ACS OMEGA

Article

Cite This: ACS Omega 2019, 4, 18803−18808

http://pubs.acs.org/journal/acsodf

Synthesis of Organometallic Oligonucleotides through Oximation with Metalated Benzaldehydes

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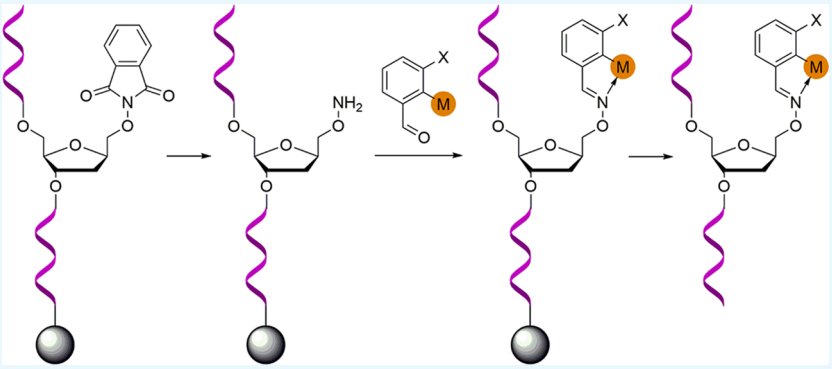
Supporting Information



ABSTRACT: A phthaloyl-protected aminooxymethyl-*C*-2′-deoxyriboside building block has been prepared and incorporated in the middle of an oligodeoxyribonucleotide. Removal of the phthaloyl protection followed by on-support oximation with either mercurated or palladated benzaldehydes yielded oligonucleotides bearing the respective benzaldoxime metallacycles.

INTRODUCTION

Oligonucleotides functionalized with transition metal ions are studied intensively for potential applications in DNA nanotechnology[1−4] as well as diagnostics and chemotherapy.[5−8] Such structures are typically obtained through coordination of the desired metal ion to a high-affinity ligand within the oligonucleotide. We, on the other hand, have recently become interested in an alternative approach in which the metal ion is held in place by an organometallic bond formed by postsynthetic metalation of an oligonucleotide, incorporating an appropriate "hot spot" in a solution.[9] The main advantage of this strategy is that the organometallic complex resists dissociation even at extreme dilution, widening the scope of metal ions usable in intracellular applications from the kinetically inert Pt(II) and Ru(II) to more labile ones, such as Pd(II) and Hg(II). For example, the high thermodynamic stability of kinetically labile metal-mediated base pairs[2,3] could be harnessed to promote hybridization of therapeutic oligonucleotides with their target sequences.[10] A notable disadvantage is that the formation of carbon−metal bonds often requires conditions hardly compatible with the solubility or chemical stability of oligonucleotides. Even when the metalation is achieved under sufficiently mild conditions, the removal of excess free transition metal ions from the product mixture can present a problem.

Herein, we describe the synthesis of a modified oligonucleotide containing an aminooxymethyl-functionalized residue for the introduction of diverse organometallic complexes through oxime coupling on a solid support. A deoxyribose scaffold bearing the aminooxymethyl function on its β face was chosen so that the resulting benzaldoxime metallacycles would resemble natural nucleosides, allowing high-affinity metal-mediated base pairing with a complementary oligonucleotide. For the same reason, the sequences flanking the metallacyclic residue were the same as in our previous studies on metal-mediated base pairing.[11,12] The method described effectively removes the limitations on the reaction conditions used in the preparation of the organometallic complex. Furthermore, the use of oximation for the introduction of conjugate groups to oligonucleotides is well established[13] and should proceed predictably, exhibiting little dependence on the structure of the organometallic complex. Finally, any excess reagent is conveniently removed by washing the solid support with appropriate solvents. The utility of the method has been demonstrated with one arylmercury(II) compound and one arylpalladium(II) compound.

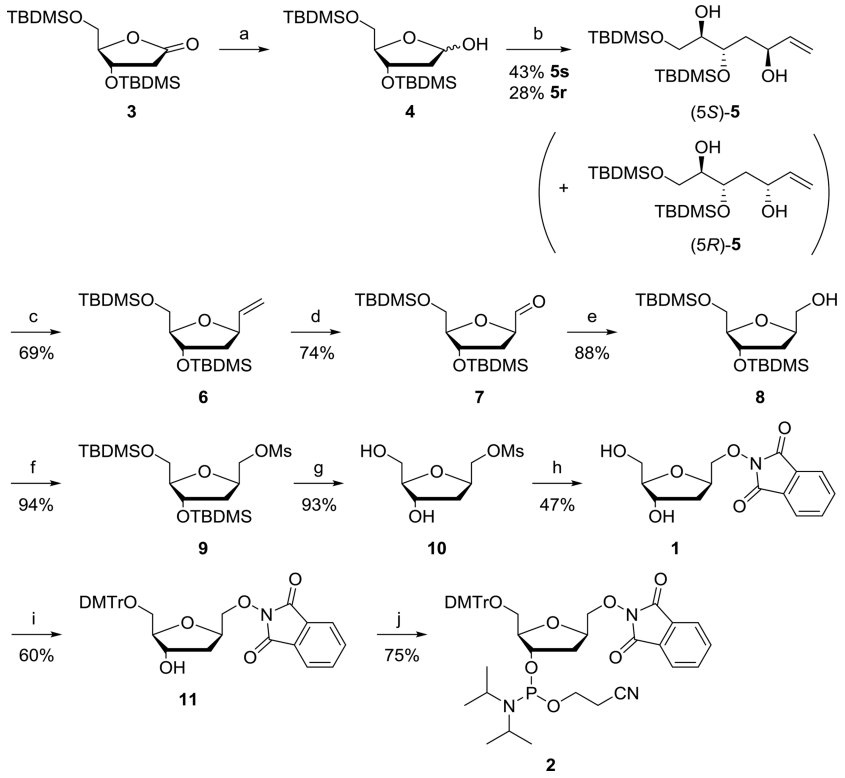
RESULTS AND DISCUSSION

Synthesis of the phthaloyl-protected aminooxy sugar **1** and the corresponding phosphoramidite building block **2** is described in Scheme 1. First, lactone **3** was transformed to the corresponding lactol **4** by treatment with DIBAL-H, as described previously.[14] The reaction with vinyl magnesium bromide then provided compound **5** as a mixture of two

Received: August 30, 2019
Accepted: October 14, 2019
Published: November 1, 2019

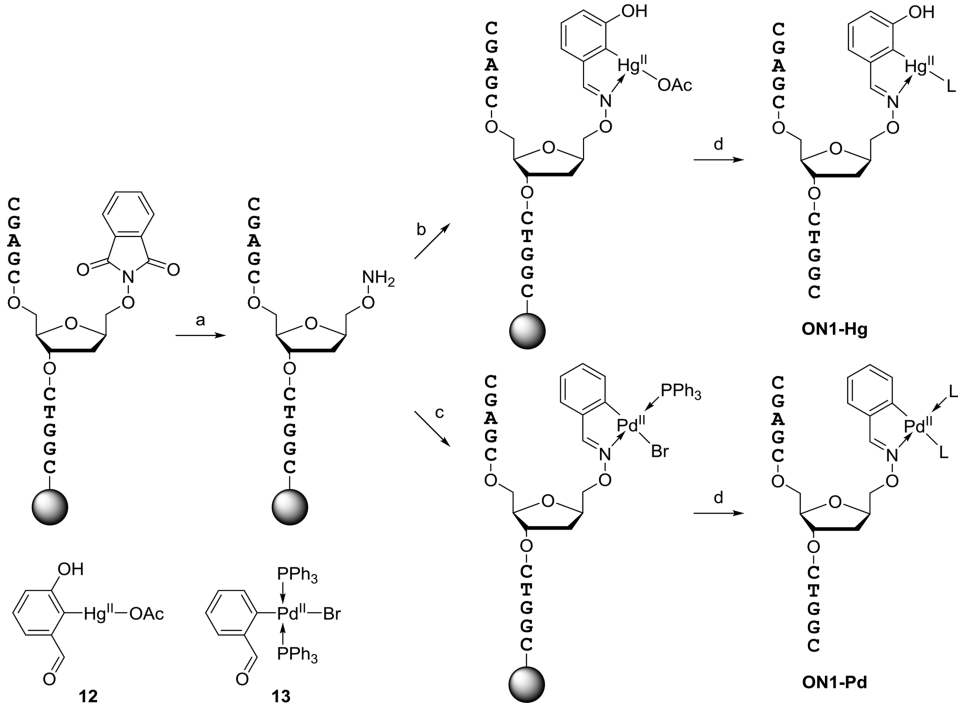
DOI: 10.1021/acsomega.9b02804
ACS Omega 2019, 4, 18803−18808

Scheme 1. Synthesis of Aminooxy Sugar 1 and the Corresponding Phosphoramidite Building Block 2[a]



[a]Reagents and conditions: (a) DIBAL-H, THF, −78 °C, 1 h; (b) C_2H_3MgBr, THF, 0 °C, 4 h; (c) MsCl, pyridine, CH_2Cl_2, −25 °C, 12 h; (d) (i) $RuCl_3$, $NaIO_4$, $CeCl_3$, MeCN, EtOAc, H_2O, 25 °C, 10 min; (ii) $NaIO_4$, THF, H_2O, Et_2O, 25 °C, 90 min; (e) $NaBH_4$, EtOH, Et_2O, 25 °C, 3 h; (f) MsCl, pyridine, CH_2Cl_2, 25 °C, 3 h; (g) $Et_3N{\cdot}3HF$, THF, 25 °C, 16 h; (h) HONPhth, DIPEA, DMF, 70 °C, 30 h; (i) DMTrCl, pyridine, 25 °C, 12 h; and (j) 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite, Et_3N, CH_2Cl_2, N_2 atmosphere, 25 °C, 2 h.

Scheme 2. Synthesis of the Organomercury and Organopalladium Oligonucleotides ON1-Hg and ON1-Pd[a]



[a]Reagents and conditions: (a) H_2NNH_2, AcOH, pyridine, 25 °C, 45 min; (b) **12**, DMSO, 25 °C, 12 h; (c) **13**, CH_2Cl_2, 25 °C, 12 h; and (d) $MeNH_2$, NH_3, H_2O, 65 °C, 10 min.

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ACS Omega 2019, 4, 18803−18808

diastereomers ((5*R*)-**5** and (5*S*)-**5**). Chromatographic separation of the desired diastereomer (5*S*)-**5** followed by mesyl chloride-promoted ring closure afforded the β-vinyl *C*-glycoside **6**. Synthesis of an analogous compound with *tert*-butyldiphenylsilyl, rather than *tert*-butyldimethylsilyl, protections by the same pathway has been described in the literature.[15] The desired β-stereochemistry was confirmed by a 1D NOESY experiment, where irradiation of the anomeric proton (H1) at 4.58 ppm lead to a single positive NOE enhancement at 4.35 ppm, corresponding to H4 (Figure S1, spectra presented in the Supporting Information).

RuO_4 (generated in situ from $RuCl_3$)-catalyzed dihydroxylation of the vinyl group to a vicinal diol[16] followed by oxidative cleavage with $NaIO_4$ gave the formyl *C*-glycoside **7** in excellent yield. Based on ^1H and ^{13}C NMR spectra, the product was identical to the authentic compound reported previously,[17] further confirming the stereochemical assignment of the two diastereomers of compound **5**. Aldehyde **7** was then reduced to the corresponding alcohol **8** by treatment with $NaBH_4$, and the newly created hydroxy function was mesylated to give compound **9**. The hydroxy functions at positions 4 and 6, in turn, were desilylated by treatment with $Et_3N{\cdot}3HF$ to yield intermediate **10**, which was further converted to the phthaloyl-protected aminooxy sugar **1** by nucleophilic displacement of the mesylate leaving group with an *N*-hydroxyphthalimide anion. It is worth pointing out that attempts to introduce the phthaloyl-protected aminooxy group to intermediate **8** by the Mitsunobu reaction failed, even though various reagent combinations were used. Similarly, the reaction between the protected mesylate **9** and *N*-hydroxyphthalimide afforded only traces of the desired product, the main product being a monodesilylated mesylate. Finally, the primary hydroxy group was protected as a 4,4′-dimethoxytrityl ether to give compound **11**, and the secondary one was phosphitylated with 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite to give the protected phosphoramidite building block **2**.

To demonstrate functionalization with organometallic complexes, an oligonucleotide incorporating a single phthaloyl-protected aminooxy sugar in the middle of the chain was first assembled on an automated DNA synthesizer by the standard phosphoramidite protocol using an extended coupling time (600 s) for incorporation of the phosphoramidite building block **2**. Normal (>99%) coupling yields were obtained throughout the synthesis. Selective removal of the phthaloyl protection was achieved by treatment with a mixture of hydrazine hydrate, pyridine, and acetic acid, as described previously (Scheme 2).[18] Immediately after liberation of the aminooxy function, the support-bound oligonucleotide was immersed in a solution of either acetato(2-formyl-6-hydroxyphenyl)mercury (**12**) in DMSO or bromo(2-formylphenyl)bis(triphenylphosphine)palladium (**13**) in CH_2Cl_2. These organometallic complexes were prepared as described previously.[19,20] The mixtures were shaken for 12 h at 25 °C, after which the solid supports were washed with appropriate solvents, and the oligonucleotide products were deprotected and released by treatment with a mixture of aqueous $MeNH_2$ and NH_3 at 65 °C. A short treatment of only 10 min was used to avoid demetalation of the organomercury or organopalladium residue. Finally, the modified oligonucleotides **ON1-Hg** and **ON1-Pd** thus obtained were purified by RP-HPLC and characterized by ESI-TOF-MS.

The mass spectra of **ON1-Hg** and **ON1-Pd** corresponded to the expected structures, showing incorporation of exactly one Hg(II) ion and one Pd(II) ion, respectively. On the other hand, no exchangeable ligands of the metal centers could be detected. In **ON1-Pd**, the oxime nitrogen probably occupies one coordination site. While oxime coupling can yield both *E* and *Z* isomers of the C=N bond, *ortho*-palladated benzaldoximes have invariably been reported as palladacycles with the oxygen and the phenyl carbon *trans* to each other and the nitrogen coordinated to palladium.[21−23] Hg(II) tends to form linear complexes, but weak N−Hg bonds in organomercury complexes similar to the one in **ON1-Hg** have nonetheless been proposed based on X-ray crystallographic evidence.[24] Possible candidates for the remaining ligands (one in **ON1-Hg** and two in **ON1-Pd**) include methylamine and ammonia used for deprotection of the oligonucleotides as well as the HPLC buffer components triethylamine and acetate ion. With **ON1-Pd**, the presence of a triphenylphosphine ligand was ruled out by ^{31}P NMR (spectrum presented in the Supporting Information).

CONCLUSIONS

In summary, we have developed a method for the preparation of covalently metalated oligonucleotides through oxime coupling between aminooxy-functionalized oligonucleotides and organometallic benzaldehyde derivatives. The scope of the method has been demonstrated with Hg(II) and Pd(II) but, in all likelihood, can be expanded to other metals. The most important improvement compared to previously reported methods is that the metalation conditions are not limited by the solubility or chemical stability of oligonucleotides. The metal as well as the organic ligand can be easily varied without the need to synthesize new phosphoramidite building blocks. We therefore believe that our method will enable rapid synthesis of previously unattainable metalated oligonucleotides.

EXPERIMENTAL SECTION

General Information. All experiments involving air- and/or moisture-sensitive compounds were performed using oven-dried glassware under an argon atmosphere. For the preparation of HPLC elution buffers, freshly distilled triethylamine was used. Other commercially available chemicals were used without further purification, unless otherwise stated. The solvents for organic synthesis were of reagent grade and were dried over 4 Å molecular sieves. All reactions were monitored by thin-layer chromatography (TLC), performed on Merck 60 (silica gel F254) plates. Chromatographic purification of products was accomplished using flash column chromatography on a silica gel (230−400 mesh). ^1H, ^{13}C, and ^{31}P NMR spectra were recorded in deuterated solvents on Bruker Biospin 500 and 600 MHz NMR spectrometers. Chemical shifts (δ, ppm) are quoted relative to the residual solvent peak as an internal standard. Mass spectra were recorded on a Bruker micrOTOF-Q ESI mass spectrometer.

Oligonucleotide Synthesis. The modified oligonucleotide scaffold was assembled on an Applied Biosystems Incorporated 3400 automated DNA/RNA synthesizer by a conventional phosphoramidite strategy. The exocyclic amino functions of adenine, cytosine, and guanine bases were protected by the standard acyl-protecting groups (benzoyl, acetyl, and isobutyryl, respectively). For the phthaloyl-protected aminooxy-*C*-deoxyriboside phosphoramidite building block **2**, the coupling time was extended to 600 s. The

DOI: 10.1021/acsomega.9b02804
ACS Omega 2019, 4, 18803−18808

support-bound oligonucleotide was then treated with a mixture of $H_2NNH_2{\cdot}H_2O$, pyridine, and AcOH (1:32:8, v/v) for 45 min. The support was washed subsequently with pyridine, methanol, and acetonitrile and dried under vacuum. The support-bound oligonucleotide was placed in two microcentrifuge tubes (approximately 0.25 μmol each), and a solution of either acetato(2-formyl-6-hydroxyphenyl)mercury[19] (**12**, 10 μmol) in dry DMSO (100 μL) or bromo(2-formylphenyl)bis(triphenylphosphine)palladium[20] (**13**, 10 μmol) in dry CH_2Cl_2 (200 μL) was added. Note that the organomercury compound **12** is potentially highly toxic and should be handled appropriately. The resulting mixtures were shaken for 12 h at 25 °C. The solutions were discarded, and the solid supports were washed with either DMSO or CH_2Cl_2 and then treated with a mixture of methylamine and 25% aqueous ammonia (1:1, v/v) for 10 min at 65 °C. The solutions were recovered and evaporated to dryness, and the crude products thus obtained were purified by RP-HPLC on a Hypersil ODS C18 column (250 × 4.6 mm, 5 μm), eluting with a linear gradient (10 to 30% over 30 min for **ON1-Hg** and 5 to 30% over 30 min for **ON1-Pd**; flow rate = 1.0 mL min^{-1}) of MeCN in 50 mM aq triethylammonium acetate. Detection was performed by UV absorbance at λ = 260 nm. Finally, the purified oligonucleotides were characterized by ESI-TOF-MS and quantified spectrophotometrically using an implementation of the nearest neighbors method. Isolated yields of oligonucleotides **ON1-Hg** and **ON1-Pd** were approximately 1 and 11%, respectively. The low yields stem from general difficulties in the chromatographic purification of covalently metalated oligonucleotides and is not an inherent property of the method reported in the present study. In particular with covalently mercurated oligonucleotides, ligand exchange of Hg(II) causes severe broadening of the peaks, making isolation of the oligonucleotide in a high yield challenging. Efforts to improve the purification methods for covalently metalated oligonucleotides are underway in our laboratory.

(2*R*,3*S*,5*S*)-1,3-Bis((*tert*-butyldimethylsilyl)oxy)hept-6-ene-2,5-diol ((5*S*)-5) and (2*R*,3*S*,5*R*)-1,3-Bis((*tert*-butyldimethylsilyl)oxy)hept-6-ene-2,5-diol ((5*R*)-5). To a stirred solution of lactol **4** (3.62 g, 9.99 mmol) in dry THF (30 mL) at 0 °C was slowly added vinylmagnesium bromide (1.0 M THF, 30.0 mL, 30.0 mmol). The resulting mixture was stirred for 4 h at 0 °C, after which the reaction was then quenched by addition of 30 mL of saturated aqueous ammonium chloride and extracted with diethyl ether (2 × 50 mL). The organic phase was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was purified by flash chromatography eluting with a mixture of MeOH and CH_2Cl_2 (1:99, v/v), yielding 1.70 g (43%) of (5*S*)-**5** and 1.10 g (28%) of (5*R*)-**5** as colorless oils. ^{1}H NMR (500 MHz, $CDCl_3$, (5*S*)-**5**): δ = 5.91 (ddd, *J* = 17.0, 10.5, 5.5 Hz, 1H), 5.30 (m, 1H), 5.10 (m, 1H), 4.43 (t, *J* = 7.0 Hz, 1H), 3.94 (dd, *J* = 11.0, 5.5 Hz, 1H), 3.79 (dd, *J* = 10.0, 4.0 Hz, 1H), 3.74 (m, 1H), 3.60 (dd, *J* = 10.0, 7.0 Hz, 1H), 3.16 (s, 1H), 2.66 (s, 1H), 1.90 (m, 1H), 1.72 (ddd, *J* = 15.0, 10.0, 4.5 Hz, 1H), 0.93 (s, 9H), 0.92 (s, 9H), 0.15 (s, 3H), 0.12 (s, 3H), 0.10 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$, (5*S*)-**5**): δ = 141.4, 113.8, 73.9, 71.5, 69.8, 64.1, 40.3, 25.9, 25.8, 18.3, 18.0, −4.4, −4.7, −5.3, −5.4. ^{1}H NMR (500 MHz, $CDCl_3$, (5*R*)-**5**): δ = 5.89 (ddd, *J* = 17.0, 10.5, 5.5 Hz, 1H), 5.27 (dt, *J* = 17.0, 1.5 Hz, 1H), 5.10 (dt, *J* = 10.5, 1.5 Hz, 1H), 4.43 (brs, 1H), 3.93 (q, *J* = 5.5 Hz, 1H), 3.76−3.72 (m, 2H), 3.62 (m, 1H), 3.33 (s, 1H), 2.92 (s, 1H), 1.89 (m, 1H), 1.77 (m, 1H), 0.92−0.91 (m, 18H), 0.10 (s, 3H), 0.09 (s, 3H), 0.08 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$, (5*R*)-**5**): δ = 141.2, 114.0, 74.5, 70.8, 69.0, 64.0, 40.5, 25.9, 25.8, 18.3, 18.0, −4.4, −4.6, −5.36, −5.41. HRMS (ESI^+): *m*/*z* calcd for $C_{19}H_{42}O_4Si_2Na$ $[M + Na]^+$, 413.2514; found, 413.2513.

3,5-Di-*O*-(*tert*-butyldimethylsilyl)-*C*-ethenyl-2-deoxy-β-D-ribofuranose (6). To a solution of (5*S*)-**5** (1.10 g, 2.82 mmol) in CH_2Cl_2 (15 mL) was added 3.4 mL of dry pyridine. The solution was cooled to −25 °C, and a solution of MsCl (502 μL, 6.49 mmol) in CH_2Cl_2 (3.0 mL) was added gradually over 30 min with stirring. After standing overnight at −25 °C, the reaction mixture was quenched by addition of 5.0 mL of saturated aqueous NH_4Cl. The aqueous phase was extracted with diethyl ether (2 × 20 mL), and the combined organic extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was purified by silica gel flash column chromatography eluting with a mixture of hexane and EtOAc (24:1, v/v), yielding 343 mg (69%) of **6** as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.84 (ddd, *J* = 17.0, 10.0, 7.0 Hz, 1H), 5.30 (d, *J* = 17.0 Hz, 1H), 5.12 (d, *J* = 10.5 Hz, 1H), 4.58 (m, 1H), 4.35 (d, *J* = 5.0 Hz, 1H), 3.87 (m, 1H), 3.68 (dd, *J* = 10.5, 4.0 Hz, 1H), 3.47 (dd, *J* = 10.5, 6.5 Hz, 1H), 1.92 (m, 1H), 1.74 (m, 1H), 0.92 (s, 18H), 0.10 (s, 6H), 0.08 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 138.8, 116.0, 87.8, 79.6, 74.3, 63.9, 41.6, 26.0, 25.8, 18.4, 18.0, −4.67, −4.69, −5.3, −5.4. HRMS (ESI^+): *m*/*z* calcd for $C_{19}H_{40}O_3Si_2K$ $[M + K]^+$, 411.2148; found, 411.2146.

3,5-Di-*O*-(*tert*-butyldimethylsilyl)-*C*-formyl-2-deoxy-β-D-ribofuranose (7). $NaIO_4$ (379 mg, 1.77 mmol) and $CeCl_3{\cdot}7H_2O$ (88.0 mg, 0.236 mmol) were taken in a 10 mL round-bottom flask. H_2O (0.54 mL) was added, and the resulting mixture was gently heated until a bright yellow suspension was formed. After cooling to 0 °C, EtOAc (1.48 mL) and MeCN (1.78 mL) were added, and the mixture was stirred for 2 min. A 0.1 M aqueous solution of $RuCl_3$ (60 μL, 5.95 μmol) was added, and the mixture was stirred for another 2 min. A solution of compound **6** (440.0 mg, 1.181 mmol) in EtOAc (0.3 mL) was then added in one portion, and the resulting slurry was stirred for 10 min. The mixture was poured into a mixture of saturated aqueous $NaHCO_3$ (10 mL) and saturated aqueous $Na_2S_2O_3$ (10 mL). Phases were separated, and the aqueous phase was extracted with EtOAc (3 × 50 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was dissolved in a mixture of THF, H_2O, and Et_2O (1:1:1, v/v, 5.0 mL), and the resulting solution was cooled to 0 °C. $NaIO_4$ (470 mg, 2.2 mmol) was added, and the solution was stirred for 90 min at 25 °C. Brine (10.0 mL) was added, and the resulting mixture was extracted with EtOAc (3 × 20 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was purified by silica gel flash column chromatography eluting with a mixture of hexane and EtOAc (17:3, v/v), yielding 351 mg (74% over two steps) of **7** as a colorless syrup. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.69 (d, *J* = 2.0 Hz, 1H), 4.48 (m, 1H), 4.37 (m, 1H), 3.99 (m, 1H), 3.71 (dd, *J* = 11.0, 3.5 Hz, 1H), 3.60 (dd, *J* = 11.0, 5.5 Hz, 1H), 2.09−2.02 (m, 2H), 0.92−0.91 (m, 18H), 0.10−0.08 (m, 12H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 202.8, 88.6, 82.6, 73.3, 63.4, 37.0, 25.9, 25.7, 18.3, 17.9, −4.7, −4.8, −5.4, −5.5. HRMS (ESI^+): *m*/*z* calcd for $C_{18}H_{38}O_4Si_2Na$ $[M + Na]^+$, 397.2201; found, 397.2202.

3,5-Di-*O*-(*tert*-butyldimethylsilyl)-*C*-hydroxymethyl-2-deoxy-β-D-ribofuranose (8). Aldehyde **7** (351 mg, 0.939

DOI: 10.1021/acsomega.9b02804
ACS Omega 2019, 4, 18803−18808

mmol) was dissolved in a mixture of EtOH and H_2O (2/1, v/v, 25.0 mL), and the resulting solution was cooled to 0 °C. $NaBH_4$ (142.1 mg, 3.760 mmol) was added, and the resulting mixture was stirred for 3 h at 25 °C. The reaction mixture was then poured into brine (20 mL) and extracted with EtOAc (2 × 20 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was purified by silica gel flash column chromatography eluting with a mixture of hexane and EtOAc (17:3, v/v), yielding 311 mg (88%) of **8** as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.43 (dt, J = 6.0, 4.0 Hz, 1H), 4.33 (ddd, J = 10.0, 7.0, 3.0 Hz, 1H), 3.85−3.81 (m, 2H), 3.73−3.67 (m, 2H), 3.46 (ddd, J = 12.0, 8.5, 3.0 Hz, 1H), 2.47 (dd, J = 8.5, 3.0 Hz, 1H), 2.11 (m, 1H), 1.87 (m, 1H), 0.93 (s, 9H), 0.91 (s, 9H), 0.10−0.09 (m, 12H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 87.0, 78.8, 73.1, 64.9, 63.3, 36.9, 25.9, 25.8, 18.4, 18.0, −4.6, −4.8, −5.48, −5.51. HRMS (ESI$^+$): m/z calcd for $C_{18}H_{40}O_4Si_2Na$ [M + Na]$^+$, 399.2357; found, 399.2359.

3,5-Di-*O*-(*tert*-butyldimethylsilyl)-*C*-methanesulfonyloxymethyl-2-deoxy-β-D-ribofuranose (9). To a solution of **8** (218.0 mg, 0.579 mmol) in CH_2Cl_2 (5 mL) was added 0.58 mL of dry pyridine, and the resulting solution was cooled to 0 °C. MsCl (85.0 μL, 0.869 mmol) was then added slowly, and the mixture was stirred for 3 h at 25 °C, after which it was poured into a saturated aqueous solution of $NaHCO_3$. The phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was purified by silica gel flash column chromatography eluting with a mixture of hexane and EtOAc (4:1, v/v), yielding 246 mg (94%) of **9** as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.46 (m, 1H), 4.36−4.33 (m, 2H), 4.20 (m, 1H), 3.89 (ddd, J = 6.0, 4.0, 2.0 Hz, 1H), 3.64 (dd, J = 11.0, 4.0 Hz, 1H), 3.49 (dd, J = 11.0, 5.5 Hz, 1H), 3.07 (s, 3H), 1.89−1.81 (m, 2H), 0.92 (s, 9H), 0.91 (s, 9H), 0.09 (s, 6H), 0.08 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 88.3, 76.0, 73.6, 71.4, 63.6, 37.7, 37.0, 25.9, 25.8, 18.3, 18.0, −4.69, −4.75, −5.38, −5.46. HRMS (ESI$^+$): m/z calcd for $C_{19}H_{42}O_6SSi_2Na$ [M + Na]$^+$, 477.2133; found, 477.2132.

***C*-Methanesulfonyloxymethyl-2-deoxy-β-D-ribofuranose (10).** To a stirred solution of compound **9** (246 mg, 0.542 mmol) in dry THF (4 mL) under argon at 0 °C was added $Et_3N{\cdot}3HF$ (265 μL, 1.63 mmol). The resulting mixture was stirred for 16 h at 25 °C, after which it was passed through a short silica gel plug eluting with EtOAc. The EtOAc filtrate was evaporated, and the residue was purified by silica gel flash column chromatography eluting with a mixture of MeOH and CH_2Cl_2 (1:9, v/v), yielding 114 mg (93%) of **10** as a white foam. ^{1}H NMR (500 MHz, MeOD): δ = 4.42 (m, 1H), 4.36 (dd, J = 11.0, 3.0 Hz, 1H), 4.26−4.21 (m, 2H), 3.87 (m, 1H), 3.60−3.54 (m, 2H), 3.13 (s, 3H), 1.95 (dd, J = 8.0, 4.5 Hz, 2H). ^{13}C NMR (125 MHz, MeOD): δ = 87.7, 76.0, 72.2, 71.5, 62.4, 36.1, 36.0. HRMS (ESI$^+$): m/z calcd for $C_7H_{14}O_6SNa$ [M + Na]$^+$, 249.0403; found, 249.0401.

***C*-Phthalimidooxymethyl-2-deoxy-β-D-ribofuranose (1).** To a solution of compound **10** (114 mg, 0.504 mmol) and *N*-hydroxyphthalimide (165 mg, 1.01 mmol) in DMF (1.5 mL) was added diisopropylethylamine (351 μL, 2.02 mmol). The resulting red solution was stirred at 70 °C for 30 h, after which it was evaporated to dryness. The residue was purified by silica gel flash column chromatography eluting with a mixture of MeOH and CH_2Cl_2 (1:19, v/v), yielding 69.2 mg (47%) of **1** as a syrup. ^{1}H NMR (500 MHz, MeOD): δ = 7.87−7.83 (m, 4H), 4.51 (m, 1H), 4.30−4.25 (m, 3H), 3.82 (dd, J = 8.0, 5.0 Hz, 1H), 3.60−3.51 (m, 2H), 2.09−1.98 (m, 2H). ^{13}C NMR (125 MHz, MeOD): δ = 163.5, 134.4, 128.9, 122.9, 87.7, 79.5, 76.1, 72.1, 62.4, 36.6. HRMS (ESI$^+$): m/z calcd for $C_{14}H_{15}O_6NNa$ [M + Na]$^+$, 316.0792; found, 316.0792.

5-*O*-(4,4′-Dimethoxytrityl)-*C*-phthalimidooxymethyl-2-deoxy-β-D-ribofuranose (11). Compound **1** (69.0 mg, 0.235 mmol) was coevaporated from dry pyridine (2 × 10 mL), and the residue was dissolved in dry pyridine (5 mL). DMTrCl (88.0 mg, 0.260 mmol) was added, and the resulting mixture was stirred at room temperature for 12 h, after which it was concentrated under reduced pressure. The residue was diluted with CH_2Cl_2 (20 mL), washed with saturated aqueous $NaHCO_3$ (20 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was evaporated to dryness, and the residue was purified by silica gel flash chromatography eluting with a mixture of hexane, EtOAc, and Et_3N (38:57:5, v/v) to yield 84.2 mg (60%) of **11** as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.83−7.80 (m, 2H), 7.75−7.72 (m, 2H), 7.42−7.40 (m, 2H), 7.32−7.28 (m, 6H), 7.21 (m, 1H), 6.88−6.84 (m, 4H), 4.61 (m, 1H), 4.35 (m, 1H), 4.33−4.28 (m, 2H), 3.95 (m, 1H), 3.81 (s, 6H), 3.21 (dd, J = 9.5, 4.5 Hz, 1H), 3.13 (dd, J = 10.0, 5.5 Hz, 1H), 2.11−2.07 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 163.3, 158.5, 144.8, 136.0, 134.4, 130.1, 130.0, 129.0, 128.1, 127.8, 126.8, 123.5, 113.1, 86.2, 79.9, 76.0, 74.1, 64.5, 55.2, 37.4. HRMS (ESI$^+$): m/z calcd for $C_{35}H_{33}O_8NNa$ [M + Na]$^+$, 618.2098; found, 618.2096.

3-*O*-[(2-Cyanoethoxy)(*N*,*N*-diisopropylamino)-phosphinyl]-5-*O*-(4,4′-dimethoxytrityl)-*C*-phthalimidooxymethyl-2-deoxy-β-D-ribofuranose (2). To a stirred solution of compound **11** (108.0 mg, 0.181 mmol) in dry CH_2Cl_2 (1.5 mL) were added Et_3N (152 μL, 1.09 mmol) and 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (49 μL, 0.22 mmol). The reaction mixture was stirred at 25 °C under a N_2 atmosphere for 2 h, after which it was diluted with CH_2Cl_2 (20 mL) and washed with saturated aqueous $NaHCO_3$ (20 mL). The organic phase was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was purified by silica gel flash column chromatography eluting with a mixture of hexane, EtOAc, and Et_3N (57:38:5, v/v) to yield 108.4 mg (75% as a mixture of R_P and S_P diastereomers) of **2** as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$, a mixture of R_P and S_P diastereomers): δ = 7.84−7.81 (m, 2H), 7.74−7.71 (m, 2H), 7.43−7.41 (m, 2H), 7.33−7.27 (m, 6H), 7.22−7.18 (m, 1H), 6.85−6.82 (m, 4H), 4.62 (m, 1H), 4.44 (m, 1H), 4.37−4.35 (m, 2H), 4.13 (m, 1H), 3.81−3.80 (m, 6H), 3.69 (m, 1H), 3.63−3.54 (m, 2H), 3.19 (m, 1H), 3.10 (m, 1H), 2.64 (t, J = 6.5 Hz, 1H), 2.45 (t, J = 6.5 Hz, 1H), 2.14−2.03 (m, 2H), 1.20−1.16 (m, 10H), 1.08−1.07 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$, a mixture of R_P and S_P diastereomers): δ = 163.31, 163.29, 158.4, 144.9, 136.1, 136.07, 136.04, 136.03, 134.4, 130.13, 130.13, 130.10, 129.0, 128.3, 128.2, 127.77, 127.76, 126.69, 126.66, 123.5, 117.6, 117.5, 113.09, 113.08, 86.19, 86.16, 86.0, 79.96, 79.86, 76.4, 76.3, 75.4, 75.2, 75.0, 74.9, 64.1, 64.0, 58.41, 58.39, 58.3, 58.2, 55.22, 55.20, 43.3, 43.22, 43.19, 43.1, 37.09, 37.06, 36.93, 36.89, 24.65, 24.63, 24.59, 24.57, 24.50, 24.46, 24.45, 24.41, 20.4, 20.3, 20.2, 20.1. ^{31}P (162 MHz, $CDCl_3$, a mixture of R_P and S_P diastereomers): δ = 148.1, 147.8. HRMS (ESI$^+$): m/z calcd for $C_{44}H_{50}O_9N_3PNa$ [M + Na]$^+$, 818.3177; found, 818.3175.

DOI: 10.1021/acsomega.9b02804
ACS Omega 2019, 4, 18803−18808

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b02804.

NMR spectra of small molecules and HPLC traces and mass spectra of oligonucleotides (PDF)

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S.K.M. carried out all of the experimental work and wrote the first draft of the manuscript. T.A.L. wrote the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Financial support from the Academy of Finland (decisions #286478 and #294008) is gratefully recognized.

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DOI: 10.1021/acsomega.9b02804
ACS Omega 2019, 4, 18803−18808