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Synthesis of a 5′-6-Locked, 1,10-Phenanthroline-Containing Nucleoside and Its Incorporation into DNA

Kristmann Gislason[a] and Snorri T. Sigurdsson*[a]

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A rigid nucleoside containing a phenanthroline ligand for metal-ion chelation was synthesized through condensation of 1,10-phenanthroline-5,6-dione with 5-amino-2′-deoxycytidine. During the condensation, a 5′-6 ether linkage was formed between the sugar and the base. The phosphoramidite of the nucleoside was used to synthesize oligodeoxynucleotides by means of automated oligonucleotide synthesis, placing the phenanthroline nucleoside on the 5′-end of the oligomers. The free nucleoside is fluorescent; however, the fluorescence of the nucleoside was effectively quenched in both single- and double-stranded DNA. Thermal denaturation experiments on DNA duplexes containing the modified nucleoside showed similar base-pairing properties as T and stronger stacking interactions with a flanking A·T base pair than with a G·C pair. CD spectra of helixes containing the modified nucleoside were characteristic of B-DNA. A model structure of a B-DNA helix, where the nucleoside was paired with A, showed only minor deviations from B-DNA parameters, except for a noticeable buckle of the modified base pair due to the constraints of the 5′-6 linkage. Due to the relative ease of the synthesis and minimal distortions of the helix structure, the phenanthroline nucleoside reported here shows promise for facile 5′-labeling of nucleic acids with metal complexes. This strategy can likely be extended to fusing other aromatic or aliphatic rings to a nucleotide base for incorporating the 5′-end of nucleic acid duplexes.

Introduction

Complexes of transition metals with polypyridine ligands, such as bipyridine and 1,10-phenanthroline, have emerged as powerful tools in structural studies of nucleic acids.[1] The usefulness of these complexes results from their properties, which organic molecules do not possess, i.e. catalytic activity, photophysical properties, such as increased photostability and luminescence lifetimes, redox activity and geometries, such as square-planar or octahedral.[1] The first nucleic acid studies using metal complexes involved incubation of the complexes in solutions containing nucleic acids, for example, in foot-printing experiments with chemical nucleases.[2] In later experiments, sensitivity and affinity of complex probes were enhanced through the use of ligands that intercalated into the base stack of the nucleic acid.[3] Also, complexes containing sterically bulky insertor ligands were found to bind to mismatch sites in DNA double helixes with certain specificity.[3] Further improvement was obtained by site-specifically incorporating the metal complex through covalent attachment to a nucleotide of interest.

Some of the first examples of polypyridine complexes covalently attached to nucleic acids were in the field of artificial nucleases. Complexes, consisting mainly of copper or iron ions with 1,10-phenanthroline, had previously been shown to arbitrarily cleave nucleic acids.[2,4] Covalently attaching these complexes to oligonucleotides complementary to a recognition site in the target nucleic acid imparted sequence specificity to the cleavage.[4] Recently, more focus has been applied to the labeling of oligonucleotides with luminescent transition metal complexes, mainly complexes of ruthenium, rhodium and osmium.[5] Although these complexes have proven to be useful probes in DNA hybridization studies based on luminescence energy transfer,[6] the main interest has been in their redox properties, i.e. their ability to act as both donors and acceptors in charge-transfer studies. Such complexes have been used to study charge transfer through the DNA double-helix structure, both in regard to detect changes in the helix structure and to evaluate the use of DNA as a conducting building block for nanomaterials.[7] The incorporation of redox-active complexes into a specific site in DNA helixes, both through covalently attached metallointercalators[8] and through complex-modified nucleotides,[9] has given valuable information about these transfers.

In addition to the use of (polypyridine)transition metal complexes as probes in nucleic acids research, recent studies have proposed the use of these complexes in conjugation with DNA to create structures for nanotechnological applications. Here, the complexes can act as structural components, such as linkers and junctions between DNA helical components, increasing the versatility in assembling struc-

[a] University of Iceland, Science Institute,
Dunhaga 3, 107 Reykjavik, Iceland
Fax: +354-552-8911
E-mail: snorrisi@hi.is

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tures through their coordination properties.[10] These complexes can also be functional, imparting properties such as catalytic activity, luminescence and redox activity to the DNA nanostructures.[10c] DNA wires have also been constructed through the use of metal-ion-mediated base pairing of artificial nucleosides.[11]

Most of the methods for attaching metal complexes to nucleic acids have utilized flexible linkers spanning a few atoms, although semirigid acetylene linkers have been used in a number of cases.[5b–5d] Our group has previously synthesised the rigid nitroxide spin-label nucleoside Ç, where the nitroxide moiety is fused to the nucleoside through a six-membered ring;[12] Ç was shown to have advantageous properties as a probe for electron paramagnetic resonance spectroscopy studies due to its rigid structure.[13] Similarly, a rigid metal complex nucleoside, where one of the ligands is fused with the base through a planar ring system, might present several advantages. First, limiting movement of the complex independent of the nucleic acid decreases uncertainty of distance and mobility measurements, for example in luminescence energy transfer experiments.[14] Second, direct coupling of the ligand to the nucleoside will make the complex more sensitive to physical changes in the microenvironment of the nucleoside, i.e. stacking, base-pairing and charge transfer. Third, in the case of nanostructures, a rigid attachment of a metal complex to DNA structural elements would extend the preset structural properties of DNA through the complex.

In this paper we report the synthesis of rigid nucleoside **1** (Figure 1A), containing a 1,10-phenanthroline moiety attached to the nucleoside base through a pyrazine ring, and its incorporation into DNA. During the synthesis, an ether linkage was formed between 5′-O of the sugar and 6-C of the base, which limits the direct incorporation of the nucleoside to the 5′-end of nucleic acids. However, given the fact that single-strand breaks have been shown to have a minor effect on the structure of B-DNA double helixes,[15] it should be possible to incorporate nucleoside **1** into an internal position of the helix through a three-piece construct. The lack of a free 5′-hydroxy group in **1** has the advantage that the preparation of the phosphoramidite building block for oligonucleotide synthesis does not require the usual 5′-protection with a trityl group. We show that the rigid nucleoside has similar base-pairing properties as T (Figure 1B) and that the nucleoside is accommodated in DNA duplexes without causing any appreciable distortion of the helix structure.

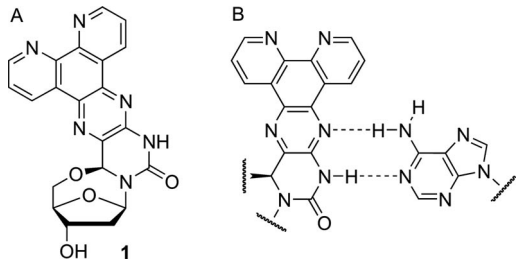


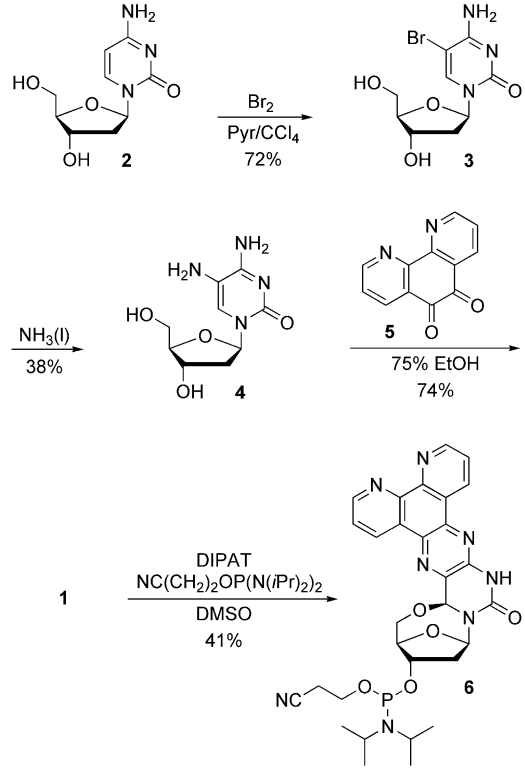
Figure 1. Structure of nucleoside **1** (A) and its base pair to adenosine (B).

Results and Discussion

Synthesis of Nucleoside 1 and Its Phosphoramidite Derivative

The strategy for incorporation of a rigid metal-ion chelator into nucleic acids was based on a nucleoside derivative prepared by Kalman and co-workers in search of new anticancer drugs.[16] The key step in the syntheses of this nucleoside was a condensation reaction of a diketone with 5-amino-2′-deoxycytidine. We employed the commercially available 1,10-phenanthroline-5,6-dione in a similar manner to obtain a rigid chelating nucleoside.

The synthesis started with the preparation of 5-amino-2′-deoxycytidine (**4**) from 2′-deoxycytidine (**2**) through a slight modification of a previously reported protocol (Scheme 1).[17] 2′-Deoxycytidine (**2**) was brominated to obtain 5-bromo-2′-deoxycytidine (**3**) and subsequently exposed to liquid ammonia to afford **4**. Compound **4** was then coupled to 1,10-phenanthroline-5,6-dione (**5**) in aqueous ethanol. During the condensation reaction between the diketone and the diamine, a nucleophilic attack of the 5′-hydroxy group on the 6 position of the base resulted in the formation of a 5′-6 ether linkage. The stereochemistry of the newly formed chiral centre in **1** was assumed to be the same as determined by Kalman and co-workers for a similar compound using NOE experiments.[16a] The pure nucleoside product **1** precipitated from the reaction mixture and was isolated in 74% yield. Nucleoside **1** proved to be insoluble in most organic solvents used in previously reported nucleoside phosphitylation protocols. However, **1** was found



Scheme 1. Synthesis of nucleoside **1** and its phosphoramidite **6** (DIPAT = diisopropylammonium tetrazolide).

to be soluble in DMSO and DMF and was subsequently phosphitylated in dry DMSO to give phosphoramidite **6** in 41% yield.

Incorporation of Nucleoside 1 into Oligonucleotides

Nucleoside **1** was incorporated into oligonucleotides by using standard automated DNA oligonucleotide synthesis. The coupling time was extended to 15 min when incorporating the modification. Two 14-mer oligodeoxyribonucleotide sequences, 5′-d(**1**GC CTC GCA TCG TG) (**X**) and 5′-d(**1**AC CTC GCA TCG TG) (**Y**), were synthesised to study the effect of the identity of the base pair flanking **1** on the stability of modified duplexes. In sequence **X**, **1** is flanked by G and in sequence **Y** by A. Incorporation of **1** into the modified oligonucleotides was verified by MALDI-ToF mass spectrometry. Denaturing polyacrylamide gel electrophoresis (DPAGE) of the modified oligomers showed a fluorescent product that moved slower than an unmodified 14-mer, presumably due to the increased bulk of the modified nucleoside (data not shown). Yields of the modified oligonucleotides were comparable to that of unmodified oligonucleotides.

Photophysical Properties of Nucleoside 1 and 1-Modified Oligonucleotides

Since nucleoside **1** displayed fluorescence, its photophysical properties, both free and after incorporation into oligonucleotides, were characterized. The UV spectrum of **1** in DMSO displays two peaks at 276 and 377 nm, with shoulders at 307 and 363 nm (Figure 2). The fluorescence maximum lays at ca. 400 nm, and the fluorescence intensity of **1** is highest when excited at 375 nm. The nucleoside exhibits a moderate fluorescence with a quantum yield of 0.05. UV spectra of modified oligonucleotides **X** and **Y** in phosphate buffer, show peaks at ca. 377 and 363 nm, resulting from the modification. However, fluorescence of the nucleoside was effectively quenched in both single- and double-stranded DNA (Figures S3 and S4).

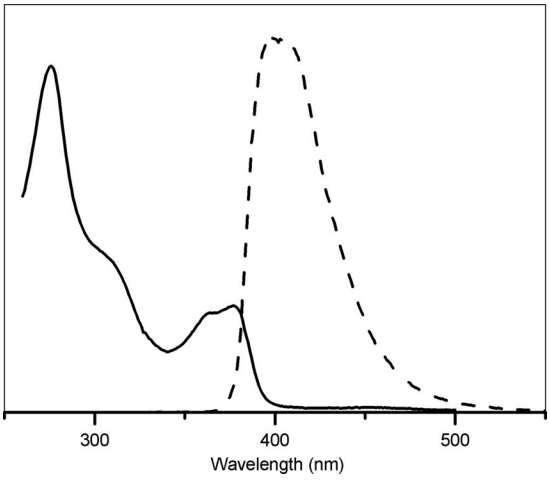


Figure 2. Absorption (solid line) and emission (dashed line; excitation wavelength 365.5 nm) spectra of nucleoside **1** in DMSO.

Thermal Denaturing Studies

To determine the effect of **1** on the stability of DNA duplexes, melting temperatures were recorded for duplexes containing modified oligonucleotides **X** (G-flank) and **Y** (A-flank) (Table 1). This included helixes where **1** is paired with all four natural nucleosides, to study the base-pairing properties of **1**. The modified duplexes were compared to unmodified duplexes containing a T·A base pair at the site of modification. In the case of the G-flank, the complementary modified duplex **GII** shows an increase of 0.5 °C in T_m

Table 1. Melting temperatures of unmodified and modified helixes.

Duplex	Sequences	T_m [°C][a]	ΔT_m [°C][b]
GI	5′-d(TGC CTC GCA TCG TG) 3′-d(ACG GAG CGT AGC AC)	62.4	–
GII	5′-d(**1**GC CTC GCA TCG TG) 3′-d(ACG GAG CGT AGC AC)	62.9	+0.5
GIII	5′-d(**1**GC CTC GCA TCG TG) 3′-d(GCG GAG CGT AGC AC)	62.7	+0.3
GIV	5′-d(**1**GC CTC GCA TCG TG) 3′-d(TCG GAG CGT AGC AC)	62.3	–0.1
GV	5′-d(**1**GC CTC GCA TCG TG) 3′-d(CCG GAG CGT AGC AC)	62.6	+0.2
GVI	5′-d(TGC CTC GCA TCG TG) 3′-d(CG GAG CGT AGC AC)	60.3	–
GVII	5′-d(**1**GC CTC GCA TCG TG) 3′-d(CG GAG CGT AGC AC)	62.1	+1.8
AI	5′-d(TAC CTC GCA TCG TG) 3′-d(ATG GAG CGT AGC AC)	60.7	–
AII	5′-d(**1**AC CTC GCA TCG TG) 3′-d(ATG GAG CGT AGC AC)	64.5	+3.8
AIII	5′-d(**1**AC CTC GCA TCG TG) 3′-d(GTG GAG CGT AGC AC)	64.1	+3.4
AIV	5′-d(**1**AC CTC GCA TCG TG) 3′-d(TTG GAG CGT AGC AC)	62.9	+2.2
AV	5′-d(**1**AC CTC GCA TCG TG) 3′-d(CTG GAG CGT AGC AC)	62.2	+1.5
AVI	5′-d(TAC CTC GCA TCG TG) 3′-d(TG GAG CGT AGC AC)	59.9	–
AVII	5′-d(**1**AC CTC GCA TCG TG) 3′-d(TG GAG CGT AGC AC)	63.4	+3.5

[a] Errors for T_m values were estimated to be ±0.5 °C. [b] Duplexes **GII** to **GV** and **AII** to **AV** are compared to duplexes **GI** and **AI**, respectively. Overhang duplexes **GVII** and **AVII** are compared to **GVI** and **AVI**, respectively.

when compared to the unmodified duplex **GI**, indicating the nucleoside forms a stable base pair with A. For the A flank, the stabilization effect is more pronounced; duplex **AII** shows an increase of 3.8 °C in T_m when compared to the unmodified **AI**. This indicates stronger stacking interactions of the modified **1**·A pair with an A·T base pair than with a G·C base pair.

The stability order for T base pairs has been reported as T·A > T·G > T·T > T·C.[18] For the A-flank (**AIII** to **AV**), the trend in T_m values is **1**·A > **1**·G > **1**·T > **1**·C and agrees well with the stability order for T base pairs, further supporting the idea that **1** has similar base-pairing properties as T. However, it should be noted that the T_m differences between different base pairs with **1** are small and flanking-sequence-dependent. The G-flank mismatched duplexes (**GIII** to **GV**) show a small degree of change in T_m values, and all were less stable than **1**·A. A flanking A·T base pair was observed to stabilize all the base pairs, relative to the G-flank. For the G-flank, only duplex **GIII**, containing a **1**·G base pair, showed a slight increase in stability.

To further investigate the stacking properties of **1**, a duplex containing **1** as an overhang was created by annealing modified oligonucleotides **X** and **Y** to complimentary 13-mers lacking the nucleotide opposite **1**. A T-overhang has been shown to have almost the same stabilization effect for a flanking A·T and a flanking G·C, increasing the T_m by 2.2 °C and 2.4 °C, respectively.[19] The stacking of nucleoside **1** had a larger stabilizing effect on the duplex than T but less than modified nucleotides containing large aromatic moieties such as pyrene that have been shown to increase the T_m of six nucleotide duplexes by up to 14 °C.[20] The degree of stabilization was flanking-sequence-dependent: the T_m increased by 1.8 and 3.5 °C, for stacking on a G·C- (**GVII**) and an A·T base pair (**AVII**), respectively, compared to a T-overhang (**GVI** and **AVI**). The T_ms of both duplexes that contain **1** as an overhang remain lower than the fully complementary duplexes **GII** and **AII**, showing that although stacking is a major contributor to the stability of the modified duplexes, they are also stabilized through base-pairing of **1**.

Effects of 1 on the Duplex Structure

To study the structural effects of incorporating a nucleoside with a 5′-6 ether linkage into a DNA duplex, circular dichroism spectra of the modified duplexes were recorded. No major deviations from right-handed B-DNA double helix were observed with the spectra showing the characteristic B-DNA molar ellipticities at ca. 250 nm (negative) and 280 nm (positive) (Figures S1 and S2). Since a structure of duplexes containing oligonucleotides with a 5′-6 ether modification has not been reported, a model of the modified helix **GII** was built to visualize how the modified nucleoside is accommodated in the duplex (Figure 3). The model structure shows that the nucleoside is able to adopt a position that enables it to form two hydrogen bonds to the opposing adenosine. In fact, when compared to an unmodified duplex, only slight deviations from the natural A·T base pair were observed. The most significant change is a ca. 15° buckle of the **1**·A base pair when compared to A·T. This can be explained by the ether linkage constraining the rotation around the *N*-glycosidic bond, not allowing the modified base to be in the same plane as the A to which it is paired. Changes in the rise and slide of the modified base pair, with regard to the flanking G·C pair, were insignificant. However, the twist is decreased by ca. 15° and the shift decreased by ca. 1 Å. Both changes result from the constraint buckling the nucleotides sugar part, pulling the base closer to the DNA backbone.

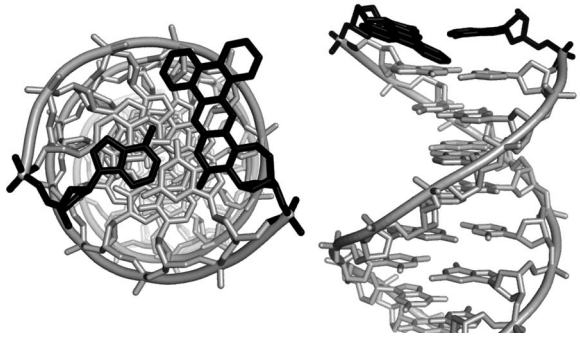


Figure 3. Model structure of helix **GII**, showing the modified nucleoside **1** base-paired with A. A top-view of the helix is shown on the left and a side-view on the right, the latter showing only a part of the **GII** helix.

Conclusions

A rigid phenanthroline nucleoside **1**, containing a 5′-6 ether linkage, has been prepared and incorporated at the 5′-end of oligonucleotides by solid phase oligonucleotide synthesis. The photophysical properties of the nucleoside and modified oligonucleotides have been determined. Thermal denaturation studies indicate that **1** has similar base-pairing properties to a T nucleotide and showed stronger stacking interactions of **1** with a flanking A·T pair than a flanking G·C pair. Circular dichroism spectra of modified oligonucleotides showed molar ellipticities characteristic of B-DNA, and a model of a **1**-containing duplex showed minor deviations from standard B-DNA. The application of this nucleoside in biophysical studies involving metal-ion binding will be reported in due course.

Experimental Section

General: Chemicals were purchased from Sigma Aldrich, Acros and Apollo Scientific Ltd. and used without further purification. Chemicals for phosphoramidite and ODN synthesis were purchased from ChemGenes. Thin layer chromatography (TLC) was carried out by using glass plates pre-coated with silica gel (0.25 mm, F-254) from Silicycle. Nucleosides were identified by staining with *p*-anisaldehyde. 1,10-Phenanthroline-containing compounds bind to the material used to coat TLC plates and thus do not move on TLC plates but can be purified by silica gel column chromatography. Flash column chromatography was performed by

using ultrapure flash silica gel from Silicycle (40–63 μm, 60 Å). Acetonitrile, dichloromethane and pyridine were freshly distilled from calcium hydride prior to use. Anhydrous triethylamine was purchased from Sigma Aldrich. DMSO was dried by vacuum distillation and stored over activated molecular sieves (3 Å) under argon until used. Liquid ammonia was dried by condensation over sodium followed by re-condensation into a pressure-reaction vial. All moisture- and oxygen-sensitive reactions were carried out in oven- or flame-dried glassware under argon. The phosphate buffer used contained 10 mM phosphate, 100 mM NaCl, 0.1 mM EDTA, pH = 7. NMR spectra were recorded with a Bruker Avance 400 spectrometer. ^{1}H NMR chemical shifts are reported in reference to undeuterated residual solvent [D_2O (δ = 4.60 ppm), $CDCl_3$ (δ = 7.26 ppm), [D_6]DMSO (δ = 2.50 ppm)]. ^{13}C NMR chemical shifts are reported in reference to undeuterated residual solvent [$CDCl_3$ (δ = 77.0 ppm), [D_6]DMSO (δ = 39.43 ppm)]. ^{31}P NMR chemical shifts were reported relative to 85% H_3PO_4 as an external standard. MALDI-ToF mass spectra were recorded with a Bruker Autoflex III by using 2,5-dihydroxybenzoic acid as the matrix. UV/Vis spectra were recorded with a Perkin–Elmer Lambda 25 UV/Vis spectrometer. Fluorescence spectra were recorded with a SPEX FluoroMax spectrometer. CD spectra were recorded with a Jasco J-810 spectropolarimeter. T_m values were recorded with a Perkin–Elmer Lambda 25 UV/Vis spectrometer equipped with a PTP-1 Peltier Temperature Programmer. DNA duplexes were formed by the following annealing protocol: 90 °C for 2 min, 60 °C for 5 min, 50 °C for 5 min, 40 °C for 5 min, 22 °C for 15 min.

5-Bromo-2′-deoxycytidine (3): A solution of Br_2 (3 mL, 58.0 mmol) in CCl_4 (125 mL) was added slowly to a suspension of 2′-deoxycytidine (**2**) (10.0 g, 44.0 mmol) in pyridine (165 mL). The resulting mixture was stirred at 22 °C for 12 h. The solvent was removed under reduced pressure and Na_2CO_3 (1.8 g) in water (150 mL) added. The water was removed and compound **3** isolated from the residue by flash column chromatography (CH_2Cl_2/MeOH, 80:20). Compound **3** was collected as a white solid (9.72 g, 72%). ^{1}H NMR (400 MHz, D_2O, 25 °C): δ = 2.20–2.27 (m, 1, H2′), 2.36–2.42 (m, 1 H, H2′′), 3.70 [dd, ^3J(H,H) = 12.6, ^3J(H,H) = 4.9 Hz, 1 H, H5′], 3.79 [dd, ^3J(H,H) = 12.6, ^3J(H,H) = 3.5 Hz, 1 H, H5′′], 3.97–4.00 (m, 1 H, H4′), 4.34–4.38 (m, 1 H, H3′), 6.13 [dd, ^3J(H,H) = 6.4 Hz, 1 H, H1′], 8.15 (s, 1 H, H6) ppm. ^{13}C NMR (400 MHz, D_2O, 25 °C): δ = 39.58, 60.85, 70.03, 86.48, 86.76, 88.74, 142.27, 156.02, 162.70 ppm.

5-Amino-2′-deoxycytidine (4): 5-Bromo-2′-deoxycytidine (**3**) (3.00 g, 9.80 mmol) was stirred in a pressure-reaction vial in dried NH_3(l) at 70 °C for 5 d. The vial was subsequently cooled in liquid N_2, opened and the cooling bath removed to allow the ammonia to evaporate. The product was purified by flash column chromatography (CH_2Cl_2/MeOH/Et_3N, 59:40:1) to give compound **4** as a pale-yellow solid (0.90 g, 38%). ^{1}H NMR (400 MHz, D_2O, 25 °C): δ = 2.19–2.26 (m, 1 H, H2′), 2.35–2.41 (m, 1 H, H2′′), 3.69 [dd, ^3J(H,H) = 12.5, ^3J(H,H) = 4.9 Hz, 1 H, H5′], 3.79 [dd, ^3J(H,H) = 12.6, ^3J(H,H) = 3.5 Hz, 1 H, H5′′], 3.96–4.00 (m, 1 H, H4′), 4.33–4.37 (m, 1 H, H3′), 6.12 (dd, $J_1 = J_2$ = 6.4 Hz, 1 H, H1′), 8.13 (s, 1 H, H6) ppm.

Nucleoside 1: 5-Amino-2′-deoxycytidine (**4**) (500 mg, 2.07 mmol) and 1,10-phenanthroline-5,6-dione (**5**) (435 mg, 2.07 mmol) were dissolved in 70% ethanol (8.25 mL) and heated in a closed vial at 95 °C for 12 h. The solution was allowed to cool to 22 °C and the precipitate collected, washed with cold 70% ethanol and dried to give compound **1** as a pale-yellow solid (620 mg, 72%). ^{1}H NMR (400 MHz, [D_6]DMSO, 25 °C): δ = 2.20–2.26 (m, 1 H, H2′), 2.52–2.58 (m, 1 H, H2′′), 4.09 (s, 2 H, H5′), 4.29–4.32 (m, 1 H, H4′), 4.42 (s, 1 H, O-H), 5.20 [d, J(H,H) = 4.3 Hz, 1 H, H3′], 6.03 [d, ^3J(H,H) = 6.5 Hz, 1 H, H1′], 6.26 (s, 1 H, H6), 7.88–7.94 (m, 2 H, Ar-H), 9.14 [dd, ^3J(H,H) = 4.4, ^3J(H,H) = 1.8 Hz, 1 H, Ar-H], 9.20–9.23 (m, 3 H, Ar-H), 11.23 (s, 1 H, NH) ppm. ^{13}C NMR (400 MHz, [D_6]DMSO, 25 °C): δ = 45.68, 71.36, 72.20, 84.53, 87.96, 90.06, 123.86, 124.03, 125.49, 126.00, 131.22, 132.32, 133.73, 134.86, 138.49, 144.26, 145.20, 146.99, 148.71, 150.58, 151.85 ppm.

Phosphoramidite 6: Compound **1** (100 mg, 0.24 mmol) and diisopropylammonium tetrazolide (100 mg, 0.58 mmol) were suspended in acetonitrile (10 mL) and the solvent removed in vacuo. After performing this procedure twice, the residue was kept under vacuum for 12 h. The solid was dissolved in anhydrous DMSO (8 mL) and $NC(CH_2)_2OP[N(iPr)_2]_2$ (0.200 mL, 0.62 mmol) added. The reaction mixture was stirred for 1 h, and another aliquot each of DIPAT and $NC(CH_2)_2OP[N(iPr)_2]_2$ were added. After stirring for another 1 h, CH_2Cl_2 (20 mL) was added and the organic layer washed with satd. $NaHCO_3$(aq) (3 × 20 mL) and brine (20 mL). The organic phase was dried with anhydrous Na_2SO_4 and the solvent removed in vacuo. The residue was dissolved in a minimum amount of dry CH_2Cl_2 and precipitated with petroleum ether. Precipitation was repeated twice and the residue dried under vacuum, to yield **6** (61 mg, 41%). ^{31}P NMR (400 MHz, $CDCl_3$, 25 °C): δ = 148.5, 148.9 ppm.

Oligonucleotide Synthesis: Oligonucleotides were synthesized on a 1000 nmol scale by using an ASM 800 DNA/RNA synthesizer with the manufacturer's standard protocols. Modified nucleotides were introduced by pausing the synthesiser program after completion of the prior cycle, removing the column from the synthesiser and running 200 μL of standard activator solution and 200 μL of a 0.1 M solution of **6** in CH_3CN back and forth through the column for 15 min. Then the column was re-mounted on the synthesiser to complete the cycle. The oligonucleotides were cleaved from the solid support, deprotected by using standard conditions (concd. aq. NH_3, 55 °C, 12 h) and purified by 20% denaturing polyacrylamide gel electrophoresis. The modified oligonucleotides were characterized by MALDI-ToF mass spectrometry: 5′-d(**1**GC CTC GCA TCG TG) (**X**) calcd. 4487, found 4487; 5′-d(**1**AC CTC GCA TCG TG) (**Y**) calcd. 4471, found 4471.

UV/Vis, Fluorescence and CD Studies: Samples of nucleoside **1** for UV/Vis and fluorescence measurements were prepared by dissolving **1** in DMSO and diluting the samples with DMSO until the absorbance at 377 nm was ca. 0.05. Fluorescence spectra were recorded with excitation at 365.5 nm and quantum yields determined by using anthracene in EtOH as a standard (Φ_F = 0.27). Samples of oligonucleotides for UV/Vis, fluorescence and CD measurements were prepared by dissolving 3 nmol of each strand in 100 μL of phosphate buffer, annealing in the case of duplex samples and diluting to the desired final concentration with phosphate buffer.

T_m Measurements: Samples for T_m measurements were prepared by annealing 3 nmol of each strand in 100 μL of phosphate buffer and diluting to 1000 μL with the same buffer. Samples were heated at a rate of 1 °C/min.

Molecular Modelling: Model helix structures were generated by using the program Hyperchem 8.0. A B-DNA helix was generated with standard parameters and the model nucleoside built into the structure. The modified base pair, along with the flanking base pair, were then minimized by using the Amber force-field, while keeping the rest of the helix frozen.

Supporting Information (see footnote on the first page of this article): CD spectra for duplexes **GI**, **GII**, **AI** and **AII**. Fluorescence spectra for oligonucleotides **X** and **Y** and helixes **GII** and **AII**. ^{1}H and ^{13}C NMR spectra of nucleoside **1**.

Acknowledgments

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