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Design and Synthesis of Novel Dinucleotide Analogs

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Syntheses of dinucleotide analogs, (*S,R*) *cis*-(4-((4-amino-2-oxopyrimidin-1(2H)-yl)methyl)-1,3-dioxolan-2-yl)methyl (*2R,3R,5R*)-2-(hydroxymethyl)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-tetrahydrofuran-3-yl hydrogen phosphate (**5a**) and (*S,R*) *cis*-(5-((4-amino-2-oxopyrimidin-1(2H)-yl)methyl)-1,3-oxathiolan-2-yl)methyl (*2R,3R,5R*)-2-(hydroxymethyl)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-tetrahydrofuran-3-yl hydrogen phosphate (**5b**), were accomplished by the use of a new strategy. The use of phenyldichlorophosphate (Method A) as the coupling reagent was shown to possess superiority relative to the reported use of di(1H-benzo[d][1,2,3]triazol-1-yl)phenyl phosphonate (Method B).

Keywords: Nucleosides, Dinucleotides, Antivirals, Anti-HIV, Phosphorotriesters

INTRODUCTION

The gene of HIV encodes three key viral enzymes for the replication of this virus that can be exploited for the development of chemotherapeutic agents [1-6]. Two of these enzymes, HIV reverse transcriptase and HIV protease, have received much attention in terms of the development of clinically useful inhibitors [4-10]. On the contrary, the third enzyme of the pol gene, HIV integrase [2,3,11-13] has received relatively less attention in terms of inhibitors, in large part because of the difficulty associated with the discovery of therapeutically significant inhibitors [14-16].

On the other hand, some oligo nucleotides are known to inhibit HIV-1 integrase [17]. Pommier and coworkers investigated natural dinucleotides and Nair and coworkers designed and studied non-natural dinucleotides as potential anti-HIV integrase inhibitors by multistep procedures including *N*-protection and deprotection steps [18-22].

Our earlier findings [23] prompted us to synthesize dinucleotide analogues **5a** and **5b** to examine their antiviral activities. We used synthetic strategy shown in Scheme 1 to synthesize the target molecules **5a** and **5b** *via* the corresponding intermediates **3a** and **3b**, which were prepared using new synthetic methodology to give 3'-5'-phosphorotriester linkages with phenyldichlorophosphate as a coupling reagent.

EXPERIMENTAL

General

All starting materials were purchased from Aldrich, TCI and Across. THF was distilled from sodium benzophenone ketyl. ^{1}H NMR spectra were recorded with Bruker AMX400, proton chemical shifts (δ) are reported in parts per million

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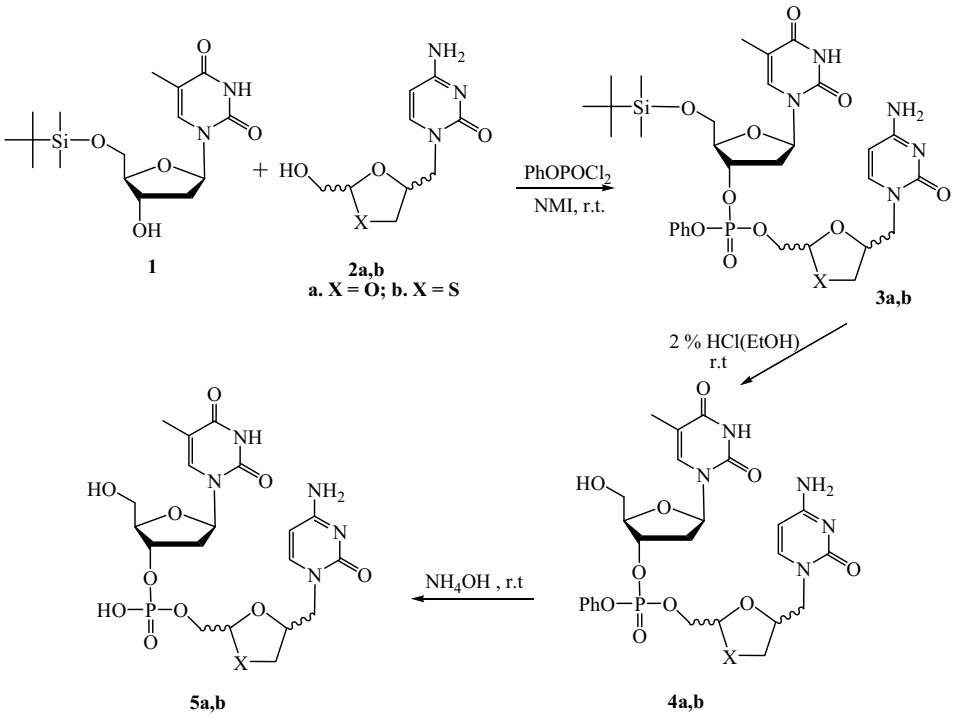

Scheme 1

(ppm) relative to the methine singlet at 7.24 ppm for the residual $CHCl_3$ in the deuteriochloroform, or the methyl pentet at 2.49 ppm for the residual $(CD_3)_2SO$ in the DMSO-d_6. Carbon chemical shifts are reported in parts per million relative to the internal ^{13}C signals in $CDCl_3$ (77.0 ppm) or DMSO-d_6 (39.5 ppm). Mass spectra were obtained with a FAB JMS-700 double focusing mass spectrometer (JEOL, Tokyo, Japan).

Purification on silica gel refers to gravity column chromatography on Merck silica gel 60 (particle size 230-400 Mesh). Analytical TLC was performed on precoated plates purchased from Merck (Silica gel 60 F_{254}). Compounds were visualized by the use of UV light.

Synthesis of Compounds (1), 2a and 2b

1-((*2R,4R,5R*)-5-((*tert*-Butyldimethylsilyloxy)methyl)-4-hydroxy-tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4 (1H,3H)-dione (1). To a stirred solution of 0.92 g (4 mmol) of thymidine in 10 ml dry pyridine was added dropwise a solution of 0.66 g (4.4 mmol) of *tert*-butyl-chlorodimethyl silane in 5 ml dry pyridine at room temperature. The reaction mixture was stirred for 24 h. Then, pyridine was removed by co-evaporation with toluene under reduced pressure. The residue was purified by column chromatography on silica gel using CH_2Cl_2-MeOH (20:1), to give 1.17 g, 85% yield of compound **1** as white crystals. m.p.: 193-195 °C. ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) = 10.06 (s, 1H), 7.53 (s, 1H), 6.38-6.34 (dd, 1H , J = 5.56 and 5.6 Hz), 4.39-4.38 (d, 1H, J = 5.4 Hz), 4.05-4.04 (d, 1H, J = 1.4 Hz), 3.87-3.75 (dddd, 3H, J = 2.1, 1.8, 2.0 and 2.1 Hz), 2.40-2.35 (dd, 1H, J = 4.6 and 5.2 Hz), 2.07-1.97 (m, 1H), 1.85 (s, 3H), 0.86 (s, 9H), 0.05 (s, 6H).

(*S,R*) *cis*-4-amino-1-((2-(hydroxymethyl)-1,3-dioxolan-4-yl)methyl)pyrimidin-2(1H)-one (2a) and (S,R) *cis*-4-amino-1-((2-(hydroxymethyl)-1,3-oxathiolan-5-yl)methyl) pyrimidin-2(1H)-one (2b). Synthesis of compounds **2a** and **2b** were described before [23].

General Procedures for the Synthesis of 3a,b

Method A. A mixture of compound **1** (1 mmol) and compound **2** in 5 ml dry *N*-methylimidazole was placed in a flame-dried 50 ml two-neck round bottom flask equipped with

a stirrer. One equivalent (1 mmol) of phenyldichlorophosphate in anhydrous THF (5 ml) was added dropwise at 0 °C and the reaction mixture was stirred at room temperature for 3 h. Water (30 ml) was added and the mixture was stirred for 30 min. Solids were isolated by filtration, dissolved in dichloromethane and dried over $MgSO_4$. Then, the solvent was evaporated and the residue was purified by column chromatography on silica gel using CH_2Cl_2-MeOH (10:1) as eluant.

Method B. A solution of phenyldichlorophosphate (1 mmol) in anhydrous THF (2 ml) was added dropwise to a solution of 1-hydroxybenzotriazole (2 mmol) and anhydrous pyridine (2 mmol) in dry THF (6 ml) at room temperature. The reaction mixture was then stirred for 1 h at the same temperature and the precipitate was filtered. The volume was reduced to 2 ml and added to a stirred solution of compound **1** (0.22 mmol) in 5 ml dry THF. The reaction mixture was stirred for 2 h. Then, compound **2** (0.22 mmol) in5 ml dry *N*-methylimidazole (NMI) was added and the reaction mixture was stirred for another 3 h. A few drops of water were added to the reaction mixture and NMI was removed in vacuum; the residue was purified by column chromatography on silica gel using CH_2Cl_2-MeOH (10:1) as eluant.

(*S,R*) *cis*-((4-Amino-2-oxopyrimidin-1(2H)-yl)methyl)-1,3-dioxolan-2-yl)methyl (*2R,3R,5R*)-2-((*tert*-butyldimethylsilyloxy)methyl)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-tetrahydrofuran-3-yl phenyl phosphate (3a). was obtained in 62% yield as a white crystals by using Method A. m.p.: 200 °C (dec.). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.45-7.42 (d, 1H, J = 8.5 Hz), 7.38-7.17 (m, 6H), 6,36-6.32 (dd, 1H, J = 4.9 and 4.4 Hz), 5.84 (br. 1H), 5.08 (br, s, 1H), 5.06-4.95 (m, 1H), 4.5-4.44 (m, 1H), 4.33-3.60 (m, 9H), 2.59- 2.41 (dddd, 1H, J = 4.9, 4.8, 5.1 and 4.4 Hz), 2.12-1.96 (m, 1H), 1.87 (s, 3H), 0.87 (s, 9H), 0.07 (s, 6H), HRMS-FAB: for $C_{31}H_{44}N_5O_{11}PSi$ $(M+H)^+$ = 722.25.

(*S,R*) *cis*-(5-((4-Amino-2-oxopyrimidin-1(2H)-yl) methyl)-1,3-oxathiolan-2-yl)methyl (*2R,3R,5R*)-2-((*tert*-butyldimethylsilyloxy)methyl)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-tetrahydrofuran-3-yl phenyl phosphate (3b). was obtained in 46% yield as a white crystals by using Method B. m.p.: 200 °C (dec.). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.4 (s, 1H), 7.46-7.24 (m, 7H), 7.14 (br, 1H), 7.07 (br, 1H), 6,2-6.17 (d, 1H, J = 3.7 Hz), 5.64-5.59 (t, 1H, J = 7.7 Hz), 5.32 (br, s, 1H), 5.02 (br, 1H), 4.30-3.65 (m, 7H), 3.08 (br, 1H), 2.75- 2.70 (t, 1H, J = 8.9 Hz), 2.48-2.33 (m, 2H), 1.78 (s, 3H), 0.86 (s, 9H), 0.06 (s, 6H). HRMS-FAB: for $C_{31}H_{44}N_5O_{10}PSSi$ $(M+H)^+$ = 738.23.

(*S,R*) *cis*-(4-((4-Amino-2-oxopyrimidin-1(2H)-yl) methyl)-1,3-dioxolan-2-yl)methyl (*2R,3R,5R*)-2-(hydroxy-methyl)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-tetrahydrofuran-3-yl phenyl phosphate(4a). To a solution of 65 mg (0.09 mmol) of compound **3a** in 5 ml THF was added 5 ml solution of 2% HCl in EtOH at room temperature and the reaction was stirred for 1 h. Reaction mixture was evaporated to dryness and residue was purified by column chromatography on silica gel using CH_2Cl_2-MeOH (10:1) to give 46.5 mg (85% yield) of compound **4a** as white crystals. m.p.: 200 °C (dec.). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.32 (s, 1H), 7.69 (s, 1H), 7.47-7.23 (m, 6H), 7.12 (br, 1H), 6.95 (br, 1H), 621-6.16 (dd, 1H, J = 6.3 and 6.9 Hz), 5.64-5.62 (d, 1H, J = 7.1 Hz), 5.31 (br, 1H), 5.11-5.07 (m, 2H), 4.35-4.28 (m, 1H), 4.19-3.51 (m, 9H), 2.44-2.31 (m, 2H), 1.78 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ = 166.03; 163.67; 155.95; 150.46; 149.49; 145.6; 135.85; 130.06 (2C); 125.51; 119.99 (2C); 109.75; 101.2; 93.18; 85.10; 83.61; 79.40; 74.25; 67.17; 67.10; 60.89; 50.67; 37.37; 12.25. HRMS-FAB for $C_{25}H_{30}N_5O_{11}P$ $(M+H)^+$ = 608.17.

(*S,R*) *cis*- (5-((4-Amino-2-oxopyrimidin-1(2H)-yl) methyl)-1,3-oxathiolan-2-yl)methyl (*2R,3R,5R*)-2-(hydroxy-methyl)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-tetrahydrofuran-3-yl phenyl phosphate (4b). The procedure was similar to the one used for preparation of compound **4a**. Compound **4b** was isolated (53 mg, 85% yield) as white crystals. m.p.: 200 °C (dec.). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.34 (s, 1H), 7.69 (s, 1H), 7.51-7.20 (m, 6H), 7.16 (br, 1H), 7.02 (br, 1H), 6.20 (br, 1H), 5.65-5.60 (t, 1H, J = 6.6 Hz), 5.31 (br, 2H), 5.09 (br, 1H), 4.30-3.61 (m, 8H), 3.07 (br, 1H), 2.74-2.69 (t, 1H, J = 8.9 Hz), 2.44-2.34 (m, 2H), 1.77 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 165.75; 163.59; 155.49; 150.39; 149.90; 146.45; 135.77; 129.96 (2C); 125.40; 119.93 (2C); 109.68; 93.23; 85.02; 83.58; 82.54; 82.14; 79.14; 69.35; 60.86; 50.38; 37.34; 33.65; 12.19. HRMS-FAB for $C_{25}H_{30}N_5O_{10}PS$ $(M+H)^+$ = 624.15.

(*S,R*) *cis*-(4-((4-amino-2-oxopyrimidin-1(2H)-yl) methyl)-1,3-dioxolan-2-yl)methyl (*2R,3R,5R*)-2-(hydroxy-methyl)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1

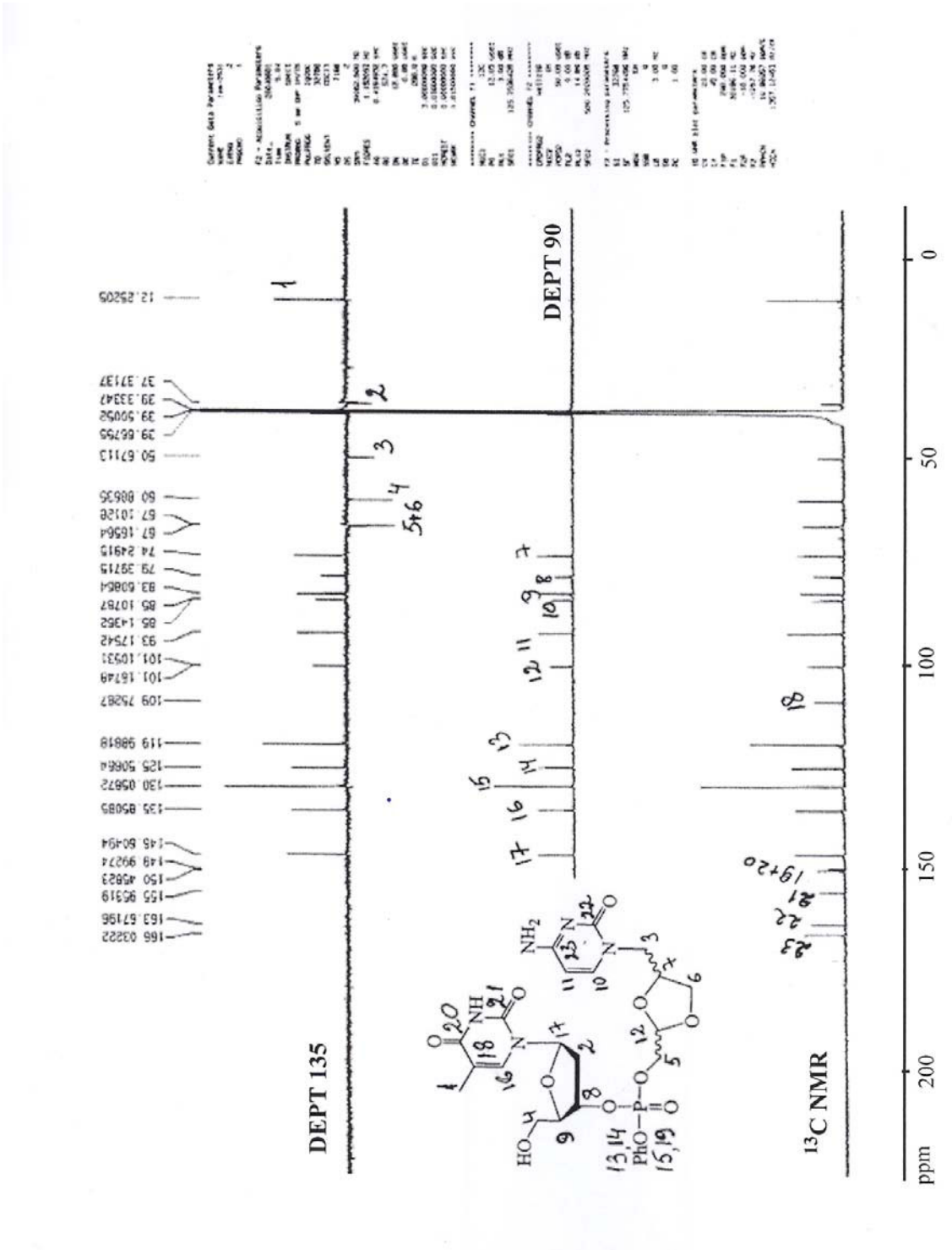

Fig. 1. 400 MHz ^{1}H NMR, ^{13}C NMR, DEPT 135 and DEPT 90 H NMR spectra of **4a**.

(2H)-yl)-tetrahydrofuran-3-yl hydrogen phosphate (5a). To a solution of 30.5 mg (0.05 mmol) of compound **4a** in 5 ml THF was added 20 ml of ammonium water (28%), and reaction mixture was shaken for a few minutes until the mixture had become almost clear (3 h). The solution was evaporated to dryness and residue was purified by short column chromatography on silica gel using CH_2Cl_2-MeOH (4:1) to give 20 mg (75% yield) of compound **5a** as white crystals. m.p.: 200 °C (dec.). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.28 (s, 1H), 7.76 (s, 1H), 7.57 (br, 1H), 7.25 (br, 1H), 6.94 (br, 1H), 6.14 (br, 1H), 5.69 (br, 1H), 5.4 (br, 1H), 4.97 (br,1H), 4.68 (br, 1H), 4.28 (br, 1H), 3.99-3.49 (m, 9H), 2.3-2.01 (m, 2H), 1.77 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ = 166.13; 162.65; 152.96; 147.46; 141.64; 132.85; 110.75; 98.22; 91.18; 86.10; 81.71; 76.40; 72.28; 66.18; 65.90; 60.52; 51.07; 36.85; 11.96. HRMS-FAB for $C_{19}H_{26}N_5O_{11}P$ $(M+H)^+$ = 532.14.

(*S,R*) *cis*-(5-((4-amino-2-oxopyrimidin-1(2H)-yl) methyl)-1,3-oxathiolan-2-yl)methyl (*2R,3R,5R*)-2-(hydroxy-methyl)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1 (2H)-yl)-tetrahydrofuran-3-yl hydrogen phosphate (5b). Compound **5b** was prepared like compound **5a** (20 mg, 74.7% yield) as white crystals. m.p.: 200 °C (dec.). ^{1}H NMR (400 MHz, D_2O) δ = 7.52-7.49 (br, 2H), 6.14-6.11 (t, 1H, J = 6.9 Hz), 5.83 (br, 1H), 5.22 (br, 1H), 4.27 (br, 1H), 4.10-3.52 (m, 8H), 3.06-3.02 (dd, 1H, J = 4.7 and 5.2 Hz), 2.70-2.66 (t, 1H, J = 10.1 Hz), 2.40-2.26 (m, 2H), 1.73(s, 3H). ^{13}C NMR (125 MHz, D_2O) δ = 166.36; 151.54; 147.74; 137.39; 137.30; 111.50; 95.42; 85.02; 83.65; 82.00; 81.94; 75.20; 75.00; 67.58; 61.10; 51.50; 37.54; 33.39; 11.53. HRMS-FAB for $C_{19}H_{26}N_5O_{10}PS$ $(M+H)^+$ = 546.11.

RESULTS AND DISCUSSION

As shown in Scheme 1, compounds **5a** and **5b** were synthesized as racemates to allow access to all stereoisomers. Compound **1** obtained in 85% yield in the reaction of thymidine with *tert*-butyl-chlorodimethyl silane in pyridine [24]. Coupling reaction of the 5'-protected thymidine, individually, with the racemates of both *cis*-4-amino-1-((2-(hydroxymethyl)-1,3-dioxolan-4-yl)methyl)pyrimidin-2(1H)-one (**2a**) and *cis*-4-amino-1-((2-(hydroxymethyl)-1,3-oxathiolan-5-yl)methyl)pyrimidin-2(1H)-one (**2b**) [23], using phenyldichlorophosphate as the coupling reagent in the presence of *N*-methylimidazole in THF afforded the respective compounds **3a** and **3b** in 60-65% yields (Method A). For the selective introduction of a 3'-5'-phosphorotriester linkage between 5'-protected deoxyribonucleoside (**1**) and dioxalane or oxathiolane nucleosides (**2a**,**b**), the coupling reagent dichlorophosphate could be applied, without formation of 3'- , or 5'- phosphorodiester intermediates. It should be noted that the compounds **3a,b** were found in 45-50% yield (Method B) by the use of a bifunctional phosphorylating agent, di(1H-benzo[d][1,2,3]triazol-1-yl)phenyl phosphonate [25].

The formation of compounds **3a** and **3b** was confirmed by their NMR and HRMS data. Deprotections with 2% hydrochloric acid in ethanol gave **4a** and **4b** in about 85% yield, which were characterized by ^{1}H NMR, ^{13}C NMR, DEPT 135, DEPT 90 (Fig. 1) and HRMS data.

Further deprotection of **4a** and **4b** with 28% NH_4OH solution in water afforded target molecules **5a** and **5b** in about 75% yield. Structures of **5a** and **5b** were characterized by ^{1}H NMR, ^{13}C NMR, and HRMS data. Compounds **5a** and **5b** will be evaluated for their antiviral activities; results will be reportedelsewhere.

In summary, we described high yield syntheses of novel nucleotide analogues **5a** and **5b** *via* the intermediates **3a** and **3b**, which in turn were prepared using the coupling reagent, phenyl dichlorophosphate.

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