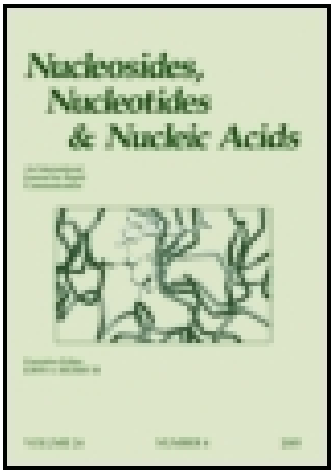


Nucleosides and Nucleotides
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lncn19

Stereospecific Synthesis and Anti-HIV Activity of (Z)2′- and (E)3′-Deoxy-2′(3′)-C-(chloromethylene) Pyrimidine Nucleosides

Elena N. Kalinichenko [a] , Elena B. Rubinova [a] , Evgueny V. Borisov [a] , Jan Balzarini [b] , Erik De Clercq [b] & Igor A. Mikhailopulo [a]

[a] Institute of Bioorganic Chemistry, Byelorussian Academy of Sciences , 220141, Minsk, Byelorussia

[b] Katholieke Universiteit Leuven, Rega Institute for Medical Research , Minderbroedersstraat, 10, B-3000, Leuven, Belgium

Published online: 16 Feb 2007.

To cite this article: Elena N. Kalinichenko , Elena B. Rubinova , Evgueny V. Borisov , Jan Balzarini , Erik De Clercq & Igor A. Mikhailopulo (1995) Stereospecific Synthesis and Anti-HIV Activity of (Z)2′- and (E)3′-Deoxy-2′(3′)-C-(chloromethylene) Pyrimidine Nucleosides, Nucleosides and Nucleotides, 14:3-5, 533-536

To link to this article: http://dx.doi.org/10.1080/15257779508012420

NUCLEOSIDES & NUCLEOTIDES, 14(3-5), 533-536 (1995)

STEREOSPECIFIC SYNTHESIS AND ANTI-HIV ACTIVITY OF (Z)2'- AND (E)3'-DEOXY-2'(3')-C-(CHLOROMETHYLENE) PYRIMIDINE NUCLEOSIDES

Elena N. Kalinichenko[1], Elena B. Rubinova[1], Evgueny V. Borisov[1], Jan Balzarini[2], Erik De Clercq[2], and Igor A. Mikhailopulo[1]*

[1]Institute of Bioorganic Chemistry, Byelorussian Academy of Sciences, 220141 Minsk, Byelorussia and [2]Katholieke Universiteit Leuven, Rega Institute for Medical Research, Minderbroedersstraat 10, B-3000, Leuven, Belgium

Abstract. Reaction of 1-[2,5(and 3,5)-di-O-trityl-β-D-*erythro*-pentofuran-3(and 2)-ulosyl]uracil derivatives **5** and **6** with (chloromethyl)triphenylphosphorane resulted in the stereoselective formation of (E)-3'- and (Z)-2'-chloromethylene derivatives **7** (69%) and **8** (53%), respectively, deprotection of which gave **9** and **10**. Transformation of the uracil nucleoside **7** into cytosine one followed by deprotection yielded **12**. The latter was converted into the arabinoside **14**. The fully deprotected chloromethylene nucleosides were tested for their activity against HIV-1 and HIV-2.

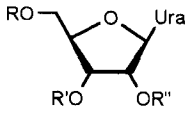
In the last few years, the 2'- and 3'-methylenenucleoside analogues have received considerable attention as valuable synthetic intermediates (*e.g.*, [1]) and as potential chemotherapeutic agents (*e.g.*, [2]) as well. The present report deals with the preparation and anti-HIV testing of (E)-3'-deoxy-3'-C-(chloromethylene) derivatives of uridine (**9**), cytidine (**12**), and ara-C (**14**) as well as (Z)-2'-deoxy-2'-C-(chloromethylene)uridine (**10**).

Tritylation of uridine (TrCl, DMAP, pyridine) followed by silica gel column chromatography afforded 2,5- and 3,5-di-O-substituted derivatives **2** (41%) and **3** (29%) as well as 5'-O-trityluridine (**4**) (18%). Compounds **2** and **3** were oxidized with CrO_3/pyridine/Ac_2O complex[3] and the respective ketones **5** and **6** thus obtained condensed with (chloromethyl)triphenylphosphorane generated from (chloromethyl)triphenylphosphonium chloride and BuLi in THF at -30 °C to give, after deblocking and chromatography, **9** and **10** in good combined yields. Only one geometric isomer of both **9**(E) and **10**(Z) was observed by ^{1}H NMR spectroscopy.

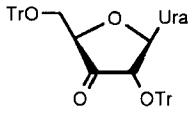
Assignment of all protons of fully deprotected nucleosides **9** and **10** was achieved by a combination of two-dimensional ^{1}H,^{1}H-COSY and NOESY experiments (data not shown). The stereochemistry of vinyl chlorides **9** and **10** was established by NOE difference spectroscopy. Comparison of NOE magnitudes for the vinylic proton H6' upon irradiation of H2' and H4' (3.8 and 1.2%, respectively) unequivocally supports the E configuration of **9**. Moreover, saturation of H2' results in NOE at H6 of 15.4% pointing to the

predominant *anti* conformation of the base at the glycosidic bond. The following data are most informative in the case of **10**: $f_{H6'}$(H3') and $f_{H3'}$(H6') are found to be 4.6 and 1.5%, respectively; no NOE at H6' was observed upon irradiation of H1' and *vice versa*. The results in NOE's at H6 of 6.9, 7.7 and 1.1% were obtained upon saturation of H1', H3' and H6', respectively. The latter data point to the predominant high-*anti* conformation of the base at the glycosidic bond. The population of the high-*anti* conformation in **10** suggests an interaction of π electrons of C5-C6 double bond and unsaturated function of the sugar. Indeed, the shielding of H6 resonance (0.43 ppm) in going from **9** to **10** reflects this interaction. Moreover, **10** displays negative Cotton effect at 259 nm, while uridine shows positive band in the B_{2u} region.

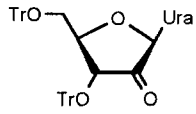
2',5'-Di-O-tritylated derivative of **7** was converted into the cytosine vinyl chloride **12** by successive treatment with $POCl_3$/1,2,4-triazole, conc. ammonia in 1,4-dioxane[4], and 80% aq. AcOH (55%; combined). Arabinoside **14** was obtained by selective 5'-O-tritylation of **12** to afford **13**, followed by reaction with DAST, O^2,2'-anhydro ring cleavage under basic conditions, and detritylation (47%; combined).



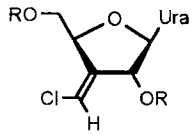
1, R = R' = R" = H
2, R = R" = Tr; R' = H
3, R = R' = Tr; R" = H
4, R = Tr; R' = R" = H



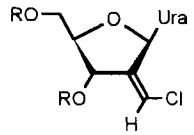
5



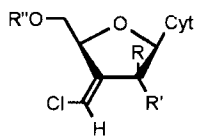
6



7, R = Tr
9, R = H



8, R = Tr
10, R = H



11, R = H; R' = OTr; R" = Tr
12, R = R" = H; R' = OH
13, R = H; R' = OH; R" = Tr
14, R = OH; R' = R" = H

Ura = uracil-1-yl
Cyt = cytosine-1-yl

None of the fully deprotected chloromethylene nucleosides showed activity against HIV-1 and HIV-2 in human T-lymphocyte (CEM/0) cells.

Experimental Section

Thin layer chromatography (TLC) was carried out on the F 1500 LS 254 silica gel plates (Schleicher & Schull, Germany). As solvent systems were used: ethyl acetate-hexane, 1:2 (A); ethyl acetate-hexane, 7:5 (B); chloroform-methanol, 9:1 (C); chloroform-methanol, 4:1 (D), n-butanol-acetic acid-water, 5:3:2 (E). Column chromatography was performed on silica gel L 40/100 μ and 100/400 μ (Chemapol, Czechoslovakia). The solutions of compounds in organic solvents were dried with anhydrous sodium sulfate for 4 h. The reactions were performed at room temperature, unless stated otherwise. All new compounds synthesized gave satisfactory FAB MS and elemental analysis.

1-[2,5(and 3,5)-di-O-trityl-β-D-*erythro*-pentofuran-3(and 2)-ulosyl]uracils (**5** and **6**) were prepared[3] from **2** and **3** in yields of 89 and 85%, respectively; mp's and ^{1}H NMR spectra for both compounds were in accord with previously published data[5].

1-[2,5(and 3,5)-di-O-trityl-3(and 2)-deoxy-3(and 2)-chloromethylene-β-D-*erythro*-pentofuranosyl]-[3'E(and 2'Z]uracils (7 and 8). A solution of butyllithium (1.5 M in hexane, 0.91 mL, 1.375 mmol) was added dropwise over 10 min to a suspension of (chloromethyl)triphenylphosphonium chloride (0.56 g, 1.65 mmol) in anh. THF (2 mL) at -30 °C under argon. The resulting clear, bright yellow mixture was stirred at 0 °C for 0.5 h, cooled to -30 °C and then a solution of **5** (0.2 g, 0.275 mmol) in anh. THF (3 mL) was added slowly. The mixture was allowed to warm to room temperature and after 2 h saturated NH_4Cl-H_2O was added. The aqueous phase was extracted with ethyl acetate (3x30 mL) and organic extracts were combined, dried and evaporated. The residue was chromatographed, using a linear ethyl acetate gradient (10→60%,v/v, 2 x 500 mL) in hexane to yield 0.15 g (69%) of **7** as a foam which was crystallized from ethyl acetate with hexane added until slight turbidity. Mp >300 °C (decomp.); R_f 0.35 (A); UV (MeOH) λ_{max} nm (ε): 261 (17,900), λ_{min} nm (ε): 245 (9,900).

In a similar way, starting from **6** (0.15 g, 0.206 mmol), 85 mg (53%) of **8** was prepared as an amorphous powder. Mp >300 °C (decomp.); R_f 0.28 (A); UV (MeOH) λ_{max} nm (ε): 260 (13,800), λ_{min} nm (ε): 245 (9,400).

[3'E(and 2'Z)]-1-(3(and 2)-deoxy-3(and 2)-chloromethylene-β-D-*erythro*-pentofuranosyl)uracils (9 and 10). A suspension of **7** (0.26 g, 3.42 mmol) in AcOH (80%, 20 mL) was heated at 80 °C for 6 h and evaporated. The residue was coevaporated with toluene and purified by column chromatography, using a linear methanol gradient (0→10%, v/v, 2 x 250 mL) in chloroform. The product containing fractions were collected, evaporated, dissolved in chloroform (0.1 mL) and precipitated in hexane (100 mL) to give 75 mg (80%) of **9** as an amorphous powder. Mp 180-182 °C; R_f 0.18 (C); UV (MeOH) λ_{max} nm (ε): 260 (11,200), λ_{min} nm (ε): 230 (2,100); CD (MeOH), λ nm ($[\Theta]\cdot10^{-3}$): 268 (+5.48), 240 sh (-10.3), 214 (-27.4), λ nm, [Θ] = 0: 282, 257. ^{1}H NMR (DMSO-d_6): 7.91 (d, 1H, $J_{5,6}$ = 8.0; H-6), 6.24 (t, 1H, $J_{2',6'}$ = $J_{4',6'}$ = 2.25; H-6'), 6.13 (d, 1H, J = 6.0; 2'-OH), 5.70 (d, 2H; $J_{1',2'}$ = 8.0; H-1' and H-5), 5.24 (br.s, 1H; 5'-OH), 4.73 (br.t, 1H, $J_{4',5'}$ = 2.25, $J_{4'5''}$ = ~1.0; H-4'), 4.57 (m, 1H, $J_{2',4'}$ = 2.25, H-2'), 3.74 (dd, 1H, $J_{5',5''}$ = 11.0; H-5').

In a similar way, starting from **8** (0.23 g, 0.303 mmol), 46 mg (55%) of **10** was prepared. Mp 122-124 °C (from MeOH); R_f 0.13 (A); UV (MeOH) λ_{max} nm (ε): 259 (11,300), λ_{min} nm (ε): 230 (1,900); CD (MeOH), λ nm ($[\Theta]\cdot10^{-3}$): 259 (-15.5), λ nm, [Θ] = 0: 285. ^{1}H NMR (DMSO-d_6): 7.48 (d, 1H, $J_{5,6}$ = 8.0; H-6), 6.52 (t, 1H; $J_{1',3'}$ = 2.25; H-1'), 6.49 (t, 1H, $J_{1',6'}$ = $J_{3',6'}$ = 2.25; H-6'), 5.64 (d, 1H, H-5), 5.90 (d, 1H, J = 6.5; 3'-OH), 4.52 (t, 1H; J = 5.5; 5'-OH), 4.64 (br.t, 1H, H-3'), 3.75-3-52 (m, 3H; H-4', H-5' and H5").

(3'E)-1-(3-deoxy-3-chloromethylene-β-D-*erythro*-pentofuranosyl)cytosine (12). Compound **7** was converted into **11** essentially as described[4] in 79% combined yield. Mp 260-262 °C (from chloroform-MeOH, 1:1); R_f 0.32 (C).

Compound **11** was detritylated as above to give **12** as acetate in 65%. Mp 118-120 °C; R_f 0.31 (D); UV (MeOH) λ_{max} nm (ε): 271 (9,500), λ_{min} nm (ε): 256 (8,400).

The solution of **12** (acetate) (26 mg, 0.078 mmol) in a mixture of methanol-H_2O (1:2) was applied to a column of Dowex 1x2 (OH^--form), which was eluted with methanol-H_2O (1:1, 100 mL). Appropriate fractions were collected, evaporated, dissolved in methanol (0.1 mL) and precipitated in ether (100 mL) to give 23 mg (96%) of **12** as a free base. Mp 114-117 °C; R_f 0.31 (D); UV (MeOH) λ_{max} nm (ε): 271 (9,700), λ_{min} nm (ε): 256 (8,300); CD (MeOH), λ nm ($[\Theta]\cdot10^{-3}$): 270 (+6.0), 220 (-11.5), λ nm, [Θ] = 0: 315, 242. ^{1}H NMR (DMSO-d_6): 7.83 (d, 1H, $J_{5,6}$ = 7.5; H-6), 7.36 (br.d, 2H, NH_2), 6.26 (t, 1H, $J_{2',6'}$ = $J_{4',6'}$ = 2.5; H-6'), 6.02 (d, 1H, J = 6.5; 2'-OH), 5.81 (d, 1H, H-5), 5.72 (d, 1H, $J_{1',2'}$ = 7.8; H-1'), 5.19 (t, 1H, J = 5.7; 5'-OH), 4.71 (t, 1H, $J_{4',2'}$ = 2.5; H-4'), 4.63 (m, 1H; H-2'), 3.67 (m, 2H; H-5' and H-5").

(3'E)-1-(5-O-Trityl-3-deoxy-3-chloromethylene-β-D-*erythro*-pentofuranosyl)cytosine (13). A mixture of **12** (0.06 g, 0.22 mmol), triphenylmethyl chloride (0.122 g, 0.44 mmol) and DMAP (1 mg, 0.002 mmol) in anh. pyridine (1 mL) was kept for 2 days and then poured into a mixture of ice and water (100 mL). The resulting precipitate was filtered off, dried and chromatographed with a linear methanol gradient (0→15%, v/v, 0.5 L) in chloroform to yield 90 mg (78%) of **13** as an amorphous powder. Mp 130-132 °C; R_f 0.43 (C); UV (MeOH) λ_{max} nm (ε): 271 (11,700), λ_{min} nm (ε): 255 (10,600).

(3'E)-1-(3-deoxy-3-chloromethylene-β-D-arabinopentofuranosyl)cytosine (14). Compound **13** (120 mg, 0.226 mmol) was dissolved in dioxane (1 mL) and (diethylamino)sulfur trifluoride (67 μL, 0.451 mmol) was added under argon. After stirring for 1 h, the reaction mixture was diluted with H_2O (1 mL) and neutralized (pH 7) with $BaCO_3$. The powder of $BaCO_3$ was filtered off, washed 3 times with di-

oxane and the filtrate was evaporated to dryness. The precipitate was dissolved in a mixture of chloroform-methanol (0.1 mL) and precipitated in ether (100 mL) to give 100 mg (92 %) of 1-(5-O-trityl-3-deoxy-3-chloromethylene-O^2,2-anhydro)-(3'E)-cytosine as an amorphous powder, which was used in the next step without purification; mp >236 °C; R_f 0.58 (E); UV (MeOH) λ_{max} nm (ε): 263 (6,700), λ_{min} nm (ε): 248 (5,000). The crude product was suspended in AcOH (80%, 10 mL) and the mixture was stirred for 2 h. The solvent was evaporated, and the residue was dissolved in ethanol (4 mL) and treated with 1N NaOH solution (2 mL) for 20 h. The reaction mixture was neutralized with AcOH in ethanol (2.3 mL, 1:1, v/v) and evaporated. The residue was purified with column chromatography, using a linear methanol gradient (5→20%, v/v, 2 x 300 mL) in chloroform to yield 34 mg (63%) of **14** as acetate. Mp 147-149 °C; R_f 0.37 (D). The solution of **14** (acetate) (34 mg, 0.102 mmol) in a mixture of methanol/H_2O (1:2) was applied to a column of Dowex 1x2 (OH^-) (10 mL) , which was eluted with H_2O (100 mL). The product containing fractions were collected, evaporated, dissolved in methanol (0.1 mL) and precipitated in ether (100 mL) to give 28 mg (91%) of **14** as a free base. Mp 129-131 °C; R_f 0.37 (D); UV (MeOH) λ_{max} nm (ε): 273 (13,300), λ_{min} nm (ε): 253 (10,000); CD (MeOH), λ nm ($[\Theta]\cdot10^{-3}$): 270 (+20.2), 216 (-68.4), λ nm, [Θ] = 0: 300, 235. ^{1}H NMR (DMSO-d_6): 7.72 (d, 1H, $J_{5,6}$ = 7.5; H-6), 7.10 (br.d, 2H, NH_2), 6.79 (d, 1H, $J_{4',6'}$ = 2.2; H-6'), 5.69 (d, 1H, H-5), 5.82 (d, 1H, $J_{1',2'}$ = 3.0; H-1'), 5.51 (d, 1H, J = 5.8; 2'-OH), 5.40 (t, 1H, J = 5.5; 5'-OH), 4.71 (m, 1H, H-4'), 4.44 (dd, 1H; H-2'), 3.71 (m, 2H; H-5' and H-5").

Acknowledgement. I.A.M. is deeply grateful to the Alexander von Humboldt-Stiftung (Bonn - Bad-Godesberg, Germany) for partial financial support.

REFERENCES

1. Samano, V.; Robins, M.J. *Can. J. Chem.* **1993**, *71*, 186-191.; Czernecki, S.; Ezzitouni, A. *Tetrahedron Lett.* **1993**, *34*, 315-318.; Svendsen, M.L.; Wengel, J.; Dahl, O.; Kirpekar, F.; Roepstorff, P. *Tetrahedron* **1993**, *49*, 11341-11352.; Ioannidis, P.; Classon, B.; Samuelsson, B.; Kvarnström, I. *Nucleosides & Nucleotides* **1993**, *12*, 449-462.; Hassan, A.E.A.; Shuto, S.; Matsuda, A. *Nucleosides & Nucleotides* **1994**, *13*, 197-211.
2. Machida, H.; Sakata, S.; Ashida, N.; Takenuki, K.; Matsuda, A. *Antiviral Chem. Chemother.* **1993**, *4*, 11-17.; Matthews, D.P.; Persichetti, R.A.; Sabol, J.S.; Stewart, K.T.; McCarthy, J.R. *Nucleosides & Nucleotides* **1993**, *12*, 115-123.; Robins, M.J.; Samano, V.; Zhang, W.; Balzarini, J.; De Clercq, E.; Borchardt, R.T.; Lee, Y.; Yuan, C.-S. *J. Med. Chem.* **1992**, *35*, 2283-2293.
3. Hansske, F.; Madej, D.; Robins, M.J. *Tetrahedron* **1984**, *40*, 125-135.
4. Divakar, R. J.; Reese C. B. *J. Chem. Soc., Perkin Trans. I.* **1982**, No. 5, 1171-1176.
5. Cook, A.F.; Moffatt, J.G. *J. Am. Chem. Soc.* **1967**, *89*, 2697-2705.