

**SYNTHESIS OF 5-SUBSTITUTED 2'-DEOXYURIDINE-5'-
PHOSPHONATE ANALOGUES AND EVALUATION OF THEIR
ANTIVIRAL ACTIVITY**

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SYNTHESIS OF 5-SUBSTITUTED 2'-DEOXYURIDINE ANALOGUES AND EVALUATION OF THEIR ANTIVIRAL ACTIVITY

A small series of 5-(hetero)aryl-modified nucleoside phosphonates was synthesized via an 8-step procedure including a Wittig reaction and Suzuki-Miyaura coupling. An unanticipated anomerisation during phosphonate deprotection allowed us to isolate both anomers of the 5-substituted 2'-deoxyuridine phosphonates and assess their antiviral activity against a broad panel of viruses.

Keywords: antiviral nucleoside analogues, pyrimidine modification, phosphonates, α -nucleosides, antiviral activity

Introduction

Antiviral drugs have become crucial in the management of several viral infections, including human HSV, HIV, HBV, HCV and cytomegalovirus (HCMV) infections. Prominent among these drugs are nucleoside analogues, which can act as potent antiviral agents owing to their ability to inhibit viral polymerases.¹ Many publications have appeared on the synthesis and antiviral activity of 5-modified 2'-deoxyuridine analogues. Several substituents have been introduced at C-5, including alkoxymethyl groups,² azoles³ and alkyloximes.³ Several analogues proved moderately to highly active against HSV. These results showed that modification at position 5 of 2'-deoxyuridine represents an interesting approach in the search for new anti-herpes agents. However, relatively few examples of 5-(hetero)aryl-modified nucleosides with promising antiviral activity have been reported.⁴ This may be due to the fact that these

nucleosides are not efficiently converted into their triphosphate form. The first step in this process, the phosphorylation of the nucleoside analogue into its 5'-monophosphate counterpart by nucleoside kinases, is often rate-limiting in the conversion to the active metabolite. One way to overcome this bottleneck is to devise prodrugs that are capable of delivering the nucleoside monophosphate intracellularly.^{5,6} Another approach to bypass the first phosphorylation step is to use phosphonate analogues, that, after intracellular conversion into their corresponding diphosphophosphonate forms, can exhibit antiviral activities. This led us to investigate a small series of 2'-deoxyuridine analogues that combine different aromatic substituents at position 5 of the base with a 5'-methylene phosphonate group.

Results and discussion

The synthesis of a series of 5-(hetero)aryl-modified nucleoside phosphonates started from 3'-*O*-*tert*-butyldimethylsilyl-2'-deoxy- β -D-uridine⁷ and is depicted in Scheme 1. Conversion of nucleoside **6** to the vinylic phosphonate **7** was accomplished following the two-step procedure described by Cosyn *et al.*⁸ Catalytic hydrogenation of **7** in the presence of Pd/C afforded phosphonate **8** which was selectively brominated at C-5 of the pyrimidine moiety using *N*-bromosuccinimide in DMF.⁹ Palladium-catalyzed cross-coupling with four commercial aryl and heteroaryl boronic acids gave access to **10-13**.¹⁰ After removing the silyl protecting group, deprotection of the phosphonate esters was performed using TMSBr in CH₂Cl₂. Concomitant anomerisation during this last step resulted for each analogue in a 2:1 mixture of the α - and β -isomer, which could be separated using RP-HPLC. Stereochemical assignment of compound **1a** was based on the results of a ROESY experiment (Figure 3). A clear rOe contact between H-4' and H-2'b (proton down) and a much weaker interaction between H-6 and H-2'b proved that

H-4' and H-6 were not positioned at the same side of the furanose ring. The β -configuration of the nucleobase was further established by the presence of a strong interaction between H-6 and H-5'a,b.

In an effort to synthesize the non-modified 2'-deoxyuridine-5'-phosphonate analogue, compound **8** was successively treated with a 1M TBAF solution in THF and TMSBr in CH₂Cl₂ (Scheme 2). In this case, attempts to separate the anomeric mixture using flash chromatography and RP-HPLC were unsuccessful. To avoid the anomerisation issue, we attempted to deprotect **18** in the presence of TMSBr under different reaction conditions. Following the reaction via ³¹P NMR, it was observed that anomerisation started immediately after addition of TMSBr. We learned by attempts at different temperatures that no reaction occurred under -20 °C, and that anomerisation started simultaneously with the phosphonate hydrolysis, even at low temperature. Also the addition of an acid scavenger (e.g., (trimethylsilyl)acetamide or 2,6-lutidine) could not prevent anomerisation.¹¹

All compounds were evaluated for their antiviral activity against a broad panel of viruses including HSV-1 (KOS), HSV-2 (G), vaccinia virus (VV), vesicular stomatitis virus (VSV), thymidine kinase deficient HSV-1 TK⁻ (KOS ACV^r), HCMV and VZV in HEL (human embryonic lung) cell cultures; and HIV-1 (III_B) and HIV-2 (ROD) in human T-lymphocyte (CEM) cell cultures. The activities of the compounds were compared with reference antiviral drugs: brivudin, cidofovir, acyclovir and ganciclovir.

None of the tested compounds showed toxicity to any of the tested cell lines. However, the final compounds **1a-4a** and **1b-4b** failed to show antiviral activity against HSV-1, HSV-2, VV, VSV, HSV-1 TK⁻ and different VZV strains. Also in the human T-lymphocyte (CEM) cell cultures, none of these compounds showed activity against

HIV-1 or HIV-2. Very weak antiviral activity was observed for the β -analogue **3a** and the α -analogue **2b** against HCMV Davies and HCMV AD-169, respectively, while compounds **2a** and **4a** showed weak activity against both HCMV strains (Table 1). The most active compound of this series, analogue **2a**, was at least 6 or 50 times less active than ganciclovir and cidofovir, respectively. The lack of biological activity of these derivatives might be attributed to several features, including 1) their inability to diffuse through the cell membrane; 2) their ineffective conversion to the corresponding diphosphosphonate analogue; or 3) their weak affinity for the target polymerases and/or lack of incorporation into viral RNA. If uptake into the cell would be the bottleneck, converting the phosphonates to an appropriate prodrug form could be considered.

Conclusion

In conclusion, this study described the synthesis, structural analysis and antiviral activity of a small series of 2'-deoxyuridine analogues that combine different aromatic substituents at position 5 of the base and a 5'-methylene phosphonate modification at the sugar moiety. All compounds were synthesized via an 8-step procedure, featuring a Wittig reaction and Suzuki-Miyaura coupling. An unexpected anomerisation during the last step of the synthesis allowed us to investigate the β - as well as the α -anomers of the corresponding phosphonates. None of these analogues exhibited significant antiviral activity

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Experimental section

Synthesis. General. All reagents were from standard commercial sources and of analytical grade. Precoated Merck silica gel F254 plates were purchased for TLC, spots were examined under ultraviolet light at 254 nm and further visualized by sulfuric acid-anisaldehyde spray. Column chromatography was performed on silica gel (63-200 μm , 60 \AA , Biosolve, Valkenswaard, The Netherlands). With the exception of the ^{13}C NMR spectrum of compound **1a**, which was recorded on a 500 MHz Bruker DRX apparatus, all NMR spectra were determined using a Varian Mercury 300 MHz spectrometer. Chemical shifts are given in ppm (δ) relative to the residual solvent signals, which in the case of $\text{DMSO-}d_6$ were 2.54 ppm for ^1H and 40.5 ppm for ^{13}C . Structural assignment was confirmed with COSY and DEPT. All signals assigned to hydroxyl groups were exchangeable with D_2O . Exact mass measurements were performed on a Waters LCT Premier XETM Time of flight (TOF) mass spectrometer equipped with a standard electrospray ionization (ESI) and modular LockSpray TM interface. Samples were infused in a $\text{CH}_3\text{CN}/\text{water}$ (1:1) mixture at 10 $\mu\text{L}/\text{min}$.

1-[3-*O*-*tert*-Butyldimethylsilyl-2,5,6-trideoxy-6-(diethoxyphosphinyl)- β -D-hex-5-enofuranosyl]-uracil (7). 2'-Iodoxybenzoic acid (550 mg, 1.97 mmol) was added to a solution of **6** (449 mg, 1.31 mmol) in CH_3CN (12 mL) and the resulted suspension was stirred at 80 $^\circ\text{C}$ for 6 h. After cooling in an ice bath (15 min), the solid was removed by filtration and washed with cold CH_3CN . The solvent was evaporated and the residue was co-distilled with toluene. The residue was dissolved in anhydrous DMSO (3.4 mL) and freshly prepared [(diethoxyphosphinyl)methyl]triphenylphosphorane in DMSO (3.4 mL) was added. After 20 h, the mixture was poured into water and extracted with CH_2Cl_2 . The combined organic layers were dried over MgSO_4 , filtered and concentrated

in vacuo. The residue was lyophilized to remove the remaining DMSO and purified on a silica gel column (CH₂Cl₂/MeOH 98:2) yielding 257 mg (41%) of **7** as a colourless solid. ¹H NMR (300 MHz, DMSO-*d*₆): δ 0.070 (6H, s, TBDMS), 0.87 (9H, s, TBDMS), 1.23 (6H, app dt, *J* = 1.5 Hz, *J* = 6.9 Hz, 2 x OCH₂CH₃), 2.13-2.22 (1H, m, H-2'a), 2.34-2.43 (1H, m, H-2'b), 3.92-4.03 (4H, m, 2 x OCH₂CH₃), 4.20-4.25 (1H, m, H-4'), 4.38-4.45 (1H, m, H-3'), 5.64 (1H, d, *J* = 7.8 Hz, CH=CH), 5.96-6.09 (1H, m, H-6'), 6.16 (1H, dd, *J* = 6.0 Hz, *J* = 7.5 Hz, H-1'), 6.60-6.75 (1H, m, H-5'), 7.68 (1H, d, *J* = 8.4 Hz, CH=CH), 11.34 (1H, s, 3-NH). ³¹P NMR (DMSO-*d*₆): δ 17.21. ¹³C NMR (75 MHz, DMSO-*d*₆): δ -5.02, -4.87 (TBDMS), 16.10, 16.18 (OCH₂CH₃), 17.60 (TBDMS), 25.57 (TBDMS), 61.21, 61.31 (OCH₂CH₃), 74.36 (C-3'), 84.27 (C-1'), 85.27 (C-4', d, *J* = 23 Hz), 102.07 (C-5), 119.70 (C-6', d, *J* = 182 Hz), 141.38 (C-6), 147.81 (C-5', d, *J* = 5 Hz), 150.29 (C-2), 162.98 (C-4). Exact mass (ESI-MS) for C₂₀H₃₆N₂O₇PSi [M+H]⁺ found, 475.2035; calcd, 475.2024.

1-[3-*O*-*tert*-Butyldimethylsilyl-2,5,6-trideoxy-6-(diethoxyphosphinyl)-β-D-hexofuranosyl]-uracil (8**)**. To a solution of compound **7** (257 mg, 0.54 mmol) in MeOH (8 mL) was added 10% Pd/C. The reaction mixture was stirred under hydrogen atmosphere overnight. The catalyst was removed by filtration through Celite and the filtrate was evaporated to yield pure compound **8** (250 mg, 97%) as a colourless solid. ¹H NMR (300 MHz, DMSO-*d*₆): δ 0.084 (6H, app d, *J* = 2.4 Hz, TBDMS), 0.87 (9H, s, TBDMS), 1.22 (6H, t, *J* = 6.9 Hz, 2 x OCH₂CH₃), 1.65-1.86 (4H, m, H-5'a, H-5'b, H-6'a and H-6'b), 2.00-2.15 (1H, m, H-2'a), 2.22-2.31 (1H, m, H-2'b), 3.68-3.72 (1H, m, H-3'), 3.92-4.04 (4H, m, 2 x OCH₂CH₃), 4.23-4.29 (1H, m, H-4'), 5.62 (1H, d, *J* = 8.1 Hz, CH=CH), 6.10 (1H, t, *J* = 6.6 Hz, H-1'), 7.59 (1H, d, *J* = 8.4 Hz, CH=CH), 11.32 (1H, s, 3-NH). ³¹P NMR (DMSO-*d*₆): δ 31.72. ¹³C NMR (75 MHz, DMSO-*d*₆): δ -5.04, -4.99 and -4.73 (TBDMS), 16.21 and 16.28 (OCH₂CH₃)₂, 17.58 (TBDMS), 20.97 (C-6',

d, $J=139$ Hz), , 25.69 (C5', d, $J=12$ Hz), 60.90, 60.97 (OCH₂CH₃)₂, 73.84 (C-3'), 84.95 (C-4', d, $J=16$ Hz), 83.61 (C-1'), 102.10 (C-5), 141.03 (C-6), 150.40 (C-2), 163.11 (C-4). Exact mass (ESI-MS) for C₂₀H₃₈N₂O₇PSi [M+H]⁺ found, 477.2119; calcd, 477.2180.

1-[3-*O*-*tert*-Butyldimethylsilyl-2,5,6-trideoxy-6-(diethoxyphosphinyl)-β-D-

hexofuranosyl]-5-bromouracil (9). To a solution of compound **8** (950 mg, 1.99 mmol) in DMF (15 mL) was added *N*-bromosuccinimide (NBS, 390 mg, 2.19 mmol) under N₂. The reaction mixture was stirred at room temperature for 16 hours. DMF was removed *in vacuo* and the residue was purified by column chromatography (CH₂Cl₂/MeOH 97:3) to afford **9** (538 mg, 49%) as a white foam. ¹H NMR (300 MHz, CDCl₃): δ 0.082 (6H, app d, $J=1.8$ Hz, TBDMS), 0.89 (9H, s, TBDMS), 1.34 (6H, t, $J=6.9$ Hz, 2 x OCH₂CH₃), 1.82-2.02 (4H, m, H-5'a, H-5'b, H-6'a and H-6'b), 2.09-2.16 (1H, m, H-2'a), 2.29-2.32 (1H, m, H-2'b), 3.81-3.84 (1H, m, H-4'), 4.07-4.19 (5H, m, 2 x OCH₂CH₃, H-3'), 6.13 (1H, t, $J=6.3$ Hz, H-1'), 7.65 (1H, s, H-6), 9.83 (1H, s, 3-NH). ³¹P NMR (CDCl₃): δ 30.90. ¹³C NMR (75 MHz, CDCl₃): δ -4.83, -4.56 (TBDMS), 16.46, 16.54 (OCH₂CH₃)₂, 17.89 (TBDMS), 22.33 (C-6', d, $J=143$ Hz), 25.68 (TBDMS), 26.56 (C-5', d, $J=4.1$ Hz), 40.85 (C-2'), 61.80, 61.83 (OCH₂CH₃)₂, 74.50 (C-3'), 85.57 (C-1'), 86.53 (C-4', d, $J=17.0$ Hz), 97.10 (C-5), 139.01 (C-6), 149.47 (C-2), 159.03 (C-4). Exact mass (ESI-MS) for C₂₀H₃₇BrN₂O₇PSi [M+H]⁺ found, 555.1323; calcd, 555.1286.

General procedure for the synthesis of 5-modified nucleoside phosphonates via Suzuki-Miyaura coupling . A mixture of compound **9** (1 equiv.), aryl boronic acid (2 equiv.), Pd(PPh₃)₄ (0.1 equiv.) and Na₂CO₃ (3.3 equiv.) in DMF and degassed H₂O was heated (± 130 °C, oil bath) under argon for 6 h or until TLC indicated consumption of all starting material. The mixture was then concentrated and co-distilled with toluene.

The residue was purified by column chromatography (CH₂Cl₂/MeOH 94:6-98:2) affording the 5-modified analogues in moderate yield.

1-[3-*O*-*tert*-Butyldimethylsilyl-2,5,6-trideoxy-6-(diethoxyphosphinyl)-β-D-hexofuranosyl]-5-phenyluracil (10). Reaction of compound **9** (153 mg, 0.27 mmol) with phenylboronic acid (68 mg, 0.55 mmol), Pd(PPh₃)₄ (32 mg, 0.027 mmol) and Na₂CO₃ (96 mg, 0.91 mmol) in DMF (6.5 mL) and degassed H₂O (0.8 mL) was performed as described in the general procedure to yield compound **10** as a colourless solid (120 mg, 79%). ¹H NMR (300 MHz, CDCl₃): δ 0.084 (6H, s, TBDMS), 0.90 (9H, s, TBDMS), 1.25-1.32 (6H, m, 2 x OCH₂CH₃), 1.73-2.04 (4H, m, H-5'a, H-5'b, H-6'a and H-6'b), 2.11-2.20 (1H, m, H-2'a), 2.31-2.39 (1H, m, H-2'b), 3.83-3.86 (1H, m, H-4'), 4.00-4.11 (5H, m, 2 x OCH₂CH₃ and H-3'), 6.23 (1H, app t, *J*= 6.6 Hz, H-1'), 7.33-7.71 (6H, m, Ph and H-6). ³¹P NMR (CDCl₃): δ 30.69. ¹³C NMR (75 MHz, CDCl₃): δ -4.73 and -4.46 (TBDMS), 16.48, 16.56 (OCH₂CH₃)₂, 18.00 (TBDMS), 22.39 (C-6', d, *J*= 143 Hz), 25.80 (TBDMS), 26.60 (C-5', d, *J*= 4.4 Hz), 40.67 (C-2'), 61.89 and 61.92 ((OCH₂CH₃)₂, d, *J*= 6.5 Hz), 74.75 (C-3'), 85.30 (C-1'), 86.42 (C-4', d, *J*= 17.0 Hz), 115.67 (C-5), 128.07-133.10 (Ph), 136.95 (C-6), 150.02 (C-2), 162.39 (C-4). Exact mass (ESI-MS) for C₂₆H₄₂N₂O₇PSi [M+H]⁺ found, 553.2485; calcd, 553.2493.

1-[3-*O*-*tert*-Butyldimethylsilyl-2,5,6-trideoxy-6-(diethoxyphosphinyl)-β-D-hexofuranosyl]-5-(naphthalen-1-yl)uracil (11). Reaction of compound **9** (122 mg, 0.22 mmol) with naphthalene-1-boronic acid (75 mg, 0.44 mmol), Pd(PPh₃)₄ (25 mg, 0.022 mmol) and Na₂CO₃ (77 mg, 0.72 mmol) in DMF (5 mL) and degassed H₂O (0.7 mL) was performed as described in the general procedure to afford compound **11** as a colourless solid (120 mg, 91%). ¹H NMR (300 MHz, CDCl₃): δ 0.056 (6H, s, TBDMS), 0.87 (9H, s, TBDMS), 1.15-1.24 (6H, m, 2 x OCH₂CH₃), 1.77-2.03 (4H, m, H-5'a, H-5'b, H-6'a and H-6'b), 2.12-2.18 (1H, m, H-2'a), 2.26-2.31 (1H, m, H-2'b), 3.82 (1H,

app s, H-4'), 3.95-4.13 (5H, m, 2 x OCH₂CH₃ and H-3'), 6.24 (1H, app t, *J*= 6.6 Hz, H-1'), 7.42-7.82 (7H, m, naphthalene), 7.98 (1H, s, H-6), 8.43 (1H, s, 3-NH). ³¹P NMR (CDCl₃): δ 30.95. ¹³C NMR (75 MHz, CDCl₃): δ -4.87 and -4.62 (TBDMS), 16.2, 16.3 (OCH₂CH₃), 17.85 (TBDMS), 22.26 (C-6', d, *J*= 143 Hz), 25.65 (TBDMS), 23.46 (C-5'), 40.50 (C-2'), 61.63, 61.71 (OCH₂CH₃), 74.63 (C-3'), 85.26 (C-1'), 86.29 (C-4', d, *J*= 17.0 Hz), 115.45 (C-5), 125.89-133.22 (naphthalene), 136.91 (C-6), 149.88 (C-2), 162.26 (C-4). Exact mass (ESI-MS) for C₃₀H₄₄FN₂O₇PSi [M+H]⁺ found, 603.2675; calcd, 603.2650.

1-[3-*O*-*tert*-Butyldimethylsilyl-2,5,6-trideoxy-6-(diethoxyphosphinyl)-β-D-

hexofuranosyl]-5-(4-fluorophenyl)uracil (12). Reaction of compound **9** (163 mg, 0.29 mmol) with 4-fluorophenylboronic acid (82 mg, 0.59 mmol), Pd(PPh₃)₄ (34 mg, 0.029 mmol) and Na₂CO₃ (102 mg, 0.97 mmol) in DMF (7 mL) and degassed H₂O (0.9 mL) was performed as described in the general procedure to yield compound **12** as a colourless solid (141 mg, 84%). ¹H NMR (300 MHz, CDCl₃): δ 0.010 (6H, s, TBDMS), 0.80-0.84 (9H, m, TBDMS), 1.21 (6H, app q, *J*= 7.2 Hz, 2 x OCH₂CH₃), 1.68-1.94 (4H, m, H-5'a, H-5'b, H-6'a and H-6'b), 2.02-2.11 (1H, m, H-2'a), 2.23-2.31 (1H, m, H-2'b), 3.74-3.78 (1H, m, H-4'), 3.93-4.02 (5H, m, 2 x OCH₂CH₃ and H-3'), 6.14 (1H, app t, *J*= 6.9 Hz, H-1'), 6.99-7.04 (2H, m, subs Ph), 7.34-7.42 (3H, m, subs Ph and H-6), 8.25 (1H, s, 3-NH). ³¹P NMR (CDCl₃): δ 30.74. ¹³C NMR (75 MHz, CDCl₃): δ -4.59, -4.33 (TBDMS), 16.64, 16.72 (OCH₂CH₃), 18.14 (TBDMS), 22.65 (C-6', d, *J*= 143 Hz), 25.91 (TBDMS), 26.89 (C-5', d, *J*= 5.0 Hz), 40.95 (C-2'), 61.89, 62.01 (OCH₂CH₃), 74.89 (C-3'), 85.60 (C-1'), 86.55 (C-4', d, *J*= 17 Hz), 115.00 (C-5), 128.42-132.40 (naphthalene), 136.74 (C-6), 149.55 (C-2), 161.72 (C-4). Exact mass (ESI-MS) for C₂₆H₄₁FN₂O₇PSi [M+H]⁺ found, 571.2430; calcd, 571.2399.

1-[3-*O*-*tert*-Butyldimethylsilyl-2,5,6-trideoxy-6-(diethoxyphosphinyl)- β -D-

hexofuranosyl]-5-(thiophen-2-yl)uracil (13). Reaction of compound **9** (155 mg, 0.28 mmol) with thiophene-2-boronic acid (71 mg, 0.56 mmol), Pd(PPh₃)₄ (32 mg, 0.028 mmol) and Na₂CO₃ (98 mg, 0.92 mmol) in DMF (6.7 mL) and degassed H₂O (0.8 mL) was performed as described in the general procedure to yield compound **13** as a colourless solid (117 mg, 75%). ¹H NMR (300 MHz, CDCl₃): δ 0.090 (6H, s, TBDMS), 0.90 (9H, s, TBDMS), 1.26-1.37 (6H, m, 2 x OCH₂CH₃), 1.83-2.08 (4H, m, H-5'a, H-5'b, H-6'a and H-6'b), 2.14-2.23 (1H, m, H-2'a), 2.32-2.40 (1H, m, H-2'b), 3.85-3.90 (1H, m, H-4'), 4.06-4.18 (5H, m, 2 x OCH₂CH₃ and H-3'), 6.23 (1H, app t, J = 6.3 Hz, H-1'), 7.01-7.04 (1H, m, thiophene), 7.25-7.29 (1H, m, thiophene), 7.39-7.44 (1H, m, thiophene), 7.69 (1H, s, H-6), 9.97 (1H, s, 3-NH). ³¹P NMR (CDCl₃): δ 30.80. ¹³C NMR (75 MHz, CDCl₃): δ -4.77 and -4.50 (TBDMS), 16.48, 16.56 (OCH₂CH₃), 17.95 (TBDMS), 22.36 (C-6', d, J = 143 Hz), 25.74 (TBDMS), 26.69 (C-5', d, J = 4.7 Hz), 40.91 (C-2'), 61.79, 61.86 (OCH₂CH₃), 74.64 (C-3'), 85.62 (C-1'), 86.55 (C-4', d, J = 16.8 Hz), 110.20 (C-5), 124.42, 125.37, 127.10 and 133.63 (thiophene), 134.32 (C-6), 149.39 (C-2), 161.27 (C-4). Exact mass (ESI-MS) for C₂₄H₄₀N₂O₇PSSi [M+H]⁺ found, 559.2058; calcd, 559.2058.

1-[2,5,6-Trideoxy-6-(diethoxyphosphinyl)- β -D-hexofuranosyl]-5-phenyluracil (14).

Compound **10** (115 mg, 0.21 mmol) was dissolved in THF (1.3 mL). A solution of 1 M TBAF in THF (0.46 mmol, 0.46 mL) was added. After stirring for 1 h at room temperature, the reaction was completed. The solvent was evaporated and the dry residue was purified by column chromatography (CH₂Cl₂/MeOH 96:4) to give pure compound **14** (65 mg, colourless solid) in 71% yield. ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.18 (6H, dt, J = 1.8 Hz, J = 6.9 Hz, 2 x OCH₂CH₃), 1.75-1.83 (4H, m, H-5'a, H-5'b, H-6'a and H-6'b), 2.06-2.14 (1H, m, H-2'a), 2.36-2.51 (1H, m, H-2'b), 3.71-3.72 (1H, m,

H-4'), 3.89-3.99 (4H, m, 2 x OCH₂CH₃), 4.06-4.12 (1H, m, H-3'), 5.29 (1H, d, *J*= 4.5 Hz, 3'-OH), 6.17 (1H, t, *J*= 6.9 Hz, H-1'), 7.29-7.40 (3H, m, Ph), 7.51-7.54 (2H, m, Ph), 7.63 (1H, s, H-6). ³¹P NMR (DMSO-*d*₆): δ 31.80. ¹³C NMR (75 MHz, DMSO-*d*₆): δ 16.13, 16.20 (OCH₂CH₃), 21.20 (C-6', d, *J*= 139 Hz), 26.01 (C-5', d, *J*= 4.7 Hz), 60.82, 60.85 ((OCH₂CH₃)₂, 2d, *J*= 6.3 Hz), 72.80 (C-3'), 84.46 (C-1'), 85.74 (C-4', d, *J*= 17.0 Hz), 113.07 (C-5), 114.76, 115.04, 129.29, 129.34, 130.23 and 130.34 (Ph), 137.87 (C-6), 149.89 (C-6), 162.05 (C-4). Exact mass (ESI-MS) for C₂₀H₂₈N₂O₇P [M+H]⁺ found: 439.1639, calcd: 439.1686.

1-[2,5,6-Trideoxy-6-(diethoxyphosphinyl)-β-D-hexofuranosyl]-5-(naphthalen-1-yl)-uracil (15). Compound **11** (120 mg, 0.20 mmol) was deprotected using the same procedure as described for the synthesis of compound **14**. Compound **15** was obtained as a colourless solid in a 65% yield (63 mg). ¹H NMR (300 MHz, CDCl₃): δ 1.17-1.25 (6H, m, 2 x OCH₂CH₃), 1.77-2.02 (4H, m, H-5'a, H-5'b, H-6'a and H-6'b), 2.12-2.21 (1H, m, H-2'a), 2.40-2.53 (1H, m, H-2'b), 3.91-4.07 (5H, m, 2 x OCH₂CH₃ and H-4'), 4.16-4.20 (1H, m, H-3'), 5.28 (1H, s, 3'-OH), 6.26 (1H, t, *J*= 6.6 Hz, H-1'), 7.40-7.46 (2H, m, naphthalene), 7.53-7.58 (2H, m, naphthalene), 7.75-7.84 (3H, m, naphthalene), 7.96 (1H, s, H-6). ³¹P NMR (CDCl₃): δ 31.46. ¹³C NMR (75 MHz, CDCl₃): δ 16.41, 16.46 ((OCH₂CH₃)₂, d, *J*= 5.9 Hz), 22.01 (C-6', d, *J*= 141 Hz), 26.66 (C-5'), 40.06 (C-2'), 61.99, 62.06 (OCH₂CH₃)₂, 73.85 (C-3'), 85.43 (C-1'), 86.25 (C-4', d, *J*= 16.2 Hz), 115.60 (C-5), 126.03, 126.31, 126.36, 127.25, 127.62, 128.09, 128.30, 130.00, 132.88 and 133.34 (naphthalene), 137.17 (C-6), 150.29 (C-6), 162.61 (C-4). Exact mass (ESI-MS) for C₂₄H₃₀N₂O₇P [M+H]⁺ found: 489.1814, calcd: 489.1785.

1-[2,5,6-Trideoxy-6-(diethoxyphosphinyl)-β-D-hexofuranosyl]-5-(4-fluorophenyl)-uracil (16). Compound **12** (135 mg, 0.24 mmol) was deprotected using the same procedure as described for the synthesis of compound **14**. Compound **16** was obtained

as a colourless solid in a 32% yield (35 mg). ^1H NMR (300 MHz, $\text{DMSO-}d_6$): δ 1.18 (6H, dt, $J= 2.4$ Hz, $J= 6.9$ Hz, 2 x OCH_2CH_3), 1.70-1.82 (4H, m, H-5'a, H-5'b, H-6'a and H-6'b), 2.05-2.13 (1H, m, H-2'a), 2.37-2.44 (1H, m, H-2'b), 3.70-3.71 (1H, m, H-4'), 3.89-3.99 (4H, m, 2 x OCH_2CH_3), 4.06-4.09 (1H, m, H-3'), 5.28 (1H, d, $J= 4.5$ Hz, 3'-OH), 6.16 (1H, t, $J= 7.2$ Hz, H-1'), 7.17-7.23 (2H, m, subs Ph), 7.55-7.60 (2H, m, subs Ph), 7.64 (1H, s, H-6), 11.53 (1H, s, 3-NH). ^{31}P NMR ($\text{DMSO-}d_6$): δ 31.80. ^{13}C NMR (75 MHz, $\text{DMSO-}d_6$): δ 16.2, 16.3 (OCH_2CH_3), 21.22 (C-6', d, $J= 139$ Hz), 26.11 (C-5', d, $J= 5$ Hz), 60.99, 61.95 ($(\text{OCH}_2\text{CH}_3)_2$, 2d, $J= 6.1$ Hz), 72.80 (C-3'), 84.61 (C-1'), 85.86 (C-4', d, $J= 16.7$ Hz), 113.07 (C-5), 114.76, 115.04, 129.29, 129.34, 130.23 and 130.34 (subs Ph), 137.87 (C-6), 149.89 (C-6), 162.05 (C-4). Exact mass (ESI-MS) for $\text{C}_{20}\text{H}_{27}\text{FN}_2\text{O}_7\text{P}$ $[\text{M}+\text{H}]^+$ found, 457.1547; calcd, 457.1534.

1-[2,5,6-Trideoxy-6-(diethoxyphosphinyl)- β -D-hexofuranosyl]-5-(thiophen-2-yl)-uracil (17). Compound **13** (117 mg, 0.21 mmol) was deprotected using the same procedure as described for the synthesis of compound **14**. Compound **17** was obtained as a colourless solid in a 63% yield (59 mg). ^1H NMR (300 MHz, CDCl_3): δ 1.31 (6H, app dt, $J= 2.1$ Hz, $J= 6.9$ Hz, 2 x OCH_2CH_3), 1.86-2.08 (4H, m, H-5'a, H-5'b, H-6'a and H-6'b), 2.18-2.27 (1H, m, H-2'a), 2.46-2.54 (1H, m, H-2'b), 3.97-3.98 (1H, m, H-4'), 4.04-4.15 (4H, m, 2 x OCH_2CH_3), 4.19-4.26 (1H, m, H-3'), 5.30 (1H, s, 3'-OH), 6.25 (1H, app t, $J= 6.6$ Hz, H-1'), 6.97-7.00 (1H, m, thiophene), 7.24 (1H, d, $J= 4.8$ Hz, thiophene), 7.36 (1H, d, $J= 3.0$ Hz, thiophene), 7.69 (1H, s, H-6). ^{31}P NMR (CDCl_3): δ 31.48. ^{13}C NMR (75 MHz, CDCl_3): δ 16.50, 16.58 (OCH_2CH_3)₂, 22.05 (C-6', d, $J= 142$ Hz), 26.67 (C-5'), 40.27 (C-2'), 62.07, 62.17 (OCH_2CH_3)₂, 73.80 (C-3'), 85.72 (C-1'), 86.40 (C-4', d, $J= 15.9$ Hz), 110.19 (C-5), 124.35, 125.45, 127.08 and 133.64 (thiophene), 134.55 (C-6), 149.77 (C-2), 161.52 (C-4). Exact mass (ESI-MS) for $\text{C}_{18}\text{H}_{26}\text{N}_2\text{O}_7\text{PS}$ $[\text{M}+\text{H}]^+$ found, 445.1210; calcd, 445.1193.

General procedure for the deprotection of 5-modified nucleoside phosphonates

The phosphonic ester (1 equiv.) was dissolved in CH₂Cl₂ under argon. TMSBr (2 equiv.) was added and the resulting solution was stirred overnight. The solvent was evaporated and the residue dissolved in a mixture of EtOAc/Et₂O (1:1) and water. The organic phase was washed with water and the water layers were combined and lyophilized. Purification of the crude using RP-HPLC (Phenomenex Luna C-18, H₂O/0.1% HCOOH in CH₃CN, 90:10 → 0:100 in 23 min, flow 17.5 mL/min) afforded 2 series of compounds: the α- (retention time ≥ 12 min) and β-isomer (retention time ≈ 10-11 min) of each phosphonate.

1-[2,5,6-Trideoxy-6-(dihydroxyphosphinyl)-β-D-hexofuranosyl]-5-phenyluracil (**1a**) and 1-[2,5,6-trideoxy-6-(dihydroxyphosphinyl)-α-D-hexofuranosyl]-5-phenyluracil (**1b**)

Reaction of compound **14** (27 mg, 0.061 mmol) with TMSBr (16 μL, 0.12 mmol) in CH₂Cl₂ (0.9 mL) as described in the general procedure affording the β- (**1a**, 0.9 mg, 4%) and α-isomer (**1b**, 3.2 mg, 14%) as white powders. Compound **1a**: ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.52-1.55 (2H, m, H-6'a and H-6'b), 1.79-1.91 (2H, m, H-5'a and H-5'b), 2.07-2.11 (1H, m, H-2'a), 2.29-2.38 (1H, m, H-2'b), 3.73 (1H, app s, H-4'), 4.06 (1H, app s, H-3'), 6.14 (1H, t, *J* = 6.6 Hz, H-1'), 7.27-7.40 (3H, m, Ph), 7.51-7.54 (2H, m, Ph), 7.63 (1H, s, H-6). ³¹P NMR (DMSO-*d*₆): δ 24.44. ¹³C NMR (125 MHz, DMSO-*d*₆): δ 24.56 (C-6', d, *J* = 146 Hz), 27.22 (C-5'), 38.75 (C-2'), 72.76 (C-3'), 84.30 (C-1'), 86.57 (C-4', d, *J* = 19.3 Hz), 113.89 (C-5), 127.31 (para), 128.10 (ortho), 128.21 (meta), 132.91 (ipsa) (Ph), 137.58 (C-6), 149.86 (C-2), 162.08 (C-4). Exact mass (ESI-MS) for C₁₆H₁₈N₂O₇P [M-H]⁻ found, 381.0836; calcd, 381.0857; Compound **1b**: ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.49-1.57 (4H, m, H-5'a, H-5'b, H-6'a and H-6'b), 1.98 (1H, app d, *J* = 14.1 Hz, H-2'a), 2.57-2.65 (1H, m, H-2'b), 4.11 (1H, app s, H-4'), 4.23 (H, app s, H-3'), 6.14 (1H, app d, *J* = 6.6 Hz, H-1'), 7.27-7.38 (3H, m, Ph), 7.51-

7.54 (2H, m, Ph), 8.16 (1H, s, H-6). ^{31}P NMR (DMSO- d_6): δ 23.62. Exact mass (ESI-MS) for $\text{C}_{16}\text{H}_{18}\text{N}_2\text{O}_7\text{P}$ $[\text{M}-\text{H}]^-$ found, 381.0851; calcd, 381.0857.

1-[2,5,6-Trideoxy-6-(dihydroxyphosphinyl)- β -D-hexofuranosyl]-5-(naphthalen-1-yl)uracil (2a) and 1-[2,5,6-trideoxy-6-(dihydroxyphosphinyl)- α -D-hexofuranosyl]-5-(naphthalen-1-yl)uracil (2b). Reaction of compound **15** (63 mg, 0.13 mmol) with TMSBr (34 μL , 0.26 mmol) in CH_2Cl_2 (1.9 mL) as described in the general procedure affording the β - (**2a**, 7.05 mg, 13%) and α -isomer (**2b**, 8.77 mg, 16%) as white powders. Compound **2a**: ^1H NMR (300 MHz, DMSO- d_6): δ 1.47 (2H, app br s, H-6'a and H-6'b), 1.80 (2H, app br s, H-5'a and H-5'b), 2.13 (1H, app br s, H-2'a), 2.27 (1H, app br s, H-2'b), 3.79 (1H, app s, H-4'), 4.09-4.14 (1H, m, H-3'), 6.14 (1H, app s, H-1'), 7.49-8.27 (8H, m, naphthalene and H-6). ^{31}P NMR (DMSO- d_6): δ 20.97. Exact mass (ESI-MS) for $\text{C}_{20}\text{H}_{20}\text{N}_2\text{O}_7\text{P}$ $[\text{M}-\text{H}]^-$ found: 431.1041, calcd: 431.1014; Compound **2b**: ^1H NMR (300 MHz, DMSO- d_6): δ 1.59 (4H, app br s, H-5'a, H-5'b, H-6'a and H-6'b), 1.99-2.06 (1H, m, H-2'a), 2.52-2.70 (1H, m, H-2'b), 4.13 (1H, app s, H-4'), 4.27 (1H, app s, H-3'), 6.18 (1H, app d, $J = 5.7$ Hz, H-1'), 7.46-7.52 (2H, m, naphthalene), 7.66 (2H, d, $J = 8.4$ Hz, naphthalene), 7.88-7.91 (3H, m, naphthalene), 8.12 (1H, s, naphthalene), 8.31 (1H, s, H-6). ^{31}P NMR (DMSO- d_6): δ 24.28. ^{13}C NMR (75 MHz, DMSO- d_6): δ 72.61 (C-3'), 85.41 (C-1'), 89.23 (C-4'), 112.48 (C-5), 125.95-132.84 (naphthalene), 139.65 (C-6), 150.02 (C-2), 162.35 (C-4). Exact mass (ESI-MS) for $\text{C}_{20}\text{H}_{20}\text{N}_2\text{O}_7\text{P}$ $[\text{M}-\text{H}]^-$ found: 431.1009, calcd: 431.1014.

1-[2,5,6-Trideoxy-6-(dihydroxyphosphinyl)- β -D-hexofuranosyl]-5-(4-fluorophenyl)uracil (3a) and 1-[2,5,6-trideoxy-6-(dihydroxyphosphinyl)- α -D-hexofuranosyl]-5-(4-fluorophenyl)uracil (3b). Reaction of compound **16** (36 mg, 0.078 mmol) with TMSBr (21 μL , 0.16 mmol) in CH_2Cl_2 (1.0 mL) as described in the general affording the β - (**3a**, 1.69 mg, 6%) and α -isomer (**3b**, 3.19 mg, 12%) as white

powders. Compound **3a**: ^1H NMR (300 MHz, $\text{DMSO-}d_6$): δ 1.43 (2H, app br s, H-6'a and H-6'b), 1.76 (2H, app br s, H-5'a and H-5'b), 2.09 (1H, app br s, H-2'a), 2.28 (1H, app br s, H-2'b), H-4' under H_2O peak, 4.08 (1H, app s, H-3'), 6.09 (1H, app s, H-1'), 7.21-7.62 (5H, m, subs Ph and H-6). ^{31}P NMR ($\text{DMSO-}d_6$): δ 20.60. Exact mass (ESI-MS) for $\text{C}_{16}\text{H}_{17}\text{FN}_2\text{O}_7\text{P}$ $[\text{M-H}]^-$ found: 399.0762, calcd: 399.0763; Compound **3b**: ^1H NMR (300 MHz, $\text{DMSO-}d_6$): δ 1.44-1.58 (4H, m, H-5'a, H-5'b, H-6'a and H-6'b), 1.99 (1H, d, $J=13.5$ Hz, H-2'a), 2.52-2.65 (1H, m, H-2'b), 4.11 (1H, app s, H-4'), 4.20-4.25 (1H, m, H-3'), 6.12 (1H, app d, $J=6.9$ Hz, H-1'), 7.19 (2H, app t, $J=8.7$ Hz, subs Ph), 7.54-7.59 (2H, m, subs Ph), 8.15 (1H, s, H-6), 8.33 (1H, s, subs Ph). ^{31}P NMR ($\text{DMSO-}d_6$): δ 22.19. ^{13}C NMR (75 MHz, $\text{DMSO-}d_6$): δ 72.22 (C-3'), 85.16 (C-1'), 111.49 (C-5), 114.67, 114.96, 129.57, 129.67 (Ph), 139.00 (C-6), 149.84 (C-2), 162.04 (C-4). Exact mass (ESI-MS) for $\text{C}_{16}\text{H}_{17}\text{FN}_2\text{O}_7\text{P}$ $[\text{M-H}]^-$ found: 399.0743, calcd: 399.0763.

1-[2,5,6-Trideoxy-6-(dihydroxyphosphinyl)- β -D-hexofuranosyl]-5-(thiophen-2-yl)uracil (4a) and 1-[2,5,6-trideoxy-6-(dihydroxyphosphinyl)- α -D-hexofuranosyl]-5-(thiophen-2-yl)uracil (4b). Reaction of compound **17** (59 mg, 0.13 mmol) with TMSBr (35 μL , 0.27 mmol) in CH_2Cl_2 (2.0 mL) as described in the general procedure affording the β - (**4a**, 5.2 mg, 10%) and α -isomer (**4b**, 9.6 mg, 19%) as white powders. Compound **4a**: ^1H NMR (300 MHz, $\text{DMSO-}d_6$): δ 1.50 (2H, app br s, H-6'a and H-6'b), 1.80 (2H, app br s, H-5'a and H-5'b), 2.04-2.12 (1H, m, H-2'a), 2.27-2.31 (1H, m, H-2'b), 3.80 (1H, app br s, H-4'), 4.09 (1H, app br s, H-3'), 6.10 (1H, d, $J=6.6$ Hz, H-1'), 7.06 (1H, t, $J=4.8$ Hz, thiophene), 7.45 (2H, app dd, $J=4.8$ Hz, $J=14.4$ Hz, thiophene), 7.89 (1H, s, H-6). ^{31}P NMR ($\text{DMSO-}d_6$): δ 23.13. ^{13}C NMR (75 MHz, CDCl_3): δ (C-6', C-5' and C-2' not visible), 73.42 (C-3'), 85.35 (C-1'), 109.21 (C-5), 123.93, 126.37, 127.43, 134.26 (thiophene), 135.74 (C-6), 149.97 (C-2), 161.94 (C-4). Exact mass (ESI-MS) for $\text{C}_{14}\text{H}_{16}\text{N}_2\text{O}_7\text{PS}$ $[\text{M-H}]^-$ found: 387.0377, calcd: 387.0421; Compound **4b**: ^1H

NMR (300 MHz, DMSO-*d*₆): δ 1.40-1.72 (4H, m, H-5'a, H-5'b, H-6'a and H-6'b), 2.02 (1H, app d, *J*= 14.4 Hz, H-2'a), 2.59-2.68 (1H, m, H-2'b), 4.13 (1H, app d, *J*= 4.8 Hz, H-3'), 4.27 (4H, app s, H-4'), 6.16 (1H, app d, *J*= 5.7 Hz, H-1'), 7.04-7.07 (1H, dd, *J*= 3.6 Hz, *J*= 4.8 Hz, thiophene), 7.37-7.38 (1H, m, thiophene), 7.44-7.45 (1H, m, thiophene), 8.46 (1H, s, H-6). ³¹P NMR (DMSO-*d*₆): δ 25.63. ¹³C NMR (75 MHz, DMSO-*d*₆): δ 24.29 (C-6', d, *J*= 135 Hz), 27.01 (C-5'), (C-2' under DMSO peak), 72.59 (C-3'), 85.51 (C-1'), 88.91 (C-4', d, *J*= 15.7 Hz), 107.71 (C-5), 122.58, 125.47, 126.54, 134.28 (thiophene), 136.98 (C-6), 149.53 (C-2), 161.87 (C-4). Exact mass (ESI-MS) for C₁₄H₁₆N₂O₇PS [M-H]⁻ found: 387.0416, calcd: 387.0421.

1-[2,5,6-Trideoxy-6-(diethoxyphosphinyl)- β -D-hexofuranosyl]-uracil (18).

Compound **8** (309 mg, 0.65 mmol) was dissolved in 4.1 mL THF and a TBAF solution in THF (1M, 1.44 mL, 1.44 mmol) was added at rt. After stirring for 3 h the reaction mixture was evaporated *in vacuo* and poured on a silica column (CH₂Cl₂/MeOH 92:8) to give compound **18** (162 mg, 69%) as a colourless solid. ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.23 (6H, t, *J*= 6.9 Hz, 2 x OCH₂CH₃), 1.68-1.82 (4H, m, H-5'a, H-5'b, H-6'a and H-6'b), 2.04-2.24 (2H, m, H-2'a and H-2''), 3.66-3.68 (1H, m, H-4'), 3.92-4.08 (5H, m, 2 x OCH₂CH₃ and H-3'), 5.30 (1H, d, *J*= 4.5 Hz, 3'-OH), 5.63 (1H, d, *J*= 8.7 Hz, CH=CH), 6.10 (1H, t, *J*= 6.6 Hz, H-1'), 7.58 (1H, d, *J*= 7.8 Hz, CH=CH), 11.31 (1H, s, 3-NH). ³¹P NMR (DMSO-*d*₆): δ 31.89. ¹³C NMR (75 MHz, DMSO-*d*₆): δ 16.26, 16.33 (OCH₂CH₃), 21.07 (C-6', d, *J*= 139 Hz), 25.95 (C-5', d, *J*= 4.1 Hz), 60.93, 60.96 (OCH₂CH₃, d, *J*= 6.3 Hz), 72.52 (C-3'), 83.65 (C-1'), 85.43 (C-4', *J*= 16.8 Hz), 102.08 (C-5), 140.79 (C-6), 150.45 (C-2), 163.10 (C-4). Exact mass (ESI-MS) for C₁₄H₂₄N₂O₇P [M+H]⁺ found, 363.1308; calcd, 363.1316.

1-[2,5,6-Trideoxy-6-(dihydroxyphosphinyl)- β -D-hexofuranosyl]-uracil (5a) and 1-[2,5,6-trideoxy-6-(dihydroxyphosphinyl)- α -D-hexofuranosyl]-uracil (5b). To a

solution of **18** (40 mg, 0.11 mmol) in CH₂Cl₂ (1.6 mL) was added TMSBr (29 μL, 0.22 mmol). After stirring overnight, the volatiles were removed *in vacuo*. The residue was dissolved in water, washed with EtOAc/Et₂O (1:1) and lyophilized. A 3:2 mixture of the α- and β-isomer (25 mg, 65%) was obtained as a yellow powder. ¹H NMR (300 MHz, D₂O): δ 1.48-1.91 (4.4H, m, H-5'a, H-5'b, H-6'a, H-6'b and minor isomer H-2'a), 2.12-2.17 (0.6H, major isomer, m, H-2'b), 2.34-2.38 (0.6H, major isomer, m, H-2'a), 2.72-2.80 (0.4 H, minor isomer, m, H-2'b), 3.94-4.00 (0.4H, minor isomer, m, H-3'), 4.31-4.37 (1.6H, m, major isomer H-3' and H-4'), 5.85 (0.6H, major isomer, d, *J*= 7.8 Hz, CH=CH), 5.89 (0.4H, minor isomer, d, *J*= 8.1 Hz, CH=CH), 6.15 (0.6H, major isomer, dd, *J*= 2.7 Hz, *J*= 7.5 Hz, H-1'), 6.25 (0.4H, minor isomer, app t, *J*= 6.9 Hz, H-1'), 7.73 (0.4H, minor isomer, d, *J*= 8.4 Hz, CH=CH), 7.93 (0.6H, major isomer, d, *J*= 8.1 Hz, CH=CH). ³¹P NMR (DMSO-*d*₆): δ 23.63. ¹³C NMR (75 MHz, DMSO-*d*₆): δ 25.53 (major isomer, C-6', d, *J*= 134 Hz), 27.24 (minor isomer, C-6', d, *J*= 128 Hz), 72.72 (major isomer, C-3'), 72.86 (minor isomer, C-3'), 83.86 (minor isomer, C-1'), 85.25 (major isomer, C-1'), 87.17 (minor isomer C-4', d, *J*= 15.2 Hz), 89.09, (major isomer, C-4', d, *J*= 13.7 Hz), 101.13 (major isomer, C-5), 102.33 (minor isomer, C-5), 140.64 (minor isomer, C-6), 141.67 (major isomer, C-6), 150.62 (minor isomer, C-2), 150.74 (major isomer, C-2), 163.29 (minor isomer, C-4), 163.56 (major isomer, C-4). Exact mass (ESI-MS) for C₁₀H₁₄N₂O₇P [M-H]⁻ found, 305.0539; calcd, 305.0544.

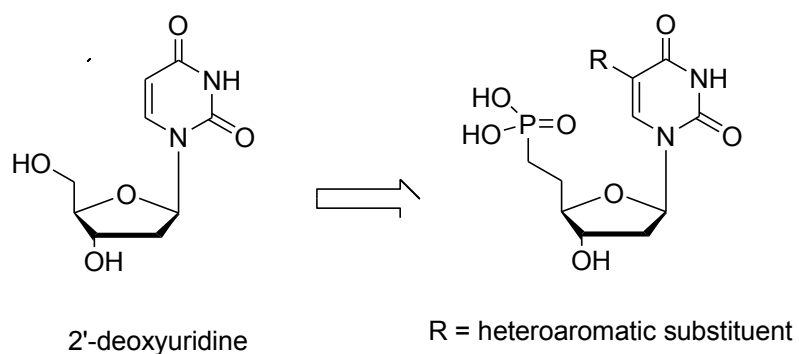
Experimental assay. Antiviral and cytotoxicity assays for compounds 1a-4b. The antiviral activity of the new compounds was determined using a cytopathogenicity assay against herpes simplex virus type 1 (HSV-1) (KOS strain), herpes simplex virus type 2 (HSV-2) (G strain), vaccinia virus, vesicular stomatitis virus, HSV-1 TK⁻ KOS ACV^r in HEL cell cultures. Stock solutions of the test compounds were prepared in DMSO at a concentration of 10 mg/mL. Cells, grown to confluency in 96-well plates, were infected

with 100 CCID₅₀ of virus, one CCID₅₀ being the 50% cell culture infective dose in the presence of varying concentrations of the test compounds. Cultures were further incubated until complete cytopathogenicity was observed in the infected and untreated virus control. The cytotoxicity of the compounds was evaluated in parallel with their antiviral activity in uninfected cells and is expressed as the minimum cytotoxic concentration (MCC) that causes a microscopically detectable alteration of normal cell morphology. The symbol “>” is used to indicate the highest concentration at which the compounds were tested and found not to be antivirally active.

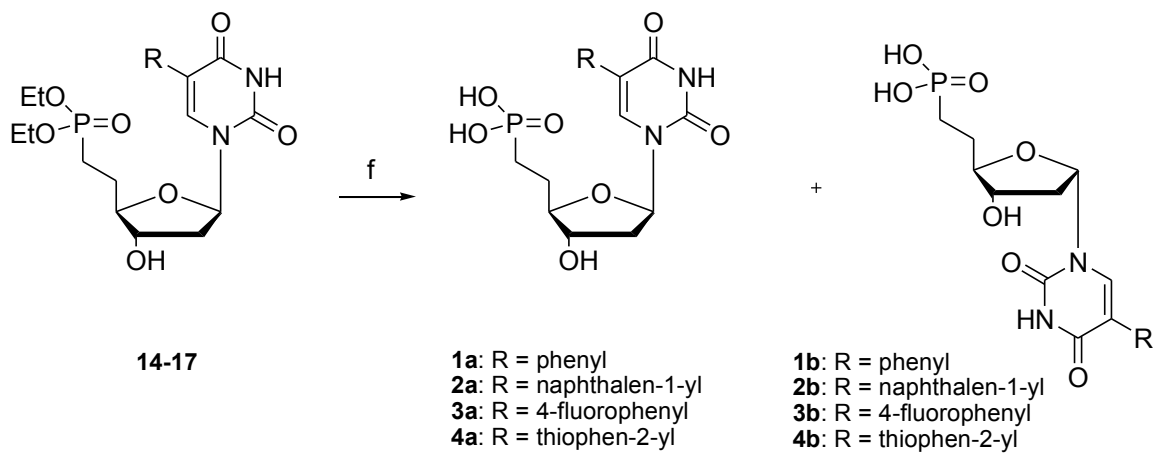
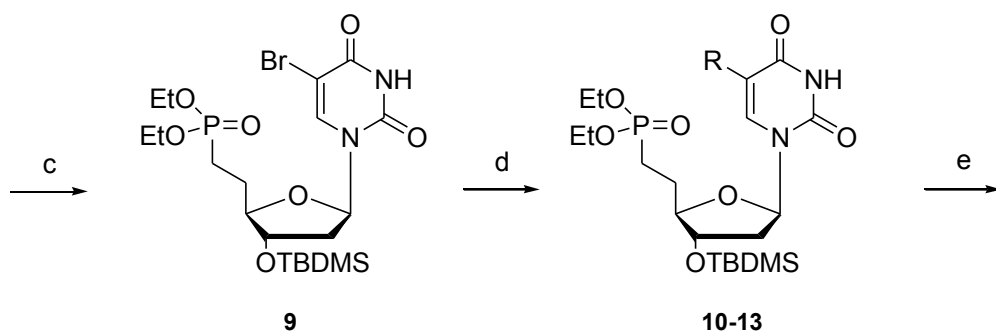
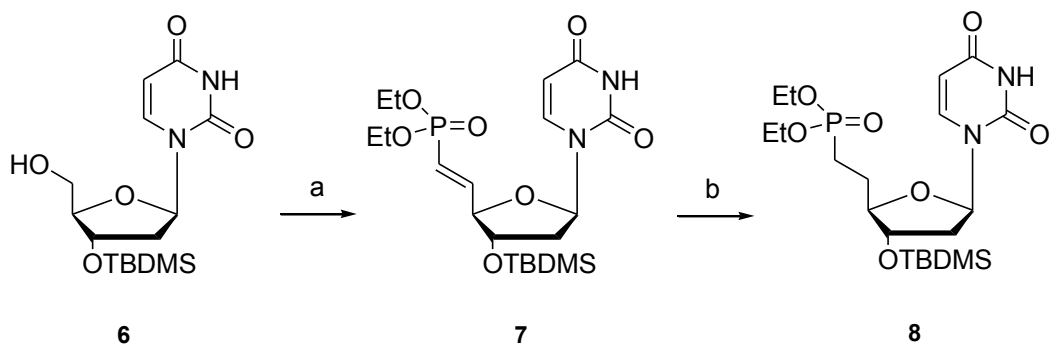
For the anti-HCMV and anti-VZV assays, HEL fibroblasts were infected with 100 PFU per well. Compounds were added after a 1 h-incubation period, and the cells were further incubated at 37 °C. After 5 (VZV) and 7 days (HCMV) of incubation, plaques (VZV) or virus-induced cytopathogenicity (HCMV) was monitored microscopically after ethanol fixation and staining with Giemsa solution. The cytotoxicity of the compounds was evaluated in parallel with their antiviral activity in uninfected cells and is expressed as the minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology (MCC) and the concentration required to reduce cell growth by 50% (CC₅₀).

Determination of the anti-HIV activity of the compounds was based on virus-induced cytopathogenicity of HIV-infected CEM cells, measured at day 4 to 5 post virus infection by microscopically estimating virus-induced syncytia formation. Results are expressed as the 50% effective concentration (EC₅₀) as reported in Table IV.3. The cytostatic activity of the compounds was evaluated in parallel with their antiviral activity in uninfected cell cultures and is expressed as the 50%-inhibitory concentration for the proliferation of the T-lymphocyte CEM cells (EC₅₀).

Figure 1. 2'-Deoxyuridine and envisaged analogues.



Scheme 1. Synthesis of 5-modified 2'-deoxyuridine phosphonate analogues. Reagents and conditions: (a) 2'-Iodoxybenzoic acid, CH_3CN , 80 °C, 6 h; (ii) [(diethoxyphosphinyl)methyl]triphenylphosphorane, DMSO , rt, 20 h, 41% over 2 steps; (b) H_2 , Pd/C, MeOH, rt, overnight, 97%; (c) NBS, DMF, rt, overnight, 49%; (d) R-B(OH)₂, Na_2CO_3 , Pd(PPh₃)₄, DMF, H_2O , reflux, 4 h, 75-91%; (e) 1M TBAF in THF, rt, 1 h, 32-71%; (f) TMSBr, CH_2Cl_2 , rt, overnight, 4-19%.



Scheme 2. Synthesis of non-modified 2'-deoxyuridine phosphonate analogue **5a**.

Reagents and conditions: (a) 1M TBAF in THF, rt, 3 h, 69%; (b) TMSBr, CH₂Cl₂, rt, overnight, 65%.

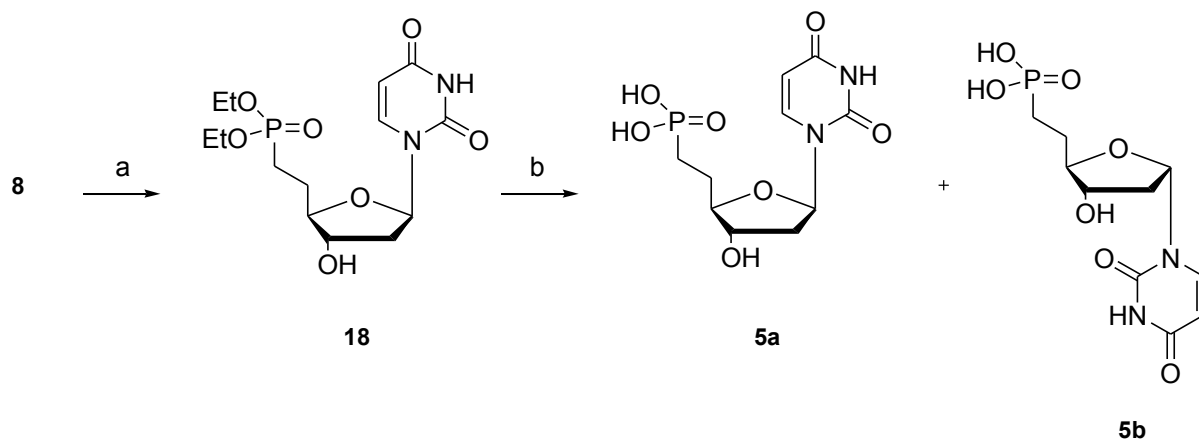


Figure 2. 2D ROESY (500.13 MHz, 298.0 K) spectrum of compound **1a**

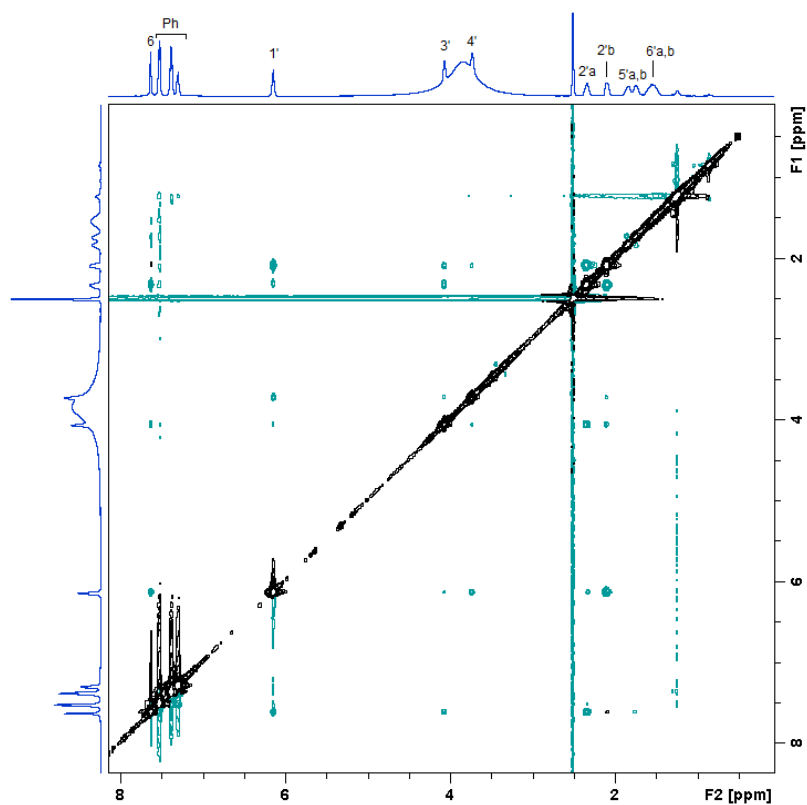


Table 1. Antiviral activity and cytotoxicity of 5-modified 2'-deoxyuridine phosphonate analogues against different HCMV and VZV strains in HEL cell cultures.

Compound	EC ₅₀ ^a (μM)				Cytotoxicity (μM)	
	HCMV AD-169	HCMV Davis	VZV OKA	VZV (TK ⁻) 07/1	Cell morphology ^b (MCC)	Cell growth ^c (CC ₅₀)
1a	> 100	> 100	> 100	> 100	> 100	
2a	45	45	100	100	> 100	> 100
3a	> 20	41	> 20	> 20	≥ 100	> 100
4a	55	63	> 100	> 100	> 100	100
1b	> 100	> 100	> 100	> 100	> 100	
2b	45	> 20	100	100	≥ 100	> 100
3b	> 100	> 100	> 100	> 100	> 100	
4b	> 100	> 100	> 100	> 100	> 100	
Acyclovir			1.3	37	> 440	
Brivudin			0.026	12	> 300	
Ganciclovir	7.9	7.0			≥ 394	141
Cidofovir	0.67	0.95			≥ 317	63

^aEffective concentration required to reduce virus-induced cytopathicity by 50%. Virus input was 100 plaque forming units (PFU).

^bMinimum cytotoxic concentration or compound concentration that caused a microscopically detectable alteration of cell morphology.

^c50%-Cytotoxic concentration or compound concentration required to reduce cell growth by 50%.

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