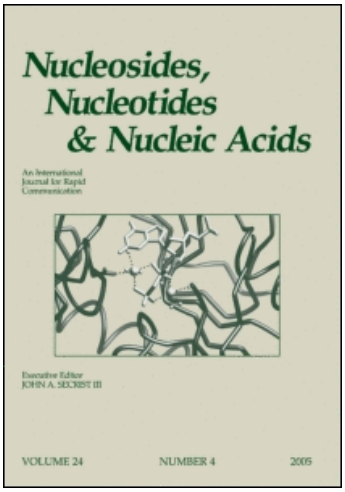

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Synthesis of Two 2'-Deoxyuridine Analogs Substituted in Position 5 with 2-Atom Tethered Nitroxide Radicals

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SYNTHESIS OF TWO 2'-DEOXYURIDINE ANALOGS SUBSTITUTED IN POSITION 5 WITH 2-ATOM TETHERED NITROXIDE RADICALS

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ABSTRACT: The synthesis of two novel spin labeled 2'-deoxyuridine analogs is described. The nucleic acid building block is substituted in position 5 with a short methylamino tether, which bears either a six- or five-membered nitroxide ring.

A variety of spin labeled 2'-deoxyuridine analogs have been incorporated enzymatically or non-enzymatically into nucleic acids, and then used as EPR probes to investigate DNA dynamics[1,2], protein - nucleic acid interaction[3], and drug - nucleic acid[4] binding. The nitroxide group of a spin labeled deoxyuridine is substituted in position 5 of the pyrimidine ring and resides in the major groove of a double-stranded DNA. In this position, a 5-atom tethered nitroxide does not distort the conformation of the lattice, as was observed with $(DUMPT,dT)_n(dA)_n$ duplexes in conjunction with the uvrABC protein complex[1]. Although it is desirable to achieve maximum coupling between nitroxide and base for the evaluation of base dynamics, attaching the nitroxide ring to a very short tether may disrupt the local DNA structure. Computer modeling on an Evans & Sutherland PS390 Computer Graphics System of a double stranded dodecamer spin labeled with the 1-atom tethered nitroxide DUTA[5] or one of the 2-atom tethered nitroxides described here is shown with Figure 1. It is obvious that the 2-atom tether in Figure 1b should prevent the nitroxide moiety from interfering with the sugar-phosphate backbone, whereas this seems to be no longer the case with DUTA (Figure 1a).

The two new 2-atom tethered analogs, 5-{[(2,2,6,6-tetramethyl-4-piperidyl-1-oxy)amino]methyl}-2'-deoxyuridine (2) [DUMTA] and 5-{[(2,2,5,5-tetramethyl-3-pyrrolidinyl-1-oxy)amino]methyl}-2'-deoxyuridine (3) [DUMPDA], are made by Schiff's base formation between 3',5'-di-0-acetyl-5-

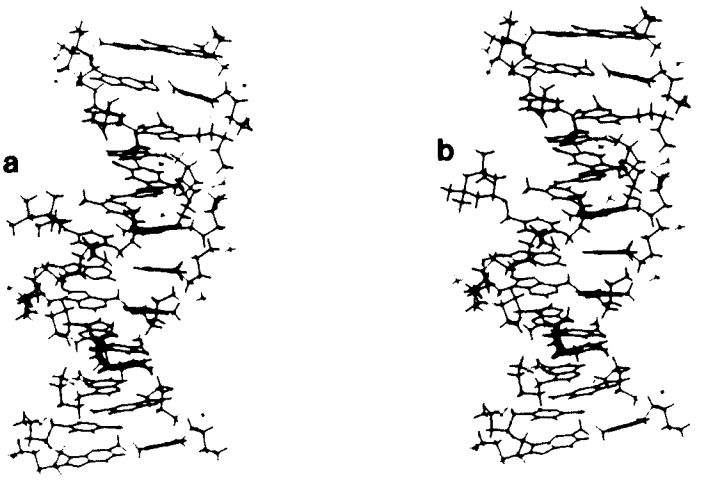
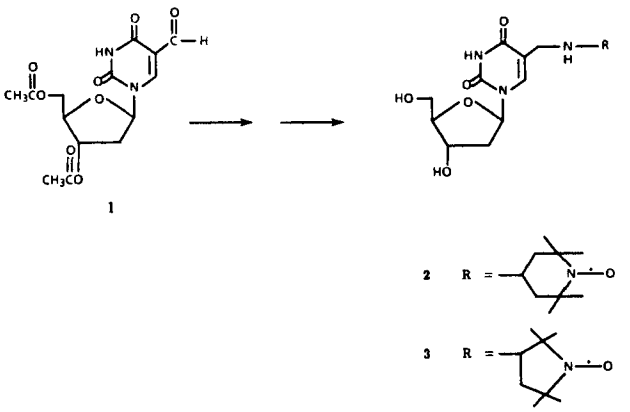


Figure 1. Model of EcoRI restriction site sequence CGCGGAATLCGCG-3' where L corresponds to the spin labeled base DUTA (a) and DUMTA (b).

formyl-2'-deoxyuridine (1) and 4-amino-2,2,6,6-tetramethylpiperidino-1-oxy or 3-amino-2,2,5,5-tetramethyl-1-pyrrolidinyloxy, respectively, followed by reduction and deacetylation. Similar reaction conditions were used for the synthesis of a putrescine probe[6].



The synthesis is facile and gives a good yield for 2 and a somewhat smaller yield for 3. HPLC is required for the final purification of both 2 and 3. Analytical HPLC after preparative HPLC purification gives a single peak for

2, while 3 shows the presence of two closely spaced peaks with similar intensity, which most likely indicate the partial resolution of the two possible stereoisomers of the five-membered nitroxide ring. The structures of 2 and 3 are substantiated by fast atom bombardment mass spectrometry and proton NMR spectroscopy. The EPR spectra of 2 and 3 show signal heights and line-shapes characteristic for six- and five-membered nitroxide labeled nucleosides (Figure 2). The hyperfine splitting constant (A) is 16.7 and 15.7 gauss for DUMTA and DUMPDA, respectively. This difference in the A values gives DUMTA and DUMPDA labeled oligomers distinct EPR signatures that should be useful in bio-assays. Work is in progress with DUMTA and DUMPDA labeled oligonucleotides to corroborate the computer modeling studies and to establish the usefulness of these two new analogs in hybridization assays.

EXPERIMENTAL

UV spectra were recorded on a Perkin-Elmer Lambda 5 UV-VIS spectrophotometer. ^{1}H-NMR spectra were recorded on a Bruker AC-250 spectrometer relative to TMS (0.0 ppm). Fast atom bombardment mass spectra (FAB-MS) were made on a Kratos MS-890 mass spectrometer using nitrobenzyl alcohol as matrix. EPR spectra were obtained using a Bruker ESP 300 spectrometer. High Pressure Liquid Chromatography was done on a Waters Delta Pak column. 3',5'-di-O-acetyl-5-formyl-2'-deoxyuridine (1) was synthesized starting with 2'-deoxythymidine according to published procedures[7,8].

5-{[(2,2,6,6-tetramethyl-4-piperidyl-1-oxy)amino]methyl}-2'-deoxyuridine (2).

To a solution of 1 (0.21 mmol, 70 mg) in methanol/water (20%, 1 ml) was added 4-amino-2,2,6,6-tetramethyl-piperidino-1-oxy (0.26 mmol, 44 mg) in 100 µl of methanol. The pH was immediately adjusted to 8.5 using aqueous acetic acid (10%, 25 µl) and stirring was continued for 30 minutes at room temperature. Aqueous sodium cyanoborohydride (0.21 mmol, 0.41 ml of 0.5 M) was then added to the mixture and the pH was immediately adjusted to 6.5 using aqueous acetic acid (10%, 85 µl). After 30 minutes stirring at room temperature the reaction mixture was dried in vacuo and purified using silica gel column chromatography eluting with the upper phase of ethyl acetate/2-propanol/water 12:1:6. Fractions containing the di-O-acetylated material were dried in vacuo. The labeled compound (0.13 mmol, 62 mg) was dissolved in a minimum volume of methanol followed by water (1 ml). To the solution was added aqueous ammonium hydroxide (7 N, 1 ml). After approximately 3 hours, the methanol, water and ammonia were removed in vacuo and the material was purified using a small silica gel chromatography column, developing with a methanol/chloroform solvent system. The fractions containing the clean material were combined and concentrated to dryness. Final purification was done using reverse phase high pressure liquid chromatography with a 15% methanol/35 mM ammonium phosphate buffer system. The yield was greater than 55%. UV (pH 7): λ_{max} 265 nm (9,000 $M^{-1}cm^{-1}$); FAB-MS: 413(M+2)+; ^{1}H-NMR (250 MHz, DMSO-d_6, ppm) [$Na_2S_2O_4$ reduced[9] in H_2O]: H-C(6), 7.8 (s); H-C(1'), 6.2 (s); H-N(3), 11.3 (s); H-C(2'), 2.1 (m); CH_2 (label), 1.9 (m); CH_3 (label), 1.4 (s).

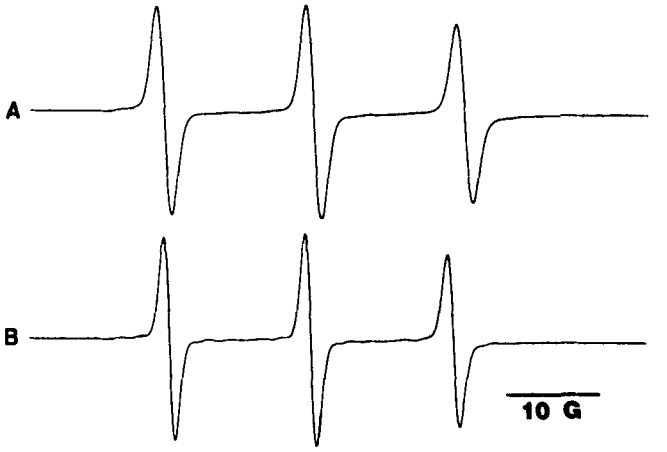


Figure 2. Normalized EPR spectra of DUMTA (A) and DUMPDA (B) in 10 mM Na-cacodylate, 10 mM NaCl, pH 7.

5-{[(2,2,5,5-tetramethyl-3-pyrrolidinyl-1-oxy)amino]methyl}-2'-deoxyuridine (3).

3 was synthesized on a larger scale using conditions similar to those used for 2. To 1 (0.51 mmol, 175 mg) was added 3-amino-2,2,5,5-tetramethyl-1-pyrrolidinyloxy (0.64 mmol, 100 mg) and the pH was lowered using aqueous acetic acid (10%, 25 µl). After 30 minutes stirring at room temperature, aqueous sodium cyanoborohydride (0.51 mmol, 1.03 ml of 0.5M) was added and the pH was lowered using aqueous acetic acid (10%, 175 µl). After 30 minutes the mixture was dried in vacuo and purified using the same procedure as that for the acetylated 2. Without further purification this compound (0.05 mmol, 24 mg) was deacetylated using the same reaction conditions and purification as for 2. The yield was greater than 15%. UV (pH 7): λ_{max} 265 nm (9,000 $M^{-1}cm^{-1}$); FAB-MS: 399(M+2)+; ^{1}H-NMR (250 MHz, DMSO-d_6, ppm) [$Na_2S_2O_4$ reduced[9] in H_2O]: H-C(6), 7.8 (s); H-C (1'), 6.2 (s); H-N(3), 11.3 (s); H-C(2'), 2.1 (m); CH_2 (label), 1.8 (m); CH_3 (label), 1.0-1.5 (m).

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