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7,5′-*O*-DIBENZYLINOSINES: SYNTHESIS AND STUDIES ON THEIR CONFORMATIONAL PROPERTIES

Elena Casanova and Leire Aguado □ *Instituto de Química Médica (CSIC), Madrid, Spain*

María-Luisa Jimeno □ *Centro de Química Orgánica Manuel Lora-Tamayo (CSIC), Madrid, Spain*

Federico Gago □ *Departamento de Farmacología, Universidad de Alcalá, Madrid, Spain.*

María-José Camarasa and María-Jesús Pérez-Pérez □ *Instituto de Química Médica (CSIC), Madrid, Spain*

□ *Reaction of 2′,3′-O-isopropylidene inosine with benzyl bromide (1 h, rt) led to the 1,5′-O-dibenzylderivative* ***4****, but by increasing the reaction time or the temperature, compound* ***4*** *is further transformed into the 1,7,5′-O-tribenzylinosine derivative* ***5****. Similarly, the 7-methyl-1,5′-O-dibenzylderivative* ***6*** *has been synthesized from* ***4****. The* ^{1} *H-NMR spectra of* ***5*** *and* ***6*** *showed peculiar chemical shifts for geminal protons (H5′ and H5″ of the ribose, and the* CH_2 *of the benzyl groups). Preliminary NMR studies have been performed, including NOESY experiments that point toward the predominant existence of conformers that are stabilized by an electrostatic interaction between the positively charged imidazole of the base moiety and the high electron density of the 5′-benzyl substituent.*

Keywords Inosine; benzylation; NMR studies; conformation; intramolecular interactions

INTRODUCTION

The N7 position of guanine has been extensively studied due to covalent bond formation at this site when double-stranded DNA is exposed to electrophiles.[1] Moreover, 7-methylguanosine (**1**) (Figure 1) is present in the mRNA 5′-cap structure that serves as a recognition site for numerous enzymes.[2] The positive charge of the imidazole ring of the guanine base

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Address correspondence to María-Jesús Pérez-Pérez, Instituto de Química Médica (CSIC), Madrid 28006, Spain. E-mail: mjperez@iqm.csic.es

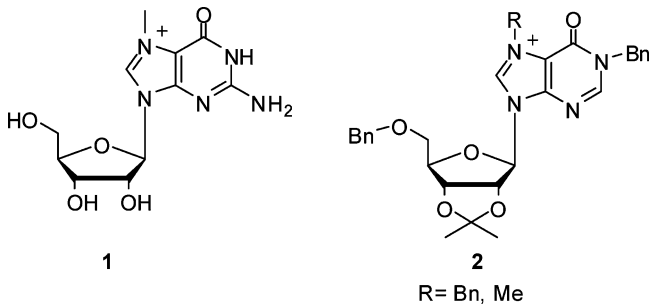


FIGURE 1

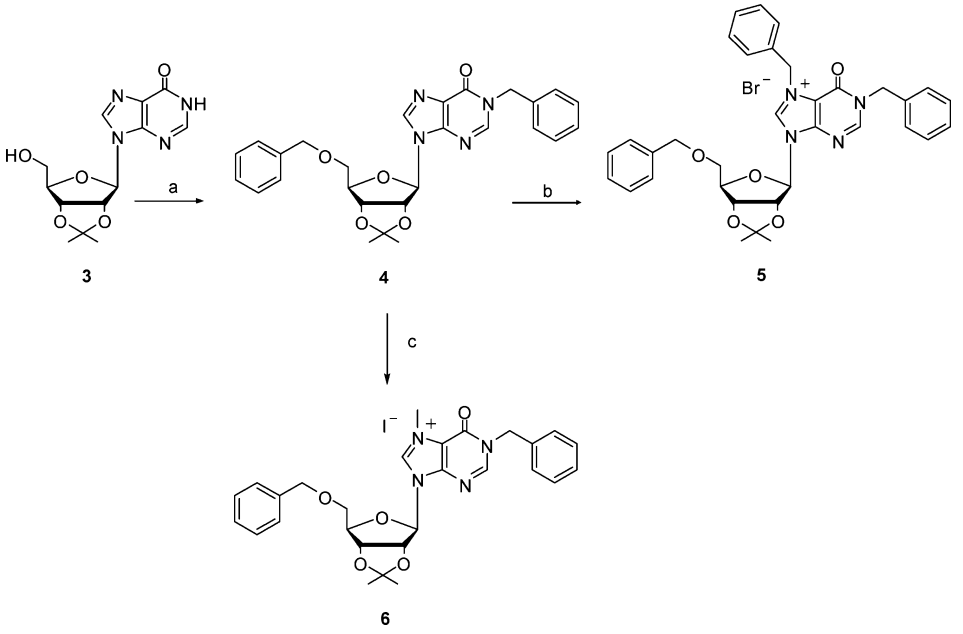
in both examples is essential for enzyme recognition. Therefore, model compounds modified at position N7 of guanine have been subjected to numerous studies to better understand the role of this site in biological interactions. Interestingly, the N7 position of inosine has received much less attention.

In this communication we report on the easy functionalization of position N7 of inosine and on the preliminary conformational properties of the resulting 5′-O-benzyl-7-susbstituted inosines of general formula **2**.

RESULTS AND DISCUSSION

When performing the reaction between 2′,3′-*O*-isopropylidene inosine (**3**) and benzyl bromide (Scheme 1), we found that the 1,5′-*O*-dibenzylderivative **4** is initially obtained (1 h, rt). However, upon increasing either the reaction time or the temperature, compound **4** is further transformed into the 1,7,5′-*O*-tribenzylinosine derivative **5**. The ^{1}H-NMR spectrum of **5** showed peculiar chemical shifts for geminal protons (H5′ and H5″ of the ribose, and the CH_2 of the benzyl groups). In order to evaluate whether this characteristic spectrum is due to the simultaneous presence of the benzyl groups at 5′ and 7 positions, or to the positive charge of the imidazole ring, we synthesized the corresponding 7-methyl-1,5′-*O*-dibenzylinosine (**6**) (Scheme 1) by reaction of **4** with MeI in DMF at 60°C in the absence of base, as recently reported for N1,N7-disubstituted purines.[3] Interestingly, the ^{1}H-NMR spectrum of the 7-methyl derivative **6** showed similar chemical shifts for geminal protons to those of the 7-benzyl derivative **5**.

Full structural assignments of compounds **5** and **6** have been performed based on mono- and bidimensional experiments (gCOSY, gHSQC, and gHMBC). Moreover, NOESY and ROESY experiments were carried out to obtain information about the conformational properties. Since the spectra of the 7-methyl derivative (**6**) are simpler than those of the 7-benzyl derivative (**5**), compound **6** was taken as a model compound, although the same features are present in the spectra of compound **5**. In the NOESY spectrum of compound **6**, particular attention has been paid to cross peaks relative to



SCHEME 1 Reagents and conditions (a) 2.2 equiv BrBn, 3.3 equiv. Nah, DMF, 1 h rt; (b) longer reaction time or heating; (c) 2.2 equiv. Mel, DMF, 24 h, 60°C.

the H-8 of the base, and the *o*-proton of the Ph ring at 5′ (Figures 2a and 2b, respectively). On the basis of the available NMR restraints, the 3-D structure of the molecule was constructed and energy minimization was run at the AM1 level using HyperChem™ 7.52. The conformers **6A** and **6B** obtained

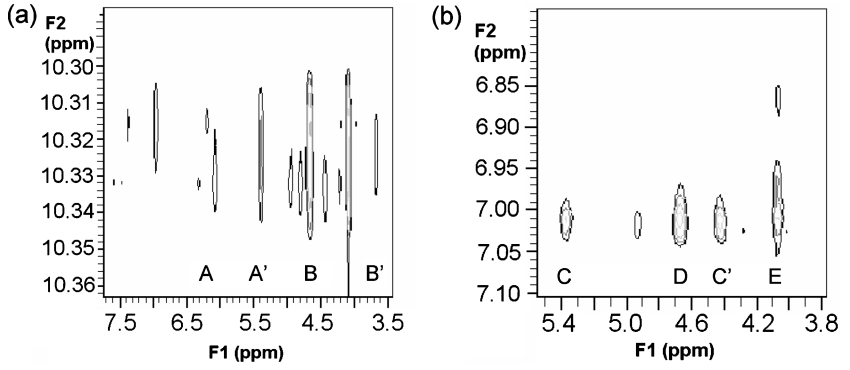


FIGURE 2 Selected peaks of the NOESY spectrum of **6**. (a) interesting peaks between the H-8 of the base and the sugar protons H1′ (A) and H5′′ (B) on one hand, and between the H-8 of the base and the sugar protons H2′ (A′) and H5′ (B′). (b) interesting peaks between the *o*-proton of the Ph ring at 5′ and the sugar protons H2′ (C) and H3′ (D), and CH-O′′ proton on one hand, and between the *o*-proton of the Ph ring at 5′ and CH-O′ (C′); a strong NOE is observed between the *o*-proton of the Ph ring at 5′ and N7-CH3 (E).

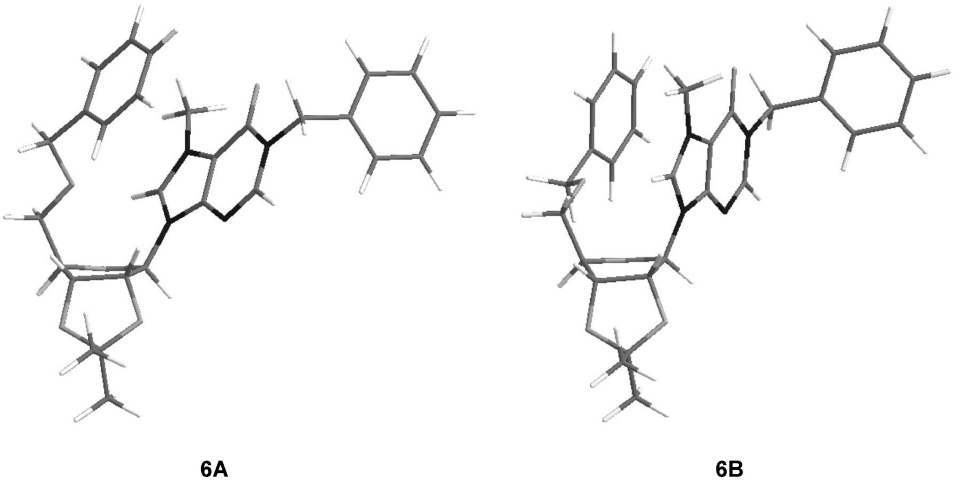


FIGURE 3 Computed molecular models showing possible conformers of **6** compatible with the NOESY cross peaks. Confomer **6A** explains the cross peaks A, B, C, and D in Figure 2 while conformer **6B** is consistent with cross peaks A′, B′, C′, and D in Figure 2.

(Figure 3) are in agreement with the observed NOE effects. The strong NOE between the *o*-proton of the Ph ring at 5′ and the 7-CH_3 could further support the spatial proximity of the aromatic ring at 5′ and the imidazole ring in both conformers.

These results allow us to propose the following conclusions:

1. In both cases (**6A** and **6B**), the conformation of the sugar ring is O(4′)-*exo*, probably due to the presence of the isopropylidene moiety, which is in agreement with the reported X-ray structure of isopropylidene inosine **3**.[4]
2. The glycosidic bond is predominantly *anti* based on the NOE effect between H8 and H5″ (conformer **6A**), and the strong NOE between H8 and H2′ (conformer **6B**). This is in agreement with the preferred *anti* conformation reported for 7-methyl guanosine.[2]
3. The proposed conformations for **6A** and **6B** are likely to result from the stabilization brought about by the electrostatic interaction between the positively charged base and the high electron density of the 5′-benzyl moiety.

A more detailed study of the nucleoside conformation is in progress.

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