Synthesis of α -L-threose nucleoside phosphonates via regioselective sugar protection.

Shrinivas G. Dumbre^{a#}, Mi-Yeon Jang^{a#}, Piet Herdewijn^{a,*}

^a Rega Institute for Medical Research, Laboratory of Medicinal Chemistry, KU Leuven,

Minderbroedersstraat 10, 3000 Leuven, Belgium

[#]These authors contributed equally.

*** Corresponding author:**

Piet Herdewijn

KU Leuven, Rega Institute for Medical Research,

Laboratory of Medicinal Chemistry,

Minderbroedersstraat 10, B-3000 Leuven, Belgium

e-mail: Piet.Herdewijn@rega.kuleuven.be

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Abstract: A new synthetic route to α -L-threose nucleoside phosphonates via 2-*O* and 3-*O*-selectively protected L-threose is developed. The key intermediates 2-*O*-benzoyl-L-threonolactone and 1-*O*-acetyl-2-*O*-benzoyl-3-*O*-*t*-butyldiphenylsilyl-L-threofuranose were functionalized to synthesize 2'-deoxy-2'-fluoro- and 3'-*C*-ethynyl L-threose 3'-*O*-phosphonate nucleosides. The key intermediates developed are important intermediates for the synthesis of new L-threose based nucleoside analogues, TNA phosphoramidites and TNA triphosphates.

INTRODUCTION

The modification of the nucleobase and/or of the sugar moiety of a natural nucleoside is an evident choice to develop new antiviral compounds and threose-based nucleosides could serve this purpose.¹⁻⁴ Moreover, (3', 2')- α -L-threose nucleic acid (TNA) is a possible RNA progenitor because of the chemical simplicity of threose relative to ribose and the ability of TNA to form duplexes with DNA and RNA of thermal stability, comparable to the natural nucleic acid associations.⁵ α -L-Threose nucleosides have been synthesized and assembled chemically^{5,6} to TNA and recently all four threo-nucleoside triphosphates (tNTPs) were successfully assembled into TNA oligomers with high fidelity by DNA polymerases.⁷⁻⁹ There are convincing reasons to develop new efficient routes for the synthesis of L-threose nucleosides and try to discover new α -L-threose nucleoside analogues that target viral polymerase as triphosphate.¹⁰⁻¹⁵

Recently, we have demonstrated that L-2'-deoxythreose nucleoside phosphonates PMDTA (**5**, EC₅₀ = 2.5 μ M) and PMDTT (**6**) selectively inhibit HIV without affecting normal cell proliferation (Figure 1).¹⁰ 4'-C-Ethynyl substitution of natural nucleosides (e.g. **4**) has a beneficial effect on anti-HIV activity.¹⁶ This has generated our interest to synthesize the 3'-C-ethynyl analogue of PMTA (**8**). Furthermore, 2'-deoxy-2'-fluoro nucleosides were found to possess interesting biological properties as fluorine mimics both H or OH to some extent.¹⁷ Particularly the cytosine based 2'-deoxy-2'-fluoro-threose phosphonate (**7**) analogue is an attractive target as several bioactive nucleosides are cytosine nucleosides^{4,18} with a 2'-mono or 2',2'-difluoro modification (e.g. **1**, **2** and **3**), the latter showing potent inhibition of HCV replication. 2'-Fluoro-2'-methyl substituted nucleosides have become lead compounds in the development of an anti-HCV

therapy and these analogues are currently under phase II/III clinical trials.⁴ Herein, we describe an easy chemical synthetic route to develop new α -L-threose nucleoside analogues via differentially protected 2-*O* and 3-*O* L-threose (**17**, Scheme 2).

Figure 1: Structures of biologically active nucleoside analogues (**1** to **6**) and current L-threose nucleoside phosphonate targets (**7** and **8**).

RESULTS AND DISCUSSION

Our initial synthetic plan for the synthesis of **7** is shown in Scheme 1. Eschenmoser *et al.*⁶ have described an efficient strategy for the synthesis of compounds **9** and **10**. The stereoselective Vorbrüggen glycosylation of silylated nucleobases requires the availability of 1-*O* and 2-*O* acylated sugars. To fulfill this requirement, the synthesis of PMDT analogue was accomplished via multistep protection, deprotection and reprotection procedures to obtain compound **14**¹⁰ (via **11** and **12**, Scheme 1). For the nucleophilic fluorination at 2' position, epimerization of the 2'-hydroxyl group in threose to an erythrose is needed which was successfully accomplished via Dess-Martin periodinane (DMP) oxidation followed by sodium borohydride reduction to afford compound **15**.

*O*⁴-methyluracil to obtain **20** (82 %). Several reaction conditions were tested for the sodium hydride mediated 3'-*O*-phosphomethylation of the desilylated compound **20** with diisopropylphosphonomethyl triflate, in order to avoid undesired side reactions resulting from the presence of 2'-*O*-benzoyl moiety. Addition of one equivalent of sodium hydride to a equimolar mixture of desilylated compound **20** and diisopropylphosphonomethyl triflate in acetonitrile at -5 to -10 °C for 45 min afforded 3'-phosphonomethylated **21** in 85% yield. Compound **24** could readily be prepared by functional group manipulation of **21**, similar to the one described for the conversion of **14** to **15** (64 % over 3 steps). The difluorination of 2'-keto nucleoside **22** was attempted under various reaction conditions with diethylaminosulfur trifluoride (DAST) and morpholinodifluorosulfonium tetraborate²⁰ (XtalFluoro-M) with or without promoter like Et₃N.3HF or Py.HF or DBU. The required difluoro compound (**23b**) could not be obtained. Under DAST, Py.HF-DBU conditions only a trace of required compound (**23b**) was formed. All the efforts to improve the yields failed in our hand. TLC of the reaction mixture showed the formation of a second product, which however disappeared to starting material after aqueous sat. NaHCO₃ workup. Thus the reaction mixture was purified (elution with 98:2; CH₂Cl₂:MeOH) without a previous reaction workup, to afford **23a** in 29% yield. The configuration of 2'-OMe in **23a** was confirmed by HMBC analysis.

Scheme 2: The synthesis of 2'-deoxy-2'-fluoro L-threose analogue.

Reagents and conditions: (a) benzoyl chloride (1 eq.), imidazole (Im, 2 eq), CH₃CN, -5 °C to rt, 8 h, 95 %; (b) TBDPSCI, cat. DMAP, Im (2 eq), 0 °C to rt, 10 h, 98 %; (c) DIBAL-H (1.2 M in toluene, 2 eq.), dry THF, -70 °C to -60 °C, 3 h, 94 %; (d) acetic anhydride, Et₃N, dry CH₂Cl₂, 0 °C to rt, 8 h, 92 %; (e) *O*⁴-methyluracil or uracil, *N,O*-bis(trimethylsilyl)acetamide (BSA, 2 eq.), TMSOTf (3 eq.) 60 °C, 2.5 h, 82-88 %. (f) LiOH, H₂O-MeOH, rt, 1 h, 83 %; (g) DMP (15% in CH₂Cl₂), 0 °C to rt, 1 h, 98 %, (h) NaBH₄, MeOH, 1 h, 0 °C to rt, 80 %; (i) TBAF (1 M in THF), 0 °C to rt, or Et₃N.3HF, 15 h, 0 °C to rt.; 84-90 %; (j) NaH, CH₃CN, -5 to -10 °C, (iPrO)₂POCH₂OTf, 45 min, 85 %; (k) DAST, Py.HF, CH₂Cl₂, -5 °C to rt 12 h, 29 % of **23a**; (l), DAST (4 eq.), Et₃N.HF (0.05 eq.), CH₂Cl₂, -10 °C to rt, 10 h, 46 %; (m) sat. NH₃ in MeOH, 60 °C, 6 h, 81 %; (n) TMSBr, Et₃N, rt, 30 h, 71 %.

Fluorination of **24** was carried out with DAST and Et₃N.3HF as a promoter at -10 °C to room temperature to furnish 2'-fluorinated nucleoside (**25**) in 45 % yield. Smooth conversion of the *O*⁴-methyluracil moiety to a cytosine base was achieved by treatment of **25** with sat. methanolic ammonia at 60 °C to obtain the analogue **25m** in 81 % yield. The final trimethylsilyl bromide mediated hydrolysis of the diisopropylesters furnished the target cytosyl nucleoside phosphonate **7**.

To synthesize the 3'-C-ethynyl analogue of PMTA (**8**), silylated *N*⁶-benzoyladenine was glycosylated with **17** using TMSOTf as a Lewis acid catalyst to give a mixture of **26** and **26b** (1.7:1, Scheme 3). The *N*⁷-isomer (**26b**) was isomerized to the *N*⁹-isomer (**26**) under acidic condition. The *N*⁷-nucleoside (**26b**) was refluxed in toluene in the presence of *p*-toluenesulfonic acid to furnish **26** in 45 % yield.²¹ The TBDPS group was removed by treatment with TBAF in THF and the oxidation of the 3'-hydroxyl with DMP provided ketone **27**. The ethynyl group was introduced using ethynylmagnesium bromide in the presence of CeCl₃ in THF at -78 °C. As could be expected, the addition reaction did show no selectivity, yielding 3'-ethynyl nucleoside (**28**) and its epimer as 1:1 mixture, which can be separated by silica gel chromatography. The configuration of both the epimers was confirmed by 2D-NMR experiments. In the ROESY measurement, a cross-peak was observed between 3'-OH and 4'-H_b (**28**) and 4'-H_a (epimer) respectively. The phosphonate function was introduced using the diisopropylphosphonomethyl triflate and NaH in THF at -15 °C. The benzoyl group was removed with ammonia in methanol and hydrolysis of the phosphonate ester function of **29** was carried out with TMSI in the presence of HMDS at 0 °C to furnish the 3'-ethynyl analogue of PMTA (**8**). The diphosphate-phosphonate **30** was prepared by the reaction with pyrophosphate, following a literature procedure.^{22,23}

Scheme 3: The synthesis of 3'-ethynyl analogue of PMTA

Reagents and conditions: (a) *N*⁶-benzoyladenine, *N*, *O*-bis(trimethylsilyl)acetamide (BSA, 2 eq.), TMSOTf (1 eq.) 70 °C, 12 h, 65 %; (b) *p*-TSA, toluene, reflux; (c) TBAF (1 M in THF), 0°C to rt, 78 %; (d) DMP (15 % in CH₂Cl₂), 0 °C to rt, 1 h, then rt, 10 h, 97 %; (e) CeCl₃, ethynylmagnesium bromide (0.5 M in THF, dry THF, -78 °C, 6 h, 32 %; (f) NaH, CH₃CN, -10 °C, (iPrO)₂POCH₂OTf, 1 h, 88 %; (g) sat. NH₃ in MeOH, 24 h, rt, 86 %; (h) TMSI, HMDS, 0 °C, 2 h, 60%. (i) Bu₃N, *N,N*-carbonyldiimidazole, tris(*tetra*-butylammonium)hydrogen pyrophosphate, DMF, 16 h, 31 %. (j) phenyl chloroformate, DMAP, CH₃CN, rt, 2 h, 91 %; (k) Bu₃SnH, AIBN, toluene, 75 °C.

Biellmann *et al* described a modified Barton-McCombie reaction for a 2'-deoxygenation of 3'-C-ethynyl nucleoside analogues.²⁴ However, in our hands, attempts to 2'-deoxygenate compound **29** were not successful. When we followed Biellmann's method, no reaction had taken place. Under standard conditions a complex mixture of by-products was obtained.

CONCLUSION

An efficient synthetic route to L-threose nucleoside analogues is developed. The key intermediates 2-*O*-benzoyl-L-threonolactone (**16**) and 1-*O*-acetyl-2-*O*-benzoyl-3-*O*-*t*-butyldiphenylsilyl-L-threofuranose (**17**) were functionalized to obtain 2'-deoxy-2'-fluoro and 3'-*C*-ethynyl (PMTA) L-threose 3'-*O*-phosphonate nucleosides (**7** and **8**) and the diphosphate-phosphonate compound **30**. Unfortunately, none of the synthesized compounds showed any significant in vitro activity against HIV, HCV and RSV, as well as cytotoxicity at the concentrations up to 100 μ M. The diphosphate-phosphonate (**30**) did not inhibit HCV polymerase ($IC_{50} > 200 \mu$ M) and was not incorporated in mtRNA. Nevertheless, the key intermediate **17** is an important synthon for the synthesis of new L-threose base nucleoside analogues. Until now²⁵ a mixture of 2'-*O*-DMTr and 3'-*O*-DMTr protected L-threose nucleosides needs to be separated⁶ to obtain TNA phosphoramidite and TNA triphosphate for construction of TNA oligomers. The accessibility of intermediates like **16** and **17** allows a more straightforward synthesis of TNA monomers.

EXPERIMENTAL SECTION:

¹H, ¹³C and ³¹P NMR spectra were recorded on 300 MHz (¹H NMR, 300 MHz; ¹³C NMR, 75 MHz; ³¹P NMR, 121 MHz) or 500 MHz (¹H NMR, 500 MHz; ¹³C NMR, 125 MHz) spectrometer. 2D NMRs (H-COSY, NOESY, ROESY, HSQC and HMBC) were used for the assignment of all the intermediate and final compounds. The specific optical rotation was measured at 589 nm and c is given in g/100 mL. Mass spectra were acquired on a quadrupole orthogonal acceleration time-of-flight mass spectrometer. Column chromatographic separations were carried out by gradient elution with suitable

combination of two/three solvents and silica gel (100-200 mesh or 230-400 mesh). Solvents for reactions were distilled prior to use: THF and toluene from Na/benzophenone; CH₂Cl₂ and CH₃CN from CaH₂; Et₃N and pyridine from KOH.

1'- α -(Uracil-1-yl)-3'-*O*-diisopropylphosphonomethyl-L-erythrose (15). Following the procedure described for **24**, 2'-hydroxy moiety in compound **18** was epimerized to **15**: ¹H-NMR (Acetone-*d*₆, 500 MHz): δ 11.25 (s, 1H, NH) 7.67 (d, *J* = 8.0 Hz, 1H, H6), 5.99 (d, *J* = 5.8 Hz, 1H, H1'), 5.56 (d, *J* = 5.3 Hz, 1H, OH), 5.47 (dd, *J* = 8.1, 2.2 Hz, 1H, H5), 4.62 [m, 2H, P(OCH(CH₃)₂)₂], 4.15 (q, *J* = 5.2 Hz, 1H, H-2'), 3.97 (dd, *J* = 9.4, 4.4 Hz, 1H, H4a'), 3.95 (dd, *J* = 13.9, 8.3 Hz, 1H, PCH_a), 3.88 (dd, *J* = 13.9 Hz, 9.1 Hz, 1H, PCH_b), 3.83 (dd, *J* = 9.4, 4.8 Hz, 1H, H4b'), 1.25 [m, 12H, P(OCH(CH₃)₂)₂]; ¹³C NMR (Acetone-*d*₆, 125 MHz): δ 163.3 (C4), 150.8 (C2), 143.3 (C6), 100.0 (C5), 84.1 (C1'), 79.5 (d, ²*J*_{P,C} = 11.9 Hz, C3'), 70.4 and 70.4 [CH(CH₃)₂], 69.4 (C2'), 68.9 (C4'), 64.2 (PCH₂, ¹*J*_{P,C} = 165.5 Hz), 23.8 and 23.9 [P(OCH(CH₃)₂)₂]; HRMS (ESI+) calcd for C₁₅H₂₅N₂O₈PNa [M+Na]⁺ 415.1246, found 415.1240.

2-*O*-Benzoyl- L-threonolactone (16). To a solution of L-threonolactone (**9**, 11.20 g, 95 mmol) and imidazole (12.91 g, 190 mmol) in dry acetonitrile at 0 to -5 °C, benzoyl chloride (11.02 mL, 95 mmol) was slowly added over a period of 10 min. The reaction mixture was stirred at room temperature for 8 h. The acetonitrile was removed under reduced pressure, the residue was taken up into 150 mL ethyl ether and 100 mL water was added. The resulting mixture was sequentially washed with 2 x 100 mL of ice-cold 1M HCl soln., 100 mL of water, 100 mL of sat. NaHCO₃ soln, and 100 mL of sat aq. NaCl soln. The organic layer was dried over MgSO₄, concentrated to obtain crude product. The residue was purified by column chromatography (9:1 to 7:3, hexane:EtOAc;

$R_f = 0.4$, 6:1 hexane:EtOAc) to obtain **16** (20 g, 90 mmol, 95 % yield): $[\alpha]^{20}_D -21.5$ ($c = 0.065$, CH_2Cl_2) ^1H NMR (500MHz, CDCl_3): δ 8.05 (d, $J = 7.4$ Hz, 2H, Ph), 7.73 (t, $J = 7.4$ Hz, 1H, Ph), 7.60 (t, $J = 7.9$ Hz, 2H, Ph), 6.9 (d, $J = 4.9$ Hz, 1H, 3'-OH), 5.74 (d, $J = 8.0$ Hz, 1H, H2'), 4.75 (ddd, $J = 5.21, 8.0$ Hz, 1H, H3'), 4.55 (appt t, $J = 8.3$ Hz, 1H, H4a'), 4.10 (appt, $J = 8.3$ Hz, 1H, H4b'); ^{13}C NMR (125 MHz, CDCl_3): δ 172.0 (C1'), 165.7 (CO), 135.0 (Ph), 130.5 (Ph), 129.9 (Ph), 129.4 (Ph), 76.0 (C2'), 70.6 (C4' and C3'); HRMS (ESI+) calcd for $\text{C}_{11}\text{H}_{10}\text{O}_5\text{Na}$ $[\text{M}+\text{Na}]^+$ 245.0421, found 245.0414.

2-O-Benzoyl-3-O-tert-butyldiphenylsilyl-L-threonolactone (16b). To a solution of lactone (**16**, 25.7 g, 116 mmol), cat. DMAP (0.1g) and imidazole (15.75 g, 231 mmol) in dry acetonitrile at 0 °C, *tert*-butyldiphenylchlorosilane (29.7 mL, 116 mmol) was added dropwise. The reaction mixture was allowed to stir at room temperature for 10 h. The acetonitrile was removed under reduced pressure, the residue was taken up into 400 mL ethyl ether and 200 mL water was added. The resulting mixture was sequentially washed with 2 x 200 mL of ice-cold 1M HCl soln., 200 mL of water, 100 mL of sat. NaHCO_3 soln, and 100 mL of sat aq. NaCl soln. The organic layer was dried over MgSO_4 , concentrated to obtain crude product. The residue was purified by column chromatography (95:5 hexane:EtOAc; $R_f = 0.4$) to obtain **16b** (52.2 g, 113 mmol, 98 % yield): $[\alpha]^{20}_D -20.2$ ($c = 0.47$, CH_2Cl_2); ^1H NMR (300 MHz, CDCl_3): δ 7.18-7.89 (m, 15H, Ph), 6.05 (d, $J = 7.8$ Hz, 1H, H2'), 4.95 (dd, $J = 7.8, 16.8$ Hz, 1H, H3'), 4.35 (appt t, $J = 7.8$ Hz, 1H, H4a'), 4.27 (appt t, $J = 8.2$ Hz, 1H, H4b'), 0.97 [s, 9H, $\text{C}(\text{CH}_3)_3$]; ^{13}C NMR (75 MHz, CDCl_3): δ 170.9 (C1'), 165.4 (PhCO), 136.0, 135.9, 135.4, 135.0, 133.0, 132.6, 131.1, 130.9, 130.4, 129.7, 129.0, 128.9, 128.9, 128.7, 128.3, 75.8 (C2'), 72.5

(C3'), 69.7 (C4'), 27.3 (CH₃), 19.5 [C(CH₃)₃]; HRMS (ESI+) calcd for C₂₇H₂₈O₅SiNa [M+Na]⁺ 483.1598, found 483.1581.

2-*O*-Benzoyl-3-*O*-*tert*-butyldiphenylsilyl-L-threofuranose (16c). To a solution of **16b** (27.7 g, 60.1 mmol) in 350 mL of dry THF at –78 °C was added 1.2M DIBAL-H (100 mL, 120 mmol) solution in toluene was added dropwise via cannula. the mixture was stirred at –78 to –60 °C for 3 h (starting material was consumed in 3 h). The reaction was quenched by dropwise addition of 15 mL of MeOH at –78 °C. The cooling bath was removed; the reaction mixture was diluted with EtOAc, 300 mL of a sat. aq. sodium potassium tartrate solution was added, and the mixture was stirred vigorously for 2 h. The reaction mixture was filtered and the organic phase was given a wash with water and sat. NaCl solution and dried over MgSO₄. The organic phase was concentrated under reduced pressure, co-evaporated with 2 x 200 mL of toluene. This crude residue was passed through a short silica column (1:1 EtOAc:hexane) to obtain **16c** as a diastereomeric mixture at anomeric position (26 g, 56.2 mmol, 94 % yield): HRMS (ESI+) calcd for C₂₇H₃₀O₅SiNa [M+Na]⁺ 485.1755, found 485.1740.

1-*O*-Acetyl-2-*O*-benzoyl-3-*O*-*tert*-butyldiphenylsilyl-L-threofuranose (17). To a solution of lactol **16c** (26.0 g, 56.2 mmol) and triethylamine (39 mL, 281 mmol) in 200mL of dry dichloromethane at 0 °C was added acetic anhydride (10.6 mL, 112 mmol) dropwise. The reaction mixture was stirred at room temperature for 8 h. The reaction mixture was given a water and brine wash. The organic layer was dried over MgSO₄ and concentrated under reduced pressure to obtain crude product. The residue was purified by column chromatography (95:5 EtOAc:hexane; *R*_f = 0.15) to obtain **17** as diastereomeric mixture at anomeric position (26 g, 51.5 mmol, 92 % yield): C1' β-isomer : ¹H-NMR

(300 MHz, CDCl₃): δ 7.89 (d, J = 7.1 Hz, 2H, Bz-H), 7.68-7.63 (m, 4H, Ph-H), 7.56 (t, J = 7.5 Hz, 1H, Bz-H), 7.43-7.30 (m, 8H, Ph-H, Bz-H), 6.17 (s, 1H, H1'), 5.43 (d, J = 1.9 Hz, 1H, H2'), 4.50 (dt, J = 2.0, 5.2 Hz, 1H, H3'), 4.06-3.96 (m, 2H, H4'), 2.18 (s, 3H, CH₃CO), 1.10 [s, 9H, C(CH₃)₃]; ¹³C NMR (75 MHz, CDCl₃): δ 169.9, 165.3, 135.9, 135.8, 133.6, 133.3, 132.8, 130.2, 130.2, 130.0, 129.9, 128.6, 128.1, 128.03, 100.0, 83.5, 75.9, 75.0, 26.9, 21.3, 19.3; HRMS: calcd for C₂₉H₃₂O₆SiNa [M+Na]⁺ 527.1860, found 527.1863; C1' α -isomer : ¹H NMR (300 MHz, CDCl₃): δ 7.92 (d, J = 7.1 Hz, 2H, Bz-H), 7.67-7.56 (m, 4H, Ph-H), 7.45 (t, J = 7.5 Hz, 1H, Bz-H), 7.43-7.25 (m, 8H, Ph-H, Bz-H), 6.50 (d, J = 4.5 Hz, 1H, H1'), 5.44 (t, J = 5.3 Hz, 1H, H2'), 4.75 (q, J = 6.0 Hz, 1H, H3'), 3.95 (dd, J = 6.5, 9.3 Hz, 1H, H4'), 3.77 (dd, J = 4.7, 9.3 Hz, 1H, H4'), 1.87 (s, 3H, CH₃CO), 1.06 [s, 9H, C(CH₃)₃]; ¹³C NMR (75 MHz, CDCl₃): δ 169.6, 165.5, 135.8, 133.5, 133.3, 132.8, 130.3, 130.1, 129.9, 129.3, 128.5, 128.1, 127.9, 94.5, 79.4, 74.3, 72.5, 26.9, 21.0, 19.2; HRMS (ESI+) calcd for C₂₉H₃₂O₆SiNa [M+Na]⁺ 527.1860, found 527.1859.

1'- α -(Uracil-1-yl)-2'-O-benzoyl-3'-O-*tert*-butyldiphenylsilyl- L-threose (18)

Following procedure described for **20**, compound **18** was obtained in 87% yield.

¹H NMR (500 MHz, CDCl₃): δ 7.93 (dd, J = 1.2, 8.4 Hz, 2H, Ph), 7.90 (d, J = 8.1 Hz, 1H, H6), 7.60-7.64 (m, 4H, Ph), 7.54-7.58 (m, 1H, Ph), 7.34-7.48 (m, 8H, Ph), 6.10 (d, J = 1.5 Hz, 1H, H1'), 5.76 (dd, J = 2.0, 8.1 Hz, 1H, H5), 5.46 (d, J = 1.0 Hz, 1H, H2'), 4.40 (dt, J = 1.0, 3.4 Hz, 1H, H3'), 4.12 (bd, J = 10.4 Hz, 1H, H4a'), 3.94 (dd, J = 3.4, 10.4 Hz, 1H, H4'), 1.10 [s, 9H, C(CH₃)₃]; ¹³C NMR (125 MHz, CDCl₃): δ 164.2 (C4), 162.0 (PhCO), 149.7 (C3), 140.4 (C6), 135.40, 135.38, 133.3, 132.1, 131.3, 130.3, 129.5, 128.4, 128.1, 127.8, 127.7, 100.1 (C5), 89.5 (C1'), 82.1 (C2'), 75.5 (C4'), 75.4 (C3'), 26.5 (CH₃), 18.7 [SiC(CH₃)₃]; HRMS (ESI+) calcd for C₃₁H₃₂N₂O₆SiNa [M+Na]⁺ 579.1922, found 579.1890.

1'- α -(Uracil-1-yl)-3'-*O*-*tert*-butyldiphenylsilyl-L-threose (18f). To a solution of compound **18** (1.0 g, 1.790 mmol) in 6 mL of methanol was added LiOH (0.107 g, 4.47 mmol) solution in 3 mL water and stirred at room temperature for 2 h. The solvent was removed and residue is subjected to column chromatography (elution with 1:1 hexane:EtOAc; R_f = 0.5 1:1 hexane:EtOAc) to obtain compound **18f** (0.7 g, 15.40 mmol, 86 % yield): ^1H NMR (500 MHz, CDCl_3): δ 7.77 (dd, J = 8.0 Hz, 1H, H6), 7.52-7.61 (m, 4H, Ph), 7.33-7.48 (m, 6H, Ph), 5.74 (d, J = 8 Hz, 1H, H5), 5.72 (s, 1H, H1'), 4.35 (s, 1H, H2'), 4.27 (d, J = 2.7, 1H, H3'), 4.09-4.17 (m, 2H, H4), 1.02 [s, 9H, $\text{C}(\text{CH}_3)_3$]; ^{13}C NMR (75 MHz, CDCl_3): δ 164.0 (C4), 151.0 (C2), 140.7 (C6), 135.66, 135.57, 132.58, 132.41, 130.20, 130.16, 127.9, 100.5 (C5), 94.0 (C1'), 81.4 (C2'), 76.9 (C3'), 77.5 (C4'), 26.8 (CH_3), 19.0 [$\text{SiC}(\text{CH}_3)_3$]; HRMS (ESI+) calcd for $\text{C}_{24}\text{H}_{28}\text{N}_2\text{O}_5\text{SiNa}$ [$\text{M}+\text{Na}$] $^+$ 475.1660, found 475.1657.

Dess-Martin oxidation reaction on compound **18f** in CH_2Cl_2 (2 h, rt) followed by sodium borohydride mediated reduction of ketone resulted in the mixtures of **19a** and **19b** (6:4).

Diisopropylphosphonomethyl trifluoromethanesulfonate. To a solution of (hydroxymethyl)diisopropylphosphonate (2.9 g, 14.78 mmol) in 50 mL of dry diethyl ether at -78°C was added a solution of 2.5M *n*-BuLi (6.12 mL, 15.29 mmol). This reaction mixture was allowed to stir at this temperature for 5 min and then trifluoromethanesulfonyl chloride (1.628 mL, 15.29 mmol) was added dropwise over 5 min. The reaction mixture was stirred at this temperature for 1 h. The reaction was quenched with sat. NH_4Cl . The organic layer was given a wash with water and brine; dried over MgSO_4 , concentrated under reduced pressure at room temperature to obtain diisopropylphosphonomethyl triflate (4.77 g, 14.53 mmol, 98 % yield). The crude

product was pure, without any traces of phosphonate dimer or any other reactant: ^1H NMR (300 MHz, CDCl_3): δ 1.35-1.40 [m, 12H, $\text{POCH}(\text{CH}_3)_2$], 4.55 (d, $^1J_{\text{P,H}} = 8.97$ Hz, 2H, PCH_2), 4.82 [sept, $J = 6.21$ Hz, 2H, $\text{POCH}(\text{CH}_3)_2$]; ^{31}P NMR (CDCl_3 , 125 MHz): δ 10.01.

1'- α -(*O*⁴-Methyluracil-1-yl)-2'-*O*-benzoyl -3'-*O*-*tert*-butyldiphenylsilyl-L-threose (20).

To a solution of **17** (12.88 g, 25.5 mmol) and *O*⁴-methyluracil (3.22 g, 25.5 mmol) in 120 mL of dry acetonitrile was treated with BSA (13.94 mL, 56.2 mmol) and stirred at 60 °C for 30 min. Then, TMSOTf (13.86 mL, 77 mmol) was added, and the heating was continued of another 2 h (the Vorbrüggen glycosylation was found to be complete). The reaction mixture was cooled to room temperature and diluted with 200 mL of EtOAc, and poured in to 100 mL of 10 % NaHCO_3 solution with stirring. The organic layer was separated and washed with water and brine. The organic layer was dried over MgSO_4 and concentrated under reduced pressure. The oily residue was purified by column chromatography (6:4, hexane:EtOAc; $R_f = 0.4$) to obtain light yellow low melting solid **20** (12 g, 21.03 mmol, 82 % yield): ^1H NMR (500 MHz, CDCl_3): δ 8.05 (d, $J = 7.5$, Hz, 1H, H6), 7.96 (dd, $J = 1.9$, 8.2 Hz, 2H, Ph), 7.51-7.64 (m, 4H, Ph), 7.51-7.56 (m, 1H, Ph), 7.34-7.46 (m, 8H, Ph), 6.22 (d, $J = 1.0$ Hz, 1H, H1'), 5.92 (d, $J = 7.5$ Hz, 1H, H5), 5.60 (d, $J = 1.0$ Hz, 1H, H2'), 4.36 (dt, $J = 1.0$, 3.5 Hz, 1H, H3'), 4.12 (d, $J = 9.9$ Hz, 1H, H4'), 4.00 (dd, $J = 3.5$, 10.2 Hz, 1H, H4'), 3.98 (s, 3H, CH_3O), 1.05 [s, 9H, $\text{C}(\text{CH}_3)_3$]; ^{13}C NMR (125 MHz, CDCl_3): δ 172.0 (C2'), 164.5 (PhCO), 155.9 (C3), 143.4 (C6), 135.9 135.8, 133.5, 132.7, 131.8, 130.34, 130.32, 130.0, 129.2, 128.5, 128.4, 128.1, 128.0, 95.5 (C5), 91.0 (C1'), 82.2 (C2'), 76.4 (C4'), 75.9 (C3'), 54.6 (OMe), 26.7 (CH_3),

19.1 [SiC(CH₃)₃]; HRMS (ESI+) calcd for C₃₂H₃₄N₂O₆SiNa [M+Na]⁺ 593.2078, found 593.2076.

1'- α -(*O*⁴-Methyluracil-1-yl)-2'-*O*-benzoyl-L-threose (20i). To a solution of compound **20** (0.330 g, 0.578 mmol) in 10 mL THF at 0 °C was added tetra-*n*-butylammonium fluoride (0.578 mL, 0.578 mmol) dropwise, reaction mixture was stirred in ice bath for 1 h, and found to be complete (clean reaction), solvent was removed, column chromatographed with 1:1 hexane:EtOAc then 100 % EtOAc to obtain **20i** (0.162 g, 0.487 mmol, 84 % yield). Note: deprotection of TBDPS with triethylamine trihydrofluoride (2 eq) in dichloromethane for 15 h at room temperature gave **20i** in 90 % yield: [α]_D²⁰ -34.5 (c = 0.185, CH₂Cl₂); ¹H NMR (500 MHz, CDCl₃): δ 8.05 (dd, *J* = 1.5, 8.4 Hz, 2H, Ph), 7.60-7.64 (m, 2H, H6 and Ph), 7.46-7.50 (appt t, *J* = 7.8 Hz, 2H, Ph), 5.98 (d, *J* = 7.5 Hz, 1H, H5), 5.65 (d, *J* = 1.8 Hz, 1H, H1'), 5.47 (s, 1H, H2'), 4.54 (m, 1H, H3'), 4.30 (bd, *J* = 10.0 Hz, 1H, H4a'), 4.24 (dd, *J* = 4.3, 10.0 Hz, 1H, H4b'), 3.99 (s, 3H, CH₃O); ¹³C NMR (125 MHz, CDCl₃): δ 172.4 (C2), 166.2 (PhCO), 155.8 (C4), 145.6 (C6), 145.6, 133.8, 129.9, 128.6, 96.2 (C5), 96.1 (C1'), 84.1 (C2'), 76.1 (C3'), 75.1 (C4'), 55.2 (CH₃O); HRMS (ESI+) calcd for C₁₆H₁₆N₂O₆Na [M+Na]⁺ 355.0906, found 355.0898.

1'- α -(*O*⁴-Methyluracil-1-yl)-2'-*O*-benzoyl-3'-*O*-diisopropylphosphonomethyl-L-threose (21). To a solution of **20i** (3.20 g, 9.63 mmol) in dry Acetonitrile (10 mL) was added triflate of diisopropylphosphonomethanol (6.32 g, 19.26 mmol). The reaction mixture was cooled to -5 to -10 °C (ice-salt bath) under argon before the addition of NaH (0.463 g, 11.56 mmol, 60 % suspension). After 45 min of stirring at 0 °C, the reaction was quenched with 5 mL EtOAc containing 0.1 mL of acetic acid, the content was further diluted with 20 mL of EtOAc and 10 mL of water was added and stirred well,

extracted, dried over MgSO_4 , then, concentrated under reduced pressure. Purification by column chromatography (3 % MeOH in CH_2Cl_2) afforded the product **21** as a colorless liquid. (4.2 g, 8.23 mmol, 85 % yield); ^1H NMR (500 MHz, CDCl_3): δ 8.06 (d, $J = 7.4$ Hz, 2H, Ph), 7.61 (d, $J = 7.6$ Hz, 1H, H6), 7.62 (t, $J = 7.6$ Hz, 1H, Ph), 7.48 (t, $J = 7.6$ Hz, 2H, Ph), 6.35 (s, 1H, H1'), 5.95 (d, $J = 7.6$ Hz, 1H, H5), 5.46 (s, 1H, H2'), 4.66-4.80 [m, 2H, $\text{CH}(\text{CH}_3)_3$], 4.46 (bd, $J = 9.17$ Hz, 1H, H4a'), 4.24-4.29 (m, 2H, H4b' and H3), 3.98 (s, 3H), 3.93 (dd, $J = 9.17, 13.6$ Hz, 1H, PCH_a), 3.87 (dd, $J = 9.17, 13.6$ Hz, 1H, PCH_b), 1.30-1.35 (m, 12H, CH_3); ^{13}C NMR (150 MHz, CDCl_3): δ 171.7 (C2), 164.9 (PhCO), 155.4 (C4), 142.8 (C6) 135.3, 129.6, 128.5, 128.2, 95.2 (C5), 90.2 (C1'), 83.1 (C3', $^3J_{\text{P,C}} = 10.4$ Hz), 79.4 (C2'), 73.6 (C4'), 71.0 [$\text{CH}(\text{CH}_3)_2$, $^2J_{\text{P,C}} = 6.4$ Hz], 70.9 [$\text{CH}(\text{CH}_3)_2$, $^2J_{\text{P,C}} = 6.4$ Hz], 64.1 (PCH_2 , $^1J_{\text{P,C}} = 169.3$ Hz), 54.1 (OMe), 23.6 [$\text{CH}(\text{CH}_3)_2$]; ^{31}P -NMR (121 MHz, CDCl_3): δ 17.9; HRMS (ESI+) calcd for $\text{C}_{23}\text{H}_{32}\text{N}_2\text{O}_9\text{P}$ $[\text{M}+\text{H}]^+$ 511.1840, found 511.1847.

1'- α -(*O*⁴-Methyluracil-1-yl)-3'-*O*-diisopropylphosphonomethyl-L-threose (21f). A solution of **21** (4.70 g, 9.21 mmol) in 10 mL acetonitrile was treated with LiOH (0.220 g, 9.21 mmol) in 4 mL water and 10 mL MeOH. The reaction mixture was stirred at room temperature. The reaction was found to be complete in 1 h, the reaction mixture was neutralized with acetic acid. The solvent was removed and purified by column chromatography (elution with 7 % MeOH in CH_2Cl_2 ; $R_f = 0.2$, 5 % methanol in CH_2Cl_2) to obtain **21f** as colorless solid. (3.11 g, 7.65 mmol, 83 % yield): $[\alpha]^{20}_{\text{D}} +33.3$ ($c = 0.045$ CH_2Cl_2); ^1H NMR (300MHz, CDCl_3): δ 7.80 (d, $J = 7.6$ Hz, 1H, H6), 5.92 (d, $J = 7.6$ Hz, 1H, H5), 5.75 (d, $J = 1.3$ Hz, 1H, H1'), 4.61-4.85 [m, 2H, $\text{CH}(\text{CH}_3)_2$], 4.22-4.37 (m, 3H, H3' and H4'), 4.09 (s, 1H, H2'), 3.90 (s, 3H, CH_3O) 3.68 (d, $J = 8.7$ Hz, 1H, PCH_2) 1.15-

1.42 (m, 12H, P[CH(CH₃)₂]₂); ¹³C NMR (75 MHz, CDCl₃): δ 172.1 (C2), 156.6 (C4), 143.1 (C6), 95.1 (C5), 94.2 (C1'), 85.6 (C3', ³J_{P,C} = 11.2 Hz), 79.0 (C2'), 73.6 (C4'), 71.3 [CH(CH₃)₂, ²J_{P,C} = 4.7 Hz], 71.2 [CH(CH₃)₂, ²J_{P,C} = 4.7 Hz], 64.2 (PCH₂, ¹J_{P,C} = 169.3 Hz), 54.4 (OMe), 24.0 [CH(CH₃)₂]; ³¹P-NMR (121 MHz, CDCl₃): δ 18.3; HRMS (ESI+) calcd for C₁₆H₂₇N₂O₈PNa [M+Na]⁺ 429.1403, found 429.1408.

(1'α,2'S)-1'-(O⁴-methyluracil-1-yl)-2'-fluoro-2'-O-methyl-3'-O-

diisopropylphosphonomethyl-L-threose (23a). A solution of **22** (0.033 g, 0.082 mmol) in dry CH₂Cl₂ was cooled in ice-salt bath to maintain temperature at -5 to -10 °C. To this was added (diethylamino)sulfur trifluoride (0.219 mL, 1.632 mmol) and py.HF (0.809 mg, 8.16 μmol) as a solution in 1 mL of dry CH₂Cl₂. The reaction mixture was allowed to stir at room temperature for 12 h. The volatiles are removed under reduced pressure to obtain crude oil. This residue was purified by silica gel flash column chromatography (98:2, CH₂Cl₂:MeOH) to obtain **23a** as a light yellow liquid (0.01 g, 0.023 mmol, 29 % yield): ¹H NMR (500MHz, CDCl₃): δ 7.89 (d, *J* = 7.6 Hz, 1H, H6), 6.44 (d, ²J_{F,H} = 16.4 Hz, 1H, H1'), 5.86 (d, *J* = 7.6 Hz, 1H, H5), 4.70-4.81 [m, 2H, CH(CH₃)₂], 4-33 (d, *J* = 11.5 Hz, 1H, H4a'), 4.14 (dd, *J* = 2.4, 7.9 Hz, 1H, H3'), 4.00-4.07 (m, 1H, H4b'), 3.97 (s, 3H, OCH₃), 3.86 (d, *J* = 9.0 Hz, 2H, PCH₂), 3.52 (s, 3H, 2'-OCH₃), 1.30-1.38 (m, 12 H, P[CH(CH₃)₂]₂); ¹³C NMR (125 MHz, CDCl₃): δ 171.4 (C2), 155.8 (C4), 144.9 (C6), 119.0 (C2', ¹J_{F,C} = 227.2 Hz), 95.3 (C5), 86.6 (C1', ²J_{F,C} = 48.6), 82.3 (C3', ³J_{P,C} = 11.1 Hz, ²J_{F,C} = 43.1), 71.1 and 71.0 [CH(CH₃)₂, ²J_{P,C} = 6.4 Hz], 69.1 (C4'), 64.1 (PCH₂, ¹J_{P,C} = 171.0 Hz), 54.1 (OCH₃), 53.27 (2'-OCH₃, ²J_{F,C} = 4.5 Hz), 29.6 [CH(CH₃)₂]; ³¹P NMR (121 MHz, CDCl₃): δ 17.6; HRMS (ESI+) calcd for C₁₇H₂₈FN₂O₈PNa [M+Na]⁺ 461.1465, found 461.1457.

1'-*O*-(*O*⁴-methyluracil-1-yl)-3'-*O*-diisopropylphosphonomethyl-L-erythrose (24). A solution of **21f** (0.129 g, 0.317 mmol) in 1 mL of dichloromethane was treated with 15% solution of Dess-Martin periodinane (DMP) in CH₂Cl₂ (1.8 mL, 0.635 mmol). A drop of water is added to accelerate the reaction; the reaction was complete in 1 h. The reaction mixture was diluted with diethyl ether and filtered off. The organic solvent was removed under reduced pressure to obtain crude keto (**22**) (*R*_f = 0.4, 7% MeOH in CH₂Cl₂). HRMS (ESI⁺) calcd for C₁₆H₂₆N₂O₈ [M+H]⁺ 405.1421, found 405.1386.

The crude ketone (**22**) was taken up in 2 mL of methanol and treated with sodium borohydride (0.635 mmol, 0.024g) at 0 °C. The reaction mixture was stirred at room temperature for 1 h, the reaction is quenched by dropwise addition of brine solution (1 mL) and stirred at room temperature for 30 min. The reaction mixture was diluted 20 mL of CH₂Cl₂ washed with 5 mL of water and 5mL of brine. The organic layer was dried and concentrated under reduced pressure. The residue was purified by column chromatography (elution with 7% MeOH in CH₂Cl₂; *R*_f = 0.3) to obtain **24** (0.1 g, 0.247 mmol, 78 % yield): ¹H NMR (300 MHz, CDCl₃): δ 7.76 (d, *J* = 7.6, Hz 1H, H₆), 6.20 (d, *J* = 3.4 Hz, 1H'), 5.91 (d, *J* = 7.6 Hz, 1H, H₅), 4.61-4.83 [m, 2H, CH(CH₃)₂], 4.60 (t, *J* = 3.9 Hz, 1H, H₂'), 4.20-4.32 (m, 1H, H₃'), 4.11 (d, *J* = 5.8 Hz, 2H, H₄'), 3.90-4.00 (m, CH₃O and PCH_a) 3.77 (dd, *J* = 8.6, 14.1 Hz, 1H, PCH_a,) 1.18-1.45 (m, 12 H, P[CH(CH₃)₂]₂); ¹³C NMR (75 MHz, CDCl₃): δ 171.6 (C₂), 156.2 (C₄), 144.1 (C₆), 94.3 (C₅), 86.7 (C₁'), 81.1 (C₃', ³*J*_{P, C} = 7.6 Hz), 71.5 [CH(CH₃)₂, ²*J*_{P, C} = 6.9 Hz], 71.3 [CH(CH₃)₂, ²*J*_{P, C} = 6.9 Hz], 69.4 (C₄'), 69.30 (C₂'), 65.3 (PCH₂, ¹*J*_{P, C} = 169.0 Hz), 54.4 (CH₃O), 23.7 [CH(CH₃)₂]; ³¹P NMR (121 MHz, CDCl₃): δ 19.3; HRMS (ESI⁺) calcd for C₁₆H₂₇N₂O₈P Na [M+Na]⁺ 429.1403, found 429.1408.

(1'α,2'R)-1'-(O⁴-Methyluracil-1-yl)-2'-deoxy-2'-fluoro-3'-O-diisopropylphosphonomethyl-L-threose (25). A solution of **24** (0.111 g, 0.273 mmol) in 1 mL of dry CH₂Cl₂ at -10 °C was treated with diethylaminosulfur trifluoride (DAST) (0.147 mL, 1.093 mmol). The reaction mixture was then allowed to warm to room temperature and stirred at room temperature for 10 h. The resulting solution was then carefully poured into an ice-cooled saturated solution of sodium bicarbonate (5 mL) and extracted with diethyl ether (4 x 20). The combined organic extracts were washed with brine (50 mL), dried over MgSO₄, filtered, and concentrated to a crude oil that was purified by silica gel flash column chromatography (elution with 3% MeOH in CH₂Cl₂ *R_f* = 0.3 in 3% MeOH in CH₂Cl₂) to obtain **25** as light yellow liquid (0.051 g, 0.125 mmol, 45.7 % yield). Note: reaction yield is poor if reaction begins at room temperature or 0 °C: ¹H NMR (300MHz, CDCl₃): δ 7.70 (d, *J* = 7.6 Hz, 1H, H6), 6.10 (d, ²*J*_{F,H} = 17.1 Hz, 1H, H1'), 5.89 (d, *J* = 7.6 Hz, 1H, H5), 5.14 (d, ¹*J*_{F,H} = 47.8 Hz, 1H, H2'), 4.62-4.76 [m, 2H, CH(CH₃)₂], 4.45 (d, *J* = 10.3 Hz, 1H, H4a'), 4.25-4.32 (m, 2H, H4b' and H3'), 3.95 (s, 3H, CH₃O), 3.69 (d, *J* = 1.48, 8.69 Hz, 2H, PCH₂), 1.27-1.32 (m, 12 H, P[CH(CH₃)₂]₂); ¹³C NMR (75 MHz, CDCl₃): δ 172.1 (C2), 155.8 (C4), 142.7 (C6), 95.5 (C2', ¹*J*_{F,C} = 186.2 Hz), 95.2 (C5), 90.8 (C1', ²*J*_{F,C} = 36.5 Hz), 82.3 (C3', ³*J*_{P,C} = 9.6 Hz, ²*J*_{F,C} = 26.9 Hz), 73.7 (C4'), 71.4 [CH(CH₃)₂, ²*J*_{P,C} = 4.6 Hz], 64.5 (PCH₂, ¹*J*_{P,C} = 169.0 Hz), 54.4 (OCH₃), 29.6 [CH(CH₃)₂]; ³¹P NMR (121 MHz, CDCl₃): δ 17.5; HRMS (ESI+) calcd for C₁₆H₂₆FN₂O₇PNa [M+Na]⁺ 431.1360, found 431.1365.

(1'α,2'R)-1'-(Cytosin-1-yl)-2'-deoxy-2'-fluoro-3'-O-diisopropylphosphonomethyl-L-threose (25m). The compound **25** (0.051 g, 0.125 mmol) was taken up in 2 mL of sat. ammonia in methanol and refluxed for 6 h. The volatiles are removed and purified by

column chromatography (elution with 7 % MeOH in CH₂Cl₂; *R_f* = 0.2) to obtain compound **25m** (0.04 g, 0.102 mmol, 81 % yield) as light yellow liquid: ¹H NMR (500 MHz, MeOD): δ 7.65 (d, *J* = 7.6 Hz, 1H, H6), 5.99 (d, ¹*J*_{F,H} = 18.3 Hz, 1H, H1), 5.89 (d, *J* = 7.6 Hz, 1H, H5), 5.16 (d, ¹*J*_{F,H} = 47.8 Hz, 1H, H2), 4.62-4.73 [m, 2H, CH(CH₃)₂], 4.50 (d, *J* = 10.9 Hz, 1H, H4a'), 4.35 (dd, *J* = 3.6, 11.2 Hz, 1H, H4b'), 4.25 (ddd, *J* = 10.9, 3.6, 2.1 Hz, 1H, H3'), 3.95 (dd, *J* = 9.7, 13.6 Hz, 1H, PCH_a), 3.88 (dd, *J* = 9.7, 13.6 Hz, 1H, PCH_b), 1.28-1.34 (m, 12 H, P[CH(CH₃)₂]₂); ¹³C NMR (125 MHz, MeOD): δ 165.3 (C2), 155.1 (C4), 141.3 (C6), 95.4 (C2', ¹*J*_{F,C} = 181.0 Hz), 93.4 (C5), 90.3 (C1', ²*J*_{F,C} = 36.1 Hz), 82.3 (C3', ³*J*_{P,C} = 10.8 Hz, ²*J*_{F,C} = 28.7 Hz), 73.1 (C4'), 71.1 [CH(CH₃)₂, ²*J*_{P,C} = 7.4 Hz], 64.2 (PCH₂, ¹*J*_{P,C} = 169.0 Hz), 23.7 [CH(CH₃)₂]; ³¹P NMR (121 MHz, MeOD): δ 17.6; HRMS (ESI+) calcd for C₁₅H₂₆FN₃O₆P [M+H]⁺ 394.1538, found 394.1555.

(1'α,2'-1'-(Cytosin-1-yl)-2'-deoxy-2'-fluoro-3'-O-phosphonomethyl-L-threose (7).

A solution of **25m** (5.00 mg, 0.014 mmol) and triethylamine (0.019 mL, 0.137 mmol) in 2 mL of dry CH₂Cl₂ was treated with bromotrimethylsilane (0.018 mL, 0.137 mmol) at room temperature for 30 h. The reaction is quenched with 1M TEAB solution and the reaction mixture was concentrated to dryness. The residue was purified by flash column chromatography (50:25:3, CH₂Cl₂:MeOH:1M TEAB) to obtain target compound **7** (3 mg, 9.70 μmol, 70.9 % yield) as off-white solid: ¹H NMR (500MHz, MeOD): δ 7.76 (d, *J* = 7.6 Hz, 1H, H6), 6.08 (d, ¹*J*_{F,H} = 20.5 Hz, 1H, H1'), 6.02 (d, *J* = 7.6 Hz, 1H, H5), 5.22 (d, ¹*J*_{F,H} = 47.8 Hz, 1H, H2), 4.50 (d, *J* = 11.2 Hz, 1H, H4a'), 4.39 (bd, *J* = 12.8, 1H, H3'), 4.19 (bd, *J* = 11.2 Hz, 1H, H4b'), 3.47 (bd, *J* = 8.6 Hz, 2H, PCH₂); ¹³C NMR (125 MHz, MeOD): δ 166.0 (C2), 157.1 (C4), 142.0 (C6), 96.1 (C2', ¹*J*_{F,C} = 181.6 Hz), 95.8 (C5),

89.7 (C1', $^2J_{F,C} = 39.0$ Hz), 81.3 (C3', $^3J_{P,C} = 9.0$ Hz, $^2J_{F,C} = 27.0$ Hz), 72.9 (C4'), 66.6 (PCH₂, $^1J_{P,C} = 152.4$ Hz); ^{31}P NMR (121 MHz, MeOD): δ 13.4; HRMS (ESI-) calcd for C₉H₁₂FN₃O₆P⁻ [M-H]⁻ 308.0453, found 308.0450

1'- α -(N⁶-Benzoyladenine-9-yl)-2'-O-benzoyl-3'-O-*tert*-butyldiphenylsilyl-L-threose

(26). To a solution of N⁶-benzyladenine (3.98 g, 16.7 mmol) in CH₃CN (80 mL), BSA (8.14 mL, 33.3 mmol) was added and the mixture stirred for 20 min at room temperature. To the mixture, a solution of **17** in CH₃CN (10 mL) was added. After cooling at 0 °C, TMSOTf (3.01 mL, 16.7 mmol) was added dropwise and the reaction mixture was stirred for 1 h at room temperature and heated at 70 °C for overnight. The reaction mixture was poured into a mixture of CH₂Cl₂ and saturated NaHCO₃ solution (5/1, v/v). The CH₂Cl₂ layer was removed and the water layer was extracted with CH₂Cl₂. The combined CH₂Cl₂ extracts were dried over Na₂SO₄. After the solvent was removed the residue was purified by column chromatography (CH₂Cl₂/MeOH 50:1) on silica gel to afford **26** (2.71 g, 51 %) and **26b** (1.71 g, 30 %) as a light yellow foam.

N⁷ to N⁹ isomerization: To a solution of **26b** (1.71 g, 2.50 mmol) in toluene (25 mL), a catalytic amount of *p*-TsOH-H₂O (48 mg, 0.25 mmol) was added and the mixture heated at 130 °C for overnight. After removing volatiles, the residue was diluted with CH₂Cl₂ and washed with saturated NaHCO₃ solution. The CH₂Cl₂ layer was removed and the water layer was extracted with CH₂Cl₂. The combined CH₂Cl₂ extracts were dried over Na₂SO₄. After the solvent was removed the residue was purified by column chromatography (CH₂Cl₂/MeOH 50:1) on silica gel to afford **26** (0.77 g, 45 %) as a light yellow foam: $[\alpha]_D^{20}$ -115.4 (*c* = 0.42 CH₂Cl₂); ^1H NMR (300 MHz, CDCl₃): δ 9.17 (s, 1H, NH), 8.84 (s, 1H, H2), 8.55 (s, 1H, H8), 8.04 (d, *J* = 7.1 Hz, 2H, Bz-H), 7.95 (d, *J* =

7.1 Hz, 2H, Bz-H), 7.66-7.29 (m, 16H, Ph-H, Bz-H), 6.37 (d, $J = 1.4$ Hz, 1H, H1'), 5.94 (s, 1H, H2'), 4.60-4.57 (m, 1H, H3'), 4.22 (dd, $J = 2.3, 10.0$ Hz, 1H, H4'), 4.11 (dd, $J = 4.4, 10.0$ Hz, 1H, H4'), 1.03 [s, 9H, C(CH₃)₃]. ¹³C NMR (75 MHz, CDCl₃): δ 165.0, 164.7, 153.1, 151.8, 149.7, 141.8, 135.9, 135.8, 134.0, 133.9, 132.9, 132.6, 131.9, 130.5, 130.4, 130.1, 129.0, 128.8, 128.7, 128.2, 128.1, 128.0, 123.2, 88.5, 82.8, 76.3, 75.8, 27.0, 19.1; HRMS: calcd for C₃₉H₃₈N₅O₅Si [M+H]⁺ 684.2637, found 684.2642.

1'- α -(N⁶-Benzoyladenin-9-yl)-2'-O-benzoyl-L-threose (26c). To a solution of **26** (4.3 g, 6.29 mmol) in THF (65 mL) were added 1M solution of TBAF in THF (7.55 mL, 7.55 mmol) and AcOH (0.5 mL) and the solution was stirred for 2 h at room temperature. After removing volatiles, the residue was diluted with CH₂Cl₂ and washed with water, brine and dried over Na₂SO₄. After evaporation, the crude residue was purified by silica gel chromatography (40:1; CH₂Cl₂:MeOH) to afford **26c** (2.18 g, 78 %) as a white foam: $[\alpha]^{20}_D -75.2$ (c = 0.34 CH₂Cl₂); ¹H NMR (300 MHz, MeOD): δ 8.68 (s, 1H, H2), 8.66 (s, 1H, H8), 8.08-8.02 (m, 4H, Bz-H), 7.64-7.45 (m, 6H, Bz-H), 6.44 (d, $J = 1.1$ Hz, 1H, H1'), 5.68 (s, 1H, H2'), 4.59 (s, 1H, H3'), 4.35 (br s, 2H, H4'); ¹³C NMR (75 MHz, MeOD): δ 166.7, 153.2, 152.9, 151.2, 144.7, 135.0, 134.9, 133.9, 130.8, 130.3, 129.7, 129.4, 124.9, 90.0, 84.0, 77.0, 75.0; HRMS: calcd for C₂₃H₂₀N₅O₅ [M+H]⁺ 446.1459, found 446.1459.

1'- α -(N⁶-Benzoyladenin-9-yl)-2'-O-benzoyl-3'-oxo-L-threose (27). Under argon, to **26c** (1.87 g, 4.2 mmol) was added to a solution of Dess-Martin periodinane (15 % in CH₂Cl₂, 13.4 mL, 6.3 mmol) in dry CH₂Cl₂ (100 mL) at 0 °C. The reaction mixture was stirred overnight at room temperature (a white solid has formed). To a reaction mixture, a solution of saturated aqueous sodium thiosulfate and saturated aqueous NaHCO₃ (1:1,

100 mL) was added and the mixture was stirred for 20 min at room temperature. After separation, the organic layer was brined, dried over Na₂SO₄, filtered and concentrated under reduced pressure to afford crude **27** (1.8 g, 97 %) as a white foam; ¹H NMR (300 MHz, CDCl₃): δ 9.00 (s, 1H, NH), 8.79 (s, 1H, H2), 8.20 (s, 1H, H8), 8.04 (d, *J* = 7.2 Hz, 4H, Bz-H), 7.66-7.44 (m, 6H, Bz-H), 6.46 (d, *J* = 5.5 Hz, 1H, H1'), 6.18 (d, *J* = 5.5 Hz, 1H, H2'), 4.76 (d, *J* = 16.9 Hz, 1H, H4a'), 4.58 (d, *J* = 16.9 Hz, 1H, H4b'); ¹³C NMR (75 MHz, CDCl₃): δ 204.5, 165.5, 165.0, 152.9, 151.9, 150.1, 142.3, 134.3, 133.5, 132.9, 130.2, 128.9, 128.7, 128.1, 127.7, 123.8, 86.3, 73.9, 72.0; HRMS: calcd for C₂₃H₁₈N₅O₅ [M+H]⁺ 444.1302, found 444.1307

(1'α,3'S)-1'-(N⁶-Benzoyladenine-9-yl)-2'-O-benzoyl-3'-ethynyl-L-threose (28). A suspension of anhydrous CeCl₃ (5.10 g, 20.7 mmol) in THF (80 mL) under Ar was stirred overnight. The solution is cooled to -78 °C. Ethynylmagnesium bromide (0.5 M in THF, 40.6 mL, 20.3 mmol) was added over 20 min. The suspension was stirred at -78 °C for 1.5 h. A solution of **27** (1.8 g, 4.06 mmol) in THF (10 mL) was added over 10 min and stirred at -78 °C for 4 h. The reaction was quenched with sat. NH₄Cl (10 mL). After being allowed to warm to room temperature, the reaction mixture was filtered and extracted with CH₂Cl₂, brine, dried over Na₂SO₄ and concentrated under reduced pressure. The residue was purified by silica gel chromatography (60:1 ; CH₂Cl₂:MeOH) to afford **28** (0.61 g, 32 %) as a white foam; ¹H NMR (500 MHz, DMSO): δ 11.24 (s, 1H, NH), 8.76 (s, 1H, H2), 8.59 (s, 1H, H8), 8.09-8.05 (m, 4H, Bz-H), 7.73 (tt, *J* = 1.3, 7.5 Hz, 1H, Bz-H), 7.66 (tt, *J* = 1.2, 7.4 Hz, 1H, Bz-H), 7.59 (t, *J* = 8.1 Hz, 2H, Bz-H), 7.56 (t, *J* = 8.0 Hz, 2H, Bz-H), 7.00 (s, 1H, OH), 6.47 (d, *J* = 2.2 Hz, 1H, H1'), 5.88 (d, *J* = 1.9 Hz, 1H, H2'), 4.48 (d, *J* = 9.1 Hz, 1H, H4a'), 4.27 (d, *J* = 9.1 Hz, 1H, H4b'), 3.70 (s, 1H,

CH); ^{13}C NMR (75 MHz, CDCl_3): δ 165.7, 164.3, 151.9, 51.8, 150.5, 142.7, 134.1, 133.4, 132.5, 129.7, 129.0, 128.5, 125.5, 87.9, 81.2, 78.5, 78.2, 73.4; HRMS: calcd for $\text{C}_{25}\text{H}_{20}\text{N}_5\text{O}_5$ $[\text{M}+\text{H}]^+$ 470.1459, found 470.1458

(1' α ,3'S)-1'-(N⁶-Benzoyladenine-9-yl)-2'-O-benzoyl-3'-ethynyl-3'-O-

(diisopropylphosphonomethyl)-L-threose (28f). To a solution of **28** (0.60 g, 1.28 mmol) and diisopropylphosphonomethyl trifluoromethanesulfonate (0.42 g, 1.28 mmol) in dry THF was added NaH (60 % in oil, 0.13 g, 3.3 mmol) at -15°C . The reaction mixture was stirred at -15°C for 1 h. The reaction was quenched with saturated NH_4Cl (5 mL) and concentrated. The residue was partitioned between water and EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by chromatography on silica gel column (50:1, CH_2Cl_2 :MeOH) to afford **28f** (0.73 g, 88 %) as a white solid: ^1H NMR (300 MHz, CDCl_3): δ 9.33 (s, 1H, NH), 8.70 (s, 1H, H2), 8.66 (s, 1H, H8), 8.01 (d, J = 7.1 Hz, 2H, Bz-H), 7.95 (d, J = 7.1 Hz, 2H, Bz-H), 7.55-7.48 (m, 2H, Bz-H), 7.44-7.38 (m, 4H, Bz-H), 6.47 (d, J = 2.1 Hz, 1H, H1'), 5.79 (s, 1H, H2'), 4.77-4.70 [m, 2H, $\text{CH}(\text{CH}_3)_2$], 4.54 (d, J = 10.5 Hz, 1H, H4a'), 4.17 (d, J = 10.5 Hz, 1H, H4b'), 4.01-3.96 (m, 2H, PCH_2), 2.71 (s, 1H, CH), 1.31-1.26 (m, 12H, CH_3); ^{13}C NMR (75 MHz, CDCl_3): δ 164.7, 164.6, 152.9, 151.6, 149.6, 142.5, 133.9, 132.6, 130.0, 128.8, 128.6, 127.9, 122.6, 87.9, 82.6 (d, $^3J_{\text{P,C}}$ = 15.5 Hz), 80.9, 80.5, 75.4, 74.3, 71.7 (d, $^2J_{\text{P,C}}$ = 6.5 Hz), 60.5 (d, $^1J_{\text{P,C}}$ = 170.8), 24.1 (d, J = 4.2 Hz), 24.0 (d, $^3J_{\text{P,C}}$ = 5.5 Hz); ^{31}P NMR (125 MHz, CDCl_3 , 25°C): δ 17.7; HRMS: calcd for $\text{C}_{32}\text{H}_{35}\text{N}_5\text{O}_8\text{P}_1$ $[\text{M}+\text{H}]^+$ 648.2218, found 648.2218.

(1' α ,3'S)-1'-(N⁶-Benzoyladenine-9-yl)-3'-ethynyl-3'-O-

(diisopropylphosphonomethyl)-L-threose (29). To a solution of **28f** (0.6 g, 0.93 mmol)

in 7N NH₃ in MeOH (5 mL) was stirred at room temperature for 24 h. After removing volatiles, the crude residue was purified by chromatography on silica gel (20:1, CH₂Cl₂:MeOH) to afford **29** (0.35 g, 86 %) as a white solid: ¹H NMR (300 MHz, CDCl₃): δ 8.27 (s, 1H, H2), 8.23(s, 1H, H8), 7.29 (s, 2H, NH₂), 6.19 (s, 1H, H1'), 4.79-4.69 [m, 2H, CH(CH₃)₂], 4.53 (s, 1H, H2'), 4.47 (d, *J* = 9.9 Hz, 1H, H4a'), 4.18 (d, *J* = 9.9 Hz, 1H, H4b'), 3.95 (quint, *J* = 9.9 Hz, 2H, PCH₂), 2.93 (s, 1H, CH), 1.34-1.24 (m, 12H, CH₃) ppm; ¹³C-NMR (75 MHz, CDCl₃): δ 156.1, 152.9, 149.1, 140.0, 119.4, 90.6, 83.8 (d, ³*J*_{P,C} = 13.9 Hz), 80.6, 80.4, 76.4, 74.4, 71.9 (d, ²*J*_{P,C} = 3.5 Hz), 71.8 (d, ²*J*_{P,C} = 2.8 Hz), 60.4 (d, ¹*J*_{P,C} = 171.4 Hz), 24.2 (d, ³*J*_{P,C} = 3.5 Hz), 24.1 (d, ³*J*_{P,C} = 4.4 Hz); ³¹P NMR (121 MHz, CDCl₃, 25 °C): δ 18.4; HRMS: calcd for C₁₈H₂₇N₅O₆P₁ [M+H]⁺ 440.1693, found 440.1687.

(1'α,3'□)-1'-(Adenin-9-yl)-3'-ethynyl-3'-O-(phosphonomethyl)-L-threose (8). The compound **29** (0.15 g, 0.34 mmol) in dry CH₂Cl₂ (2 mL) was treated with HMDS (1.07 mL, 5.12 mmol), and then TMSI (0.29 mL, 2.05 mmol) was added dropwise under stirring at 0 °C. The mixture was stirred for 2 h at 0 °C and quenched with 1M TEAB (1 mL). The mixture was concentrated, and the residue was purified by RP-HPLC running a gradient of CH₃CN in 0.1M TEAB buffer solution to afford **8** (0.115 g, 60 %, triethylamine salt) as a white solid: [α]_D²⁰ -65.9 (c = 0.22 H₂O); ¹H-NMR (300 MHz, D₂O): δ 8.41 (s, 1H, H2), 8.07(s, 1H, H8), 6.04 (s, 1H, H1'), 4.68 (s, 1H, H2'), 4.58 (d, *J* = 10.0 Hz, 1H, H4a'), 4.21 (d, *J* = 10.0 Hz, 1H, H4b'), 3.74 (d, *J* = 10.5 Hz, 2H, PCH₂), 3.27 (s, 1H, CH); ¹³C-NMR (75 MHz, D₂O): δ 154.9, 152.2, 148.1, 140.5, 117.8, 88.9, 82.4 (CH(CH₃)₂, ³*J*_{P,C} = 13.8 Hz), 80.4, 79.2, 75.8, 74.3, 61.7 (PCH₂, ¹*J*_{P,C} = 154.6 Hz);

^{31}P -NMR (121 MHz, D_2O): δ 14.1; HRMS: calcd for $\text{C}_{12}\text{H}_{13}\text{N}_5\text{O}_6\text{P}$ $[\text{M}-\text{H}]^-$ 354.0609, found 354.0613.

(1' α ,3' S)-1'-(Adenin-9-yl)-3'-ethynyl-3'- O -(diphosphatephosphonomethyl)-L-threose (30). The compound **8** (70 mg, 0.13 mmol) was dissolved in anhydrous DMF (1 mL) and Bu_3N (0.09 mL, 0.38 mmol) was added. The mixture was concentrated in vacuo and then redissolved in anhydrous DMF (1 mL) and treated with *N,N*-carbonyldiimidazole (0.2 g, 1.25 mmol). The mixture was stirred for 30 min and tris(tetrabutylammonium) hydrogen pyrophosphate (0.9 g, 1.0 mmol) in DMF (1 mL) was added. The mixture was stirred for overnight. Excess aqueous ammonia (25 % in water, 2 mL) was added and the mixture concentrated by lyophilization. The residue was purified by RP-HPLC running a gradient of CH_3CN in 0.1M TEAB buffer solution to afford **30** (0.02 g, 31 %) as a white solid: ^1H NMR (500 MHz, D_2O): δ 8.37(s, 1H, H2), 8.06 (s, 1H, H8), 5.97 (d, $J = 3.3\text{ Hz}$, 1H, H1'), 4.60 (s, 1H, H2'), 4.45 (d, $J = 10.0\text{ Hz}$, 1H, H4a'), 4.07 (d, $J = 10.0\text{ Hz}$, 1H, H4b'), 3.87 (d, $J = 10.5\text{ Hz}$, 2H, PCH_2), 3.12 (s, 1H, CH); ^{13}C NMR (125 MHz, D_2O): δ 155.3, 152.4, 148.5, 140.5, 118.1, 88.7, 82.5 (d, $^3J_{\text{P,C}} = 15.4\text{ Hz}$), 80.9, 79.2, 74.8, 74.3, 61.2 (d, $^1J_{\text{P,C}} = 165.7\text{ Hz}$); ^{31}P NMR (200 MHz, D_2O): δ 7.5 (d, $J = 24\text{ Hz}$, P_α), -7.3 (br s, P_γ), -22.2 (t, $J = 21\text{ Hz}$, P_β); HRMS: calcd for $\text{C}_{12}\text{H}_{16}\text{N}_5\text{O}_{12}\text{P}_3$ $[\text{M}-\text{H}]^-$ 513.9935, found 513.9933.

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Supporting Information Available.

Supporting information associated with this note is available free of charge via the Internet at <http://pubs.acs.org>

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