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Facile, chemoenzymatic synthesis of the potent antiviral compound, 2-acetonylinosine

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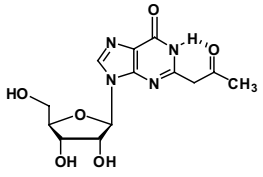
Abstract—A facile and efficient methodology for the chemoenzymatic synthesis of the antiviral compound, 2-acetonylinosine has been developed. The present synthetic strategy, which has generality, is a dramatic improvement on the methodologies currently available for the synthesis of functionalized purine nucleosides of therapeutic interest.

Functionalized purine nucleosides have exhibited both antiviral and anticancer chemotherapeutic activity.[1–7] They may serve also as biological probes for the inhibition of key viral enzymes that may lead to the discovery of new therapeutic agents. Purine nucleosides with surrogate nucleobases bearing functionalization at the 2-, 6- and 8-positions are of special interest, not only because of their expected stability towards enzymatic degradation, but also because such substitution may dramatically alter their base-pairing ability, receptor binding properties as well as in their possible ability to differentiate in their mode of interaction between human and viral enzymes.[1,3] Consequently, the development of efficient and facile synthetic routes to generate structurally diverse functionalized purine nucleoside analogues is of significance in organic and medicinal chemistry and chemical biology. This communication describes an example of a general approach to functionalized purine nucleosides that combines both organic chemistry and enzymology.

2-Acetonylinosine (**1**), a functionalized purine nucleoside, exhibits spectacular antiviral activity (therapeutic index of >1000!) against a RNA virus of the genus, *Bunyavirus*. However, our previous approach to the synthesis of this compound[8] was cumbersome and gave low yields in some of the steps. In addition, removal of the protecting group at the 6-position to reach the target compound was difficult. In order to develop a synthetic route that was more efficient and general, we designed a more concise synthesis that allowed regiospecific introduction of an acetonyl functionality at the C-2 position combined with a clean and efficient enzymatic step in the last conversion of the synthesis.



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The synthesis was approached through the protected 6-chloro-2-iodo-tri-*O*-acetylpurine riboside (**4**) as the key intermediate (Scheme 1). This compound can be prepared in three steps from guanosine,[9] by protection of the hydroxyl groups via selective acetylation with acetic anhydride, triethylamine and 4-(dimethyl-amino)pyridine in acetonitrile (93% yield), treatment of the triacetylated guanosine with phosphorus oxychloride and *N*,*N*-diethylaniline (90%) and conversion of the resulting compound **3** to the 6-chloro-2-iodopurine ribonucleoside **4** (66%) through a radical deamination–halogenation reaction.[10] Synthesis of the 2-acetonylpurine nucleoside from **4** involved a palladium-catalyzed cross-coupling reaction with regiospecificity for the 2-position. Palladium-catalyzed cross-coupling is an

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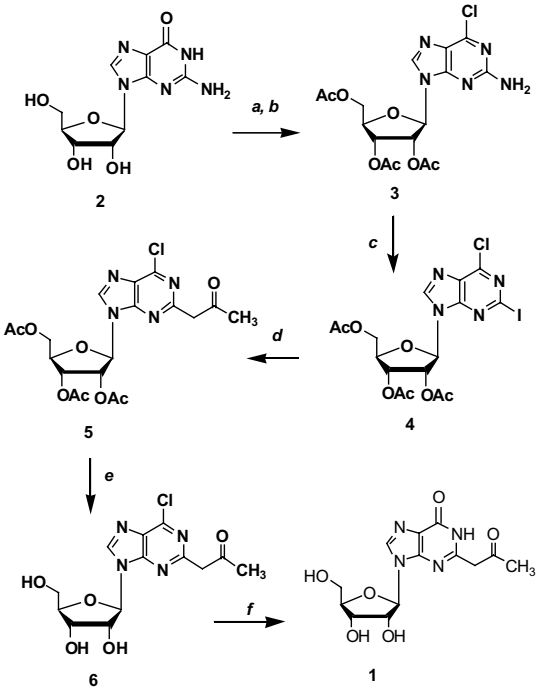
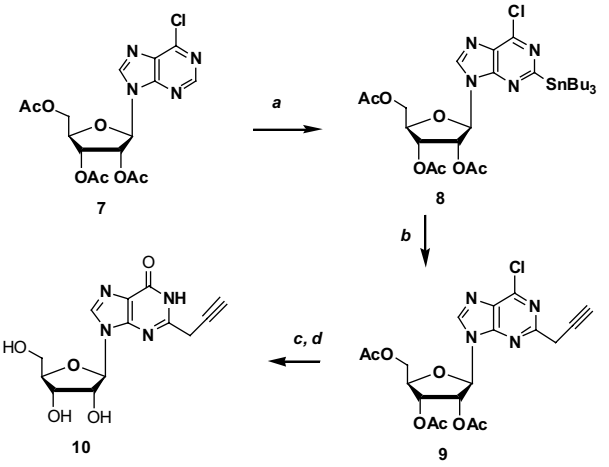


Scheme 1. Reagents and conditions: (a) Ac_2O, Et_3N, DMAP, CH_3CN, rt, 0.5 h; (b) $POCl_3$, *N*,*N*-diethylaniline, 70 °C, 1 h; (c) *n*-pentyl nitrite, CH_2I_2, 60 °C, 4 h; (d) tributyltin methoxide, isopropenyl acetate, $Pd(PPh_3)_4$, DMF, 95 °C, 2.5 h; (e) NH_3/MeOH, rt, 4 h; (f) adenosine deaminase, 0.1 M phosphate buffer (pH 7.4), 2 days, 25 °C.



Scheme 2. Reagents and conditions: (a) LTMP, Bu_3SnCl, THF, −75 °C, 0.5 h; (b) $Pd(PPh_3)_4$, CuI, propargyl bromide, DMF, 95 °C, 9 h; (c) NH_3/MeOH, rt, 4 h; (d) adenosine deaminase, 0.1 M phosphate buffer (pH 7.4), 4 days, 25 °C.

important method for the introduction of specific functionality into the purine nucleus; this methodology was described earlier by Nair et al.[8–11] The best palladium catalyst and solvent for this reaction were $Pd(PPh_3)_4$ and DMF and these conditions furnished superior yields of pure product **5** (85%) compared to other palladium catalysts and solvents.[8] Reaction times were also reduced. Ammonolysis of **5** gave the deacetylated compound **6**.

The final conversion involved the masking group at the 6-position. Protecting groups, such as *O*-alkyl or *O*-aralkyl commonly used at the 6-position of the purine ring, are difficult to remove as the conditions required for their removal are harsh and result in considerable decomposition of the substrate. Introduction of the chloro group at the 6-position as a masking group was a key strategy in this synthesis as its removal could be carried out by mild enzymatic methods.[12] Hydrolytic dechlorination of **6** using bovine intestinal adenosine deaminase was achieved in 2 days at 25 °C and was monitored by observing the UV spectral shift of λ_{max} from 265 to 250 nm. The target compound **1** was isolated in 82% yield following dialysis and purification by reversed-phase HPLC on a C_{18} column with methanol–water as the eluting solvent. 2-Acetonylinosine exists in a single tautomeric form, the keto isomer, which is stabilized by intramolecular hydrogen bonding.[13]

Another approach to regiospecific functionalization at the C-2 position, while significantly less efficient, is through the C-2-tributylstannyl derivative[14] of 6-chloropurine nucleosides. This method represents the reverse mode of introduction of functionality compared to the approach described above which relied on the cross-coupling reagent being utilized as its tributylstannyl derivative. This second approach can be exemplified by the case shown in Scheme 2 (overall yield of **10** from **7** is <5%).

In summary, 2-acetonylinosine can be synthesized in a facile and efficient manner using a chemoenzymatic methodology. The synthesis is far superior to that previously reported by us with respect to the reduced number of steps, a 250% increase in overall yield and generally shorter reaction times for the key steps. The final enzymatic step produces the target compound with minimal impurity, which renders purification by by reversed-phase HPLC relatively straightforward. The palladium-catalyzed methodology combined with the enzymatic step with adenosine deaminase is general and can be extended for the synthesis of many other C-2 functionalized nucleosides of antiviral interest.

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13. Spectral data for key intermediates and the target compound are given below.
Compound **5**: mp 189–191 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.48 (s, 1H), 6.41 (d, 1H), 4.78 (t, 1H), 4.69 (m, 1H), 4.38 (m, 1H), 4.10–4.12 (m, 2H), 3.72–3.78 (m, 2H), 2.23 (s, 3H), 2.18 (s, 3H), 2.14 (s, 3H), 2.11 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 20.6, 20.8, 21.0, 26.0, 49.0, 63.5, 70.7, 71.5, 75.0, 84.8, 127.3, 142.2, 143.1, 151.5, 154.3, 169.7, 169.9, 170.5, 203.5. Compound **6**: mp 140–141 °C. UV(H_2O): λ_{max} 265 nm (ε 12,700). ^{1}H NMR (Me_2SO-d_6, 500 MHz): δ 8.41 (s, 1H), 5.90 (d, J = 5 Hz, 1H), 5.61 (d, J = 5.5 Hz, 1H), 5.37 (d, J = 5.2 Hz, 1H), 5.12–5.14 (t, 1H), 4.45–4.46 (m, 1H), 4.11–4.12 (m, 1H), 3.94 (br s, 3H), 3.59–3.60 (m, 2H), 2.23 (s, 3H). ^{13}C NMR (Me_2SO-d_6, 125 MHz): δ 29.4, 49.0, 61.8, 70.8, 74.6, 86.1, 87.8, 122.9, 138.9, 149.0, 154.0, 157.1, 203.2. HRMS (FAB) calcd for $C_{13}H_{15}ClN_4O_5$ (M^++H): 343.0802, found 343.0803. For **1**: mp 114–116 °C. UV(H_2O): λ_{max} 250 nm (ε 11,900). ^{1}H NMR (Me_2SO-d_6, 500 MHz): δ 12.01 (s, 1H), 8.32 (s, 1H), 5.84 (d, J = 5 Hz, 1H), 5.51 (d, J = 5.5 Hz, 1H), 5.23 (d, J = 5.2 Hz, 1H), 5.10–5.11 (t, 1H), 4.47–4.48 (m, 1H), 4.10–4.11 (m, 1H), 3.92 (br s, 3H), 3.58–3.59 (m, 2H), 2.23 (s, 3H). ^{13}C NMR (Me_2SO-d_6, 125 MHz): δ 30.0, 49.2, 61.4, 70.6, 74.1, 85.8, 87.1, 122.7, 138.7, 148.4, 152.7, 157.1, 202.9. HRMS (FAB) calcd for $C_{13}H_{16}N_4O_6$ (M^++H): 325.1171, found 325.1173.
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