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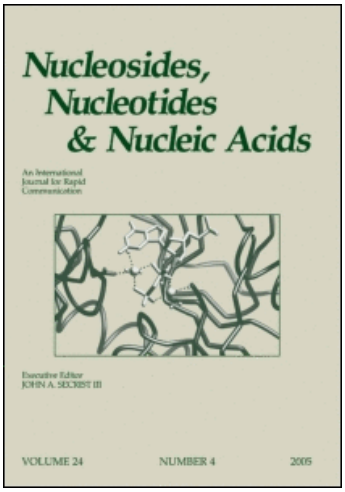


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# Synthesis and Stability Studies of 2',3',5'-tri-O-Acetyl-2-Amino(-N6-Cyclopentyl)-1-Deazaadenosines

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# SYNTHESIS AND STABILITY STUDIES OF 2′,3′,5′-TRI-*O*-ACETYL-2-AMINO(-N$^6$-CYCLOPENTYL)-1-DEAZAADENOSINES

**Sauro Vittori, Ram Chandra Mishra, Diego Dal Ben, Dhuldeo Kachare, Catia Lambertucci, Rosaria Volpini, and Gloria Cristalli** □ *Dipartimento di Scienze Chimiche, Università di Camerino, Camerino (MC), Italy*

□ *In this article, we report on the synthesis of 2′,3′,5′-tri-O-acetyl-2-amino-1-deazaadenosine and of 2′,3′,5′-tri-O-acetyl-2-amino-N$^6$-cyclopentyl-1-deazaadenosine, which are very versatile intermediates for the preparation of 2-substituted 1-deazaadenosine derivatives. The two synthesized compounds showed to be quite unstable, with the N$^6$-substituted derivatives being less stable than the N$^6$-unsubstituted counterpart, according to the calculated HOMO-LUMO energy gap. Stability studies were performed through HPLC-MS analysis.*

**Keywords** Stability studies; I-deazaadenosine; HOMO-LUMO energy gap

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## INTRODUCTION

1-Deazaadenosine analogues have shown to possess biological activity as inhibitors of viral replication, as adenosine receptor ligands, antiproliferative agents, and inhibitors of different classes of enzymes. In particular, 2-substituted-1-deazapurine derivatives are endowed with high antiviral efficacy.[1,2] Availability of 2-functionalized 1-deazaadenosine analogues is of paramount importance for the preparation of variously substituted 1-deazaadenosines. Very often, the presence of protecting groups on the -OH of sugar moiety is necessary to avoid side reactions in the conditions needed for substitution at C-2 position. In this work, we synthesized for the first time the 2′,3′,5′-tri-*O*-acetyl-2-amino-1-deazaadenosine (**6**) and the 2′,3′,5′-tri-*O*-acetyl-2-amino-N$^6$-cyclopentyl-1-deazaadenosine (**3**). Since their evident lack of stability in the laboratory operating conditions, we decided to study this characteristic of the two compounds, both from theoretical and experimental point of view. Computational analysis of energy levels of

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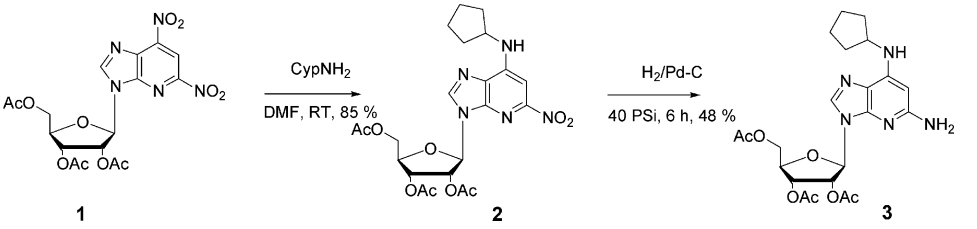



**SCHEME 1** Synthesis of 2-amino-$N^6$-cyclopentyl-1-deazaadenosine.

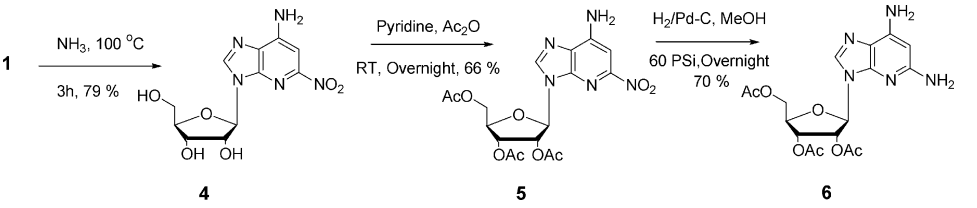
HOMO and LUMO orbitals, and HPLC-MS study of stability of compounds in methanolic solution at room temperature were performed.

## CHEMISTRY

Title nucleosides **3** and **6** were synthesized according to Schemes 1 and 2, respectively. Compound **1** was reacted with a small excess of cyclopentyl amine in dry triethylamine as solvent, to afford compound **2**, as previously described.[3] Catalytic hydrogenation of **2** in presence of Pd-C, followed by flash chromatography, gave 2′,3′,5′-tri-*O*-acetyl-2-amino-6-cyclopentylamino-1-deazaadenosine (**3**) in 48% yield (Scheme 1).

Compound **4** was obtained in very good yield (79%) by reacting **1** with ammonia at 100°C in a sealed bomb. **4** was in turn treated with $Ac_2O$/pyridine to give the protected nucleoside **5** in 66% yield. Synthesis of **4** and **5** has already been reported,[3] but our procedure gave better yields and was made by a lower number of steps. Reduction of the 2-nitro substituent of **5** at 60 psi of $H_2$ in methanol in presence of Pd-C afforded compound **6** in good yield (70%, Scheme 2).

During laboratory purification steps an evident lack of stability for **3** and **6** became clear. Hence, we performed stability studies of **3** and **6,** both from theoretical and experimental point of view.




**SCHEME 2** Synthesis of 2-amino-1-deazaadenosine.

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**TABLE 1** HOMO and LUMO energy levels (eV)

| Compound | HOMO | LUMO | ΔE (HOMO-LUMO) |
|---|---|---|---|
| **3** | –7.98355 | 3.59190 | 11.57546 |
| **6** | –8.11607 | 3.55816 | 11.67423 |

## STABILITY STUDIES

Quantum mechanical calculations were carried out to study the molecular geometry and electronic structure of **3** and **6**. The lower energy conformations and electronic structure of the two molecules were assessed by ab initio, high level theory. Molecular calculations started with molecular mechanics method and the low-level Hartree-Fock model STO-3G. The resulting geometry of the molecules were used and submitted for a second calculation with the basis set 6-31G. All the calculations were performed with Gamess software[4] using MOE (Molecular Operating Environment) interface.[5]

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The energy, spatial distribution, positions of highest occupied molecular orbital (HOMO), and lowest unoccupied molecular orbital (LUMO) of the two molecules yield information on their reactivity. The results of the simulations are reported in Table 1.

Compound **3** presents a decreased HOMO-LUMO energy gap compared to $N^6$-unsubstituted compound (**6**), and this is due to a strong increase of HOMO energy, while LUMO energy increases slightly. The reduced HOMO-LUMO energy gap can be read as a prediction of enhanced reactivity, hence reduced stability, of the $N^6$-cyclopentyl substituted compound.

Stability of **3** and **6** was assessed through HPLC-MS analysis. After chromatographic purification, a small amount of the two nucleosides was dissolved in methanol and analyzed in an HPLC-DAD-MS apparatus (C18 Lichrospher 125-4 column; injection volume of 10 $\mu$l; DAD, $\lambda = 54$ nm; ESI-MS: positive ions; programmed elution: MeOH:$H_2O$ 50:50 for 10 minutes, then gradient to 95:5 in 5 minutes), recording their chromatograms at 0, 45, and 180 minutes after dissolution. Table 2 reports the peak area of analyzed nucleosides and the corresponding percentage, taking T = 0 as 100%, at the indicated time. As shown, both compounds

**TABLE 2** Peak area of analyzed nucleosides at the reported time after dissolution

| | Stability (% of unchanged compound) | | | |
|---|---|---|---|---|
| Compound | T = 0 min | T = 45 min | T = 180 min | Half Life (min) |
| **3** | 837 (100%) | 775 (92.6%) | 379 (45.3%) | 168 |
| **6** | 2702 (100%) | 2482 (91.8%) | 1751 (64.8%) | 255 |

showed to be quite unstable, with 2′,3′,5′-tri-O-acetyl-2-amino-$N^6$-cyclopentyl-1-deazaadenosine (**6**) being significantly less stable. In fact, after 3 hours only 45.3% of compound **3** remained unchanged, while for compound **6** the corresponding value was 64.8%. Hence, both compounds are quite unstable and need to be handled very quickly, with compound **3** showing a stability significantly lower than **6**, according to the differences in calculated HOMO-LUMO energy gap.

## CONCLUSION

In this study we prepared protected 2-amino-($N^6$-substituted)-1-deazaadenosines, which are versatile intermediates for the preparation of 2-substituted-1-deazaadenosine derivatives. Stability studies demonstrated that $N^6$-unsubstituted derivatives have higher stability. These results are in agreement with theoretical calculations.

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