Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 8, pp. 981-986, 1994

0960-894X/94 $6.00+0.00

0960-894X(94)E0087-U

Toward the Synthesis of a Second-Generation Nitroxide Spin Probe for DNA Dynamics Studies

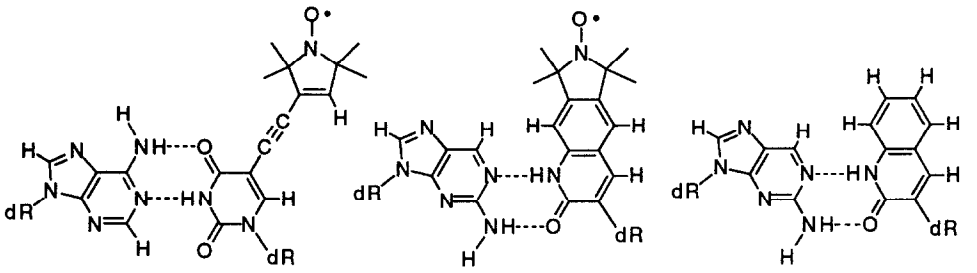
Todd R. Miller and Paul B. Hopkins*

Department of Chemistry, University of Washington, Seattle, Washington 98195

Abstract: A synthesis of the 2'-deoxyribonucleoside **5** is reported. This substance possesses the carbon skeleton and functional groups appropriate for the synthesis of phosphoramidite **4**, which is required for the incorporation of base pair **2** into double-helical DNA. Base pair **2** is of interest as a spectroscopic probe for DNA dynamics.

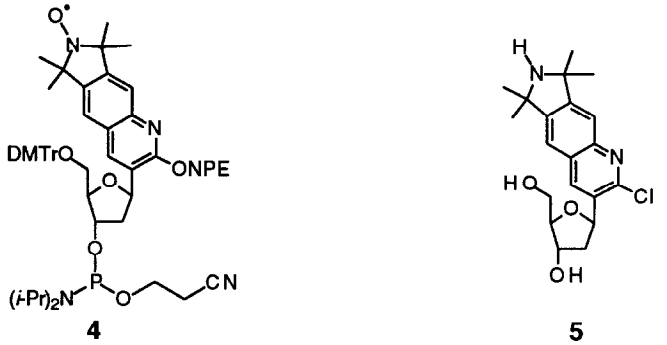
It is increasingly appreciated that the textbook structural model of B DNA, with its graceful and regular double helix of sugar-phosphate chains, is greatly oversimplified. This fiber diffraction-based model has recently given way to models built on single crystal x-ray and solution NMR measurements, which reveal conformational variation of the sugar-phosphate backbone as a function of nucleotide sequence. The importance of these *structural* differences in DNA's biological function remain unknown. By analogy, it seems probable that there will be differences in the conformational *dynamics* of nucleic acid structures as a function of sequence, and that these differences may be biologically relevant. For example, it is now well-accepted that the ground-state structure of DNA in the presence and absence of a DNA-binding protein can be distinct, for example, the DNA being bent or otherwise reorganized by binding to the protein. Available data suggest that the energetic cost of such reorganization is a function of nucleotide sequence. For example, in one case where significant bending of DNA occurs on protein binding, the affinity of the protein for DNA has been shown to correlate with the ability of the DNA to bend.[1] In another case, it has been concluded that the dynamic flexibility of a DNA sequence may dominate its sensitivity to cutting by DNase I.[2] Studies of DNA dynamics may eventually occupy a position similar in importance to the studies of DNA structure.

For several years we have been involved in a collaborative study of nucleic acid dynamics using electron paramagnetic resonance (EPR) spectroscopy.[3] Of several spectroscopic approaches which have provided the bulk of our knowledge concerning nucleic acid dynamics, EPR possesses advantages in terms of the large range of accessible time scales and the small quantities of material required. Nevertheless, the method has been relatively sparingly applied to the problem of nucleic acid dynamics, as a result of a number of obstacles. A significant effort is necessary to prepare "spin-labeled" DNA with the spin label at a precisely defined location, and with the motion of the spin label and biopolymer coupled to one another. Furthermore, interpretation of the resulting EPR spectra is not straightforward. There have been recent advances on both fronts.[3,4] The overall task remains complex, but surmountable.



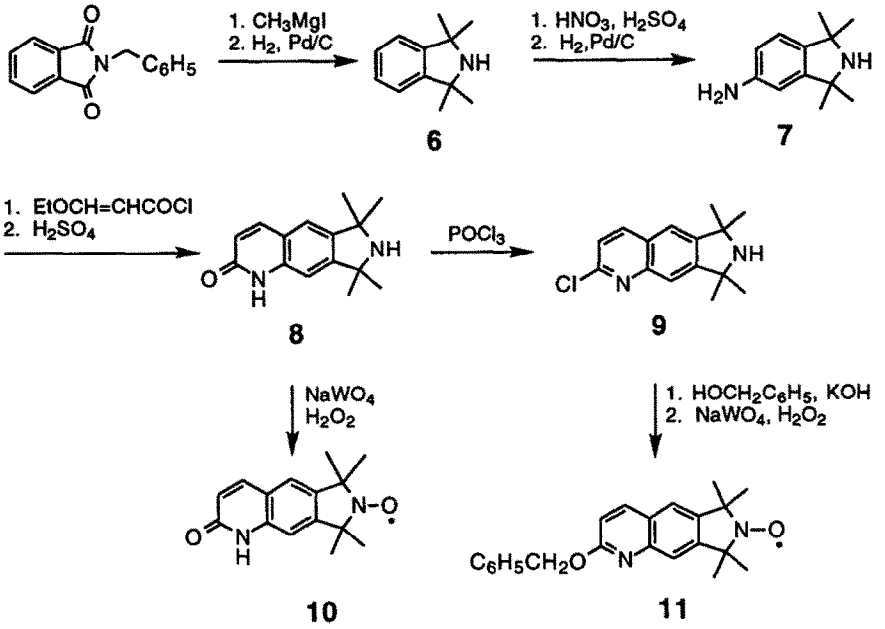
1 2 3

We have previously studied base pair **1** in several duplex DNAs.[3,4] From the perspective of meeting the challenges noted above, those studies constituted a major advance. The motion of this nitroxide was almost identical to that expected for the attached DNA, but retained a residual motion of the nitroxide independent of the attached biopolymer, likely rotation about the acetylene function, restricted by collision with the neighboring sugar-phosphate backbone.[5] For this reason, we have proposed to prepare the base pair **2**, which should eliminate the "wobble" problem inherent in **1**.[6,7] EPR data from such a probe would confirm previous dynamic analyses using probe **1**. We anticipate that the EPR spectra of DNAs spin-labeled with **2** will succumb more readily to spectroscopic analysis by virtue of elimination of the wobble. Because there were several uncertainties implicit in the proposed installation of base pair **2** into B DNA, we did not pursue this goal directly. Instead, we have previously reported a model study in which base pair **3** was incorporated into duplex DNA.[7] That study clearly revealed that base pair **3** could be incorporated into the center of short double helices (e.g. 11 to 13-mer) without appreciable alteration of the thermodynamics of melting of the resulting biopolymer relative to the corresponding duplexes containing an A-T pair. That accomplished, we have embarked upon the synthesis of duplex DNA containing the base pair **2**. The previous studies suggest that the availability of phosphoramidite **4** will prove critical in this regard. We report herein a synthesis of the 2'-deoxyribonucleoside **5**, which contains the complete carbon skeleton of **4** and functional groups appropriate for installation of the remaining protective and activating groups.



4 5

The synthesis of **5** commenced by conversion of N-benzylphthalimide by the literature procedure[8] to the isoindoline **6**,[9] which was in turn aminated in 94% yield by sequential nitration and reduction, affording the aminoisoindoline **7**. This substance was then converted to a quinolone by sequential acylation with 3-ethoxyacryloyl chloride[10] and cyclized[11] as shown, to afford the substituted quinolone **8** in 64% overall yield from **7**. To prepare this substance for installation of the 2'-deoxyribosyl residue, it was converted to the corresponding chloroquinoline **9** in 62% yield. (We have previously used an analogous fluoroquinoline for this purpose.[6,7] In the present case the fluoroquinoline could not be obtained in acceptable yield, and as described below, the chloroquinoline was used herein.)



10 **11**

Before proceeding with the synthesis, we used substances **8** and **9** to explore the installation of the nitroxide function. Preliminary experiments revealed that the aminoquinolone **8** could be converted to the nitroxide quinolone **10** in good yield by exposure to sodium tungstate and hydrogen peroxide, without oxidation elsewhere in the molecule. Likewise, the displacement of the chloro-substituent by a benzyloxy group afforded an intermediate which could be oxidized to the nitroxide **11**. The accompanying figure shows the EPR spectrum of **11**. The 300 MHz ^{1}H NMR spectrum of nitroxide **11** revealed the expected line broadening of the proton resonances as a function of distance from the nitroxide function. The two pairs of methyl groups, which in spectra of **8** and **9** had appeared as a pair of sharp singlets separated by some 0-5 Hz, appeared in **11** as a broad singlet of line width 50 Hz, as a result of their 2.5 Å distance from the nitroxide nitrogen. The aromatic protons of the heterocyclic ring distal to the nitroxide-containing ring,

that in spectra of **8** and **9** had each appeared as a doublet of ca. J=9 Hz, appeared in **11** as broad signals of line widths 30 Hz and 14 Hz, respectively, for the protons 6.5 Å and 8 Å distant from the nitroxide nitrogen. These reactions suggest that the quinolone or quinoline nucleus will not interfere with preparation of the nitroxide, auguring well for installation of the nitroxide at a late stage in the synthesis.

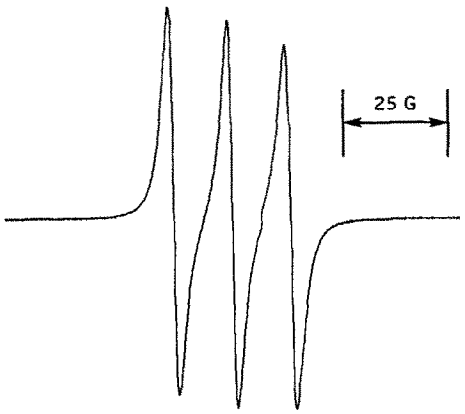
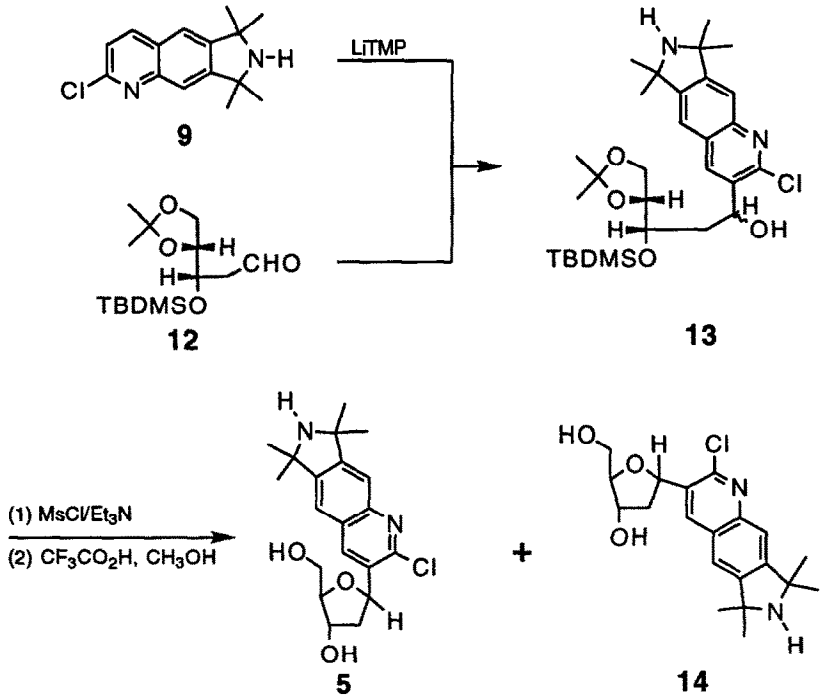


Figure. EPR spectrum of nitroxide **11** in $CDCl_3$.

The 2'-deoxyribosyl residue was now installed.[6] The chloroquinoline **9** was metallated by treatment with 7.5 equivalents of lithium tetramethyl piperidide, and in turn quenched with 2 equivalents of the protected 2'-deoxyribose derivative **12**[6] to afford **13** as a mixture of anomers in 82% yield. The mixture of anomers (admixed with some residual chloroquinoline **9**) was carried forward without separation by preparation of the corresponding mesylate, followed by simultaneous deprotection and cyclization to afford the 2'-deoxyriboside epimers **5** and **14.** This mixture was separated on alumina to afford **5** and **14** in a 32:68 ratio in 64% overall yield from **13.** In our previous studies, this loss of material to the undesired anomer was avoided by an expedient which could presumably be applied in the present case. In that instance,[6,7] it was shown that the two epimers analogous to the epimeric alcohols **13** cyclized stereospecifically. The epimers analogous to **13** were thus separated, and the isomer which afforded the undesired anomer (the analog of **14**) was converted by the Mitsunobu reaction to its more useful isomer. An adequate quantity of **5** can probably be prepared without resort to this recycling operation.

The stereochemistries at the anomeric centers in **5** and **14** were assigned based upon their ^1H NMR spectra. The spectra of these two substances were quite different from one another, especially with regard to both chemical shift and coupling constants of the deoxyribosyl protons. However, each of these two spectra corresponded closely with respect to chemical shift and coupling constants to the spectrum of one of the two isomers of the previously reported quinolines.[6,7] The stereochemistry of those epimers had been unequivocally assigned by nuclear

Overhauser enhancement measurements. Analogous stereochemistries were thus assigned to **5** and **14**.



The studies described herein set the stage for completion of the synthesis of phosphoramidite **4**. Because phosphoramidite **4** is essentially a hybrid of a nitroxide and a 2'-deoxyribosyl-protected quinolone, both of which have independently been previously incorporated into synthetic DNA,[4,7] we anticipate no problems with the use of phosphoramidite **4** to install the nitroxide-bearing residue into synthetic DNA. The completion of the synthesis of **4**, its incorporation into DNA, and spectroscopic characterization of this DNA will be reported in due course.

Acknowledgement. We thank the National Institutes of Health (GM32681) for generous financial support. We thank Professor B. Robinson for his encouragement, Dr. M. Solomon and Mr. S. Sigurdsson for valuable technical advice, Ms. A. Reese for recording the EPR spectrum of **11**, and Ms. K. Bennett for preparation of the manuscript.

References and Endnotes

1. Gartenberg, M.R.; Crothers, D.M. *Nature* (London) **1988**, *333*, 824.
2. Hogan, M.E.; Roberson, M.W.; Austin, R.H. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 9273.
3. Hustedt, E.J.; Spaltenstein, A.; Kirchner, J.; Hopkins, P.B.; Robinson, B.H. *Biochemistry* **1993**, *32*, 1774 and references therein.
4. (a) Spaltenstein, A.; Robinson, B.H.; Hopkins, P.B. *J. Am. Chem. Soc.* **1988**, *110*, 1299; (b) Spaltenstein, A.; Robinson, B.H.; Hopkins, P.B. *J. Am. Chem. Soc.* **1989**, *111*, 2303; (c) Spaltenstein, A.; Robinson, B.H.; Hopkins, P.B. *Biochemistry* **1989**, *28*, 9484.
5. Kirchner, J.J.; Hustedt, E.; Robinson, B.H.; Hopkins, P.B. *Tetrahedron Lett.* **1990**, *31*, 593.
6. Solomon, M.S.; Hopkins, P.B. *Tetrahedron Lett.* **1991**, *32*, 3297.
7. (a) Solomon, M.S.; Hopkins, P.B. *J. Org. Chem.* **1993**, *58*, 2232; (b) Solomon, M. S. *Chemical Synthesis and Characterization of Duplex DNA Containing a New Base Pair*, Ph.D. thesis, University of Washington, 1992.
8. Griffiths, P.G.; Moad, G.; Rizzardo, E.; Solomon, D.H. *Aust. J. Chem.* **1983**, *36*, 397.
9. Key intermediates were characterized by 300 MHz ^{1}H NMR and low resolution MS. Spectroscopic data on key compounds follows: **7**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.41 (6H, s, CH_3); 1.51 (6H, s, CH_3); 6.43 (1H, d, J=2 Hz, H4); 6.58 (1H, dd, J=2, 8 Hz, H6); 6.89 (1H, d, J=8 Hz, H7); LRMS (ei): m/e 190 (M^+), 175 (M^+-CH_3), 160 (M^+-2CH_3); **8**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.50 (6H, s, CH_3); 1.52 (6H, s, CH_3); 6.68 (1H, d, J=9 Hz, H7); 7.09 (1H, s, H4); 7.28 (1H, s, H9); 7.80 (1H, d, J=9 Hz, H8); LRMS (ei): m/e 242 (M^+), 227 (M^+-CH_3), 212 (M^+-2CH_3); **9**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.55 (12H, s, CH_3); 7.33 (1H, d, J=8 Hz, H7); 7.50 (1H, s, H4); 7.73 (1H, s, H9); 8.07 (1H, d, J=8 Hz, H8); LRMS (ei): m/e 245/247 (M^+-CH_3); **5**: ^{1}H NMR (300 MHz, $CDCl_3/D_2O$): δ 1.54 (12H, s, CH_3); 1.92 (1H, ddd, J=6, 10, 13 Hz, H2' or 2''); 2.67 (1H, ddd, J=2, 6, 13 Hz, H2' or 2''); 3.88 (1H, dd, J=5, 11 Hz, H5' or 5''); 3.94 (1H, dd, J=4, 11 Hz, H5' or 5''); 4.12 (1H, ddd, J=3, 4, 5 Hz, H4'); 4.46 (1H, ddd, J=2, 3, 6 Hz, H3'); 5.53 (1H, dd, J=6,10 Hz, H1'); 7.54 (1H, s, H4); 7.72 (1H, s, H9); 8.32 (1H, s, H8); LRMS (ei): m/e 377 ($M+H^+$), 361 (M^+-CH_3); **14**: ^{1}H NMR (300 MHz, $CDCl_3/D_2O$); δ 1.54 (12H, s, CH_3); 1.98 (1H, ddd, J=6, 7, 13 Hz, H2' or 2''); 3.00 (1H, ddd, J=7, 7, 13 Hz, H2' or 2''); 3.77 (1H, dd, J=5, 11 Hz, H5' or 5''); 3.87 (1H, dd, J=4, 11 Hz, H5' or 5''); 4.29 (1H, ddd, J=4, 5, 5 Hz, H4'); 4.46(1H, ddd, J=5, 6, 7 Hz, H3'); 5.53 (1H, dd, J=7, 7 Hz, H1'); 7.56 (1H, s, H4); 7.72 (1H, s, H9); 8.40 (1H, s, H8); LRMS (ei): m/e 377 ($M+H^+$), 361 (M^+-CH_3).
10. Shaw, G.; Warrener, R.N. *J. Chem. Soc.* **1958**, 153.
11. Effenberger, F.; Hartmann, W. *Chem. Ber.* **1969**, *102*, 3260.

(*Received* 20 *September* 1993)