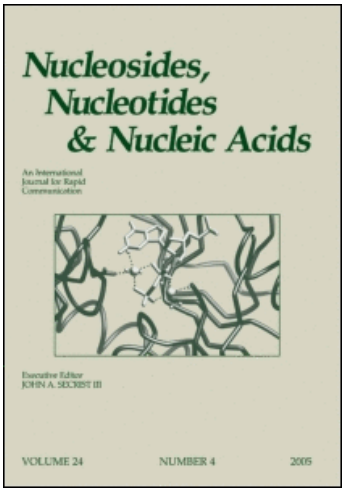

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Guo, Ruoling and Zheng, Qinguo(2005) 'Chemical Synthesis of Oligonucleotides Containing (M 4) Guanine', Nucleosides, Nucleotides and Nucleic Acids, 24: 5, 1081 — 1083
To link to this Article: DOI: 10.1081/NCN-200059175
URL: http://dx.doi.org/10.1081/NCN-200059175

Nucleosides, Nucleotides, and Nucleic Acids, 24 (5–7):1081–1083, (2005)

ISSN: 1525-7770 print/ 1532-2335 online
DOI: 10.1081/NCN-200059175

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CHEMICAL SYNTHESIS OF OLIGONUCLEOTIDES CONTAINING (M + 4) GUANINE

Ruoling Guo and Qinguo Zheng □ *School of Life and Health Sciences, Aston University, Birmingham, UK*

□ *HPLC MS/MS has shown great potential in the measurement of DNA oxidative damage. Its accuracy depends on the use of multiply isotopically labelled internal standards. In this report, multiply isotopically labelled (M + 4) guanine internal standards were prepared in the form of base, nucleoside, as well as DNA oligomer. To our knowledge, this is the first chemical synthesis of oligomers containing (M + 4) guanine, and we believe that they can be used to develop a procedure that can make further improvement to the existing analytical procedures.*

Keywords DNA Oxidative Damage, Isotopically Labelled Guanine, Internal Standard, Oligonucleotide

INTRODUCTION

DNA oxidative damage has been associated with several important health issues, including cancer and aging.[1,2] Numerous analytical methods have been established to measure the level of DNA oxidative damage, but measurement of the generally accepted biomarker, 8-oxo-2′-deoxyguanosine (8-oxo-dG) in vivo has proven to be problematic. Oxidation of intact guanine during the sample preparation stages leads to the overestimation of 8-oxo-dG.[3] One of the most promising techniques is HPLC-MS/MS. However, its potential has been limited by the lack of appropriate internal standards, such as oligomers containing multi-isotopically labelled guanine and those containing labelled 8-oxo-guanine. Here, we describe the synthesis of multiply isotopically labelled (M+4) guanine internal standards in the form of base nucleoside, as well as DNA oligomer for use in the analysis of oxidative DNA damage.

The synthesis of (M+4) guanine (**5**) is illustrated in Scheme 1. Compound **1** was obtained readily from the hydrolysis of commercially available 2,4,6-trichloropyrimidine.[4] Two ^{15}N were then introduced simultaneously by the

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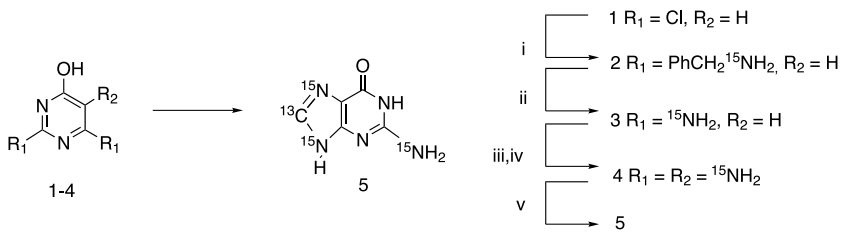
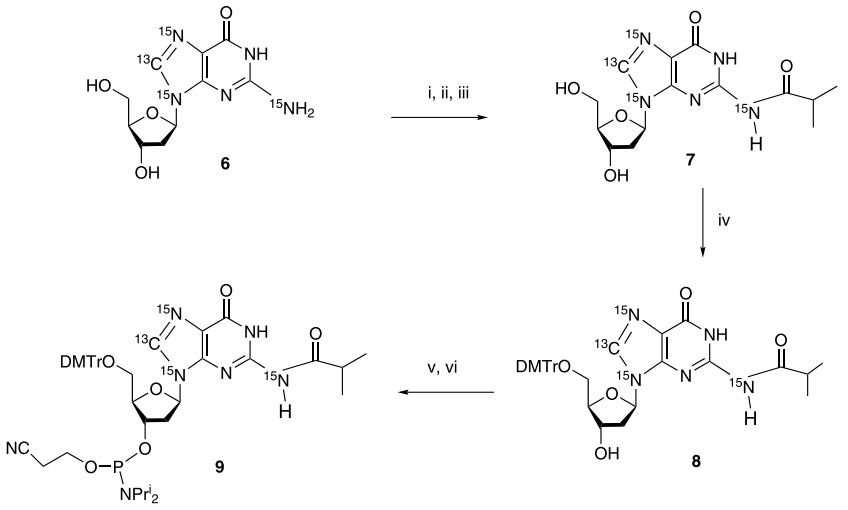


SCHEME 1 The synthesis of (M+4) guanine. i) [^{15}N]benzylamine, 145°C; ii) 10% Pd-C/H_2, 45–50°C; iii) $Na^{15}NO_2$; iv) $Na_2S_2O_4$; v) [^{13}C]formamide.

displacement of the two chlorine atoms of **1** with [^{15}N]-benzylamine to give **2**. Subsequent hydrogenolysis removed the benzyl groups of **2**, resulting in **3**. The third ^{15}N at 5-position was introduced by nitrosation followed by reduction with sodium dithionite. Labelled (M+4) guanine (**5**) was obtained by cyclization of the 4-hydroxy-[2,5,6-$^{15}N_3$] triaminopyrimidine sulfate (**4**) with [^{13}C] formamide, and because of our efforts to optimize the procedure, inspired by the high cost of [^{13}C] formamide, a better yield than that shown in the published method[5] was obtained.

The labelled base was then converted into its corresponding 2′-deoxynucleoside (**6**) by enzymatic glycosylation. This involved the use of thymidine phosphorylase (TPase) and purine nucleoside phosphorylase (PNPase).[6] We developed a gel filtration system to purify the target nucleoside directly after glycosylation, which greatly increased the efficiency of the purification.

2′-Deoxyguanosine phosphoramidite (**9**) was then prepared as shown in Scheme 2. 2-Amino group of **6** was protected by isobutyryl group, which was



SCHEME 2 The preparation of (M+4) 2′-deoxyguanosine phosphoramidite. i) trimethylsilyl chloride (TMS-Cl)/pyridine; ii) isobutyric anhydride; iii) H_2O/NH_4OH; iv) DMTr-Cl/pyridine; v) diisopropylamine, 5-ethyl-tetrazole/pyridine/dichloromethane; vi) 2-cyanoethyl (N,N,N′,N′)-tetraisopropylphosphorodiamidite.

introduced using transient protection scheme.[7] The resulting compound **7** was tritylated with 4,4-dimethoxytritylchloride (DMTr-Cl) to give **8**. Using 2-cyanoethyl (N,N,N′,N′)-tetraisopropylphosphorodiamidite as phosphitylating reagent, the desired phosphoramidite monomer **9** was obtained. It was then incorporated into an 18-mer DNA oligonucleotide: 5′-GTA TGA CXA TCG CGC CAT-3′, where X = (M+4) dG, using standard phosphoramidite chemistry.

A number of multiply labelled compounds, including (M+4) 8-oxo-dG and (M+3) guanine have been prepared and used as internal standards in Mass Spectrometry based analysis.[5,8] However, as monomers, they cannot account for the errors that can occur during the early stages of sample preparation. Therefore, oligomeric internal standards are advantageous over their corresponding bases or nucleosides. In the future, we will be synthesizing other oligomers containing (M+4) 8-oxo-guanine and using them as internal standards in the measurement of oxidative DNA damage in cells.

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