

Syntheses of (2')3'-¹⁵N-Amino-(2')3'-deoxyguanosine and Determination of Their pK_a Values by ¹⁵N NMR Spectroscopy

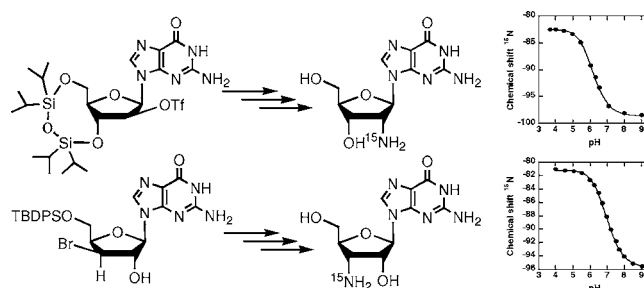
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Received May 15, 2007

ABSTRACT



2'-Amino-2'-deoxyguanosine and 3'-amino-3'-deoxyguanosine are valuable probes for investigating the metal ion interactions at the active site of the group I ribozyme. However, these experiments require a thorough understanding of the protonation state of the amino group at a specific pH. Here, we describe the first syntheses of 2'-¹⁵N-amino-2'-deoxyadenosine, 2'-¹⁵N-amino-2'-deoxyguanosine, and 3'-¹⁵N-amino-3'-deoxyguanosine. The ¹⁵N-enriched nucleus allows convenient and accurate determination of the amine pK_a by ¹⁵N NMR.

2'-Amino-2'-deoxynucleosides and 3'-amino-3'-deoxynucleosides (herein referred as 2'(3')-amino-2'(3')-deoxynucleosides) and oligonucleotides containing them have received much attention in recent years as potential therapeutic agents,¹ chemogenetic agents,² and diagnostic and biochemical probes. For example, 3'-amino-3'-deoxyadenosine derivatives inhibit replication of HIV-1 and serve as tailored agonists for activation of an engineered adenosine receptor.³ As components of antisense oligonucleotides or small

interfering RNAs,⁴ 2'(3')-amino-2'(3')-deoxynucleosides have the potential to improve drug efficacy by imparting resistance to chemical and enzymatic degradation.⁵ In addition to their therapeutic potential, the distinct physiochemical properties of 2'(3')-amino-2'(3')-deoxynucleosides render them especially powerful tools for exploring RNA structure, function, and dynamics,⁶ particularly in defining the roles and environments of specific 2'- or 3'-hydroxyl groups within structured and catalytic RNA molecules.^{5,7}

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Our interest in 2'(3')-amino-2'(3')-deoxyguanosine originates from its value in mechanistic investigations of the group I ribozyme, which efficiently catalyzes nucleotidyl transfer between an oligonucleotide substrate and guanosine.⁸ Previous metal ion rescue experiments⁹ and crystal structures¹⁰ have implicated metal ions in the catalytic mechanisms of exon-ligation and intron-excision. However, whether three metal ions (Figure 1A) or two metal ions (Figure 1B)

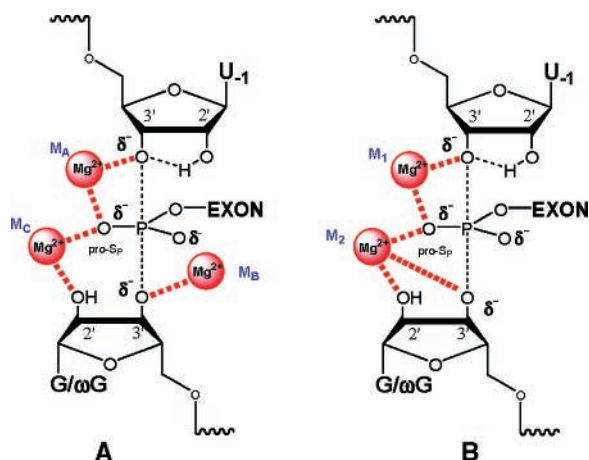


Figure 1. Models of the transition state for both step 1 and step 2 of the group I intron RNA splicing reaction with (A) three catalytic metals and (B) two catalytic metals. In both models, there is a metal ion, M_A or M_1 , which coordinates with the 3'-hydroxyl group of the U_{-1} residue. However, in the three-metal ion model, two distinct metal ions, M_B and M_C , coordinate with the 3'- and 2'-hydroxyl groups of guanosine, respectively, whereas in the two-metal ion model, M_2 coordinates with both the 3'- and 2'-hydroxyl groups.¹⁰

contribute to transition-state interactions remains unclear. The crystal structure of the *Azoarcus* intron suggests that the 3'-oxygen of the guanosine cofactor interacts with M_2 in the ground state (Figure 1B). The guanosine analogue, 3'-amino-3'-deoxyguanosine, provides a probe to test this interaction via metal ion rescue experiments in the same manner that studies using 2'-amino-2'-deoxyguanosine have established the interaction between M_C and the guanosine 2'-hydroxyl group as shown in Figure 1A.¹¹

Defining the interactions between 3'-amino-3'-deoxyguanosine and the ribozyme requires knowledge of the amine pK_a , as protonation may compete with metal ion coordinations and influence binding of the guanosine analogue to the ribozyme. Eckstein et al. have used ^{13}C NMR to determine the ribofuranosyl amine pK_a of 2'-amino-2'-deoxycytidine in a dinucleotide.¹² The corresponding pK_a value for 2'-amino-2'-deoxyguanosine has not been determined by NMR but was inferred from biochemical experiments.¹¹ We wanted to use ^{15}N NMR to determine the pK_a values of 2'(3')-amino-2'(3')-deoxyguanosine, which requires the installation of a ^{15}N -enriched amino group at the 2'(3')- α position of guanosine. The low natural abundance of ^{15}N (0.36%) allows facile chemical shift assignment of ^{15}N -enriched (98%) nuclei, which yield one singlet in the ^{15}N NMR spectrum. Moreover, the ionization state of the amine influences the ^{15}N chemical shift, allowing a sensitive and convenient way to determine the pK_a of the amino group.¹³ Herein, we describe the first syntheses of 2'- ^{15}N -amino-2'-deoxyadenosine, 2'- ^{15}N -amino-2'-deoxyguanosine, and 3'- ^{15}N -amino-3'-deoxyguanosine and determination of their pK_a values using ^{15}N NMR.

To synthesize 2'(3')- ^{15}N -amino-2'(3')-deoxyguanosine, we considered strategies that introduce the ^{15}N atom into the C-2'- or C-3' position of guanosine using the commercially available ^{15}N -labeled reagents. Some of these include $^{15}\text{NH}_3$, $\text{PhCH}_2^{15}\text{NH}_2$, and phthalimide- ^{15}N potassium salt. Considering the nucleophilicity of these ^{15}N -labeled reagents, the most straightforward approach would involve $\text{S}_\text{N}2$ displacement reactions with the 2'- or 3'- β -triflate derivative of guanosine. Iodide or bromide has been reported to displace the 2'- β -triflate from the 3',5'-O-disilyl protected guanosine derivative (**1b**) to give the corresponding 2'- α -halo-2'-deoxyguanosine derivatives.¹⁴ We recently reported that *t*-butylthiol¹⁵ reacts with **1b** smoothly to generate a 2'-*t*-butylthio-2'-deoxyguanosine derivative. Direct $\text{S}_\text{N}2$ displacement reaction of **1b** with amines, however, has not been reported.

We first tried to introduce the 2'-amino group directly by treating **1b** with ammonia but obtained no desired product under various conditions.¹⁶ The 2'- β -triflate derivatives of adenosine, cytosine and uridine react with phthalimide to afford the corresponding 2'- α -phthalimido analogues.¹⁷ However, we observed no reaction for guanosine analogue **1b** under similar conditions. Based on the previous work showing that the 2'- β -triflate derivative of adenosine (**1a**) reacts with methylamine and dimethylamine,¹⁸ we attempted

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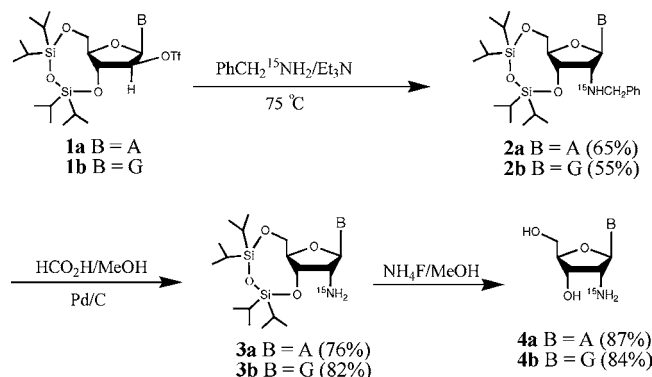
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to use $\text{PhCH}_2^{15}\text{NH}_2$ for the synthesis of 2'- ^{15}N -benzylamino-2'-deoxyadenosine and 2'- ^{15}N -benzylamino-2'-deoxyguanosine, anticipating that the benzyl group could be subsequently removed by catalytic transfer hydrogenation¹⁹ (Scheme 1).

Scheme 1. Syntheses of 2'- ^{15}N -Amino-2'-deoxypurine Nucleosides



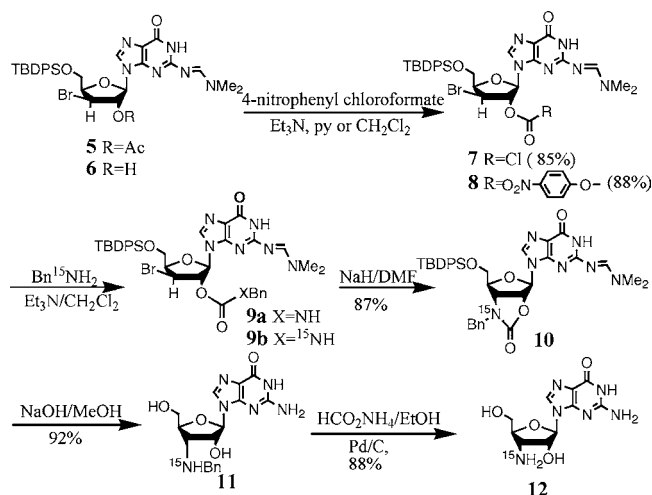
Reaction of **1a**²⁰ with ^{15}N -labeled benzylamine in THF in the presence of triethylamine²¹ at 75 °C for 2 days gave **2a** in 65% yield. Hydrogenolysis with formic acid and Pd/C in methanol for 6 h removed the benzyl protecting group to afford **3a** in 76% yield. Removal of the 3',5'-silyl protecting group with TBAF generated product **4a**. It was purified by reversed-phase column chromatography but the *tetra*-butylammonium salt could not be removed completely (as indicated by ^1H NMR). To circumvent this problem, we treated **3a** with ammonium fluoride (NH_4F) in methanol²² and obtained **4a** in 87% yield, which showed no contamination of NH_4F (as revealed by the absence of a ^{19}F NMR signal) after purification by reversed-phase chromatography.

We synthesized 2'- ^{15}N -amino-2'-deoxyguanosine (**4b**) using the same approach. Although **1b** behaved differently from **1a** in its reaction with phthalimide, we found that **1b** reacted smoothly with ^{15}N -benzylamine in DMF²³ at 75 °C to generate **2b** in 55% yield. Direct displacement of the 2'- β -triflate from guanosine by an amine to generate the corresponding 2'- α -amino-2'-deoxyguanosine analogue has not been reported previously. We expect that other primary amines may react similarly. Removal of benzyl (82%) and silyl protecting groups (84%) in the same manner as described for the adenosine derivative afforded 2'- ^{15}N -amino-2'-deoxyguanosine **4b** (Scheme 1).

Encouraged by the successful introduction of the 2'- ^{15}N -benzylamino group into adenosine and guanosine, we reasoned that the 3'- β -triflate derivative of guanosine would

react similarly with ^{15}N -benzylamine to give the corresponding 3'- ^{15}N -benzylamino-3'-deoxyguanosine derivative. However, we had difficulty in preparing the 3'- β -triflate of guanosine due to poor selectivity during protection of the 2'- and 5'-hydroxyl groups.²⁴ As an alternative, we prepared the corresponding 3'- β -bromo-3'-deoxyguanosine derivative (**5**, Scheme 2) from 5'-*O*-TBDPS-guanosine.²⁵ However, we

Scheme 2. Synthesis of 3'- ^{15}N -Amino-3'-deoxyguanosine



found that treatment of **5** with benzylamine formed the 2',3'-epoxide derivative exclusively. Probably, benzylamine first removes the 2'-acetyl group, releasing the 2'-hydroxyl group, which then attacks the C-3' from the α -face.

Subsequent attempts to synthesize 3'- ^{15}N -amino-3'-deoxyguanosine were carried out by modifying Zhang's recent synthesis of 3'-amino-3'-deoxyguanosine.²⁵ The key steps of their synthesis include the intramolecular $\text{S}_{\text{N}}2$ reaction of **9a** (Scheme 2), followed by ring opening under basic conditions to introduce the 3'-benzylamino group. The intermediate **9a** was prepared by treating 3'-bromoguanosine derivative **6** with benzyl isocyanate. Unfortunately, ^{15}N -benzyl isocyanate is not commercially available and preparing it from expensive ^{15}N -benzylamine seemed impractical. To use ^{15}N -benzylamine directly, we adopted a two-step strategy to introduce a ^{15}N -labeled benzylaminocarbonyl group at the 2'- α -C position of **6**. Reaction of **6** with 4-nitrophenyl chloroformate²⁶ first gave an intermediate, which then reacted with ^{15}N -benzylamine to give the 2'- O - ^{15}N -benzylaminocarbonyl-guanosine analogue. We found that the reaction of **6** with 4-nitrophenyl chloroformate gave different major products depending upon the solvent. In dichloromethane, the reaction gave chloroformate **7** as the major product, while in pyridine, carbonate **8** formed as the major product (Scheme 2). Both **7** and **8** reacted readily with ^{15}N -benzylamine in dichloromethane in the presence of triethylamine to give the key

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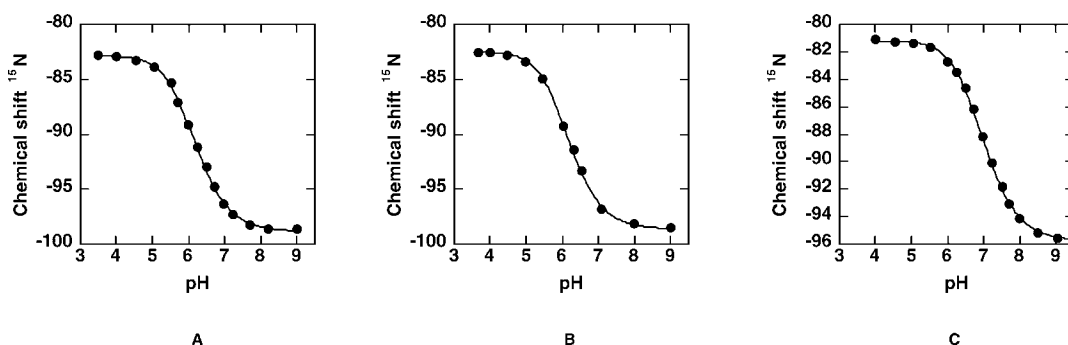


Figure 2. Determination of aminonucleoside pK_a values by following ^{15}N NMR chemical shifts versus pH. (A) $2'\text{-}^{15}\text{N}$ -amino- $2'$ -deoxyadenosine, $pK_a = 6.2$; (B) $2'\text{-}^{15}\text{N}$ -amino- $2'$ -deoxyguanosine, $pK_a = 6.2$; (C) $3'\text{-}^{15}\text{N}$ -amino- $3'$ -deoxyguanosine, $pK_a = 7.0$. Data were fit according to eq 1.

intermediate **9b**. Cyclization with NaH in DMF gave intermediate **10** in 87% yield.²⁷ Removal of the protecting groups²⁵ gave $3'\text{-}^{15}\text{N}$ -amino- $3'$ -deoxyguanosine **12**.

To determine the pK_a values by ^{15}N NMR, we dissolved each ^{15}N -labeled nucleoside in deionized water and combined the solutions with an equal volume of buffer.²⁸ We kept the final concentration of nucleoside in each sample (17 mM) well below the buffer concentration (250 mM).¹² We acquired ^{15}N NMR spectra using 90% formamide in $\text{DMSO-}d_6$ as the reference. In all cases, ^{15}N chemical shifts moved upfield with increasing pH as shown in Figure 2. The chemical shifts were plotted against pH and fit to eq 1, which was derived from the Henderson–Hasselbalch equation,²⁹

$$\delta = \delta_{\text{acid}} + \delta_{\text{base}} 10^{(\text{pH}-pK_a)} / [1 + 10^{(\text{pH}-pK_a)}] \quad (1)$$

where δ is the ^{15}N chemical shift and δ_{acid} and δ_{base} represent the chemical shift values at the low and high extremes of pH, respectively. The influence of pH on the measured ^{15}N chemical shifts in Figure 2 follows the above equation, where the pK_a value corresponds to the midpoint of the curve. In all cases, the fits gave very good correlation coefficients ($R > 0.999$). Our data indicate that both $2'\text{-}^{15}\text{N}$ -amino- $2'$ -deoxyadenosine and $2'\text{-}^{15}\text{N}$ -amino- $2'$ -deoxyguanosine ionize with $pK_a = 6.2$ (Figure 2A,B), identical to that obtained for $2'$ -amino- $2'$ -deoxycytidine by ^{13}C NMR. These observations

suggest that the nucleobase exerts little influence on the pK_a of the $2'$ -amino group, as observed for the $2'$ -hydroxyl group pK_a in natural RNA.³⁰ The regioisomer $3'\text{-}^{15}\text{N}$ -amino- $3'$ -deoxyguanosine ionizes with $pK_a = 7.0$ (Figure 2C), higher than its $2'$ -counterpart.

In conclusion, we have efficiently synthesized $2'\text{-}^{15}\text{N}$ -amino- $2'$ -deoxyadenosine and $2'\text{-}^{15}\text{N}$ -amino- $2'$ -deoxyguanosine by $\text{S}_{\text{N}}2$ displacement of the $2'\text{-}\beta$ -triflate analogues of adenosine and guanosine with ^{15}N -benzylamine. We prepared $3'\text{-}^{15}\text{N}$ -amino- $3'$ -deoxy-guanosine using a two-step strategy to introduce an ^{15}N -labeled benzylaminocarbonyl group at the $2'\text{-}\alpha\text{-C}$ position by first treating **6** with 4-nitrophenyl chloroformate followed by treatment with ^{15}N -benzylamine. We determined the pK_a values for the ribofuranosyl amines in these nucleoside analogues by monitoring the chemical shifts of their ^{15}N NMR spectra as a function of pH. The pK_a value for $3'$ -amino- $3'$ -deoxyguanosine allows us to calculate the relative concentrations of the ionized and neutral species at specific pH values. This fundamental physicochemical information will help to define the interaction of $3'$ -amino- $3'$ -deoxyguanosine with the group I intron.

Acknowledgment. Q.D. is a Research Assistant Professor at the University of Chicago, J.L. is a Research Associate, and J.A.P. is an Investigator of the Howard Hughes Medical Institute. We thank the following members of the Piccirilli laboratory for critical comments on the manuscript: J. Ye, N-S. Li, R. Fong, J. Frederiksen, T. Novak, R. Sengupta, K. Sundaram, and S. Koo.

Supporting Information Available: Full experimental and analytical data and the ^1H and ^{13}C NMR spectra for compounds **2a,b**, **3a,b**, **4a,b**, and **7–12**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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