The Synthesis of a Novel *C*-Nucleoside Designed as Guanosine Analogue

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Abstract: The syntheses of a novel *C*-nucleoside which can be viewed as 8-aza-3,9-dideazaguanosine, as well as of the corresponding heterocyclic base, are described. *N*-[4-(2,3,5-tri-*O*-Acetyl-β-D-ribofuranosylmethyl)-2-methoxypyridin-3-yl]acetamide was regiospecifically nitrated and upon reduction and protection of the amino group underwent ring closure to the corresponding pyrazolopyridine derivative. The guanosine analogue was obtained via successive cleavage of the protecting groups.

Key words: *C*-nucleosides, heterocycles, pyrazolo[3,4-*c*]pyridine, guanosine, lithiation, ring annulation, proton–deuterium exchange

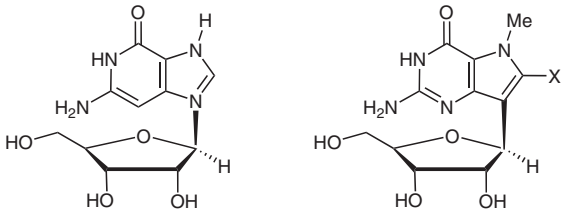


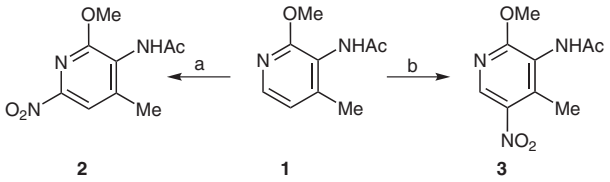
Figure 1 Structures of biologically active guanosine analogues.

The importance of nucleoside analogues, which are in clinical use for a long time as anticancer and antiviral agents,[1] has attracted wide interest for the preparation and study of new structurally modified compounds. Common and very simple alterations of the purine or pyrimidine nucleobase are the isosteric replacement of a hydrophilic nitrogen atom with a hydrophobic methylene unit, resulting in different deazanucleosides, or on the contrary, the replacement of a carbon atom with nitrogen, resulting in azanucleoside derivatives. As all these heterocyclic nitrogens can be involved in a variety of biological interactions, the preparation of the abovementioned molecules, which imitate the shape of natural nucleosides, but have different hydrogen-bonding abilities, can provide important information for nucleic acid and medicinal chemistry studies.[2] Within this concept, different guanosine analogues have been reported, for example 1- or 7-deaza-2′-deoxyguanosines have been incorporated into oligonucleotides,[3] whereas 3-deazaguanosine (Figure 1) and its derivatives were found to exhibit strong and broad-spectrum inhibitory activity against various RNA and DNA viruses and potent anticancer activity in mice.[4] Furthermore, 9-deazaguanine derivatives (**I**, Figure 1), which are *C*-nucleosides, have shown good prophylactic activity against a lethal Semliki Forest virus infection in mice.[5]

C-Nucleosides represent a unique class of compounds, which are characterized by the presence of a chemically and enzymatically stable carbon-to-carbon bond between the heterocyclic and the carbohydrate part of the molecule. The natural occurrence and the biological importance of a number of *C*-nucleosides, such as pseudouridine, showdomycin, oxazinomycin, and the antibiotics formycin A and formycin B has stimulated much research effort towards the synthesis of this class of compounds.[6]

As a continuation of our ongoing research efforts involving the design and synthesis of *C*-nucleoside derivatives,[7] we present here the preparation and antiviral activity evaluation of a new *C*-nucleoside, namely 5-amino-3-(1-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*c*]pyridine-7(6*H*)-one, which can be viewed as 8-aza-3,9-dideazaguanosine.

For the synthesis of the target nucleoside we have used as precursor the acetamide **10** (see Scheme 4), which was previously reported by us. This has been prepared from lithiated 3-acetamido-2-methoxy-4-methylpyridine (**1**, Scheme 1),[8] through condensation with the suitably protected D-ribonolactone, dehydration of the resulting hemiacetal to provide an intermediate olefin, which was subsequently hydrogenated so as to provide the β-nucleoside analogue upon separation from the corrersponding α-isomer.[7a]



Scheme 1 *Reagents and conditions*: (a) Ac_2O, fuming HNO_3, 60 °C, 15 min, 45%; (b) TFAA, fuming HNO_3, r.t., 90 min, 85%.

During the early stages of the present work we realized that it was necessary to insert a suitable functionality on the pyridine ring in order to elaborate the guanine skeleton later on. Consequently, we decided to study the appropriate reaction sequences on the pure heterocyclic ring system and then to apply the method on the nucleoside

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precursor. The 6-nitroderivative of the picoline **1** would be a convenient intermediate and we have thus attempted the nitration of this acetamide.

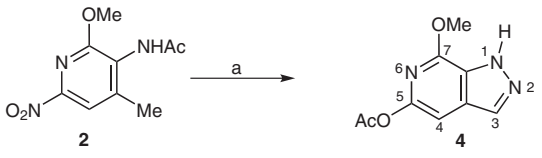
We found that the ideal reaction conditions for the insertion of the nitro group at the 6-position were the addition at 0 °C of a mixture of fuming nitric acid and acetic anhydride into a solution of the acetamide in acetic anhydride, followed by heating at 60 °C for 15 minutes. Raising the temperature, or elongation of the reaction time, resulted in significantly lower yields. Through the abovementioned conditions the 6-nitro isomer **2** was selectively obtained at 45% yield. The structure was confirmed by heteronuclear 2D NMR experiments (HMBC), where we observed a clear cross-peak between the nonsubstituted aromatic carbon atom and the protons of the methyl group (J_3 coupling). On the contrary, the use of trifluoracetic anhydride instead of acetic anhydride resulted even at room temperature selectively in the 5-nitro isomer **3** at 85% yield. It seems that the use of the more powerful nitronium trifluoracetate results in the protonation of the pyridine and thus, the nitration occurs *para* to the methoxy group.

The nitroacetamide **2** was then refluxed in benzene in the presence of isoamyl nitrite, potassium acetate, and acetic anhydride[9] in order to prepare through the rearrangement of the intermediate *N*-nitroso compound, the corresponding pyrazolopyridine. However, from this reaction we isolated the 5-acetyloxy derivative **4** (Scheme 2) providing evidence for the lability of the nitro group of one of the intermediates involved in the cyclization reaction.[8]

Consequently, we reduced the nitro group of the nitroacetamide **2**, and the free amino group of the resulting compound **5** (Scheme 3) was protected through conversion into the corresponding phthalimide **6**. This protecting group allowed the preparation of a derivative which was stable at the ring-closure reaction conditions, consequently **6** was successfully cyclized according to the already mentioned methodology to provide a mixture of the 1- and 2-acetylpyrazolopyridines **7** in very good yield (87%). The 1-acetyl-isomer[10] was clearly the major component of this mixture. Both the acetyl and the phthaloyl groups were easily removed upon treatment with methanolic ammonia to provide almost quantitatively the pyrazolopyridine **8**.[11] The methyl group was cleaved by treatment at reflux of a solution of **8** in acetonitrile with trimethylsilylchloride in the presence of sodium iodide,[12] to give the pyrazolopyridinone **9**.[13]

The preparation of the target nucleoside according to the developed procedure is depicted in Scheme 4. Compound **10** was nitrated to provide selectively the 6-nitro isomer **11**. This was first hydrogenated to yield the amine **12**, which was then converted into the phthalimide **13**. The phthalimide was ring-closed through reaction with isoamyl nitrite, and the resulting isomeric pyrazolopyridines **14** were subjected to deprotection of the acetyl groups as well as of the 5-phthaloyl group to give the *C*-nucleoside **15**.[14] The guanosine analogue **16**[15] resulted from the demethylation of **15**, as described above.

An interesting remark resulting from the study of the spectroscopic data of the base **9** and the nucleoside **16** is the observed acidity of the 4-aromatic proton of these molecules. The resonance peak corresponding to H4 was gradually disappearing in CD_3OD solution, and this effect should be attributed to the rapid H–D exchange of the aromatic proton. The H–D exchange of aromatic and heteroaromatic substrates has been previously studied.[16] More interestingly this phenomenon was not produced in the case of the previously prepared 1*H*-pyrazolo[3,4-*c*]pyridine-7(6*H*)-one[8] and pyrazolo[3,4-*c*]pyridin-5-ylamine.[17] Simple AM1 semi-empirical calculations[18] predict a significant difference between the partial charges of C4 and H4 of **9** (calculated to be –0.280 and +0.125, respectively) compared to the corresponding C–H bonds of



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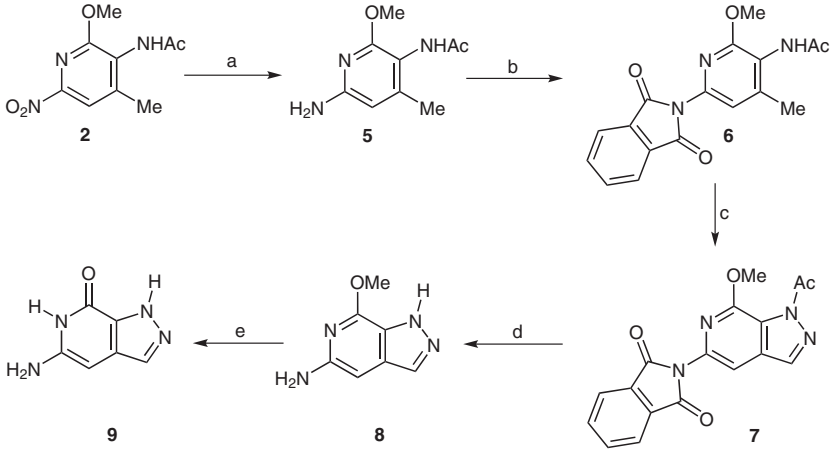
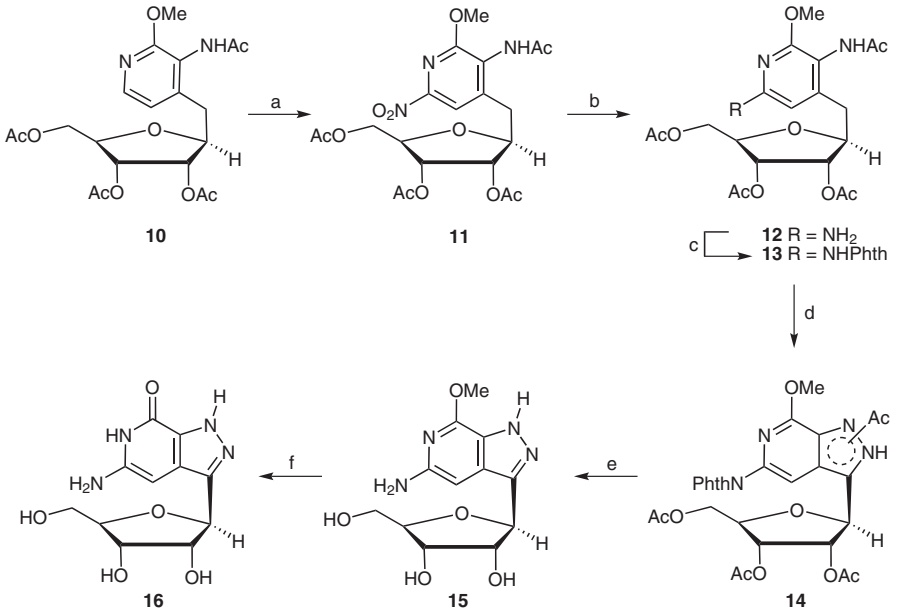
Scheme 2 *Reagents and conditions*: (a) KOAc, Ac_2O, isoamyl nitrite, benzene, reflux, 15 h, 75%.



2 5 6 7 8 9

Scheme 3 *Reagents and conditions*: (a) H_2, Pd/C (10 mol%), EtOH, r.t., 45 psi, 4 h, 95%; (b) phthalic anhydride, benzene, reflux, 8 h, 83%; (c) KOAc, Ac_2O, isoamyl nitrite, benzene, reflux, 10 h, 87%; (d) NH_3–MeOH, r.t., 4 h, 92%; (e) NaI, TMSCl, MeCN, 65 °C, 3 h, 77%.



Scheme 4 *Reagents and conditions* (a) Ac_2O, fuming HNO_3, 60 °C, 20 min, 40%; (b) H_2, Pd/C (10 mol%), EtOH, r.t., 45 psi, 5 h, 92%; (c) phthalic anhydride, benzene, reflux, 12 h, 75%; (d) KOAc, Ac_2O, isoamyl nitrite, toluene, 100 °C, 10 h, 90%, (e) NH_3–MeOH, r.t., 5 h, 95%, (f) NaI, TMSCl, MeCN, 70 °C, 5 h, 70%.

the other two compounds. More specifically, the gap between the negative partial charge of C4 and the positive of H4 is –0.405e for **9**, –0.323e for 1*H*-pyrazolo[3,4-*c*]pyridine-7(6*H*)-one, and –0.357e for pyrazolo[3,4-*c*]pyridin-5-yl-amine. These calculations suggest that the presence of both C=O at position 7 and NH_2 at position 5 increase the C4–H4 polarization enhancing the tendency of H4 to dissociate.

Antiviral evaluation revealed that the reported *C*-nucleosides **15** and **16** did not have significant activity (IC_{50} values >100 μM) against a broad panel of viruses tested (HSV-1, HSV-2, HIV-1, HIV-2, vaccinia, vesicular stomatitis, respiratory syncytial, parainfluenza-3, reovirus-1, Sindbis, coxsackie B4, Punta Toro and Feline Corona viruses). The inhibitory effects of the compounds on the proliferation of murine leukemia cells (L1210) and human T-lymphocyte cells (Molt4/C8) were also determined. Compounds **15** and **16** exhibited a slight antiproliferative activity against L1210 and Molt4/C8 cells with IC_{50} values 260 μM and 303 μM against L1210, and 232 μM and 287 μM against Molt4/C8, respectively.

In conclusion, we have prepared a novel *C*-nucleoside which could be viewed as 8-aza-3,9-dideazaguanosine. We have also developed a method for the synthesis of the corresponding heterocyclic base and have studied the nitration conditions for the preparation of suitable regioisomers as crucial synthetic intermediates.

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(10) **Preparation of 1-Acetyl-5-pthalimido-7-methoxy-1*H*-pyrazolo[3,4-*c*]pyridine (7)**
Potassium acetate (77 mg, 0.78 mmol) and Ac_2O (0.15 mL, 1.56 mmol) were added under argon to a solution of the acetamide **6** (170 mg, 0.52 mmol) in dry benzene (40 mL). The reaction mixture was heated at 80 °C, isoamyl nitrite (0.07 mL, 0.52 mmol) was added, and the resulting mixture was refluxed for 10 h. The insoluble material was then filtered off, the solvent was vacuum evaporated, and the residue was purified by column chromatography (silica gel) using a mixture of cyclohexane–EtOAc (60:40, v/v) as the eluent to give **7** as a white solid (153 mg, 87%); mp >300 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.84 (s, 3 H, $COCH_3$), 4.13 (s, 3 H, OCH_3), 7.82 (m, 3 H, H-4, H-4′, H-5′), 7.98 (m, 2 H, H-3′, H-6′), 8.17 (s, 1 H, H-3). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 23.9 (*C*H_3CO), 54.8 (OCH_3), 106.3 (C-4), 123.9 (C-3′, C-6′), 124.7 (C-3a), 131.8 (C-2a′, C-6a′), 134.5 (C-4′, C-5′), 135.6 (C-7a), 138.1 (C-3), 145.5 (C-5), 151.2 (C-7), 166.9 [CO(Phth)], 168.5 (*C*OCH_3). Anal. Calcd for $C_{17}H_{12}N_4O_4$: C, 60.71; H, 3.60; N, 16.66. Found: C, 60.82; H, 3.45; N, 16.88.

(11) **Preparation of 7-Methoxy-1*H*-pyrazolo[3,4-*c*]pyridin-5-amine (8)**
Compound **7** (120 mg, 0.73 mmol) was dissolved in a sat. solution of NH_3 in MeOH. The solution was stirred at r.t. for 4 h, the solvent was vacuum evaporated, and the residue was purified by column chromatography (silica gel) using a mixture of cyclohexane–EtOAc (20:80, v/v) as the eluent to give **8** (54 mg, 92%) as white crystals; mp 162–164 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.07 (s, 3 H, OCH_3), 5.20 (br s, 2 H, NH_2, D_2O exch.), 6.29 (s, 1 H, H-4), 7.82 (s, 1 H, H-3). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 53.3 (OCH_3), 86.8 (C-4), 122.9 (C-7a), 132.0 (C-3a), 132.7 (C-3), 149.1 (C-5), 149.6 (C-7). Anal. Calcd for $C_7H_8N_4O$: C, 51.21; H, 4.91; N, 34.13. Found: C, 51.43; H, 4.80; N, 34.26.

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(13) **Preparation of 5-Amino-1*H*-pyrazolo[3,4-*c*]pyridin-7 (6*H*)-one (9)**
Sodium iodide (81 mg, 0.54 mmol) and TMSCl (68 μL, 0.54 mmol) were added under argon to a solution of **8** (85 mg, 0.52 mmol) in dry MeCN (5 mL). The resulting mixture was heated at 65 °C for 3 h, the precipitate was filtered, washed with EtOAc, and it was purified by column chromatography (silica gel) using a mixture of EtOAc–MeOH (98:2, v/v) as the eluent to give **9** (60 mg, 77%); mp >300 °C (EtOH). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 5.09 (br s, 2 H, NH_2, D_2O exch.), 5.40 (s, 1 H, H-4, D_2O exch.), 7.54 (s, 1 H, H-3), 10.50 (br s, 1 H, N^{6}H, D_2O exch.), 13.38 (br s, 1 H, N^{1}H, D_2O exch.). Anal. Calcd for $C_6H_6N_4O$: C, 48.00; H, 4.03; N, 37.32. Found: C, 47.83; H, 3.95; N, 37.17.

(14) **Data for 7-Methoxy-3-(β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*c*]pyridin-5-amine (15)**
Mp 216–218 °C (EtOH). ^{1}H NMR (400 MHz, CD_3OD): δ = 3.72 (dd, 1 H, H-5′, $J_{4',5'}$ = 4.70 Hz, $J_{5',5'}$ = 12.13 Hz), 3.84 (dd, 1 H, H-5′, $J_{4',5'}$ = 3.52 Hz, $J_{5',5'}$ = 12.13 Hz), 4.01 (m, 1 H, H-4′), 4.04 (s, 3 H, OCH_3), 4.18 (m, 1 H, H-3′), 4.31 (m, 1 H, H-2′), 5.04 (d, 1 H, H-1′, $J_{1',2'}$ = 6.65 Hz), 6.46 (s, 1 H, H-4, D_2O exch.). ^{13}C NMR (50 MHz, CD_3OD): δ = 53.7 (CH_3O), 63.5 (C-5′), 72.7 (C-3′), 76.4 (C-2′), 80.4 (C-1′), 86.6 (C-4′), 87.9 (C-4), 124.0 (C-7a), 131.0 (C-3a), 143.2 (C-3), 150.7 (C-7). Anal. Calcd for $C_{12}H_{16}N_4O_5$: C, 48.65; H, 5.44; N, 18.91. Found: C, 48.45; H, 5.28; N, 18.83.

(15) **Data for 5-Amino-3-(β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*c*]pyridin-7 (6*H*)-one (16)**
Mp 158–160 °C (EtOH). ^{1}H NMR (400 MHz, CD_3OD): δ = 3.72 (dd, 1 H, H-5′, $J_{4',5'}$ = 4.70 Hz, $J_{5',5'}$ = 12.13 Hz), 3.82 (dd, 1 H, H-5′, $J_{4',5'}$ = 3.52 Hz, $J_{5',5'}$ = 12.13 Hz), 3.99 (m, 1 H, H-4′), 4.16 (m, 1 H, H-3′), 4.26 (m, 1 H, H-2′), 4.97 (d, 1 H, H-1′, $J_{1',2'}$ = 6.26 Hz), 5.81 (s, 1 H, H-4, D_2O exch.). Anal. Calcd for $C_{11}H_{14}N_4O_5$: C, 46.81; H, 5.00; N, 19.85. Found: C, 46.75; H, 5.12; N, 19.97.

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(18) The AM1 calculations were performed in combination with RHF method and a convergence criterion of 0.01 kcal mol^{-1}, using the Polak-Ribiere (conjugate gradient) geometry optimization method as implemented in the HyperChem 5.0 software (Hypercube Inc).