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Synthesis and Characterization of Abasic β-Diol-*C*-nucleosides

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Abstract: Modified nucleobases are potentially useful building blocks when containing catalytically active functionalities and could be introduced in chiral tridimensional molecules such as nucleic acids, which creates the premises for the development of novel catalytic species. Herein, we describe the synthesis of a novel β-*C*-nucleoside bearing a diol group at anomeric position, amenable as a metal ligand or organocatalyst. An abasic ligand was successfully prepared and inserted into a complementary DNA strand.

Keywords: *C*-nucleosides; abasic nucleosides; functionalized DNAs

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1. Introduction

DNA has a central role in chemical evolution due to its ability to store and transfer genetic information. This feature is due to the specific Watson–Crick hydrogen bond exerted by nucleobases (C:G, A:T) [1] that allows for DNA interstrand molecular recognition. In order to obtain novel materials that could be used for storage of information, synthetic chemists engaged in the design and development of alternative base pairs that could exert the same role in a new, unnatural genetic code [2–4]. The design of an unnatural base pair can be based on different hydrogen bonding patterns [5,6] or on shape complementarity [7]. Among the variety of approaches to DNA mimetic supramolecular chemistry, the strategy of replacing DNA natural bases using alternative heterocyclic moieties capable of metal complexation is of particular interest, as metals inserted in a chiral environment, such as DNA, may open the opportunity to use artificial DNAs as catalysts [8–10]. In these new molecular objects, the hydrogen bond base pairing is replaced by metal coordination that supplies the energy required for interstrand pairing. Hence, by choosing an appropriate ligand nucleoside and a metal ion, duplexes or other higher order complexes were formed, paving the way to metal-responsive functional DNAs, DNA nanomachines, and DNA-based nanomaterials, wires, and magnetic devices [11,12]. Natural nucleobases (C, G, T, A) could form metal mediated base pairs; [13–16] however, most of the unnatural nucleosides used for the generation of artificial DNAs contained unnatural bases, i.e., imidazole [17], salen [18], 6-substituted purines [19], Dipic/pyridine [20], and hydroxypyridone [21]. For example, pyridine-2,6-dicarboxylate nucleobase (Dipic) **1** [20] was reported to form a copper-mediated complex with a pyridine nucleobase (Py) (Figure 1). Dipic and Py formed, in the presence of Cu^{2+}, a (3 + 1) coordination compound possessing a square planar geometry (**1**, Figure 1). When inserted in double strand of complementary DNAs, the dipic–Cu^{2+}–Py pairing furnished a duplex characterized by higher thermal stability compared to the native natural DNA [20].

Shionoya reported the Cu^{2+}-mediated base pairing of hydroxypyridone **2** (Figure 1) [21]. Interestingly, in the absence of Cu^{2+}, the hydroxypyridone (H) bases inserted in complementary DNA strands behaved as a mis-pair. However, following deprotonation, a square planar complex with Cu^{2+} was formed that gave rise to a stabilized duplex. The artificial

DNA strands described by Shionoya were extremely efficient and formed double helices quantitatively through H–Cu^{2+}-H pairing, where H stands for a hydroxypyridone. It was also demonstrated that several consecutive H–Cu^{2+}-H pairings could be introduced in a sequence providing the opportunity to assemble a one-dimensional array of metals inserted in a double strand of DNAs [4,21]. The same authors reported, the enzymatic polymerization of dHTP, an activated form of nucleotide H recognized by the cell enzymatic machinery, that furnished unnatural DNA strands containing up to five H nucleotides at 3′ [4]. These strands successfully formed copper-mediated metal DNA duplexes through the formation of the pair H–Cu^{2+}-H. Based on these findings and intrigued by the potential applications of DNA as molecular wires, organic catalysts, and magnets, we posed the question of whether or not a nucleobase should be indispensable for the formation of metal bound complexes [4]. The assumption was that an abasic nucleoside, having a simpler functionality capable of coordinating divalent metal ion, could be sufficient to work as a ligand, then generating a new class of DNA based materials capable of asymmetric catalysis.

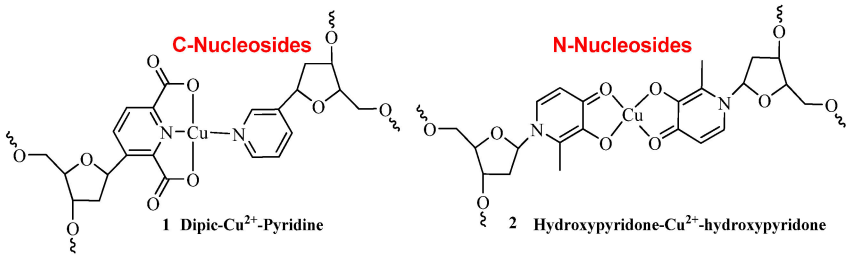


Figure 1. Representative examples of metal-mediated base pairs.

Importantly, for catalysis, the formation of a double helix would not be indispensable for the creation of an asymmetric environment around the metal center. With this in mind, we set out to synthesize a new β-*C*-nucleoside bearing a β-diol group at the anomeric position and derive preliminary results regarding its suitability for being inserted in oligomeric materials.

2. Materials and Methods

2.1. General Experimental

This section is part of the Ph.D. thesis submitted by G.B. [22]. ^{1}H, ^{13}C, NMR spectra were recorded using a Varian AS 300 and Bruker 400 and 600 spectrometer. ^{1}H- and ^{13}C-NMR for compounds **4**–**18** can be found in Supplementary Materials. Chemical shifts (δ) are reported in ppm relative to residual solvent signals for ^{1}H and ^{13}C NMR (1H NMR: 7.26 ppm for $CDCl_3$; ^{13}C NMR: 77.0 ppm for $CDCl_3$. ^{13}C NMR spectra were acquired using ^{1}H broadband decoupled mode. DMSO-d6 was used (referenced to 2.52 and 3.35 ppm for 1H and 40.0 for ^{13}C). Coupling constants (*J*) are in Hz. Multiplicities are reported as follows: s—singlet; d—doublet; dd—doublets of doublets; t—triplet; q,—quartet; m—multiplet; c—complex; and br—broad. ^{1}H-NMR spectral assignments are supported by ^{1}H-^{1}H COSY and ^{13}C-^{1}H-COSY where necessary. Carbon spectra are supported by DEPT analysis where necessary. Infrared spectra (IR) were obtained in CCl_4 using a Bruker Tensor 27 FT-IR instrument. Absorption maximum (ν_{max}) was reported in wave numbers (cm^{-1}) and only selected peaks are reported. High resolution mass spectra were obtained using a Waters Micro mass LCT and low-resolution mass spectra were recorded using Waters Micro mass Quattro LCMS spectrometers at 70 eV. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Tetrahydrofuran was freshly distilled over sodium benzophenone prior to use according to standard procedure. All other reagents and solvents were used as purchased from Aldrich. Reactions were checked for completion using TLC (EM Science, silica gel 60 F254), which were visualized by quenching of u.v. fluorescence (λ_{max} = 254 nm) or by staining with either 10% *w*/*v* ammonium molybdate in 2M sulfuric acid or basic potassium permanganate solution (followed by heat) as appropriate. Flash chromatography

was performed using silica gel 60 (0.040–0.063 mm, 230–400 mesh). Retention factors (R_f) are reported to ±0.05.

2.2. Synthesis of (2R,3S)-2-(Hydroxymethyl)-5-methoxytetrahydrofuran-3-ol **4**

Acetyl chloride (690 µL, 6.5 mol%) was added To a stirred solution of 2-deoxy-D-ribose (20.0 g, 149.0 mmol) in methanol (240 mL). The reaction mixture was stirred at room temperature for 1 h; then, sodium bicarbonate (7.70 g) was added and the reaction stirred for a further 10 min. The solid formed was filtered through celite, and the filtrate was evaporated in vacuo to afford 4 as a mixture of two diastereoisomers as an orange oil (22.0 g, >99% yield). This product did not require any further purification. (α + β anomers): R_f = 0.53 (chloroform/methanol 8:2). δ_H (400 MHz, $CDCl_3$): 5.19–4.95 (m, 2H), 4.39–4.32 (m, 1H), 4.11–4.08 (m, 1H), 3.99 (q, *J* = 4.4, 1H) 3.92 (q, *J* = 4.4, 1H), 3.70–3.55 (m, 4H), 3.32 (s, 3H, -CH_3), 3.30 (s, 3H, -CH_3), 2.19–2.10 (m, 2H), 2.06–2.00 (m, 1H), 1.92–1.88 (m, 1H). δ_C (100.6 MHz, $CDCl_3$): 105.60, 105.56, 87.7, 87.5, 72.9, 72.3, 63.6, 63.2, 55.5, 54.9, 42.7, 41.6. HRMS (ESI): calculated for $[M + Na]^+$, $C_6H_{12}O_4Na$: 171.0633; found: 171.0638.

2.3. Synthesis of (2R,3S)-3-(Benzyloxy)-2-((benzyloxy)methyl)-5-methoxytetrahydrofuran **5**

The reaction was split in two round-bottom flasks. To a stirred solution of **4** (22.7 g, 153.4 mmol) in THF (160 mL), powdered KOH (77.0 g, 1380.0 mmol, 9.0 eq.), and benzyl chloride (247.0 mL, 2148.0 mmol, 14.0 eq.) were added sequentially, and the reaction mixture was heated to reflux conditions for 24 h. The reaction mixture was allowed to cool to room temperature; then, the solution was filtered, and the solvent removed in vacuo. The residue was purified using flash chromatography on silica gel eluting the first time with petroleum ether to eliminate excess benzyl chloride, the second time with petroleum ether/ethyl acetate 8:2 to afford the title compound **5** as a yellow oil (42.6 g, 85% yield); (α + β anomers): R_f = 0.39 and 0.57 (petroleum ether/ethyl acetate 8:2). δ_H (400 MHz, CDCl3): 7.45–7.20 (m, 20H), 5.13–5.07 (m, 2H), 4.63–4.47 (m, 8H), 4.29–4.21 (m, 2H), 4.16–4.12 (m, 1H), 4.00–3.92 (m, 1H), 3.57–3.43 (m, 4H), 3.41 (s, 3H, -CH3), 3.31 (s, 3H, -CH3), 2.26–2.19 (m, 2H), 2.19–2.15 (m, 1H), 2.05–2.00 (m, 1H). δ_C (100.6 MHz, CDCl3):138.3, 138.23, 138.20, 138.1, 128.5, 128.4, 127.9, 127.8, 127.7, 127.6, 105.5, 105.3, 82.9, 82.2, 80.0, 78.6, 73.5, 73.4, 72.1, 71.7, 71.6, 70.2, 55.3, 55.0, 39.5, 39.0. HRMS (ESI): calculated for $[M + Na]^+$, $C_{20}H_{24}NaO_4$: 351.1572; found: 351.1568.

2.4. Synthesis of (4S,5R)-4-(Benzyloxy)-5-((benzyloxy)methyl)tetrahydrofuran-2-ol **6**

The reaction was split in two round-bottom flasks. A stirred solution of **5** (25.0 g, 76.0 mmol) in AcOH/H_2O 80:20 (740 mL) was heated to 49 °C (external temperature) for 24 h. A solution of AcOH/H_2O (80:20, 500 mL) was then added, and the reaction mixture was allowed to stir at the same temperature for another 24 h. The reaction was cooled to room temperature and the solvent was removed in vacuo. Heptane was added to the resulting crude mixture and then removed under reduced pressure to eliminate the residual acetic acid. The crude residue was then purified using column chromatography eluting with petroleum ether/ethyl acetate 9:1. The title compound **6** was obtained as yellow oil (19.9 g, 83% yield). (α + β anomers): R_f = 0.18 (petroleum ether/ethyl acetate 8:2). IR: υ_{max} (neat)/cm^{-1}: 3300, 3032, 2937, 1590, 1310, 1042, 870. δ_H (400 MHz, $CDCl_3$): 7.42–7.27 (m, 20H), 5.56–5.49 (m, 2H), 4.62–4.47 (m, 11H), 4.29–4.22 (m, 2H), 4.13–4.10 (m, 1H), 3.67–3.62 (m, 1H), 3.59–3.50 (m, 2H), 3.39–3.35 (m, 1H), 2.28–2.20 (m, 2H), 2.17–2.10 (m, 2H). δ_C (100.6 MHz, $CDCl_3$): 138.1, 138.0, 137.9, 137.5, 137.3, 128.7, 128.6, 128.5, 128.48, 128.46, 128.44, 128.3, 128.1, 128.0, 127.98, 127.96, 99.4, 83.3, 82.7, 79.83, 79.8, 79.0, 78.8, 73.2, 72.1, 71.9, 71.8, 41.8, 39.2. HRMS (ESI): calculated for $[M + Na]^+$, $C_{19}H_{22}NaO_4$: 337.1416; found: 337.1421.

2.5. Synthesis of (2R,3S)-1,3-Bis(benzyloxy)hept-6-ene-2,5-diol **7**

The reaction was split in two round-bottom flasks. A solution of vinyl magnesium bromide (1M in THF, 190 mL, 190 mmol, 3.0 eq.) was added to a stirred solution of **6**

(19.9 g, 63.3 mmol) in dry THF (220 mL) at 0 °C under controlled atmosphere. The reaction mixture was allowed to reach room temperature and stirred for a further 24 h. The reaction mixture was cooled to 0 °C and quenched with ammonium chloride-saturated solution (50 mL), then stirred for a further 10 min at room temperature. The solvent was then evaporated under reduced pressure and the salts formed were filtered off; water (30 mL) was added, and the product was extracted using EtOAc (3 × 100 mL). The organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified using column chromatography on silica gel eluting with dichloromethane/ethyl acetate 8:2 to afford the mixture of two diastereoisomers **7** as a yellow oil (19.8 g, 91% yield). (α + β anomers): R_f = 0.30 and 0.46 (dichloromethane/ethyl acetate 8:2). IR: v_{max} (neat)/cm^{-1}: 3422, 3064, 3031, 2868, 1737, 1643, 1497, 1454, 1371, 1245, 1208, 1092, 923, 737, 699. δ_H (400 MHz, $CDCl_3$): 7.45–7.23 (m, 20H), 5.93–5.84 (m, 2H), 5.30–5.20 (m, 2H), 5.12–5.08 (m, 2H), 4.67–4.54 (m, 8H), 4.40–4.32 (m, 2H), 4.05–3.95 (m, 2H), 3.81–3.70 (m, 2H), 3.62–3.55 (m, 4H), 1.90–1.70 (m, 4H). δ_C (100.6 MHz, $CDCl_3$): 141.1, 140.8, 138.0, 137.9, 128.7, 128.6, 128.2, 128.1, 128.09, 128.06, 114.6, 114.2 78.4, 77.7, 77.4, 73.6, 72.6, 72.2, 71.8, 71.7, 71.0, 70.9, 70.6, 69.8, 37.2, 37.1. HRMS (ESI): calculated for $[M + Na]^+$, $C_{21}H_{26}NaO_4$: 365.1729; found: 365.1741.

2.6. Synthesis of (2R,3S,5R)-3-(Benzyloxy)-2-((benzyloxy)methyl-5-vinyltetrahydrofuran **8β** *and (2R,3S,5S)-3-(Benzyloxy)-2-((benzyloxy)methyl-5-vinyltetrahydrofuran* **8α**

Toluene-*p*-sulphonyl chloride (5.49 g, 28.8 mmol, 1.1 eq.) and KOH (5 M in H_2O, 13 mL, 2.5 eq.) were sequentially added to a stirred solution of **7** (9.00 g, 26.2 mmol) in acetone (250 mL). The reaction was stirred for 52 h at 35 °C (external temperature). The reaction mixture was then diluted using water and extracted using ethyl acetate (3 × 100 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The two diastereoisomers were separated using column chromatography eluting with petroleum ether/diethyl ether 85:15 to afford the title compounds **8α** and **8β** as pale yellow oils (**8α** 1.93 g, 23% yield; **8β** 3.03 g, 36% yield).

(2R,3S,5R)-3-(benzyloxy)-2-((benzyloxy)methyl-5-vinyltetrahydrofuran **8β**: $[\alpha]_D^{20}$ = +21.4 (c = 4.3 in CH_2Cl_2). R_f = 0.56 (petroleum ether/ethyl acetate 8:2). δ_H (400 MHz, $CDCl_3$): 7.39–7.21 (m, 10H), 6.01–5.93 (m, 1H), 5.25–5.08 (m, 2H), 4.65–4.39 (m, 4H), 4.37–4.31 (m, 1H), 4.16–4.13 (m, 1H), 4.04–4.02 (m, 1H), 3.86–3.82 (m, 1H), 3.77–3.73 (m, 1H), 2.35–2.28 (m, 1H), 1.91–1.86 (m, 1H). δ_C (100.6 MHz, $CDCl_3$): 139.4, 138.5, 138.4, 128.5, 128.4, 127.9, 127.6, 127.5, 116.0, 81.5, 79.4, 79.2, 73.6, 71.3, 69.3, 38.5. HRMS (ESI): calculated for $[M + H]^+$, $C_{21}H_{25}O_3$: 325.1804; found: 325.1808.

(2R,3S,5S)-3-(benzyloxy)-2-((benzyloxy)methyl-5-vinyltetrahydrofuran **8α**: $[\alpha]_D^{20}$ = +34.0 (c = 6.0 in CH_2Cl_2). R_f = 0.63 (petroleum ether/ethyl acetate 8:2). IR: v_{max} (neat)/cm^{-1}: 2864, 1454, 1094, 925, 787, 697. δ_H (400 MHz, $CDCl_3$): 7.39–7.21 (m, 10H), 5.91–5.81 (m, 1H), 5.30–5.25 (m, 1H), 5.13–5.10 (m, 1H), 4.65–4.46 (m, 5H), 4.25–4.19 (m, 2H), 3.79 (dd, J_1 = 10.0, J_2 = 5.6, 1H), 3.72 (dd, J_1 = 9.6, J_2 = 6.4, 1H), 2.32–2.26 (m, 1H), 1.78–1.71 (m, 1H). δ_C (100.6 MHz, $CDCl_3$): 138.33, 138.29, 138.2, 128.51, 128.46, 127.8, 127.74, 127.7, 116.6, 83.7, 81.5, 80.0, 77.4, 73.5, 71.2, 38.8. HRMS (ESI): calculated for $[M + H]^+$, $C_{21}H_{25}O_3$: 325.1804; found: 325.1810.

*2.7. Synthesis of 1-((2R,4S,5R)-4-(Benzyloxy)-5-((benzyloxy)methyl)tetrahydrofuran-2-yl)ethane-1,2-diol (***9a**/**b***)*

N-methylmorpholine-*N*-oxide (540 mg, 4.60 mmol, 1.5 eq.) and OsO_4 (78 mg, 0.31 mmol, 0.1 eq.) were sequentially added to a solution of **8β** (1.00 g, 3.08 mmol) in THF/H_2O (1:1, 80 mL). After stirring at room temperature for 3 h, the reaction mixture was quenched using $Na_2S_2O_5$/$NaHSO_3$ (765 mg, 1.3 eq.) and stirred 1 h at the same temperature. The reaction was then extracted using ethyl acetate (3 × 50 mL). The combined organic extracts were washed with 1 N HCl (1 × 50 mL), followed by H_2O (1 × 50 mL) and brine (1 × 50 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo to afford **9a**/**b** as a mixture of two diastereoisomers (*dr* 80:20) as a brown oil. This product did not require any further purification for the next step (1.10 g, 99% yield). R_f = 0.24 (dichloromethane/methanol 9:1). IR: v_{max} (neat)/cm^{-1}: 3384, 2936, 1445, 1064. δ_H (400 MHz, $CDCl_3$): 7.42–7.22 (m, 20H),

4.69–4.51 (m, 6H), 4.42–4.39 (m, 2H), 4.27–4.11 (m, 4H), 3.99–3.97 (m, 2H), 3.91–3.89 (m, 2H), 3.79–3.61 (m, 8H), 2.29–2.20 (m, 2H), 2.14–2.08 (m, 2H). δ_C (100.6 MHz, $CDCl_3$): 138.1, 137.7, 128.6, 128.54, 128.52, 128.0, 127.93, 127.9, 127.8, 127.7, 81.5, 80.9, 79.5, 78.8, 78.73, 78.67, 78.5, 73.6, 73.1, 72.8, 71.5, 71.4, 68.9, 68.8, 64.9, 64.0, 33.7, 32.0. HRMS (ESI): calculated for $[M + Na]^+$, $C_{21}H_{26}NaO_5$: 381.1678; found: 381.1681.

2.8. Synthesis of (R)-1-((2R,4S,5R)-4-(Benzyloxy)-5-((benzyloxy)methyl)tetrahydrofuran-2-yl) ethane-1,2-diyl Diacetate **10a** *and (S)-1-((2R,4S,5R)-4-(Benzyloxy)-5-((benzyloxy)methyl) tetrahydrofuran-2-yl)ethane-1,2-diyl Diacetate* **10b**

Acetic anhydride (6.2 mL, 65.0 mmol, 18.3 eq.), pyridine (3.1 mL, 38.5 mmol, 10.7 eq.), and a catalytic amount of *N*,*N*-dimethylaminopyridine (22 mg, 0.18 mmol, 0.05 eq.) were sequentially added to a stirred solution of **9a**/**b** (1.30 g, 3.60 mmol) in CH_2Cl_2 (40 mL). The reaction was stirred at room temperature for 2 h, then diluted using CH_2Cl_2 and washed using HCl 10% (2 × 30 mL), followed by $NaHCO_3$-saturated solution (2 × 30 mL). The organic phase was dried over Na_2SO_4 and then concentrated in vacuo. The reaction afforded a mixture of two diastereoisomers that were separated using column chromatography eluting with petroleum ether/diethyl ether 8:2 to afford **10a** and **10b** as yellow oils (**10a** 1.01 g, 60% yield; **10b** 0.25 g, 20% yield).

(R)-1-((2R,4S,5R)-4-(benzyloxy)-5-((benzyloxy)methyl)tetrahydrofuran-2-yl)ethane-1,2-diyl diacetate **10a***:* $[\alpha]_D^{20}$ = +26.6 (c = 6.17 in CH_2Cl_2). R_f = 0.76 (petroleum ether/ethyl acetate 6:4). IR: v_{max} (neat)/cm^{-1}: 1748, 1224, 793. δ_H (400 MHz, $CDCl_3$): 7.36–7.28 (m, 10H, Ar), 5.18 (ddd, J_1 = 6.4, J_2 = 3.6, J_3 = 2.8, 1H, H-8), 4.61–4.50 (m, 4H, H-6, and H-7), 4.36 (d, J = 12, 1H, H-9), 4.18–4.01 (m, 4H, H-3, H-1, H-9′, and H-4), 3.78 (dd, J_1 = 10, J_2 = 4.8, 1H, H-5), 3.68 (dd, J_1 = 10, J_2 = 6.4, 1H, H-5′), 2.20–2.04 (m, 2H, H-2, and H-2′), 2.07 (s, 3H, H-11), 2.05 (s, 3H, H-10). δ_C (100.6 MHz, $CDCl_3$): 171.0, 170.4, 138.3, 138.1, 128.5, 127.9, 127.82, 127.8, 127.76, 127.72, 82.3, 78.3, 76.2, 73.6, 73.4, 71.3, 68.9, 63.0, 33.7, 21.2, 21.0. HRMS (ESI): calculated for $[M + Na]^+$, $C_{25}H_{30}NaO_7$: 465.1889; found: 465.1883.

(S)-1-((2R,4S,5R)-4-(benzyloxy)-5-((benzyloxy)methyl)tetrahydrofuran-2-yl)ethane-1,2-diyl diacetate **10b***:* $[\alpha]_D^{20}$ = +21.4 (c = 5.43 in CH_2Cl_2). R_f = 0.68 (petroleum ether/ethyl acetate 6:4). IR: v_{max} (neat)/cm^{-1}: 1748, 1224, 793. δ_H (400 MHz, $CDCl_3$): 7.34–7.27 (m, 10H, Ar), 5.24 (ddd, J_1 = 6.4, J_2 = 3.6, J_3 = 2.8, 1H, H-8), 4.62–4.52 (m, 4H, H-6, and H-7), 4.42 (m, 1H, H-9), 4.37–4.33 (m, 1H, H-3), 4.18–4.10 (m, 2H, H-1, and H-9′), 4.04–4.00 (m, 1H, H-4), 3.80 (dd, J_1 = 10, J_2 = 4.8, 1H, H-5), 3.70 (dd, J_1 = 10, J_2 = 6.4, 1H, H-5′), 2.20–2.14 (m, 1H, H-2), 2.04 (s, 3H, H-11), 2.02 (s, 3H, H-10), 1.94–1.89 (m, 1H, H-2′). δ_C (100.6 MHz, $CDCl_3$): 170.8, 128.54, 128.5, 127.9, 127.8, 127.74 127.7, 127.52, 127.5, 81.6, 78.4, 76.3, 73.6, 72.5, 71.6, 69.0, 63.6, 33.7, 21.2, 20.9. HRMS (ESI): calculated for $[M + Na]^+$, $C_{25}H_{30}NaO_7$: 465.1889; found: 465.1891.

2.9. Synthesis of (2S,4R)-2-(Anthracen-9-yl)-4-((2R,4S,5R)-4-(benzyloxy)-5-((benzyloxy)methyl) tetrahydrofuran-2-yl)-1,3-dioxolane **12a** *and (2R,4R)-2-(Anthracen-9-yl)-4-((2R,4S,5R)-4-(benzyloxy)-5-((benzyloxy)methyl)tetrahydrofuran-2-yl)-1,3-dioxolane* **12b**

Anthraldehyde dimethyl acetal (59 mg, 0.23 mmol, 1.25 eq.) and *p*-toluensulfonic acid (0.8 mg, 2 mol%) were added to a stirred solution of **9a** (67 mg, 0.19 mmol) in CH_3CN (1.7 mL). The reaction was stirred at room temperature 48 h; then, it was neutralized using Et_3N and the solvent was evaporated. The crude material was purified using column chromatography on silica gel eluting using petroleum ether/ethyl acetate 9:1 to afford the title compound **12a** and **12b** (*dr* 78:22) as a yellow oil (18 mg, 18%) and as individual compounds. (4*R*)-2-(anthracen-9-yl)-4-((2*R*,4*S*,5*R*)-4-(benzyloxy)-5-((benzyloxy)methyl)tetrahydrofuran-2-yl)-1,3-dioxolane.

12a: $[\alpha]_D^{25}$ = +8.6 (c = 1.4 in CH_2Cl_2). R_f = 0.44 (petroleum ether/ethyl acetate 8:2). IR: v_{max} (neat)/cm^{-1}: 3012, 1592, 1220, 780. δ_H (400 MHz, $CDCl_3$): 8.59–8.52 (m, 2H), 8.44 (s, 1H), 7.97–7.92 (m, 2H), 7.60–7.36 (m, 5H), 7.32–7.11 (m, 9H), 7.05 (s, 1H), 4.61–4.50 (m, 4H), 4.42–4.32 (m, 3H), 4.32–4.23 (m, 1H), 4.23–4.11 (m, 2H), 3.84–3.70 (m, 2H), 2.20–2.15 (m, 2H). δ_C (100.6 MHz, $CDCl_3$): 138.4, 138.3, 131.6, 131.1, 130.6, 129.2, 128.5, 128.0, 127.8,

127.6, 126.3, 125.0, 124.7, 101.8, 82.3, 79.34, 79.28, 79.0, 73.7, 71.3, 69.2, 68.5, 35.1. HRMS (ESI): calculated for $[M + H]^+$, $C_{36}H_{35}O_5$: 547.2484; found: 547.2491.

12b: $[\alpha]_D^{25} = -20.0$ (c = 0.4 in CH_2Cl_2). R_f = 0.36 (petroleum ether/ethyl acetate 8:2). IR: v_{max} (neat)/cm^{-1}:3012, 1592, 1220, 780. δ_H (400 MHz, $CDCl_3$): 8.53–8.40 (m, 3H), 8.10–7.91 (m, 2H), 7.52–7.40 (m, 4H), 7.40–7.17 (m, 10H), 7.15 (s, 1H), 4.73–4.50 (m, 4H), 4.42–4.17 (m, 4H), 4.20–4.10 (m, 2H), 3.90–3.71 (m, 2H), 2.29–2.15 (m, 2H). δ_C (100.6 MHz, $CDCl_3$): 138.4, 138.2, 131.6, 131.0, 130.5, 129.3, 128.6, 128.0, 127.8, 127.79, 127.7, 127.6, 126.4, 125.1, 124.9, 124.4 101.4, 82.3, 79.5, 79.0, 78.3, 73.7, 71.3, 70.0, 69.3, 35.1. HRMS (ESI): calculated for $[M + H]^+$, $C_{36}H_{35}O_5$: 547.2484; found: 547.2488.

2.10. Synthesis of (R)-1-((2R,4S,5R)-4-Hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)ethane-1,2-diyl Diacetate **13**

Pd/C 10% (144 mg, 1.36 mmol, 2.0 eq.) was added to a stirred solution of **10a** (300 mg, 0.678 mmol) in methanol/formic acid 9:1 (24 mL). The reaction mixture was vigorously stirred at room temperature under atmospheric hydrogen pressure (balloon) for 16 h. The solution was then filtered on celite, and the solvent evaporated under reduced pressure to afford a colorless oil. This product did not require any further purification for the next step (163 mg, 92% yield). $[\alpha]_D^{25}$ = +53.3 (c = 0.3 in CH_2Cl_2). R_f = 0.2 (dichloromethane/methanol 9:1). IR: v_{max} (neat)/cm^{-1}: 3584, 2946, 1740. δ_H (400 MHz, $CDCl_3$): 5.26–5.23 (m, 1H), 4.53–4.48 (m, 2H), 4.17–4.10 (m, 2H), 4.00–3.91 (m, 2H), 3.88–3.85 (m, 1H), 2.37–2.30 (m, 1H), 2.11 (s, 3H, $CH_3C(O)$-), 2.07 (s, 3H, $CH_3C(O)$-), 1.97–1.95 (m, 1H). δ_C (100.6 MHz, $CDCl_3$): 170.9, 170.4, 77.3, 76.4, 73.5, 72.6, 62.5, 61.8, 37.2, 21.1, 21.0. HRMS (ESI): calculated for $[M + Na]^+$, $C_{11}H_{18}NaO_7$: 285.0950; found: 285.0954.

2.11. Synthesis of (R)-1-((2R,4S,5R)-5-((Bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-hydroxytetrahydrofuran-2-yl)ethane-1,2diyl Diacetate **14**

4,4′-dimethoxytrityl chloride (274 mg, 0.810 mmol, 1.3 eq.) was added to a stirred solution of **13** (163 mg g, 0.621 mmol) in dry pyridine (3.3 mL) under an inert atmosphere. The reaction was stirred at room temperature for 16 h, then quenched using a solution of chloroform/methanol 9:1 (8 mL), diluted using CH_2Cl_2 (5 mL) and washed using H_2O (1 × 10 mL). The organic phase was dried over $MgSO_4$, concentrated in vacuo and purified using column chromatography on silica gel eluting with petroleum ether/ethyl acetate 7:3 containing 3% of Et_3N to afford **14** as a yellow oil (210 mg, 60% yield). $[\alpha]_D^{25}$ = +1.8 (c = 21.0 in CH_2Cl_2). R_f = 0.86 (petroleum ether/ethyl acetate 1:1). IR: v_{max} (neat)/cm^{-1}: 3584, 3029, 2946, 1740. δ_H (400 MHz, $CDCl_3$): 7.39–7.28 (m, 4H), 7.45–7.21 (m, 5H), 6.90–6.79 (m, 4H), 5.26–5.23 (m, 1H), 4.53–4.46 (m, 2H), 4.21–4.10 (m, 1H), 3.95–3.91 (m, 1H), 3.79 (s, 6H, $ArOCH_3$), 3.39–3.37 (m, 2H), 2.66–2.65 (m, 1H), 2.33–2.25 (m, 1H), 2.10 (s, 3H, $CH_3C(O)$-), 2.04 (s, 3H,—$CH_3C(O)$-), 1.97–1.91 (m, 1H). δ_C (100.6 MHz, $CDCl_3$): 170.9, 170.3, 158.7, 144.7, 135.8, 130.1, 128.08, 128.04, 127.0, 113.4, 86.8, 81.7, 76.1, 73.0, 72.7, 62.9, 62.6, 55.4, 37.1, 21.2, 21.0. HRMS (ESI): calculated for $[M + Na]^+$, $C_{32}H_{36}NaO_9$: 587.2257; found: 587.2263.

2.12. Synthesis of (1R)-1-((2R,4S,5R)-5-((Bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-(((2-cyanoethoxy)(diisopropylamino)phosphanyl)oxy)tetrahydrofuran-2-yl)ethane-1,2-diyl Diacetate **15**

N,*N*-diisopropylethylamine (181 µL, 1.04 mmol, 2.5 eq.) and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (204 µL, 0.915 mmol, 2.2 eq.) were sequentially added to a stirred solution of **14** (235 mg, 0.416 mmol) in dry CH_2Cl_2 (10 mL) under an inert atmosphere. The reaction was stirred at room temperature overnight, then poured into ice-cold water (15 mL) and extracted using CH_2Cl_2 (3 × 15 mL). The combined organic extracts were washed using water (1 × 10 mL), dried over $MgSO_4$, and evaporated under reduced pressure. The crude residue was purified using silica gel column chromatography (petroleum ether/ethyl acetate 8:2 containing 3% Et_3N) to afford **15** as a yellow oil (303 mg, 95% yield). IR: v_{max} (neat)/cm^{-1}: 3029, 2946, 1740; 1380. δ_H (400 MHz, $CDCl_3$): 7.49–7.57 (m, 2H), 7.38–7.19 (m, 7H), 6.91–6.77 (m, 4H), 5.11–5.07 (m, 1H), 4.61–4.53 (m, 1H), 4.47–4.05 (m, 4H), 3.79 (s, 3H), 3.78 (s, 3H), 3.65–3.12 (m, 5H), 2.58–2.51 (m, 1H), 2.45–2.20 (m, 2H), 2.15–2.12 (m, 1H), 2.08 (s, 3H), 2.06 (s, 3H), 1.97–1.91 (m, 1H), 1.21–0.90 (m, 12H).

δ_C (100.6 MHz, $CDCl_3$): 171.0, 170.9, 170.3, 170.1, 158.47, 158.45, 145.13, 145.1, 136.47, 136.46, 136.24, 136.21, 130.31, 130.25, 128.5, 128.4, 127.8, 126.8, 126.7, 117.9, 117.7, 113.09, 113.06, 86.23, 86.21, 83.2, 83.1, 76.01, 76.0, 73.3, 73.1, 64.3, 64.0, 62.9, 62.7, 58.7, 58.5, 58.1, 58.0, 55.33, 55.29, 43.4, 43.3, 43.2, 43.1, 36.7, 36.5, 24.74, 24.67, 24.63, 24.60, 24.55, 24.4, 24.3, 21.2, 21.1, 21.0, 20.97. HRMS (ESI): calculated for $[M + Na]^+$, $C_{41}H_{53}N_2NaO_{10}P$: 787.3336; found: 787.3340.

2.13. Synthesis of (S)-1-((2R,4S,5R)-4-Hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)ethane-1,2-diyl Diacetate **16**

Pd/C 10% (96 mg, 0.90 mmol, 2.0 eq.) was added to a stirred solution of **10b** (200 mg, 0.452 mmol) in methanol/formic acid 9:1 (19 mL). The reaction mixture was vigorously stirred at room temperature under atmospheric hydrogen pressure (balloon) for 16 h. The solution was then filtered on celite, and the solvent evaporated under reduced pressure to afford a colorless oil. This product did not require any further purification for the next step (51 mg, 43% yield). $[\alpha]_D^{25}$ = +40.0 (c = 0.6 in CH_2Cl_2). R_f = 0.2 (dichloromethane/methanol 9:1). IR: v_{max} (neat)/cm^{-1}: 3584, 2946, 1740. δ_H (400 MHz, $CDCl_3$): δ_H 5.27–5.23 (m, 1H), 4.47–4.44 (m, 1H), 4.29–4.26 (m, 1H), 4.17–4.10 (m, 2H), 3.93–3.79 (m, 3H), 2.30–2.26 (m, 1H), 2.10 (s, 3H), 2.03 (s, 3H), 1.85-1.80 (m, 1H). δ_C (100.6 MHz, $CDCl_3$): 171.7, 171.2, 81.5, 77.4, 73.8, 73.7, 63.3, 62.0, 38.3, 21.4, 21.0. HRMS (ESI): calculated for $[M + Na]^+$, $C_{11}H_{18}NaO_7$: 285.0950; found: 285.0954.

2.14. Synthesis of (S)-1-((2R,4S,5R)-5-((Bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-hydroxytetrahydrofuran-2-yl)ethane-1,2-diyl **17**

4,4'-dimethoxytrityl chloride (83 mg, 0.25 mmol, 1.4 eq.) was added to a stirred solution of **16** (46 mg g, 0.175 mmol) in dry pyridine (1.0 mL) under an inert atmosphere. The reaction was stirred at room temperature for 16 h, then quenched using a solution of chloroform/methanol 9:1 (2 mL), diluted using CH_2Cl_2 (5 mL) and washed using H_2O (1 × 5 mL). The organic phase was dried over $MgSO_4$, concentrated in vacuo and purified using column chromatography on silica gel eluting with petroleum ether/ethyl acetate 7:3 containing 3% of Et_3N to afford **17** as a yellow oil (42 mg, 43% yield). $[\alpha]_D^{25}$ = +2.0 (c = 2.0 in CH_2Cl_2). R_f = 0.86 (petroleum ether/ethyl acetate 1:1). IR: v_{max} (neat)/cm^{-1}: 3584, 3029, 2946, 1740. δ_H (400 MHz, $CDCl_3$): 7.47–7.21 (m, 9H), 6.84 (d, J = 8.8, 4H), 5.26–5.24 (m, 1H), 4.42–4.38 (m, 2H), 4.26–4.15 (m, 2H), 3.89–3.88 (m, 1H), 3.79 (s, 6H), 3.42 (dd, J_1 = 10, J_2 = 5.2, 1H), 3.32 (dd, J_1 = 10, J_2 = 4.8, 1H), 2.87–2.85 (m, 1H), 2.36–2.29 (m, 1H), 2.05 (s, 3H), 2.04 (s, 3H), 1.87–1.75 (m, 1H). δ_C (100.6 MHz, $CDCl_3$): 170.84, 170.82, 158.7, 144.8, 135.9, 135.8, 130.1, 128.2, 128.1, 113.4, 86.8, 81.3, 75.9, 72.7, 72.3, 63.5, 62.7, 55.4, 37.4, 21.1, 21.0. HRMS (ESI): calculated for $[M + Na]^+$, $C_{32}H_{36}NaO_9$: 587.2257; found: 587.2261.

2.15. Synthesis of (1S)-1-((2R,4S,5R)-5-((Bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-(((2-cyanoethoxy)(diisopropylamino)phosphanyl)oxy)tetrahydrofuran-2-yl)ethane-1,2diyl Diacetate **18**

N,*N*-diisopropylethylamine (20 µL, 0.12 mmol, 2.5 eq.) and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (24 µL, 0.449 mmol, 2.2 eq.) were sequentially added to a stirred solution of **17** (27 mg, 0.048 mmol) in dry CH_2Cl_2 (1.2 mL). The reaction was stirred at room temperature overnight under argon atmosphere, then poured into ice-cold water (5 mL) and extracted using CH_2Cl_2 (3 × 5 mL). The combined organic extracts were washed using water, dried over $MgSO_4$, and evaporated under reduced pressure. The crude residue was purified using silica gel column chromatography (petroleum ether/ethyl acetate 8:2 containing 3% Et_3N) to afford **18** as a yellow oil (15 mg, 41% yield). IR: v_{max} (neat)/cm^{-1}: 3029, 2946, 1740; 1380. δ_H (400 MHz, $CDCl_3$): 7.29–7.20 (m, 18H), 6.76–6.74 (m, 8H), 5.27–5.22 (m, 2H) 4.45–3.98 (m, 8H), 3.71 (s, 6H), 3.70 (s, 6H), 3.65–3.12 (m, 10H), 2.54–2.50 (m, 2H), 2.78–2.55 (m, 6H), 2.39–2.32 (m, 2H), 2.09 (s, 6H), 2.01 (s, 6H), 1.92–1.81 (m, 2H), 1.25–0.79 (m, 24H). δ_C (100.6 MHz, $CDCl_3$): 170.84, 170.76, 170.5, 158.43, 158.4, 145.1, 136.6, 136.3, 132.5, 131.0, 130.4, 130.31, 130.28, 130.22, 128.9, 128.5, 128.4, 127.8, 126.8, 126.7, 117.8, 116.7, 113.1, 86.2, 82.1, 76.3, 76.0, 73.3, 72.7, 63.8, 63.6, 63.4, 58.2, 58.2, 55.31,

55.27, 45.42, 45.4, 43.2, 43.1, 35.9, 24.84, 24.63, 24.6, 24.59, 24.55, 24.4, 24.3, 21.2, 21.1, 21.0, 20.97. HRMS (ESI): calculated for $[M + Na]^+$, $C_{41}H_{53}N_2NaO_{10}P$: 787.3336; found: 787.3341.

2.16. Preparation of Oligonucleotides A and B

Oligonucleotides **A** and **B** were synthesized on an AB 3400 DNA synthesizer using standard *β*-cyanoethyl phosphoramidite chemistry. Reagents and concentrations applied were the same as those for syntheses of natural DNA oligomers. DNA solid phase synthesis was performed on 1 µmol dA^{Bz} 500 A CPG resin and 1 µmol dG^{iBU} 500A CPG (Applied Biosystem) and using scale standard protocol. Syntheses were performed using a 1 µmol scale in trityl-on mode, according to the manufacturer's protocol. The only change made to the usual synthesis cycle for the monomer **15** was the prolongation of the coupling time to 3 min. Coupling efficiency during the automated synthesis was estimated spectrophotometrically using the DMT cation, released during the detritylation steps. The oligomers, were removed from the support and deprotected using treatment with 35% NH_3 16 h at 60 °C. The crude oligonucleotides were submitted to the protocol for PoliPak II (Glen Research) where first the DMT was removed and then the oligo purified (attached HPLC profile after Poli-Pak II treatment). After the treatment using Poli-Pak II, the sequences were submitted to RP-HPLC using a C12 Jupiter Proteo column and a gradient of 20% of B (CH_3CN) in A (H_2O, 0.1M TEEA, pH = 7). The products were characterized using matrix-assisted laser desorption ionization (MALDI) mass spectra using the Applied Biosystems Voyager DE-PRO spectrometer with 3-hydroxy picolinic acid matrix. Sequence **A** (MALDI): calculated for $[M - H + Na^+]^-$ 4575.61; found: 4575.66. Sequence **B** (MALDI): calculated for $[M - H + Na]^-$ 4610.67; found: 4610.71.

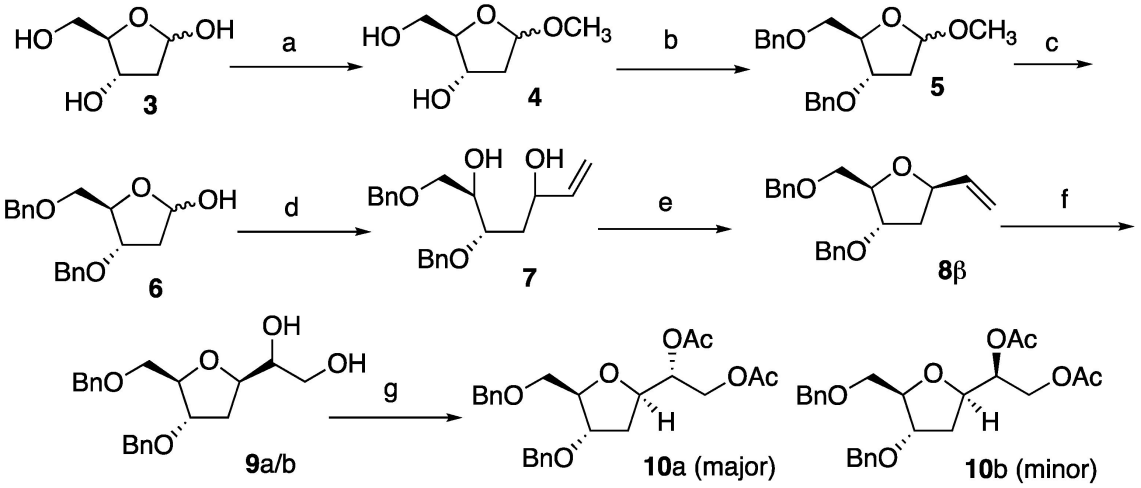
2.17. Procedure for UV Absorption Measurements and UV-Melting Experiments

UV measurements were obtained using a JASCO V-550 UV/VIS spectrophotometer equipped with a Peltier block by using 1 cm quartz cells of both 0.5 and 1 mL internal volume (Hellma). Oligomer quantification was achieved by measuring the absorbance (l = 260 nm) at 80 °C, using the molar extinction coefficients calculated for the unstacked oligonucleotides. The molar extinction coefficients used for the calculations were A: 15.4; T: 8.8; G: 11.7; C: 7.3 $m^{-1}M^{-1}$ (for the DNA monomers). The epsilons used for the quantification of the oligonucleotide are ε_{260} = 152.6 $m^{-1}M^{-1}$ for sequence **A** ($A_4C_2G_2T_6$) and 165.6 $m^{-1}M^{-1}$ for sequence **B** ($A_6C_2G_2T_4$). UV quantification of the oligos provided the following values: a = 135 nmol (0.62 mg, 13% yield); b = 125 nmol (0,58 mg, 12% yield). Annealing of all the duplexes was performed by dissolving equimolar amounts of the two complementary strands in milliQ water, heating the solution at 85 °C (5 min), and then allowing to cool slowly to room temperature. Melting curves (at 260 nm) were recorded for a consecutive heating (10–85 °C)–cooling–heating protocol with a linear gradient of 0.5 °C/min.

3. Results and Discussion

A large number of synthetic approaches towards *C*-nucleosides have been established to date [23,24]. Our group has developed a diversity-oriented strategy to provide access to a range of unnatural *C*-nucleosides [25,26]. Taking advantage of this methodology, we set out to prepare a number of abasic nucleosides holding nonheterocyclic metal ligand templates, for example, β-diols, β-aminoalcohols, β-diamines, or β-hydroxamic acid. We set out with the synthesis of β-diol *C*-nucleoside **15** (Scheme 1) since naturally occurring nucleosides possess the β-anomeric configuration. Desired target **15** contains the protecting group required for its introduction into an oligonucleotide using solid phase synthesis. Hence, starting from commercially available 2-deoxy-D-Ribose **3**, treatment with methanol in presence of catalytic AcCl generated compound **4** with a 99% yield. Subsequent exhaustive benzylation produced **5** that, in turn, was selectively deprotected on the anomeric position to provide the desired **6**. Compound **6** was obtained in overall 70% yields for the steps (a)–(c) (Scheme 1). Compound **6** was treated with an excess of vinylmagnesium bromide at room

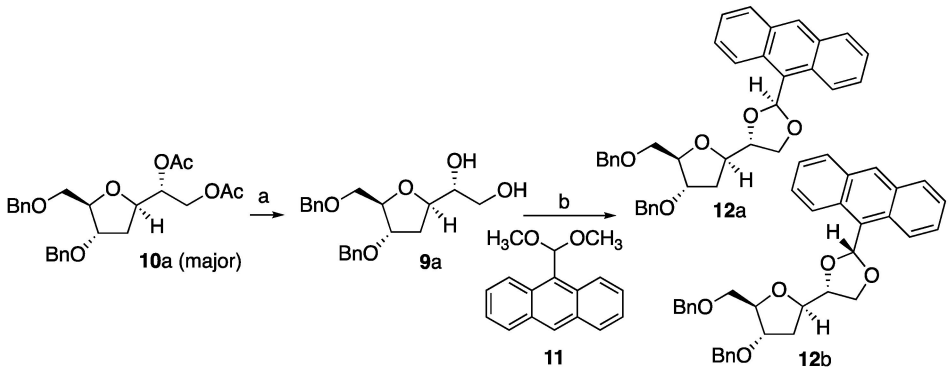
temperature to provide the corresponding ring that opened product **7** as a diastereoisomeric mixture in overall 91% isolated yields.



Scheme 1. Reagents and conditions: (**a**) AcCl, CH_3OH, r.t. 1 h, 99%; (**b**) BnCl, KOH, THF reflux 24 h, 85%; (**c**) AcOH/H_20 8/2, 49 °C, 48 h, 83%; overall for (**a**)–(**c**) 70% yield; (**d**) $CH_2 = CH_2MgBr$, THF, 0 °C, 24 h, 91%; (**e**) TsCl, KOH, 35 °C, 48 h, 70%; (**f**) OsO_4 10%, NMO (1,5 eq) THF/H_2O 1:1, 2 h, r.t. 99%; (**g**) Ac_2O, DMAP, pyridine, CH_2Cl_2, **10a**, 60%; **10b**, 20%; dr 3:1; **10a** + **10b** 80%.

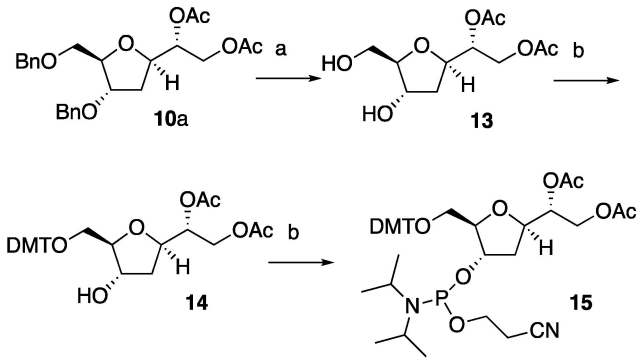
Diastereoisomeric mixture **7** was treated using *p*-toluenesulfonyl chloride and KOH resulting in the formation of **8α/β** (*dr* 1:1.5), which were successfully separated using column chromatography to obtain enantiomerically pure **8β**. The ^{1}H-NMR spectroscopy data of **8β** (and therefore the stereochemistry at the anomeric position) were consistent with those already reported in the literature [27]. The next step involved the dihydroxylation of **8β** to afford diols **9a/b**. Hence, treatment of **8β** with OsO_4 (10 mol%) and NMO as the terminal oxidant provided **9** in near to quantitative yield and as an inseparable mixture of two diastereoisomers. The same result was also obtained when the reaction was carried out at −78 °C. In order to increase the diastereoisomeric ratio of compound **9** and obviate to the separation of a single diastereomer, compound **8β** was subjected to the condition reported by Sharpless for asymmetric dihydroxylation [28]. Therefore, **8β** was reacted in the presence of *cinchona* alkaloid ligand hydroquinidine 1,4-phthalazinediyl diether $(DHQD)_2PHAL$ [21] (10 mol%), NMO (2.2 eq.), and OsO_4 (10 mol%). This experiment furnished **9a/b** with an inseparable mixture of two diasteroisomers. However, diols **9a/b** were then reacted with Ac_2O, pyridine and in the presence of 5% of *N*,*N*-dimethylaminopyridine (DMAP) to provide **10a/b** as a mixture of diastereoisomers, which, satisfactorily, could be separated using column chromatography in pure compounds **10a** and **10b**, respectively. Noteworthy, the preparation of compounds possessing the same scaffold as 9 and 10 has been reported using an alternative route [29–31]. Compound **10a** (major isomer) was tested for configurational stability under the standard reaction conditions adopted in oligonucleotide-automated synthesis. Hence, a solution of 7 μmol of **10a** in CD_3CN (0.75 mL) was submitted to cycle reactants, including ammonia, and the progression of reaction monitored using ^{1}H-NMR. We were delighted to observe that **10a** underwent acetyl hydrolysis to provide expected **9a** as a single diastereoisomer, hence proving its configurational stability under oligonucleotide synthesis conditions. The stereochemistry of the *C6–O* bond of **9a** and **10a** was determined by converting **9a** to acetal **12a** and **12b** and conducting n.O.e. studies on these derivatives. 9-anthraldehyde dimethyl acetal **11** has been reported as a protecting group for diols as a means to obtain crystalline structures [21]. 9-anthraldehyde dimethyl acetal **11** (Scheme 2) [32] was synthesized according to the procedure reported then reacted with **9a** (Scheme 2) in MeCN under the catalysis of p-TSA to provide expected compound **12a/b** as a mixture of two diastereoisomers (*dr* 78:22). Compounds **12a/b** could not be crystallized;

however, it was possible, once again, to separate **12a** and **12b** as a single diastereoisomer using column chromatography.



Scheme 2. Reagents and conditions: (**a**) 35% NH_3, 60 °C, 16h, 70%; (**b**) **9a**, p-TSA (2% mol), CH_3CN, r.t., 18 h, 18% mixture of two diastereoisomers.

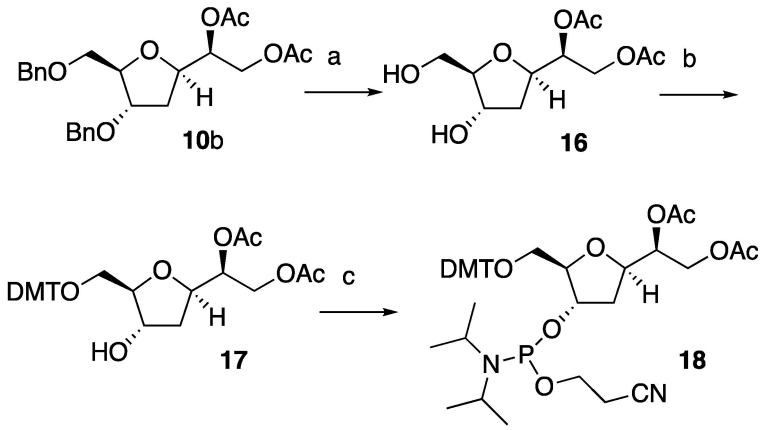
With pure compounds **12a** and **12b** in hand, we carried out n.O.e experiments aimed at elucidating the stereochemistry of the *C4–O* bond of the 1,3-dioxolane nucleus. While n.O.e. experiments carried out on **10a** were unconclusive, n.O.e. run on conformationally locked **12a** and **12b** pointed out at the spatial orientation of the *C4–O* bond in compounds compatible with an absolute (*R)* stereochemistry, which can be extended to the parent compounds **9a**, **10a**, **12a**, and **12b**. In particular, upon irradiation of *C6–H* in **12a**, no enhancement was observed for *C1′–H* but significant enhancement was observed for *C2′–H*, therefore confirming a *trans* relationship between *C6–H* and *C1′–H*; lack of enhancement of benzylic C–H upon irradiation of *C6–H* was observed for compound **12a**, which was in contrast to that evidenced for compound **12b**. Major diastereoisomer **10a** was therefore employed to obtain desired compound **15** (Scheme 3). Firstly, hydrogenation of **10a** using an excess of Pd/C (2.0 eq.) in methanol and 10% of HCOOH under an H_2 atmosphere removed the benzylic groups providing expected diol **13** in 92% isolated yields. The *5′–O* was then functionalized with a 4,4′-dimethoxytrityl group (DMT), to provide **14** at a 60% yield. In turn, compound **14** was converted to the correspondent phosphoramidite **15**, which was obtained in 95% isolated yield (Scheme 3).



Scheme 3. Reagents and conditions: (**a**) Pd/C, H_2, MeOH/HCOOH, r.t., 92%; (**b**) DMT-Cl, pyridine, r.t., 60%; (**c**) 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite, iPr_2EtN, CH_2Cl_2, r.t., 95%.

With compound **10b** in hand, we repeated the synthetic route highlighted above to prepare solid phase synthesis-activated nucleoside **18** (Scheme 4). Hence, **10b** was first debenzylated under reductive conditions to generate diol **16**. In turn, **16** was reacted with DMTr to provide intermediate **17** that was finally converted to the desired **18**. We noted that the reaction yields for each of the steps leading to **18** were significantly lower compared to those observed for the synthesis of diastereoisomeric compound **15**. These data may

account for the steric hindrance provided by the C6-acetoxy group that in compounds **16** and **17** may slow the reaction of the *5′–O* and *3′–O* with their electrophilic counterparts.



Scheme 4. Reagents and conditions: (**a**) Pd/C, H_2, MeOH/HCOOH (9:1), r.t., 16h, 43%; (**b**) DMT-Cl, pyridine, r.t., 43%; (**c**) 2-cyanoethyl *N*,*N*-diisopropylchorophosphoramidite, iPr_2EtN, CH_2Cl_2, r.t., 41%.

In order to evaluate the ability of abasic nucleoside **15** to be introduced on single and double strands of unnatural DNAs, compound **15** was inserted in a sequence of DNA. Hence, two strands of complementary DNAs, namely **A** and **B** (Figure 2), were prepared, in which compound **15** was located in the central portion of each strand. This was achieved using standard automated DNA synthesis, demonstrating that compound **15** could efficiently be introduced in a DNA framework. This was a significant milestone, as it was shown that **15** could be used nested in a biomolecule with the prospect of becoming a catalyst upon introduction in a DNA and their subsequent deacetoxylation to become diol **19** (Figure 2). The sequence of **A** and **B** was selected as reported for similar studies [16].

5’- d(CACATTA**19**TGTTGTA)-3’ **A**
3’-d(GTGTAAT**19**ACAACAT)-5’ **B**

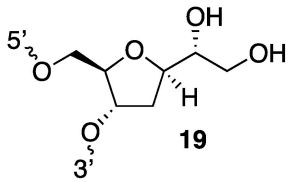


Figure 2. Unnatural DNA strands **A** and **B** containing abasic nucleoside **19**.

Unnatural strands A and B were then mixed and allowed to hybridize using established thermal protocols; then, the thermal stability of duplex **A**/**B** was recorded by carrying out UV-monitored thermal denaturation. The results obtained (Figure 3) showed duplex **A**/**B** possessing a melting temperature (T_m) of 24 °C. It should be noted that in a natural-type duplex, in which the **15**/**15** base pair was replaced by A-T base pair, T_m was 44.2 °C [16]. Thus, these data show that the introduction of **15** in a natural sequence of DNA perturbed the overall stability of the duplex, resulting in a significant decrease in melting temperature (ΔT_m = 20.2 °C). The data were significant, as the lower meting temperature obtained by introducing nucleobase **15** indicated the formation of a new groove with potential for nucleophilic catalysis or for metal coordination.

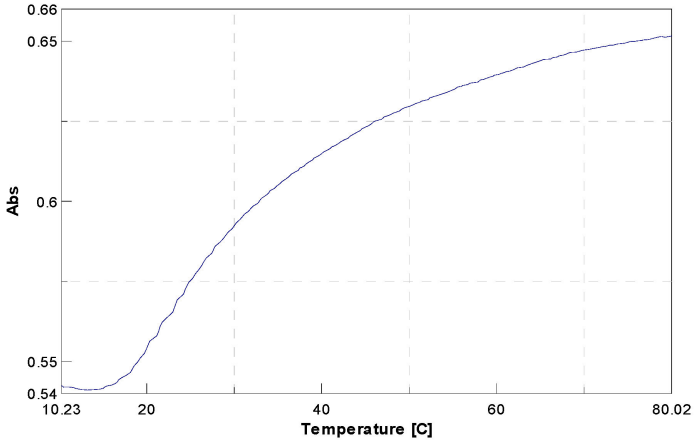


Figure 3. Melting curve of the duplex **A**/**B**: 2 μM, 25 mM NaCl, 10 mM phosphate buffer pH = 7.

4. Conclusions

In conclusion, we herein reported the synthesis of a novel abasic, unnatural *C*-nucleoside bearing a β-diol at the anomeric position. We also demonstrated that (*i*) β-diol **15** could be efficiently incorporated into DNA strands; (*ii*) DNA strands bearing **15** do hybridize, forming a double helix that, according to the melting point, holds a new type of groove containing polyhydroxylated functionalities. Studies regarding the ability of single strand DNAs and double strands including **15** and their diastereoisomeric analogues in catalysis are ongoing.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/chemistry5020091/s1: Electronic Supplementary Information (ESI) available: Copies of ^{1}H- and ^{13}C- NMR for compounds **4**–**18**. This includes: Figure S1: 1H-NMR and 13C-NMR of compound **4**; Figure S2: 1H-NMR and 13C-NMR of compound **5**; Figure S3: 1H-NMR and 13C-NMR of compound **6**; Figure S4: 1H-NMR and 13C-NMR of compound **7**; Figure S5: 1H-NMR and 13C-NMR of compound **8β**; Figure S6: 1H-NMR and 13C-NMR of compound **8α**; Figure S7: 1H-NMR and 13C-NMR of compounds **9a**/**b**; Figure S8: 1H-NMR and 13C-NMR of compound **10a**; Figure S9: 1H-NMR and 13C-NMR of compound **10b**; Figure S10: 1H-NMR and 13C-NMR of compound **12a**; Figure S11: 1H-NMR and 13C-NMR of compound **12b**; Figure S12: 1H-NMR and 13C-NMR of compound **13**; Figure S13: 1H-NMR and 13C-NMR of compound **14**; Figure S14: 1H-NMR and 13C-NMR of compound **15**; Figure S15: 1H-NMR and 13C-NMR of compound **16**; Figure S16: 1H-NMR and 13C-NMR of compound **17**; Figure S17: 1H-NMR and 13C-NMR of compound **18**; Figure S18: Mass Spectrum of oligomer **A**; Figure S19: Mass Spectrum of oligomer **B**; Figure S20: HPLC profile oligomer **A** after Poli-Pak II purification; Figure S21: HPLC profile oligomer **B** after Poli-Pak II purification.

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