

Article

Synthesis of a Novel Benzoyl Adenosine Analog Containing a 1, 4-Dioxane Sugar Analog and the Synthesis of a Corresponding Uracil Adenine Dinucleotide

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Received: 3 March 2011; in revised form: 28 April 2011 / Accepted: 6 May 2011 /

Published: 12 May 2011

Abstract: Adenosine analogs in which the sugar unit was replaced by a 1,4-dioxane sugar equivalent, were prepared by coupling the 1,4-dioxane sugar analog as its anomeric acetates, with *N*6-benzoyl protected adenine. The 1,4-dioxane system was obtained in an enantioselective synthesis from (*R,R*)-dimethyl tartrate. Using standard phosphorimidite methodology, the adenine analog was further reacted with a 1,4-dioxane uridine analog to give the corresponding, protected dinucleotide, set-up for further condensation into larger oligonucleotides.

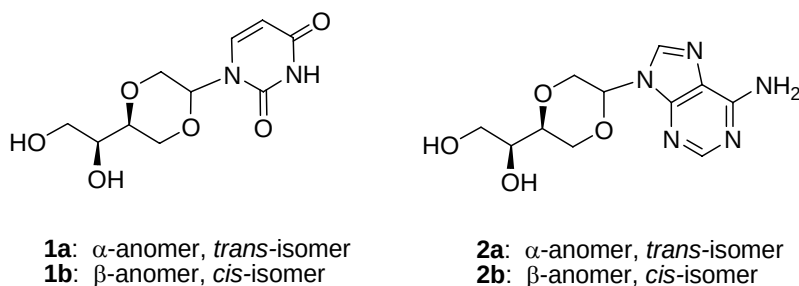
Keywords: nucleoside analogs; heterocyclic; adenosine; 1,4-dioxane; uracil-adenine

1. Introduction

Pursuing development of new antiviral and antitumor agents, a number of new nucleosides analogs have been synthesized in which of the sugar structures were modified [1]. The sugar unit has for example been replaced by a 1,4-dioxane moiety [2–6]. Some of these structures were reported to exhibit interesting biological activities [2,3], which may be ascribed to their particular flexible conformational properties [4]. Earlier we reported the synthesis of new optically active uridine analogs **1a** or **1b** where the sugar was substituted by an optically active 1,4-dioxane moiety [7]. These uridine analog was further elaborated into the corresponding dinucleotide using standard phosphorimidite

methodology [8]. However, we also reported [7] that preparation of the related adenosine analog failed. In this communication we wish to report our recent findings dealing with the synthesis of adenosine analogs **2a** and **2b**, which as the *N*-benzoyl protected compounds were then tested in dinucleotide formation with uridines **1a**.

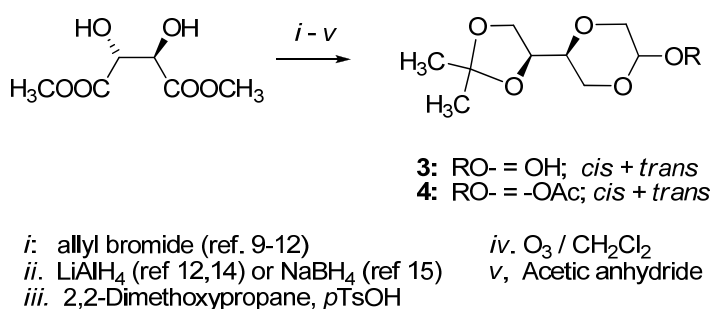
Figure 1. Uridine and adenosine analogs containing a 1,4-dioxane sugar equivalent.



2. Results and Discussion

The synthesis of 1,4-dioxane sugar analog **3** from (2*R*,3*R*)-dimethyl tartrate was reported earlier [7]. The tartrate was first converted into the corresponding enantiomerically pure diethyl (2*R*,3*R*)-2-*O*-allyltartrate either by the reaction with allyl bromide in the presence of silver oxide or in a tin assisted reaction with dibutyltin oxide [9–11]. The allyl ether was reduced by LiAlH₄ [12–14] or NaBH₄ [15,16] to give a triol which was protected as an acetal with 2,2-dimethoxy-propane in the presence of *p*-toluene sulfonic acid. Subsequent ozonolysis afforded 1,4-dioxane sugar analog **3** which was further transformed into acetate **4** with acetic anhydride (Scheme 1). All the tartrate stereoisomers are readily available from the chiral pool, conveniently allowing for the synthesis of all the possible stereoisomers of the nucleoside analogs.

Scheme 1. Synthesis of 1,4-dioxane sugar analogs **3** and **4** [7].

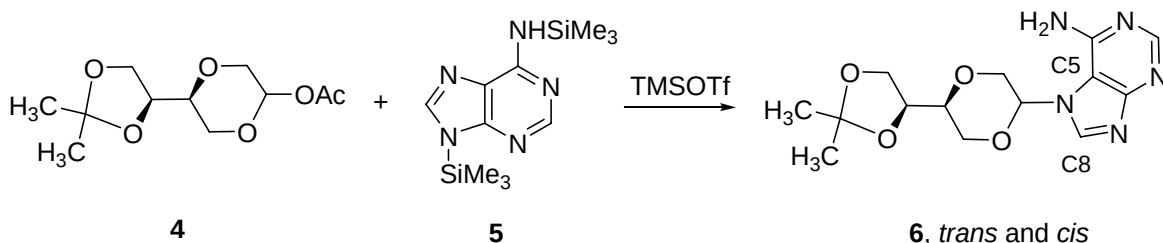


2.1. Synthesis of Adenosine Analogs

The corresponding adenine nucleoside analog from **4** was attempted prepared using a Vorbrüggen procedure [17,18]. Thus, acetate **4** was coupled with silylated adenine **5**, in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) to give **6** as a *cis/trans* mixture in 23% yield after flash chromatography. However, HMBC-NMR spectroscopic analysis showed that the product was the

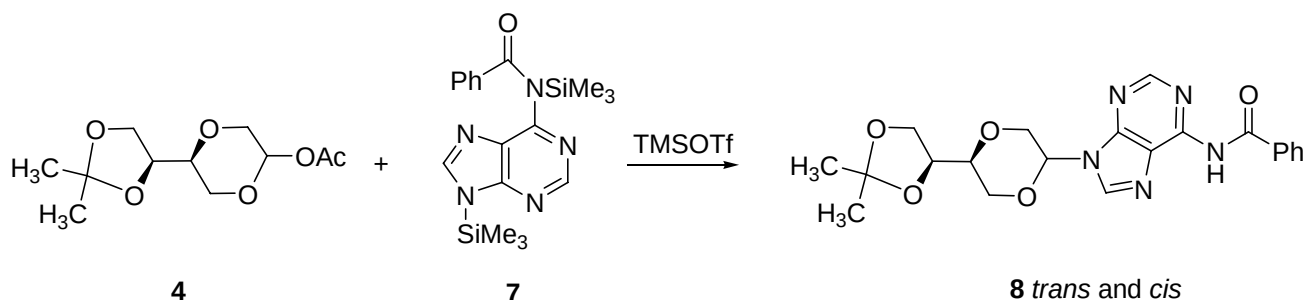
undesired *N*-7 regioisomer **6**, as three bond correlations were observed for the anomeric proton with C5 as well as C8 (Scheme 2).

Scheme 2. Synthesis of *N*-7 adenine nucleoside analogs **6**.



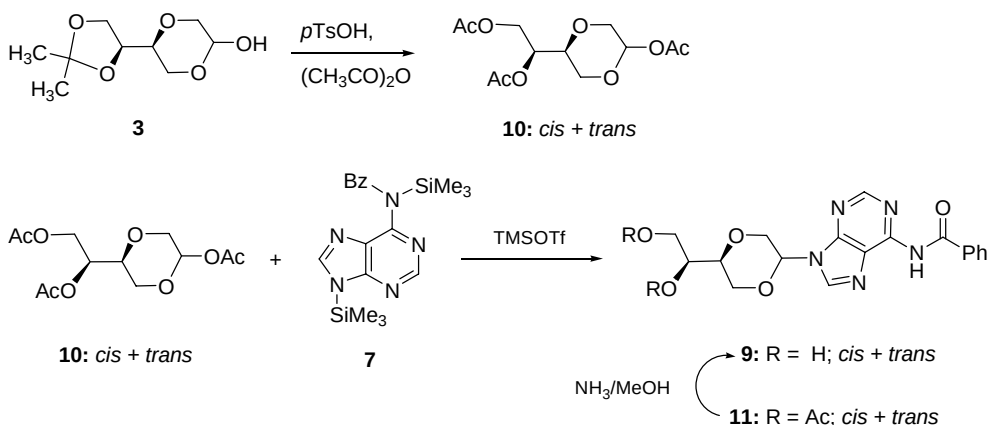
For reasons of forcing the adenine to react at the 9-position, **4** was instead reacted with the silylated *N*6-benzoyl protected adenine, **7**, in the presence of TMSOTf, affording the desired benzoyl protected adenine nucleoside analogues **8** as a *cis/trans* mixture in 42 % isolated yield after chromatographic purification, Scheme 3. The structure was confirmed by NMR analysis.

Scheme 3. Synthesis of benzoyl adenine nucleoside analogues **8**.



Attempts to remove the acetal protection group in **8** failed, as depurination was observed to take place in all cases. A number of literature methods described were tested [19,20], such as cleavage using 80% acetic acid [21] or CSA as catalyst, reaction with Amberlyst 15, HCl [22] or trifluoroacetic acid [23]. Other reagents, such as ferric chloride on silica gel [24], iodine in methanol [25] or alumina or silica gel catalysis in all cases gave no or similar results. The desired benzoyl protected nucleoside analog **9** was finally obtained by the reaction sequence shown in Scheme 4.

Scheme 4. Synthesis of benzoyl protected adenine nucleoside analogue **9**.

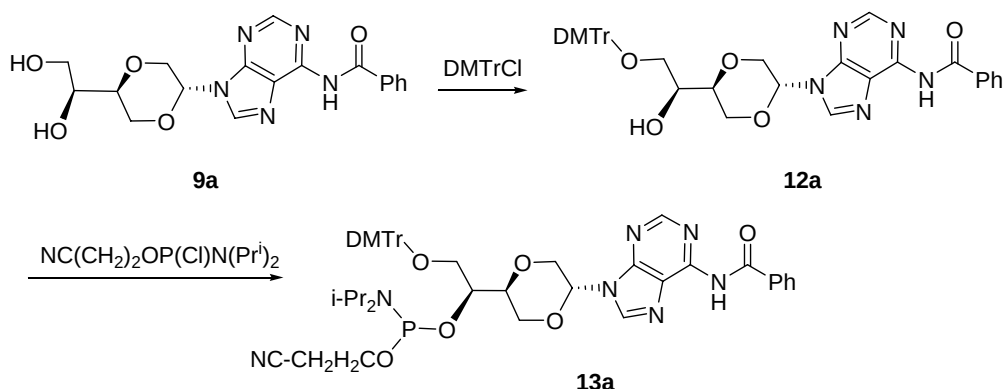


Conversion of **3** to the corresponding triacetate **10** by the reaction with acetic anhydride in the presence of catalytic amount of H_2SO_4 [26,27] gave the desired product though in a poor yield. Product **10** was, however, obtained in a satisfactory yield when **3** were reacted with acetic anhydride in the presence of 1.1 equivalent of *p*-TsOH [28]. Subsequently the triacetate was smoothly coupled with the silylated benzoyl adenine **7** in the presence of TMSOTf to afford benzoyl adenine nucleoside analogue **11**. The acetate groups were selectively removed by ammonia in methanol [29] leaving the desired, benzoyl-protected nucleoside analog **9** (Scheme 4).

2.2. Synthesis of Dinucleotide Analogs

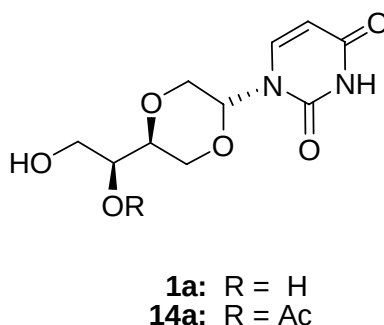
The pure *trans*-nucleoside analog **9a** was separated by flash chromatography from the mixture of diastereomers of compound **9**. Treatment of **9a** with 4,4'-dimethoxytrityl chloride (DMTrCl) in pyridine [30] afforded the primary hydroxyl group protected compound **12a** in 40% yield after flash chromatography. Compound **12a** was next reacted with *N,N*-diisopropyl-2-cyanoethylphosphor-amidic chloride [31,32] in the presence of *N,N*-diisopropylethylamine to give the desired phosphoramidite **13a** in 51% isolated yield, Scheme 5.

Scheme 5. Synthesis of phosphoramidite **13a**.



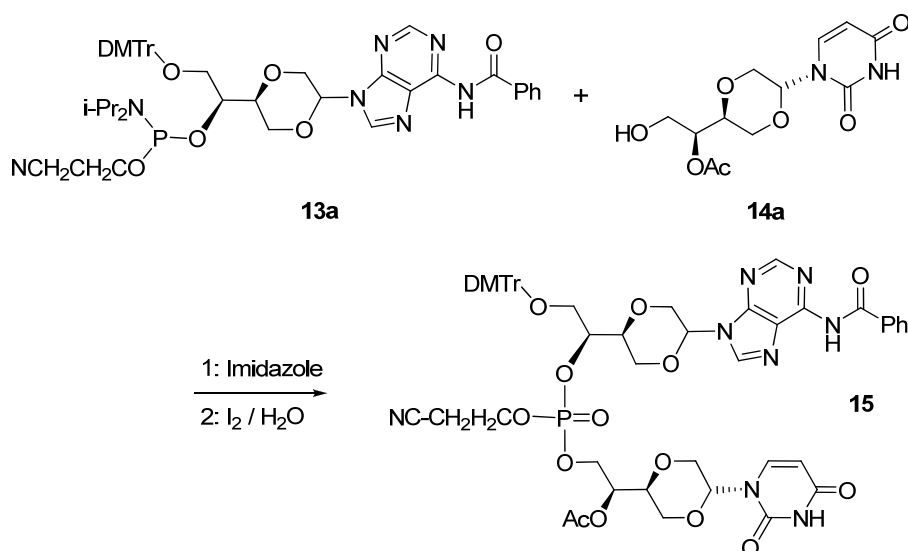
We have previously reported the synthesis of the uridine analog **1**, obtained from acetates **4**. The *trans*-anomer **1a** was isolated in 42% yield after recrystallization from acetonitrile, and subsequently converted to the secondary acetate **14a** [7].

Figure 2. *Trans*-uridine analogs **1a** and **14a**.



The coupling of adenosine phosphoramidite **13a** with uridine analog **14a** was carried out in the presence of 1*H*-tetrazole in dry acetonitrile [33], followed by iodine oxidation and purification by flash chromatography on silica gel. This afforded the uracil adenine dinucleotide analog **15** in 62% yield, Scheme 6. To obtain the analytically pure dinucleotide **15**, the product was purified by multi elution preparative TLC.

Scheme 6. Synthesis of 1,4-dioxane uracil adenine dinucleotide analog **15**.



Further elaboration of product **15** was not pursued, as it at this stage was demonstrated that the 1,4-dioxane nucleoside analogs can function in the standard reaction scheme for oligonucleotide preparations, used in for example automatic nucleotide synthesis machines. Thus **15** represent a standard intermediate for the further preparation of oligonucleotides.

3. Experimental

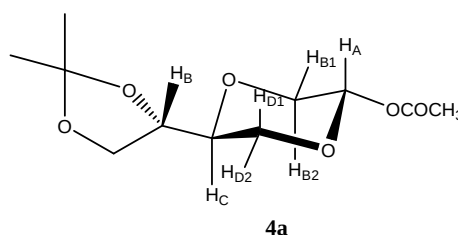
3.1. General

NMR spectra were recorded on Bruker Avance DPX 300 or DPX 400 instruments. Chemical shifts are reported in ppm using TMS (δ = 0.0) as the internal standard in CDCl₃ or relative to 2.50 ppm for ¹H and 39.99 ppm for ¹³C in [*d*₆-DMSO] or 3.31 ppm for ¹H and 49.15 ppm for ¹³C in CD₃OD. Structural assignments were based on ¹H, ¹³C, DEPT135 and 2D-spectra, COSY, HSQC, HMBC, and NOESY. EI-Mass and ESI spectra were recorded on a Finnigan MAT 95XL spectrometer. IR spectra were obtained on a Thermo Nicolet FT-IR Nexus spectrometer using a Smart Endurance reflection cell. For ozonolysis was used an OZ-500 Ozone Generator produced by Fishcer Technology. Silica gel Kieselgel 60G (Merck) was used for Flash Chromatography. The solvents were purified by standard methods. All reactions were carried out in inert atmospheres (nitrogen). The synthesis of compounds **1a** and **14a** has been described elsewhere [7].

(2*S*,5*S*)-5-[(4*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl]-2-acetyloxy-1,4-dioxane (**4a**) and (2*R*,5*S*)-5-[(4*S*)-2,2-dimethyl-1,3-dioxolan-4-yl]-2-acetyloxy-1,4-dioxane (**4b**). To a solution of **3** (1.0 g, 4.9 mmol) in

dry pyridine (15 mL) was added acetic anhydride (0.62 g, 6 mmol) at 0–5 °C and the reaction mixture was stirred for 6 hours. The solution was concentrated under reduced *in vacuo* overnight, yielding the crude product in 87% yield as an oily solid material, which was used in the subsequent reaction step without further purification. The anomeric ratio **4a**:**4b** (*trans*-*cis* ratio) was determined to be 4:1 by NMR. The products exhibited the following spectroscopic properties: The *trans*-product **4a** (Figure 1) was assigned the following signals: ¹H-NMR (CDCl₃, 400 MHz): δ 1.36, 1.43 (s, 2 × 3H, (CH₃)₂C), 2.11 (s, 3H, CH₃COO), 3.48 (dd, *J* = 8.0 Hz, 11.4 Hz, 1H, H_{B1}), 3.63 (m, 1H, H_C), 3.69 (dd, *J* = 9.4 Hz, 11.4 Hz, 1H, H_{D1}), 3.81 (dd, *J* = 6.8 Hz, 8.0 Hz, 1H, (CH₃)₂C-O-CH₂), 3.89 (dd, *J* = 2.6 Hz, 11.4 Hz, 1H, H_{D2}), 3.93 (dd, *J* = 2.8 Hz, 11.4 Hz, 1H, H_{B2}), 4.00 (dd, *J* = 6.8 Hz, 8.4 Hz, 1H, (CH₃)₂C-O-CH₂), 4.15 (m, 1H, (CH₃)₂C-O-CH-), 5.74 (dd, *J* = 2.8 Hz, 8.4 Hz, 1H, H_A) ppm. ¹³C-NMR (CDCl₃, 100 MHz): δ 20.9, 25.2, 26.3, 65.2, 65.6, 66.9, 74.2, 74.2, 89.4, 109.7, 169.0 ppm. The protons NMR spectrum of the corresponding *cis*- compound, **4b**, could not be fully assigned due to the peaks overlap with **4a**, however, the carbon NMR spectrum of the *cis*- compound was assigned the following signals: ¹³C-NMR (CDCl₃, 100 MHz): δ 21.1, 25.2, 26.2, 61.0, 64.9, 67.6, 74.9, 75.1, 88.4, 109.7, 169.8 ppm. The mixture exhibited the following mass spectrum: MS (EI) *m/z*: 247 (M⁺+1), 231(M⁺-CH₃), 187(M⁺-OAc), 145 (C₆H₉O₄). Elem. Anal. calcd. for C₁₁H₁₈O₆: C 53.65, H 7.37; found C, 53.84, H 7.45.

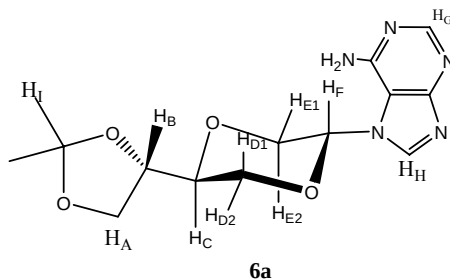
Figure 1. Structure of compound **4a**.



N-7-{(2*R*,5*S*)-5-[(4*S*)-(2,2-Dimethyl-1,3-dioxolan-4-yl)]-1,4-dioxan-2-yl}adenine (**6a**) and *N*-7-{(2*S*,5*S*)-5-[(4*S*)-(2,2-dimethyl-1,3-dioxolan-4-yl)]-1,4-dioxan-2-yl}adenine (**6b**). The mixture of adenine (1.35 g, 10 mmol) and ammonium sulfate (124 mg, 0.9 mmol) in hexamethyldisilazane (HMDS, 35 mL) was refluxed overnight. The solvent was evaporated and the residue was dissolved in dry dichloroethane (20 mL). To this solution, compound **4** (0.85 g, 3.5 mmol) was added. The solution was cooled to 0 °C and TMSOTf (0.75 mL, 4 mmol) was added. The solution was stirred for 8 hours at room temperature. The chloroform (50 mL) was added and the solution was washed twice with saturated NaHCO₃ solution (15 mL). The aqueous phase was extracted twice with chloroform (50 mL), and the combined organic layers was dried over anhydrous MgSO₄ and filtered and concentrated under reduced pressure. The residue was purified by flash chromatography using a mixture of dichloromethane and methanol (19:1) as the eluent. A white solid (0.26 g, 23%) was obtained which was identified as a 3:2 mixture of the *trans*- and *cis*- products **6a** and **6b**. From the mixture of isomers was extracted the following spectroscopic properties for isomer **6a** (Figure 2): ¹H-NMR (CDCl₃, 400 MHz): δ 1.47, 1.48 (s, 2 × 3H, H_I), 3.87–3.91 (m, 3H, H_B), 3.97–4.03 (m, 3H, H_A and H_E), 4.04 (dd, *J* = 12 Hz, 6 Hz, 1H, H_{D2}), 4.07 (dd, *J* = 8.4 Hz, 6.8 Hz, 1H, H_A), 4.21–4.27 (m, 2H, H_C and H_D),

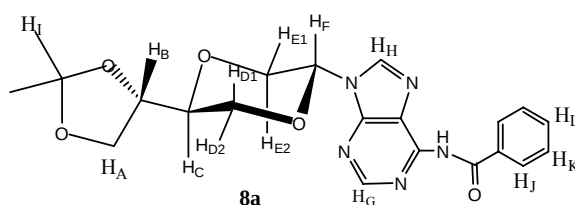
5.72 (dd, $J = 8.8$ Hz, 4 Hz, H_F), 5.99 (br.s, 2H, NH_2), 8.11 (s, 1H, H_G), 8.54 (s, 1H, H_H) ppm. ^{13}C -NMR ($CDCl_3$, 100 MHz): δ 25.2, 26.4, 66.1, 68.5, 60.4, 74.1, 82.0, 110.3, 111.1, 144.0, 151.3, 154.0, 161.6 ppm. For the mixture of **6a** and **6b**: IR (neat): 3418, 3290, 3149, 2979, 2905, 1625, 1595, 1062 cm^{-1} . HRMS (ESI): Calcd. for $C_{14}H_{19}N_5O_4$ $[M+Na]^+$ 322.1516, Found 322.1513.

Figure 2. Structure of compound **6a**.



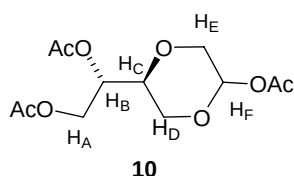
N-6-Benzoyl-*N*-9- $\{ (2R,5S)$ -5- $\{ (4S)$ -(2,2-dimethyl-1,3-dioxolan-4-yl)-1,4-dioxan-2-yl $\}$ adenine (**8a**) and *N*-6-benzoyl-*N*-9- $\{ (2S,5S)$ -5- $\{ (4S)$ -(2,2-dimethyl-1,3-dioxolan-4-yl)-1,4-dioxan-2-yl $\}$ -adenine (**8b**). *N*6-benzoyladenine (2.065 g, 8.7 mmol) and ammonium sulfate (120 mg, 0.9 mmol) in HMDS (50 mL) was refluxed overnight. The solution was cooled and concentrated under reduced pressure. The residue was dissolved in dry acetonitrile (20 mL) and added a solution of **6** (0.77 g, 3.1 mmol) in dry acetonitrile (5 mL). The mixture was then cooled to 0 °C and added TMSOTf (0.6 mL, 3.3 mmol). The mixture was stirred for four hours. Then chloroform (50 mL) was added and the solution washed twice with saturated $NaHCO_3$ solution (20 mL). The organic phase was dried over anhydrous $MgSO_4$ filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography using a gradient eluent system, first diethyl ether, followed by a mixture of dichloromethane and methanol (19:1) to afford the product as a yellow solid (0.55 g, 42%) as a 3:1 mixture of *trans*- and *cis* products **8a** and **8b**. Pure **8a** was obtained by repeated chromatography. Product **8a** (Figure 3) exhibited the following spectroscopic properties: 1H -NMR ($CDCl_3$, 400 MHz): δ 1.41, 1.52 (s, $2 \times 3H$, H_G), 3.48 (dd, $J = 11.4$ Hz, 9.4 Hz, 1H, H_{E2}), 3.79–3.84 (m, 1H, H_C), 3.94–3.97 (m, 1H, H_A), 4.00 (dd, $J = 11.6$ Hz, 10.8 Hz, 1H, H_{D1}), 4.09 (dd, $J = 8.4$ Hz, 6.8 Hz, 1H, H_A), 4.17 (dd, $J = 11.6$ Hz, 2.6 Hz, 1H, H_{D2}), 4.20–4.25 (m, 1H, H_B), 4.54 (dd, $J = 11.4$ Hz, 2.6 Hz, H_{E1}), 6.64 (dd, $J = 9.2$ Hz, 2.8 Hz, 1H, H_F), 7.43–7.49 (m, 2H, H_K), 7.50–7.54 (m, 1H, H_L), 8.23–8.28 (m, 2H, H_J) ppm. ^{13}C -NMR ($CDCl_3$, 100 MHz): δ 25.4, 26.4, 62.8, 65.0, 66.6, 74.1, 74.4, 81.9, 110.3, 115.2, 128.4, 130.1, 132.4, 137.5, 142.1, 144.9, 149.3, 157.2, 175.6 ppm. For the mixture of **8a** and **8b**: IR (neat): 3214, 2985, 1635, 1597, 1480, 1116, 1067, 1048 cm^{-1} . HRMS (ESI): Calcd. for $C_{21}H_{23}N_5O_5$ $[M+H]^+$ 426.1778, Found 426.1776.

Figure 3. Structure of compound **8a**.



(2*R*,5*S*)-5-[(1*S*)-(1,2-Diacetyloxy)]-2-acetyloxy-1,4-dioxane (**10a**) and (2*S*,5*S*)-5-[(1*S*)-(1,2-diacetyloxy)]-2-acetyloxy-1,4-dioxane (**10b**). Compound **3** (2.2 g, 10.8 mmol) and *p*-TsOH (2.26 g, 11.9 mmol) was dissolved in acetic anhydride (50 mL). The mixture was stirred for 7 hours at room temperature and then poured into ice water. To the mixture was neutralized with sodium bicarbonate, and then extracted with ethyl acetate. The organic phase was dried over anhydrous MgSO₄ filtration and evaporated under reduced pressure. The residue was purified by flash chromatography using a mixture of diethyl ether and *n*-hexane (2:1) as the eluent. The product appeared as yellow oil, (2.35 g, 75%). The diastereomers were not separated, however the NMR of the one diastereomer (Figure 4) was assigned the NMR signals: ¹H-NMR (CDCl₃, 400 MHz): δ 2.07, 2.14, 2.15 (s, 3 × 3H, CH₃), 3.59–3.61(m, 1H, H_D), 3.83 (dd, *J* = 12.8 Hz, 2H, 1H, H_E), 3.89–3.98 (m, 3H, H_E, H_D and H_C), 4.16 (dd, *J* = 12 Hz, 6.8 Hz, 1H, H_A), 4.36 (dd, *J* = 11.8 Hz, 4.2 Hz, 1H, H_A), 5.15–5.18 (m, 1H, H_B), 5.87 (d, 2Hz, 1H, H_F) ppm. ¹³C-NMR (CDCl₃, 100 MHz): δ 20.7, 20.8, 21.1, 61.2, 62.1, 68.1, 69.8, 73.6, 88.2, 169.8, 170.2, 170.5 ppm. The NMR data for the other diastereomer were the following: ¹H-NMR (CDCl₃, 400 MHz): δ 2.10, 2.11, 2.16 (s, 3 × 3H, CH₃), 3.78–3.82(m, 1H, H_C), 3.83 (dd, 12.8Hz, 2Hz, 1H, H_E), 3.93 (d, 12.8Hz, 1H, H_E), 4.13–4.26 (m, 5H, H_B, H_D and H_A), 5.87 (d, 2Hz, 1H, H_F) ppm. ¹³C-NMR (CDCl₃, 100 MHz): δ 20.7, 20.8, 21.4, 63.1, 63.4, 67.6, 68.8, 74.2, 89.0, 169.7, 170.5, 170.6 ppm. IR (neat): 2959, 1737, 1219, 1044, 1014 cm^{−1}. MS (*m/z*): 289.3, 243.3, 231.3, 217.3.

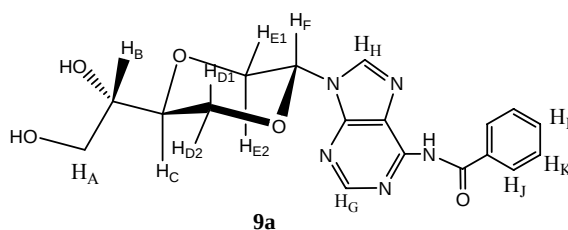
Figure 4. Structure of compound **10**.



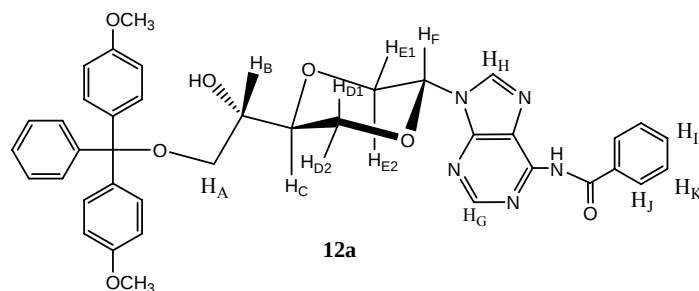
N-6-Benzoyl-*N*-9-{(2*R*,5*S*)-5-[(1*S*)-(1,2-diacetyloxy)]-1,4-dioxan-2-yl}adenine (**11a**) and *N*-6-benzoyl-*N*-9-{(2*S*,5*S*)-5-[(1*S*)-(1,2-diacetyloxy)]-1,4-dioxan-2-yl}adenine (**11b**). *N*-6-benzoyladenine (1.09 g, 4.6 mmol) and ammonium sulfate (62 mg, 0.47 mmol) in HMDS (50 mL) was refluxed overnight. The mixture containing the silylated *N*-6-benzoyladenine **7** was concentrated under reduced pressure, dissolved in dry dichloroethane (24 mL) and then added a solution of **10** (0.74 g, 2.6 mmol) in dry dichloromethane (6 mL). This mixture was added TMSOTf (0.94 mL, 5.2 mmol) at 0 °C and stirred overnight at room temperature. Chloroform (50 mL) was added to the mixture which was washed with saturated NaHCO₃ solution (25 mL). The aqueous phase was extracted with chloroform (50 mL). The combined organic layers were dried over anhydrous Na₂SO₄ and filtered and concentrated under reduced pressure. The residue was purified by flash chromatography using a mixture of dichloromethane and methanol (9/1) as the eluent to afford the product (0.67 g, 56%) as a mixture of the *trans* and *cis* products, **11a** and **11b**. The product was used for the next reaction without further purification. The NMR was too complex to allow reasonable assignments of the signals. IR (neat): 2958, 1737, 1636, 1216.1045, 1023 cm^{−1}. HRMS (ESI): Calcd. for C₂₂H₂₃N₅O₇ [M+H]⁺ 470.1677, Found 470.1663.

N-6-Benzoyl-*N*-9- $\{$ (2*R*,5*S*)-5- $\{$ (1*S*)-(1,2-dihydroxyl)-1,4-dioxan-2-yl $\}$ adenine (**9a**) and *N*-6-benzoyl-*N*-9- $\{$ (2*S*,5*S*)-5- $\{$ (1*S*)-(1,2-dihydroxyl)-1,4-dioxan-2-yl $\}$ adenine (**9b**). Compound **11** (1.27 g, 30 mL) was dissolved in 7*N* ammonia in methanol (30 mL) and stirred for 7 hours at room temperature. The solution was concentrated and purified by flash chromatography using a mixture of dichloromethane and methanol (19/1) as the eluent. The pure *trans*- product **9a** was isolated (140 mg, 13.5%, Figure 5). Product **9a** exhibited the following spectroscopic properties: $^1\text{H-NMR}$ (CD_3OD , 400 MHz): δ 3.59 (dd, $J = 11.2$ Hz, 9.4 Hz, 1H, $\text{H}_{\text{E}2}$), 3.60–3.70 (m, 3H, H_{A} and H_{B}), 3.89 (dt, $J = 10.6$ Hz, 2.8 Hz, 1H, H_{C}), 4.13 (dd, $J = 11.8$, 10.6 Hz, 1H, $\text{H}_{\text{D}1}$), 4.24 (dd, $J = 11.8$, 2.8 Hz, 1H, $\text{H}_{\text{D}2}$), 4.37 (dd, $J = 11.2$ Hz, 2.6 Hz, 1H, $\text{H}_{\text{E}1}$), 6.55 (dd, $J = 9.4$, 2.6 Hz, 1H, H_{F}), 7.41–7.45 (m, 1H, H_{I}), 7.49–7.53 (m, 2H, H_{K}), 8.21–8.24 (m, 1H, H_{C}), 8.24 (s, 1H, H_{G}), 8.67 (s, 1H, H_{H}) ppm. $^{13}\text{C-NMR}$ (CD_3OD , 100 MHz): δ 63.8, 70.4, 70.8, 72.1, 76.3, 82.6, 115.5, 129.3, 131.0, 133.4, 138.2, 144.2, 145.2, 149.7, 156.9, 176.6 ppm. IR (neat): 3286, 2876, 1635, 1424, 1285, 1115, 1063, 1021 cm^{-1} . HRMS (ESI): Calcd. for $\text{C}_{18}\text{H}_{19}\text{N}_5\text{O}_5$ $[\text{M}+\text{H}]^+$ 470.1677, Found 470.1668.

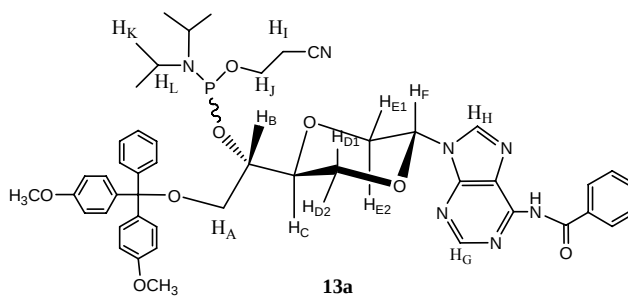
Figure 5. Structure of compound **9a**.



N-6-Benzoyl-*N*-9- $\{$ (2*S*,5*S*)-5- $\{$ (1*S*)-hydroxyl-2-*O*-(4,4-dimethoxytrityl)-ethyl-1-yl $\}$ -1,4-dioxan-2-yl $\}$ adenine (**12**). Compound **9** (mixture of **9a** and **9b**, 139 mg, 0.36 mmol) and dimethoxytrityl chloride (277 mg, 0.79 mmol) were dissolved in dry pyridine (10 mL). The solution was stirred at room temperature for 3 hours. The solution was concentrated *in vacuo* and residue was purified by flash chromatography using first diethyl ether and then ethyl acetate as the eluent to provide the product (**12a** and **12b**) as a white solid (100 mg, 40%). Product **12a** (Figure 6) was obtained upon repeated chromatography and exhibited the following spectroscopic properties: $^1\text{H-NMR}$ (CDCl_3 , 400 MHz): δ 2.82 (d, $J = 4.8$ Hz, 1H, OH), 3.25 (dd, $J = 9.6$ Hz, 5.4 Hz, 1H, H_{A}), 3.34 (dd, $J = 9.6$ Hz, 5.6 Hz, H_{A}), 3.43 (dd, $J = 11.2$ Hz, 9.2 Hz, $\text{H}_{\text{E}2}$), 3.75–3.82 (m, 1H, H_{B}), 3.77 (s, $2 \times 3\text{H}$, OCH_3), 3.86–3.90 (m, 1H, H_{C}), 4.00 (dd, $J = 12$ Hz, 10.2 Hz, $\text{H}_{\text{D}1}$), 4.13 (dd, $J = 12$ Hz, 2.8 Hz, $\text{H}_{\text{D}2}$), 4.43 (dd, $J = 11.2$ Hz, 2.8 Hz, H_{E}), 6.60 (dd, $J = 9.2$ Hz, 2.8 Hz, H_{F}), 6.82–6.86 (m, 5H, aromatic protons), 7.20–7.24 (m, 1H, aromatic proton), 7.26–7.37 (m, 5H, aromatic protons), 7.40–7.53 (m, 5H, aromatic protons), 8.13 (s, 1H, H_{G}), 8.15–8.25 (m, 2H, aromatic protons), 8.64 (s, 1H, H_{H}) ppm. $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz): δ 55.2, 63.8, 69.3, 69.4, 70.0, 75.5, 81.0, 86.5, 113.2, 126.9, 129.8, 130.0, 132.3, 135.7, 135.8, 137.1, 142.1, 143.0, 144.6, 148.4, 157.1, 158.6, 175.4 ppm. HRMS (ESI): Calcd. for $\text{C}_{39}\text{H}_{37}\text{N}_5\text{O}_7$ $[\text{M}+\text{Na}]^+$ 710.2591, Found 710.2588.

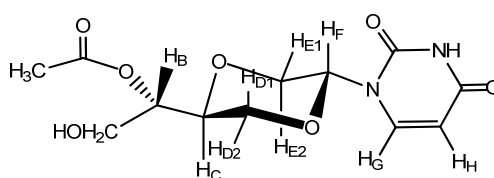
Figure 6. Structure of compound **12a**.

N-6-Benzoyl-*N*-9- $\{(2S,5S)$ -5- $\{(1S)$ -*O*-[2-cyanoethoxy(diisopropylamino)phosphino]-2-*O*-(4,4'-dimethoxytrityl)-ethyl-1-yl}-1,4-dioxan-2-yl}adenine (**13a**). Compound **12** (mixture of **12a** and **12b**) (99 mg, 0.14 mmol) was dissolved in dry dichloromethane (6 mL) was then added *N,N*-diisopropylethylamine (50 μ L, 0.29 mmol). Then *N,N*-diisopropyl-2-cyanoethyl phosphoramidic chloride (105 μ L, 0.47 mmol) was added. The resulting solution was stirred for 6 hours at room temperature. The solution was then concentrated and purified by flash chromatography using a mixture of ethyl acetate and n-hexane (4:1) as the eluent to give the *trans* isomer **13a** (65 mg, 51%) as a mixture of diastereomers due to the stereogenic phosphorus. Major diastereomer **13a** (Figure 7) had the following spectroscopic properties: $^1\text{H-NMR}$ (CDCl_3 , 400 MHz): δ 1.14 (d, $J = 7.2$ Hz, $2 \times 3\text{H}$, H_K), 1.19 (d, $J = 7.2$ Hz, $2 \times 3\text{H}$, H_K), 2.64 (dt, $J = 6.4, 2.4$ Hz, 2H, H_I), 3.27 (dd, $J = 9.4, 5.2$ Hz, 1H, H_A), 3.36 (dd, $J = 9.4, 6$ Hz, 1H, H_A), 3.45 (dd, $J = 11.2, 9.4$ Hz, 1H, H_E2), 3.56–3.70 (m, $2 \times 1\text{H}$, H_L), 3.771, 3.768 (s, $2 \times 3\text{H}$, OCH_3), 3.72–3.82 (m, 1H, H_J), 3.87–3.95 (m, 1H, H_J), 4.00–4.09 (m, $3 \times 1\text{H}$, H_B , H_D , H_C), 4.24 (d, $J = 10$ Hz, 1H, H_D), 4.40 (dd, $J = 11.2, 2.6$ Hz, 1H, H_E1), 6.61 (dd, $J = 9.4, 2.6$ Hz, 1H, H_F), 6.81–6.85 (m, 4H, aromatic H), 7.19–7.22 (m, 1H, aromatic H), 7.27–7.30 (m, 2H, aromatic H), 7.34–7.36 (m, 4H, aromatic H), 7.41–7.48 (m, 4H, aromatic H), 7.52–7.56 (m, 4H, aromatic H), 8.14 (s, 1H, H_G), 8.22–8.25 (m, 2H, aromatic H), 8.69 (s, 1H, H_H), 12.59 (s, 1H, NH) ppm. $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz): δ 20.4, 20.5, 24.56, 24.64, 24.67, 24.74, 43.2, 43.4, 55.2, 58.0, 58.2, 60.4, 69.55, 69.62, 72.1, 75.3, 75.4, 81.3, 86.4, 113.1, 114.4, 117.7, 126.8, 127.8, 128.2, 128.3, 129.9, 130.05, 130.09, 132.3, 135.95, 136.01, 137.2, 141.8, 143.3, 144.8, 148.4, 157.2, 158.5, 175.5 ppm. $^{31}\text{P-NMR}$ (CDCl_3): 151.0, 152.0 (small) ppm. IR (neat): 3239, 3056, 2965, 2929, 2362, 2338, 1637, 1507, 1251, 1083, 788, 754, 719 cm^{-1} . HRMS (ESI): Calcd. for $\text{C}_{48}\text{H}_{54}\text{N}_7\text{O}_8\text{P} [\text{M}+\text{Na}]^+$ 910.3669, Found 910.3639.

Figure 7. Structure of compound **13a**.

1-[(2R,5S)-5-[(1S)-Acetyloxy-2-hydroxyethyl-1-yl]-1,4-dioxan-2-yl]uracil (14a). The synthesis of this compound has been reported earlier [7]. Compound **14a** (Figure 8) exhibited the following spectroscopic properties: $^1\text{H-NMR}$ (CD_3OD , 400 MHz): δ 2.07 (s, 3H, CH_3), 3.58 (dd, $J = 11.4$ Hz, 9.8 Hz, 1H, $\text{H}_{\text{E}2}$), 3.70–3.74 (m, 1H, H_{B}), 3.77–3.82 (m, 1H, H_{C}), 3.92–3.98 (m, 2H, H_{E} and $\text{H}_{\text{D}1}$), 4.06 (dd, $J = 11.6$, 2.8 Hz, 1H, $\text{H}_{\text{D}2}$), 4.13 (dd, $J = 11.2$, 6.4 Hz, 1H, H_{A}), 4.16 (dd, $J = 11.2$ Hz, 5.4 Hz, 1H, H_{A}), 5.69 (dd, $J = 10$ Hz, 2.8 Hz, 1H, H_{F}), 5.70 (d, $J = 8$ Hz, 1H, H_{H}), 7.71 (d, $J = 8$ Hz, 1H, H_{G}) ppm. $^{13}\text{C-NMR}$ (CD_3OD , 100 MHz): δ 20.9, 66.4, 69.2, 69.4, 69.8, 76.0, 80.0, 103.1, 142.3, 151.9, 166.0, 172.8 ppm. IR (neat): 3477, 3190, 3110, 3074, 2996, 2879, 1697, 1268, 1105 cm^{-1} . MS (EI): 230.3, 197($\text{M}^+ - (\text{CH}_3\text{COCHCH}_2\text{OH})$), 189($\text{M}^+ - \text{uracil}$). HRMS (ESI): Calcd. for $\text{C}_{12}\text{H}_{16}\text{N}_2\text{O}_7$ [$\text{M} + \text{Na}$] $^+$ 323.0856, Found 323.0862.

Figure 8. Structure of compound **14**.

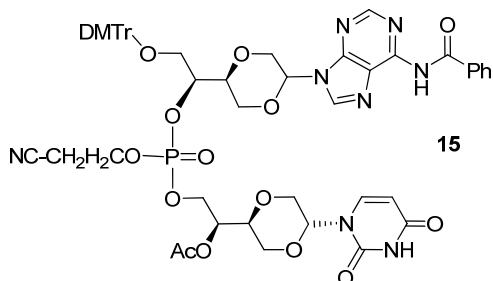


14

N6-Benzoyladenine uracil dinucleotide (15). Compound **13a** (30 mg, 0.039 mmol) and **14a** (8 mg, 0.027 mmol) in a reaction flask with a magnetic stirring bar were dried under high vacuum for 4 hours. Then a mixture 1-*H* tetrazole (0.3 mL, 0.45 M, 0.135 mmol) in dry acetonitrile (6 mL) was added under a nitrogen atmosphere. The solution was stirred overnight at room temperature. Then was added a few drops of iodine in THF (1M solution), 2,6-lutidine and H_2O (2:2:1) until an orange color persisted. The solution was then quenched with saturated sodium thiosulfate solution (4 mL). Then the two phases were treated with saturated NaHCO_3 solution (4 mL). The separated aqueous phase was extracted with 4×8 mL of dichloromethane. The combined organic phase was dried over anhydrous Na_2SO_4 and filtered and the solvent evaporated. Flash chromatographic purification, using mixtures of dichloromethane and methanol in the ratio from 25:1 to 15:1 as gradient solvents, afforded a product (16 mg, 61.5%), which was assigned structure **15** (Figure 9) based on the following spectroscopic properties: $^1\text{H-NMR}$ (CDCl_3 , 400 MHz): δ 2.02 (s, 3H, CH_3COO), 2.09 (s, 3H, CH_3COO), 2.51–2.62 (m, 2H, CH_2CN), 2.71–2.74 (m, 2H, CH_2CN), 3.15 (dd, 1H, $J = 11.2$ Hz, 9.6 Hz), 3.21–3.28 (m), 3.38 (dd, $J = 11.4$ Hz, 9.8 Hz), 3.45–3.58 (m), 3.62–3.96 (m), 4.01 (dd, 1H, $J = 11.2$ Hz, 2.8 Hz), 4.03–4.13 (m), 4.14–4.35 (m), 4.37–4.47 (m), 4.63–4.74 (m), 5.58 (dd, 1H, $J = 9.8$ Hz, 3 Hz, anomeric proton), 5.66–5.71 (m, 3H, two protons in uracil and one anomeric proton), 6.62–6.68 (m), 6.84–6.89 (m, aromatic protons), 7.20 (d, $J = 8$ Hz, 1H, a proton in uracil), 7.22–7.28 (m, aromatic protons), 7.30–7.37 (m, aromatic protons and one proton in uracil), 7.42–7.49 (m, aromatic protons), 7.52–7.58 (m, aromatic protons), 8.143 (s, 1H, a proton in adenine), 8.148 (s, 1H, a proton in adenine), 8.23–8.25 (m, aromatic protons), 8.33 (br. 1H, NH), 8.63 (s, a proton in adenine), 8.64 (s, a proton in adenine) ppm. $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz): δ 20.76, 20.82, 55.3, 62.0–62.5 (m), 67.6, 68.1, 68.4, 69.4, 73.2, 74.4, 74.8, 78.4, 78.5, 80.9, 86.8, 86.9, 102.7, 102.8, 128.06, 128.10, 128.3, 129.9, 130.0, 132.4, 134.98, 135.02, 135.2, 137.1, 139.2, 139.3, 141.9, 142.9, 148.4, 149.5, 157.2, 158.8, 162.1, 170.4,

175.6 ppm. ^{31}P -NMR (CDCl_3): -1.28 , -1.49 ppm. HRMS (ESI): Calcd. for $\text{C}_{54}\text{H}_{55}\text{N}_8\text{O}_{16}\text{P}$ $[\text{M}+\text{Na}]^+$ 1125.3372, Found 1125.3354.

Figure 9. Structure of compound 15.



4. Conclusions

The synthesis of the adenosine analogs containing an optically active 1,4-dioxane sugar equivalent was achieved by coupling of *N*-silylated *N*-6-benzoyl protected adenine with the sugar acetate equivalents. Applying conventional phosphorimidite methodology the adenine analog was further coupled to the related uridine analog to give the corresponding protected dinucleotide, thus making it feasible that larger oligonucleotides can be prepared, including the application of conventional automated procedures.

Acknowledgments

The authors wish to thank the Norwegian Research Council, NFR, for financial support.

References and Notes

1. Ichikawa, E.; Kato, K. Sugar-modified nucleosides in past 10 years, a review. *Curr. Med. Chem.* **2001**, *8*, 385-423.
2. Szarek, W.A.; Pinto, B.M.; Iwakawa, M. Synthesis and biological activity of nucleoside analogues involving modifications in the carbohydrate ring. *Can. J. Chem.* **1985**, *63*, 2149-2161.
3. Hronowski, L.J.; Szarek, W.A. Synthesis and biological evaluation of novel pyrimidine nucleoside analogues of 1,4-oxathiane, 1,4-dithiane, and 1,4-dioxane. *J. Med. Chem.* **1982**, *25*, 522-526.
4. Prisbe, E.J. Synthesis and antiviral evaluation of 1,4-dioxane nucleoside analogues related to nucleoside dialdehydes. *J. Med. Chem.* **1986**, *29*, 2445-2450.
5. Mitsuaki, M.; Naoko, K.; Yamaizumi, Z.; Okamoto, Y.; Nagahara, K.; Takayanagi, H. Synthesis of a novel dioxane nucleoside having two bases, 2(R)-(5-fluorouracil-1-yl)-5(R)-hydroxymethyl-3(R)-(uracil-1-yl)-1,4-dioxane and its 2(S)-isomer, from uridine. *Tetrahedron Lett.* **1997**, *38*, 6841-6844.
6. Szarek, W.A.; Vyas, D.M.; Achmatowicz, B. Synthesis of nucleoside analogues of 1,4-oxathiane, 1,4-dithiane, and 1,4-dioxane. *J. Heteroc. Chem.* **1975**, *12*, 123-127.

7. Qiang, Y.; Carlsen, P. Synthesis of a Novel, Optically Active Uridine Analog Containing a 1,4-dioxane Sugar Moiety. Synthesis of the Corresponding Dinucleotide. *Nucleos. Nucleot. Nucl. Acids* **2009**, *28*, 165-174.
8. Sinha, N.D.; Biernat, J.; Mcmanus, J. Köster, H. Polymer support oligonucleotide synthesis. XVIII: use of -cyanoethyl-N,N-dialkylamino-/N-morpholino phosphoramidite of deoxynucleosides for the synthesis of DNA fragments simplifying deprotection and isolation of the final product. *Nucl. Acids Res.* **1984**, *12*, 4539-4557.
9. Nagashima, N.; Ohno, M. Selective monoalkylation of acyclic diols by means of dibutyltin oxide and fluoride salts. *Chem. Pharm. Bull.* **1991**, *39*, 1972-1982.
10. Nagashima, N.; Ohno, M. An efficient O-monoalkylation of dimethyl L-tartrate via O-stannylene acetal with alkyl halides in the presence of caesium fluoride. *Chem. Lett.* **1987**, *1*, 141-144.
11. Baskaran, S.; Aurich, H.G.; Biesemeier, F.; Harms, K. Cycloadducts from highly functionalized nitrones and oximes as ligands in the enantioselective addition of diethylzinc to benzaldehyde. *J. Chem. Soc. Perkin Trans. 1* **1998**, *22*, 3717-3724.
12. Marshall, J.A.; Andersen, N.H.; Hochstetler, A. Reduction of malonic enolates with lithium aluminum hydride. *J. Org. Chem.* **1967**, *32*, 113-118.
13. Shi, M.; Jiang, J.-K.; Feng, Y.-S. Chiral C_2 -symmetric 2,3-disubstituted aziridine and 2,6-disubstituted piperidine as chiral ligands in the addition reaction of diethylzinc with arylaldehydes. *Tetrahedron Asymmetry* **2000**, *11*, 4923-4933.
14. Marshall, J.A.; Beaudion, S. Stereoselective Synthesis of Higher Sugars by Homologation of Carbohydrate-Derived Enals with Nonracemic γ -(Silyloxy) Allylic Stannanes and Substrate-Directed Hydroxylation. *J. Org. Chem.* **1994**, *59*, 6614-6619.
15. Albizati, K.F.; Babu, S. A synthesis of the HIV-protease inhibitor nelfinavir from D-tartaric acid. *Tetrahedron Lett.* **2001**, *42*, 6481-6485.
16. Takano, S.; Kurotaki, A.; Sekiguchi, Y.; Satoh, S.; Hiram, M.; Ogasawara, K. Selective manipulation of hydroxy groups in (2S,3S)-threitol. *Synthesis* **1986**, *10*, 811-817.
17. Vorbrüggen, H.; Krolkiewicz, K. Nucleoside syntheses. XXII. Nucleoside synthesis with trimethylsilyl triflate and perchlorate as catalysts. *Chem. Ber.* **1981**, *114*, 1234-1255.
18. Vorbrüggen, H.; Höfle, G. Nucleoside syntheses. XXIII. On the mechanism of nucleoside synthesis. *Chem. Ber.* **1981**, *114*, 1256-1268.
19. Green, T.W.; Wuts, P.G.M. *Protective Groups in Organic Synthesis*; Wiley & Sons: New York, NY, USA, 1999.
20. Magdalena, J.; Fernandez, S.; Ferrero, M.; Gotor, V. Difficulties in the deprotection of 1,2-ketals in nucleosides containing alkylidencarbazoyl groups. *Nucleos. Nucleoti. Nucl. Acids* **2002**, *21*, 55-64.
21. Van Cleve, J.W.; Rist, C.E. Ethylidene derivatives of D-erythrose. I. 2,3-O-Ethylidene- β -D-erythrofuranoose. *Carbohydr. Res.* **1967**, *4*, 82-92.
22. Angyal, S.J.; Beveridge, R.J. Conformational analysis in carbohydrate chemistry. Part IV. Intramolecular acetal formation by primary versus secondary hydroxyl groups. *Carbohydr. Res.* **1978**, *65*, 229-234.

23. Shing, T.K.M.; Wan, L.H. Facile Syntheses of Valiolamine and Its Diastereomers from (-)-Quinic Acid. Nucleophilic Substitution Reactions of 5-(Hydroxy-methyl)cyclohexane-1,2,3,4,5-pentol. *J. Org. Chem.* **1996**, *61*, 8468-8479.
24. Kim, K.S.; Song, Y.H.; Lee, B.H.; Hahn, C.S. Efficient and selective cleavage of acetals and ketals using ferric chloride adsorbed on silica gel. *J. Org. Chem.* **1986**, *51*, 404-406.
25. Szarek, W.A.; Zamojski, A. A new, facile method for cleavage of acetals and dithioacetals. *Tetrahedron Lett.* **1986**, *27*, 3827-3830.
26. Hall, D.M.; Lawler, T.E.; Childress, B.C. A practical synthesis of 1,2,3,6-tetra-*O*-acetyl- α - and β -D-glucopyranose, and their use to prepare trisaccharides. *Carbohydr. Res.* **1974**, *38*, 359-363.
27. Saito, Y.; Zevaco, T.A.; Agrofoglio, L.A. Chemical synthesis of ^{13}C labeled anti-HIV nucleosides as mass-internal standards. *Tetrahedron* **2002**, *58*, 9593-9603.
28. Cao, Y.; Okada, Y.; Yamada, H. Facile and regioselective preparation of partly *O*-benzylated D-glucopyranose acetates via acid-mediated simultaneous debenzylation-acetolysis. *Carbohydr. Res.* **2006**, *341*, 2219-2223.
29. Neilson, T.; Werstiuk, E.S. Oligoribonucleotide synthesis. II. Preparation of 2'-*O*-tetrahydropyranol derivatives of adenosine and cytidine necessary for insertion in stepwise synthesis. *Can. J. Chem.* **1971**, *49*, 493-499.
30. Fisher, E.F.; Caruthers, M.H. Color coded triarylmethyl protecting groups useful for deoxypolynucleotide synthesis. *Nucl. Acids Res.* **1983**, *11*, 1589-1599.
31. Stütz, J.A.R.; Richert, C. Synthesis of a formamidine-protected 5'-amino-2',5'-dideoxy-guanosine phosphoramidite and preparation of 5'-acylamidooligonucleotides. *Tetrahedron Lett.* **2004**, *45*, 509-513.
32. Eisenfuhr, A.; Arora, P.S. A ribozyme with michaelase activity: synthesis of the substrate precursors. *Bioorg. Med. Chem.* **2003**, *11*, 235-249.
33. Ferris, J.P.; Peyser, J.R. Rapid and efficient syntheses of phosphorylated dinucleotides. *Nucleos. Nucleot.* **1994**, *13*, 1087-1111.

Sample Availability: Not available

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