Novel O^2-Nucleoside Analogues with an Optically Active Bicyclo[2.2.1]Heptane Sugar Moiety, Obtained by Mitsunobu Reaction

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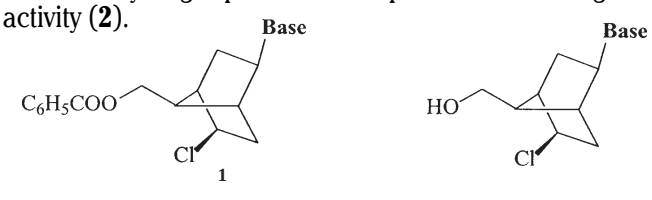
*Novel O^2-pyrimidine nucleosides with a functionalized bicyclo[2.2.1]heptan sugar moiety were synthesized starting from a pure optically active alcohol (**4**). This alcohol was coupled by Mitsunobu reaction to pyrimidine bases: N^4-benzoylcytosine, 5-fluorouracil and N^3-benzoyl-thymine, then the ester protected nucleosides were debenzoylated to the coresponding carbocyclic nucleosides, which looks to be O^2-linked compounds. The compounds were characterized by UV, IR, MS, ^{1}H-NMR and ^{13}C-NMR spectra. The preliminary in vitro preclinical results show that ent-27-5-FU (**13**) and ent-27-T (**19**) present cytotoxic or cytototxic activity in Jurkat lymphoblasts and/or U937 monocytic blasts.*

Keywords: Mitsunobu synthesis, bicyclo[2.2.1]heptane nucleoside, carbocyclic nucleoside, anti-neoplastic activity

We have previously [1] synthesized the cytosine derivative *ent*-27-cytosine-dibenzoate, (**1a**), that was shown to exert moderate cytotoxic effects on Jurkat and U937 tumor cells, in association with a decrease of uridine and thymidine uptake. Meanwhile, *ent*-27-cytosine-dibenzoate is only cytostatic for human normal mononuclear cells (PBMC), but inhibits both uridine and thymidine uptake by proliferating PBMC. In these experiment we believed that the benzoate protective groups could be enzymatically hydrolized to the free nucleoside *ent*-27-cytosine (**2a**), the dibenzoate is acting as a prodrug. Another nucleoside analogue with 5-fluoro-uracil, (**1b**), was also obtained in the same way. Although *ent*-27-cytosine-dibenzoate proved to have anti-proliferative activity, our study was switched over towards the unprotected nucleoside analogues which, at least theoretically might prove a more pronounced biological activity (**2**).



1a, Base = N^4-benzoylcytosine
1b, Base = 5-Fluoro-uracil

Experimental part

Melting points were determined in open capillary on a OptiMelt melting point apparatus and the values are uncorrected. Progress of the reaction was monitored by TLC on Merck silica gel 60 or $60F_{254}$ plates (Merck) eluted with the solvent system presented for each compound. Spots were developed in UV light or with sulfuric acid (15% in etanol) or phosphomolybdic acid (5% in etanol). IR spectra were recorded on a FT-IR- 100 Perkin Elmer spectrometer, in solid phase by ATR and frequences are expressed in cm^{-1}, with the following abbreviations were used: w weak, m medium, s strong, v very, br broad. MS spectra were recorded on 1200 L/MS/MS triple-quadrupole Varian with ESI interface, ^{1}H-NMR and ^{13}C-NMR spectra are recorded on Varian Gemini 300 BB and INOVA-400 spectrometers (300 and 400 MHz for ^{1}H and 75 and/or 100 MHz for ^{13}C), chemical shifts are given in ppm relative to TMS as internal standard. Complementary spectra: COSY, HETCOR and trifluoroacetic acid added, were done for correct assignement of NMR signals. The numbering of the atoms in compounds is presented in Schemes. THF was anhydrized on sodium wire, the other reagents were of reagent grade.

*Synthesis of the intermediates (**4**) and (**5**), Benzoic acid, 2-chloro-5-hydroxy-bicyclo[2.2.1]hept-7-ylmethyl ester.*

Sodium borohydride reduction

83.55g (0.3 moles) intermediate (**3**) were reduced with $NaBH_4$ as in [1], giving 85.5 g oily mixture of alcohol isomers (**4**) and (**5**), which was purified by multiple pressure chromatography on silica gel column (ethyl acetate-hexane, 1:1). Resulted:

-71 g (84.3%) pure less polar alcohol (**4**), as colourless oil, with $[\alpha]_D^{20}$= +2.12°(c=1% in THF), MS spectra for $C_{15}H_{17}ClO_3$, M=280.75: (M+1): 281/283 for two Cl-isotopes, and fragments of the molecular peak: 263/265 $(M-H_2O)^+$, 159 and 161 (Hydroxybicyclo)$^+$, 141 and 143 (bicycloalchene)$^+$, 123(Hydroxybicyclo-HCl)$^+$, 105$(C_6H_5CO)^+$.

IR: 3429br, 2963w, 1715.5s, 1698s, 1602w, 1585w, 1451m, 1407w, 1358w 1315m, 1271vs, 1206w, 1176m, 1140m, 1112s, 1083s, 1070s, 1026m, 1002s, 942m, 904m, 864w, 806w, 709vs, 685s.

^{1}H-NMR($CDCl_3$, δ ppm, *J* Hz): **8.05**(dd, 2H, H-*o*, 1.4, 7.4; 7.8); **7.57**(dt, 1H, H-*p*, 1.4, 7.4); **7.55**(dt, 2H, H-*m*, 7.8, 7.4); **4.73**(dd, 1H, H-8, 9.1, 11.4); **4.57**(dd, 1H, H-8, 6.4, 11.4); **4.23**(dddd, 1H, H-5, $^3J(H^5\text{-}H^1)$=1.4, $J(H^5\text{-}H^{6A})$=3.0, $J(H^5\text{-}H^3)$=4.4, $J(H^5\text{-}H^{6B})$=9.8); **4.04**(ddd, 1H, H-2, 0.9, 3.8, 8.0); **2.90**(brs, 1H, OH); **2.84**(dd, 1H, H-3, 8.0, 14.4); **2.50**(d, 1H, H-4, 5.7); **2.48**(d, 1H, H-1, 4.3); **2.16**(m, 1H, H-7), **2.13**(ddd, 1H, H-6, 5.1, 9.8, 13.7); **2.07**(dt, 1H, H-3, 4.3, 14.4); **0.94**(dd,

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1H, H-6, 3.1, 13.7), ^{13}C-NMR($CDCl_3$, δ ppm): **166.55**(COO), **132.93**(C-*p*), **130.15**(C_q); **129.50**(C-*o*), **128.31**(C-*m*), **69.92**(C-5), **63.02**(C-8); **60.17**(C-2), **48.25**(C-4 *or C-1*), **48.05**(C-7), **45.29**(C-1 or C-4), **39.26**(C-6), **32.45**(C-3).

-7.45g, mixture of alcohols (~7:3, **4**/**5**)

-2.59g (3.08%) pure more polar alcohol (**5**), obtained crystallized, m.p. 64-64.8°C, with $[\alpha]_D^{20}$= +2.50° (c=1% in THF).

IR: 3230br, 2961w, 2935w, 2887w, 1713vs, 1603w, 1584w, 1490w, 1462m, 1449m, 1440m, 1347s, 1313m, 1265vs, 1180m, 1115vs, 1096s, 1070s, 1050s, 1026s, 1017m, 979m, 946m, 934m, 907s, 897m, 795m, 708vs, 687m, 675m, 663s.

^{1}H-NMR-400MHz($CDCl_3$, δ ppm, *J* Hz): **8.06**(dd, 2H, H-*o*, 1.3, 7.8); **7.56**(tt, 1H, H-*p*, 1.3, 7.4); **7.55**(dd, 2H, H-*m*, 7.8, 7.4); **4.75**(dd, 1H, H-8, 9.1, 11.4); **4.61**(dd, 1H, H-8, 6.8, 11.4); **3.81**(t, 1H, H-5, 7.0); **3.80**(d, 1H, H-2, 7.4); **2.59**(t, 1H, H-7, ~7.9); **2.55**(d, 1H, H-1, 4.6); **2.32**(d, 1H, H-4, 4.4); **2.14**(dt, 1H, H-3, 4.4, 14.8); **1.96**(dd, 1H, H-3, 8.0, 14.8); **1.72**(dd, 1H, H-6, 7.0, 13.8); **1.50**(broad dt, 1H, H-6, 4.6, 13.8). ^{13}C-NMR-100MHz($CDCl_3$, δ ppm): **166.61**(COO), **132.93**(C-*p*), **130.36**(C_q); **129.63**(C-*o*), **128.36**(C-*m*), **72.98**(C-5), **63.08**(C-8); **59.51**(C-2), **47.14**(C-4), **46.73**(C-1), **45.49**(C-7) , **40.86**(C-6), **36.31**(C-3).

9-BBN reduction

1.12 g (4 mmoli) *ent*-27-Benzoate, (**3**), were dissolved in 8 mL anh. THF and 8 mL 0.5M 9-BBN solution în THF were dropwise added at room temperature under argon. TLC (ethyl acetate-hexane-acetic acid, 5:4:0.1, $R_{f(3)}$=0.68, $R_{f(4)}$=0.53, $R_{f(5)}$ = 0.46) indicate that the reaction is slowly and from the first part, a ratio of alcohols (**4**)/(**5**) of about 4:1 is observed. After 2 h, another 2 mL, then another 2 mL (+2h) 0.5M 9-BBN solution în THF were added and the reaction mixture was stirred for 18h. The reaction mixture was worked-up (20 mL 20% $KHCO_3$ added, stirred 1.5 h, 50 mL benzene added, phases separated, organic phase washed with 30 mL water, dried and concentrated under reduced pressure), resulting 1.74 g oil which was purified as previously described. 0.81g Alcohol (**4**) and ~0.19g of alcohol (**5**) were obtained, in a ratio of ~4:1.

General procedure for the Mitsunobu reaction [2] of bicyclo[2.2.1]heptane alcohol (4) with pirimidine bases.

a). Bicyclo[2.2.1]heptane alcohol (**4**) was linked to N^4-benzoyl cytosine and 5-fluoro-uracil by Mitsunobu reaction [2] as previously described (with DEAD or DIAD) giving the *ent*-27-C-dibenzoate (**7**) and *ent*-27-5-FU-benzoate (**11**).

b). A modified procedure, with first formation of DEAD-(or DIAD)-triphenylphosphine complex in the presence of alcohol (**4**) and then adding the pyrimidine base, is presented at 2.1. and 2.4 [3].

ent-27-5-Fluorouracil-dibenzoate, (11a), Benzoic acid 2-chloro-5-[4-(5-chloro-7-benzoyloxymethyl-bicyclo[2.2.1]hept-2-yloxy)-5-fluoro-pyrimidin-2-yloxy]-bicyclo[2.2.1]hept-7-ylmethyl ester; 2,4-Bis-(5-chloro-7-benzoyloxymethyl-bicyclo[2.2.1]hept-2-yloxy)-5-fluoro-pyrimidine

The synthesis of the compound is given with identification of a secondary compound and their full characterization.

a). The procedure a) was followed: Starting from 2.42 g (8.6 mmoles) alcohol (**4**) resulted ~3.7 g impure product used in the next step.

b). The procedure b) was followed: 2.88 g (10 mmoles) intermediate (**4**), twice coevaporated with benzene, and 3.96 g (15mmoles) triphenylphosphine were dissolved in 84 mL anh. THF, then 7 mL 40% DEAD solution in toluene is added dropwise under stirring at r.t.(16°C) in 23 min. The yellow solution is stirred for 10 min., 1.952 g (15 mmoles) powdered 5-fluorouracil added, stirred 1h at r.t. (all 5-FU is dissolved), then refluxed on the night, monitoring the reaction by tlc (Silicagel, hexan-ethyl acetate-acetic acid, 5:2:0.1, twice, $R_{f(4)}$ = 0.25, $R_{f(11)}$ = 0.46). The reaction mixture was concentrated and purified by pressure chromatography on a silica gel column (eluent: extraction benzine-ethyl acetate, 2:1), resulting 1.256 g (51.07%) pure product as oil which hardened at r.t, and was analysed by: elemental analysis, calc. for $C_{34}H_{33}FCl_2N_2O_6$, C:62.30, H: 5.07, N: 4.27, found C:62.40, H: 5.15, N: 4.27, $[\delta]_D$ = +13.20°(2% in THF), IR: 3671wb, 2969m, 2501m, 2160vs, 2028vs, 1977vs, 1713vs, 1603m, 1584s, 1466s, 1451s, 1413vs, 1347s, 1315s, 1266vs, 1203s, 1176s, 1144m, 1112vs, 1069s, 1026s, 908s, 780s, 709vs, 687s.

MS presents for $C_{34}H_{33}FCl_2N_2O_6$ $(M+H)^+$ 655 (for both Cl-atoms 35), 657 (for one Cl-35 and the other Cl-37) and 659 (for both Cl-atoms 37), showing that two Cl-atoms are present in the molecule. By fragmentation of molecular peak, resulted: 393 and 395 (for the two Cl-isotopes) [(M+1)-263/265(one bicyclo-benzoate moiety)], 263 and 265 (for the two Cl-isotopes) $[(M+1)^+$ for (one bicyclo-benzoate moiety)], 141/143, 105.

^{1}H-NMR-**400**MHz($CDCl_3$, δ ppm, J Hz): **8.06**(m, 4H, H-*o*); **8.01**(d, 1H, H-6', 2.3); **7.58-7.53**(m, 2H, H-*p*); **7.44**(t, 4H, H-*m*, 7.8); **4.93**(dd, 1H, H-5, 2.8, 7.0); **4.78**(dd, 1H, H-8, 8.6, 11.5); **4.77**(dd, 1H, H-8, 8.6, 11.5); **4.67**(dd, 1H, H-5, 3.1, 7.0); **4.64**(2dd, 2H, H-8, 7.2, 11.5); **3.92**(dd, 1H, H-2, 3.3, 7.0); **3.91**(dd, 1H, H-2, 3.5, 7.0); **2.73-2.64**(m, 6H, H-7-1-4); **2.29**(dt, 2H, H-3, 4.4,14.8); **2.14**(dt, 2H, H-3, 8.2, 14.8); **1.96**(dd, 1H, H-6, 7.2, 14.3), **1.90**(dd, 1H, H-6, 6.9, 14.3), **1.81-1.74**(m, 2H, H-6), ^{13}C-NMR-100MHz($CDCl_3$, δ ppm): **166.50**(COO); **166.47**(COO); **159.00(C-2'); 158.91**(d, C-4, J=11.7Hz); **143.03**(C_5-5, J=252,7 Hz); **143.35**(d, C-6, J=20.5Hz); **132.94; 132.93**(C-*p*), **130.39; 130.33**(C_q), **129.66; 129.64**(C-*o*), **128.40; 128.36**(C-*m*), **78.77, 78.23**(C-5), **62.79; 62.64**(C-8); **59.16, 58.80**(CH-Cl), **46.65; 46.56**(C-7 or 1); **46.44; 46.30**(C-1 or 7); **44.30, 44.22**(C-4), **38.95; 38.83**(C-6), **36.44; 36.16**(C-3).

A fraction of 159 mg of more polar secondary product (**12a**), 1-(5-Chloro-7-benzoyloxymethyl-bicyclo[2.2.1]hept-2-yl)-4-(5-chloro-7-benzoyloxymethyl-bicyclo[2.2.1]hept-2-yloxy)-5-fluoro-1H-pyrimidin-2-one, was isolated (at longer reaction time this compound is formed in greater quantity), and crystallized from ethyl ether-hexane, m.p. 159.2-161.7°C, $[\alpha]_D$ = +38.97 (1% in THF), which was characterized by: elemental analysis for th. $C_{34}H_{33}FCl_2N_2O_6$, C: 62.30, H: 5.07, N: 4.27, found: C: 62.11, H: 5.21, N: 4.19, IR: 2971m, 2518bw, 2159m, 2030m, 1976m, 1713vs, 1695vs, 1627m, 1560vs,1452m, 1408s, 1354m, 1333s, 1316s, 1284vs, 1274vs, 1232s, 1158s, 1117vs, 1094s, 1072s, 1025s, 1010s, 941m, 906s, 798m, 762m, 717vs, 690s,

MS: presents for $[M+1]^+$ the same values: 655/657/659 for 2 Cl-atoms and by fragmentation of molecular peak, resulted: 533/535/537 $[(M+1) - C_6H_5CO- H_2O]^+$, 393/395 $[(M+1) - C_6H_5CO- H_2O$ - one bicyclo moiety$]^+$, 271/273 $[(M+1)- 2C_6H_5CO- H_2O$- one bicyclo moiety $]^+$, 263/265 $[(M+1)- C_6H_5CO- H_2O$- one bicyclo moiety -5FU$]^+$, 105 for $C_6H_5CO^+$,

^{1}H-NMR-**400**MHz($CDCl_3$, δ ppm, J Hz): **8.07-8.03**(m, 4H, H-*o*); **7.58**(tt, 1H, H-*p*, 1.4, 7.4); **7.54** (tt, 1H, H-*p*, 1.4, 7.4); **7.50-7.40**(m, 5H, 4H-m, H-6'); **4.87**(dd, 1H, H-5, 2.7, 7.0); **4.80**(dd, 1H, H-8, 9.8, 11.5); **4.69**(dd, 1H, H-8, 8.0, 11.5); **4.62**(dd, 1H, H-8, 5.4, 11.5); **4.56**(dd, 1H, H-8, 7.6, 11.5); **4.30**(m, 1H, H-5); **3.89**(dd, 1H, H-2, 3.6, 7.8); **3.83**(dd, 1H, H-2, 4.5, 7.2); **3.23**(t, 1H, H-7, 8.0); **2.75**(d, 1H, H-4, 4.3);

2.66(d, 1H, H-4, 4.5); **2.63**(d, 1H, H-1, 4.1); **2.59**(d, 1H, H-1, 4.5); **2.55**(dd, 1H, H-7, 5.4, 9.8); **2.38**(dt, 1H, H-3, 5.1, 13.3); **2.31**(dt, 1H, H-3, 4.5, 14.8); **2.13**(dd, 1H, H-3, 8.2, 14.8); **2.13**(m, 1H-H-6); **2.00**(dd, 1H, H-6, 7.8, 14.6); **1.94**(dd, 1H, H-6, 7.0, 14.6), **1.77**(dd, 1H, H-3, 8.8, 13.3); **1.67** (dt, 1H, H-6, 3.5, 14.3), ^{13}C-NMR - 100MHz ($CDCl_3$, δ ppm): **166.45**(COO); **166.39**(COO); **157.15**(d, C-4', J=24.5Hz); 151.20(Cq, C-2'); **146.51**(C_q-5, J=245,3Hz); **133.36; 132.89**(2C-*p*); **132.65**(C-6', 21.9); **130.34; 130.00**(2Cq), **129.64; 129.46**(2C-*o*); **128.462; 128.34**(2C-*m*); **80.51**(C-5); **63.05; 62.51**(2C-8); **60.40**(C-5); **58.87, 58.57**(2C-2); **48.05; 47.38**(2C-7); **47.03; 46.94**(2C-1); **44.40, 44.29**(2C-4), **40.69; 39.04**(2C-6); **35.94; 35.90**(2C-3).

ent-27-5-Fluorouracil- Trityl, (15), 2,4-Bis-(5-chloro-7-trityloxymethyl-bicyclo[2.2.1]hept-2-yloxy)-5-fluoro-pyrimidine

To 4.26 g (10.2 mmoles) 8-O-trityl protected derivative (**4a**) in 103 mL dioxane, 1.99 g (15.3 mmoles) 5-fluorouracil and 4.02 g (15.3 mmoles) triphenylphosphine were added, then 3.01 mL (15.3 mmoles) DIAD were slowly (10 h) added at r.t. under stirring in argon atmosphere and stirred then for 4 days, monitoring the reaction by tlc (Silica gel, hexan-ethyl acetate-acetic acid, 5:2:0.1, twice, $R_{f(4)}$ = 0.59, $R_{f(15)}$ = 0.27). The reaction mixture was filtered, recovering 0.57g unreacted trityl-alcohol (4), the filtrate concentrated and purified by pressure chromatography as for benzoate, resulting 2.28g impure product, used as so in the next reaction for deprotection of trityl group.

ent-27-Thymine-Benzoat, (**11b**), Benzoic acid 2-chloro-5-[4-(5-chloro-7-benzoyloxymethyl-bicyclo[2.2.1]hept-2-yloxy)-5-methyl-pyrimidin-2-yloxy]-bicyclo[2.2.1]hept-7-ylmethyl ester; 2,4-Bis-(5-chloro-7-benzoyloxymethyl-bicyclo[2.2.1]hept-2-yloxy)-5-methyl-pyrimidine

Thymine carbocyclic nucleoside (**11b**) was obtained by the same procedure (a), starting from 2g (7.13 mmoles) bicyclo[2.2.1]heptane alcohol (**4**), refluxing until dissapearance of the alcohol (TLC, silica gel Merck, hexane-ethyl acetate-acetic acid, 5:2:0,1, twice eluted $R_{f(4)}$ = 0.69; $R_{f(11b)}$ = 0.84).

After removing the solvent, the crude product, was purified by pressure chromatography, resulting 1.07 g a little impure product, which was used in the next step.

Benzoic acid 2-chloro-5-(5-methyl-6-oxo-1,6-dihydro-pyrimidin-2-yloxy)-bicyclo[2.2.1]hept-7-ylmethyl ester (**18**)

To a solution of 2.81 g(10 mmoles) alcohol (**4**) and 3.934 g(15 mmoles) triphenylphosphine in 120 mL anh. THF, in argon atmosphere, 6.9 mL 40% soln. DEAD in toluene were dropwise added in 40 min. under stirring and, after another 25 min. stirring, 3.453 g (15 mmoles) N^3-benzoyl-thymine were added and refluxed 6 days monitoring the evolution of the reaction by tlc (Merck silica gel plates, hexane-ethyl acetate - acetic acid, 5:2:0,1, twice eluted, $R_{f(4)}$ = 0.53; $R_{f(18)}$ = 0.37). The solution was filtered, filtrate concentrated and used in the next reaction.

MS: (M+1) 493, 495, 105$(C_6H_5CO)^+$.

General procedure for deprotection of the benzoate groups for obtaining carbocyclic bicyclo[2.2.1]heptane nucleosides

The crude benzoate nucleoside was dissolved in metanol, anh. K_2CO_3 added and the reaction mixture stirred on the night at room temperature. Methanol was removed under reduced pressure, water and ethyl acetate added, organic phase washed with water, brine, dried and concentrated under reduced pressure. (The aqueous phases were extracted until no more product remains).

[2-(4-Amino-pyrimidin-2-yloxy)-5-chloro-bicyclo[2.2.1]hept-7-yl]-methanol (**8**)

Starting from 3.82 g (13.6 mmoles) alcohol (**4**), 2.33 g impure dibenzoylated O^2-nucleoside (**7**) resulted, which was debenzoylated by transesterification in 100 mL methanol with 2g anh. K_2CO_3. After work-up, 1.21 g crude carbocyclic O^2-cytosine nucleoside (**8**) resulted, which crystallized 0.54 g pure product (dichloromethane-methanol, 95:5), m.p. 168.1-171.3°C, with $[\alpha]_D^{20}$= +16.60° (c=1% in THF). The filtrate was concentrated and purified by pressure chromatography, resulting another 0.34 g of pure compound (24.26%). For $C_{12}H_{16}ClN_3O_2$, M = 269.73,

MS: (M+1) 270 (for Cl-35) and 272(for Cl 37). By fragmentation of both peaks resulted (M+1) for cytosine, 112.

IR: 3314w, 3205w, 3107w, 2971m, 1641m, 1593 vs, 1557s, 1489m, 1442w, 1413vs, 1375m, 1359vs, 1314w, 1297vs, 1259w, 1134m, 1111m, 1097m, 1043m, 1027m, 989m, 911m, 819s, 636m, 590m.

^{1}H-NMR(DMSO-d_6, δ ppm, *J* Hz): **7.84**(d, 1H, H-6', 5.6); **6.77**(br singlet, 2H, NH_2); **6.07**(d, 1H, H-5', 5.6); **4.64**(dd, 1H, H-5, 3.7, 7.9); **4.04**(m, 1H, H-2); **3.72**(dd, 1H, H-8, 9.3, 11.1); **3.66**(dd, 1H, H-8, 6.0, 11.1); **2.46**(d, 1H, H-4, 3.2); **2.35**(d, 1H, H-1, 3.8); **2.21**(m, 1H, H-7)**; 2.07**(dd, 1H, H-3, 7.7, 14.2); **1.96**(dt, 1H, H-3, 3.7, 14.2); **1.89**(dd, 1H, H-6, 7.1, 14.0), **1.49**(bdt, 1H, H-6, 14.0).^{13}C-NMR(DMSO-d_6, δ ppm):, **165.25**(C4'), **164.28**(C-2'), **156.13**(C-6'), **99.32**(C-5'), **76.20**(C-5), **60.39**(C-2); **58.52**(C-8), **50.10**(C-7), **45.86**(C-1), **43.49**(C-4), **38.53**(C-6), **35.73**(C-3).

{2-Chloro-5-[4-(5-chloro-7-hydroxymethyl-bicyclo[2.2.1]hept-2-yloxy)-5-fluoro-pyrimidin-2-yloxy]-bicyclo[2.2.1]hept-7-yl}-methanol (**13a**)

a). Starting from 2.42 g (8.6 mmoles) alcohol (**4**) resulted ~3.7 g impure product used in the next step (as mentioned at 3.1). After transesterification (TLC, silica gel, dichloromethane-methanol, 9:1, $R_{f(11a)}$ = 0.93; $R_{f(13a)}$ = 0.51), K_2CO_3 was neutralized with 7.3 mL 2N HCl, the methanol was distilled, and worked-up in the same way. 1.57 g crude product resulted that was purified by pressure chromatography, giving 0.63 g pure O^2-carbocyclic 5-fluoro-uracil nucleoside (**13a**), m.p. 82.2-85°C(dec.), with $[\alpha]_D^{20}$= +14.61° (c=1% in THF) and 0.24g slightly impure fraction (26.42% overall yield).

b). 1.398g(2.13 mmoles) Pure product (**11a**) were dissolved in 30 mL THF and 30 mL methanol, 320 mg anh. K_2CO_3 were added and the mixture stirred at r.t. on the night, monitoring the reaction by CSS. The reaction mixture was neutralized with 2N HCl, solvents removed under reduced pressure, the crude product purified by pressure chromatography, resulting 629 mg (65.9%) pure product (**13a**).

Elemental analysis, th. for $C_{20}H_{25}FCl_2N_2O_4$, C:53.70, H: 5.63, N: 6.26, found C:53.69, H: 5.78, N: 5.92, IR: 3324br, 2972m, 2943m, 2497bs, 2159vs, 2026vs, 1976vs, 1585s, 1466s, 1445m, 1444m, 1415vs, 1355s, 1342s, 1265s, 1232m, 1206m, 1164w, 1091m, 1078m, 1032vs, 999vs, 905s, 780m, 667m.

MS: (M+1) 447 (for both Cl-atoms 35), 449 (for one Cl-35 and the other 37) and 451 (for both Cl-atoms 37). By fragmentation of molecular peak, resulted: 289 and 291 (for the two Cl-isotopes) [(M+1)-158(one bicyclo-moiety)], 271[289-H_2O], 158 (one bicyclo-moiety)], 141, 131[(M+1) for 5-FU]. MS presents for (M-1) the values 445 (for both Cl-atoms 35), 447 (for one Cl-35 and the other Cl-37) and

449 (for both Cl-atoms 37) and by fragmentation of molecular peak 287, 251, 142, 127, etc.

$^{-1}$H-NMR-300 MHz(DMSO-d_6, δ, ppm, J Hz): **8.32**(d, 1H, H-6', 2.8); **4.83**(dd, 1H, H-5, 2.5, 7.0); **4.60**(dd, 1H, H-5, 2.8, 7.0); **4.52**(bs, 2H, OH); **4.07**(dd, 1H, H-2, 3.6, 7.4); **4.06**(dd, 1H, H-2, 3.8, 7.6); **3.74**(dd, 1H, H-8, 9.1, 11.0); **3.73**(dd, 1H, H-8, 8.8, 11.0); **3.66**(dd, 1H, H-8, 5.8, 11.0); **3.65**(dd, 1H, H-8, 5.8, 11.0); **2.56**(d, 1H, H-4, 4.4); **2.52**(d, 1H, H-4 in DMSO); **2.38**(d, 1H, H-1, 4.7); **2.35**(d, 1H, H-1, 4.7); **2.22-2.16**(m, 2H, H-7); **2.12**(dt, 2H, H-3, 7.7, 14.3); **2.03-1.98**(m, 2H, H-3); **1.98-1.85**(m, 2H, H-6); **1.63-1.52**(m, 2H, H-6), ^{13}C-RMN-100MHz(DMSO-d_6, δ, ppm): **158.81**(C-2'); (**158.20**(d, C-4',11.7); **143.95**(d, C-6', J=20.6Hz); **142.49**(d, C-5', 250.2Hz); **78.66, 78.48**(2CH-5); **60.16; 59.92**(2C-2); **58.56; 58.44**(2C-8); **50.35; 50.24**(2C-7); **45.90; 45.82**(2C-1); **43.37**(2C-4); **38.36**(2C-3); **35.57; 35. 35**(2C-6).

{2-Chloro-5-[4-(5-chloro-7-hydroxymethyl-bicyclo[2.2.1]hept-2-yloxy)-5-methyl-pyrimidin-2-yloxy]-bicyclo[2.2.1]hept-7-yl}-methanol (**13b**)

1.07 g Crude thymine carbocyclic nucleoside (**18**), obtained as above, were used in the next step (as mentioned at 3.1). After transesterification (TLC, silica gel, dichloromethane-methanol, 9:1, $R_{f-(18)}$ = 0.88; $R_{f-(19)}$ = 0.40) and worked-up, the crude product was crystallised from ethyl acetate-hexane, resulting 0.640 g (31.5%) *ent*-27-T nucleoside (**19**), m.p. 97.4-105.8°C, with $[\alpha]_D^{20}$= +18.80° (c=1% in THF).

IR: 3241w, 2977.7w, 2946w, 1741vw, 1606.3m, 1578s, 1432s, 1362.6s, 1330s, 1299.9m, 1239w, 1286.6m, 1186m, 1079.6m, 1038.6vs, 1027vs, 1003.7vs, 976w, 921.5w, 900m, 785.4m, 661.9m .

MS: (M+1) 443, 445, 447(for Cl-isotopes of 2 atoms), 285,287[(M-one bicycle as alkene)+1], 159, 161(Bicyclo)$^+$, 141, 143(BC-H_2O), 127(Thy +1)$^+$.

^{1}H-NMR(DMSO-d_6, δ ppm, J Hz): **8.08**(s, 1H, H-6'); **4.80**(dd, 1H, H-5, 2.5, 7.0); **4.64**(dd, 1H, H-5, 2.5, 6.8); **4.05**(m, 2H, H-2); **3.73**(dd, 1H, AB syst., H-8, 9.1, 10.7); **3.66**(dd, 1H, AB syst., H-8, 5.5, 10.7); **2.54**(m, 2H, H-4, in DMSO, from HETCOR); **2.39**(d, 1H, H-1, 4.8); **2.36**(d, 1H, H-1, 4.6); **2.25-2.19**(m, 2H, H-7); **2.14**(dt, 2H, H-3, 8.0, 14.9), **2.04-1.98**(m, 2H, H-3); **1.98**(s, CH_3); **1.92**(dd, 1H, H-6, 7.2, 13.8); **1.90**(dd, 1H, H-6, 7.2, 13.8), **1.56**(bt, 2H, H-6, 13.8), ^{13}C-NMR(DMSO-d_6, δ ppm):, **167.87**(C4'); **162.48**(C-2'); **157.60**(C-6'); **110.53**(C-5'); **77.54; 77.11**(2C-5), **60.48; 60.19**(2C-2); **58.47; 58.36**(2C-8), **50.16**(2C-7), **45.90; 45.74**(2C-1), **43.38**(2C-4), **38.43**(2C-6), **35.67; 35.51**(2C-3); **11.39**(CH_3).

2-(5-Chloro-7-hydroxymethyl-bicyclo[2.2.1]hept-2-yloxy)-5-methyl-3H-pyrimidin-4-one (**19**)

The concentrate obtained at 2.4. was taken in 70 mL anh. methanol, 1.5 g anh. K_2CO_3 added and stirred on the night, and then concentrated to dryness. The residue was distributed between 100 mL toluene and 80 mL water, phases separated and organic phase washed with 4x50 mL water. Water phases were concentrated and residue extracted with ethyl acetate, organic extract concentrated, purified by pressure chromatography (silicagel, eluent DCM-methanol, 9:1), resulting 550 mg of nucleoside (**19**), which was crystallized from acetone.. The compound has m.p. 213.2°C(dec.), with $[\alpha]_D^{20}$ = 43.3°(c=1% in THF), Elemental anal.: th for $C_{13}H_{17}ClN_2O_3$: C:54.84, H: 6.02, Cl: 12.45, N: 9.83, found: C:54.58, H: 6.66; N: 9.85.

IR: 3351.2, 3097.6, 2974, 2943.4, 2531.3, 2159.7s, 2028.4s, 1976.7s, 1662.9vs, 1611.1s, 1598.4vs, 1445.4m, 1385.9m, 1316.6s, 1295.4vs, 1280.3s, 1163.2s, 1094.6m, 1072.9m, 1045.3vs, 1018.8vs, 1003.2s, 980.2s, 932.3m, 899.2vs, 852.8s, 801.8m, 770.4s.

MS: *[M=284.73]*, (M+1) 285, 287(for both Cl-isotopes), 127(Thy+1)$^+$; (M-1) 283, 285(for both Cl-isotopes), 247(-HCl), 217, 151, 124(Thy-2).

^{1}H-NMR-300 MHz(DMSO-d6, δ, ppm, J Hz): **7.35**(q, 1H, H-6', 1.1); **4.70**(dd, 1H, H-5, 2.8, 7.1); **4.02**(dd, 1H, H-2, 4.4, 7.6); **3.72**(dd, 1H, H-8, 8.8, 11.0); **3.64**(dd, 1H, H-8, 5.8, 11.0); **2.52**(m in DMSO, 1H, H-4); **2.35**(d, 1H, H-1, 4.9); **2.15**(m, 1H, H-7); **2.06**(dd, 1H, H-3, 7.7, 14.3); **1.94**(dt, 1H, H-3, 4.4, 14.3); **1.87**(dd, 1H, H-6, 7.1, 14.0); **1.80**(d, 3H, CH_3, 1.1); **1.51**(dt, 1H, H-6, 4.1, 14.0), ^{13}C-RMN-75MHz(DMSO-d6, δ, ppm): **164.16**(C-4'); **155.71**(C-2'); **148.89**(C-6'); **116.43**(C-5'); **78.27**(C-5); **59.92**(C-2); **58.40**(C-8); **50.10**(C-7); **45.73**(C-4); **43.29**(C-1); **38.18**(C-3); **35.38**(C-6); **12.29**(CH_3).

Deprotection of trityl group of nucleoside (**15**)

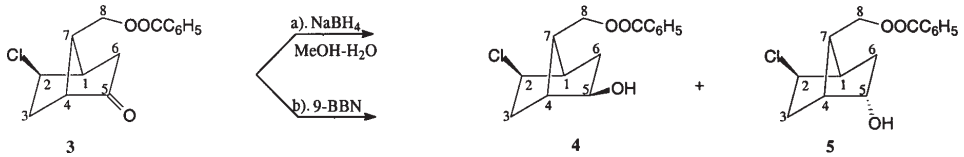
2.28g of Crude product (**15**) was dissolved in 50 mL dichlorometane(DCM)-methanol (9:1), 1.01g $NaHSO_4$/silicagel catalyst added and the mixture stirred on the night at r.t. The catalyst was filtered, washed with solvent, concentrated and purified by pressure chromatography (eluent: DCM-MeOH (9:1). Resulted 352 mg (12%) of pure product (**13a**) as solid foam, identical with the product obtained above, using benzoate as protecting group, characterized by:

^{1}H-NMR-400MHz (DMSO-d_6, δ ppm, J Hz): **8.32**(d, 1H, H-6', 2.9); **4.83**(dd, 1H, H-5, 2.4, 7.0); **4.60**(dd, 1H, H-5, 2.4, 7.0); **4.55**(bs, 2H, OH); **4.09**(dd, 1H, H-2, 4.1, 7.7); **4.07**(dd, 1H, H-2, 3.8, 7.4); **3.75**(dd, 1H, H-8, 9.1, 11.0); **3.72**(dd, 1H, H-8, 9.1, 11.0); **3.65**(2dd, 2H, H-8, 2.5, 5.5, 11.0); **2.56**(d, 1H, H-4, 4.5); **2.52**(m in DMSO, 1H, H-4); **2.38**(d, 1H, H-1, 4.5); **2.35**(d, 1H, H-1, 4.3); **2.22-2.17**(m, 2H, H-7); **2.15-1.97**(m, 4H, H-3); **1.94**(dd, 1H, H-6, 7,2, 14.0); **1.89**(dd, 1H, H-6, 7.2, 14.0); **1.63-1.53**(m, 2H, H-6), ^{13}C-NMR-100MHz (DMSO-d_6, δ ppm): **158.77**(C_q-2); **158.13**(d, C-4, 11.0Hz); **143.87**(d, C-6, 20.5), **142.42**(d, C-5, 249Hz); **78.59, 78.42**(2C-5); **60.08; 59.84**(2C-2); **58.49; 58.38**(2C-8); **50.27; 50.17**(2C-7); **45.83; 45.75**(2C-1); **43.31**(2C-4); **38.30**(2C-6); **35.54; 35.31**(2C-3).

Results and discussion

Synthesis of the nucleoside analogues, (**2**), started from the same optically active bicyclo[2.2.1]heptane key intermediate (**3**), which was reduced with sodium borohydride as previously described [1]. From the reaction resulted the alcohol (**4**), as a major product, and also a small quantity of 5α-OH regioisomer, (**5**). It is this mixture of alcohols (**4**) and (**5**) which was used in the preceeding paper [1] for the synthesis of nucleoside analogues *ent*-**27**-Cytosine dibenzoate and *ent*-**27**-5-Fluorouracil benzoate.

Now, by multiple column chromatography purifications, we separate the crude reaction mixture and obtained the regioisomer alcohols (**4**) and (**5**), in pure form, the compound with 5β-OH as a colourless oil and the other



3 4 5

Scheme 1. $NaBH_4$ and 9-BBN reduction of ketone group of compound 3

Compound **4**, 5-ß-OH				Compound **5**, 5-α-OH			
Hydrogen atom	δ (ppm)	Carbon atom	δ (ppm)	Hydrogen atom	δ (ppm)	Carbon atom	δ (ppm)
5-α	4.23	5	69.92	5-ß	3.80	5	72.98
3-α	2.84	3	32.45	3-α	2.14	3	36.31
2-α	4.04	2	60.17	2-α	3.80	2	59.51
6-α	2.13	6	36.26	6-α	1.72	6	40.86

Table 1
CHEMICAL SHIFTS FOR THE SELECTED PROTONS AND CARBON ATOMS OF THE ALCOHOLS (**4**) AND (**5**)

one, with 5α-OH, as a crystallised compound. The ratio of the purified alcohols was ~ 94:6, which means that the borohydride reduction was highly selective.

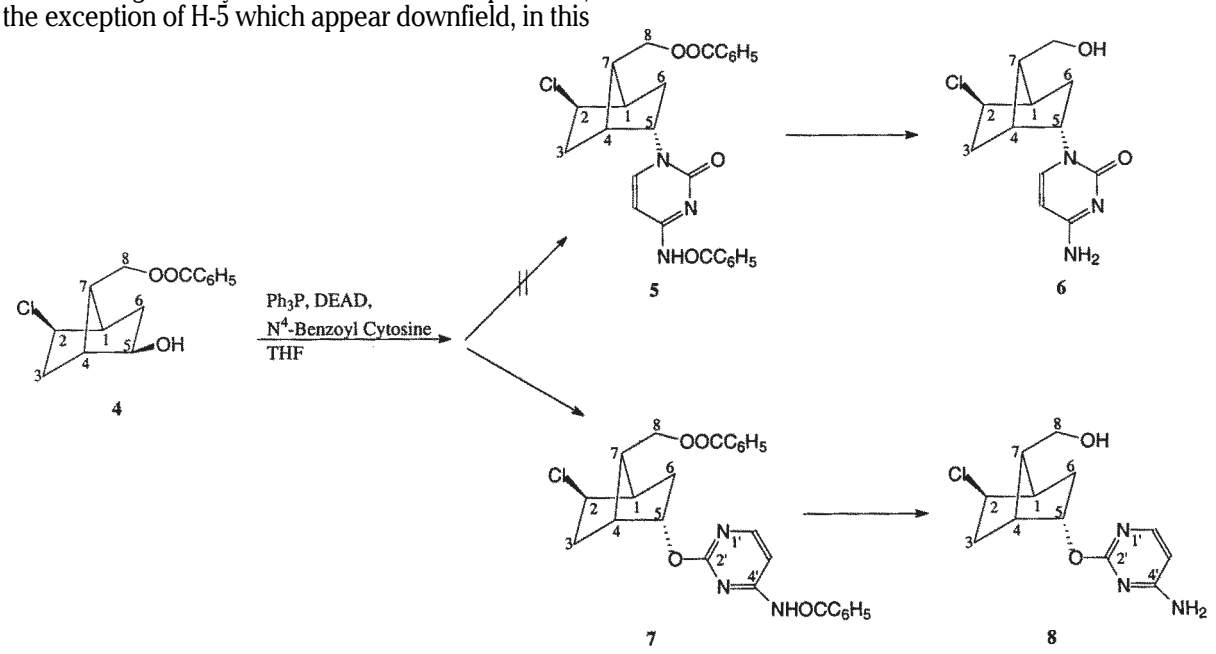
Using the bulky reagent 9-BBN instead of sodium borohydride, the reduction was slowly and required an excess of the reagent, but the ratio of the alcohols was only ~8:2.

The position of the newly formed 5-OH determine different steric conformations for the compounds (**4**) and (**5**), which are reflected in ^{1}H- and ^{13}C-NMR spectra. For the compound (**4**) with *exo*-OH, the 2_{α}, 3_{α}, 5_{α} and 6_{α} protons are shifted to higher field than those of the compound (**5**) with *endo*-OH. A net difference is observed also for the carbon atoms (table 1.)

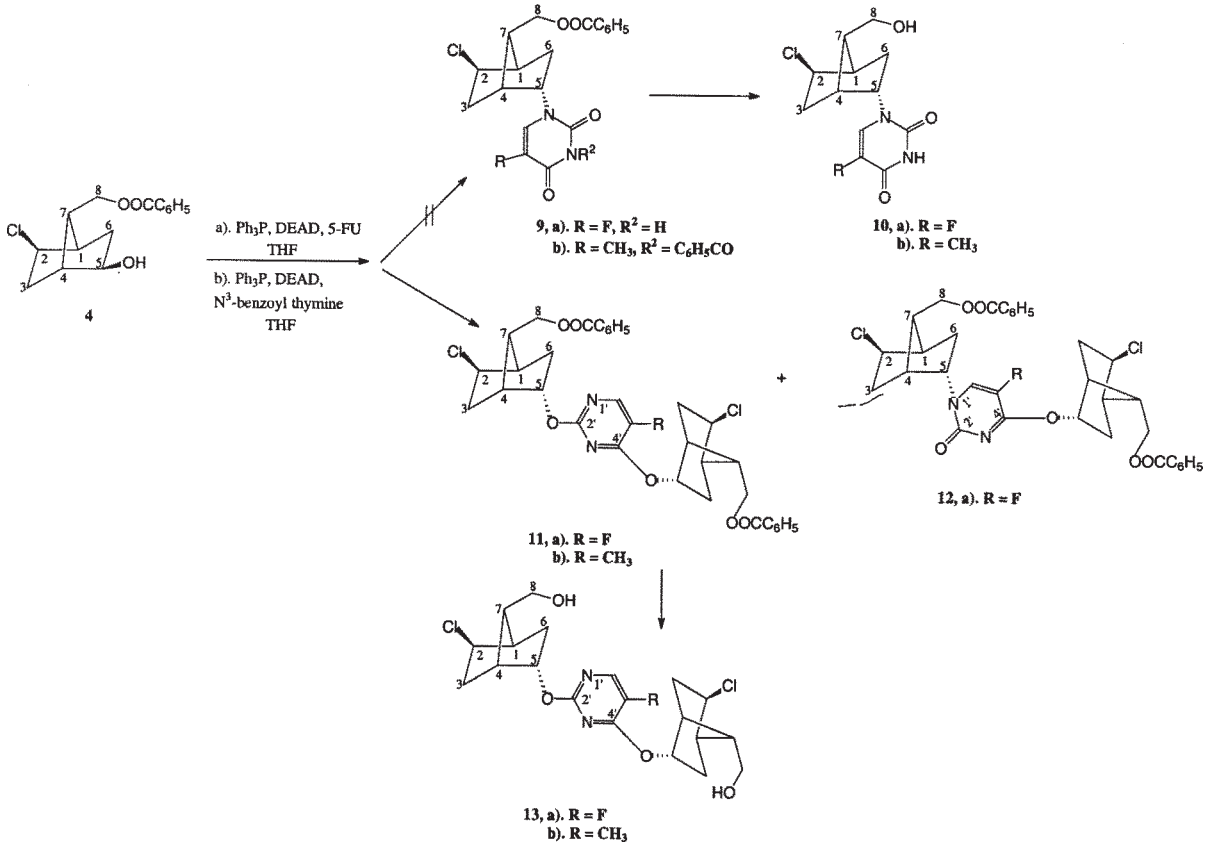
The configuration of the alcohol (**4**) was proved also on its 5-O-mesilated derivative (the result will be publised separatelly) obtained as a crystallized compound.

The pure alcohol (**4**) was used now for the synthesis of new bicyclo[2.2.1]heptane carbocyclic nucleoside analogues. As in the previous paper this alcohol was linked to pyrimidine bases: N^{4}-benzoyl cytosine and 5-fluoro uracil by the same Mitsunobu reaction [2] (procedure a.). After a rough column chromatography purification, the cytosine nucleoside was benzoate deprotected by trans-esterification (K_2CO_3 in anh. methanol) and pure compound (m.p. 168.1-171.3°C) was obtained by crystallization (dichloromethane-methanol). But, in ^{13}C-NMR, the chemical shift for C-5 atom have a higher value (76.20 ppm) which, according to the literature data [4-9] correspond to a C-O^{2} bond and this is also similar with that existing in 5β-hidroxy alcohol (**5**) of 72.98 ppm. Other differences between the nucleosides linked to N^{1} atom and O^{2}-atom are generally not observed in NMR-spectrum, with the exception of H-5 which appear downfield, in this case at α 4.64 ppm. In heterocyclic base, the alkylation at O^{2} introduce a conjugated double bond in the ring and this makes carbon atoms C-6 and C-5 to be shifted downfield from abot 146 and 93 ppm to 156.13 and 99.32 ppm [10]. MS for (M+1) gave 270 (for Cl-35) and 272 (for Cl-37), indicating a single heterocyclic moiety coupled on cytosine base, but does not give an indication of a C-O or a C-N bond; for fragmentation of both peaks resulted (M+1) for cytosine. This means that the structure of the cytosine nucleoside appear to be that corresponding to an O^{2}-linked compound (**8**) and not to an N^{1}-linked compound (**6**) [11] (scheme 2).

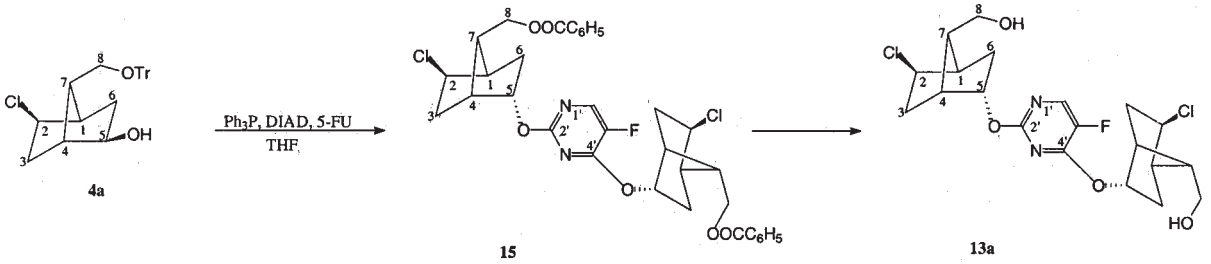
In the case of benzoyl 5-fluorouracil nucleoside, two compounds are formed. The less polar compound (**11a**), presents in ^{13}C-NMR spectrum doublets for all carbon atoms of bicyclic moiety and, like in the case of cytosine nucleoside (**7**), the chemical shift for C-5 atoms have a similar higher value (78.77 and 78.23 ppm). H-5 proton appear at two different values, a higher value at 4.93 ppm and a smaller value, similar with that for cytosine compound (**7**) at 4.67 ppm. This fact could indicate a compound linked to O^{2}- and O^{4}- of the heterocyclic base, like in (**11a**) (scheme 3). This structure is confirmed by MS, where $(M+1)^{+}$ has three values: 655 (for both Cl-atoms 35), 657 (for one Cl-35 and the other 37) and 659 (for both Cl-atoms 37), showing that two Cl-atoms and of course two bicyclic fragments are present in the molecule. By fragmentation of molecular peak, resulted: 393 and 395 (for the two Cl-isotopes) for the fragment missing one bicyclo-benzoate moiety and 263, respectivelly 265 (for the two Cl-isotopes) for bicyclo-benzoate carbocation moiety.



Scheme 2. Synthesis of ent-27-O^{2}-cytosine nucleoside analogue (**ent-27-C**, **8**)



Scheme 3. Synthesis of Thymine and 5-fluorouracil O-nucleoside analogues



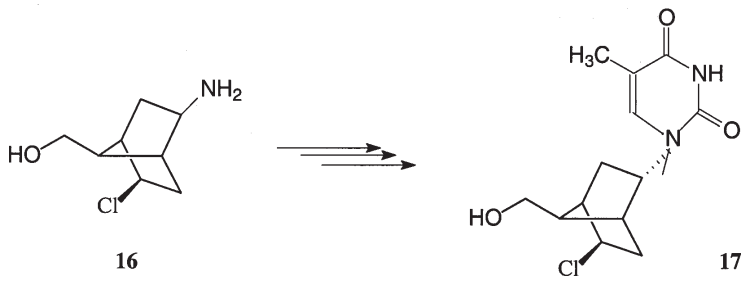
Scheme 4. Synthesis of **ent-27-5-FU** from trityl protected alcohol (**4a**)

The more polar compound (**12a**) presents also doublets for all carbon atoms of bicyclic moiety in ^{13}C-NMR spectrum, but in this case there is a great difference between the chemical shifts of C-5 atoms, 80.51 and 60.46 ppm, indicating a C-N link for C-5 carbon of one bicyclic moiety with the lower value and probably a C-O^4 bond for C-5 carbon for the other moiety with 80.51 value. ^{1}H-NMR spectrum presents a higher value, 4.87; ppm, for H-5 linked to C-O^4 (80.51 pm) and a lower value, 4.30 ppm for H-5 in the moiety linked to N^1-atom. MS: presents for $[M+H]^+$ the same values: 655/657/659 for 2 Cl-atoms and by fragmentation of molecular peak, resulted: 533/535/537 $[(M+H)- C_6H_5CO- H_2O]^+$, 393/395 $[(M+H)- C_6H_5CO- H_2O$ - one bicyclo moiety$]^+$, 271/273 $[(M+H)- 2C_6H_5CO- H_2O$- one bicyclo moiety $]^+$, 263/265 $[M+H- C_6H_5CO- H_2O$ - one bicyclo moiety -5FU$]^+$, 105 for $C_6H_5CO^+$.

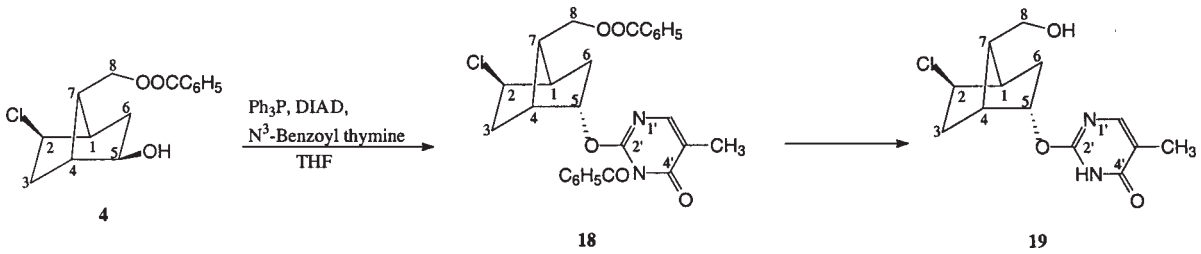
In the deprotection of benzoate groups of compound (**11a**) by transesterification a lot of compound decomposed, but resulted in 24.62% yield the compound (**13a**) [an improved procedure for deprotection of benzoate groups increases the yield to 65.9%] which presents similar characteristics with (**11a**) in ^{13}C- and ^{1}H-NMR spectra: 1). ^{13}C-NMR spectrum presents doublets for all carbon atoms of bicyclic moiety, 2). both C-5 atoms have higher value for δ: 78.66 and 78.48 ppm, 3). H-5 protons appear at two different values, 4.83 and 4.60 ppm, 4). the C-6' atom is shifted downfield from ~123.5 ppm in N^1-nucleosides to 143.95 ppm, indicating an aromacity of the ring. It is to be mentioned that the product (**13a**) named ***ent*-27-5-FU**, has the same characteristics with the product obtained from 8-O-Tritil-alcohol (**4a**), whose synthesis will be presented in a separate paper, by the same sequence of reactions (scheme 4). The remove of the trityl group in acid catalysis ($NaHSO_4$/silicagel) [12] produces also a great degradation of O^2-linked nucleoside (**15**), and the yield of whole sequence is reduced (12%).

MS presents for (M+1) the values 447 (for both Cl-atoms 35), 449 (for one Cl-35 and the other 37) and 451 (for both Cl-atoms 37). By fragmentation of molecular peak, resulted: 289 and 291 (for the two Cl-isotopes) [(M+1)-158(one bicyclo-moiety), 271/ 289-H_2O, 158(one bicyclo-moiety)], 141, 131[(M+1) for 5-FU]. MS presents for (M-1) the values 445 (for both Cl-atoms 35), 447 (for one Cl-35 and the other 37) and 449 (for both Cl-atoms 37) and similar 287,etc. This means that the compound must have molecular weight of 476; it means that all the NMR and MS data confirmed the structure (**13a**) for ***ent*-27-5-FU**.

The deprotection of the benzoate groups of the N^1,O^4-compound (**12a**) in the same conditions as for O^2,O^4-compound (**11a**) takes place with decomposition of the product, and this will be presented in a separate paper.



Scheme 5. Synthesis of N^1-thymine nucleoside (**17**) by construction of pyrimidine ring



Scheme 6. Synthesis of O^2-thymine nucleoside by procedure b)

Using N^3-benzoyl-thymine instead of 5-fluorouracil, by the same sequence of synthesis, we obtained a similar compound (**13b**), which presents very similar characteristics with ***ent*-27-5-FU** (**13a**): 1). ^{13}C-NMR spectrum presents doublets for all carbon atoms of bicyclic moiety, with the exception of C-3, C-4 and C-7, 2). both C-5 atoms have higher value for δ: 77.54 and 77.11 ppm, 3). H-5 protons appear at two different higher values, 4.80 and 4.64 ppm (4.84 and 4.61 ppm for ***ent*-27-5-FU**), 4). the C-6' atom is shifted downfield from ~137 ppm in N^1-nucleosides to 157.60 ppm, indicating an aromacity of the ring [8, 10]. For this compound it is clear that the values for C-5 and H-5 atoms are for a O^2-nucleoside, because the N^1- thymine nucleoside (**17**), obtained by construction of the thymine ring on an 5α-amine (**16**) (scheme 7) by Hrebabecky protocol [13] presents for C-5 a chemical shift of 57.39 ppm and for H-5, 4.04 ppm (the synthesis will be presented in a separate paper).

By modification of Mitsunobu reaction [3] as in procedure b), with first formation of DEAD-triphenylphosphine complex in the presence of alcohol (**4**) and then addition of N^3-benzoyl thymine (scheme 6), the compound formed was only an O^2-thymine nucleoside (**18**), with only one bicyclic moiety linked to thymine. N^3-Benzoate hindered in this case the link of a second bicyclic moiety to O^4, by contrast with that of unprotected 5-fluorouracil. The unprotected compound (**19**) presents in NMR only one signal for carbon atoms in the bicyclic moiety, as in the cytosine analogue (**8**), and specific higher values for C-5 atom (78.27 ppm) and H-5 atom (4.70 ppm). In this case, the C-6' atom is shifted downfield only to an intermediary value of 148.89 ppm, indicating no aromacity of the ring [8]. Mass spectrum presents for (M+1) 285 and for (M-1) 283, indicating a molecular weight of 284, this being, like NMR, in agreement with formula (**19**).

This means that, by procedure a), in the Mitsunobu reaction is obtained an O^2,O^4-bis-alkylated nucleoside and by procedure b), an O^2-alkylated compound.

By tlc and HPLC, the nucleoside compounds looks to be very pure and were used immediately in biological testing for their antineoplastic activity, before there full characterization. [In fact, at the beginning we believed that 5-FU and thymine nucleosides (**13a**) and (**13b**) were a mixture of isomers at C-5].

Our in vitro preclinical results, that are intended to be published elsewhere, show that *ent*-27-T and *ent*-27-5-FU exhibited mainly a cytostatic activity in Jurkat T lymphoblasts and on U937 monocytic blasts. The compounds inhibited uridine and thymidine uptake by cancer cells, even at non-cytostatic concentrations. Furthermore, the action of *ent*-27-T and *ent*-27-5-FU was different from that of the standard chemotherapeutical 5-FU. Thus, 5-FU was cytotoxic both for Jurkat and U937 cells, did not alter uridine uptake, but significantly enhanced thymidine incorporation, probably due to depletion of intracellular thymidine stores.

We also showed that the cytosine-containing compound *ent*-27-C was less active: it did not alter the viability of Jurkat or U937 cells, decreased uridine uptake only in the case of Jurkat lymphoblasts and did not interfere with thymidine metabolism.

Conclusions

The enantiomeric pure bicyclo[2.2.1]heptane ketone intermediate (**3**) were reduced selectively with sodium borohydride to the *exo* alcohol (**4**) in a ratio of 94:6 to the *endo* alcohol (**5**). Comparatively, the reduction with the bulky borane 9-BBN gave the alcohols (**4**) and (**5**) in a significantly slower ratio of ~4:1. Both alcohols were purified by multiple column chromatography separations and characterized.

From the pure alcohol (**4**), by Mitsunobu reaction with N^4-benzoyl-cytosine and N^3benzoyl-thymine, we obtained, instead N^1-linked nucleosides, O^2-carbocyclic nucleoside analogues (***ent*-27-C**, **8**) and (**19**). With 5-fluoro-uracil and N^3benzoyl-thymine we obtained also O^2,O^4- carbocyclic nucleosides with two bicyclic moieties on a pyrimidine bases, (***ent*-27-5-FU**, **13a**) and (***ent*-27-T**, **13b**).

The structure of the compounds was confirmed by elemental analysis, IR, MS, ^{1}H- and ^{13}C-NMR and complementary 2D spectra: COSY and HETCOR.

Preliminary biological testing results indicate that the investigated compounds might present anti-neoplastic activitiy, but more detailed investigations must be performed.

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