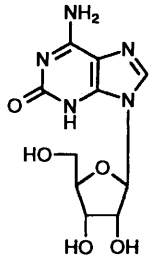
Improved Synthesis of Isoguanosine and 6-Substituted Xanthosine Derivatives

Lorenzo De Napoli, Daniela Montesarchio, Gennaro Piccialli,* Ciro Santacroce and Michela Varra
Dipartimento di Chimica Organica e Biologica, Universita' di Napoli "Federico II", via Mezzocannone 16, I-80134 Napoli, Italy

Isoguanosine **1** was obtained in 76% overall yield starting from 2',3',5'-tri-*O*-acetylxanthosine **3** in a reaction involving the chloro derivative **4** and the *N*-(purin-6-yl)pyridinium salt derivative **8** which also proved to be new and valuable synthetic intermediates in the obtainment of C-6-substituted xanthosine derivatives.

Isoguanosine **1**, known as crotonoside or 2-hydroxyadenosine, is a naturally occurring isomer of guanosine and was first isolated from *Croton tiglium*.[1] Interesting biological activities have been reported for this guanosine analogue, such as incorporation into mammalian, but not bacterial, nucleic acids,[2] stimulation of the accumulation of cyclic-AMP in the brain,[3] and inhibition of inosine monophosphate (IMP) pyrophosphorylase[4] and glutamic acid dehydrogenase.[5]

Several methods for the synthesis of compound **1** have been



reported in the literature. Some of these use 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide[6] as precursor of purine nucleoside base formation. Others start from a preformed purine nucleoside such as 2-aminoadenosine,[7] adenosine *N*-1-oxide[8] or 2-amino-6-chloropurine,[9] and involve a number of transformations of the base functional groups.

The last methods need several steps to circumvent the non-availability of a direct synthetic route to the 6-chloroxanthosine **5** which is undoubtedly the key intermediate in the synthesis of isoguanosine **1**. In fact the previously reported chlorination procedures[10] on sugar-protected xanthosines led only to the 2,6-dichloro derivative, never giving monohalogenation at the C-6 base position. On the other hand, partial basic hydrolysis of the 2,6-dichlorinated compounds led exclusively to the corresponding 2-chloro-6-oxo derivative as a result of the higher reactivity of the C-6 position towards nucleophiles.[10]

We report here an easy synthesis of the 6-chloroxanthosine derivative **4** from which isoguanosine **1** and C-6-substituted analogues **6**–**10** could be obtained in a one-pot reaction in very high yields. Our chlorination procedure is related to a previously reported method,[11] which uses the adduct triphenylphosphine–carbon tetrachloride (PPh_3–CCl_4)[12] to introduce a chlorine atom at the C-4 base position of pyrimidine nucleosides and at the C-6 base position of inosine under very mild conditions (Scheme 1).

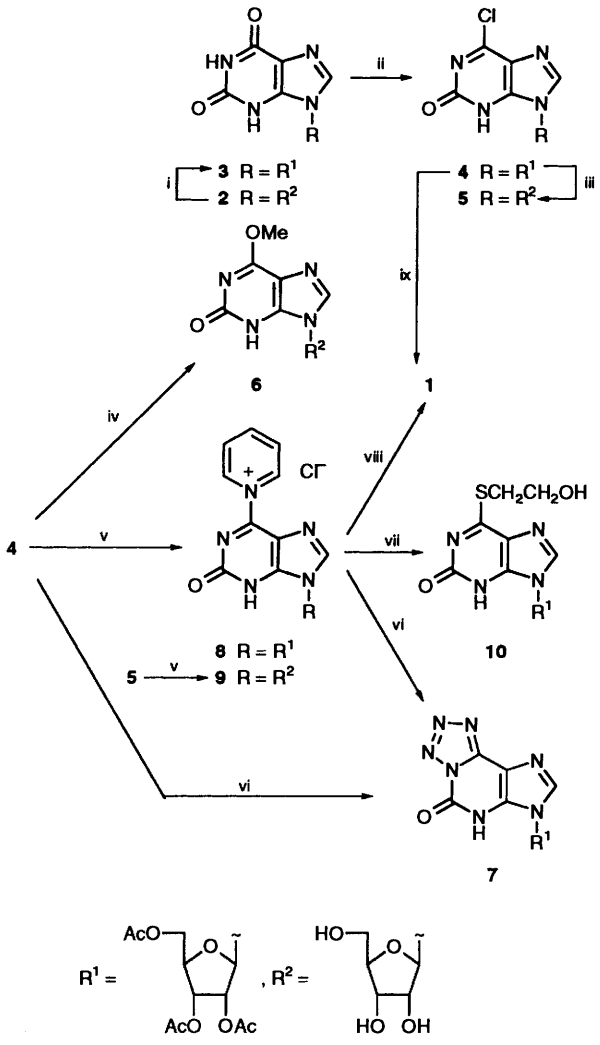
As a starting product in the chlorination we used 2',3',5'-tri-*O*-acetylxanthosine **3**, synthesized by reaction of xanthosine **2** with Ac_2O in pyridine. This product was allowed to react with 2 mol equiv. of PPh_3 in CH_2Cl_2–CCl_4 (1:1, v/v) at reflux for 3 h to obtain the desired target compound **4** as the main product. The reaction mixture was dried *in vacuo* and the residue, dissolved in $CHCl_3$, was purified on a silica gel column eluted with increasing amounts of MeOH in $CHCl_3$ (up to 6%) and thus furnished pure chloride **4** (84% yield) whose structure was confirmed by spectral data. This reaction proceeds speedily and in higher yields than does the chlorination of 3',5'-di-*O*-acetyl-2'-deoxyinosine[11] which requires, in addition, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as catalyst to increase the nucleophilicity of the O-6 function towards the adduct $[Ph_3P$–$CCl_3]^+$ Cl^-. Using this adduct, the chlorination on the 2,6-dioxopurine nucleoside seems to be comparable to the C-4 chlorination of the 2,4-dioxopyrimidine derivative (thymidine and uridine),[11] assuring high yields and a selective, fast reaction.

In order to explore the reactivity of compound **4** towards nucleophiles we treated it with 6 mol equiv. of MeOK in MeOH (20 h; room temp.) and with 10 mol equiv. of NaN_3 [dimethylformamide (DMF); 1.5 h; room temp.] and obtained the 6-methoxy derivative **6** (85% yield) and the tetrazolo-[5,1-*i*]purine derivative **7** (90% yield), respectively. On the other hand, treatment with conc. aq. ammonia (3 h; 50 °C) gave the sole deacetylated product **5** (90% yield), with no apparent C-6 substitution taking place; only with a prolonged reaction time (3 days; 55 °C) was isoguanosine formed, and then in low yield (20%). Reaction of chloride **4** with aq. pyridine proceeded rapidly (2 h; 50 °C) to afford *N*-[2-oxo-9-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)-2,3-dihydropurin-6-yl]pyridinium chloride **8** which was obtained pure as a yellow glass in almost quantitative yield after repeated evaporation of water. The structure **8** was assigned on the basis of its spectral data. As reported for some *N*-(purin-6-yl)monopyridinium salts,[13] compound **8** showed a pH-dependent UV spectrum, indicative of the occurrence of a prototropic equilibrium, and exhibited fluorescence in water at room temperature with the emission maximum at 445 nm (exc. 303 nm).

When the salt **8** was treated with aq. ammonia (5 h; 50 °C), isoguanosine **1** was obtained as the sole product. Analogously, reactions of compound **8** with propylamine or benzylamine afforded, in both cases, isoguanosine **1** in high yields, thus confirming that these reactions proceed *via* a nucleophilic attack on the pyridinium α-carbons with subsequent opening of the ring (Zincke reaction).[14]

Isoguanosine **1** was purified on a reversed-phase HPLC column using MeOH–water (7:3) as eluent (91% yield on isolated product). Crystallization from water–MeOH (95:5) furnished crystals of isoguanosine **1**, m.p. 236–241 °C (lit.,[15] 237–241 °C). The structure of compound **1** was confirmed by comparison of its spectroscopic data with those already reported.[6,9,15]

On the other hand, when the salt **8** was treated with a weak nucleophile such as NaN_3 (DMF; 1.5 h; room temp.) or 2-sulfanylethanol (10 h; room temp.), then nucleophilic attack took place on the C-6 purine carbon to give compounds **7** and **10**, isolated in 87 and 70% yield, respectively, after silica gel chromatography.



Scheme 1 *Reagents and conditions:* i, Ac_2O–pyridine, 5 h, room temp.; ii, PPh_3 (2 mol equiv.), CCl_4, 3 h, reflux; iii, aq. NH_3 (32%), 3 h, 50 °C; iv, MeOK (1 mol dm^{-3}), MeOH, 20 h, room temp.; v, pyridine–water (1 : 1), 2 h, 50 °C; vi, NaN_3 (10 mol equiv.), DMF, 1.5 h, room temp.; vii, $HSCH_2CH_2OH$, 10 h, room temp.; viii, aq. NH_3 (32%), 5 h, 50 °C; ix, aq. NH_3 (32%), 3 days, room temp.

The reported procedure proposes a new and easily accessible method of activation towards nucleophiles, under very mild conditions, of the C-6 position of xanthosine derivatives. The halogenation furnishes, in high yields, the valuable synthetic intermediate **4**, which can be converted into the new and more reactive compound **8**, a useful precursor for a convenient synthesis of isoguanosine **1** and other C-6-substituted xanthosine analogues.

Experimental

General Procedures.—TLC plates (Merck, silica gel 60, F254) were developed in solvent systems: A [$CHCl_3$–MeOH (9 : 1, v/v)]; B [butan-1-ol–acetic acid–water (60 : 15 : 25, v/v)]. HPLC was carried out on a Lichrosorb RP-18 column (Merck, 7 μm, 250-10). Macherey-Nagel silica gel 60 was used for column chromatography. Liquid chromatography was carried out on a Lobar Lichroprep RP-18 column (Merck, 40–63 μm, 310-25). PPh_3 was dried under reduced pressure at 50 °C for 15 h. CH_2Cl_2 and CCl_4 were dried by treatment with P_2O_5 and were then distilled. The ^1H NMR and ^{13}C spectra were recorded on a Bruker WM 270 instrument (270 MHz); *J* values are given in Hz. FAB mass spectra (positive) were determined with a double-focusing mass spectrometer (ZAB 2SE). UV spectra were taken on a Perkin-Elmer lambda 7 spectrophotometer. M.p.s were determined on a Reichert Thermovar apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 141 polarimeter at 25 °C, and are quoted in units of 10^{-1} deg cm^2 g^{-1}.

6-*Chloro*-9-(2′,3′,5′-*tri*-O-*acetyl*-β-D-*ribofuranosyl*)-2,3-*dihydropurin*-2-*one* **4**.—2′,3′,5′-Tri-*O*-acetylxanthosine **3** (410 mg, 1 mmol) and triphenylphosphine (524 mg, 2 mmol) were dissolved in CH_2Cl_2–CCl_4 [(1 : 1, v/v); 12 cm^3] and the resulting mixture was stirred and kept at reflux for 3 h. Then the mixture was cooled, concentrated under reduced pressure, and purified on a silica gel column eluted with increasing amounts of MeOH in $CHCl_3$ (up to 6%) to afford a pure *chloride* **4** as a solid (360 mg, 84%), R_f 0.7 (system A); m.p. 130–132 °C (from $CHCl_3$–C_6H_6) (Found: C, 44.8; H, 3.9; N, 13.3. $C_{16}H_{17}ClN_4O_8$ requires C, 44.8; H, 4.0; N, 13.1%); $[\alpha]_D$ −4.6 (*c* 0.10, $CHCl_3$); λ_{max}($CHCl_3$)/nm 284 (ε/dm^3 mol^{-1} cm^{-1} 7500) and 241 (3300); *m*/*z* (FAB) 429 (MH^+); δ_H($CDCl_3$) 8.13 (1 H, s, 8-H), 6.18 (1 H, d, *J* 5.0, 1′-H), 5.80 (1 H, t, *J* 5.0, 2′-H), 5.53 (1 H, dd, *J* 5.0 and 4.6, 3′-H), 4.42 (3 H, overlapped signals, 4′-H and 5′-H_2) and 2.07, 2.11 and 2.13 (3 H each, s, Ac); δ_C($CDCl_3$) 170.4, 169.5, 169.4, 160.9, 154.2, 151.1, 142.3, 132.1, 85.9, 80.4, 73.1, 70.5, 62.9, 20.7, 20.5 and 20.3.

6-*Chloro*-9-(β-D-*ribofuranosyl*)-2,3-*dihydropurin*-2-*one* **5**.—Triacetate **4** (214 mg, 0.5 mmol) was treated with conc. aq. NH_3 (10 cm^3) in a closed vessel at 50 °C for 3 h. The mixture was dried, concentrated under reduced pressure, and purified on an RP 18 Lobar column, eluted with increasing amounts of MeOH in water (up to 20%) to afford pure *compound* **5** as an amorphous powder (136 mg, 90%). When recrystallized, the compound decomposed without prior melting when heated; R_f 0.75 (system B) (Found: C, 39.5; H, 3.6; N, 18.5. $C_{10}H_{11}ClN_4O_5$ requires C, 39.7; H, 3.7; N, 18.5%); $[\alpha]_D$ −37.2 (*c* 0.08, water); λ_{max}(water)/nm 309 (5000) and 244 (3100); *m*/*z* (FAB) 303 (MH^+); δ_H(CD_3OD) 8.25 (1 H, s, 8-H), 5.92 (1 H, d, *J* 5.8, 1′-H), 4.74 (1 H, t, *J* 5.6, 2′-H), 4.36 (1 H, dd, *J* 5.6 and 3.3, 3′-H), 4.16 (1 H, m, 4′-H) and 3.84 (2 H, m, 5′-H_2).

6-*Methoxy*-9-(β-D-*ribofuranosyl*)-2,3-*dihydropurin*-2-*one* **6**.—Chloride **4** (214 mg, 0.5 mmol) was treated with a stirred solution of MeOK in MeOH (1 mol dm^{-3}; 3 cm^3). After 20 h at room temp. the mixture was neutralized with acetic acid and purified on an RP 18 column as reported for product **5**, to afford pure *compound* **6** as a solid (126 mg, 85%). The recrystallized compound decomposed without prior melting when heated; R_f 0.6 (system B) (Found: C, 44.1; H, 4.6; N, 18.9. $C_{11}H_{14}N_4O_6$ requires C, 44.3; H, 4.7; N, 18.8%); $[\alpha]_D$ −42.1 (*c* 0.09, water); λ_{max}(water)/nm 282 (5500) and 241 (3800); *m*/*z* (FAB) 299 (MH^+); δ_H(D_2O) 7.95 (1 H, s, 8-H), 5.90 (1 H, d, *J* 6.0, 1′-H), 4.73 (1 H, t, *J* 6.0, 2′-H), 4.40 (1 H, dd, *J* 6.0 and 3.4, 3′-H), 4.26 (1 H, m, 4′-H), 4.06 (3 H, s, MeO) and 3.85 (2 H, m, 5′-H_2).

7-(2′,3′,5′-*Tri*-O-*acetyl*-β-D-*ribofuranosyl*)-5,6-*dihydrotetrazolo*[5,1-i]*purin*-5(7H)-*one* **7**.—(a) *From chloride* **4**. Compound **4** (250 mg, 0.6 mmol) was treated in DMF (4 cm^3) with 10 mol equiv. of NaN_3 and the stirred mixture was kept at room temp. for 1.5 h. Then the mixture, dried *in vacuo*, was suspended in MeCN and this mixture was filtered. The filtrate was concentrated under reduced pressure and purified on a silica gel column with increasing amounts of MeOH in $CHCl_3$ (up to 25%) as eluent. Concentration of the appropriate fractions gave *tricycle* **7** as an amorphous powder (235 mg, 90%).

(b) *From salt* **8**. Compound **8** (203 mg, 0.4 mmol) was treated in stirred DMF (4 cm^3) with 10 mol equiv. of NaN_3 at room

temp. After 1.5 h the mixture was dried *in vacuo* and purified as described above to furnish pure tricycle **7** (151 mg, 87%), R_f 0.5 (system B) (Found: C, 43.9; H, 4.0; N, 22.3. $C_{16}H_{17}N_7O_8$ requires C, 44.1; H, 3.9; N, 22.5%); $[\alpha]_D$ −9.6 (*c* 0.07, MeCN); λ_{max}(MeCN)/nm 268 (8200) and 296 (7200); *m/z* (FAB) 436 (MH^+); $\delta_H(CD_3OD)$ 8.05 (1 H, s, 8-H), 6.21 (1 H, d, *J* 5.2, 1'-H), 5.93 (1 H, dd, *J* 5.4 and 5.2, 2'-H), 5.62 (1 H, m, 3'-H), 4.42 (3 H, complex signal, 5'-H_2 and 4'-H) and 2.11, 2.08 and 2.06 (3 H each, s, Ac); $\delta_C(CD_3OD)$ 172.7, 171.9, 171.6, 150.9, 148.7, 145.3, 138.0, 111.1, 87.7, 81.6, 74.8, 72.2, 64.7, 20.9, 20.7 and 20.6.

N-[2-*Oxo*-9-(2',3',5'-*tri*-O-*acetyl*-β-D-*ribofuranosyl*)-2,3-*dihydropurin*-6-*yl*]*pyridinium Chloride* **8**.—Compound **4** (342 mg, 0.8 mmol) was treated with a solution of pyridine–water [1 : 1 (v/v); 5 cm^3]. After 2 h at 50 °C the mixture was dried, and evaporation of water under reduced pressure three times, afforded pure compound **8** (394 mg, 97%) as a yellow glass, R_f 0.15 (system B) (Found: C, 49.4; H, 4.2; N, 14.0. $C_{21}H_{22}ClN_5O_8$ requires C, 49.7; H, 4.4; N, 13.8%); $[\alpha]_D$ −45.1 (*c* 0.13, water); λ_{max}(water)/nm 374 (4100) and 262 (11 000); λ_{max}(0.1 mol dm^{-3} HCl)/nm 329 (3900) and 268 (4100); fluorescence (water) λ_{max} 445 nm (exc. 303 nm); FAB mass spectroscopy failed to give the (MH^+) ion peak; $\delta_H(D_2O)$ 9.99 (2 H, d, *J* 6.6, pyridinium α-H), 8.94 (1 H, t, *J* 6.6, pyridinium γ-H); 8.58 (1 H, s, 8-H), 8.41 (2 H, t, *J* 6.6, pyridinium β-H), 6.40 (1 H, d, *J* 4.5, 1'-H), 6.01 (1 H, t, *J* 4.5, 2'-H), 5.78 (1 H, t, *J* 4.5, 3'-H), 4.66 (1 H, m, 4'-H), 4.50 (2 H, m, 5'-H_2) and 2.23, 2.18 and 2.12 (3 H each, s, Ac); $\delta_C(D_2O$; 1,4-dioxane as internal reference, δ_C 67.4) 174.4, 173.6 173.5, 162.5 158.3, 151.2, 148.3, 146.9, 143.9, 129.2, 120.8, 87.8, 80.8, 74.1, 71.1, 63.8, 21.0, 20.8 and 20.7.

N-[2-*Oxo*-9-(β-D-*ribofuranosyl*)-2,3-*dihydropurin*-6-*yl*]*pyridinium Chloride* **9**.—The chloride **5** (121 mg, 0.4 mmol) was treated with a solution of pyridine–water [1 : 1 (v/v); 4 cm^3]. After 2 h at 50 °C the mixture was dried, and evaporation of water three times afforded pure *compound* **9** as a yellow oil (146 mg, 96%), R_f 0.1 (system B) (Found: C, 46.9; H, 4.1; N, 18.5. $C_{15}H_{16}ClN_5O_5$ requires C, 47.2; H, 4.2; N, 18.3%); λ_{max}(water)/nm 374 (4000), 262 (10 500); λ_{max}(0.1 mol dm^{-3} HCl)/nm 330 (3800) and 268 (4900); FAB mass spectroscopy failed to give the (MH^+) ion peak; $\delta_H(D_2O)$ 9.77 (2 H, d, *J* 8.0, pyridinium α-H), 8.83 (1 H, t, *J* 6.6, pyridinium γ-H), 8.32 (2 H, t, *J* 8.0, pyridinium β-H), 8.28 (1 H, s, 8-H), 5.97 (1 H, d, *J* 6.0, 1'-H), 5.78 (1 H, dd, *J* 6.0 and 4.0, 3'-H), 4.83 (1 H, t, *J* 6.0, 2'-H), 4.25 (1 H, m, 4'-H) and 3.86 (2 H, m, 5'-H_2).

6-(2-*Hydroxyethylsulfanyl*)-9-(2',3',5'-*tri*-O-*acetyl*-β-D-*ribofuranosyl*)-2,3-*dihydropurin*-2-*one* **10**.—The salt **8** (214 mg, 0.5 mmol) was treated with an excess of 2-sulfanylethanol. After 10 h at room temp. the mixture was dried *in vacuo* and purified on silica gel column eluted with increasing amounts of MeOH in $CHCl_3$ (up to 20%) to afford pure *sulfide* **10** as a oil (132 mg, 70%), R_f 0.6 (system A) (Found: C, 46.0; H, 4.5; N, 12.0. $C_{18}H_{22}N_4O_9S$ requires C, 45.9; H, 4.7; N, 11.9%); $[\alpha]_D$ −6.1 (*c* 0.03, MeOH); λ_{max}(MeOH)/nm 301 (6000), 293 (6050) and 250 (3350); *m/z* (FAB) 471 (MH^+); $\delta_H(CD_3OD)$ 8.20 (1 H, s, 8-H), 6.15 (1 H, d, *J* 6.0, 1'-H), 5.94 (1 H, t, *J* 6.0, 2'-H), 5.62 (1 H, dd, *J* 6.0 and 3.9, 3'-H), 4.41 (3 H, overlapped signals, 4'-H and 5'-H_2), 3.86 and 3.49 (2 H each, t, *J* 6.8, SCH_2CH_2OH) and 2.13, 2.09 and 2.06 (3 H each, s, Ac).

Isoguanosine **1**.—The salt **8** (253 mg, 0.5 mmol) was treated with conc. aq. ammonia (32%; 10 cm^3) at 50 °C. After 5 h the red solution (colour indicative of the Zincke reaction) was evaporated under reduced pressure and the residue, dissolved in water, was purified by HPLC on a reversed-phase C_{18} column eluted with MeOH–water (7 : 3, v/v). Concentration of the appropriate fractions gave isoguanosine **1** (128 mg, 91%), R_f 0.3 (system B), identical (TLC, UV and ^1H NMR)[6,9,15] with authentic material.

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