)silatrane.

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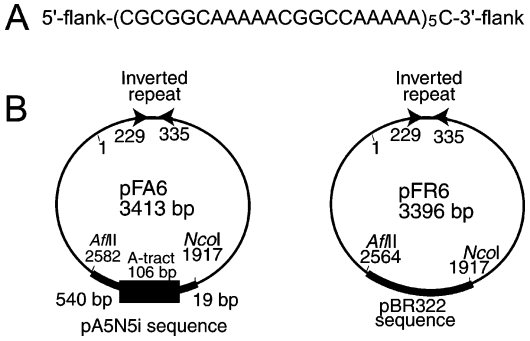

FIGURE 1: Schematics of the plasmids used in this study. (A) Intrinsically bent DNA fragment from pA_5N_5i used to create plasmid pFA6. (B) In plasmid pFA6, the inverted repeat and bent sequence are diametrically positioned, whereas in plasmid pFR6, the inverted repeat and a mixed sequence from pBR322 that is not bent are diametrically opposite.

pUC8F14C containing a 106-bp inverted repeat at the *Eco*RI site (*12*). Plasmid pFA6 contains an A tract from pA5N5i, which creates a bend of 180° (*6*) (Figure 1A) placed diametrically opposite to the cruciform-forming inverted repeat. Similarly, plasmid pFR6 contains an inverted repeat and a mixed sequence DNA without any bends from pBR322.

AFM Procedure. Freshly cleaved mica functionalized with 1-(3-aminopropyl)silatrane (APS−mica) was used as a substrate (*8*, *13*, *14*). For imaging, 10 μL of plasmid DNA (0.5 μg/mL) in 100 mM NaCl, maximizing plectonemic structure visualization with AFM (*15*), was deposited onto pieces of APS−mica for 2 min, thoroughly rinsed with deionized water (ModuPure Plus, ContinentalWater System Corp., San Antonio, TX), and argon dried. Olympus Standard Silicon OMCL-AC160TS series (Olympus Optical Co., Ltd., Tokyo, Japan) tips were used for imaging. The typical tapping frequency was 300−350 kHz, and the scanning rate was 1.97 Hz.

Angle Measurements. The schematic for this procedure is shown in Figure 2. In unbranched plectonemic plasmids with the cruciform located at one apex, the exact position of another apex relative to the cruciform structure was determined from the DNA contour length relative to the last crossover of the superhelix. This point is indicated in Figure 2B with a large black dot. The curvature of a terminal loop was characterized by the angle between two tangents applied at 10 nm from the apex (small black dots in Figure 2B). The tangents (white lines) were drawn from the tallest point of a cross section to 10 nm in each direction away from the apex. The value of this angle and the contour lengths of the molecules were determined by the Femtoscan Online software (version 1.6 (4.4); Moscow State University and Advanced Technologies Center, Moscow, Russia).

RESULTS

Plasmid Design. Two structural elements were used to position a curved DNA sequence or a mixed DNA sequence with no phased axial curvature into the apex of a plectonemic superhelix. For this, we utilized a 106-bp inverted repeat (*12*) capable of forming a cruciform with 53-bp arms, which can be easily visualized with AFM (*7*, *8*). The cruciform in the X conformation creates a bend in DNA that is predominantly (>95%) localized to the apex of a superhelix. We also utilized a 106-bp curved segment containing five copies of the sequence CGCGGCAAAAACGGCCAAAAA that creates a permanent 180° bend (*6*), which also prefers apical localization (*16*). The plasmid constructs are schematically shown in Figure 1. Relative positions of the A tract and the inverted repeat are shown on the circles. The diametrical positioning of the A tract and the inverted repeat (plasmid

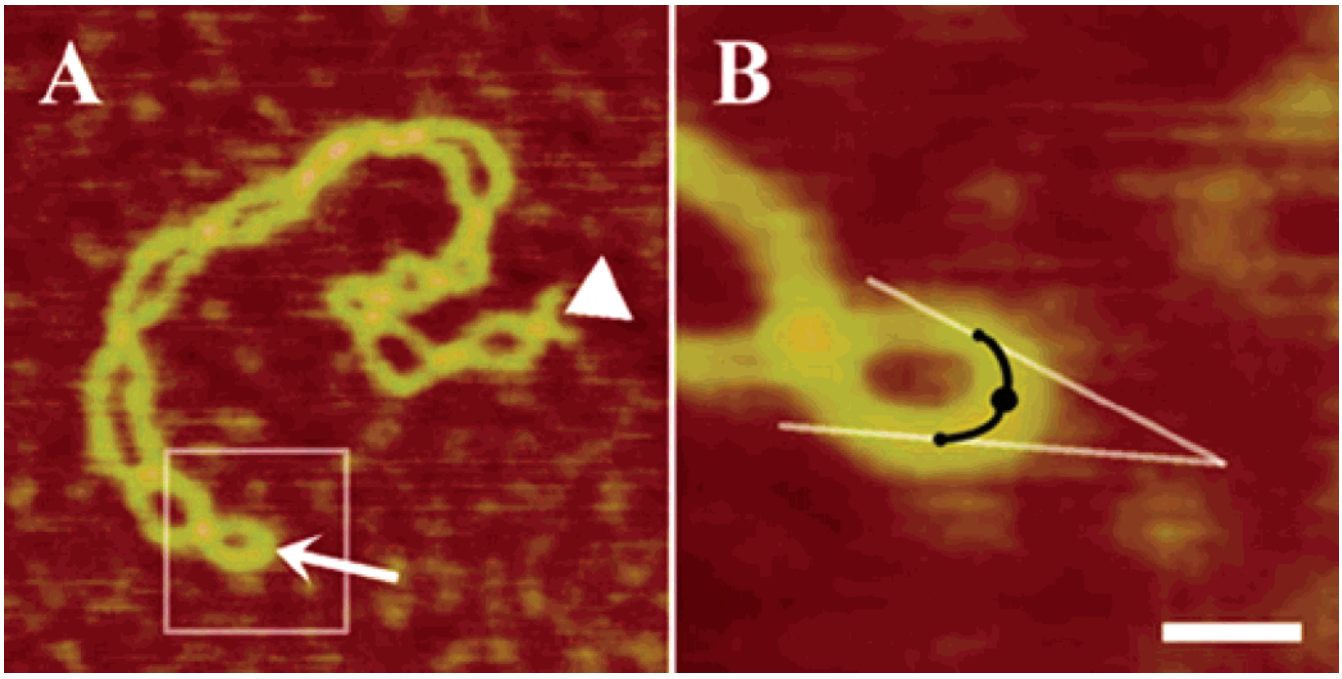

FIGURE 2: Schematics of the end loop bend-angle measurements. (A) Plectonemically wound molecules with cruciforms (shown with an arrowhead) located at an apex were used to determine the degree of bending in terminal-end loops (arrow). The boxed part of the DNA molecule was magnified and used for angle measurements as shown in (B). An exact apex location (large black dot) was determined from the DNA contour length relative to the last crossover of the superhelix. A distance of 10 nm was measured clockwise and counterclockwise from this apical location, giving a 20-nm arc. Angles were generated by taking the tangents to the DNA contour at the ends of the 20-nm arc (small black dots). For this plasmid, the bend angle is 24°. The bar length is 15 nm.

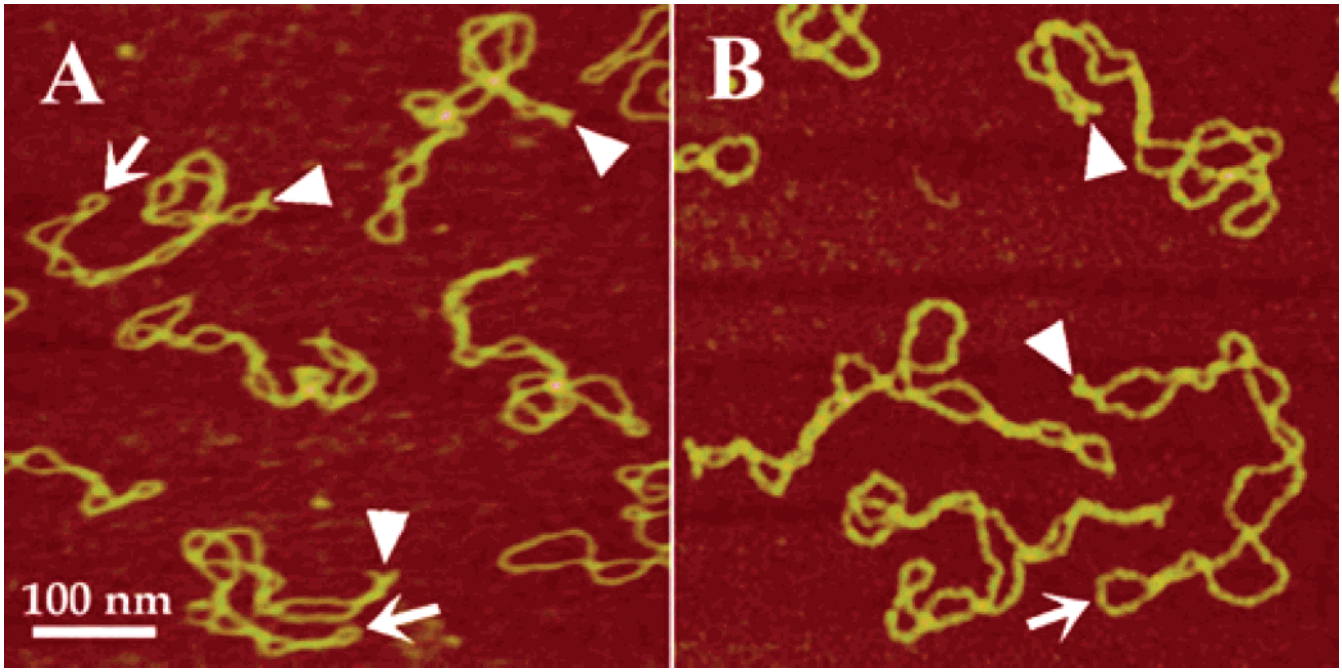

FIGURE 3: AFM images of plasmids (A) pFA6 containing an Atract region and (B) pFR6 containing a mixed sequence diametrically opposite of a cruciform. DNA was deposited from a solution containing 100 mM NaCl. Some of the cruciforms and terminal-end loops containing bent or mixed sequences are indicated with arrowheads and arrows, respectively.

pFA6) should lead to the formation of linear plectonemic superhelices. The control plasmid pFR6 was obtained by inserting the mixed DNA sequence instead of the A tract (*11*).

AFM images (Figure 3) show that compared with control plasmid pFR6, the diametrical positioning of the cruciform and the A tract in plasmid pFA6 leads to a decrease in DNA branching, with the cruciform and the A tract situated at the apexes of the superhelix (*11*). The availability of these molecules provides a unique opportunity to test the hypothesis that DNA supercoiling can increase the bend of the DNA helix located at the apex of a supercoil. Moreover, this allows us to estimate the magnitude of this effect by measuring the curvature of the loop opposite the cruciform.

Curvature of Superhelical Loops. The curvature of terminal loops containing the A tract was characterized by the angle between the tangents to the molecule trace applied at 10 nm in each direction from the apex (see Figure 2). AFM images of DNA typically appear as thick filaments; therefore, the tangent was applied to the tallest section of the filament. The results of the multiple measurements are shown in Figure 4A. The mean value of the bend angle at the apically located A tract is 27.4 ± 20.8°, and a large population of molecules has a very acute angle. For the unconstrained 20-nm segment of the A tract, an expected bend angle calculated according to the scheme shown in Figure 2 (using the 18° bend angle per one helical turn (*17*) and the periodicity of DNA of 3.57 nm) is 79.2°. This bend-angle value is considerably higher than the measured angle in supercoiled DNA, suggesting that the A tract is substantially bent under the stress of supercoiling.

For comparison, Figure 4B shows similar measurements for control plasmid pFR6 with a mixed sequence insertion instead of the A tract. The mean value of 69.5 ± 30.3° is considerably less than the 145° angle calculated for unconstrained DNA with a persistence length of 50 nm (*18*). Such

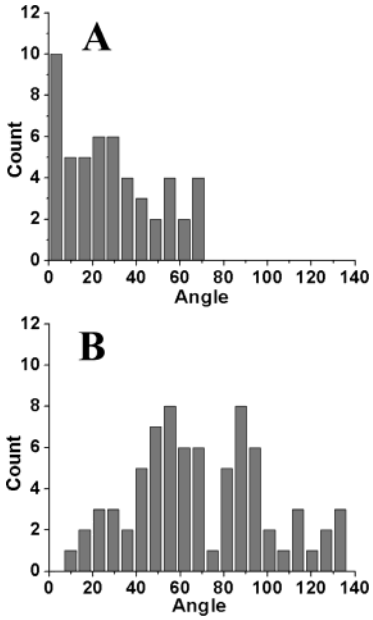

FIGURE 4: Distribution of the end-loop angles measured for plasmid pFA6 (A) and the control plasmid pFR6 (B). All angles are from molecules with linear plectonemic shape, where the A tract or mixed sequence could be identified. Enhanced bending of the A tract over nonconstrained inherent curvature was observed. Significant bending was also observed for the mixed sequence. See Figure 2 for the explanations of the measuring procedure.

a 2-fold difference in the DNA curvature suggests that the mixed sequence DNA region in the apex of the superhelix is also under substantial deformation stress.

Bend-angle measurements were also performed for apically located cruciforms in plasmids pFA6 and pFR6. Data in Table 1 show that the average bend angle at the cruciform in pFA6 is larger than the bend angle at the A tract. The cruciform bend angle in pFR6 is less than the bend angle in the mixed sequence DNA region. The average bend angle

Table 1: End Loop and Cruciform Angles for Plasmids pFA6 and pFR6[a]

plasmid	number of molecules analyzed	mean angle value at the loop	mean angle value at the cruciform
pFR6	75	69.5 ± 30.3°	54.1 ± 30.1°
pFA6	51	27.4 ± 20.8°	45.8 ± 33.6°

[a] Angles are derived from the Gaussian fits for end-loop angle (Figure 4) and the cruciform angle data (data not shown). Mean angles with standard deviations are shown.

induced by the cruciform in plasmid pFA6 (45.8 ± 33.6°) is slightly less than in plasmid pFR6 (54.1 ± 30.1°). However, these data are not significantly different ($p = 0.19$).

DISCUSSION

Superhelical Loop-Induced Bending. It is expected that DNA supercoiling deforms the double helix. Because it becomes organized into a plectonemic superhelix, it is expected that the DNA at the apexes will necessarily be bent. The possibility of A tract distortion by DNA supercoiling has been suggested earlier based on the gel-mobility data analysis (*9*), Monte Carlo modeling (*10*), and kinetics of knot formation (*6*). We have been able to measure the curvature of the apical loop occupied by the A tract and directly estimate the deformation effect induced by DNA supercoiling. The question we asked here is whether the static curvature observed for a bent sequence localized at the apex is greater than expected for the static curve. In addition, we asked if the mixed sequence DNA is bent at the apex to a greater degree than expected from the DNA persistence length. First, we analyzed plectonemically wound molecules formed by the diametrically positioned cruciform and the A tract and measured the angle at which parts of the DNA contour converge at the A tract-containing terminal loop. The angle determined from the AFM data (27.4 ± 20.8°) is considerably less than can be inferred from the A tract curvature in linear DNA (79.2°). A similar bending effect induced by DNA supercoiling was observed for the random sequence, the curvature of which can be estimated from the DNA persistence length. We observed more than a 2-fold difference between an apical curvature and unconstrained DNA (69.5 ±30.3° at the apex versus 145° calculated for linear DNA), assuming that the DNA persistence length value is 50 nm. The persistence length determined from the AFM data may vary depending on the deposition procedure. For the AP−mica procedure that is close to APS−mica used in this paper, the persistence length value is between 20 and 40 nm. The bend angles calculated for these values of persistence length using an equation from Rivetti et al. (*18*) are in the 127−141° range, corresponding to a 2-fold difference with the experimentally determined values for the apically located sequences. The similar, 2−3-fold differences between the experimentally measured angles at the apexes and the corresponding angles in unconstrained parts of DNA molecules for both the A tract and random sequences indicate that these two double-stranded DNA structures are distorted upon their apical positioning to a similar extent. Presumably, the energy for these distortions derives from the free energy of supercoiling, which drives the plectonemic winding and induces bending at the apex. This distortion can clearly be used to drive biological reactions because we have recently shown that the apical localization of an inverted repeat can reduce the energy needed to drive the cruciform formation (*11*).

Theoretical Considerations of the Energetics of Bending. We also analyzed the angle distribution that determines the radius of curvature of the terminal loop with (Figure 4A) and without (Figure 4B) the A tract. The radius of curvature R is related to the measured angle θ by $R = 20$ nm/[$\pi - \theta$ (rad)]. We expressed the changes of plasmid energy as a function of the terminal-loop radius and explored how this radius changes when the loop contains a 180° A tract of the length ℓ_A (36 nm). A detailed description on modeling the energetics and radius of curvature of the A tract end loop is given in the Supporting Information. This qualitative analysis does not take into account the differences in DNA flexibility because it has been previously shown that intrinsically curved DNA gives a very small contribution to its persistence length (*19*). The plasmid is simplified to contain a circular-terminal loop, a long superhelical plectonemic midsection, and a constant-length loop at the opposite end, where the cruciform is located. The total plasmid length, L, is constant, whereas the contour length of the terminal end loop, ℓ_1, is variable. The radius of the terminal loop varies as $R = \ell_1/2\pi$. The total energy of the plasmid can be expressed as $E_{tot} = E_1(\ell_1) + E_2(L - \ell_1)$. E_1 is the energy of the circular-end loop and accounts for the terminal-loop bending. E_2 includes the energy of bending and writhe of the superhelical midsection and the cruciform energy at the superhelix end opposite of the A tract. As shown in parts A and B of Figure 4, the measured average angles at the plasmid apexes are 69.5° ($R = 10.4$ nm) without the A tract and 27.4° ($R = 7.5$ nm) with the A tract. Figure 4A shows that the distribution for the A tract has a maximum near $\theta = 0°$ ($R = 6.4$ nm). The model suggests that this result is significant. It is intuitively clear that R should decrease with the insertion of an A tract into the end loop, and this effect is included in the model for $E_1(\ell_1)$, which incorporates the bend energy of a circular-end loop.

The model produces the following picture. Starting with a long segment with $\ell_1 \gg \ell_A$, the lowering of the energy E_2 drives base pairs outside the 106-bp A tract out of the end loop with little energy penalty cost coming from the end loop, E_1. The fact that E_1 produces little energy cost is counter-intuitive and results from the 180° A tract (as described in the Supporting Information). The loop shrinks until it is near the point where $\ell_1 \ell_A$, and *only* the A tract remains in the end loop. A "hard wall" is encountered if the A tract base pairs are attempted to be removed from the end loop. Of course, this simplified scenario based only on energetic considerations is not the complete picture; entropy and thermal fluctuations produce a distribution, which as this analysis suggests, peaks around the hard-wall position near $\ell_1 \ell_A$. The distribution curve of θ for pFA6 in Figure 4A shows a peak at 0°, which corresponds to $R = 6.4$ nm and $\ell_1 = 40$ nm. This is reasonably close to $\ell_A = 36$ nm. Evaluating the root-mean-square deviation about 0° instead of the mean, gives

$$\sigma = \sqrt{(20.8)^2 + (27.4)^2} = 34.4°$$

which is now similar to the 30.3° fluctuation of the pFR6 plasmid.

Biological Implications of Superhelical Loop-Induced Bending. Altogether, the data obtained show that terminal ends of supercoiled DNA are substantially more bent than unconstrained regions. Such an effect of DNA supercoiling may play a role in various genetic processes in which DNA bending is essential. Supercoiled molecules are very dynamic; therefore, various DNA regions can be brought to the apical position and become bent. However, such alternative regions as the A tracts or cruciforms, because of their preferential positioning at apexes, dramatically change global DNA dynamics. Moreover, DNA regions located diametrically opposite to these local structures have an elevated propensity to be placed at the apex and thus to undergo additional bending. If these regions are involved in protein−DNA interactions and regulation of gene activity, this effect is regulated indirectly by alternative DNA structures. In addition to A tracts and cruciforms used here, a similar effect should be observed for formations of intramolecular triplexes (H-DNA), which induces a strong kink in the double helix (*13*). An interesting interplay can be observed if two or more alternative structures (H-DNA, Z-DNA, and the cruciform) compete with each other in the plasmid, whose global conformation is defined by the bend (cruciform or H-DNA). Altogether, the data obtained provide a novel view on the interplay between local and global DNA structure and dynamics and regulation of gene activity entirely based on complex DNA structural dynamics.

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SUPPORTING INFORMATION AVAILABLE

Detailed description on modeling the energetics and radius of curvature of the A tract end loop. This information is available free of charge via the Internet at http://pubs.acs.org.

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