New Methodologies for the Synthesis of C-2 Functionalized Hypoxanthine Nucleosides

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Under palladium-catalyzed conditions, newly synthesized 2-iodohypoxanthine systems undergo efficient cross-coupling with organostannanes to furnish rare C-2 functionalized congeners of inosine. This represents the first examples of the use of organotin reagents in palladium-catalyzed cross-coupling involving nucleosides. 2-Acetonyl-, 2-(cyanomethyl)-, and 2-vinylinosines not only are readily accessible by this approach but can also be elaborated further through regiospecific hydroboration, osmylation, and reduction reactions. The reaction with enol acetates has considerable generality. In addition, this methodology for functionalized carbon–carbon bond formation can be extended to include 2-iodoadenosine. The products of the latter modification are exceedingly poor substrates for the enzyme adenosine deaminase.

The recent surge of interest in the synthesis of rare and previously inaccessible purine nucleosides is the result of observations that some compounds belonging to this family have potent antiviral therapeutic activity as well as being of enzymological usefulness as biological probes for the study of key viral-encoded enzymes.[1-6] Our interest in new antiviral nucleosides has focused attention on functionalized C-2 alkylated hypoxanthine systems. Although several C-2 substituted hypoxanthine nucleosides are known,[7-9] very few functionalized alkylated derivatives have been reported.[10] This is largely because of limitations in synthetic methodologies that allow access to this class of compounds. Virtually all of the 2-substituted inosines known have been synthesized from imidazole nucleosides through ring-closure reactions.[7,8] Other methods known for entry into this general class of compounds appear to be of more limited scope.[11,12] This paper reports on the development of a general methodology for the introduction of functionalized carbon–carbon bonding at the 2-position of the hypoxanthine ring and elaboration of the synthons introduced for the preparation of new congeners of inosine.[13] Also reported are the application of this methodology to an adenine nucleoside and the behavior of the resulting system toward the enzyme adenosine deaminase.

The key intermediate for the synthesis of these rare nucleosides was protected 2-iodo-6-methoxypurine **5** (Scheme I). This precursor can be prepared from guanosine in five steps. The first two steps are well-known and involve selective acetylation in 93% yield with acetic anhydride, triethylamine, and 4-(dimethylamino)pyridine in acetonitrile,[14] followed by treatment of the triacetylated guanosine with phosphorus oxychloride and *N,N*-diethylaniline at 70 °C for 1 h to give the 6-chloro compound **2** in about 89% yield.[15,16] It should be emphasized that consistently high yields in the conversion of the triacetylated guanosine to **2** were obtained when the reaction time was kept to 1 h under these reaction conditions. Considerable decomposition of both starting material and product occurred when longer reaction times were used. Preparation of the dihalogenated compound **3** from **2** involved a radical deamination–halogenation reaction.[16-18] The presence of an electron-withdrawing group at the 2-position in **3** renders the 6-position extremely susceptible to nucleophilic attack. Thus, nucleophilic displacement of the 6-chloro group in **3** with methoxide ion not only occurred with considerable ease but was accompanied by the desired deprotection of the acetate groups (96%). The carbohydrate moiety was then protected with *tert*-butyldimethylsilyl groups to give the key precursor **5** (96%). The silyl protecting groups[19] were deemed synthetically more appropriate for subsequent further elaboration of the base moiety (Scheme I). Also, when the 6-methoxy-2-iodopurine nucleoside formed in the treatment of **3** with sodium methoxide was demethylated with trimethylsilyl iodide, 2-iodoinosine was isolated. 2-Iodoinosine (**4**) has not been previously reported.

Synthesis of the novel C-2 functionalized hypoxanthine nucleosides described in this paper required, as the crucial step, a palladium-catalyzed cross-coupling reaction of a masked 2-iodoinosine system with an organostannane containing the desired synthon. Transfer of the synthon to the hypoxanthine ring, as for example in the conversion of **5** to **6** (70% yield), presumably involves oxidative insertion of palladium into the carbon–iodine bond of the masked iodohypoxanthine followed by cross-coupling of the derived Pd(II) complex with the tin enolate of acetone and reductive elimination (via the cis intermediate) to give the desired product (Scheme II).[20] The tributyltin enolate

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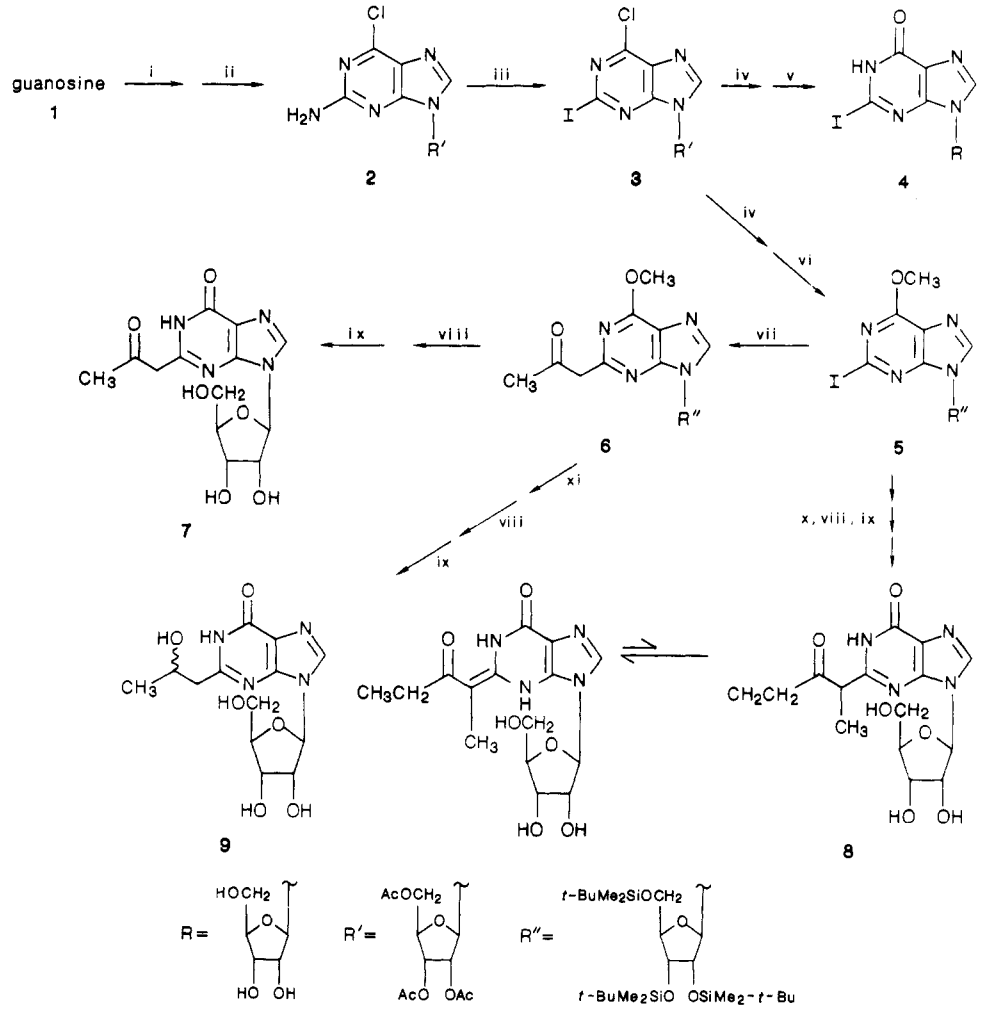
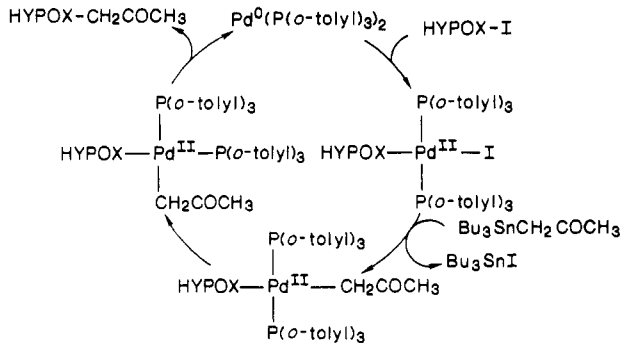
Scheme I[a]



[a] Conformation about glycosidic bond not implied by these structural representations. (i) Ac_2O, $(C_2H_5)_3N$, 4-(dimethylamino)pyridine, CH_3CN; (ii) $POCl_3$, *N,N*-diethylaniline, Δ; (iii) *n*-$C_5H_{11}ONO$, CH_2I_2, CH_3CN, Δ; (iv) $NaOCH_3$, CH_3OH; (v) $(CH_3)_3SiI$, CH_3CN, DMF; (vi) *t*-$Bu(CH_3)_2SiCl$, imidazole, DMF; (vii) $Pd(OAc)_2$, (*o*-tolyl)$_3$P, *n*-Bu_3SnOMe, $CH_2{=}C(CH_3)OAc$, toluene, Δ; (viii) $(CH_3)_3SiI$, CH_3CN; (ix) Et_4NF, CH_3CN; (x) $Pd(OAc)_2$, (*o*-tolyl)$_3$P, *n*-Bu_3SnOMe, $CH_3CH{=}C(C_2H_5)OAc$, toluene, Δ; (xi) $NaBH_4$, THF.

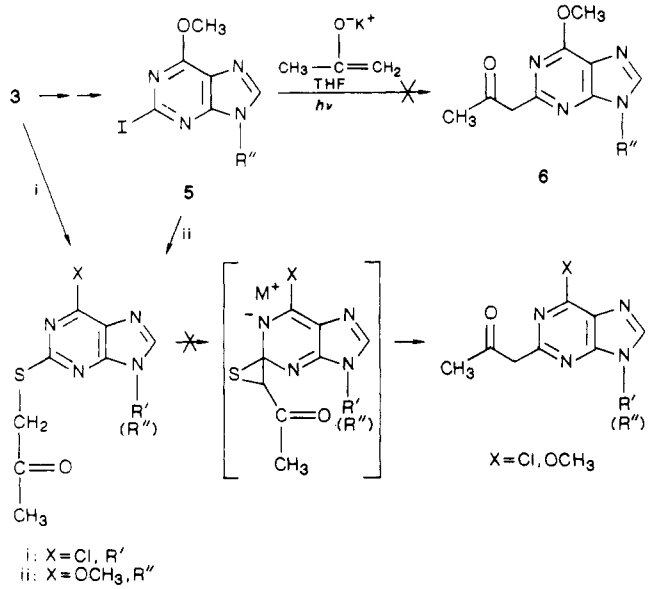
Scheme II[a]



[a] HYPOX-I = 2-iodohypoxanthine system.

of acetone, required for the cross-coupling step, is generated in situ from isopropenyl acetate and tributyltin methoxide. The palladium catalyst appears to be regenerated in this reaction as only very small amounts are needed. This work represents the first examples of the use of an organostannane in palladium-catalyzed cross-coupling involving nucleosides.

Scheme III



Other methods for the introduction of functionalized alkyl groups at the 2-position of the hypoxanthine ring are also possible. For example, the $S_{RN}1$ reaction[21] has been

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used successfully by us for the modification of the purine ring at the C-6 position.[22] However, the photoinduced $S_{RN}1$ reaction of **5** with the potassium enolate of acetone was unsuccessful (Scheme III). Purine nucleosides bearing a thioketone group at the 6-position are known to undergo the Eschenmoser sulfide contraction reaction.[23] In theory, such a reaction should also be possible with a thioacetonyl group at the 2-position. Compounds **2** and **5** can be converted to their 2-thioacetonyl derivatives through a thermal deamination–thiolation (*n*-pentyl nitrite, diacetonyl disulfide) and a photochemical reaction (diacetonyl disulfide), respectively. However, when the sulfur extrusion reaction was carried out on these compounds under a variety of conditions that involved changes in solvent, base, and phosphine, no 2-acetonyl product was isolated (Scheme III). This is consistent with the lack of any precedence in the literature for the success of the sulfide contraction at the C-2 position of the purine ring. Other approaches attempted, such as the Meerwein reaction[24] on the 2-amino compound **2**, were also unsuccessful.

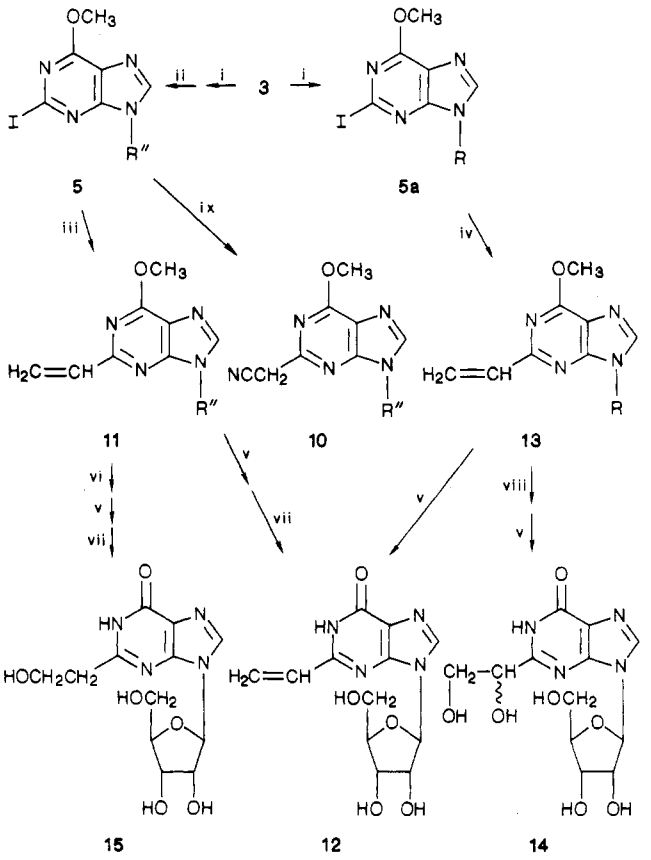
The acetonylated nucleoside **6** is the protected form of 2-acetonylinosine (**7**). The methoxy group is an effective way of protecting the amide carbonyl group in the hypoxanthine system. This protective group is relatively stable and can be easily removed at the end of a reaction sequence. Accordingly, in the case of **6**, conversion to the target molecule **7** was achieved in two steps by reaction first with trimethylsilyl iodide (64%), and subsequently with tetrabutylammonium fluoride (93%). The overall yield of **7** starting from guanosine was 18% (Scheme I). Purification of compound **7** and all other free nucleosides described in this paper was carried out by reversed-phase HPLC on Amberlite XAD-4 resin (styrene–divinylbenzene copolymer) with ethanol/water as the eluting solvent. Characterizations were carried out by UV, FTIR, and high-field NMR spectroscopy. 2-Acetonylinosine exists in a single tautomeric form (i.e., the keto isomer), which is apparently stabilized by intramolecular hydrogen bonding. The spectral data (particularly the UV and FTIR) supported the presence of the keto tautomer. The high-field carbon-13 NMR data also provided qualitative information on the preferred glycosidic bond conformation in solution through the magnitude of the ^{13}C NMR chemical shift difference between C-2′ and C-3′. A small difference (<0.5 ppm) is correlated with a preferred syn conformation in solution whereas a larger difference (>3.0 ppm) suggests a preferred anti conformation.[25] The high-field carbon spectrum of **7** shows this chemical shift difference to be 3.4 ppm, which suggests a preferred anti conformation about the glycosidic bond. This was found to be the case for all of the C-2 substituted nucleosides described in this paper.

The palladium-catalyzed cross-coupling methodology with enol acetates described above appears to have some generality. For example, the 2-iodopurine **5** undergoes coupling under these conditions with 3-acetoxypent-2-ene[26] in the presence of tri-*n*-butyltin methoxide to give the expected keto product, which can be deprotected to **8**. Other enol acetates, both symmetrical and unsymmetrical (except the parent vinyl acetate) are also reactive under the conditions of cross-coupling. Interestingly, compound **8** appears to prefer the tautomeric structure shown with an exocyclic double bond ("xanthosine-like" arrangement) as evidenced from ^1H NMR data (singlet methyl adjacent to keto group), ^{13}C NMR data (carbon adjacent to carbonyl at 79.8 ppm, ketone carbonyl at 209.2 ppm), and the FTIR spectrum (overlapping ketone and lactam carbonyls at 1685 cm^{-1}). The difference in the preferred tautomeric form in the case of **8** compared with **7** is not entirely clear.

The protected 2-acetonyl compound **6** could be reduced readily by sodium borohydride to give, after deprotection, the new nucleoside **9** (Scheme I).

The palladium-catalyzed cross-coupling reaction described above can be extended further to include other functionalized organostannanes. For example, tri-*n*-butyl(cyanomethyl)stannane[27] can be prepared from the reaction of tributyltin methoxide and (trimethylsilyl)acetonitrile. Reaction of this reagent with **5** under our cross-coupling procedure gave the 2-(cyanomethyl)inosine **10** in 55% yield (Scheme IV). 2-Vinylinosine (**12**) (or **11**), potentially a key precursor for the synthesis of a variety of functionalized alkylated purine nucleosides, is also readily available via the aforementioned methodology. Thus, the thermal reaction of **5** with tri-*n*-butylvinylstannane in the presence of palladium chloride afforded **11** in excellent yields (>90%). *Interestingly, the vinylation reaction can be carried out in almost quantitative yields with the 2-*

Scheme IV[a]



[a] (i) $NaOCH_3$, CH_3OH; (ii) *t*-Bu$(CH_3)_2SiCl$, imidazole, DMF; (iii) *n*-$Bu_3SnCH{=}CH_2$, $PdCl_2(CH_3CN)_2$, toluene, Δ; (iv) $PdCl_2(CH_3CN)_2$, *n*-$Bu_3SnCH{=}CH_2$, DMF, Δ; (v) $(CH_3)_3SiI$, CH_3CN; (vi) 9-BBN, THF, Δ; (vii) Et_4NF, CH_3CN; (viii) OsO_4, pyridine; (ix) $Pd(OAc)_2$, (*o*-tolyl)$_3P$, *n*-Bu_3SnCH_2CN, toluene, Δ.

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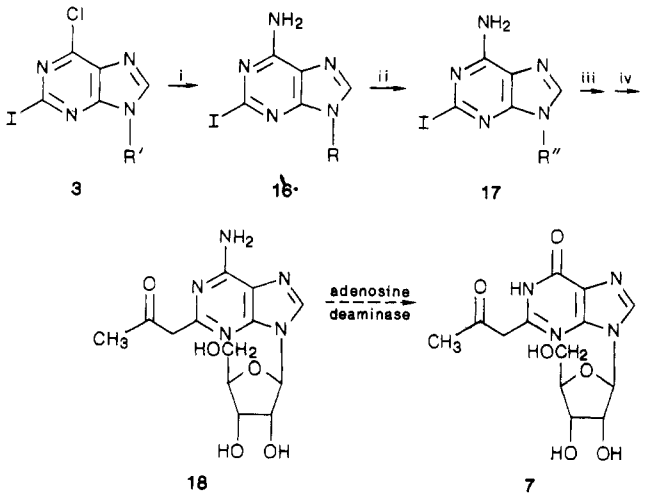
Scheme V[a]



[a] (i) NH_3, C_2H_5OH; (ii) *t*-$Bu(CH_3)_2SiCl$, imidazole, DMF; (iii) $Pd(OAc)_2$, (*o*-tolyl)$_3$P, *n*-Bu_3SnOMe, $CH_2{=}C(CH_3)OAc$, toluene, Δ; (iv) Et_4NF, CH_3CN.

iodo nucleoside ***5a****, where the carbohydrate moiety is unprotected!*

Oxidative elaboration of the vinyl group in the aforementioned 2-vinyl nucleoside was carried out by two methods. The first one involved reaction of compound **13** (the partially deprotected form of **11**) with osmium tetraoxide (65% yield). Demethylation of the product with trimethylsilyl iodide gave the highly hydroxylated compound **14**. The second method of elaboration was hydroboration of the vinyl compound **11** with 9-borabicyclo-[3.3.1]nonane (9-BBN). Oxidative workup of the organoborane intermediate resulted in the regiospecific formation (52% yield, 65% conversion) of the terminal alcohol. Deprotection of this compound afforded **15** (Scheme IV). It is of interest to mention that hydroboration reactions have rarely been used previously to elaborate structures in purine nucleoside chemistry.

Finally, it should be mentioned that we have applied the synthetic chemistry developed above for the preparation of C-2 functionalized adenosines. The starting material for this synthesis was the dihalogenated nucleoside **3**. Treatment of **3** with ethanolic ammonia resulted in both the displacement of the 6-chloro group and deprotection of the acetate protecting groups to give 2-iodoadenosine (**16**) (90%). Silylation of **16** with *tert*-butyldimethylsilyl chloride and imidazole afforded **17**, the immediate precursor for the functionalization reaction, in 94% yield. When compound **17** was treated with palladium acetate, tri-*o*-tolylphosphine, tributyltin methoxide, and isopropenyl acetate in toluene at 100 °C, smooth conversion to the acetonyl compound occurred. Deprotection gave the target molecule **18**, which exists almost entirely in the keto form depicted as evidenced from spectral data. The overall yield of purified product **18** for the two steps was 40% (Scheme V).

A fundamental reason for the synthesis of the 2-acetonyladenosine system was to examine if compounds bearing functionalized alkylation at this position would be deaminated by the ubiquitous mammalian enzyme adenosine deaminase. This enzyme normally catalyzes the hydrolytic deamination of adenosine to inosine.[28-31] Synthetically, this information would be useful because of the possibility of entering both the adenine and hypoxanthine series of nucleosides by initially synthesizing the adenosine analogues and subsequently deaminating these to the corresponding inosine compounds with adenosine deaminase. Biologically, the results would contribute to the design of prodrugs or of molecules that would be resistant to deamination. The deamination studies with adenosine deaminase were followed by UV spectral methods with adenosine as the standard, using procedures previously described by us.[29] The target molecule, 2-acetonyladenosine (**18**), was found to be resistant to deamination by this enzyme. Deamination does occur eventually but only after prolonged periods (about 24 h) and with large excesses of enzyme. This provides experimental evidence that substrate binding and significant substrate activity involving this enzyme requires the adenine ring system to be relatively unhindered at N-1, apparently one of the binding sites for the enzyme.

In summary, the new halogenated nucleoside 2-iodoinosine has been synthesized. The palladium-catalyzed cross-coupling reactions of protected or partially protected 2-iodoinosine with organostannanes provide highly efficient approaches to the synthesis of new and rare functionalized congeners of the natural nucleoside inosine. These studies were extended to the corresponding adenosine system. The cross-coupling methodology appears to have generality with respect to both the organostannane reagent and the halogenated nucleoside. Biological studies assessing the antiviral activities of the target molecules against RNA viruses are currently under investigation, and preliminary in vitro results suggest that some of the compounds described are active. The complete biological studies will be reported elsewhere.

Experimental Section

Melting points reported are uncorrected and were determined on a Thomas-Hoover melting point apparatus fitted with a microscope. Nuclear magnetic resonance spectra were recorded on JEOL Model FX90Q and Bruker Model WM360 pulse Fourier transform spectrometers. Mass spectra were determined on a Hewlett-Packard 5985 GC/MS system of a VG Analytical Model ZAB-HF instrument with high-resolution FAB capability. Ultraviolet spectra were recorded on a Varian Cary Model 219 or a Gilford Response spectrophotometer. Infrared spectra were recorded on an IBM Model 98 Fourier transform instrument. Lyophilizations were performed with a Virtis Freezemobile 3 unit. Preparative layer chromatography plates were prepared by coating six 20 cm × 20 cm plates with a slurry made from 150 g of E. Merck PF_{254} silica gel in 400 mL of water. The silica gel plates were allowed to dry slowly and were then activated for 3 h at 135 °C. Flash chromatography was carried out by using glass columns packed with 230–400-mesh silica gel. High-performance liquid chromatography was done by using Altex columns packed with Amberlite XAD-4 resin (Rohm and Haas) which was ground and sieved to 40–60 μm. Samples were injected with a gas-tight syringe through an Altex four-way slide valve. Separations were carried out at 20–80 psi by using an FMI RRPSY-SS $^1/_4$ in. piston pump. Fractions were monitored by a Pharmacia UV-2 ultraviolet monitor, and products were collected on a Gilson FC-100 fraction collector. Satisfactory elemental analyses were obtained for all final products. They were determined by Galbraith Laboratories, Inc., Knoxville, TN.

6-Chloro-2-iodo-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-purine (3). The protected nucleoside **2** (5.549 g, 12.97 mmol), prepared as described previously,[15,16] was taken up in acetonitrile (40 mL). Diiodomethane (5 mL) and *n*-pentyl nitrite (12 mL) were added. The solution was purged with nitrogen for 15 min and heated to a light reflux for 20 h under N_2. Concentration

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of the solution in vacuo was followed by purification on a silica gel flash column, first using hexane and then a 2:1 mixture of hexane and ethyl acetate as the eluting solvents. The product **3** was isolated as a light orange glass (3.852 g, 7.15 mmol, 55% yield). Crystallization from ethanol provided white needles: mp 181–183 °C (lit.[15] mp 181–183 °C); ^{1}H NMR ($CDCl_3$) δ 2.11 (s, 3 H), 2.13 (s, 3 H), 2.18 (s, 3 H), 4.43 (m, 3 H), 5.65 (t, 1 H), 5.81 (t, 1 H), 6.23 (d, 1 H), 8.27 (s, 1 H); UV (EtOH) λ_{max} 222.5 (ϵ 2.1 × 10^4), 258 (6.6 × 10^3), 281 nm (9.3 × 10^3).

2-Iodo-6-methoxy-9-[2,3,5-tri-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]purine (5). Dry 6-chloro-2-iodo triacetylated nebularine **3** (1.780 g, 3.30 mmol) was taken up in dry (Omnisolve) methanol (50 mL) to which sodium methoxide (1.045 g, 19.35 mmol) was subsequently added. The solution was fitted with a septum and stirred at 25 °C for 12 h. The reaction was quenched by heating (50 °C) with 1 g of NH_4Cl. After filtration, the solution was concentrated in vacuo. The residue was adsorbed on 5 g of 230–400-mesh silica gel and added to the top of a 40-mm silica gel flash chromatography column. The product was eluted off with 1:10 methanol/chloroform. The eluant was concentrated to give 1.059 g (2.60 mmol, 78.6%) of product, which was silylated with *tert*-butyldimethylsilyl chloride (1.56 g, 10.40 mmol) and imidazole (1.414 g, 20.8 mmol) in dimethylformamide (20 mL). The product **5** was isolated as a low-melting gum (1.560 g, 80% yield) after flash chromatography on silica gel: ^{1}H NMR ($CDCl_3$) δ –0.02–0.13 (m, 18 H), 0.90 (m, 27 H), 3.71–4.63 (m, 5 H), 4.13 (s, 3 H), 5.93 (d, 1 H), 8.20 (s, 1 H); UV (EtOH) λ_{max} 262 nm (ϵ 7.8 × 10^3); mass spectrum, *m/z* (relative intensity) 751 (M^+, 1.2), 693 (M^+ – *t*-Bu, 100), 433 (26.8), 285 (5.7), 261 (27.7), 231 (19.0), 211 (18.0), 175 (4.1), 155 (7.0), 147 (47.5), 129 (21.9), 115 (19.4).

2-Iodoinosine (4). 2-Iodo-6-methoxy-9-(β-D-ribofuranosyl)-purine (1.560 g, 3.800 mmol), prepared from the reaction of **3** with sodium methoxide as described above, was dissolved in a mixture of dry DMF (4.0 mL) and dry acetonitrile (6.5 mL), and to this was added potassium iodide (2.770 g, 16.7 mmol). This mixture was purged with nitrogen, treated with trimethylsilyl chloride (1.75 mL, 15.8 mmol), and then stirred at 25 °C under nitrogen for 24 h. Dilute aqueous NaOH was then added to the reaction mixture, and the pH was adjusted to 7.0. The solvents and water were removed under reduced pressure, and the residue was flash chromatographed on silica gel with 1:4 methanol/dichloromethane as the eluting solvent. The crude product was purified by HPLC on Amberlite XAD-4 resin with ethanol/water as the eluting solvent. Pure 2-iodoinosine (**4**) was obtained as off-white crystals (0.221 g, 0.561 mmol, 15%). The low yield is the result of considerable decomposition of product under the reaction conditions. Compound **4** gave the following data: mp 255 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 3.58 (m, 2 H), 3.93 (m, 1 H), 4.13 (m, 1 H), 4.46 (m, 1 H), 5.00 (t, 1 H), 5.17 (d, 1 H), 5.46 (d, 1 H), 5.80 (d, 1 H), 8.24 (s, 1 H); ^{13}C NMR (Me_2SO-d_6) δ 61.1, 70.2, 73.9, 85.7, 87.0, 109.6, 123.8, 138.3, 148.2, 156.8; UV (EtOH) λ_{max} 253 (ϵ 14 900), 272 nm (sh, 11 900); FAB HRMS obsd (M^+ + H) 394.9874, calcd for $C_{10}H_{11}N_4O_5I$ 394.9852.

2-Acetonyl-6-methoxy-9-[2,3,5-tri-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]purine (6). Silylated 2-iodo-6-methoxynebularine (**5**) (1.208 g, 1.611 mmol) was placed in a 100-mL round-bottomed flask already containing palladium acetate (0.036 g, 0.161 mmol) and tri-*o*-tolylphosphine (0.098 g, 0.322 mmol), and the flask was placed on the vacuum pump to remove residual oxygen and moisture. Toluene (25 mL), freshly distilled from sodium hydride, was added under nitrogen. In a separate flask, toluene (10 mL), isopropenyl acetate (0.28 mL, 2.42 mmol), and tributyltin methoxide (0.70 mL, 2.42 mmol) were combined. This solution was kept at 40 °C for 30 min. The nucleoside solution was then transferred by double-tipped needle to the flask containing the tin reagent. After heating of this solution under N_2 for 6 h at 95 °C, no starting material remained (TLC, 2:1 hexane/ether). The solution was cooled to room temperature and partitioned between ether (70 mL) and 5% aqueous disodium ethylenediaminetetraacetic acid (Na_2EDTA). The ether layer was then extracted with water (30 mL). The aqueous phases were back extracted with ether (30 mL), and the combined ether layers were dried (Na_2SO_4) and concentrated. The crude product was purified by flash chromatography, using 2:1 hexane/ether as the eluting solvent, to give the protected acetonylated product **6** (0.7952 g, 1.17 mmol, 73%) as a low-melting solid: ^{13}C NMR ($CDCl_3$) δ –5.3, –4.9, –4.7, –4.3, 17.9, 18.1, 18.6, 25.7, 25.8, 26.1, 29.8, 54.1, 54.3, 62.2, 71.3, 76.1, 84.9, 88.6, 120.4, 141.0, 152.3, 158.4, 160.7, 204.3; ^{1}H NMR ($CDCl_3$) δ –0.02–0.13 (m, 18 H), 0.92 (m, 27 H), 2.23 (s, 3 H), 3.78–4.05 (m, 3 H), 3.98 (s, 2 H), 4.13 (s, 3 H), 4.30 (t, 1 H), 4.52 (t, 1 H), 6.03 (d, 1 H), 8.27 (s, 1 H); UV (EtOH) λ_{max} 250 (ϵ 9.0 × 10^3), 290 nm (ϵ 2.7 × 10^3). (**Note**: All experimental work with organostannanes must be carried out in efficient fume hoods.)

2-Acetonyl-9-(β-D-ribofuranosyl)hypoxanthine (7). The protected acetonylated product **6** (0.850 g, 1.25 mmol) was taken up in 25 mL of acetonitrile, which had been freshly distilled from calcium hydride. Potassium iodide (0.293 g, 1.75 mmol), which had been dried on the vacuum pump at 50 °C, was added, followed by trimethylsilyl chloride (0.223 mL, 1.75 mmol) via a gas-tight syringe. The solution was stirred for 8 h at room temperature and then filtered, and the precipitate was washed with ether. The filtrate and ether washings were concentrated and purified by flash chromatography using ether as the eluting solvent to give the demethylated product (0.5305 g, 0.7965 mmol, 64% yield). This compound was dissolved in dry tetrahydrofuran (50 mL) and treated with 3.186 mmol of tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (1 M solution). The solution was stirred until no starting material remained (2 h). An aqueous solution of a 10-fold excess of ammonium chloride was added, and the tetrahydrofuran was removed in vacuo. The remaining aqueous solution was heated at 50–60 °C for 45 min. The aqueous phase was then extracted with chloroform (2 × 25 mL) and then with ether (2 × 25 mL). The aqueous phase was then concentrated under reduced pressure, and the residue was purified by reversed-phase HPLC on Amberlite XAD-4 resin (40–60 μm) using ethanol/water as the mobile phase. The combined fractions containing the product were lyophilized, and compound **7** was obtained as white crystals (0.180 g, 0.557 mmol, 70%): mp 114–116 °C; ^{13}C NMR (Me_2SO-d_6) δ 30.0, 49.2, 61.5, 70.6, 74.0, 85.8, 87.1, 122.8, 138.7, 148.5, 152.7, 157.0, 202.9; ^{1}H NMR (Me_2SO-d_6) δ 2.23 (s, 3 H), 3.59 (m, 2 H), 3.92 (br s, 3 H), 4.11 (m, 1 H), 4.47 (m, 1 H), 5.11 (t, 1 H), 5.23 (d, 1 H), 5.50 (d, 1 H), 5.84 (d, 1 H), 8.32 (s, 1 H); UV (H_2O) λ_{max} 250 (ϵ 1.2 × 10^4), 266 nm (6.8 × 10^3); FTIR (KBr) 3317, 2961, 2934, 1701, 1692, 1687, 1585, 1558 cm^{-1}; FAB HRMS obsd (M^+ + H) 325.1171, calcd for $C_{13}H_{16}N_4O_6$ 325.1148.

2-(1-Methyl-2-oxobutyl)-9-(β-D-ribofuranosyl)hypoxanthine (8). Palladium acetate (0.178 g, 0.793 mmol) and tri-*o*-tolylphosphine (0.483 g, 1.587 mmol) were added to a flask containing 3.774 g (5.026 mmol) of **5**. Freshly distilled toluene (80 mL) was added under N_2. In a separate flask, 2.20 mL (7.64 mmol) of tributyltin methoxide and 2.25 g (17.55 mmol) of 3-acetoxypent-2-ene[26] were warmed at 45 °C for 40 min. This solution was then transferred via double-tipped needle to the flask containing **5**. The solution was stirred for 9 h at toluene reflux under N_2. Upon cooling, the reaction mixture was filtered and diluted with 75 mL of ethyl ether. The organic solution was washed with 10% Na_2EDTA (3 × 30 mL) and water (50 mL) and was then dried (Na_2SO_4). The solvents were removed under reduced pressure. The residue was flash chromatographed on silica gel by using hexane followed by 3:1 hexane/ether to provide 2.763 g (3.896 mmol, 77.5%) of product as an oil: ^{1}H NMR ($CDCl_3$) δ –0.21 to –0.05 (m, 18 H), 0.62–0.76 (m, 27 H), 0.82 (t, 3 H, *J* = 7.3 Hz), 1.32 (d, 3 H, *J* = 7.3 Hz), 2.25 (q, 2 H, *J* = 7.3 Hz), 3.64 (m, 2 H), 3.82 (m, 1 H), 3.93 (m, 4 H), 4.14 (m, 1 H), 4.37 (m, 1 H), 5.85 (d, 1 H, *J* = 3.9 Hz), 8.12 (s, 1 H); UV (EtOH) λ_{max} 250, 295 nm; mass spectrum, *m/z* (relative intensity) (30 eV) 708 (M^+, 0.6), 651 (M – *tert*-butyl, 57.8), 391 (28.5), 261 (22.5), 235 (59.4).

The two-step deprotection of the above-mentioned product (2.564 g, 3.615 mmol) was carried out as described for the synthesis of **7** except that tetraethylammonium fluoride (TEAF) was used for the desilylation step instead of TBAF. (Fewer difficulties were encountered with replacement of the tetraethylammonium counterion by a proton than the tetrabutylammonium ion.) The final product was purified as described for **7** to give **8** (0.440 g, 1.249 mmol, 34.6%) as a white solid: mp 104–106 °C; ^{13}C NMR (Me_2SO-d_6) δ 8.0, 23.6, 29.6, 61.7, 70.7, 74.0, 79.8, 85.8, 87.5, 123.3, 139.5, 148.0, 156.8, 158.4, 209.2; ^{1}H NMR (Me_2SO-d_6) δ 0.95 (t, 3 H, *J* = 7.33 Hz), 1.63 (s, 3 H), 2.65 (q, 2 H, *J* = 7.32 Hz), 3.58 (m, 2 H), 3.94 (m, 1 H), 4.14 (m, 1 H), 4.51 (m, 1 H), 4.99 (m, 1 H), 5.19 (m, 1 H), 5.45 (m, 1 H), 5.84 (d, 1 H, *J* = 5.86 Hz), 6.55

(br s, 1 H), 8.32 (s, 1 H); UV (H_2O) λ_{max} 251.6 (ϵ 10.5 × 10^4), 272 nm (sh, 5.6 × 10^3); FTIR (KBr) 3200–3500, 1695, 1685, 1554 cm^{-1}; FAB HRMS obsd (M^+ + H) 353.1987, calcd for $C_{15}H_{21}N_4O_6$ 353.1466.

2-(2-Hydroxypropyl)-9-(β-D-ribofuranosyl)hypoxanthine (9). In a 100-mL round-bottomed flask, the protected 2-acetonylinosine (**6**) (0.790 g, 1.166 mmol) was dissolved in dry tetrahydrofuran (30 mL). Sodium borohydride (0.208 g, 5.520 mmol) was added, and the reaction mixture was stirred at 25 °C for 4 h. The reaction was worked up by addition of 0.02 M HCl followed by extraction with dichloromethane (3 × 30 mL). The combined organic layers were dried (Na_2SO_4), concentrated, and purified on silica gel plates to give the protected reduced compound (496 mg, 62%). The latter was deprotected in two steps and purified as described for compound **7**. The target compound **9** was obtained as a white crystalline solid: mp 115–117 °C (sealed cap); ^{13}C NMR (Me_2SO-d_6) δ 23.3, 43.7, 61.4, 65.0, 70.5, 73.8, 85.7, 87.2, 122.5, 138.4, 148.5, 156.9, 157.0; ^1H NMR (Me_2SO-d_6) δ 1.12 (d, 3 H), 2.68 (d, 2 H), 3.59 (m, 2 H), 3.93 (m, 1 H), 4.13 (m, 2 H), 4.35 (m, 1 H), 4.51 (m, 1 H), 5.11 (m, 1 H), 5.21 (m, 1 H), 5.45 (m, 1 H), 5.84 (d, 1 H), 8.26 (s, 1 H), 12.10 (s, 1 H); UV (H_2O) λ_{max} 267 (ϵ 5994), 250 nm (11 420); FAB HRMS obsd (M^+ + H) 327.1332, calcd for $C_{13}H_{18}N_4O_6$ 327.1304.

2-Vinyl-9-(β-D-ribofuranosyl)hypoxanthine (12). The palladium catalyzed coupling reaction of **5** to give **11** was carried out as described for **6** but with the following modifications: (i) bis(acetonitrile)palladium chloride replaced palladium acetate, (ii) vinyltri-*n*-butyltin replaced the mixture of tributyltin methoxide and isopropenyl acetate, and (iii) the reaction time was reduced to 3 h. The yield of **11** from this reaction was 90%. The two-step deprotection of **11** and purification of the resulting product were executed as described for **7**. Compound **12** was obtained in pure form as white crystals (50% yield, from **11**) after HPLC separations: mp 225–230 °C dec; ^{13}C NMR (Me_2SO-d_6) δ 61.3, 70.4, 73.6, 85.6, 87.2, 123.2, 125.2, 129.5, 139.3, 148.5, 151.7, 156.8; ^1H NMR (Me_2SO-d_6) δ 3.62 (m, 2 H), 3.96 (m, 1 H), 4.17 (m, 1 H), 4.56 (m, 1 H), 5.05 (t, 1 H), 5.25 (d, 1 H), 5.50 (d, 1 H), 5.83 (dd, 1 H), 5.91 (d, 1 H), 6.50 (m, 2 H), 8.35 (s, 1 H), 12.41 (s, 1 H); UV (H_2O) λ_{max} 292 (ϵ 6486), 260 (7850), 207 nm (19 891); FTIR (KBr) 3300, 3080, 2900, 1686, 1640, 1554 cm^{-1}; FAB HRMS obsd (M^+ + H) 295.1034, calcd for $C_{12}H_{14}N_4O_5$ 295.1042.

2-Vinyl-9-(β-D-ribofuranosyl)hypoxanthine (12) from Unprotected Nucleoside 5a. To a solution of **5a** (9.000 g, 22.050 mmol) and bis(acetonitrile)palladium chloride (0.249 g, 0.96 mmol) in DMF (200 mL) was added vinyltri-*n*-butyltin (7.09 mL, 24.260 mmol). The reaction mixture was stirred at 90 °C for 3 h under N_2, then cooled to ambient temperature, and filtered. The filtrate was concentrated, and the resulting residue was flash chromatographed on silica gel with 3% followed by 10% methanol/chloroform as the eluting solvent. The product **13** was obtained as a tan solid (6.530 g, 21.180 mmol, 96%). It was deprotected with trimethylsilyl chloride and KI as described for **7**. Compound **12** was characterized as shown above.

2-(2-Hydroxyethyl)-9-(β-D-ribofuranosyl)hypoxanthine (15). In a 125-mL three-neck round-bottomed flask was placed 7.750 g (11.90 mmol) of the protected vinyl compound **11** in dry tetrahydrofuran (80 mL). The flask was fitted with septa and a nitrogen bubbler. 9-Borabicyclo[3.3.1]nonane (26.2 mL of a 0.5 M solution in tetrahydrofuran, 13.10 mmol) was added via syringe and needle. The reaction mixture was heated under nitrogen at 60 °C for 72 h, during which time three additional portions of the hydroborating agent (3 × 15 mL) were added. The reaction mixture was then cooled to 0 °C and treated with 3 M sodium hydroxide (10 mL, 30 mmol) followed by 30% hydrogen peroxide (10 mL, 98 mmol). The reaction mixture was then stirred at room temperature for 2 h. Water (20 mL) was added, and the solution was extracted with ether (3 × 100 mL). The organic extract was washed with a 20% solution of ferrous sulfate (25 mL), then dried (Na_2SO_4), and concentrated. Flash chromatography on silica gel gave the product as an oil (4.128 g, 52%). This compound was deprotected and purified as described for **7**. Compound **15** was obtained in 60% yield as a white crystals: mp 153–155 °C; ^{13}C NMR (Me_2SO-d_6) δ 37.7, 58.8, 61.4, 70.5, 73.8, 85.7, 87.7, 122.5, 138.4, 148.5, 157.0; ^1H NMR (Me_2SO-d_6) δ 2.78 (t, 2 H), 3.59 (m, 2 H), 3.92 (m, 2 H), 4.09 (m, 1 H), 4.47 (m, 1 H), 4.73 (m, 2 H), 5.12 (m, 2 H), 5.38 (m, 1 H), 5.84 (d, 1 H), 8.23 (s, 1 H), 12.19 (s, 1 H); UV (H_2O) λ_{max} 250 nm (ϵ 11 124); FAB HRMS obsd (M^+ + H) 313.1149, calcd for $C_{12}H_{16}N_4O_6$ 313.1148.

2-(1,2-Dihydroxyethyl)-9-(β-D-ribofuranosyl)hypoxanthine (14). Compound **13** (the desilylated form of **11**) served as the starting material for the preparation of **14**. Compound **13** (0.163 g, 0.535 mmol) was placed in a 50-mL round-bottomed flask and dissolved in pyridine (4 mL). Osmium tetraoxide (0.123 g, 0.484 mmol) in pyridine (3 mL) was then added to the solution. The reaction mixture was stirred at room temperature for 4 h and then treated with sodium bisulfite (0.5 g in 10 mL of H_2O) for 45 min. The solution was concentrated and flash chromatographed on silica gel to give the partially protected hydroxylated product (0.119 g, 65%). Demethylation of this product was carried out as described for **7**. Purification was done by HPLC as described for **7**. Compound **14** was obtained in 83% yield as white crystals: mp 133–135 °C; ^{13}C NMR (Me_2SO-d_6) δ 61.3, 64.1, 70.4, 72.0, 73.8, 85.6, 87.0, 122.9, 138.7, 148.3, 156.5, 158.8; ^1H NMR (Me_2SO-d_6) δ 3.37 (m, 1 H), 3.67 (m, 2 H), 3.79 (m, 1 H), 3.96 (m, 1 H), 4.17 (m, 1 H), 4.62 (m, 2 H), 5.05 (m, 1 H), 5.12 (m, 1 H), 5.20 (m, 1 H), 5.28 (m, 1 H), 5.55 (m, 1 H), 5.86 (d, 1 H), 8.32 (s, 1 H), 11.74 (s, 1 H); UV (H_2O) λ_{max} 250 nm (ϵ 11 194); FAB HRMS obsd (M^+ + H) 329.1132, calcd for $C_{12}H_{16}N_4O_7$ 329.1097.

Preparation of Tri-*n*-butyl(cyanomethyl)stannane. A solution of 2.80 mL (20.450 mmol) of (trimethylsilyl)acetonitrile and 3 mL (10.420 mmol) of tributyltin methoxide in 20 mL of DMF was heated under reflux under N_2 for 15 h. Fractional distillation (130–148 °C, 1.5 Torr)[27] gave 2.854 g (83%) of the tin reagent.

2-(Cyanomethyl)-9-[2,3,5-tri-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]hypoxanthine (10). 2-Iodo-6-methoxy-9-[2,3,5-tri-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-purine (**5**) (0.864 g, 1.15 mmol), palladium acetate (0.040 g, 0.178 mmol), and tri-*o*-tolylphosphine (0.115 g, 0.378 mmol) were dried in vacuo overnight. Toluene (20 mL) and tri-*n*-butyl(cyanomethyl)stannane (0.295 mL, 1.32 mmol) were added, and the reaction mixture was stirred under N_2 and at toluene reflux for 6 h. Upon cooling, ethyl ether (30 mL) was added and the reaction mixture was extracted with 10% Na_2EDTA solution (10 mL). The solvent was removed under reduced pressure. The residue was purified on silica gel plates with 11:9 ethyl ether/hexane as the developing solvent. The band at R_f 0.53 provided 0.351 g (0.526 mmol, 46%) of **10** as an orange oil: ^{13}C NMR ($CDCl_3$) δ 27.9, 54.3, 62.0, 71.3, 75.9, 84.9, 88.6, 115.9, 120.8, 141.6, 151.9, 153.8, 160.9; ^1H NMR ($CDCl_3$) δ −0.02 to 0.12, 0.80 (s), 0.91 (s), 0.94 (s), 3.82 (m, 1 H) 3.98 (s, 3 H), 4.17 (s, 4 H), 4.28 (m, 1 H), 4.56 (t, 1 H), 6.02 (d, 1 H), 8.42 (s, 1 H); UV (ethyl ether) λ_{max} 252, 287 nm; FTIR (KBr) 2290 cm^{-1}.

6-Amino-2-iodo-9-(β-D-ribofuranosyl)purine (16). Compound **3** (0.390 g, 0.724 mmol) was taken up in absolute ethanol (50 mL) and saturated with ammonia for 45 min at 0 °C. The solution was stirred overnight at room temperature and was then concentrated in vacuo. The residue was azeotroped with *o*-xylene (40 mL) to remove the byproduct, acetamide. The crude product was then adsorbed onto silica gel and purified by flash chromatography starting with 1:10 methanol/chloroform and increasing the polarity of the eluant up to 1.5:10. The fractions from the column were concentrated to give 0.269 g (0.652 mmol, 90%) of compound **16** as a white crystalline product: mp 142–145 °C (lit.[32] mp 142–144 °C); ^1H NMR (Me_2SO-d_6) δ 3.65 (m, 2 H), 3.92 (m, 1 H), 4.07 (m, 1 H), 4.56 (m, 1 H) 5.62 (d, 1 H), 7.45 (s, 2 H), 7.89 (s, 1 H); UV (H_2O) λ_{max} 264.5 nm (ϵ 1.2 × 10^4).

2-Acetonyl-6-amino-9-(β-D-ribofuranosyl)purine (18). 2-Iodoadenosine (**16**) (0.125 g, 0.318 mmol) was silylated by using the general silylating procedure as described above for the preparation of **5**. Flash chromatography using 1:1 ether/hexane as the eluant gave 0.219 g (0.299 mmol, 94%) of product **17** as a low-melting solid: ^1H NMR ($CDCl_3$) δ −0.14 to −0.12 (m, 18 H), 0.90 (m, 27 H), 3.70–4.09 (m, 3 H), 4.28 (t, 1 H), 4.66 (t, 1 H), 5.87 (d, 1 H), 6.33 (s, 2 H), 8.03 (s, 1 H); UV (EtOH) λ_{max} 222 (ϵ 1.9 × 10^4), 265 nm (1.3 × 10^4).

Silylated 2-iodoadenosine **17** (0.733 g, 0.998 mmol) was added to a flask charged with palladium acetate (0.336 g, 0.150 mmol) and tri-*o*-tolylphosphine (0.091 g, 0.300 mmol). The flask was

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fitted with a septum, and toluene (25 mL, freshly distilled from sodium hydride and purged with nitrogen) was added via a Leur needle. Isopropenyl acetate (2 mL) and 1 equiv (0.290 mL, 1.00 mmol) of tributyltin methoxide were added via a gas-tight syringe. The solution was stirred in an oil bath under N_2 and at 100 °C. After 4 h and 7 h, additional aliquots (1 mL and 0.15 mL, respectively) of isopropenyl acetate and tributyltin methoxide were added. After 10 h, the solution was cooled, taken up in hexane (50 mL), and extracted first with 5% aqueous solution of Na_2EDTA (15 mL) and then with H_2O (10 mL). The aqueous layers were back extracted with hexane (20 mL). The combined organic extracts were dried (Na_2SO_4), concentrated, and purified by flash chromatography with 1:1 ether/hexane as the eluting solvent. The desired acetonylated product was isolated as a low-melting solid (0.354 g, 0.532 mmol, 53%): ^{13}C NMR ($CDCl_3$) δ -5.4, -4.9, -4.7, -4.3, 17.9, 18.1, 18.5, 25.7, 25.8, 26.1, 29.5, 54.6, 62.3, 71.6, 75.6, 84.8, 88.6, 118.6, 139.6, 150.4, 155.5, 159.0, 204.3; ^1H NMR ($CDCl_3$) δ -0.3 to 0.12 (m, 18 H), 0.91 (m, 27 H), 2.19 (s, 3 H), 3.90-4.09 (m, 3 H), 3.85 (s, 2 H), 4.29 (t, 1 H), 4.61 (t, 1 H), 5.64 (br s, 2 H), 5.95 (d, 1 H), 8.13 (s, 1 H); UV (EtOH) λ_{max} 262 (ϵ 1.3 × 10^3), 300 nm (sh, 2.5 × 10^2); mass spectrum, m/z (relative intensity) 666 (M^+, 2.0), 665 (2.3), 650 (4.0), 608 (100), 552 (3.1), 476 (7.2), 462 (12.3), 436 (3.1), 417 (1.7), 348 (21.8), 306 (3.3), 285 (3.5), 261 (9.6), 248 (8.1), 231 (11.0), 220 (10.3), 211 (9.9), 192 (30.1).

The desilylation procedure of the protected 2-acetonyladenosine (0.514 g, 0.773 mmol) was carried out as described for **7**. 2-Acetonyladenosine (**18**) was obtained as a white crystalline hygroscopic compound in 75% yield (0.187 g, 0.580 mmol) after purification by HPLC: mp 113-115 °C; ^{13}C NMR (Me_2SO-d_6) δ 29.1, 57.7, 61.4, 70.5, 73.2, 85.7, 88.4, 117.3, 140.5, 148.3, 154.9, 157.8, 209.8; ^1H NMR (Me_2SO-d_6) δ 2.15 (s, 3 H), 3.61 (br s, 1 H), 3.77 (s, 2 H), 3.97 (m, 1 H), 4.15 (m, 1 H), 4.60 (m, 1 H), 5.23 (s), 5.43 (br s, 3 H), 5.78 (d), 5.84 (d, 1 H), 8.31 (s, 1 H); UV (EtOH) λ_{max} 262.5 (ϵ 12050), 300 nm (4650); FAB HRMS obsd (M^+ + H) 324.1299, calcd for $C_{13}H_{17}N_5O_5$ 324.1308.

Enzymatic Deamination Studies. All assays with adenosine deaminase (Type I from calf intestinal mucosa, Sigma) were followed spectrophotometrically at 25 °C by using a Gilford Response UV-visible spectrometer. Adenosine was used as the standard. Solutions of substrates of appropriate concentrations in 0.05 M phosphate buffer (pH 7.40) were used, and deamination reactions were initiated by addition of the enzyme. For example, the conversion of **18** to **7** could be monitored quantitatively by the change in the major absorption band in the UV spectrum (263 nm → 250 nm). Details of the procedure for the assays have been previously described by us.[29]

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The Reduction of Steroid 2α-Fluoro 4-En-3-ones

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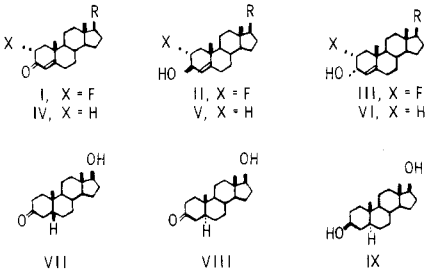
Reduction of testosterone with potassium tri-(*R,S*)-*sec*-butylborohydride gives predominantly the allylic 3β-alcohol, while 2α-fluorotestosterone is converted solely to 2α-fluoro-4-androstene-3α,17β-diol, and 2α-fluoro-4-androstene-3,17-dione to 2α-fluoro-3α-hydroxy-4-androsten-17-one. Reduction of testosterone with (*R,R*)- or (*S,S*)-Rh-DIOP and dihydrosilanes give predominantly allylic alcohols, while with the same catalysts and monohydrosilanes no allylic alcohols are found, the 4-double bond being instead reduced. The chirality of the DIOP reagents contributes only to a minor extent to stereoselectivity of 3-ketone reduction.

Introduction

As a test of the existence of a 2β-hydroxylation step in the enzymic conversion of testosterone to estrogens, we examined the aromatization of 2α-fluorotestosterone (I, R = β-OH) by placental microsomes.[1] Early products of the microsomal transformation appeared to be 2α-fluoro-allylic alcohols which were not found at later time periods; they are probably transformed back to 2α-fluoro 4-en-3-ones and then to the 2-fluoroestrogens. In order to characterize these allylic alcohols, it was necessary to obtain authentic samples of 2α-fluoroandrost-4-ene-3β,17β-diol (II, R = β-OH), its 3α-epimer (III, R = β-OH), and 2α-fluoro-3α-hydroxy-4-androsten-17-one (II, R = O). The previous preparation[2] of the diols II and III (R = β-OH), distinguished only by their melting points, had been effected by reduction of 2α-fluorotestosterone with NADH in presence of a mixture of enzymes obtained as the supernatant on disruption of cells of testosterone-induced *Pseudomonas testosteroni*. The present paper describes easy routes to the 2α-fluoro-4-androstene-3,17β-diols (II, R = β-OH, and III, R = β-OH) and reports a selective reduction of 2α-fluoro-4-androstene-3,17-dione (I, R = O) to 2α-fluoro-3α-hydroxy-4-androsten-17-one (III, R = O), an important early product in the aromatization incubations. Corresponding reduction of testosterone is also reported.



Chiral reducing agents of the dihydrosilane–rhodium–(*R,R*)-DIOP and dihydrosilane–rhodium–(*S,S*)-DIOP type converted testosterone to the 3-hydroxy 4-ene system

(1) McGirr, L. G.; Orr, J. C. *Abstracts of Papers*, 11th International Union of Biochemistry, Toronto, Ontario; National Research Council of Canada: Ottawa, 1979; Abstract 11-1-565.

(2) Ringold, H. J.; Graves, J.; Hayano, M.; Lawrence, H. *Biochem. Biophys. Res. Commun.* **1963**, *13*, 162.

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