Tetrahedron 71 (2015) 5414–5419

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Synthesis of 5-*N*-*tert*-butylaminoxylcytidine and EPR studies on its base pairing properties

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ARTICLE INFO

Article history:
Received 10 April 2015
Received in revised form 21 May 2015
Accepted 22 May 2015
Available online 4 June 2015

Keywords:
Spin-label
Cytidine
EPR spectroscopy
Base pair
Aminoxyl

ABSTRACT

5-*N*-*tert*-Butylaminoxylcytidine was synthesized as a new spin-labelled cytidine to detect guanosine by EPR spectroscopy. Metal-halogen exchange reaction of TBS-protected *N*-benzoyl-5-iodocytidine followed by a reaction with 2-methyl-2-nitrosopropane afforded 5-*N*-*tert*-butylhydroxylaminocytidine with considerable affinity for guanosine and its oxidation gave 5-*N*-*tert*-butylaminoxylcytidine. Changes in EPR parameters monitored its selective base pairing with guanosine derivatives and the formation of a 2:2 complex with 8-oxoguanosine.

1. Introduction

Electron paramagnetic resonance (EPR) spectroscopy is a powerful tool for investigating the structures and dynamics of nucleic acids in solution. Spin-labelled nucleic acids have been used in such studies, which are important for understanding biological functions of nucleic acids using EPR.[1–3] In this regard, numerous spin-labelled nucleosides have been reported. Recently, **^{T}C**, in which 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) is conjugated to the exocyclic amino group of 2′-deoxycytidine, is used for detecting mismatches in duplex DNA by EPR spectroscopy.[4] As opposed to fluorescent probes,[5] only a limited number of spin-probes have been reported for base mismatch detection. Therefore, the development of new spin-labelled nucleosides might contribute significantly to DNA studies by EPR spectroscopy. We have designed spin-labelled nucleosides in which the nitrogen atom of an *N*-*tert*-butylaminoxyl group is directly attached to the nucleobase as a spin source, for accurate monitoring of nucleobase motion, in contrast to nucleosides connected to cyclic aminoxyl such as TEMPO by a flexible linker.[6–8] We have reported the synthesis and EPR studies of 5-*N*-*tert*-butylaminoxyluridine[7] (**3**, Fig. 1) and 2-*N*-*tert*-butylaminoxylpurines.[8] In this study, we designed 5-*N*-*tert*-butylaminoxylcytidine (**1**), which selectively forms a base pair with guanosine and it derivatives such as 8-oxoguanosine. We expected **1** and its 2′-deoxy derivative to form base pairs when introduced into nucleic acids and thus aid in monitoring structures of nucleic acids and detecting base mismatch. The electron-withdrawing nature of the 5-*N*-*tert*-butylaminoxyl group[8] might not change the base pairing property of

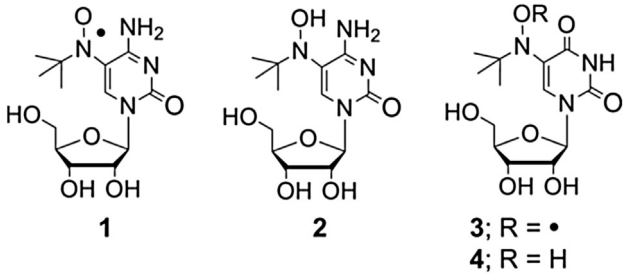

Fig. 1. 5-*N*-*tert*-butylaminoxylcytidine (**1**) and 5-*N*-*tert*-butylhydroxylaminocytidine (**2**), and the corresponding uridine derivatives (**3** and **4**).

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http://dx.doi.org/10.1016/j.tet.2015.05.080
0040-4020/

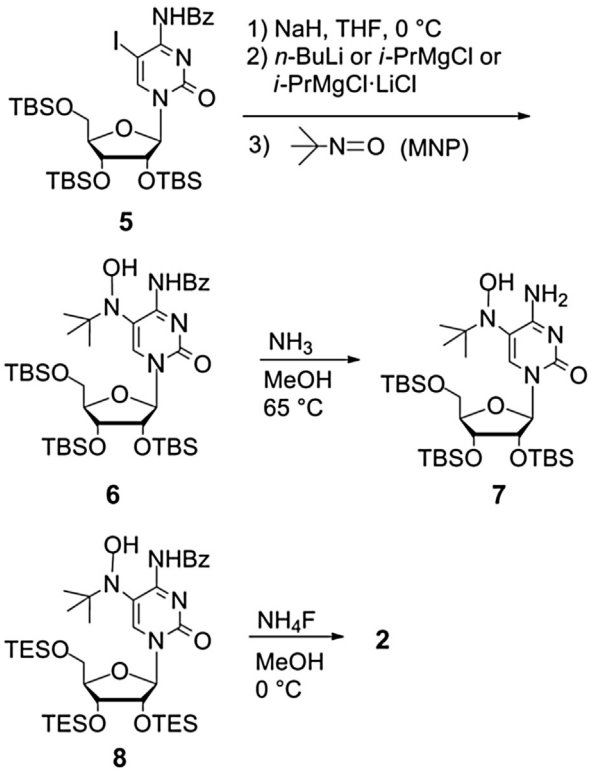
cytidine, similar to the case of 5-formylcytidine and 5-carboxylcytidine.[9] Owing to conjugation, changes in electron distribution in the nucleobase might be transmitted to an aminoxyl group. Such changes caused by base pairing and their effect on EPR spectra should be studied since the said changes cannot occur in TEMPO-carrying spin-labels, where the spin is isolated. Here, we focused on the synthesis of **1** via metal-halogen exchange and its base pairing properties using the TBS-protected derivative; we carried out EPR spectroscopic analyses for accurate monitoring of the base pairing properties and the molecular size of the complex. We also studied base pairing properties of TBS-protected 5-*N*-*tert*-butylhydroxylaminocytidine.

2. Results and discussion

2.1. Synthesis of 5-*N*-*tert*-butylhydroxylaminocytidine and its base pairing with guanosine

Previously, we synthesized 6-*N*-*tert*-butylhydroxylaminocytidine by site-selective lithiation of TBS-protected *N*-benzoylcytidine at the 6-position with LDA and the following reaction with 2-methyl-2-nitrosopropane (MNP)[7] and its oxidation afforded 6-*N*-*tert*-butylaminoxylcytidine. However, 5-substituted cytidine derivative **2** was not synthesized by similar lithiation strategy and synthesis of 5-substituted cytidine and cytosine via lithiation has rarely been reported. In contrast, functionalization of uridine and uracil via 5-lithiated species has been extensively investigated.[10–12] We synthesized *5-N-tert*-butylhydroxylaminouridine **4**[7] and its 2′-deoxy derivative[13] by the reaction of 5-lithiated uridines with MNP. In the synthesis of the 2′-deoxy derivative, halogen-lithium exchange reaction of the 5-iodo-2′-deoxyuridine sodium salt (at the 3-position) allowed us to introduce the *N*-*tert*-butylhydroxylamino group into the 5-position of uridine in good yield. The use of the sodium salt prevented 'self-protonation' in the reaction of 5-iodo-2′-deoxyuridine with *n*-BuLi, thus leading mainly to deiodination, since metal-halogen exchange and deprotonation of the imide moiety are competitive and the 5-lithiated uridine is protonated with residual imide proton. 5-Substituted cytidines can be synthesized from the corresponding uridine derivatives. However, attempted conversion of **4** to **2** was failed.[14] For the synthesis of 5-substituted cytidines, Pd-catalyzed reaction of 5-iodocytidine has been successfully applied for introducing carbon functionalities,[15–18] halogen-metal exchange reaction of 5-iodocytidine, too, might be beneficial for the introduction of non-carbon functionalities, thus complementing the existing synthetic methods. Thus, synthesis of **6** was investigated by halogen-lithium exchange of **5** after deprotonation of the amide in analogy to the corresponding reaction of 5-iodouridine.

Successive treatment of **5** with NaH (1.5 equiv, rt) and *n*-BuLi (1.7 equiv, −78 °C), followed by the reaction with MNP, gave **6** in 26% yield (31% based on recovery of **5**, Scheme 1). A similar reaction without NaH afforded **6** only in 9% yield, indicating that NaH is effective in improving the product yield, although the improvement was less significant as compared to that in the reaction of the 5-iodouridine derivative (72%). Halogen-magnesium exchange using *i*-PrMgCl also resulted in the formation of **6** with comparable efficiency. The use of *i*-PrMgCl·LiCl[12] yielded **6** in better yield (51%). Debenzoylation of **6** by heating in ammoniacal methanol afforded hydroxylamine **7** in 82% yield. For synthesis of **2**, desilylation of **7** with TBAF proceeded, but separation of **2** from excess TBAF and materials derived from TBAF was difficult. TES-protected hydroxylamine **8** was prepared similarly by the metal-halogen exchange reaction of TES-protected 5-iodocytidine (44%) and its treatment with ammonium fluoride in MeOH efficiently provided **2** (80%).[19]



Scheme 1. Synthesis of 5-*N*-*tert*-butylhydroxylaminocytidine.

The base-pair formation of the obtained hydroxylamine **7** with guanosine was examined by NMR. Job plots obtained from the changes in chemical shift of N(1)H of guanosine indicated a 1:1 binding stoichiometry (Supplementary data). To determine the association constant (K_a), titration experiments were performed by adding **7** to 2 mM solution of **G** in $CDCl_3$. The chemical shift of N(1)H of base paired **G** became almost constant when more than 2 mM of **7** were added and accurate K_a was not obtained from NMR experiments under these conditions (Supplementary data).[20] In contrast, the presence of 16 mM TBS-protected cytidine **C** did not give 100% binding to **G** and K_a of 2.4×10^4 M^{-1} was provided (reported K_a for **G**:**C** pair in chloroform; $10^4\sim10^5$ M^{-1}).[20,21] These data indicated that complex **7**:**G** might be more stable than **G**:**C**.

2.2. Synthesis of TBS-protected 5-*N*-*tert*-butylaminoxylcytidine and EPR studies of its base pairing properties

For EPR studies of base pairing properties of 5-*N*-*tert*-butylaminoxylcytidine, TBS-protected derivative **9** was prepared by the treatment of **7** with PbO_2 in CH_2Cl_2 (Fig. 2). After column chromatography, the fractions containing **9** were partially concentrated and used for measurements since the purity of radical was slightly decreased upon concentration.[22] The EPR spectrum of **9**

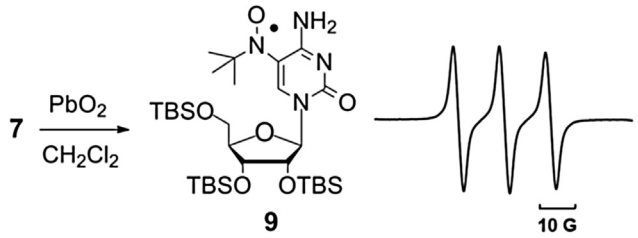


Fig. 2. Preparation of **9** and its EPR spectrum in CH_2Cl_2.