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HIV-1 non-nucleoside reverse transcriptase inhibitors: incorporation of benzylphosphonate moiety for solubility improvement

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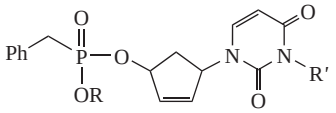
Benzylphosphonates of 5′-norcarbocyclic analogue of 2′,3′-dideoxy-2′,3′-didehydrouridine and its N^3-benzyl derivatives with different substituents at the phosphorus atom were designed and synthesized in attempt to improve solubility of potential non-nucleoside reverse transcriptase inhibitor. Solubility of the most hydrophilic representative was 400 mg in 100 ml of 1% DMSO solution in water. The target phosphonates were examined against HIV-1 reverse transcriptase with K_I for all compounds being higher than 100 μM.



R = H, Et, Bn
R′ = H, Bn

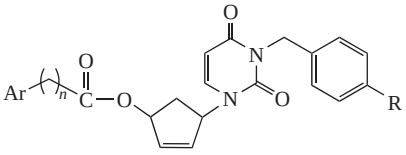
According to WHO to date, AIDS has killed more than 39 million lives, in late 2013 the number of people infected with HIV was about 36 million.[1] Currently, the main technique in the treatment of HIV infection is the so-called highly active antiretroviral therapy (HAART), wherein a pharmacological agent is applied as a cocktail of several antivirals [commonly reverse transcriptase (RT) and protease inhibitors]. There are two main classes of HIV RT inhibitors, the most studied and widely used in anti-HIV therapy target enzyme.[2] Nucleoside RT inhibitors (NRTIs) are analogues of natural nucleosides lacking free 3′-OH group and mechanism of their action consists in the intracellular triphosphorylation, followed by incorporation into a growing strand of proviral DNA and, consequently, the termination of its synthesis. Non-nucleoside RT inhibitors (NNRTIs), in contrast to the NRTIs, do not require intracellular triphosphorylation. Once in the cell, they bind to hydrophobic 'pocket' of HIV RT, alter the structure of the active centre and thus effectively inhibit viral replication *via* a noncompetitive mode. Currently there are more than 50 classes of compounds with different chemical structures, emerged as NNRTIs, but only five approved for therapy by FDA (efavirenz, delaverdin, nevirapine, etravirine and rilpivirine).[3] The main disadvantages of NNRTI are drug toxicity, side effects and the rapid emergence of resistant strains of the virus, resulting in the need to replace drug. Thus, new effective low-toxic NNRTIs with resistance profile, different from the existing ones are in demand.

A major problem of many NNRTI candidates is low solubility which results in almost zero oral bioavailability. The reason is that the molecules required for binding to the hydrophobic pocket of HIV RT should possess high lipophilicity. A lot of different modifications of potential HIV NNRTI molecules were tried with varying effectiveness to overcome this problem; various hydrophilic groups (hydroxyl, amino, nitro, sulfo, sulfamino, *etc*.) were used.[3] This work is devoted to assessing the prospects of phosphonate fragment introduction into HIV NNRTI molecule which was not applied for this purpose previously.

The 'phosphonate strategy' has been developed for HIV NRTIs. One of the major problems of the latter is the low efficiency of the three-stage intracellular phosphorylation required for the manifestation of the inhibitory activity of the drug. Introduction of phosphonate moiety which is isosteric mimic of natural phosphate residue allows one to overcome the first stage (of three ones), and to obtain a nucleoside monophosphate analogue which is resistant to dephosphorylating enzymes. This analogue is suitable for subsequent phosphorylation. The most successful example of such a strategy is the Tenofovir.[4] Strategy of introduction of the phosphonate moiety into AZT molecule led to a design of its depot forms. It was shown that the 5′-phosphonates of AZT are able to generate AZT in the body. In this case, pharmacokinetic parameters of released nucleoside are significantly better than when AZT is administered itself (intact). Hence depot forms allow one to significantly reduce the toxicity of the drug while maintaining its anti-HIV activity.[5–9] Other examples of successful biological applications of the phosphonate moiety are phosphonoxins and polyoxin phosphonate analogues with antimicrobial activity.[10–13] Methylene phosphonate analogues of adenine dinucleoside can inhibit inosine monophosphate dehydrogenase.[14] Thus, the presence of the phosphonate moiety in different biologically active molecules leads to improved efficiency and/or reduced toxicity.

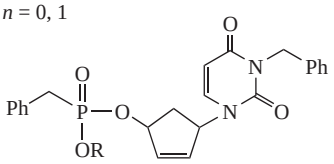
Recently, we reported that 3,4-substituted 5′-norcarbocyclic analogues of 2′,3′-dideoxy-2′,3′-didehydrouridine **A** act as NNRTIs (IC_{50} 5–50 μM) on enzyme of the wild and mutant virus strains.[15,16] However, the antiviral potential of these compounds is substantially reduced because of poor solubility, which was the prerequisite for the design of their phosphonate analogues **B**.

In this work, commercially available diethyl benzylphosphonate was used as a donor of the phosphonate moiety. Two methods of the phosphonate fragment administration into the 5′-norcarbocyclic uracil analogues were evaluated (Scheme 1). Treatment of diethyl benzylphosphonate with phosphorus pentachloride gave the corresponding dichloride whose subsequent reaction with



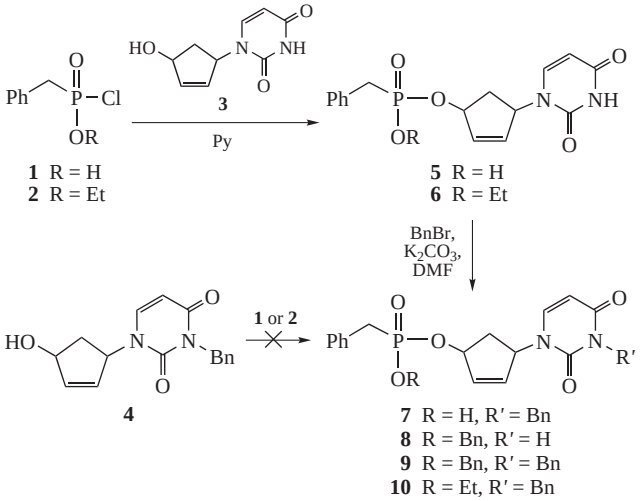
A

Ar = Ph, 3,5-$Me_2C_6H_3$, 3,5-$Cl_2C_6H_3$
R = H, Me
n = 0, 1



B

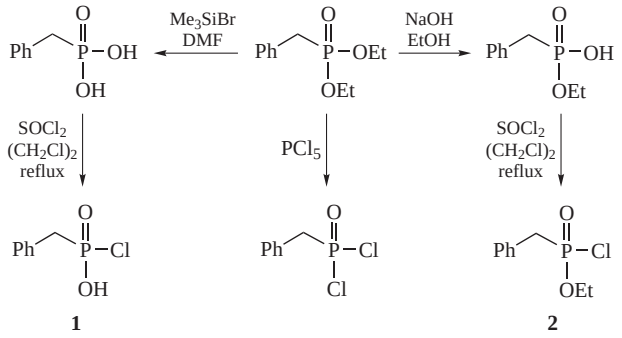
R = H, Et, Bn



Scheme 2

1-(4-hydroxycyclopent-2-en-1-yl)uracil afforded target product in 10–15% yield.

The more effective was the use of monochlorides **1** and **2**, which were prepared by treatment of diethyl benzylphosphonate with bromotrimethylsilane or sodium hydroxide followed by refluxing the resulting phosphonic acid or its monoethyl ester with thionyl chloride in 1,2-dichloroethane.[†,16] The target reagents were used without further purification in the reaction with 1-(4-hydroxycyclopent-2-en-1-yl)uracil **3**[17] in pyridine (Scheme 2).[‡] After purification by anion exchange chromatography on DEAE-cellulose and then by reverse phase chromatography (C_{18} column), the yields of target phosphonates **5** and **6** were 30 and 37%, respectively. Attempts to synthesize benzylphosphonates of 3-benzyl-1-(4-hydroxycyclopent-2-en-1-yl)uracil **4** by reaction with phosphonyl chlorides **1** and **2** were unsuccessful. However, products **7**–**10** were accessed by treatment of compounds **5** and **6** with benzyl bromide and potassium carbonate in dry DMF.[15]



Scheme 1

† Commercial reagents (Acros, Aldrich, and Fluka) were used; anhydrous solvents were purified according to the standard procedures. Column chromatography was performed on Silica Gel 60 0.040–0.063 mm (Merck) columns. Preparative liquid chromatography (PLC) was performed on Silica Gel 60 F_{254}. TLC was performed on Silica Gel 60 F_{254}. NMR spectra were recorded on a Bruker Avance 400 spectrometer (400 MHz for ^{1}H and 162 MHz for ^{31}P). HRMS spectra were measured on Bruker micrOTOF II or maXis instruments using electrospray ionization.

Benzylphosphonic acid and ethyl benzylphosphonate chlorides **1** *and* **2**. Benzylphosphonic acid (800 mg, 4.6 mmol) or ethyl hydrogen benzylphosphonate (500 mg, 2.5 mmol) were refluxed with thionyl chloride (20–40 ml) in 1,2-dichloroethane (30–50 ml) for 4 h. The solvent was then removed, the residue was dissolved in tetrachloromethane and twice evaporated. Phosphonyl chlorides were used without further purification.

1-(4-Hydroxycyclopent-2-en-1-yl)uracil **3** was obtained according to published procedures.[18]

‡ *4-[2,4-Dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]cyclopent-2-en-1-yl benzylphosphonate* **5**. Benzylphosphonyl chloride (1.3 mmol) was added to a solution of 1-(4-hydroxy-2-cyclopent-2-en-1-yl)uracil **3** (200 mg, 1.03 mmol) in dry pyridine (10 ml). The mixture was stirred for 18 h at room temperature. After the solvent was evaporated and the residue was separated by column chromatography on DEAE-cellulose (aq. NH_4HCO_3, 0–0.2 M) followed by column reversed phase chromatography (silica gel C_{18}, eluted with water), product **5** was obtained as a colourless syrup (107 mg, 0.31 mmol, 30%). R_f = 0.35 (dioxane–aq. NH_3, 4 : 1). ^{1}H NMR (DMSO-d_6) δ: 1.42–1.38 (m, 1H, H_b-5′), 2.63–2.59 (m, 1H, H_a-5′), 2.79–2.74 (m, 2H, CH_2P), 4.96–4.93 (m, 1H, H-4′), 5.33–5.30 (m, 1H, H-1′), 5.56–5.54 (d, 1H, H-5, *J* 7.98 Hz), 5.79–5.77 (m, 1H, H-3′), 6.08–6.06 (m, 1H, H-2′), 7.11–7.09 (m, 1H, H-6), 7.20–7.18 (m, 2H, 2″,6″-H_{Ph}), 7.23–7.26 (m, 2H, 3″,5″-H_{Ph}), 7.23–7.26 (m, 1H, 4″-H_{Ph}). ^{31}P NMR (DMSO-d_6) δ: 18.37. HRMS, *m*/*z*: 347.0799 $[M-H]^-$ (calc. for $C_{16}H_{17}N_2O_5P$, *m*/*z*: 347.0802).

*4-[2,4-Dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]cyclopent-2-en-1-yl ethyl benzylphosphonate* **6** *(mixture of diastereomers)*. *O*-Ethyl benzylphosphonyl chloride (0.75 mmol) was added to a solution of 1-(4-hydroxy-2-cyclopent-2-en-1-yl)uracil **3** (145 mg, 0.75 mmol) in dry pyridine (10 ml). The mixture was stirred for 18 h at room temperature, then the solvent was evaporated and the residue was subjected to column chromatography ($CHCl_3$–MeOH, 98 : 2) to give **6** as a colourless syrup (104 mg, 0.27 mmol, 37%). R_f = 0.4. ^{1}H NMR ($CDCl_3$) δ: 1.28–1.23 (m, 3H, Me), 1.50–1.46, 1.65–1.62 (2m, 1H, H_b-5′), 2.75–2.72, 2.88–2.84 (2m, 1H, H_a-5′), 3.20–3.13 (m, 2H, CH_2), 4.06–4.01 (m, 2H, CH_2P), 5.29–5.26 (m, 1H, H-4′), 5.58–5.57 (m, 1H, H-1′), 5.56–5.54 (m, 1H, H-5), 5.87–5.82 (m, 1H, H-3′), 6.02–6.01, 6.18–6.16 (2m, 1H, H-2′), 7.07–7.05 (m, 1H, H-6), 7.26–7.22 (m, 5H, Ph), 9.49 (s, 1H, NH). ^{31}P NMR (DMSO-d_6) δ: 24.31, 24.20. HRMS, *m*/*z*: 399.1073 $[M+Na]^+$ (calc. for $C_{18}H_{21}N_2O_5P$, *m*/*z*: 399.1080).

*4-[2,4-Dioxo-3-benzyl-4-hydropyrimidin-1(2*H*)-yl]cyclopent-2-en-1-yl benzylphosphonate* **7**. Potassium carbonate (18 mg, 0.13 mmol) and BnBr (17 μl, 0.13 mmol) were added to a solution of **5** (35 mg, 0.1 mmol) in dry DMF (10 ml). The reaction mixture was stirred for 2 h at room temperature, the solvent was evaporated, and the residue was separated by PLC (dioxane–aq. NH_3, 4 : 1) to give **7** as a pale yellow oil (11 mg, 0.025 mmol, 25%). R_f = 0.31. ^{1}H NMR (DMSO-d_6) δ: 1.46–1.37 (m, 1H, H_b-5′), 2.68–2.64 (m, 1H, H_a-5′), 2.91–2.87 (m, 2H, CH_2P), 4.98 (s, 2H, CH_2N), 5.03–5.00 (m, 1H, H-4′), 5.39–5.36 (m, 1H, H-1′), 5.75–5.73 (d, 1H, H-5, *J* 7.98 Hz), 5.86–5.85 (m, 1H, H-3′), 6.11–6.09 (m, 1H, H-2′), 6.63 (s, 1H, OH), 6.87 (m, 1H, H-6), 7.30–7.24 (m, 8H, 2Bn), 7.59–7.52 (m, 2H, 4″,4‴-Bn). ^{31}P NMR (DMSO-d_6) δ: 19.99. HRMS, *m*/*z*: 461.1233 $[M+Na]^+$ (calc. for $C_{23}H_{23}N_2O_5P$, *m*/*z*: 461.1237).

*4-[2,4-Dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)]cyclopent-2-en-1-yl benzylphosphonate* **8** *(mixture of diastereomers)* was obtained as a side product in the synthesis of **7**. The purification by PLC ($CHCl_3$–MeOH, 9 : 1) gave product **8** as a pale yellow oil (9 mg, 20%). R_f = 0.36. ^{1}H NMR ($CDCl_3$) δ: 1.43–1.35 (m, 1H, H_b-5′), 2.64–2.60 (m, 1H, H_a-5′), 3.15–3.09 (m, 2H, CH_2P), 5.01–4.93 (m, 2H, CH_2O), 5.15–5.12 (m, 1H, H-4′), 5.46–5.44 (m, 1H, H-1′), 5.54–5.52 (d, 1H, H-5, *J* 8.05 Hz), 5.75–5.73 (m, 1H, H-3′), 5.95–5.91 (m, 1H, H-2′), 6.93–6.89 (m, 1H, H-6), 7.29–7.20 (m, 10H, 2Ph), 8.52 (s, 1H, NH). ^{31}P NMR (DMSO-d_6) δ: 24.74, 24.91. HRMS, *m*/*z*: 461.1236 $[M+Na]^+$ (calc. for $C_{23}H_{23}N_2O_5P$, *m*/*z*: 461.1237).

Reaction of phosphonate **5** with excess of benzyl bromide (1.3 equiv.) gave a mixture of products **7**–**9**. Phosphonates **7**–**9** were isolated by preparative liquid chromatography, elution with chloroform–methanol (9 : 1); yields of products **8** and **9** were 20 and 15%, respectively. To isolate target product **7** (yield 15%), we used additional elution with aqueous ammonia–dioxane (4 : 1). Treatment of compound **6** with two-fold excess of benzyl bromide furnished product **10** in 31% yield. Due to chirality of the phosphorus atom, compounds **6**, **8**, **9** and **10** were mixtures of diastereomers.

Thus, we have synthesized benzylphosphonates of 5′-norcarbocyclic analogue of 2′,3′-dideoxy-2′,3′-didehydrouridine and its N^3-benzyl derivatives with different substituents at the phosphorus atom. Compounds **8** and **9** carrying benzyl residue were more hydrophobic (solubility was 400 mg in 100 ml of 30 and 33% DMSO in water, respectively) in comparison with **6** and **10** bearing ethoxy substituent at the phosphorus atom (solubility was 400 mg in 100 ml of 18 and 20% DMSO in water, respectively). The most hydrophilic were phosphonates **5** and **7** carrying free hydroxyl group at the phosphorus atom (solubility was 400 mg in 100 ml of 1 and 2% DMSO in water, respectively). Note that solubility in water was poor even for compounds **5** and **7**.

The inhibitory properties of the synthesized phosphonates **5**–**10** were evaluated *in vitro* against wild type HIV-1 reverse transcriptase. Experiments to determine the inhibition (K_I) were performed according to previously published method,[15,16] where activated DNA was used as the primer-template and the efficiency of the enzyme in the elongation was estimated by incorporation of radioactively labeled substrate, followed by calculation of K_I by Dixon method.[19] K_I for all of the tested compounds was more than 100 μM, which means that no significant activity was found in this experiment.

*4-[2,4-Dioxo-3-benzyl-3,4-dihydropyrimidin-1(2*H*)-yl]cyclopent-2-en-1-yl benzylphosphonate* **9** *(mixture of diastereomers)* was obtained as a side product in the synthesis of **7**. The purification by PLC ($CHCl_3$–MeOH, 9 : 1) gave product **9** as a pale yellow oil (8 mg, 15%). R_f = 0.48. ^{1}H NMR ($CDCl_3$) δ: 1.39–1.36 (m, 1H, H_b-5′), 2.62–2.58 (m, 1H, H_a-5′), 3.13–3.03 (m, 2H, CH_2P), 5.05–5.03 (m, 2H, CH_2O), 5.00–4.94 (m, 2H, CH_2O), 5.14–5.11 (m, 1H, H-4′), 5.59–5.44 (m, 1H, H-1′), 5.46–5.42 (d, 1H, H-5, *J* 8.00 Hz), 5.72–5.71 (m, 1H, H-3′), 5.93–5.87 (m, 1H, H-2′), 6.88–6.84 (m, 1H, H-6), 7.24–7.21 (m, 15H, 3Ph). ^{31}P NMR (DMSO-d_6) δ: 24.68, 24.86. HRMS, *m*/*z*: 551.1702 $[M+Na]^+$ (calc. for $C_{30}H_{29}N_2O_5P$, *m*/*z*: 551.1706).

*4-[2,4-Dioxo-3-benzyl-3,4-dihydropyrimidin-1(2*H*)-yl)]cyclopent-2-en-1-yl ethyl benzylphosphonate* **10** *(mixture of diastereomers)* was prepared from reactant **6** using the procedure described for **7**. Purification by column chromatography on silica gel ($CHCl_3$–MeOH, 98 : 2) afforded product **10** as a colourless oil (25 mg, 31%). R_f = 0.42. ^{1}H NMR ($CDCl_3$) δ: 1.28–1.26 (m, 6H, 2Me), 1.47–1.45 (m, 1H, H_b-5′), 1.64–1.63 (m, 1H, H_b-5′), 2.74–2.70 (m, 1H, H_a-5′), 2.84–2.82 (m, 1H, H_a-5′), 3.19–3.13 (m, 4H, 2CH_2), 4.07–4.02 (m, 4H, 2CH_2P), 5.14–5.09 (m, 4H, 2CH_2Bn), 5.26–5.23 (m, 2H, 2H-4′), 5.60–5.55 (m, 2H, 2H-1′), 5.73–5.70 (m, 2H, 2H-5), 5.86–5.82 (m, 2H, 2H-3′), 6.00–5.98 (m, 1H, H-2′), 6.17–6.15 (m, 1H, H-2′), 7.03–7.01 (m, 2H, 2H-6), 7.26–7.22 (m, 16H, 2Ph), 7.48–7.46 (m, 4H, 2Ph). ^{31}P NMR (DMSO-d_6) δ: 24.26, 24.14. HRMS, *m*/*z*: 489.1548 $[M+Na]^+$ (calc. for $C_{25}H_{27}N_2O_5P$, *m*/*z*: 489.1550).

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