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A NEW WAY TO NUCLEOSIDES WITH AN OXABICYCLO[3.3.0]OCTANE SCAFFOLD FROM AN ADVANCED INTERMEDIATE IN THE SYNTHESIS OF THE PROSTAGLANDIN ANALOG, CLOPROSTENOL

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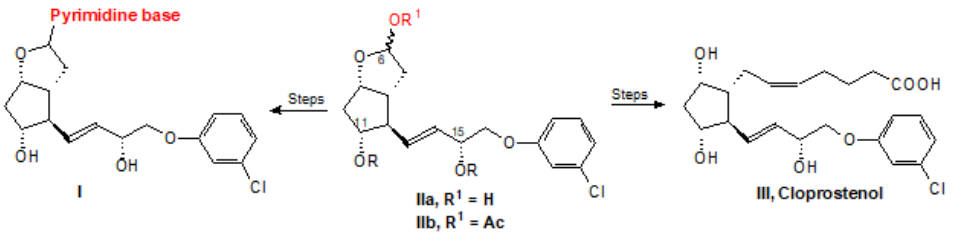
A different synthesis was performed for obtaining nucleoside analogs with an oxabicyclo[3.3.0]octane fragment containing the ω-side chain of PG analog Cloprostenol. The key intermediate **1** was acetylated to the lactol group for activation in Vorbuggen glycosylation, concomitant with the acetylation of 5,11-hydroxyls. The route gave the acetylated nucleoside analogs together with their anomers in near 2 :1 ratio. All acetylated nucleosides and anomers were separated and the pure compounds were hydrolyzed in high yields. Uracil nucleoside was also hydrogeneted to the 13,14-double bond and we obtained a new nucleoside analog **7**.



INTRODUCERE

For a long time the nucleoside, nucleotides and free nucleobase are a recognized class of drugs used in the treatment of viral and cancer deseases.[1-6] This is proved by the fact that in 2013, more than 50% of the FDA-approved antiviral and anticancer drugs belong to this chemical class.[7] We found near 47 active substances presently used in different formulated drugs.[8] The recent approved remdesivir[9] (the only approved drugs) for treatment in COVID-19 is a nucleotide. Molnupiravir[10] (uridine, 5'-(2methyppropanoate)) was the second nucleoside approved for oral administration in the treatment of COVID-19; a few other nucleosides and nucleotides are intensively studied against coronavirus SARS-CoV-2.[11] Due to their secondary effect (toxicity, stability of the nucleosides to the enzyme systems) and resistance of the viruses on prolonged use, many modifications have been done, on the nucleobase moiety, sugar moiety or on both moieties. The replace of enolic oxygen with a methylene group (but also with N and S) increased the stability of the nucleosid(t)es to hydrolysis (made by hydrolases, phosphorylases).[12] The most modifications with beneficial on the activity were performed on sugar moiety. Some of the fragments used to replace the usual sugar were mentioned in our previous papers.[13-17] The oxabicyclo[3.3.0]octane fragment in the compounds **I** generated from an advance intermediate, **II**, used in the synthesis of the prostaglandin (PG) analog, Cloprostenol, **III** (Fig. 1):

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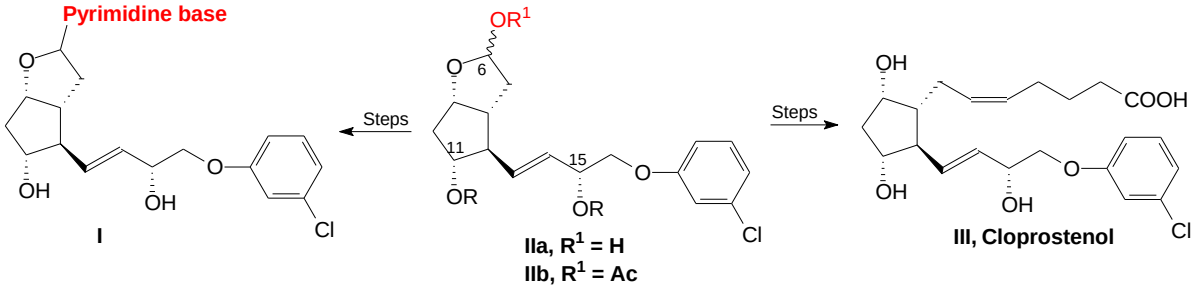


Fig. 1 – Synthesis of the pyrimidine nucleoside analogs **I** and the PG analog, **III**, Cloprostenol, from the common intermediate **II**.

A few of the analogs **I**, with uracil and 5-iodouracil as nucleobase, presented anti-cancer activity against Jurkat T lymphoblasts cancer cells.[13,14] For Cloprostenol, the intermediate **IIa** (R = TMS) is used in a selective *Z*-olefination by Wittig reaction with the requested ylide. Of course, a more direct olefination of the intermediate **II**, with R = H and R^1 = H is also used, but the first method requires about half the quantity of the phosphonium salt used for generation of the ylide. In the Vorbruggen synthesis[18] of the nucleoside analogs **I**, the activated intermediate **IIb** (R = TMS, R^1 = Ac) in this reaction was obtain by acetylation of **IIa** (R = TMS). In this paper we present the synthesis of uracil and 5-iodouracil analogs of type **I**, which use another intermediate, **II**, with all hydroxyl groups protected as acetate (R = R^1 = Ac).

RESULTS AND DISCUSSION

The synthesis of the nucleoside analogs started from the key intermediate, **1**, used in the sequence for obtaining PG analog Cloprostenol (Scheme 1).

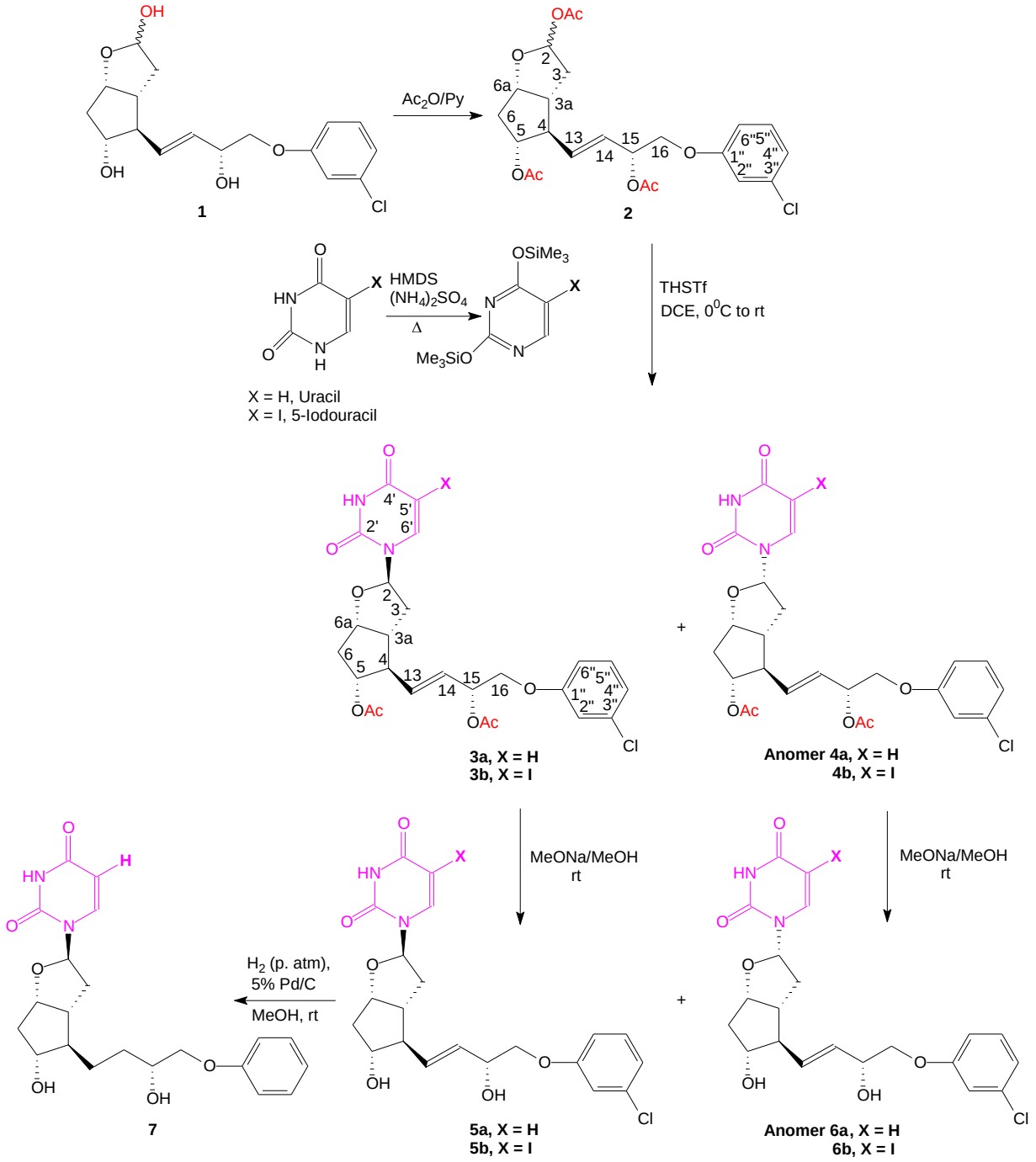
The difference between our procedure[13,14] consists in the acetylation not only of the lactol group (required for activation in the Vorbuggen reaction[18]), but also of the 5,15-hydroxyls from the ω-side chain of the intermediate **1**. The intermediate **IIa** (Fig. 1) was previously obtained in two steps from the corresponding lactone (silylation and DIBAL-reduction of the lactone to the lactol group). The acetylation was performed in quantitative yield and, after co-evaporation with anh. toluene, was directly used in the next step. The N^1 substitution of the O^2,O^4-silylated uracil with lactol-acetate **2**, in the presence of trimethylsilyltriflate (TMSTf) gave a mixture of the compound **3a** with the anomer **4a**, in a ratio of ~ 2 :1 (hardly to be exactly established in TLC, because of the small diference between the R_f of the compounds). The compounds were separated by three LPC (low pressure chromatography) purifications and we obtained the pure **3a** in 20.3% yield, and **4a** in 23.7% yield. The iodo-uracil compounds **3b** and **4b** were obtained in near the same ratio (by TLC ; the same observations as for uracil compounds **3a** and **4a**). The nucleoside **3b** was isolated pure in 46.4% yield, and the anomer **4b** was isolated pure in 10.5% yield. In both cases, a fraction of the mixture of **3** and **4** remained un-purified.

The removal of the diacetate gruops of **3a** and **4a** was performed by transesterification in methanol with MeONa as base and the nucleoside analog **5a** and the anomer nucleoside **6a** were obtained in good yield : 92.7%, respectively 91.1%.

In the case of 5-iodouracil nucleoside **3b** and **4b**, we used a milder base (anh. K_2CO_3) than MeONa and obtained the corresponding nucleoside **5b** and the anomer **6b**, also in good yields : 89.1%, respectively 83%.

In the previous procedure,[13,14] the deprotection of the TMS groups was performed during acid work-up, and the nucleosides **5a** and **5b** were directly obtained. The sequence presented in the Scheme 1 require a supplementary step for the removal of the acetate groups, performed here with the pure nucleosides **3a** and **3b**, and also with the pure anomers **4a** and **4b**, all deprotection by transesterification were performed in very good yields.

The uracil nucleoside **5a** was then hydro-genated (5% Pd/C as catalyst at atmospheric pressure of H_2 and rt) at the 13,14 double bond, to the compound **7**, but also dehydrochlorination tooks plase, as we previously mentioned for hydrogenation of other similar PG intermediates;[19] the product was obtained crystallized. The anti-cancer activity of the compound **7** was intended to be compared with that of the un-hydrogenated nucleoside, but this was not done until now.



Scheme 1 – Synthesis of oxabicyclo nucleozides (**3**-**7**) starting from an intermediate (**1**) used in the building the prostaglandin analog, Cloprostanol.

A clear difference between the ratio of the nucleosides **3**/**4** was not put in evidence, due to the small differences between their R_f.

Previously,[11] we did not succeed to isolate pure the anomers **5a** and **5b**; now, the pure isolated anomers were characterized by HR-NMR and their structure was established.

^{1}H-and ^{13}C-NMR are in agreement with their structural formula and are presented at the Experimental part. Some differences are observed in ^{1}H and ^{13}C- NMR spectra for some chemical groups and in the Table 1 are presented only the slight differences between the signals of the atoms of tetrahydrofurane ring (atoms: 2, 3, 3a and 6a) and nucleobase of the nucleosides **3** and **5** and their anomers **4** and **6**; in the Table are also included the 5-chloro- and 5-bromouracil nucleosides and their anomers, obtained previously[14]. In ^{1}H-NMR, a

slight shielding of about 0.25 ppm is observed for H-6a protons of anomers, and of 0.2-0.3 ppm is observed for protons H-6', compared with that of the corresponding nucleosides **3** and **5**. The protons H-3 and H-3a appeared as multiplets, and this difference could not be clearly observed. The ^{13}C-NMR spectra shows a shielding of the carbon atoms C-6a of all anomers, ranging from 0.49 ppm for 5-bromouracil anomer to 1.2 ppm for 5-iodouracil anomer, compared with that of the corresponding nucleoside analogs; a deshielding of 0.4-0.7 ppm is observed for the carbon atoms C-2 of anomers of 5-chlorouracil, 5-bromouracil, 5-iodouracil and uracil, compared with the that of the corresponding nucleoside analogs.

It is noticeable that the α-configuration of the nucleobase in anomers shields some protons and carbon atoms of the base and of the tetrahydrofurane ring.

Interesting, a coupling between the protons N^3-H and H-5' (J = 1.9 Hz) was observed in ^{1}H-NMR for anomer **4a**, which dissapeared after TFA addition in the NMR-tube; the same was observed for the compounds **5a** and **6a**, where the H-5' has a coupling of J = 2.1 Hz with N^3-H, but the H-N protons appeared as a broad doublet. This coupling was not observed for other nucleosides.

Table 1

^{1}H-and ^{13}C-NMR signals (in ppm) of the atoms of tetrahydrofurane ring (2, 3, 3a and 6a) and nucleobase (2', 4', 5' and 6')

		2	**3**	**3a**	**6a**	**2'**	**4'**	**5'**	**6'**
5ClU-nucleoside	^{1}H	6.09	2.18-2.07	2.44m	4.68				7.97
	^{13}C	**87.05**	**36.11**	**46.42**	**82.87**	**150.18**	**160.18**	**107.86**	**138.58**
Anomer 5ClU-nucleoside	^{1}H	5.92	1.88-1.80	2.50-2.40	4.44				8.22
	^{13}C	**85.76**	**37.28**	**45.67**	**82.01**	**149.46**	**159.60**	**107.51**	**138.00**
5BrU-nucleoside	^{1}H	6.09	2.18-2.07	2.47	4.68				8.01
	^{13}C	**87.05**	**36.11**	**45.89**	**82.49**	**149.71**	**159.66**	**95.65**	**140.49**
Anomer 5BrU-nucleoside	^{1}H	5.92	1.89-1.78		4.44				8.29
	^{13}C	**85.72**	**37.32**	**45.66**	**82.00**	**149.66**	**159.57**	**95.07**	**140.48**
U-nucleoside-diacetate, 3a	^{1}H	6.13	2.58-2.36 2.04	2.58-2.36	4.71			5.69	7.28
	^{13}C	**87.33**	**37.32**	**46.60**	**83.65**	**150.47**	**163.72**	**102.58**	**139.64**
Anomer U-nucleoside-diacetate, 4a	^{1}H	5.90	2.63-2.52 1.98	2.63-2.52	4.50			5.72	7.57
	^{13}C	**87.19**	**37.90**	**46.67**	**82.77**	**150.46**	**162.94**	**102.86**	**139.46**
5IU-nucleoside diacetate, 3b	^{1}H	6.16	2.65-2.47 2.13	2.65-2.47	4.83				7.75
	^{13}C	**86.67**	**37.95**	**46.40**	**83.84**	**149.81**	**159.83**	**68.19**	**144.04**
U-nucleoside, 5a	^{1}H	6.13	2.19-2.03	2.46	4.64			5.59	7.63
	^{13}C	**86.00**	**35.83**	**45.83**	**81.98**	**159.60**	**163.15**	**101.57**	**141.02**
Anomer U-nucleoside, 6a	^{1}H	5.94	2.49-2.35 1.75	2.49-2.35	4.38			5.68	7.81
	^{13}C	**85.42**	**36.58**	**45.33**	**81.19**	**159.59**	**163.04**	**102.00**	**141.52**
5-IU-nucleoside, 5b	^{1}H	5.83	2.36-2.23 2.19-1..50	2.37	4.51				7.95
	^{13}C	**88.29**	**38.71**	**45.87**	**81.30**	**159.71**	**160.91**	**67.90**	**145.45**
Anomer 5-IU-nucleoside, 6b	^{1}H	5.91	2.59-2.40 1.87-1.78	2.59-2.40	4.44				8.30
	^{13}C	**85.74**	**37.42**	**45.76**	**82.09**	**159.73**	**160.63**	**69.94**	**144.85**

EXPERIMENTAL

The progress of the reactions was monitored by TLC on Merck silica gel 60 or 60F$_{254}$ plates eluted with the solvent systems: I, ethyl acetate-hexane-acetic acid, 5:4:0.1; II, dichloromethane-methanol, 9:1, III, ethyl acetate-hexane-acetic acid, 5:1:0.1; IV, chloroform-methanol, 95:5. Spots were visualized in UV using a UV lamp and with 15% H_2SO_4 in MeOH (heating at 110˚C, 10 min.). IR spectra were recorded on FT-IR Bruker Vertex 70 spectrometer by ATR and frequencies were expressed in cm^{-1}, with the following abbreviations: w = weak, m = medium, s = strong, v = very, br = broad. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker 300 MHz and 500 MHz spectrometers, chemical shifts are given in ppm relative to TMS as internal standard. Complementary spectra: 2D-NMR and decoupling were done for the correct assignment of NMR signals. The numbering of the atoms in the compounds is presented in Scheme 1.

1. Synthesis of the triacetate 2

Compound **1** (10.59 g, 31 mmol) was dissolved in pyridine (50 mL) and toluene (30 mL), the solution was cooled on an ice-bath and acetic anhydride (16.23 g, 15 mL, 53 mmol; the ratio of acetic anhydride/OH = 1.71:1) was added dropwise under stirring. The stirring was continued overnight, monitoring the end of the reaction by TLC (I, $R_{f\,1}$ = 0.16, $R_{f\,2}$ = 0.56). The reaction mixture was poured on crushed ice, the product was extracted with toluene (2×100 mL), the extract was washed with sat. soln. $NaHCO_3$ (120 mL), brine (50 mL), dried (Na_2SO_4), filtered, concentrated and co-evaporated with toluene to remove traces of pyridine, resulting in quantitative yield (14.7 g) the tri-acetylated compound **2**, (3aR,4R,5R,6aS)-4-((R,E)-3-acetoxy-4-(3-chlorophenoxy)but-1-en-1-yl)hexahydro-2H-cyclopenta[b] furan-2,5-diyl diacetates, as an oil, IR: 2943w, 1733vs, 1594s, 1581m, 1481m, 1371m, 1285m, 1226vs, 1070s, 1042s, 1017s, 994s, 972s, 858m, 774m, 681m, 606m, ^{1}H-NMR-500 MHz ($CDCl_3$, δ ppm, *J* Hz): 7.20 (t, 1H, H-5”, 8.1), 6.95 (dd, 1H, H-4”, 1.9, 8.1), 6.91 (t, 1H, H-2”, 1.9), 6.79 (dt, 1H, H-6”, 2.1, 8.1), 6.39 (dd, ~0.8H, H-2Major, 1.6, 5.2), 6.36 (d, 0.2H, H-2 minor, 5.2), 5.74 (dd, 1H, H-14, 7.1, 14.8), 5.66 (dd, 1H, H-14 or 13, 6.3, 14.8), 5.56 (m, 1H, H-15, 5.9), 4.93 (q, 1H, H-5, 6.7), 4.67 (dt, 1H, H-6a, 2.6, 6.7), 4.03 (m, 2H, H-16, 5.9), 2.62-2.36 (m, 3H, H-3, H-4, H-6), 2.23 (m, 1H, H-4), 2.18-2.15 (m, 2H, H-3, H-3a), 2.09 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 1.90 (ddd, 1H, H-6, 2.6, 6.3, 14.7), ^{13}C-NMR-125 MHz ($CDCl_3$, δ ppm): 170.52, 170.42, 170.04 (COO), 159.18 (C-1”), 134.94 (Cq, C-3”), 134.15 (C-13 or 14), 130.34 (C-5”), 126.50 (C-14 or 13), 121.48 (C-4”), 115.19 (CH, C-2”), 113.16 (C-6”), 100.04 (C-2), 82.95 (C-6a), 79.09 (C-5), 71.82 (C-15), 69.24 (C-16), 53.39 (C-4), 45.01 (C-3a), 38.20 (C-6), 37.02 (C-3), 21.30, 21.15, 21.06 (CH_3COO).

2. Synthesis of 11,15 acetylated nucleosides, 3a and anomer 4a

a) Uracil (1.12 g, 10 mmol) was silylated with hexamethyldisilazane (HMDS) (22 mL) in the presence of several crystals of $(NH_4)_2SO_4$, as in our previous work [11] to O^2,O^4-bis-silylated uracil. The concentrated product was taken in dichloroethane (DCE) (20 mL) and used in the next reaction.

b) Tris-acetylated compound **2** (2.35 g, 5 mmol) was dissolved in DCE (60 mL), the previous solution of the bis-silylated uracil was added, the mixture was cooled under inert anhidrous atmosphere (Ar) on an ice bath, and trimethylsilyl triflate (TMSTf) (2.7 mL) was added slowly (1.5 hrs.) under stirring, monitoring the end of the reaction by TLC (II, 9:1, $R_{f\,2}$ = 0.68, $R_{f\,3a+4a}$ = 0.56). The reaction mixture was poured onto 10% $KHCO_3$ (40 mL) and ice under vigorous stirring, stirred more 10 min., the phases were separated, organic phase was washed with water (2×100 mL), brine (20 mL) (the aqueous phases were extracted with 2×100 mL dichloromethane), dried (Na_2SO_4), filtered and concentrated to dryness, resulting 2.83 g of crude product, as a mixture of **3a** with anomer **3b** (TLC III, 5:1:0;1, $R_{f\,3a}$ = 0.52, $R_{f\,4a}$ = 0.59; I, $R_{f\,3a}$ = 0.11, $R_{f\,4a}$ = 0.15). The crude product was purified by LPC (eluent: dichloromethane-methanol, 10:0.5, three purifications), resulting a pure fraction (0.9 g, 20.3%) of anomer **4a**, *rac*-(R,E)-4-((2S,3aR,4R,5R,6aS)-5-acetoxy-2-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)hexahydro-2H-cyclopenta[b] furan- 4-yl)-1-(3-chlorophenoxy)but-3-en-2-yl acetate, as an oil, ^{1}H-RMN-300 MHz ($CDCl_3$, δ ppm, *J* Hz): 9.50 (s, 1H, NH, deuterable), 7.57 **(**d, 1H, H-6’, 8.1), 7.13 (t, 1H, H-5”, 8.1), 6.88 (ddd, 1H, H-4”, 0.9, 2.0, 8.1), 6.82 (t, 1H, H-2”, 2.0), 6.71 (ddd, 1H, H-6”, 0.9, 2.4, 8.1), 5.90 (dd, 1H, H-2, 6.0, 7.5), 5.72 (dd, 1H, H-5’, 1.3, 8.1), 5.60 (dd, 1H, H-13, 6.3, 14.7), 5.54 (dd, 1H, H-14, 6.2, 14.7), 5.48 (m, 1H, H-15), 4.97 (dt, 1H, H-5, 4.8, 6.6), 4.50 (dt, 1H, H-6a, 2.0, 6.5), 3.98 (dd, 1H, H-16, 5.7, 10.1), 3.93 (dd, 1H, H-16, 4.6, 10.1), 2.63-2.52 (m, 3H, H-3, H-3a, H-4), 2.36 (dt, 1H, H-6, 6.7, 15.2), 2.02, 1.99 (2s, 6H, CH_3COO), 1.98 (m, 1H, H-3), 1.68 (m, 1H, H-6), ^{13}C-RMN-75 MHz ($CDCl_3$, δ ppm): 170.06 (COO), 162.94 (C-4’), 159.83 (C-1”), 150.46 (C-2’), 139.46 (C-6’), 135.01 (C-3”), 134.19 (C-13 or 14), 130.65 (C-5”), 127.40 (C-14 or C-13), 122.07 (C-4”), 116.04 (C-2”), 113.85 (C-6”), 102.86 (C-5’), 87.19 (C-2), 82.77 (C-6a), 80.19 (C-5), 72.26 (C-15), 70.00 (C-16), 54.51 (C-4), 46.67 (C-3a), 38.43 (C-6), 37.90 (C-3), 21.22 (CH_3COO) and 1.05 g (23.7 **%**) of **3a**, acetic acid 4-[3-acetoxy-4-(3-chloro-phenoxy)-but-1-enyl]-2-(2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-hexahydro-cyclopenta[b]furan-5-yl ester, as an oil, FT-IR: 3179w, 2941w, 1731s, 1685vs, 1594s, 1581m, 1477m, 1461m, 1372s, 1285m, 1227vs, 1172m, 1067s, 1040s, 969m, 858m, 811m, 771s, 681m, ^{1}H-NMR-300MHz ($CDCl_3$, δ ppm, *J* Hz): 9.52 (s, 1H, NH, deuterable), 7.28 (d, 1H, H-6’, 8.2), 7.13 (t, 1H, H-5”, 8.1), 6.93 (ddd, 1H, H-4”, 1.0, 2.0, 8.1), 6.89 (t, 1H, H-2”, 2.0), 6.77 (ddd, 1H, H-6”, 1.0, 2.0, 8.1), 6.13 (t, 1H, H-2, 6.3), 5.69 (d, 1H, H-5’, 8.2), 5.65 (d, 1H, H-13 or 14, 6.3), 5.63 (d, 1H, H-14 or 13, 5.7), 5.52 (q, 1H, H-15, 5.7), 4.84 (q, 1H, H-5, 6.6), 4.71 (dt, 1H, H-6a, 2.5, 6.3), 3.99 (dd, 1H, H-16, 5.7, 10.2), 3.94 (dd, 1H, H-16, 4.6, 10.2), 2.58-2.36 (m, 4H, H-3, H-4, H-3a, H-6), 2.04 (m, 1H, H-3), 2.03 (s, 3H, CH_3), 1.99 (s, 3H, CH_3), 1.82 (ddd, 1H, H-6, 2.4, 6.3, 14.8), ^{13}C-NMR-75 MHz ($CDCl_3$, δ ppm): 170.66, 170.18 (COO), 163.72 (C-4’), 159.19 (Cq, C-1”), 150.47 (C-2’), 139.64 (CH, C-6’), 135.01 (Cq, C-3”), 133.76 (C-13 or 14), 130.42 (C-5”), 127.16 (C-14 or 13), 121.56 (C-4”), 115.26 (C-2”), 113.21 (C-6”), 102.58 (C-5’), 87.33 (C-2), 83.65 (C-6a), 78.24 (C-5), 71.80 (C-15), 69.21 (C-16), 52.83 (C-4), 46.60 (C-3a), 38.36 (C-6), 37.72 (C-3), 21.22, 21.13 ($2CH_3$).

The fractions containing the mixture of the compounds **3a** and **4a** were not separated.

3. Synthesis of 11,15 acetylated nucleosides, 3b and 4b

a) 5-Iodouracil (1.79 g, 7.5 mmol) was silylated with hexamethyldisilazane (HMDS) (15 mL) in the presence of several crystals of $(NH_4)_2SO_4$, as in our previous work[13] to O^2,O^4-bis-silylated iodouracil. The concentrated product was taken in dichloroethane (DCE) (20 mL) and used in the next reaction.

b) Tris-acetylated compound **2** (2.35 g, 5 mmol) was dissolved in DCE (60 mL), the previous solution of the bis-silylated iodouracil was added, the mixture was cooled under inert anh. atmosphere (Ar) on an ice bath, and trimethylsilyl triflate (TMSTf) (2.7 mL) was added slowly (1.5 hrs.) under stirring, monitoring the end of the reaction by TLC (II, 9:1, $R_{f\,2}$ = 0.68, $R_{f\,3b+4b}$ = 0.60). The reaction mixture was poured onto 10% $KHCO_3$ (40 mL) and ice under vigorous stirring, stirred more 10 min., the phases separated, organic phase was washed with water (2×100 mL), brine (20 mL) (the aqueous phases were extracted with 2×100 mL dichloromethane), dried (Na_2SO_4), filtered and concentrated to dryness, resulting 3.53 g of crude product, as a near 2:1 mixture of **3b** and anomer **4b** (TLC I, $R_{f\,3b}$ = 0.35, $R_{f\,4b}$ = 0.40). The crude product was purified by LPC (eluent: dichloromethane-methanol, 10:0.5, three purifications), resulting a pure fraction (0.53 g, 10.5%)) as an oil of the anomer **4b**, (R,E)-4-((2S,3aR,4R,5R,6aS)-5-acetoxy-2-(5-iodo-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)hexahydro-2H-cyclopenta[b]furan-4-yl)-1-(3-chlorophenoxy)but-3-en-2-yl acetate, used directly in the next step, and a pure fraction (1.33 g) (and another slightly impure fraction of 1.02 g; total yield 46.4%) as an oil, (for optically active compound, $[\alpha]_D$ = -15.3˚ (1% in MeOH), of

3b, Acetic acid 4-[3-acetoxy-4-(3-chloro-phenoxy)-but-1-enyl]-2-(5-iodo-2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-hexahydro-cyclopenta[b]furan-5-yl ester, ^{1}H-NMR-300 MHz ($CDCl_3$, δ ppm, *J*Hz): 9.02 (s, 1H, NH), 7.75 (s, 1H, H-6'), 7.20 (t, 1H, H-5", 8.1), 6.95 (dd, 1H, H-4", 1.9, 7.9), 6.90 (t, 1H, H-2", 2.2), 6.79 (dd, 1H, H-6", 2.3, 8.4), 6.16 (t, 1H, H-2, 6.3), 5.74 (dd, 1H, H-13 or 14, 5.9, 15.4), 5.67 (dd, 1H, H-14 or 13, 4.5, 15.4), 5.58 (dt, 1H, H-15, 4.8, 5.6), 4.92 (dt, 1H, H-5, 6.7, 6.9), 4.83 (dt, 1H, H-6a, 2.3, 6.3), 4.07 (dd, 1H, H-16, 5.8, 10.2), 4.02 (dd, 1H, H-16, 4.3, 10.2), 2.65-2.47 (m, 4H, H-3, H-3a, H-4, H-6), 2.13 (m, 1H, H-3), 2.10 (s, 3H, CH_3), 2.06 (s, 3H, CH_3), 1.90 (ddd, 1H, H-6, 2.7, 6.3, 14.8), ^{13}C-NMR-75 MHz ($CDCl_3$, δ ppm): 170.42, 169.99 (COO), 159.83 (C-4'), 159.04 (Cq, C-1"), 149.81 (CO, C-2'), 144.04 (C-6'), 134.88 (Cq, C-3"), 133.48 (C-13 or 14), 130.28 (C-5"), 127.11 (C-14 or 13), 121.44 (C-4"), 115.12 (C-2"), 113.07 (C-6"), 87.67 (C-2), 83.84 (C-6a), 78.10 (C-5), 71.72, 71.62 (C-15), 69.06 (C-16), 68.19 (Cq, C-I), 52.73 (C-4), 46.40 (C-3a), 38.23 (C-6), 37.95 (C-3), 21.09, 21.99 ($2CH_3$).

4. Hydrolysis of Uracil diacetat nucleoside 3a to 5a

The pure uracil-diacetate nucleoside **3a** (0.8 g, 1.54 mmol) was dissolved in anh. methanol (30 mL), a solution of 0.156 M MeONa in MeOH (30 mL) was added and stirred at rt, monitoring the end of the reaction by TLC (II, $R_{f\ 3a}$ = 0.66, $R_{f\ 5a}$ = 0.36). Sodium methoxide was neutralized with 2N HCl, methanol was distilled under reduced pressure, the residue was purified by LPC (eluent: dichloromethane-methanol, 95:5), resulting 0.62 g (92.7 %) of pure nucleoside analog **5a**, mp 96.2-98.7˚C (mp 126.7-131.6˚C for optically active compound), with the same characteristics mentioned in our previous paper[13], R: 3372 large band, 3025w, 2936w, 2873w, 2820w, 1773w, 1667vs, 1585m, 1466m, 1426m, 1388m, 1280s, 1229s, 1027s, 814m, ^{1}H-RMN-500MHz (DMSO-d_6; δ ppm; *J* Hz): 11.28 (brd, 1H, NH, deuterable. 0.9), 7.63 (d, 1H, H-6', 8.0), 7.29 (t, 1H, H-5", 8.2), 7.00 (t, 1H, H-2", 2.0), 6.97 (dd, 1H, H-4", 8.0, 2.2), 6.91 (dd, 1H, H-6", 8.2, 2.0), 6.13 (t, 1H, H-2, 6.4), 5.68 (dd, 1H, H-13, 7.2, 15.6), 5.63 (dd, 1H, H-14, 6.3, 15.6), 5.59 (dd, 1H, H-5', 2.1, 8.1), 5.17 (d, 1H, OH-15, 4.9), 4.85 (d, 1H OH-5, 5.7), 4.64 (dt, 1H, H-6a, 3.6, 6.8), 4.31 (tt, 1H, H-15, 4.6, 6.9), 3.92 (dd, 1H, H-16 3.5, 9.9), 3.86 (dd, 1H, H-16, 7.1, 9.9); 3.72 (qv, 1H, H-5, 8.1), 2.46 (m_q, 1H, H-3a, 8.3), 2.28 (dt, 1H, syst. AB, H-6A, 7.1, 13.7), 2.19-2.03 (m, 3H, 2H-3, H-4), 1.54 (ddd, 1H, syst. AB, H-6B, 3.5, 8.3, 13.7), ^{13}C-RMN-125 MHz (DMSO-d_6; δ ppm): 163.15 (C=O, C-4'), 159.60 (C=O, C-2'), 150.41 (C-1"), *139.66 (C-3"),* 141.02 (C-6'), 133.66 (C-3"), 131.89 (C-14), 130.94 (C-13 or C-5"), 130.79 (C-5" or 13), 120.44 (C-4"), 114.63 (C-2"), 113.71 (C-6"), 101.57 (C-5'), 86.00 (C-2), 81.98(C-6a), 76.00 (C-5), 72.37 (C-16), 69.12 (C-15), 54.94 (C-4), 45.83 (C-3a), 41.12 (C-6), 35.82 (C-3).

The natural compound **5a** was obtained as an oil, which crystallized in time, mp 126.7-131.6˚C.

5. Hydrolysis of anomer nucleoside diacetate 4a to 6a

The pure anomer uracil-diacetate nucleoside **4a** (260 mg, 0.5 mmol) was dissolved in anh. methanol (10 mL), a solution of 0.156 M MeONa in MeOH (10 mL) was added and stirred at rt, monitoring the end of the reaction by TLC (II, $R_{f\ 3a}$ = 0.70, $R_{f\ 5a}$ = 0.39). After work-up and LPC purification as in example 4, 198 mg (91.1%) of pure nucleoside anomer analog **6a** *rac*-1-((2R,3aS,4S,5S,6aR)-4-((S,E)-4-(3-chlorophenoxy)-3-hydroxybut-1-en-1-yl)-5-hydroxyhexahydro-2H-cyclopenta[b]furan-2-yl)pyrimidine-2,4(1H,3H)-dione, resulted, as an foam, IR: 3423m, 3200m, 1668vs, 1593s, 1459s, 1420m, 1278s, 1243s, 1076s, 1025s, 784m, ^{1}H-RMN (DMSO-d_6, δ ppm, *J* Hz): ^{1}H-RMN-500MHz (DMSO-d_6; δ ppm; *J* Hz):11.31 (s, 1H, NH, deuterable), 7.81 (d, 1H, H-6', 8.1), 7.28 (t, 1H, H-5", 8.1), 7.00 (s, 1H, H-2"), 6.97 (d, 1H, H-4", 8.1), 6.91 (d, 1H, H-6", 8.1), 5.94 (t, 1H, H-2, 6.9), 5.68 (dd, 1H, H-5', 2.1, 8.1), 5.65 (dd, 1H, H-13, 7.2, 15.6), 5.55 (dd, 1H, H-14, 6.3, 15.6), 5.15 (d, 1H, OH-15, 4.5), 5.00 (d, 1H OH-5, 4.2), 4.38 (brt, 1H, H-6a), 4.28 (brt, 1H, H-15), 3.90 (dd, 1H, H-16 4.4, 9.7), 3.85 (dd, 1H, H-16, 3.8, 9.7), 3.84 (m, 1H, H-5), 2.49-2.35 (m, 3H, H-3, H-3a, H-4), 2.18 (dt, 1H, H-6, 6.3, 13.9), 1.75 (m, 2H, H-3, H-6), ^{13}C-RMN-125 MHz (DMSO-d_6; δ ppm): 163.04 (C=O, C-4'), 159.59 (C=O, C-2'), 150.33 (C-1"), 141.52 (C-6'), 133.66 (C-3"), 131.91 (C-14), 130.78 (C-13 or C-5"), 130.33 (C-5" or 13), 120.44 (C-4"), 114.62 (C-2"), 113.72 (C-6"), 102.00 (C-5'), 85.42 (C-2), 81.19 (C-6a), 77.24 (C-5), 72.31 (C-16), 69.10 (C-15), 55.63 (C-4), 45.33 (C-3a), 39.92 (C-6), 36.58 (C-3). The compound was not isolated pure previously.[13]

6. Hydrolysis of IUracil diacetat nucleoside 3b to 5b

The pure iodouracil-diacetate nucleoside **3b** (1.1 g, 1.7 mmol) was dissolved in anh. methanol (70 mL), K_2CO_3 (1 g, 7.2 mmol) was added and stirred at rt overnight, monitoring the end of the reaction by TLC (II, $R_{f\ 3b}$ = 0.60, $R_{f\ 5b}$ = 0.45). Methanol was distilled under reduced pressure, water (40 mL) and ethyl acetate (80 mL) were added, phases were separated, organic phase was washed with water (2× 50 mL), brine (50 mL) (the aqueous phases were extracted with 2×50 mL), dried (Na_2SO_4), concentrated and purified by LPC (95.5:0.5, then 95:5), resulting 852 mg (89.1%) of pure **5b**, with the same characteristics with that mentioned in our previous paper,[13] ^{1}H-RMN-300MHz (DMSO-d_6; δ ppm; *J* Hz): 11.54 (s, 1H, NH), 7.95 (s, 1H, H-6'), 7.27 (dt, 1H, H-5", 2.1, 8.0), 6.97, 6.82 (m, 3H, H-2", H-3", H-4"), 5.83 (m, 1H, H-2), 5.61-5.51 (m, 2H, H-13, H-14), 5.25 (m, 1H, OH-15, deuterable), 4.91 (m, 1H, OH-5, deuterable), 4.51 m, 1H, H-6a), 4.25 (m, 1H, H-15), 3.85-3.36 (m, 3H, H-5, 2H-16), 2.37 (m, 1H, H-3a), 2.36-2.23 (m, 2H, H-3, H-4), 2.19-1.50 (m, 3H, H-3, 2H-6), ^{13}C-RMN-75 MHz (DMSO-d_6, δ ppm): 160.91 (C-4'), 159.71 (C-2'), 150.54 (C-1"), 145.45, (C-6'), 133.85 (C-3"), 132.97 (C-13 or C-14), 131.08 (C-5"), 130.73 (C-14 or C-13), 120.73 (C-4"), 114.86 (C-2"), 113.87 (C-6"), 88.29 (C-2), 81.30 (C-6a), 77.56 (C-5), 72.59 (C-16), 69.51 (C-15), 67.90 (C-5'), 54.76 (C-4), 45.87 (C-3a), 41.48 (C-6), 38.71 (C-3).

7. Hydrolysis of anomer nucleoside diacetate 4b to 6b

The pure anomer iodouracil-diacetate nucleoside **4b** (193.6 mg, 0.3 mmol) was dissolved in anh. methanol (20 mL), K_2CO_3 (166 mg, 1.2 mmol) was added and stirred at rt overnight, monitoring the end of the reaction by TLC (II, $R_{f\ 3b}$ = 0.67, $R_{f\ 5b}$ = 0.49). After work-up and LPC purification as in example 6, 140 mg (83%) of pure nucleoside anomer analog **6b**, 1-((2S,3aR,4R,5R,6aS)-4-((R,E)-4-(3-chlorophenoxy)-3-hydroxybut-1-en-1-yl)-5-hydroxyhexahydro-2H-cyclopenta[b]furan-2-yl)-5-iodopyrimidine-2,4(1H,3H)-dione, as an oil, ^{1}H-RMN-300MHz (DMSO-d_6; δ ppm; *J* Hz): 11.70 (s, 1H, NH, deuterable), 8.30 (s, 1H, H-6'), 7.30 (t, 1H, H-5", 8.1), 7.00 (m, 1H, H-2"), 6.98 (d, 1H, H-4", 8.1), 6.91 (dd, 1H, H-6", 1.6, 8.1), 5.91 (dd, 1H, H-2, 1.66, 6.2), 5.73 (s, 1H, H-6'), 5.66 (dd, 1H, H-13, 7.2, 15.6), 5.54 (dd, 1H, H-14, 5.8, 15.6), 5.23 (d, 1H, OH-15, 4.5), 5.20 (d, 1H OH-5, 4.0), 4.44 (dt, 1H, H-6a, 4.2, 6.5), 4.36 (m, 1H, H-15), 3.91 (dd, 1H, H-16 4.5, 10.0), 3.85 (dd, 1H, H-16, 6.9, 10.0), 3.90 (m, 1H, H-5), 2.59-2.40 (m, 3H, H-3, H-3a, H-4), 2.82 (dt, 1H, H-6, 6.1, 13.9), 1.87-1.78 (m, 2H, H-3, H-6), ^{13}C-RMN-75 MHz (DMSO-d_6; δ ppm): 160.63 (C=O, C-4'), 159.73 (C=O, C-2'), 150.21 (C-1"), 144.85 (C-6'), 133.66 (C-3"), 132.38 (C-14), 131.03 (C-5"), 130.22 (13), 120.66 (C-4"), 114.77 (C-2"),

113.87 (C-6"), 85.74 (C-2), 82.09 (C-6a), 77.53 (C-5), 72.38 (C-16), 69.84 (C-5'), 69.31 (C-15), 55.92 (C-4), 45.76 (C-3a), 40.33 (C-6), 37.42 (C-3).

8. The hydrogenation of the nucleoside 5a to 7

The nucleoside **5a** (1.2 g, 2.2 mmol) was dissolved in ethyl acetate (25 mL) and methanol (25 mL), 5% Pd/C as the catalyst, (240 mg) and $NaHCO_3$ (100 mg) were added and hydrohenated (atm prresure, rt), monitoring the end of reaction by consumption of the requested H_2 by TLC (IV, eluted three times, $R_{f\ 5a} = 0.25$, $R_{f\ 7} = 0.23$). The catalyst was filtered off, washed with ethyl acetate, the filtrate was concentrated to dryness under reduced pressure, the residue was taken in ethyl acetate (30 mL) and water (30 mL), the phases were separated, organic phase was washed with brine (10 mL) (aqueous phases were extracted with 3×30 mL ethyl acetate), dried (Na_2SO_4) and concentrated to dryness. The crude product (1.06 g) was crystallized from acetone, resulting 600 mg of pure nucleoside **7**, 1-((2R,3aR,4R,5R,6aS)-4-((R)-4-(phenoxy)-3-hydroxybutyl)-5-hydroxyhexahydro-2H-cyclopenta[b]furan-2-yl)pyrimidine-2,4(1H,3H)-dione, mp 180.8-183.2˚C, IR: 3494m, 3359m, 3030m, 1661vs, 1607s, 1470m, 1427s, 1380m, 1265s, 1233s, 1088s, 1019s, 754s, ^{1}H-RMN-300 MHz (DMSO-d_6, δ ppm, *J* Hz): 11.27 (d, 1H, NH, 1.9, deuterable), 7.59 (d, 1H, H-6', 8.1), 7.27 (t, 2H, H-*m*,7.4), 7.00-6.88 (m, 3H, 2H-*o*, H-*p*), 6.13 (t, 1H, H-2, 6.0), 5.59 (dd, 1H, H-5' 1.9, 8.1), 4.89 (d, 1H, HO-15, 4.7, deuterable), 4.80 (d, 1H, HO-5, 5.3, deuterable), 4.63 (td, 1H, H-6a, 7.0, 4.3), 3.83 (m, 2H, H-16), 3.77 (m, 1H, H-15), 3.61 (m, 1H, H-5), 2.32 (m, 1H, H-3a), 2.23-2.05 (m, 2H, H-3, H-6), 1.66-1.40 (m, 6H, H-4, H-6, 2H-13, 2H-14), 1.23 (m, 1H, H-3), ^{13}C-RMN-75 MHz (DMSO-d_6, δ ppm): 162.76 (C-4'), 158.64 (C-2'), 150.09 (C-1"), 140.35 (C-6'), 129.03 (C-*m*), 120.18 (C-*p*); 114.55 (C-*o*), 101.20 (C-5'), 86.41 (C-2), 82.74 (C-6a), 76.32 (C-5), 72.21 (C-16), 68.62 (C-15), 52.41 (C-4), 46.04 (C-3a), 41.12 (C-6), 37.59 (C-3), 31.40 (C-14,; 28.21 (C-13).

CONCLUSIONS

A synthesis of oxabicyclo[3.3.0]octane nucleosides was performed, starting from the key PG intermediate **1**, with the ω-side chain of PG analog, Cloprostenol. In this variant, the acetylation of the lactol group for Vorbugger glycosylation, gave a different protection of 5,11-hydroxyl group of compound **1** with an ester group (acetyl), from the silylether (TMS) groups, used previously. The silylation at an earlier stage of 5,15-hydroxyls was replaced by acetylation, but this required a supplementary (indeed, high yield) step for the deprotection of the acetyl groups ; previously, the silyl groups were easily removed during acid work-up. In the LPC purification, the nucleosides **3a**, **3b** and the anomers **4a**, **4b** were dificulty obtained pure (by three LPC purifications) and characterized. The hydrolysis of the acetyl groups were performed with the above pure compounds in high yields. The α-configuration of the nucleobase in anomers shields some protons and carbon atoms of the base and of the tetrahydrofurane ring and are clearly observed in ^{1}H- and ^{13}C-NMR spectra. The nucleoside **5a** was also hydrogenated to the new nucleoside analog **7**.

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