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Synthesis of 3′-*S*-Phosphonomethyl-Modified Nucleoside Phosphonates with a 3′-Deoxy-3′-thio-α-L-threosyl Sugar Moiety

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The synthesis and antiviral evaluation of 3′-deoxy-3′-*S*-phosphonomethyl-3′-thio-α-L-threosyl nucleosides as well as the 2′-deoxy analogues related to PMDTT are described. The key transformations involved the synthesis of the 3′-deoxy-3′-thio-L-threosyl and the 2′,3′-dideoxy-3′-thio-L-threosyl derivatives by the Mitsunobu reaction. The phosphonate function was introduced by nucleophilic substitution and the glycosylation was carried out under Vorbrüggen conditions. None of these synthesized compounds showed significant in vitro activity against HIV, HCV, and RSV, or cytotoxicity.

Introduction

Nucleoside analogues with a thiosugar moiety (e.g., 4′-thionucleosides[1]) exhibit interesting biological activities, such as antibacterial,[2] antiviral,[3] and antitumor[4] activity, owing to the presence of the sulfur atom in the sugar moiety. Despite the development of new synthetic strategies,[5] practical and facile preparation of thiosugar derivatives remains a challenge for synthetic organic chemists. Nucleoside phosphonates with a phosphonomethoxy group on the sugar moiety, such as (*S*)-HPMPC,[6] PMEA,[7] (*R*)-PMPA,[8] PMDTA,[9] and d4AP,[10] are of biological interest. Herein we describe the synthesis of thionucleoside analogues with a bioisosteric phosphonate function on the sugar moiety. To the best of our knowledge little attention has been paid to the synthesis of thionucleoside phosphonates primarily due to the synthetic challenges involved.

PMDTA analogues (Figure 1) bearing a phosphonomethoxy group at the 3′-position of the sugar moiety have been described as highly selective and potent anti-HIV agents.[9] As part of our ongoing program exploring the properties and scope of the phosphonate linker at the 3′-position of the sugar moiety in relation to antiviral activity, we replaced the 3′-phosphonomethoxy group in the PMDTA analogues with a phosphonomethylthio congener. Thus, compounds **1a**, **1b**, and **1c** (Figure 1) with a 3′-*S*-phosphonomethyl-modified linker related to PMDTA analogues were synthesized and evaluated for their in vitro activity against HIV, HCV, and RSV in cell assays. Compounds **1a** and **1b** were obtained by synthesis of the 3′-deoxy-3′-thio-L-threosyl sugar moiety by Mitsunobu reaction followed by introduction of the base moiety by Vorbrüggen glycosylation. Following the same strategy, the 2′-deoxygenated analogue **1c** was prepared as an α and β anomeric mixture.

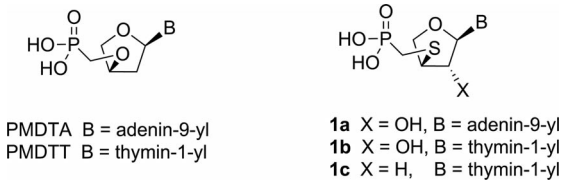

Figure 1. Structures of PMDTA, PMDTT, and the synthesized nucleoside phosphonates **1a**–**1c**.

Results and Discussion

Scheme 1 shows the synthetic strategies for obtaining the target compounds **1b** and **1c** by using the disconnection approach. Compounds **1b** and **1c** were obtained by hydrolysis of the phosphonate ester groups of **11** and **20**, respectively. However, all attempts to synthesize **20** proved unsuccessful by synthetic methods such as substitution of the 3′-hydroxy group of 2′-deoxynucleoside **2a** with thiol reagent **3** and thioacetic acid by Mitsunobu reaction,[11] nucleophilic substitution of **2b** with the nucleophile **3** under basic conditions, and reduction of the 2′-hydroxy group of **11** by Barton deoxygenation.[12] Compounds **11** and **20** were prepared by glycosylation of the thiosugars **7** and **19**, respectively, under Vorbrüggen conditions.[13] Compounds **7** and **19** were derived from the related compounds **5** and **17** by nucleophilic substitution after removal of the 3-*S*-acetyl protecting group. Introduction of the acetylthio group at the 3-position of **4** and **16** was achieved by Mitsunobu reaction giving **5** and **17**, respectively, with a reverse configuration at the 3-position.

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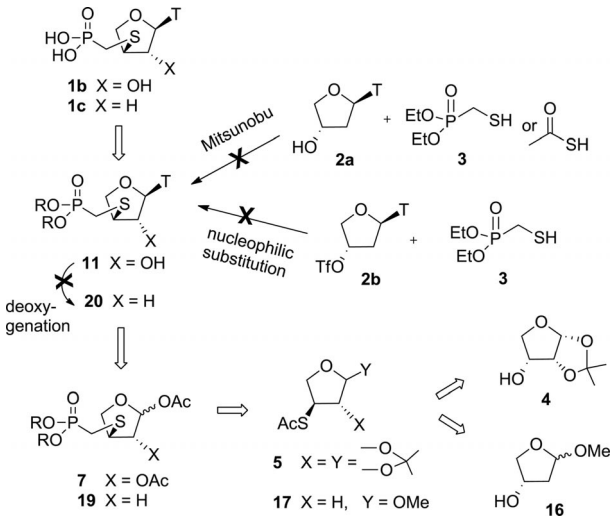
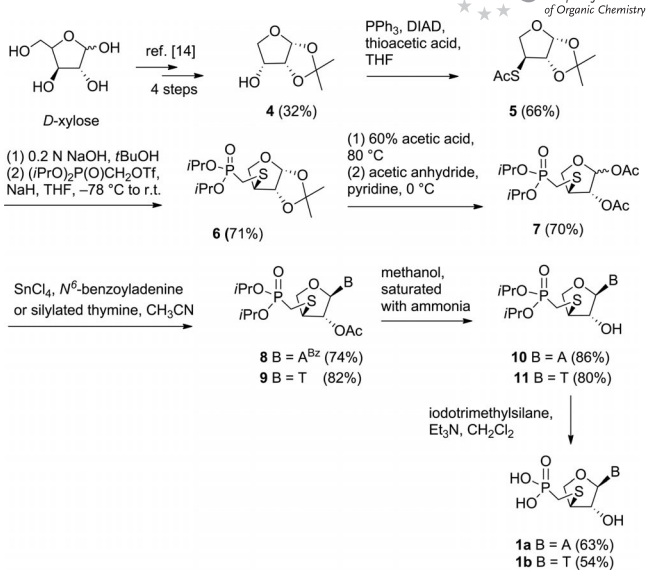


Scheme 1. Synthetic strategies towards the target compounds **1b** and **1c**.



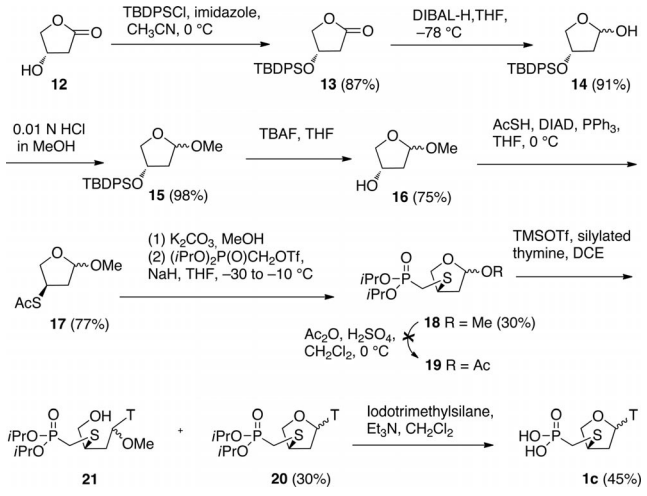
Scheme 2. Synthesis of target compounds **1a** and **1b**.

As shown in Scheme 2, nucleoside phosphonates **1a** and **1b** were synthesized starting from D-xylose. 1,2-Isopropylidene-D-erythrose (**4**) was derived from D-xylose according to reported methods.[14] Substitution of the free hydroxy group of **4** with thioacetic acid under Mitsunobu conditions provided compound **5** (with a reverse configuration at the 3-position) in 66% yield. The acetyl protecting group of **5** was removed by treatment with 0.2 N NaOH under argon followed by lyophilization to give a yellow powder, which easily forms the disulfide by oxidative coupling upon exposure to air. The intermediate obtained was directly treated with the triflate of diisopropylphosphonomethanol and NaH in THF at –78 °C under argon to provide compound **6** (71% yield for two steps). Removal of the isopropylidene protecting group of **6** and replacement with two acetyl protecting groups gave compound **7** as an α/β anomeric mixture in 70% yield. The presence of the 2-*O*-acetyl group in compound **7** allowed selective introduction of the base moiety with an α configuration. The nucleobase N^6-benzoyladenine and silylated thymine were introduced using $SnCl_4$ as Lewis catalyst under Vorbrüggen glycosylation conditions (giving **8** and **9**). Compounds **8** and **9** were deprotected in two steps: Removal of the benzoyl and acetyl groups with ammonia in methanol (yielding **10** and **11**) followed by hydrolysis of the diisopropyl protecting groups with TMSI at room temperature. After purification by silica gel chromatography, C_{18} reversed-phase HPLC, and Dowex sodium ion-exchange resin, nucleoside phosphonic diacids **1a** and **1b** were obtained as the sodium salts (63 and 54%, respectively). In an effort to obtain the 2′-deoxygenated analogues of **1a** and **1b**, Barton deoxygenation was attempted to remove the 2′-OH group of **10** and **11**. Unfortunately, under these conditions the 3′-*S*-diisopropylphosphonomethyl linker was also reduced and no selectivity in the radical reduction of the 2′- or 3′-position was observed.

2D gHMBC NMR experiments were performed to illustrate the correct connection of the nucleobase to the sugar moiety. For compound **1a**, the gHMBC spectrum shows that the proton of C-1′ of the sugar moiety is coupled to C-4 and C-8 of the adenine moiety, which proves that C-1′ is linked to the N-9 of the adenine through a C–N bond. Likewise, for compound **1b**, the gHMBC spectrum shows that the proton of C-1′ of the sugar moiety is coupled to C-6 of the thymine moiety, which suggests that C-1′ is linked to N-1 of the thymine moiety through a C–N bond.

The 2′-deoxy analogue **1c** was synthesized following the same strategy as described for **1a** and **1b**. We proposed to first synthesize the 2′,3′-dideoxy-3′-thio-L-threosyl sugar moiety and then introduce the base moiety under Vorbrüggen glycosylation conditions. Based on this strategy, the α/β anomeric mixture of **1c** needed to be separated. As shown in Scheme 3, commercially available (*S*)-β-hydroxy-γ-butyrolactone (**12**) was used as the starting material. Protection of the free hydroxy group of **12** with a TBDPS protecting group provided compound **13**. Compound **14** was obtained by selective reduction of the lactone function of **13** with DIBAL-H at –78 °C. The free 1-hydroxy group of **14** was protected with a methyl group to give **15**. Removal of the TBDPS protecting group of **15** by treatment with TBAF provided **16**. Substitution of the 3-hydroxy group of **16** with thioacetic acid was achieved under Mitsunobu conditions to give the expected compound **17** with a reverse configuration at the 3-position. The 3-*S*-acetyl protecting group of **17** was cleaved by treatment with K_2CO_3 in absolute MeOH under argon. The intermediate obtained, without purification, was treated with the triflate of diisopropylphosphonomethanol and NaH in THF at –78 °C under argon to give **18**. Note that, by removing the acetyl group of **17**, the intermediate obtained easily forms disulfides by oxidative coupling upon exposure to air. Attempts to convert the 1-*O*-methyl group of **18** to a 1-*O*-acetyl group were not successful under acid catalysis. Gly-

cosylation of **18** with silylated thymine under Vorbrüggen conditions (TMSOTf as Lewis catalyst) provided an α/β anomeric mixture of **20** as well as the unexpected ring-opened nucleoside **21**. Note that the glycosylation reaction of **18** was not successful when using silylated N^6-benzoyladenine and $SnCl_4$ as Lewis catalyst. Unfortunately, we were unable to separate the α and β anomers of **20** by various purification methods, including preparative TLC and normal-phase (silica gel column) and reversed-phase (C_{18} column) HPLC. Hydrolysis of the diisopropyl protecting groups of the anomeric mixture **20** was achieved by treatment with TMSI at 0 °C. After purification by silica gel chromatography, reversed-phase (C_{18} column) HPLC, and Dowex sodium ion-exchange resin, the expected nucleoside phosphonic diacid **1c** was obtained as an anomeric mixture in the sodium form. The gHMBC spectrum of **1c** shows that the proton of C-1′ of the sugar moiety of both anomers is coupled with C-6 of the thymine moiety, which suggests that C-1′ is linked to the N-1 of the thymine moiety for both the α and β anomers.



Scheme 3. Synthesis of target compound **1c**.

Conclusions

Starting from D-xylose, a synthetic scheme has been developed for the synthesis of 3′-*S*-phosphonomethyl-modified 3′-deoxy-3′-thio-α-L-threosyl nucleoside analogues **1a** and **1b**. Unfortunately, attempts to reduce the 2′-hydroxy group of the related nucleoside analogues under Barton deoxygenation led to loss of the 3′-*S*-phosphonomethyl linker of the sugar moiety. The 2′-deoxy analogue **1c** with a thymine moiety was obtained as a mixture of α and β anomers. The key transformations in the synthetic scheme leading to **1c** are the introduction of the acetylthio group at the sugar moiety using the Mitsunobu reaction and glycosylation with the silylated thymine base under Vorbrüggen conditions. Unfortunately, none of the synthesized compounds (**1a**, **1b** and **1c**) shows in vitro activity against HIV, HCV, and RSV, or cytotoxicity at concentrations up to 50 μM. Thus, the replacement of the 3′-oxygen by a sulfur atom in the 3′-phosphonomethoxy group of PMDTT results in the loss of antiviral activity. The lack of antiviral activity in these compounds could result from poor metabolic activation to the related diphosphates and/or the lack of affinity of the diphosphates towards viral polymerases (in this case, HIV RT, HCV NS5B RdRp, and RSV RNA polymerase).

Experimental Section

General: Analytical grade solvents were used for all reactions. All moisture-sensitive reactions were carried out in oven-dried glassware (135 °C) under nitrogen. Anhydrous THF was heated at reflux over sodium/benzophenone and distilled. Varian Unity 500 MHz and Gemini 300 MHz spectrometers were used for ^1H, ^{31}P, and ^{13}C NMR spectroscopy. 2D NMR (H,H-COSY, gHQSC, and gHMBC) was used for structural assignment of the final compounds. Exact mass measurements were performed with a quadrupole time-of-flight mass spectrometer (Q-Tof-2, Micromass, Manchester, UK) equipped with a standard electrospray ionization (ESI) interface; samples were infused in *i*PrOH/H_2O (1:1) at 3 μL/min. Precoated aluminium sheets (Fluka silica gel/TLC cards, 254 nm) were used for TLC analysis; the spots were examined under UV light and visualized with CAM (ceric ammonium molybdate) stains. Column chromatography was performed on ICN silica gel (mesh 63–200, 60 Å). For the sake of clarity, the NMR signals of sugar protons and carbon atoms are indicated with a prime and the signals of base protons and carbon atoms are given without a prime.

3-*S*-Acetyl-3-deoxy-1,2-*O*-isopropylidene-3-mercapto-β-L-threofuranose (5): DIAD (652 μL, 3.28 mmol) was added dropwise to a solution of PPh_3 (859 mg, 3.28 mmol) and compound **4** (238 mg, 1.49 mmol) in dry THF (15 mL) at 0 °C. After stirring for 30 min, a solution of thioacetic acid (233 μL, 3.28 mmol) was added dropwise to the resulting yellow suspension and the reaction mixture was continuously stirred for 3 h at 0 °C. The reaction mixture was quenched with sat. sodium hydrogen carbonate, concentrated, and partitioned between EtOAc and water. The organic layer was washed with water and brine, dried with anhydrous sodium sulfate, filtered, and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/hexane = 1:4) to afford **5** (214 mg, 0.98 mmol) in 66% yield as yellow crystals. ^1H NMR (300 MHz, [D_6]DMSO): δ_H = 1.24 (s, 3 H, CH_3), 1.41 (s, 3 H, CH_3), 2.36 (s, 3 H, Ac CH_3), 3.73 (d, J = 10.0 Hz, 1 H, 4-H_a), 3.83 (d, J = 4.1 Hz, 1 H, 3-H), 4.16 (dd, J_1 = 10.0, J_2 = 4.2 Hz, 1 H, 4-H_b), 4.54 (d, J = 3.6 Hz, 1 H, 2-H), 5.82 (d, J = 3.6 Hz, 1 H, 1-H) ppm. ^{13}C NMR (75 MHz, [D_6]DMSO): δ_C = 26.54 (CH_3), 26.77 (CH_3), 31.07 (Ac CH_3), 47.86 (C-3), 69.91 (C-4), 84.86 (C-2), 105.17 (C-1), 111.51 [$(O)_2C(CH_3)_2$], 194.83 (Ac, CO) ppm. HRMS: calcd. for $C_9H_{15}O_4S$ [M + Na]$^+$ 219.0691; found 219.0683.

3-Deoxy-3-*S*-(diisopropylphosphonomethyl)-1,2-*O*-isopropylidene-3-mercapto-β-L-threofuranose (6): Compound **5** (160 mg, 0.735 mmol) was stirred in degassed NaOH (0.2 N, 8 mL) solution at room temperature under Ar for 7 h. The solvent was removed by lyophilization to give a white solid. A suspension of NaH (147 mg, 3.677 mmol) in dry and degassed THF (20 mL) was added to the flask with the white solid under Ar. The mixture was cooled to –78 °C and the triflate of diisopropylphosphonomethanol (2.941 mmol) in THF (5 mL) was added dropwise. The reaction mixture was slowly warmed to room temperature and stirred overnight. Aqueous NH_4Cl was added to quench the reaction and the

mixture was concentrated under reduced pressure. The residue was partitioned between EtOAc and H_2O, the organic layer was dried with Na_2SO_4, concentrated, and purified by column chromatography (EtOAc/hexane = 1:1) to afford **6** (185 mg, 0.522 mmol) as a colorless oil in 71% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H = 1.32–1.36 (m, 15 H, CH_3), 1.49 (s, 3 H, CH_3), 2.72 (d_{AB}, J_{AB} = 13.5, $J_{P,H}$ = 13.8 Hz, 2 H, PCH_2), 3.61 (d, J = 4.5 Hz, 1 H, 3-H), 3.90 (d, J = 9.9 Hz, 1 H, 4-H_a), 4.28 (dd, J_1 = 9.9, J_2 = 4.5 Hz, 1 H, 4-H_b), 4.67 (d, J = 3.6 Hz, 1 H, 2-H), 4.70–4.81 [m, 2 H, OC*H*-$(CH_3)_2$], 5.93 (d, J = 3.6 Hz, 1 H, 1-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ_C = 23.93 (d, $J_{P,C1}$ = 1.6 Hz, CH_3), 24.00 (d, $J_{P,C}$ = 1.6 Hz, CH_3), 24.04 (d, $J_{P,C}$ = 1.6 Hz, CH_3), 24.09 (d, $J_{P,C}$ = 1.6 Hz, CH_3), 24.76 (d, $J_{P,C}$ = 150.6 Hz, PCH_2), 26.34 (CH_3), 26.70 (CH_3), 50.16 (d, $J_{P,C}$ = 3.4 Hz, C-3), 70.08 (C-4), 71.37 [d, $J_{P,C}$ = 13.6 Hz, $OCH(CH_3)_2$], 71.46 [$OCH(CH_3)_2$], 84.77 (C-2), 105.52 (C-1), 111.79 [$(O)_2C(CH_3)_2$] ppm. ^{31}P NMR (121.5 MHz, $CDCl_3$): δ_P = 21.38 ppm. HRMS: calcd. for $C_{14}H_{28}O_6PS$ [M + H]$^+$ 355.1344; found 355.1346.

1,2-*O*-Diacetyl-3-deoxy-3-*S*-(diisopropylphosphonomethyl)-3-mercapto-L-threofuranose (7): Compound **6** (330 mg, 0.931 mmol) in 60% acetic acid (6 mL) was heated at 80 °C under Ar for 19 h. The starting material was completely consumed. The excess acetic acid and water were evaporated in vacuo followed by co-evaporation with dry CH_3CN twice and drying in vacuo. The resulting yellow oil was dissolved in dry pyridine (8 mL) at 0 °C under N_2. Acetic anhydride (1.05 mL, 11.17 mmol) was added dropwise to this solution. The reaction mixture was slowly warmed to room temperature and continuously stirred overnight. Aqueous NH_4Cl was added to quench the reaction and the mixture was concentrated and co-evaporated with toluene (10 mL × 2) and the residue was purified by column chromatography on silica gel (EtOAc/hexane = 2:1) to give **7** (259 mg, 0.651 mmol) in 70% yield as a colorless oil containing an anomeric mixture of α and β anomers. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H = 1.32–1.34 (m, 24 H, CH_3), 2.05–2.08 (m, 12 H, Ac CH_3), 2.69 (t, J = 15.0 Hz, 1 H, PCH_a), 2.72 (t, J = 15.0 Hz, 1 H, PCH_a), 2.80 (dd, J_1 = 15.1, J_2 = 12.7 Hz, 1 H, PCH_b), 2.96 (dd, J_1 = 15.2, J_2 = 11.7 Hz, 1 H, PCH_b), 3.67–3.75 (m, 1 H, 3-H), 3.77–3.88 [m, 3 H (3-H, 1 H), (4-H, 2 H)], 4.42–4.54 (m, 2 H, 4-H), 4.69–4.77 [m, 4 H, OC*H*$(CH_3)_2$, α and β anomer], 5.05 (dd, J_1 = 7.7, J_2 = 4.3 Hz, 1 H, 2-H), 5.19 (d, J = 2.0 Hz, 1 H, 2-H), 6.15 (s, 1 H, 1-H), 6.34 (d, J = 4.3 Hz, 1 H, 1-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ_C = 20.58 (Ac CH_3), 20.90 (Ac CH_3), 21.02 (Ac CH_3), 21.08 (Ac CH_3), 23.96 (CH_3), 24.02 (CH_3), 24.12 (CH_3), one signal of CH_3 was overlapped, 24.55 (d, $J_{P,C}$ = 150.4 Hz, PCH_2), 25.11 (d, $J_{P,C}$ = 149.8 Hz, PCH_2), 44.41 (d, $J_{P,C}$ = 3.4 Hz, C-3), 47.41 (d, $J_{P,C}$ = 2.6 Hz, C-3), 71.21 (C-4), 71.35 (OCH_2), 71.49 (OCH_2), 71.56 (OCH_2), 71.66 (OCH_2), 73.29 (C-4), 77.49 (C-2), 82.84 (C-2), 94.39 (C-1), 100.51 (C-1), 169.54 (Ac C=O), 169.77 (Ac C=O), 169.83 (Ac C=O) ppm, one Ac C=O signal was overlapped. ^{31}P NMR (121.5 MHz, $CDCl_3$): δ_P = 21.07, 21.53 ppm. HRMS: calcd. for $C_{15}H_{28}O_8PS$ [M + H]$^+$ 399.1242; found 399.1243; calcd. for $C_{15}H_{27}O_8PSNa$ [M + Na]$^+$ 421.1062; found 421.1055.

2-*O*-Acetyl-1-(N^6-benzoyladenin-9-yl)-3-deoxy-3-*S*-(diisopropylphosphonomethyl)-3-mercapto-α-L-threose (8): $SnCl_4$ (177 μL, 1.506 mmol) was added dropwise to a solution of **7** (150 mg, 0.376 mmol) and N^6-benzoyladenine (180 mg, 0.753 mmol) in dry CH_3CN (15 mL) at room temperature under N_2. The reaction mixture was stirred for 4 h. Then the reaction was quenched with aqueous NH_4Cl and concentrated. The residue was partitioned between H_2O (10 mL) and $CHCl_3$ (50 mL × 4). The organic layer was washed with water and brine, dried with Na_2SO_4, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (CH_2Cl_2/MeOH = 29:1, 19:1) to give **8** (165 mg, 0.278 mmol, 74%) as an amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H = 1.32–1.34 (m, 12 H, CH_3), 2.14 (s, 3 H, Ac CH_3), 2.77 (t, J = 15.1 Hz, 1 H, PCH_a), 2.98 (dd, J_1 = 15.3, J_2 = 11.6 Hz, 1 H, PCH_b), 3.97–4.01 (m, 1 H, 3′-H), 4.27 (dd, J_1 = 10.0, J_2 = 4.5 Hz, 1 H, 4′-H_a), 4.50 (dd, J_1 = 10.0, J_2 = 6.7 Hz, 1 H, 4′-H_b), 4.69–4.82 [m, 2 H, OC*H*$(CH_3)_2$], 5.81 (t, J = 2.3 Hz, 1 H, 2′-H), 6.27 (d, J = 2.2 Hz, 1 H, 1′-H), 7.39–7.60 (m, 3 H, Ar-H), 8.01–8.08 (m, 2 H, Ar-H), 8.46 (s, 1 H, A 2-H), 8.79 (s, 1 H, A 8-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ_C = 20.81 (Ac CH_3), 23.88 (CH_3), 23.94 (CH_3), 24.03 (CH_3), 24.07 (CH_3), 24.25 (d, $J_{P,C}$ = 150.74 Hz, PCH_2), 48.42 (d, $J_{P,C}$ = 2.2 Hz, C-3′), 71.53 [d, $J_{P,C}$ = 6.8 Hz, $OCH(CH_3)_2$], 71.67 [d, $J_{P,C}$ = 6.9 Hz, $OCH(CH_3)_2$], 73.34 (C-4′), 81.66 (C-2′), 89.29 (C-1′), 123.29 (A C-5), 128.21 (Bz C), 128.61 (Bz C), 129.85 (Bz C), 130.43 (Bz C), 141.83 (A C-8), 149.89 (A C-4), 151.61 (A C-2), 152.48 (A C-6), 165.21 (Bz C=O), 169.87 (Ac C=O) ppm. ^{31}P NMR (121.5 MHz, $CDCl_3$): δ_P = 21.40 ppm. MS (ESI): calcd. for $C_{25}H_{33}N_5O_7PS$ [M + H]$^+$ 578.1838; found 578.2.

2-*O*-Acetyl-3-deoxy-3-*S*-(diisopropylphosphonomethyl)-3-mercapto-1-(thymin-1-yl)-α-L-threofuranose (9): Thymine (103 mg, 0.816 mmol), ammonium sulfate (2.6 mg, 0.018 mmol), and HMDS (4 mL) were added to a dried flask. The mixture was heated at reflux overnight under N_2. HMDS was removed in vacuo. A solution of compound **7** (130 mg, 0.326 mmol) in dried CH_3CN (16 mL) was added to the flask with the residue followed by the dropwise addition of $SnCl_4$ (153 μL, 1.305 mmol) at room temperature under N_2. The reaction mixture was stirred for 4 h. The reaction was then quenched with saturated aq. NH_4Cl and concentrated to a small volume. The residue was partitioned between H_2O (10 mL) and EtOAc (80 mL). The organic layer was washed with water and brine, dried with Na_2SO_4, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (CH_2Cl_2/MeOH = 29:1) to afford **9** (128 mg, 0.267 mmol) as a colorless amorphous solid in 82% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H = 1.29–1.31 (m, 12 H, CH_3), 1.91 (s, 3 H, T CH_3), 2.08 (s, 3 H, Ac CH_3), 2.75 (t, J = 14.9 Hz, 1 H, PCH_a), 2.93 (dd, J_1 = 15.3, J_2 = 11.9 Hz, 1 H, PCH_b), 3.78–3.80 (m, 1 H, 3′-H), 4.10 (dd, J_1 = 10.2, J_2 = 2.6 Hz, 1 H, 4′-H_a), 4.30 (dd, J_1 = 10.2, J_2 = 6.2 Hz, 1 H, 4′-H_b), 4.65–4.78 [m, 2 H, OC*H*$(CH_3)_2$], 5.24 (s, 1 H, 2′-H), 5.95 (d, J = 2.6 Hz, 1 H, 1′-H), 7.43 (s, 1 H, T 6-H), 9.44 (br., 1 H, NH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ_C = 12.58 (T CH_3), 20.84 (Ac CH_3), 23.90 (CH_3), 23.96 (CH_3), 24.04 (CH_3), 24.09 (CH_3), 24.36 (d, $J_{P,C}$ = 150.4 Hz, PCH_2), 48.54 (d, $J_{P,C}$ = 2.6 Hz, C-3′), 71.42 [d, $J_{P,C}$ = 7.2 Hz, $OCH(CH_3)_2$], 71.61 [$OCH(CH_3)_2$], 72.72 (C-4′), 81.56 (C-2′), 90.19 (C-1′), 111.06 (T C-5), 135.87 (T C-6), 150.52 (T C-2), 163.92 (T C-4), 170.0 (Ac C=O) ppm. ^{31}P NMR (121.5 MHz, $CDCl_3$): δ_P = 21.40 ppm. MS (ESI): calcd. for $C_{18}H_{30}N_2O_8PS$ [M + H]$^+$ 465.146; found 465.2.

1-(Adenin-9-yl)-3-deoxy-3-*S*-(diisopropylphosphonomethyl)-3-mercapto-α-L-threofuranose (10): A solution of **8** (330 mg, 0.557 mmol) in methanol saturated with ammonia (15 mL) was stirred at room temperature for 7 h. The mixture was concentrated and the residue was purified by chromatography on a silica gel column (CH_2Cl_2/MeOH = 19:1 and 9:1) to afford **10** (215 mg, 0.481 mmol) as a white powder in 86% yield. ^{1}H NMR (300 MHz, [D_6]DMSO): δ_H = 1.25–1.27 (m, 12 H, CH_3), 3.05 (s, 1 H, PCH_a), 3.09 (s, 1 H, PCH_b), 3.59–3.66 (m, 1 H, 3′-H), 4.11 (t, J = 8.8 Hz, 1 H, 4′-H_a), 4.30 (dd, J_1 = 8.3, J_2 = 8.0 Hz, 1 H, 4′-H_b), 4.59–4.65 [m, 2 H, OC*H*$(CH_3)_2$], 4.82 (d, J = 6.1 Hz, 1 H, OH), 5.81 (d, J = 4.6 Hz, 1 H, 2′-H), 6.06 (d, J = 4.9 Hz, 1 H, 1′-H), 7.29 (br., 2 H, NH_2), 8.15 (s, 1 H, A 2-H), 8.35 (s, 1 H, A 8-H) ppm. ^{13}C NMR (75 MHz, [D_6]DMSO): δ_C = 23.66 (d, $J_{P,C}$ = 146.11 Hz, PCH_2), 24.13 (CH_3), 24.20 (CH_3), 24.26 (CH_3), 24.31 (CH_3), 49.18 (d, $J_{P,C}$ = 4.5 Hz, C-3′), 70.86 [$OCH(CH_3)_2$], 70.95 [$OCH(CH_3)_2$], 72.20 (C-4′), 78.69

(C-2′), 90.07 (C-1′), 119.76 (A C-5), 140.50 (A C-8), 149.71 (A C-4), 153.05 (A C-2), 156.55 (A C-6) ppm. ^{31}P NMR (121.5 MHz, [D$_6$]DMSO): δ_P = 22.52 ppm. HRMS: calcd. for $C_{16}H_{27}N_5O_5PS$ $[M + H]^+$ 432.1470; found 432.1476.

3-Deoxy-3-*S*-(diisopropylphosphonomethyl)-3-mercapto-1-(thymin-1-yl)-α-L-threofuranose (11): A solution of **9** (195 mg, 0.406 mmol) in methanol saturated with ammonia (10 mL) was stirred at room temperature for 7 h. The mixture was concentrated and the residue was purified by chromatography on a silica gel column (CH_2Cl_2/MeOH = 29:1 and 9:1) to afford **11** (143 mg, 0.327 mmol) as a white powder in 80% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H = 1.33–1.34 (m, 12 H, CH_3), 1.92 (s, 3 H, T CH_3), 2.72 (dd, J_1 = 15.2, J_2 = 12.5 Hz, 1 H, PCH_a), 2.89 (dd, J_1 = 14.8, J_2 = 13.9 Hz, 1 H, PCH_b), 3.59–3.66 (m, 1 H, 3′-H), 3.98 (dd, J_1 = 8.8, J_2 = 7.9 Hz, 1 H, 4′-H_a), 4.39–4.46 [m, 2 H (4′-H_b, 1 H), (2′-H, 1 H)], 4.70–4.81 [m, 2 H, OC*H*$(CH_3)_2$], 5.73 (d, J = 4.3 Hz, 1 H, 1′-H), 7.23 (s, 1 H, T 6-H), 9.93 (br., 1 H, NH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ_C = 12.52 (T CH_3), 23.89 (d, $J_{P,C}$ = 3.3 Hz, CH_3), 23.96 (d, $J_{P,C}$ = 2.7 Hz, CH_3), 23.99 (d, $J_{P,C}$ = 3.8 Hz, CH_3), 24.07 (d, $J_{P,C}$ = 3.6 Hz, CH_3), 25.01 (d, $J_{P,C}$ = 151.73 Hz, PCH_2), 50.41 (C-3′), 71.84 [d, $J_{P,C}$ = 7.0 Hz, $OCH(CH_3)_2$], 72.13 [d, $J_{P,C}$ = 6.9 Hz, $OCH(CH_3)_2$], 72.17 (C-4′), 81.52 (C-2′), 92.81 (C-1′), 110.73 (T C-5), 136.26 (T C-6), 151.02 (T C-2), 164.15 (T C-4) ppm. ^{31}P NMR (121.5 MHz, $CDCl_3$): δ_P = 22.32 ppm. HRMS: calcd. for $C_{16}H_{28}N_2O_7PS$ $[M + H]^+$ 423.1354; found 423.1346.

1-(Adenin-9-yl)-3-deoxy-3-mercapto-3-*S*-(phosphonomethyl)-α-L-threofuranose Sodium Salt (1a): Iodotrimethylsilane (93 μL, 0.681 mmol) was added to a solution of compound **10** (38 mg, 0.085 mmol) and Et_3N (116 μL, 0.851 mmol) in dry CH_2Cl_2 (8 mL) at room temperature under nitrogen. The reaction mixture was continuously stirred for 2 h. The reaction was quenched with 0.5 M TEAB solution. The mixture was concentrated in vacuo and the residue was purified by chromatography on a silica gel column (CH_2Cl_2/MeOH = 3:1 and $CHCl_3$/MeOH: H_2O = 5:4:1) to give crude **1a**. Purification by HPLC using a reversed-phase C18 column (isocratic mobile phase: 1% MeCN and 99% H_2O) and ion exchange with Dowex Na^+ resin offered **1a** (21 mg, 0.053 mmol) as a colorless solid after lyophilization in 63% yield. ^{1}H NMR (500 MHz, D_2O): δ_H = 2.76 (d_{AB}, J_{AB} = 13.9, $J_{P,H}$ = 13.8 Hz, 2 H, PCH_2), 3.71 (dt, $J_{3′,2′}$ = 6.1, $J_{3′,4′}$ = 7.4 Hz, 1 H, 3′-H), 4.21 (dd, J_1 = 9.5, J_2 = 7.4 Hz, 1 H, 4′-H_a), 4.56 (dd, J_1 = 9.5, J_2 = 7.5 Hz, 1 H, 4′-H_b), 4.83 (dd, $J_{2′,1′}$ = 4.8, $J_{2′,3′}$ = 6.0 Hz, 1 H, 2′-H), 6.02 (d, J = 4.6 Hz, 1 H, 1′-H), 8.21 (s, 1 H, A 2-H), 8.37 (s, 1 H, A 8-H) ppm. ^{13}C NMR (125 MHz, D_2O): δ_C = 26.53 (d, $J_{P,C}$ = 132.7 Hz, PCH_2), 48.87 (d, $J_{P,C}$ = 6.3 Hz, C-3′), 72.05 (C-4′), 78.68 (C-2′), 89.20 (C-1′), 118.51 (A C-5), 140.09 (A C-8), 148.37 (A C-4), 152.41 (A C-2), 155.17 (A C-6) ppm. ^{31}P NMR (121.5 MHz, D_2O): δ_P = 16.11 ppm. HRMS: calcd. for $C_{10}H_{13}N_5O_5PS$ $[M - H]^-$ 346.0375; found 346.0381.

3-Deoxy-3-*S*-(phosphonomethyl)-3-mercapto-1-(thymin-1-yl)-α-L-threofuranose Sodium Salt (1b): This compound was prepared as described for **1a** using **11** (68 mg, 0.152 mmol) as the starting material and iodotrimethylsilane (166 μL, 1.218 mmol). Compound **1b** (31 mg, 0.082 mmol) was obtained as a colorless solid after lyophilization in 54% yield. ^{1}H NMR (500 MHz, D_2O): δ_H = 1.90 (d, J = 1.2 Hz, 3 H, T CH_3), 2.73 (d_{AB}, J_{AB} = 13.9, $J_{P,H}$ = 13.8 Hz, 2 H, PCH_2), 3.61 (dt, $J_{3′,2′}$ = 6.2, $J_{3′,4′}$ = 7.3 Hz, 1 H, 3′-H), 4.15 (dd, J_1 = 9.5, J_2 = 7.2 Hz, 1 H, 4′-H_a), 4.43 (dd, $J_{2′,1′}$ = 4.8, $J_{2′,3′}$ = 6.0 Hz, 1 H, 2′-H), 4.47 (dd, J_1 = 9.5, J_2 = 7.4 Hz, 1 H, 4′-H_b), 5.80 (d, J = 4.8 Hz, 1 H, 1′-H), 7.61 (d, J = 1.1 Hz, 1 H, T 6-H) ppm. ^{13}C NMR (125 MHz, D_2O): δ_C = 11.11 (T CH_3), 26.43 (d, $J_{P,C}$ = 132.9 Hz, PCH_2), 48.68 (d, $J_{P,C}$ = 6.3 Hz, C-3′), 72.23 (C-4′), 78.51 (C-2′), 91.04 (C-1′), 110.85 (T C-5), 137.58 (T C-6), 151.43 (T C-2), 166.24 (T C-4) ppm. ^{31}P NMR (121.5 MHz, D_2O): δ_P = 16.03 ppm. HRMS: calcd. for $C_{10}H_{14}N_2O_7PS$ $[M - H]^-$ 337.0260; found 337.0253.

(*S*)-β-(*tert*-Butyldiphenylsiloxy)-γ-butyrolactone (13): *tert*-Butyldiphenyl(chloro)silane (7.8 mL, 30 mmol) was added dropwise to a solution of (*S*)-β-hydroxy-γ-butyrolactone (**12**; 2.04 g, 20 mmol) and imidazole (2.72 g, 40 mmol) in dry DMF (20 mL) at 0 °C. The reaction mixture was warmed to room temperature, stirred overnight, and concentrated. The residue was partitioned between H_2O (20 mL) and EtOAc (100 mL). The organic layer was washed with water and brine, dried with anhydrous Na_2SO_4, and concentrated in vacuo. The residue was crystallized in *n*-hexane to afford **13** (5.98 g, 17.4 mmol) as a white solid in 87% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H = 1.06 (s, 9 H, CH_3), 2.49 (d, J = 4.5 Hz, 2 H, 2-H), 4.13–4.22 (m, 2 H, 4-H), 4.53–4.58 (m, 1 H, 3-H), 7.37–7.46 (m, 6 H, Ar-H), 7.60–7.62 (m, 4 H, Ar-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ_C = 19.11 [$SiC(CH_3)_3$], 26.85 (CH_3), 38.00 (C-2), 69.14 (C-3), 75.73 (C-4), 128.13 (Ar C), 130.33 (Ar C), 132.83 (Ar C), 132.96 (Ar C), 135.67 (Ar C), 135.71 (Ar C), 175.68 (C-1, C=O) ppm. HRMS: calcd. for $C_{20}H_{25}O_3Si$ $[M + H]^+$ 341.1573; found 341.1576.

3-*O*-(*tert*-Butyldiphenylsilyl)-2-deoxy-D-erythrofuranose (14): A 1.2 M solution of diisobutylaluminium hydride (14.5 mL, 17.43 mmol) in toluene was slowly added dropwise to a solution of **13** (4.96 g, 14.53 mmol) in dry THF (45 mL) at –78 °C under argon. The reaction mixture was stirred at –78 °C until the starting material was completely consumed (TLC, 5 h). Methanol (2 mL) was slowly added to quench the reaction at –78 °C. The cooling bath was removed, a saturated aq. sodium potassium tartrate solution (40 mL) and EtOAc (150 mL) were added, and the mixture was stirred vigorously for 3 h. The organic layer was washed with water and brine, dried with anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (*n*-hexane/EtOAc = 4:1) to afford **14** (4.56 g, 13.22 mmol) as an anomeric mixture of β and α anomers as a colorless oil in 91% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H = 1.05 (s, 9 H, CH_3, minor), 1.08 (s, 9 H, CH_3, major), 1.84–1.91 (m, 1 H, 2-H_a, major), 1.92–2.02 (m, 1 H, 2-H_a, minor), 2.08 (d, J = 13.5 Hz, 1 H, 2-H_b, major), 2.57 (d, J = 2.7 Hz, 1 H, 2-H_b, minor), 3.67 (dd, J_1 = 9.7, J_2 = 3.9 Hz, 1 H, 4-H_a, major), 3.74 (dd, J_1 = 9.2, J_2 = 2.6 Hz, 1 H, 4-H_a, minor), 3.89 (dd, J_1 = 9.2, J_2 = 4.7 Hz, 1 H, 4-H_b, minor), 3.93 (d, J = 11.3 Hz, 1 H, OH, major), 4.05–4.09 (m, 1 H, 4-H_b, major), 4.10 (d, J = 7.1 Hz, 1 H, OH, minor), 4.43–4.46 (m, 1 H, 3-H, major), 4.55–4.61 (m, 1 H, 3-H, minor), 5.39 (dd, J_1 = 11.4, J_2 = 4.9 Hz, 1 H, 1-H, major), 5.65 (t, J = 2.6 Hz, 1 H, 1-H, minor), 7.37–7.47 (m, 12 H, Ar-H, α + β anomer), 7.62–7.67 (m, 8 H, Ar-H, α + β anomer) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ_C = 19.06 [$SiC(CH_3)_3$], 19.17 [$SiC(CH_3)_3$], 27.00 (CH_3), 42.46 (C-2), 43.55 (C-2), 72.92 (C-3), 73.45 (C-3), 74.28 (C-4), 75.74 (C-4), 98.97 (C-1), 99.49 (C-1), 127.85 (Ar C), 128.06 (Ar C), 129.91 (Ar C), 130.24 (Ar C), 132.82 (Ar C), 132.92 (Ar C), 135.80 (Ar C), 135.84 (Ar C) ppm. HRMS: calcd. for $C_{20}H_{26}O_3SiNa$ $[M + Na]^+$ 365.1549; found 365.1556.

3-*O*-(*tert*-Butyldiphenylsilyl)-2-deoxy-1-*O*-methyl-D-erythrofuranose (15): Compound **14** (270 mg, 0.786 mmol) was dissolved in a 0.01 M solution of hydrogen chloride in MeOH (2.5 mL) and the reaction mixture was stirred for 2 h. Et_3N was added to neutralize the excess acid and the mixture was concentrated in vacuo and purified by chromatography on a silica gel column (EtOAc/*n*-hexane = 1:29) to give **15** (277 mg, 0.77 mmol) in 98% yield as an anomeric mixture (2.3:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ_H = 1.05 (s, 18 H, CH_3,

α + β anomer), 1.85–1.99 (m, 2 H, 2-H_a, α + β anomer), 2.04–2.20 (m, 2 H, 2-H_b, α + β anomer), 3.27 (s, 3 H, OCH_3, major), 3.36 (s, 3 H, OCH_3, minor), 3.71–3.77 (m, 4 H, 4-H, α + β anomer), 4.31–4.37 (m, 1 H, 3-H, minor), 4.53–4.56 (m, 1 H, 3-H, major), 4.86 (dd, J_1 = 5.7, J_2 = 2.5 Hz, 1 H, 1-H, minor), 5.11 (dd, J_1 = 5.3, J_2 = 2.3 Hz, 1 H, 1-H, major), 7.34–7.42 (m, 12 H, Ar-H, α + β anomer), 7.62–7.64 (m, 8 H, Ar-H, α + β anomer) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ_C = 19.16 [$SiC(CH_3)_3$], 19.20 [$SiC(CH_3)_3$], 26.96 (CH_3), 41.70 (C-2), 42.79 (C-2), 55.00 (OCH_3), 55.03 (OCH_3), 71.90 (C-3), 72.36 (C-4), 72.85 (C-3), 73.97 (C-4), 104.97 (C-1), 105.39 (C-1), 127.83 (Ar C), 129.87 (Ar C), 133.88 (Ar C), 133.91 (Ar C), 133.97 (Ar C), 135.77 (Ar C), 135.79 (Ar C), 135.83 (Ar C) ppm. HRMS: calcd. for $C_{21}H_{29}O_3SiNa$ $[M + Na]^+$ 357.1886; found 357.1882.

2-Deoxy-1-*O*-methyl-D-erythrofuranose (16): A 1.0 M solution of TBAF (9.8 mL, 9.78 mmol) in THF was added dropwise to a solution of **15** (7.0 g, 19.58 mmol) in dry THF (100 mL) at 0 °C. The reaction mixture was stirred at room temperature for 0.5 h and concentrated under reduced pressure. The residue was purified by chromatography on a silica gel column (EtOAc/*n*-hexane = 2: 1) to give **16** (1.74 g, 14.68 mmol) in 75% yield as an anomeric mixture (5.4:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ_H = 2.02–2.13 (m, 4 H, 2-H, α + β anomer), 3.34 (s, 3 H, OCH_3, major), 3.37 (s, 3 H, OCH_3, minor), 3.77 (dd, J_1 = 9.8, J_2 = 1.1 Hz, 1 H, 4-H_a, major), 3.90 (dd, J_1 = 9.8, J_2 = 4.1 Hz, 1 H, 4-H_b, major), 3.95–3.97 (m, 1 H, 4-H_a, minor), 4.02 (dd, J_1 = 9.8, J_2 = 4.7 Hz, 1 H, 4-H_b, minor), 4.32–4.35 (m, 1 H, 3-H, minor), 4.49–4.53 (m, 1 H, 3-H, major), 5.06 (d, J = 4.1 Hz, 1 H, 1-H, minor), 5.15 (dd, J_1 = 4.8, J_2 = 3.2 Hz, 1 H, 1-H, major) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ_C = 41.41 (C-2), 42.76 (C-2), 54.88 (OMe), 55.15 (OMe), 71.16 (C-3), 71.47 (C-3), 73.80 (C-4), 76.54 (C-4), 105.02 (C-1), 105.11 (C-1) ppm. HRMS: calcd. for $C_5H_{10}O_3Na$ $[M + Na]^+$ 141.0528; found 141.0526.

3-*S*-Acetyl-2,3-dideoxy-3-mercapto-1-*O*-methyl-L-threofuranose (17): DIAD (5.0 mL, 25.39 mmol) was added dropwise to a solution of PPh_3 (6.66 g, 25.3 mmol) and compound **16** (1.36 g, 11.54 mmol) in dry THF (60 mL) at 0 °C under Ar. After stirring for 30 min at the same temperature a solution of thioacetic acid (1.8 mL, 25.39 mmol) was added dropwise to the resulting yellow suspension and the reaction mixture was continuously stirred for 3 h at 0 °C. The reaction mixture was quenched with saturated sodium hydrogen carbonate, concentrated, and partitioned between EtOAc and water. The organic layer was washed with water and brine, dried with anhydrous sodium sulfate, filtered, and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/*n*-hexane = 1:19) to afford a crude product, which was diluted with *n*-hexane. The precipitate was removed by filtration and the filtrate was evaporated to dryness to obtain pure **17** (1.58 g, 8.88 mmol) in 77% yield as an anomeric mixture (3.3:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ_H = 1.81–1.88 (m, 1 H, 2-H_a, major), 1.92–2.00 (m, 1 H, 2-H_a, minor), 2.31 (s, 6 H, CH_3, α + β anomer), 2.45–2.59 (m, 2 H, 2-H_b, α + β anomer), 3.33 (s, 3 H, OCH_3, minor), 3.35 (s, 3 H, OCH_3, major), 3.66 (dd, J_1 = 9.0, J_2 = 6.8 Hz, 1 H, 4-H_a, major), 3.71 (dd, J_1 = 9.4, J_2 = 4.3 Hz, 1 H, 4-H_a, minor), 3.91–4.01 (m, 1 H, 3-H, major), 4.08–4.12 (m, 1 H, 3-H, minor), 4.26 (dd, J_1 = 9.4, J_2 = 6.5 Hz, 1 H, 4-H_b, minor), 4.28 (dd, J_1 = 9.0, J_2 = 7.4 Hz, 1 H, 4-H_b, major), 5.04 (dd, J_1 = 5.2, J_2 = 1.5 Hz, 1 H, 1-H, major), 5.08 (d, J = 1.8 Hz, 1 H, 1-H, minor) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ_C = 30.39 (Me), the two Me signals overlapped, 38.54 (C-2), 38.87 (C-2), 38.92 (C-3), 40.08 (C-3), 54.93 (OMe), 55.06 (OMe), 72.21 (C-4), 72.43 (C-4), 104.62 (C-1), 104.69 (C-1), 196.00 (Ac C=O) ppm, one Ac C=O signal was obscured by the noise. HRMS: calcd. for $C_7H_{12}O_3SNa$ $[M + Na]^+$ 199.0405; found 199.0409.

2,3-Dideoxy-3-*S*-(diisopropylphosphonomethyl)-3-mercapto-1-*O*-methyl-L-threofuranose (18): K_2CO_3 (69 mg, 0.5 mmol) was added to a solution of **17** (71 mg, 0.4 mmol) in dry and degassed MeOH (3 mL) under Ar. The reaction mixture was stirred for 5 h. The volatiles were removed under high vacuum and exchanged with Ar (to avoid the formation of the disulfide by carrying out the reaction and following work-up under an inert atmosphere). The resulting residue was charged with dried and degassed THF under Ar and NaH (24 mg, 0.6 mmol) was added at –30 °C. The reaction mixture was stirred for 10 min. The triflate of (diisopropylphosphono) methanol (397 mg, 1.2 mmol) was added to this suspension at –30 °C under Ar. The reaction mixture was stirred at the same temperature for 1 h. The reaction was quenched with a saturated $NaHCO_3$ solution and extracted with EtOAc (×2). The organic phases were washed with water and brine, dried with anhydrous sodium sulfate, filtered, and concentrated. The residue was purified by chromatography on a silica gel column (EtOAc/*n*-hexane = 1:1 and 3:2) to give **18** (38 mg, 0.12 mmol) in 30% yield as an anomeric mixture (4.3:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ_H = 1.33–1.35 (m, 24 H, CH_3, α + β anomer), 1.78–1.87 (m, 2 H, 2-H_a, α + β anomer), 2.49–2.58 (m, 2 H, 2-H_b, α + β anomer), 2.69 (d, J = 1.3 Hz, 1 H, PCH_a, minor), 2.71 (s, 1 H, PCH_a, major), 2.74 (d, J = 1.2 Hz, 1 H, PCH_b, minor), 2.75 (s, 1 H, PCH_b, major), 3.32 (s, 3 H, OMe, minor), 3.35 (s, 3 H, OMe, major), 3.50–3.59 (m, 2 H, 3-H, α + β anomer), 3.67 (t, J = 8.6 Hz, 1 H, 4-H_a, major), 3.76–3.82 (m, 1 H, 4-H_a, minor), 4.17 (dd, J_1 = 8.3, J_2 = 7.5 Hz, 1 H, 4-H_b, major), 4.29–4.31 (m, 1 H, 4-H_b, minor), 4.72–4.78 [m, 4 H, $OCH(CH_3)_2$, α + β anomer], 5.02 (dd, J_1 = 5.4, J_2 = 2.1 Hz, 1 H, 1-H, major), 5.07 (d, J = 5.2 Hz, 1 H, 1-H, minor) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ_C = 24.05 (Me), 24.11 (Me), 24.15 (Me), 24.20 (Me), 25.01 (d, $J_{P,C}$ = 150.1 Hz, PCH_2), 25.37 (d, $J_{P,C}$ = 149.9 Hz, PCH_2), 39.94 (C-2), 40.29 (C-2), 41.46 (d, $J_{P,C}$ = 3.5 Hz, C-3), 42.38 (d, $J_{P,C}$ = 4.2 Hz, C-3), 54.81 (OMe), 55.00 (OMe), 71.29 [$OCH(CH_3)_2$], 71.33 [$OCH(CH_3)_2$], 71.38 [$OCH(CH_3)_2$], 71.42 [$OCH(CH_3)_2$], 71.79 (C-4), 72.15 (C-4), 105.04 (C-1) ppm, the two signals of C-1 overlapped. ^{31}P NMR (121.5 MHz, $CDCl_3$): δ_P = 21.72, 21.93 ppm. HRMS: calcd. for $C_{12}H_{26}O_5PS$ $[M + H]^+$ 313.1238; found 313.1241.

2,3-Dideoxy-3-*S*-(diisopropylphosphonomethyl)-3-mercapto-1-(thymin-1-yl)-L-threofuranose (20): Thymine (63 mg, 0.5 mmol), ammonium sulfate (1.6 mg, 0.012 mmol), and HMDS (3 mL) were added to a dried flask. The mixture was heated at reflux overnight under Ar and then HMDS was removed in vacuo. A solution of **18** (62 mg, 0.2 mmol) in dry DCE (8 mL) was added to the flask with the residue followed by dropwise addition of TMSOTf (120 μL, 0.6 mmol) at room temperature under Ar. The reaction mixture was stirred for 0.5 h. The reaction was quenched with cold saturated $NaHCO_3$ and extracted with $CHCl_3$ (×2). The organic layer was washed with H_2O and brine, dried with Na_2SO_4, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (CH_2Cl_2/MeOH = 50:1) to give **20** (25 mg, 0.06 mmol) in 30% yield as an anomeric mixture (2:1). ^{1}H NMR (300 MHz, MeOD): δ_H = 1.32–1.35 (m, 24 H, CH_3, α + β anomer), 1.88–1.89 (m, 6 H, T CH_3, α + β anomer), 2.00–2.09 (m, 1 H, 2′-H_a, minor), 2.30–2.39 (m, 1 H, 2′-H_a, major), 2.46–2.55 (m, 1 H, 2′-H_b, major), 2.77–2.87 (m, 1 H, 2′-H_b, minor), 2.92 (s, 1 H, PCH_a, major), 2.93 (d, $J_{P,H}$ = 13.9 Hz, 1 H, PCH_a, minor), 2.96 (s, 1 H, PCH_b, major), 2.97 (d, $J_{P,H}$ = 13.8 Hz, 1 H, PCH_b, minor), 3.78–3.90 [m, 3 H (3′-H, 2 H, α + β anomer), (4′-H_a, 1 H, major)], 4.09 (dd, J_1 = 9.3, J_2 = 5.4 Hz, 1 H, 4′-H_a, minor), 4.21 (dd, J_1 = 9.3, J_2 = 6.4 Hz, 1 H, 4′-H_b, minor), 4.51 (dd, J_1 = 8.7,

J_2 = 5.8 Hz, 1 H, 4′-H_b, major), 4.66–4.75 [m, 4 H, OC*H*$(CH_3)_2$, α + β anomer], 5.99 (dd, J_1 = 6.8, J_2 = 5.2 Hz, 1 H, 1′-H, minor), 6.08 (dd, J_1 = 6.7, J_2 = 4.8 Hz, 1 H, 1′-H, major), 7.44 (d, J = 1.2 Hz, 1 H, T 6-H, major), 7.60 (d, J = 1.2 Hz, 1 H, T 6-H, minor) ppm. ^{13}C NMR (75 MHz, MeOD): δ_C = 12.47 (T CH_3, major), 12.56 (T CH_3, minor), 24.26 (Me), 24.32 (Me), 24.36 (Me), 24.41 (Me), 25.25 (d, $J_{P,C}$ = 150.1 Hz, PCH_2, major), 25.59 (d, $J_{P,C}$ = 149.6 Hz, PCH_2, minor), 39.79 (C-2′, minor), 39.83 (C-2′, major), 43.92 (d, $J_{P,C}$ = 4.1 Hz, C-3′, major), 44.10 (d, $J_{P,C}$ = 4.4 Hz, C-3′, minor), 73.28 [$OCH(CH_3)_2$, major], 73.37 [$OCH(CH_3)_2$, minor], the two signals of $OCH(CH_3)_2$ overlapped, 75.29 (C-4′, minor), 75.58 (C-4′, major), 87.95 (C-1′, minor), 88.12 (C-1′, major), 111.14 (T C-5, minor), 111.49 (T C-5, major), 137.86 (T C-6, major), 138.01 (T C-6, minor), 152.42 (T C-2), 166.49 (T C-4) ppm, the signals of C-2 and C-4 of the thymine moiety were obscured by noise. ^{31}P NMR (121.5 MHz, MeOD): δ_P = 22.72, 22.84 ppm. HRMS: calcd. for $C_{16}H_{28}N_2O_6PS$ $[M + H]^+$ 407.1405; found 407.1411.

2,3-Dideoxy-3-mercapto-3-*S*-(phosphonomethyl)-1-(thymin-1-yl)-L-threofuranose (1c): Iodotrimethylsilane (612 μL, 4.5 mmol) was added to a solution of compound **20** (183 mg, 0.45 mmol) and Et_3N (3 mL, 21.64 mmol) in dry CH_2Cl_2 (25 mL) at 0 °C under nitrogen. The reaction mixture was continuously stirred for 3.5 h. The reaction was quenched with a 0.5 M TEAB solution. The mixture was concentrated at room temperature in vacuo and the residue was purified by chromatography on a silica gel column (CH_2Cl_2/MeOH = 9:1 and $CHCl_3$/MeOH/H_2O = 5:4:1) to give crude **1c**. Purification by HPLC using a reversed-phase C18 column (isocratic mobile phase: 1% MeCN and 99% H_2O) and ion exchange with Dowex Na^+ resin offered **1c** (75 mg, 0.205 mmol) as a colorless solid after lyophilization in 45% yield as an anomeric mixture (α/β). ^{1}H NMR (600 MHz, D_2O): δ_H = 1.89 (s, 3 H, T CH_3, major), 1.91 (s, 3 H, T CH_3, minor), 2.04 (ddd, J_1 = 13.5, J_2 = 6.4, J_3 = 6.4 Hz, 1 H, 2′-H_a, minor), 2.42 (ddd, J_1 = 13.9, J_2 = 6.7, J_3 = 6.7 Hz, 1 H, 2′-H_a, major), 2.52 (ddd, J_1 = 13.9, J_2 = 7.2, J_3 = 4.8 Hz, 1 H, 2′-H_b, major), 2.69 (d_{AB}, $J_{P,H}$ = 14.1 Hz, 2 H, PCH_2, minor), 2.70 (d_{AB}, $J_{P,H}$ = 14.3 Hz, 2 H, PCH_2, major), 2.85 (ddd, J_1 = 14.4, J_2 = 7.3, J_3 = 7.3 Hz, 1 H, 2′-H_b, minor), 3.74–3.77 (m, 1 H, 3′-H, minor), 3.77–3.82 (m, 1 H, 3′-H, major), 3.91 (dd, J_1 = 9.3, J_2 = 5.8 Hz, 1 H, 4′-H_a, major), 4.07 (dd, J_1 = 9.0, J_2 = 6.3 Hz, 1 H, 4′-H_a, minor), 4.28 (dd, J_1 = 9.1, J_2 = 6.6 Hz, 1 H, 4′-H_b, minor), 4.51 (dd, J_1 = 9.3, J_2 = 6.2 Hz, 1 H, 4′-H_b, major), 6.08 (dd, J_1 = 6.5, J_2 = 6.2 Hz, 1 H, 1′-H, minor), 6.17 (dd, J_1 = 6.3, J_2 = 5.2 Hz, 1 H, 1′-H, major), 7.51 (s, 1 H, T 6-H, major), 7.71 (s, T 6-H, minor) ppm. ^{13}C NMR (150 MHz, D_2O): δ_C = 11.12 (T CH_3, major), 11.16 (T CH_3, minor), 26.89 (d, $J_{P,C}$ = 158.3 Hz, PCH_2, major), 27.07 (d, $J_{P,C}$ = 158.1 Hz, PCH_2, minor), 37.22 (C-2′, minor), 37.34 (C-2′, major), 41.63 (d, $J_{P,C}$ = 8.4 Hz, C-3′, minor), 41.91 (d, $J_{P,C}$ = 8.8 Hz, C-3′, major), 73.64 (C-4′, minor), 74.02 (C-4′, major), 86.15 (C-1′, minor), 86.53 (C-1′, major), 110.70 (two signals overlapped, T C-5), 137.23 (T C-6, major), 137.46 (T C-6, minor), 151.14 (T C-2, major), 151.23 (T C-2, minor), 166.21 (two signals overlapped, T C-4) ppm. ^{31}P NMR (121.5 MHz, D_2O): δ_P = 14.42, 14.49 ppm. HRMS: calcd. for $C_{10}H_{14}N_2O_6PS$ $[M - H]^-$ 321.0310; found 321.0322.

Supporting Information (see footnote on the first page of this article): ^{1}H and ^{13}C NMR spectra of **1**–**20** and HPLC spectra of **1a**–**1c**.

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