ORGANIC LETTERS

2005
Vol. 7, No. 2
303−306

Synthesis and EPR Studies of 2-*N*-*tert*-Butylaminoxylpurine Derivatives

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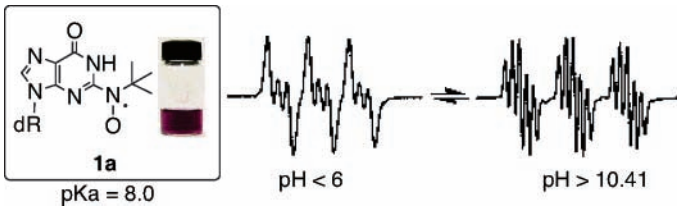
Received November 12, 2004

ABSTRACT



2′-Deoxyribofuranosylpurine derivatives bearing an *N*-*tert*-butylaminoxyl group (1a and 2a) were synthesized via oxidation of the corresponding *N*-*tert*-butylhydroxylamines (1b and 2b), which were synthesized by lithiation of 8-TIPS-6-chloropurine (3) at the 2-position and the following reaction with 2-methyl-2-nitrosopropane. Treatment of 1b and 2b with 1 equiv of $NaIO_4$ resulted in efficient formation of 1a and 2a, which were isolated as purple and red solids, respectively. The EPR spectra of 1a showed pH dependency due to structural change of purine moiety.

We designed spin-labeled nucleosides, in which a spin source, *N*-*tert*-butylaminoxyl, is directly attached to nucleobases.[1] We expected that direct introduction of a spin source into a nucleobase might result in a better correlation between the motions of the spin probe and DNA compared to spin-labeled nucleosides bearing stable radicals such as TEMPO via a linker.[2] Direct connection of the nitrogen atom of the aminoxyl to a nucleobase also leads to interactions between the unpaired electron of the aminoxyl radical and electrons of the nucleobase, which may give us information about base-pairing since it is presumed that electronic properties of the base moieties affect the electron paramagnetic resonance (EPR) spectra. It is known that C8-substituted purine derivatives prefer the syn conformation of the glycosylic bond[3] which might disturb the stable duplex formation of oligonucleotides containing substituted nucleosides. Therefore, we planned to synthesize 2-*N*-*tert*-butylaminoxyl purines **1a** and **2a**. Compounds **1a** and **2a** with directly attached aminoxyls retain hydrogen bonding site to possibly form base pair with natural bases.[2c] Here, we report the synthesis of 2-*N*-*tert*-butylhydroxylamino-2′-deoxyinosine **1b** and 2-*N*-*tert*-butylhydroxylamino-2′-deoxyadenosine **2b** (Scheme 2) by the reaction of 2-lithiated purine with 2-methyl-2-nitrosopropane and their conversion into stable aminoxyls, **1a** and **2a**, and that the EPR spectrum of **1a** showed pH dependency.

Introduction of the 2-*N*-*tert*-butylhydroxylamino group into the purine nucleus at the 2-position was carried out by lithiation of 6-chloro-8-(triisopropylsilyl)purine (**3**) with LTMP,[4] and the following reaction with a nitrogen electrophile, 2-methyl-2-nitrosopropane, and the hydroxylamine **4** was obtained in 75% yield (Scheme 1). Desilylation at the 8-position proceeded efficiently after acetylation of the hydroxylaminogroup of **4** and the following deprotection of

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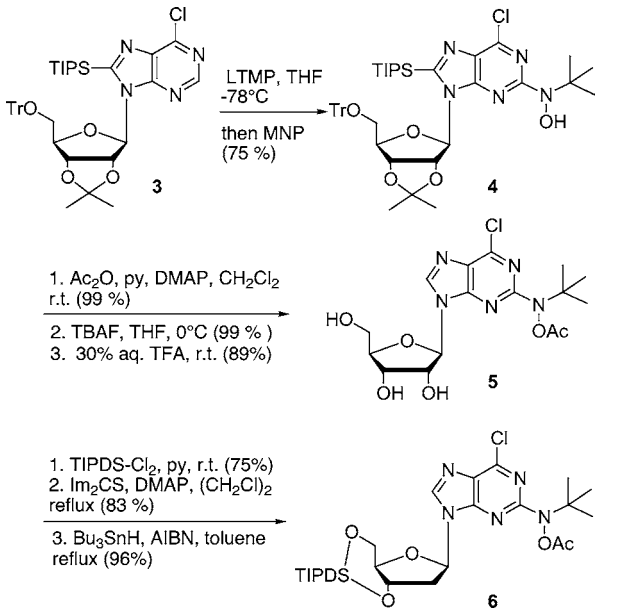
(4) LTMP, lithium tetramethyl piperidide: Kumamoto, H.; Tanaka, H.; Tsukioka, R.; Ishida, Y.; Nakamura, A.; Kimura, S.; Hayakawa, H.; Kato, K.; Miyasaka, T. *J. Org. Chem.* **1999**, *64*, 7773.

10.1021/ol047668t CCC: $30.25
Published on Web 12/23/2004

Scheme 1



hydroxyl groups gave 6-chloro-2-*N*-*tert*-butylacetoxyamino-purine **5**. The 3′,5′-hydroxyl groups of **5** were protected as a TIPDS ether and 2′-hydroxyl group was removed by Barton's procedure[5] after thioacylation. The *N*-*tert*-butyl-acetoxyamino group was stable under the reaction conditions and **6** was obtained in good yield.

Compound **6** was a key intermediate for the synthesis of **1b** and **2b**. Treatment of **6** with CsOAc in the presence of 1,4-diazabicyclo[2.2.2]octane (DABCO) and Et_3N and the following treatment with Ac_2O[6] afforded **7** as the sole product (Scheme 2). It is reported that the reaction of 6-chloropurines with DABCO gives the "DABCO-purines".[7] It was presumed that the corresponding "DABCO-purine" of **6** might be involved in the reaction and its displacement reaction with the less nucleophilic acetate ion, compared to the alkoxide ion,[7] and hydrolysis of the resultant 6-acetate under the reaction conditions afforded **7**. Deprotection of the sugar moiety of **7** with NH_4F (MeOH, reflux) was accompanied by removal of the acetyl group and **1b** was obtained in 77% yield. For the synthesis of **2b**, **6** was converted to **8** via azide (Scheme 2). Desilylation and hydrolysis of the acetyl group gave **2b**.

Conversion of **1b** and **2b** into aminoxyls proceeded with ease by treatment with $NaIO_4$ in water (Scheme 2). Upon addition of 1 equiv of $NaIO_4$ to an aqueous solution of **1b**, the color of the solution turned purple immediately. TLC analysis of the reaction mixture showed that oxidation completed in 10 min at room temperature and purification by silica gel chromatography gave **1a** as purple solids in 98% yield. The HRMS and EPR data agreed with the

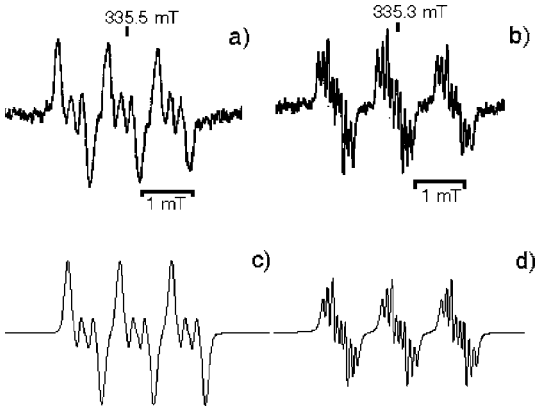


Figure 1. EPR spectra of (a) **1a** (g = 2.0064) and (b) **2a** (g = 2.0064) and (c) simulated spectra of **1a** (a_N = 9.78, 2.22, and 0.62 (G)) and (d) **2a** (a_N = 11.79, 0.95, 0.95, a_H = 2.58 (G)).

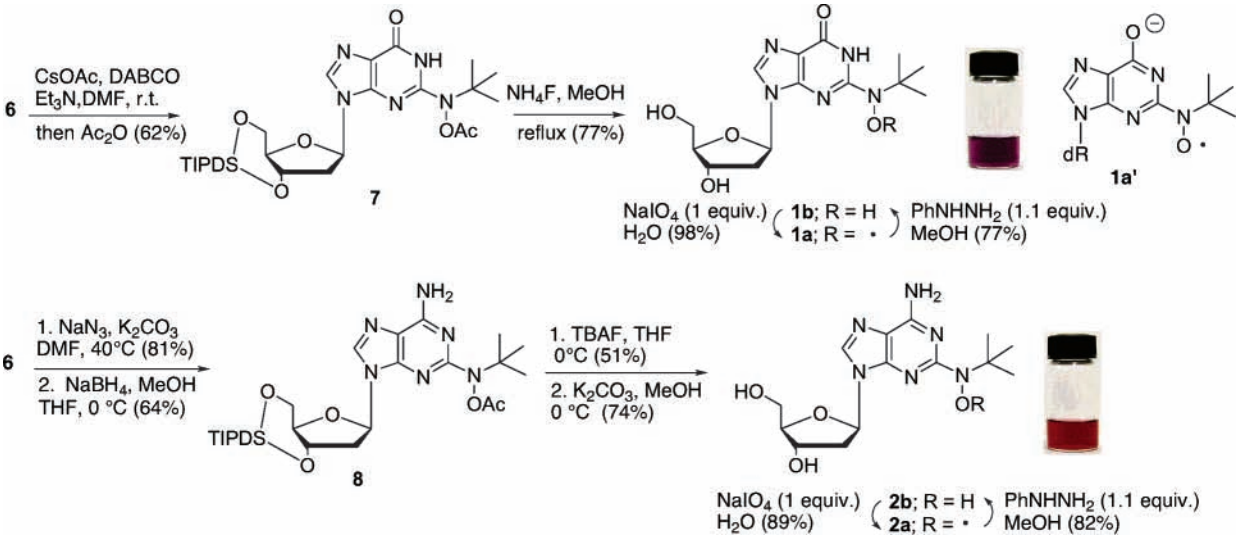
structure of **1a**. The EPR spectrum of an aqueous solution of **1a** showed a triplet, in which each peak was split into a triplet and the obtained product was assigned to be the aminoxyl radical **1a** (g value = 2.0064, Figure 1a). The EPR spectrum of **1a** could be simulated by assuming hyperfine coupling constants (a_N = 9.78, 2.22, and 0.62 (G)) (Figure 1c).[8] Aminoxyl **1a** was reduced to the parent **1b** by treatment with 1.1 equiv of phenylhydrazine in 77% yield.

Oxidation of **2b** with $NaIO_4$ (1 equiv) proceeded easily in water (room temperature, 10 min), and aminoxyl **2a** was isolated as red solids after silica gel chromatography (89%). The EPR spectrum of an aqueous solution of **2a** showed a triplet, in which each peak was split into an octet (g value = 2.0064, Figure 1b). The EPR spectrum of **2a** could be simulated by assuming hyperfine coupling constants (a_N = 11.79, 0.95, 0.95 and a_H = 2.58 (G)) (Figure 1d).[9] Aminoxyl **2a** was also reduced to **2b** with phenylhydrazine (82%). The half-life periods of **1a** and **2a** in H_2O were estimated to be about a week at room temperature.[10]

It was presumed that electronic properties of the purine moiety affected the EPR spectra of **1a** and **2a**, so the EPR spectra of **1a** and **2a** were studied as a function of pH. The EPR spectra of **1a** at lower pH between 3.43 and 6.10 were similar to that in water and were simulated (spectrum A, a_N = 9.83, 2.22, and 0.60 (G)) (Figure 2). Upon increase of pH by addition of KOH solution, the EPR spectra of **1a** changed. The EPR spectra at higher pH between 10.41 and 11.47 similarly displayed a triplet in which each of the three lines was split into a quintet (g value = 2.0064). The spectra were simulated and hyperfine coupling constants were assumed (spectrum B, a_N = 13.02, 1.80, and 1.50 (G)) as shown in Figure 2. Increase of the largest a_N value (a_N = 9.83 G at pH 6.10 and 13.02 G at pH 10.41, Δa_N = 3.2 G),

(5) Barton, D. H. R., McCimbiem S. W. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1574.
(6) Treatment of **10** with CsOAc in the presence of DABCO and Et_3N formed **11** and deacetylated form of **11** as a minor product.
(7) Lembicz, N. K.; Grant, S.; Clegg, W.; Griffin, R. J.; Heath, S. L.; Golding, B. T. *J. Chem. Soc., Perkin Trans. 1* **1997**, 185.
(8) Duling, D. R. *J. Magn. Reson., Ser. B* **1994**, *104*, 105.
(9) EPR spectrum of **2a** was well simulated by hyperfine coupling constants which include a_H = 2.584 with WinSin program by Duling (ref 8). The hydrogen which is responsible for the coupling constant has not been assigned.
(10) Compounds **1a** and **2a** were more stable in solids.

Scheme 2



which was due to interaction between an unpaired electron and the nitrogen nucleus of the aminoxyl radical, suggested spin density on the nitrogen atom of aminoxyl increased at basic pH. The UV spectrum of **1a** at pH 10.41 caused absorption decrease at 259 nm similarly to deprotonated guanosine[11] (Supporting Information). Presumably, **1a** was deprotonated and tautomerized to the enolate form **1a′** (Scheme 2), and the EPR spectra at pH 10.41 or greater were attributed to **1a′**.[12] A change in pH between 11.47 and 3.36 resulted in a reversible change of the EPR spectra of **1a** without loss of peak intensities.

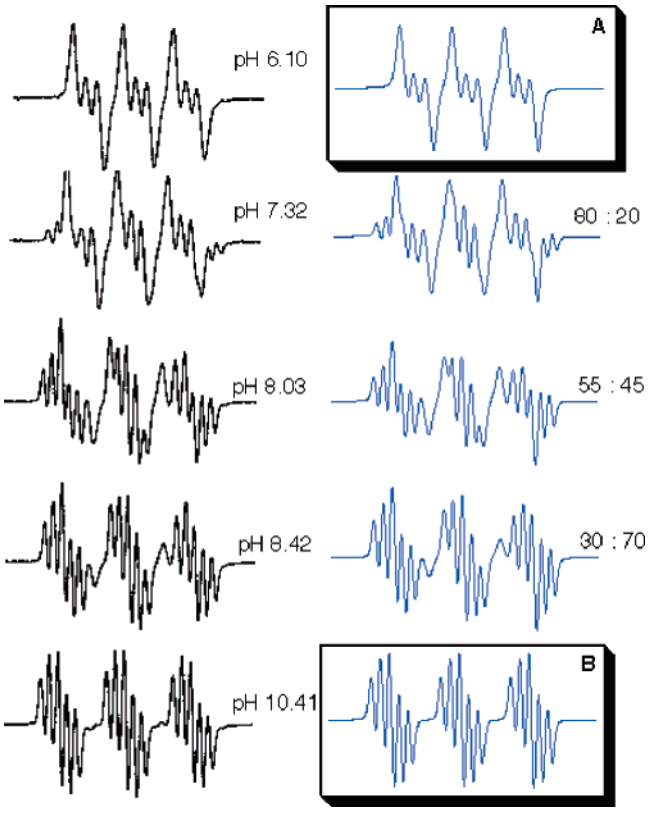


Figure 2. pH dependence of EPR spectra of **1a**. The EPR spectra of **1a** at various pH (in black) and their simulated spectra prepared by the sum of spectra **A** and **B** in the indicated ratio (**A**/**B**) (in blue).

It is reported that protonation/deprotonation of functional groups β to the aminoxyl nitrogen nucleus influences aminoxyl nitrogen hyperfine splitting constants (a_N values) due to dipolar effects between the β-substituent and the aminoxyl function.[13d] Imidazoline and imidazolidine aminoxyls were proposed as pH probes.[13] Lifetimes of protonated (RH^+) and nonprotonated (R) forms relate to pH of the medium and pK_a of the probes.[14] In the case of fast proton exchange between RH^+ and R forms on the EPR time scale, the EPR spectrum at certain pH is a weighed average of that of RH^+ form and that of R form. The a_N value changes as a function of pH in this case.[13c] In the case of slow exchange, the EPR spectrum is a weighed summation of that of RH^+ form and that of R form.[13b,14] In contrast to previously reported pH-sensitive EPR probes,[13e,f] aminoxyl **1a** showed a different EPR spectra at pH below 6.10 and above 10.41 due to change of structure/electronic properties of the purine

(11) Bloomfield, V. A.; Crothers, D. M.; Tinoco, I., Jr. *Physical Chemistry of Nucleic Acids*; Harper & Row: New York, **1974**.

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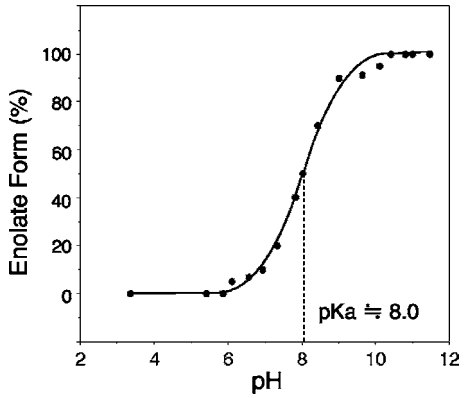

Figure 3. Plot between pH and percentage of enolate form (**1a′**) to give pK_a of **1a**.

moiety. Interestingly, the EPR spectra of **1a** at a pH between 6.10 and 10.41 could be simulated by summation of spectra A and B in an appropriate ratio. For example, the EPR spectra at pH 8.03 was an exact match of the simulation which was produced by using spectra A and B in a 55:45 ratio, respectively (Figure 2). It is due to slow exchange between **1a** and deprotonated **1a′** on the EPR time scale in this pH region. This analysis gave titration plot and the pK_a of **1a** was estimated to be approximately 8.0 (Figure 3) though attempted pK_a determination by UV spectroscopy was not successful. In **1a**, the aminoxyl group at the 2 position of purine had electron-withdrawing character[15] which resulted in decrease of pK_a value compared to that of inosine (pK_a of N1 = 8.60[16]).

The EPR spectrum of **2a** was also measured at various pHs. At pH between 3.93 and 11.74, the EPR spectra of **2a** were similar to that in water. It is estimated that pK_a of N1 of **2a** may be close to that of adenosine (pK_a of N1 = 3.60[16]). Over this pH range, electronic properties of the purine moiety of **2a** did not change because protonation/deprotonation might not take place.

In summary**,** we have synthesized stable aminoxyls **1a** and **2a**. Smooth oxidation of *N*-*tert*-butylhydroxylaminopurines **1b** and **2b** with $NaIO_4$ proceeded to give **1a** and **2a**, which were also converted to **1b** and **2b** by reduction. The EPR spectra of **1a** showed pH dependency in the physiologically important pH range. Contrary to previously reported pH-sensitive probes, the EPR spectra of **1a** reflected the structure of the purine moiety. That can be a unique character of **1a** in comparison with imidazoline and imidazolidine aminoxyls together with large Δa_N (3.2 G) ($\Delta a_N \sim 1.1$ for the imidazoline aminoxyl[13e]). The possible applications of **1a** and **2a** as new types of spin-labeled nucleosides will be studied with oligonucleotides containing **1a** and **2a**.

Supporting Information Available: Experimental procedures and characterization for compounds **1a**,**b**, **2a**,**b**, and **4**−**8**; ^{1}H NMR spectra for compounds **1b**, **2b**, and **4**−**8**; UV spectra of **1a** at pH 6.10 and 10.41. This material is available free of charge via the Internet at http://pubs.acs.org.

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