Synthesis of Aucubovir II, a New Carbocyclic Nucleoside Analog[‡]

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The synthesis of a new carbocyclic nucleoside starting from aucubin, a natural methylcyclopentanoid monoterpene, has been performed, allowing the preparation of aucubovir II, a carbocyclic nucleoside analog with a highly functionalized cyclopentane ring. The stereocontrol of the coupling reaction was complete, utilizing the procedure described by Vorbrüggen with a purinic base.

Introduction

There has been an increasing interest by synthetic organic chemists in carbocyclic nucleoside analogs since the synthesis of carbovir (Figure 1) which a showed similar potency as AZT in inhibiting viral reverse transcriptase of the Human Immunodeficiency Virus (HIV) by acting as a DNA chain terminator.[1−2] Furthermore carbovir appears to be less toxic and has a longer half life than other nucleoside analogs such as AZT[3] (Figure 1). The higher metabolic stability of carbovir, as well as of other carbocyclic nucleoside analogs, is due to the stability of the bond between the base and the cyclopentanoid moiety. In fact, while the hydrolysis of the *N*-acetylic glycosidic bond in furanose nucleosides is a relatively facile process,[4] the cleavage of the amino bond in carbocyclic nucleosides under the same conditions is more difficult. The observed antiviral efficacy can be explained by considering that the modification on the five-atom ring is well tolerated by the cellular kinase, as the methylene group is a bio-isostere with oxygen.

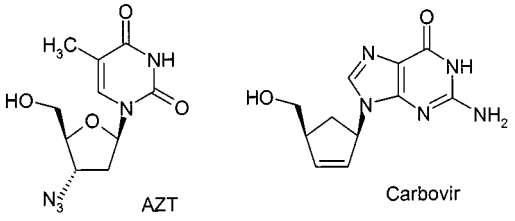

Figure 1. Nucleoside analogs

For all these reasons numerous syntheses of carbovir and other carbocyclic nucleosides have been described.[5−15] The most common approach to carbocyclic nucleosides is a convergent synthesis which couples a purine or pyrimidine base with a cyclopentane or cyclopentene moiety. The latter is generally not functionalized.

Results and Discussion

We decided to prepare a highly functionalized carbovir analog to verify if, in the presence of several functions on the cyclopentane ring, the nucleoside analog could be accepted as a substrate by the cellular kinase, and so could inhibit the transcription process.

We have chosen iridoids, methyl-cyclopentanoid glucosides belonging to the family of monoterpenoids, as the starting compound for the synthesis of the enantiomerically pure cyclopentanoid moiety. The choice of this class of compounds arises because these products are characterized by a cyclopentanoid residue. An easy elaboration of iridoids allows generation of a wide collection of cyclopentanoid derivatives, variously and generally highly substituted, and enantiomerically pure. Starting from aucubin **1**, a methyl-cyclopentanoid glucoside present in large quantities in plants of the *Aucuba* genus, we prepared a cyclopentanoid synthon. After the insertion of uracil we obtained a new carbocyclic nucleoside analog, functionalized at the C-5 carbon of the cyclopentane ring: we named it aucubovir[16] (Figure 2).

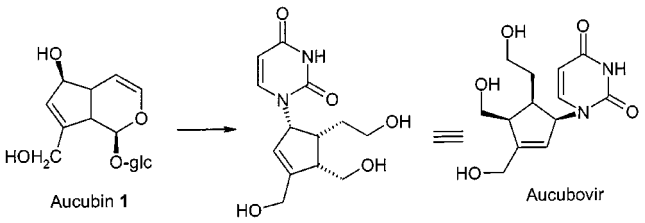

Figure 2. Synthesis of Aucubovir

The coupling between the aucubin synthon and uracil was performed using Vorbrüggen's procedure,[17−18] which allowed us to isolate the expected α-diastereoisomer in a 3:1 ratio with respect to the β-diastereoisomer.[16] The low stereoselectivity of the coupling reaction was attributed to

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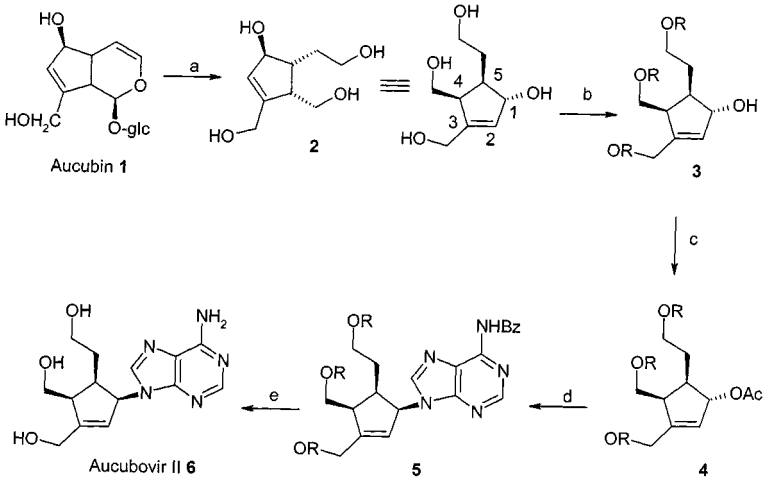

Scheme 1. R = isobutyroyl; B*z* = benzoyl; (a) (i) $Hg(OAc)_2$, $NaBH_4$, or (ii) β-glucosidase, $NaBH_4$, 90%; (b) isobutyric anhydride, pyridine, 0 °C→25 °C, 1.5 h, 90%; (c) Ac_2O, pyridine, 1 h, 99%; (d) (i) N^6-benzoyl-adenine, CH_3CN, TMSCl, HMDS, (ii) $CF_3(CF_2)_3Si(CH_3)_3$, CH_2Cl_2, reflux, 8 h, 20%; (e) DIBAL, CH_2Cl_2, −78 °C, 95%

the presence of a cyclopentanoid moiety instead of the furanose one.

We therefore tried to use a different base, a purinic one, to verify the stereoselectivity of the coupling reaction and to prepare a new carbocyclic nucleoside analog. The synthetic strategy is described in Scheme 1 and is similar, in the first steps, to that described for the preparation of aucubovir.[16]

With adenine, although we did not get very satisfactory yields, as is typical of Vorbrüggen's procedure (coupling reaction, about 20%), we observed complete stereoselectivity with formation, as the sole product, of the α-diastereoisomer, in which the hydroxymethyl chain and adenine are *syn* with respect to the cyclopentane plane.

The new nucleoside **6** is characterized, as in the case of carbovir, by a double bond in the cyclopentane moiety which, besides the required hydroxymethyl function, shows additional hydroxylated functions. Compound **6**, which we named aucubovir II, is (1β,4β,5β)-1[5-hydroxyethyl-3,4-di(hydroxymethyl)cyclopent-2-en-1-yl]adenine (carbocyclic 2′,3′-didehydro-5-′hydroxyethyl-3′,4′-di(hydroxymethyl)-adenine).

Two typical synthetic procedure were employed, starting from aucubin **1**, for the preparation of the first synthon **2**. The first one consists of the opening of the dihydropyran ring of **1** and was performed by enzymatic hydrolysis followed by reduction of the hemiacetalic structure; the second one involves mercuriation/demercuriation. From the synthetic point of view, the enzymatic route is preferable, affording a yield of 92%, instead of 87% for the mercuriation/demercuriation process.

The obtained intermediate **2** contains three primary alcoholic functions, which were regioselectively protected with an isobutyroyl residue to give the triester **3**. The insertion of a purinic base into the intermediate **3** has to be performed with Vorbrüggen's procedure on the allylic acetate **4**; therefore the secondary alcoholic function of **3** was acetylated with acetic anhydride and pyridine to give **4** in quantitative yield. The coupling of **4** was performed with the previously silylated adenine derivative, according to the procedure proposed by Vorbrüggen.[17−18]

Assignment of the configuration at the C-1 center of **5** was achieved by ^{1}H NMR spectroscopy. A positive NOE effect is present between H-5 and H-1, demonstrating the *syn* relationship between the two substituents. Because the configuration of the C-5 center is not affected by the above reactions, its absolute configuration is the same as in the starting aucubin **1**. In this way the absolute configuration of the C-1 center is also defined. The successive hydrolysis of the protective groups in **5** was performed by a classical hydrolysis with DIBAL, obtaining the target carbocyclic nucleoside **6**.

Experimental Section

General: ^{1}H NMR spectra were measured with a Bruker 500 MHz spectrometer and chemical shifts are expressed in ppm relative to TMS. Optical rotation was registered with a Jasco DIP-370 polarimeter. Product purification was obtained by solid-liquid column chromatography on Merck 0.063−0.20 nm silica gel; the elution mixtures were determined case by case. Merck TLC plates coated with Kiesel-Gel 60 F_{254} were employed to monitor the reactions using 2 N H_2SO_4, heating at 120 °C.

5-Hydroxyethyl-3,4-di(hydroxymethyl)cyclopenta-2-en-1-ol (2): Enzymatic hydrolysis of aucubin **1** (100 mg, ca. 0.29 mmol) was performed in a citrate buffer at pH 5.5 (3 mL) at 30 °C for 8 h. The aglycone was then extracted with EtOAc (10 × 10 mL) and, after removal in vacuo of volatile material, dissolved in water (5 mL) and treated with excess $NaBH_4$ for 10 min. at 25 °C. Excess $NaBH_4$ was destroyed by bubbling CO_2 into the reaction mixture until it reached pH = 7 and decolorizing charcoal (500 mg) was added to adsorb the organic material. The suspension was then stratified in a Gooch funnel and the charcoal washed with water until elimination of the salts was complete. Successive elutions with methanol allowed the recovery of pure **2** (50 mg, 92% yield).

Alternatively, mercuriation/demercuriation was performed by dissolving **1** in water (3 mL) together with $Hg(OAc)_2$ (138 mg, ca. 0.44 mmol). After 10 min. an excess of $NaBH_4$ (330 mg, ca. 8.7 mmol) was added over the course of 30 min. at 25 °C. The successive workup was similar to that previously described. In this case recovered crude **2** required a second chromatography on silica gel with $CHCl_3$/MeOH (8:2) to afford pure **2** (46 mg, ca. 87% yield).

2: ^{1}H NMR (D_2O): δ = 1.90 (m, 2 H, 2H-5′), 2.13 (m, 1 H, H-5), 2.90 (m, 1 H, H-4), 3.71 (m, 2 H, 2H-4′), 3.75 (m, 2 H, 2H-5′′), 4.24 (m, 2 H, 2H-3′), 4.60 (m, 1 H, H-1), 5.80 (m, 1 H, H-2). − ^{13}C NMR (D_2O): δ = 147.3 (C-3), 130.8 (C-2), 81.8 (C-1), 62.0 (C-3′), 60.4 (C-4′), 60.2 (C-5′′), 48.7 (C-4), 48.5 (C-5), 31.1 (C-5′). See also ref.[19]

5-Hydroxyethyl-3,4-di(hydroxymethyl)cyclopenta-2-en-1-ol 3′,4′,5′′-Triisobutyroyl Ester (3): The reaction was accomplished by dissolving **2** (50 mg) in pyridine (0.7 mL) followed by the addition of isobutyric anhydride (0.2 mL) at 0 °C. After 2.5 h the starting product was no longer present in the reaction mixture, which was then diluted with EtOAc (100 mL). After washing with 2 N HCl and successively with brine solution until neutrality, the residue obtained after evaporation in vacuo of volatile material, was chromatographed on silica gel with hexane/EtOAc (9:1), affording pure **3** (96 mg, ca. 91% yield), the rest being a tetrabutyroyl derivative.

3: ^{1}H NMR ($CDCl_3$): δ = 1.10, 1.11, 1.13 [d, J = 7.5 Hz, 3 × 6 H, 3 × $(CH_3)_2$CH], 1.89 (m, 2 H, 2H-5′), 2.13 (m, 1 H, H-5), 2.45, 2.50, 2.51 [septuplet, J = 7.5 Hz, 3 × 1 H, 3 × $(CH_3)_2CH$], 2.90 (m, 1 H, X part of an ABX system, H-4), 3.92, 4.34 (AB part of an ABX system, J_{AB} = 11.0, J_{AX} = 2.5, J_{BX} = 4.0 Hz, 2 H, 2H-4′), 4.20 (m, 2 H, 2H-5′′), 4.56 (m, 1 H, H-1), 4.62 (m, 2 H, 2H-3′), 5.76 (m, 1 H, H-2). − ^{13}C NMR ($CDCl_3$): δ = 19.2 [$(CH_3)_2$CHCO], 27.2 [$(CH_3)_2CH$CO], 34.8 (C-5′), 44.9 (C-5), 45.6 (C-4), 61.6 (C-3′), 62.0 (C-5′′), 63.2 (C-4′), 82.1 (C-1), 129.2 (C-2), 143.7 (C-3), 177.1, 177.2, 177.4 [3 × $(CH_3)_2$CHCO]. − $[\alpha]_D^{25}$ = −87 (MeOH, c = 0.1). − $C_{21}H_{34}O_7$ (398.49): C 63.30, H 8.60; found C 63.18, H 8.68.

5-Hydroxyethyl-3,4-di(hydroxymethyl)cyclopenta-2-en-1-ol 3′,4′,5′′-Triisobutyroyl-1-acetyl Ester (4): The reaction was accomplished by dissolving **3** (50 mg) in pyridine (0.7 mL) followed by the addition of acetic anhydride (0.35 mL) at room temperature. After 2 h the starting product was no longer present in the reaction mixture, which was then diluted with EtOAc (100 mL). After a simple workup (washing with 2 N HCl and successively with brine solution until neutrality) the residue, obtained after evaporation in vacuo of volatile material, was chromatographed on silica gel with $CHCl_3$/Et_2O (9:1) to afford pure **4** (66 mg, about 100% yield).

4: ^{1}H NMR ($CDCl_3$): δ = 1.11, 1.12, 1.13 [d, J = 7.5 Hz, 3 × 6 H, 3 × $(CH_3)_2$CH], 1.87 (m, 2 H, 2H-5′), 2.02 (s, 3 H, CH_3COO), 2.4−2.6 [4 H, H-5 superimposed to 3 × $(CH_3)_2CH$], 2.93 (m, 1 H, X part of an ABX system, H-4), 3.96, 4.40 (AB part of an ABX system, J_{AB} = 11.0, J_{AX} = 2.5, J_{BX} = 4.0 Hz, 2 H, 2H-4′), 4.10 (m, 2 H, 2H-5′′), 4.64 (m, 2 H, 2H-3′), 5.49 (m, 1 H, H-1), 5.79 (m, 1 H, H-2). − ^{13}C NMR ($CDCl_3$): δ = 19.1 [$(CH_3)_2$CHCO], 21.4 (CH_3COO), 27.5 [$(CH_3)_2CH$CO], 34.1 (C-5′), 44.4 (C-5), 45.7 (C-4), 61.6, 62.0 (C-3′, C-5′′), 63.2 (C-4′), 83.3 (C-1), 129.5 (C-2), 143.7 (C-3), 171.7 (CH_3COO), 177.2, 177.3, 177.6 [3 × $(CH_3)_2$CHCO]. − $[\alpha]_D^{25}$ = −81 (MeOH, c = 0.1). − $C_{23}H_{36}O_8$ (440.53): C 62.71, H 8.24; found C 62.65, H 8.33.

Coupling Reaction with 4: Under an argon atmosphere, *N*-benzoyladenine (108 mg) was dissolved in anhydrous CH_3CN (10 mL). Subsequently, 1,1,1,3,3,3-hexamethyldisilazane (HMDS) (1.6 mL) and trimethylsilyl chloride (TMSCl) (0.5 mL) were added. The solution was refluxed for 7 h. Volatile materials were evaporated under reduced pressure (50 °C at 0.1 Torr) and to the residue was added **4** (200 mg) dissolved in 1,2-dichloroethane (1.5 mL). After 5 min., $(CH_3)_3SiOSO_2C_4F_9$ (trimethylsilylnonaflate) (12μL) was added whilst stirring and the solution was refluxed for 8 h until complete disappearance of **4**. The solution was neutralized with $NaHCO_3$ (sat. sol.), diluted with CH_2Cl_2 (100 mL) and the resulting residue, after evaporation of volatile materials, was chromatographed on silica gel with hexane/EtOAc (6:4 →1:1), furnishing the expected intermediates (39 mg, 20% yield) which consisted of the α-diastereoisomer.

5: ^{1}H NMR ($CDCl_3$): δ = 1.11, 1.12, 1.14 [d, J = 7.5 Hz, 3 × 6 H, 3 × $(CH_3)_2$CH], 1.84 (m, 2 H, 2H-5′), 2.40 (m, 1 H, H-5), 2.49 [septulet, J = 7.5 Hz, 3 × 1 H, 3 × $(CH_3)_2CH$], 2.89 (m, 1 H, H-4), 3.98, 4.33 (m, 2 H, 2H-4′), 4.22 (m, 2 H, 2H-5′′), 4.42 (m, 2 H, 2H-3′) 4.44 (m, 1 H, H-1), 5.99 (m, 1 H, H-2), 8.66 (s, 1 H, H-2 adenine), 8.14 (s, 1 H, H-8 adenine), 8.06 (m, 2 H, aromatics 2,6), 7.72−7.31 (m, 3 H, aromatics 3, 4, 5). − ^{13}C NMR ($CDCl_3$): δ = 19.0 [$(CH_3)_2$CHCO], 24.7 [$(CH_3)_2CH$CO], 34.2 (C-5′), 42.2 (C-5), 46.0 (C-4), 61.6, 61.8 (C-4′, C-5′′), 63.6 (C-3′), 68.4 (C-1), 131.9 (C-2), 128.7 (CH-aromatics, C-5 adenine), 129.4, 132.9, 134.1 (C-H-aromatics), 143.4 (C-8 adenine), 151.6 (C-4 adenine), 151.8 (C-2 adenine), 153.6 (C-6 adenine). − $[\alpha]_D^{25}$ = −77 (MeOH, c = 0.1). − $C_{33}H_{41}N_5O_7$ (619.71): C 63.96, H 6.67, N 11.30; found C 63.80, H 6.70, N 11.24.

Aucubovir II (6): Compound **5** (30 mg) was dissolved in CH_2Cl_2 (2 mL) and treated with DIBAL in hexane (1.3 mL of a 1 M solution) at −78 °C for 2 h. After bubbling CO_2 through the mixture for a few minutes volatile materials were evaporated in vacuo and the residue was chromatographed on silica gel with $CHCl_3$/MeOH (6:4) to give pure aucubovir II **6** (16 mg, 91%) as a colorless powder.

Aucubovir II **6:** ^{1}H NMR ([D_6]DMSO): δ = 1. 81 (m, 2 H, 2H-5′), 2.38 (m, 1 H, H-5), 3.81 (m, 2 H, H-4′), 3.98, 4.33 (m, 2 H, 2H-4′), 3.76 (m, 2 H, 2H-5′′), 4.04 (m, 2 H, 2H-3′) 4.40 (m, 1 H, H-1), 6.08 (m, 1 H, H-2), 8.18 (s, 1 H, H-2 adenine), 8.08 (s, 1 H, H-8 adenine). ^{13}C NMR ([D_6]DMSO): δ = 32.0 (C-5′), 43.6 (C-5), 44.7 (C-4), 60.1, 60.2 (C-4′, C-5′′), 61.2 (C-3′), 69.4 (C-1), 131.7 (C-2), 128.7 (C-5 adenine), 143.7 (C-8 adenine), 152.6 (C-4 adenine), 152.0 (C-2 adenine), 151.4 (C-6 adenine). − $[\alpha]_D^{25}$ = −69 (MeOH, c = 0.1). − $C_{14}H_{19}N_5O_3$ (305.33): C 55.07, H 6.27, N 22.94; found C 54.96, H 6.33, N 22.80.

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