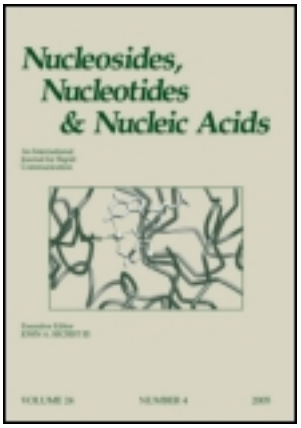

Nucleosides, Nucleotides and Nucleic Acids

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Synthesis and Properties of DNA Containing Cyclonucleosides

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SYNTHESIS AND PROPERTIES OF DNA CONTAINING CYCLONUCLEOSIDES

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□ *Here, we present efficient syntheses of the* R *and* S *diastereomers of 8,5′-cyclo-2′-deoxyadenosine and 6,5′-cyclo-2′-deoxyuridine. We incorporated these interesting nucleosides into DNA to study how the cyclo linkage affects the stability of duplex formation.*

Keywords Cyclonucleosides; 8-5′-cyclo-2′-deoxyadensine; 6,5′-cyclo-2′-deoxyuridine; TATA binding protein

INTRODUCTION

DNA damage is a ubiquitous part of the cellular life cycle. Oxidative stress is particularly problematic for nucleic acids. In many cases, this stress is initiated by a free radical, commonly the hydroxyl radical (·OH). Hydroxyl radicals are generated by both exogenous agents such as carcinogenic molecules and ionization radiation, as well as endogenous sources.[1] DNA damage by free radicals is commonly termed as "oxidatively induced damage to DNA" in living cells. Such damage has been shown to result in mutagenesis, carcinogenesis, and aging.[2] In terms of specific reactivity between these reactive oxygen species and DNA, two main adducts are likely: the addition of oxygen to the unsaturated bonds of the heterocyclic bases and abstraction of hydrogen atoms from the sugar backbone. H-abstraction has also been shown to occur at the methyl group of thymine, generating thymine dimers. As a result of these various damage pathways, a number of nucleobase and deoxyribose

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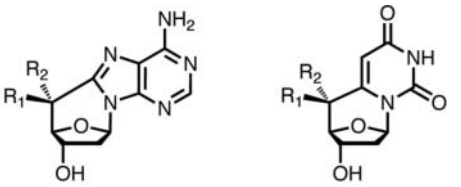


FIGURE 1 The *R* and *S* diastereomers of 8,5′-cyclo-2′-deoxyadenosine and 6,5′-cyclo-2′-deoxyuridine.

perturbations lead to single and double strand breaks, formation of abasic sites, DNA—protein, and carbohydrate-nucleobase cross-links.[3–7]

In this study, we focus on 8,5′-cyclo-2′-deoxyadenosine and 6,5′-cyclo-2′-deoxyuridine nucleosides (Figure 1). The 8,5′-cyclo-2′-deoxyadenosine represents a unique class of naturally occurring, helix-distorting DNA lesions that result in a second covalent linkage between the sugar and base moieties of a single nucleoside.[8] Unnatural 6,5′-cyclo-2′-deoxyuridine derivatives were also investigated, as they have similar structural features compared to the cyclo-deoxyadenosine moieties.

These lesions are initiated by H-abstraction at the C5′ by the hydroxyl radical species. This is followed by attack of the C5′-centered radical at the C8 of the purine base, leading to C8–C5′-cyclization. Subsequent oxidation of the resulting N7-centered radical results in the intramolecular cyclized product with a covalent bond between the C5′ and C8 positions of the purine nucleoside.[9]

Both *R* and *S* diastereomers (Figure 1) are possible and although they result in similar puckering and distortion, the unique position of the 5′ OH causes them to have different properties.[8,10–13] It has been shown that the *R*-diastereomer is more prevalent under γ-radiation of an anaerobic water solution of ss-DNA, whereas the *R*/*S* ratio is reversed in favor of the *S*-diastereomer in ds-DNA.[14] Moreover, it is postulated that the *R*- and *S*-diastereomers of cyclo-dA may be recognized differently during nucleoside excision repair due to the stereospecificity of the repair enzymes. Studies have shown that the *R*-diastereomer is more efficiently repaired than the *S*-diastereomer.[15,16] These differences demand that each diastereomer needs to be prepared separately, which lead to our synthetic efforts in this paper.

Cyclonucleoside lesions have major biological implications. These include blocking transcription and mutagenesis.[8] Studies have proven that the lesions can alter the binding of transcription factors to their cognate DNA recognition elements.[16] Cyclonucleosides can block the TATA binding protein (TBP) from associating with its target region, causing a transcriptional roadblock, which in turn shuts off gene expression. The exact mechanism of inhibition is not yet known.[8] TBP binds in the minor groove, causing a major local conformational distortion.[17] One of the reasons that nature

may have chosen the TATA box instead of a GCGC box for transcriptional control is the ease of A·T minor groove deformation rather than the stronger G·C base-pairs.[17] Because cyclonucleosides block TBP binding, several factors may be operating; the rigidity imparted by the extra covalent bond could deform the secondary structure of the oligonucleotide duplex, the cyclonucleoside could be forming a stronger base-pair with its Watson and Crick partner, or some combination of the two.

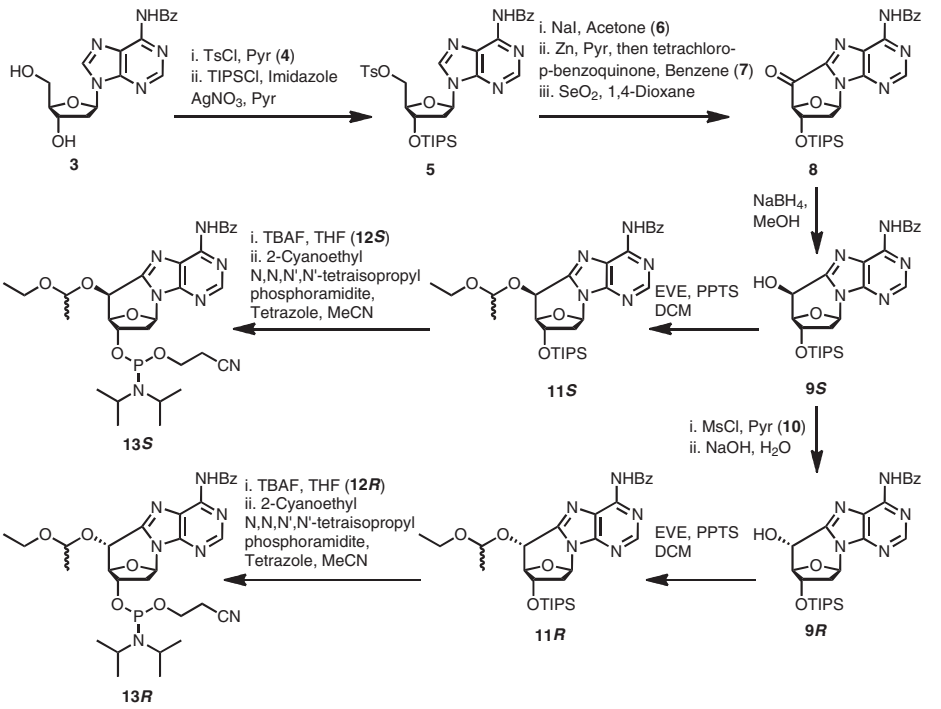
With stereochemically pure samples of these cyclic nucleosides, we hope to address the question of whether or not this lesion results in stronger hydrogen bond interactions with its Watson–Crick pair. The restricted flexibility of the C5′–C8 covalent bond might help to pre-organize and thereby reduce entropic loss while forming a base pair. On the other hand, if these lesions destabilize the duplex formation, it will confirm that the deformation caused by the extra bond overpowers the effect of the weak A·T base pair of the TATA box, and results in TBP inhibition. Because 6,5′-cyclo-2′-deoxyuridine features a similar lesion, it was also synthesized to aid in the study of duplex stability. Due to synthetic constraints, the deoxyuridine monomer was produced instead of thymidine in order to retain aromaticity after introducing the C6–C5′ bond.

RESULTS AND DISCUSSION

Chemistry

The focus of this study was to prepare four nucleosides (two sets of diastereomers) based upon dA and dU. Introducing a second bond between the sugar and the heterocycle would rigidify the nucleosides. These cyclonucleosides have a second bond between C5′ and C8 for the 2′deoxyadenosine and C5′ and for 2′-deoxyuridine. Model building studies conducted in our lab suggested that cross-links at these locations would result in glycosidic bond angles similar to those observed in double-stranded nucleic acids.

The cyclo-2′-deoxyadenosine monomer was initiated from N-benoyl-2′-deoxyadenosine (**3**) with two protection steps to generate compound **5** (Scheme 1). Synthesis of **8** was carried out in three steps. Substitution of the 5′-tosyl by iodide permitted a zinc-mediated cyclization to generate 8,5′-cyclo-2′,5′-dideoxyadenosine. Oxidation with SeO_2 yielded the ketone **8**, which upon reduction with $NaBH_4$ formed the *S* diastereomer exclusively. To obtain the *R* isomer, **9*S*** was mesylated and then inverted with hydroxide. From this point forward the two diastereomers were processed in parallel. The newly formed 5′ hydroxyls of both the *R* and *S* diastereomers were unable to be protected with the standard 4,4′-dimethoxytrityl chloride (DMTrCl) protecting group due to steric interactions. Instead we opted to use ethyl vinyl ether, which has similar deprotecting conditions as DMTrCl, to form 1-ethylethoxy protected **11*S*** and **11*R***. The ethyl vinyl ether



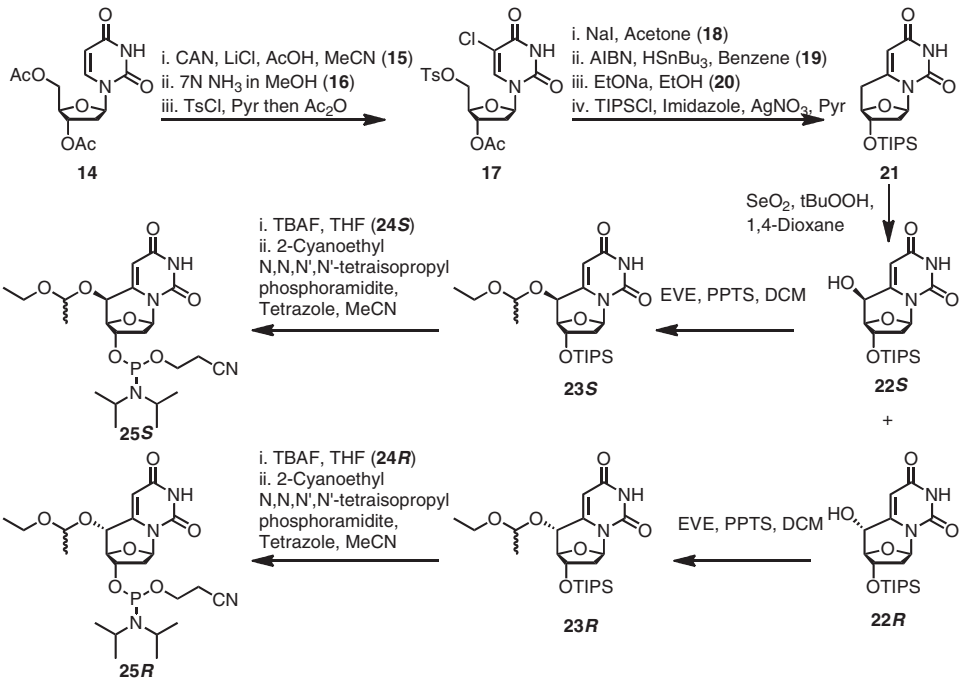
SCHEME 1 Synthesis of the *R* and *S* 8,5′-cyclo-2′-deoxyadenosine phosphoramidites.

protection resulted in two additional diastereomers at the newly formed methyl position, which co-migrated during silica gel chromatography. The final steps involved removal of the 3′ silyl protecting group and conversion to the 3′-phosphoramidites (**13*S***, **13*R***). The presence of the two phosphorus diastereomers and the two EVE diastereomers could be confirmed by the presence of four phosphorus resonances in the final *R* and *S* products (e.g., for **13*R***, ^{31}P δ 147.4, 147.6, 147.7, 147.9 ppm).

The 3′,5′-Diacetyl-2′-deoxyuridine (**14**) was converted to the fused derivative **20** using an established protocol (Scheme 2) with the exception of the chlorination reaction on the C5 position, which was more efficiently accomplished using CAN (cerium (IV) ammonium nitrate)/LiCl in 88% yield.[18] The 3′-hydroxyl protected 6,5′-cyclo-2′,5′-dideoxyuridine, **21**, was converted to the 5′-hydroxyl compounds, **22*S*** and **22*R*,** using 0.5 eq. SeO_2 and 1.5 eq. tBuOOH.[19] Column chromatography separated the two derivatives in approximately 40% yield each, for an overall 80% yield.

Crystal Structure and Thermal Behavior

The four cyclonucleosides were incorporated into 12-mer or 14-mer sequences containing a core d(AAAA)/d(TTTT) sequence. In the purified oligos, the presence of the cyclonucleosides had no effect on the activity of



SCHEME 2 Synthesis of the *R* and *S* 6,5′-cyclo-2′-deoxyuridine phosphoramidites.

nuclease enzymes, allowing us to digest the oligos and confirm the incorporation by HPLC.

After synthesizing these compounds and obtaining crystal structural information (Figure 2), it became clear that these nucleosides would not enhance duplex stability. The reasoning behind this is that the C5′–C8 (dA) and C5′–C6 (dU) bonds "pull" the heterocycle away from the center of the DNA double helix, preventing it from forming a Watson–Crick base pair.

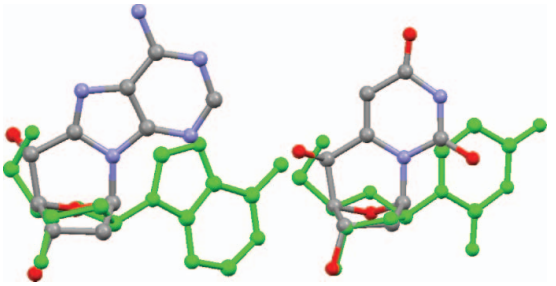


FIGURE 2 The crystal structures of **10*R***, with protecting groups hidden, and deprotected **24*S*** overlayed with 2′-dA and 2′-dU[20,21] (Color figure available online).

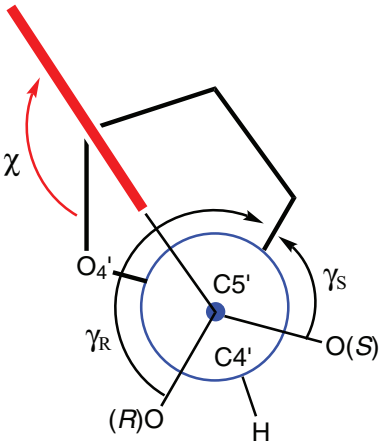


FIGURE 3 An overhead view of a cyclonucleoside highlighting the relevant torsion angles (Color figure available online).

Torsion angles can help to explain the nature of the duplexes containing rigid nucleosides. By introducing the bond between C8 and C5′ in cyclo-2′-deoxyadenosine and C6 and C5′ in cyclo-2′-deoxyuridine the glycosidic torsion angles, χ, became fixed at $-146.7°$ and $-152.1°$ respectively (Figure 3). The dA-dT core in the Dickerson Dodecamer has χ values ranging from near $-80°$ to near $-115°$ while the corresponding RNA sequence exhibits χ values around $-160°$. These values suggest that the fused nucleosides might be better accommodated in an RNA A-like duplex; however, even these RNA/DNA heteroduplexes were destabilized (see Table 1).

The torsion angle for the C4′–C5′ bond (γ) is also problematic. The γ torsion angle prefers a value close to $70°$ for RNA and a value near $40°$ for DNA. In these conformations, the 5′O is positioned above the sugar and angled toward the heterocycle. In the case of the modified nucleosides, that position is occupied up by the C8–C5′ bond in cyclo-2′-deoxyadenosine or the C6–C5′ bond in cyclo-2′-deoxyuridine. The fused 5′OH is left in a position normally inhabited by the C5′ hydrogens. The *S* diastereomer results in a γ torsion angle near $-50°$ while the *R* produces a torsion angle of about $-170°$.

CONCLUSIONS

Our effort toward preparing pure diastereomers of nucleosides of cyclo-2′-deoxyadenosine and cyclo-2′-deoxyuridine in appreciable yields has been successful. We incorporated these nucleosides into DNA sequences and found that the T_m in most of the cases is reduced. These results are in accordance with theoretical studies performed in the labs of John Miller.[12] Our data proves that cyclonucleoside lesions destabilize duplex formation.

TABLE 1 Thermal denaturation studies of DNA containing cyclonucleosides (cyclonucleosides denoted by underline and bold text)

	Sequence	T_M
1	5′-d(C C G G A A A A C G C C)/5′-d(G G C G T T T T C C G G)	49
2	5′-d(C C G G **A**$_S$ A **A**$_S$ A C G C C)/5′-d(G G C G T T T T C C G G)	40
3	5′-d(C C G G **A**$_R$ A **A**$_R$ A C G C C)/5′-d(G G C G T T T T C C G G)	36
4	5′-d(C C G G A A A A C G C C)/5′-d(G G C G **U**$_S$ T **U**$_S$ T C C G G)	28
5	5′-d(C C G G A A A A C G C C)/5′-d(G G C G **U**$_R$ T **U**$_R$ T C C G G)	36
6	5′-d(C C G G A A A A A C G C C)/5′-d(G G C G T T T T T T C C G G)	51
7	5′-d(C C G G A **A**$_S$ **A**$_S$ **A**$_S$ **A**$_S$ A C G C C)/5′-d(G G C G T T T T T T C C G G)	17
8	5′-d(C C G G A **A**$_R$ **A**$_R$ **A**$_R$ **A**$_R$ A C G C C)/5′-d(G G C G T T T T T T C C G G)	28
9	5′-d(C C G G A A A A A C G C C)/5′-d(G G C G T **U**$_S$ **U**$_S$ **U**$_S$ **U**$_S$ T C C G G)	28
10	5′-d(C C G G A A A A A C G C C)/5′-d(G G C G T **U**$_R$ **U**$_R$ **U**$_R$ **U**$_R$ T C C G G)	16
11	5′-d(C C G G A **A**$_S$ **A**$_S$ **A**$_S$ **A**$_S$ A C G C C)/5′-d(G G C G T **U**$_S$ **U**$_S$ **U**$_S$ **U**$_S$ T C C G G)	14
12	5′-d(C C G G A **A**$_S$ **A**$_S$ **A**$_S$ **A**$_S$ A C G C C)/5′-d(G G C G T **U**$_R$ **U**$_R$ **U**$_R$ **U**$_R$ T C C G G)	*
13	5′-d(C C G G A **A**$_R$ **A**$_R$ **A**$_R$ **A**$_R$ A C G C C)/5′-d(G G C G T **U**$_S$ **U**$_S$ **U**$_S$ **U**$_S$ T C C G G)	*
14	5′-d(C C G G A **A**$_R$ **A**$_R$ **A**$_R$ **A**$_R$ A C G C C)/5′-d(G G C G T **U**$_R$ **U**$_R$ **U**$_R$ **U**$_R$ T C C G G)	*
15	5′-d(C C G G A A A A C G C C)/5′-G G C G U U U U C C G G	43
16	5′-d(C C G G **A**$_S$ A **A**$_S$ A C G C C)/5′-G G C G U U U U C C G G	29
17	5′-d(C C G G **A**$_R$ A **A**$_R$ A C G C C)/5′-G G C G U U U U C C G G	30

*= Duplex formation was not observable.

This supports the hypothesis that duplex deformation caused by this lesion overpowers the effects of the weaker TATA box base pairing, thus inhibiting TBP binding. Our crystal structures show that the nucleobase is "pulled" back from its Watson and Crick position, explaining why these molecules form weaker hydrogen bonds as a base pair. These compounds are of continued interest due to the fact that they are resistant toward enzymatic repair and may be valid drug targets.[22]

EXPERIMENTAL

General Information

Reagents were purchased from Sigma-Aldrich, Acros, Oakwood, Glen Research, Lancaster, MP Biomedical, Chem-Impex International, Fisher, Chem Genes and Molekula. Flash column were performed using dynamic adsorbents silica gel (60 Å, particle size 32–63 μm) and TLC monitoring with TLC Silica Gel with F-254 Indicator (Dynamic Adsorbents). TLCs were visualized by 260 nm UV light and stained by 10% sulfuric acid. All reactions were carried out under a nitrogen atmosphere with dry solvent under anhydrous conditions unless indicated otherwise. Dry tetrahydrofuran (THF), diethyl ether (Et_2O), *N*,*N*-dimethylformamide (DMF), pyridine (pyr), acetonitrile (MeCN), and dichloromethane (DCM) were obtained by passing commercially available pre-dried, oxygen-free formulations through activated alumina columns. Dry ethanol was purchased from Sigma–Aldrich and used

directly. NMR spectra were taken by Varian VNMRS400, VNMRS500, or INOVA 500 instruments and calibrated using residual undeuterated solvent (CDCl3: δ H = 7.24 ppm, δ C = 77.23 ppm, DMSO-*d*6: δ H = 2.50 ppm, δ C = 39.51 ppm). Abbreviations of multiplicities were designated as follow: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and b = broad. High-resolution mass spectra (HRMS) were recorded on a waters LCT or JEOL AccuTOF mass spectrometer using ESI (electrospray ionization) or DART (direct analysis in real time). UV spectra were acquired by Beckman (Fullerton, CA, USA) DU650 spectrophotometer. HPLC was performed on Waters (Milford, MA, USA) Delta 600 controllers with a 2487 dual wavelength detector. DNA oligomers were prepared using an Applied Biosystems 394 DNA/RNA synthesizer with standard solid phase phosphoramidite techniques and reagents purchased from Glenn Research (Sterling, VA, USA). RNA oligomers were purchased from Integrated DNA Technologies (Coralville, IA, USA). Polyacrylamide Gel Electrophoresis was performed by using a Hoefer Scientific (San Francisco, CA, USA) apparatus. Gel imaging was performed on Bio-Rad (Hercules, CA, USA) FXpro Molecular Imager with ethidium bromide staining or Kodak-K radioisotope screens.

Synthesis

N-Benzoyl-5′-tosyl-2′-deoxyadenosine (4)

N-benoyl-2′-deoxyadenosine (10.2 g, 28.7 mmol) was dissolved in dry pyridine (140 mL) and cooled to 0°C. *p*-tosylsulfonyl chloride (8.20 g, 43.0 mmol) was added and the reaction mixture was allowed to warm to ambient temperature and stir overnight. The reaction was quenched with water at 0°C and solvents were removed under reduced pressure. The residue was dissolved in DCM and washed with water three times and once with brine. The organic layer was dried over sodium sulfate and concentrated in a rotary evaporator. The crude product was purified by flash chromatography (DCM/MeOH 95:5) to yield the **4** as a white solid (12.1 g, 83%). TLC R_f = 0.32 (DCM/MeOH 90:10). ^{1}H NMR (400 MHz; $CDCl_3$): δ 2.43 (s, 3H), 2.49 (d, 1H), 2.58 (m, 1H), 2.97 (m, 1H), 4.23–4.3 (m, 3H), 4.83 (bm, 1H), 6.49 (t, 1H), 7.31 (d, 2H), 7.54 (t, 2H), 7.63 (t, 1H), 7.74 (d, 2H), 8.03 (dd, 2H), 8.18 (s, 1H), 8.75 (s, 1H), 8.98 (s, 1H). ^{13}C NMR (100 MHz; CD_3OD): δ 41.6, 63.4, 72.8, 86.8, 89.9, 125.6, 129.6, 129.6, 129.9, 129.9, 134.0, 134.2, 134.3, 135.1, 144.7, 146.5, 151.3, 153.0, 153.1, 168.3. ESI-MS (pos.): 510.1455 $[M+H]^+$ (HSMS calc. 510.1447).

N-Benzoyl-3′-triisopropylsilyl-5′-tosyl-2′-deoxyadenosine (5)

4 (5.60 g, 11.0 mmol), imidazole (2.30 g, 33.4 mmol), and silver nitrate (3.78 g, 22.2 mmol) were dissolved in dry pyridine (56 mL) and cooled to 0°C. Triisopropylsilyl chloride (4.70 ml, 22.0 mmol) was added and the

reaction was warmed to ambient temperature. After stirring for two days the reaction was quenched with water while on an ice bath. The solvents were removed under reduced pressure and the residue dissolved in DCM and washed with water three times and once with brine. The organic layer was dried over sodium sulfate and concentrated under vacuum. The crude product was purified by flash chromatography (DCM/MeOH 97:3) to yield **5** as a white foam (5.2 g, 72%). TLC $R_f = 0.65$ (DCM/MeOH 97:3). ^{1}H NMR (400 MHz; $CDCl_3$): δ 1.07–1.12 (m, 21H), 2.42 (s, 3H), 2.47–2.51 (m, 1H), 2.93 (m, 1H), 4.22 (s, 1H), 4.19–4.3 (m, 2H), 4.73 (t, 1H), 6.47 (t, 1H), 7.29 (d, 2H), 7.52 (t, 2H), 7.6 (q, 1H), 7.74 (d, 2H), 8.03 (d, 2H), 8.20 (s, 1H), 8.73 (s, 1H). ^{13}C NMR (100 MHz; $CDCl_3$): δ 11.9, 17.9, 21.6, 40.3, 68.8, 73.0, 85.0, 85.4, 123.4, 125.8, 127.9, 128.8, 129.9, 132.2, 132.8, 133.4, 141.9, 145.2, 149.4, 151.4, 152.1, 164.7. ESI-MS (pos.): 666.2762 $[M+H]^+$ (HSMS calc. 666.2782).

N-Benzoyl-3′-triisopropylsilyl-5′-iodo-2′-deoxyadenosine (6)

5 (4.26 g, 6.40 mmol) and sodium iodide (3.84 g, 2.56 mmol) were dissolved in dry acetone (32 mL) and refluxed for four hours. The solid suspension filtered through Celite and the filtrate was concentrated under vacuum. The crude was purified by flash chromatography (DCM/MeOH 97.5/2.5) to yield **6** as a white foam (3.9 g, 98%) TLC $R_f = 0.42$ (DCM/MeOH 97.5/2.5). ^{1}H NMR (400 MHz; $CDCl_3$): δ 1.04–1.14 (m, 21H), 2.23 (s, 1H), 2.47 (m, 1H), 3.08 (m, 1H), 3.36–3.53 (m, 2H), 4.07 (m, 1H), 4.70 (t, 1H), 6.44 (t, 1H), 7,47 (t, 2H), 7.56 (t, 1H), 7.99 (d, 2H), 8.24 (s, 1H), 8.74 (s, 1H), 9.22 (s, 1H). ^{13}C NMR (100 MHz; $CDCl_3$): δ 6.3, 12.0, 18.0, 39.9, 75.4, 85.2, 86.7, 123.7, 127.8, 128.8, 132.7, 133.6, 142.2, 149.6, 151.4, 152.5, 164.6. ESI-MS (pos.): 622.1715 $[M+H]^+$ (HSMS calc. 622.1710).

N-Benzoyl-3′-triisopropylsilyl-8,5′-cyclo-2′,5′-dideoxyadenosine (7)

6 (1.25 g, 2.00 mmol) and zinc powder (2.63 g, 40.2 mmol) were dissolved in dry pyridine (70 mL) and heated to 80°C for 4 hours. The zinc powder was removed by filtration and the filtrate was concentrated under reduced pressure. The residue and tetrachloro-1,4-benzoquinone (0.490 g, 2.00 mmol) were dissolved in benzene (40 mL) and refluxed for 2 hours. The solvent was then evaporated under vacuum and the crude product was purified by flash chromatography (DCM/MeOH 98:2) to yield the cyclized product **7** as a yellow foam (0.42 g, 42%). TLC $R_f = 0.32$ (DCM/MeOH 97:3). ^{1}H NMR (400 MHz; $CDCl_3$): δ 0.98–1.07 (m, 21H), 2.36 (m, 1H), 2.66 (m, 1H), 3.11 (d, 1H), 3.58 (m, 1H), 4.51 (m, 1H), 4.80 (d, 1H), 6.58 (d, 1H), 7.50 (t, 2H), 7.59 (t, 1H), 7.99 (t, 2H), 8.68 (s, 1H), 8.74 (s, 1H). ^{13}C NMR (100 MHz; $CDCl_3$): δ 11.8, 17.8, 31.2, 48.1, 75.3, 83.2, 84.8, 127.8, 128.8, 132.8, 133.5, 146.8, 147.4, 148.1, 148.3, 151.8, 164.5. ESI-MS (pos.): 516.2425 $[M+Na]^+$ (HSMS calc. 516.2407).

N-Benzoyl-3′-triisopropylsilyl-5′-keto-8,5′-cyclo-2′-deoxyadenosine (8)

7 (0.186 g, 0.377 mmol) and selenium dioxide (90.0 mg, 0.814 mmol) were dissolved in 1,4-dioxane (50 mL) and refluxed for 1 hour. The reaction mixture was filtered through Celite and the solvent removed under vacuum. The product was purified by flash chromatography (DCM/MeOH 97:3) to yield **8** as a yellow foam (0.170 g, 89%). TLC $R_f = 0.23$ (DCM/MeOH 95:5). ^{1}H NMR (400 MHz; $CDCl_3$): δ 1.02–1.07 (m, 21H), 2.14 (s, 1H), 2.56 (m, 1H), 2.72 (m, 1H), 4.76 (dd, 1H), 4.96 (s, 1H), 6.83 (d, 1H), 7.52 (t, 2H), 7.61 (t, 1H), 7.97 (d, 2H), 8.92 (s, 1H), 9.19 (s, 1H). ^{13}C NMR (100 MHz; $CDCl_3$): δ 11.8, 17.8, 44.8, 72.7, 85.9, 92.3, 122.9, 127.9, 129.0, 133.2, 140.8, 147.9, 148.3, 152.3, 156.1, 164.3, 184.6. ESI-MS (pos.): 530.2186 $[M+Na]^+$ (HSMS calc. 530.2200).

N-Benzoyl-3′-triisopropylsilyl-8,5′ (S)-cyclo-2′-deoxyadenosine (9S)

Compound **8** (0.454 g, 0.890 mmol) was dissolved in methanol (27 mL) and cooled to 0°C, 0.1 M sodium borohydride aqueous solution (5 mL) was added and the stirring reaction was allowed to warm to ambient temperature. After 1.5 hours the reaction was neutralized with 1N hydrochloric acid and the solvents were removed under vacuum. The crude product was purified by flash chromatography (DCM/MeOH 95:5) to yield **9*S*** as a white powder (0.425 g, 93%). TLC $R_f = 0.22$ (DCM/MeOH 97:3). ^{1}H NMR (400 MHz; $CDCl_3$): δ 0.95–1.03 (m, 21H), 2.31 (m, 1H), 2.55 (s, 1H), 4.56 (d, 1H), 4.88 (q, 1H), 5.03 (s, 1H), 5.46 (d, 1H), 6.50 (d, 1H), 7.50 (t, 2H), 7.59 (t, 1H), 7.97 (d, 2H), 8.69 (s, 1H), 8.93 (s, 1H). ^{13}C NMR (100 MHz; $CDCl_3$): δ 11.8, 17.9, 47.0, 64.8, 69.3, 85.5, 86.4, 122.6, 127.8, 128.9, 132.9, 133.4, 148.6, 149.9, 151.5, 152.5, 164.8. ESI-MS (pos.): 510.2534 $[M+H]^+$ (HSMS calc. 510.2537).

N-Benzoyl-3′-triisopropylsilyl-5′-mesyl-8,5′ (S)-cyclo-2′-deoxyadenosine (10)

Compound **9*S*** (0.570 g, 0.570 mmol) was dissolved in pyridine (5.7 mL) and methanesulfonyl chloride (110 μL, 1.43 mmol) was added at 0°C. The reaction was slowly warmed to ambient temperature and stirred overnight before being quenched with water. The solvents were removed under vacuum and the residue was dissolved in DCM and washed with water three times and once with brine. After removing the DCM under reduced pressure, the crude product was purified by flash chromatography (DCM/MeOH 97.5:2.5) to yield the mesylated product **10** as a white powder (0.31 g, 91%). TLC $R_f = 0.38$ (DCM/MeOH 97.5:2.5). ^{1}H NMR (400 MHz; $CDCl_3$): δ 1.01–1.10 (m, 21H), 2.40 (m, 1H), 2.66 (m, 1H), 3.57 (s, 3H), 4.89–4.93 (m, 2H), 6.12 (d, 1H), 6.55 (d, 1H), 7.52 (t, 2H), 7.61 (t, 1H), 7.94 (d, 2H), 8.68 (s, 1H), 8.73 (s, 1H). ^{13}C NMR (100 MHz; $CDCl_3$): δ 11.8, 17.9, 40.0, 46.7, 70.1, 70.4, 85.1, 85.5, 122.8, 127.8, 129.0, 133.0, 133.4, 145.2, 149.2, 150.0, 152.9, 164.5. ESI-MS (pos.): 610.2136 $[M+Na]^+$ (HSMS calc. 610.2132).

N-Benzoyl-3′-triisopropylsilyl-8,5′ (R)-cyclo-2′-deoxyadenosine (9R)

Compound **10** (0.370 g, 0.630 mmol) was dissolved in a DMF/water mixture (50:17.6 mL) and 0.1 M sodium hydroxide (7.55 mL) was added over 5 hours and after complete addition stirred for an additional hour. The reaction mixture was neutralized with 1 N hydrochloric acid and the solvents were removed under vacuum. The crude product was purified by flash chromatography (DCM/MeOH 97:3) to yield **9*R*** as a white powder (0.26 g, 80%). TLC $R_f = 0.17$ (DCM/methanol = 30/1). ^{1}H NMR (400 MHz; $CDCl_3$): δ 1.01–1.08 (m, 21H), 2.35 (m, 1H), 2.54 (m, 1H), 4.42 (q, 1H), 4.67 (s, 1H), 4.94 (d, 1H), 6.19 (s, 1H), 6.61 (d, 1H), 7.53 (dt, 2H), 7.59 (dt, 1H), 8.07 (dd, 2H), 8.75 (s, 1H), 9.32 (s, 1H). ^{13}C NMR (100 MHz; $CDCl_3$): δ 11.8, 17.9, 45.9, 65.8, 71.7, 85.2, 89.0, 121.6, 128.2, 128.8, 132.9, 133.5, 148.0, 149.1, 149.5, 153.1, 165.0. ESI-MS (pos.): 532.2351 $[M+Na]^+$ (HSMS calc. 532.2356).

N-Benzoyl-3′-triisopropylsilyl-5′-(1-ethoxyethyl)-8,5′ (S)-cyclo-2′-deoxyadenosine (11S) and N-Benzoyl-3′-triisopropylsilyl-5′-(1-ethoxyethyl)-8,5′ (R)-cyclo-2′-deoxyadenosine (11R)

Compound **9*S*** (0.150 g, 0.300 mmol) and pyridinium-*p*-toluenesulfonate (75.0 mg, 0.300 mmol) were dissolved in DCM (3 mL) followed by the addition of ethyl vinyl ether (0.175 mL, 1.80 mmol) and stirred overnight at ambient temperature. The solvents were removed under vacuum and the crude product was purified by flash chromatography (DCM/MeOH/Et_3N 96:3:1) to yield **11*S*** as a yellow foam (0.12 g, 86%). TLC $R_f = 0.3$ (DCM/MeOH 97:3). ^{1}H NMR (400 MHz; $CDCl_3$): δ 1.04–1.26 (m, 21H), 1.25–1.30 (bm, 3H), 1.50 (dd, 3H, $J = 40.2, 5.4$ Hz), 2.34 (m, 1H), 2.60 (m, 1H), 3.62–3.88 (bm, 2H), 4.74 (dd, 1H, $J = 18.0, 6.4$ Hz), 4.95 (m, 1H), 5.21–5.56 (dq, 1H, $J = 132.4, 5.2$ Hz), 5.33 (dd, 1H, $J = 14.4, 6.0$ Hz), 6.52 (q, 1H), 7.52 (m, 2H), 7.61 (dt, 1H), 7.97 (m, 2H), 8.74 (s, 1H). ESI-MS (pos.): 582.3085 $[M+H]^+$ (HSMS calc. 582.3112).

11*R* was prepared using the same procedure as **11*S*** with the exception of chromatographic conditions (DCM/MeOH/Et_3N 96.4/2.5/1) to yield the product as a yellow foam (0.12 g, 90%). TLC $R_f = 0.45$ (DCM/MeOH 97:3). ^{1}H NMR (400 MHz; $CDCl_3$): δ 1.01–1.08 (m, 21H), 1.21–1.28 (bm, 3H), 1.40 (dd, 3H, $J = 60.8, 5.6$ Hz), 2.32 (m, 1H), 2.48 (m, 1H), 3.59–3.77 (bm, 2H), 4.38 (q, 1H), 4.82 (d, 1H), 4.87 (s, 1H), 5.14–5.44 (dq, 1H, $J =$ 109.6, 5.6 Hz), 6.60 (d, 1H), 7.52 (dt, 2H), 7.60 (dt, 1H), 7.97 (dd, 2H), 8.76 (s, 1H), 8.86 (d, 1H). ESI-MS (pos.): 604.2913 $[M+Na]^+$ (HSMS calc. 604.2931).

N-Benzoyl -5′-(1-ethoxyethyl)-8,5′ (S)-cyclo-2′-deoxyadenosine (12S) and N-Benzoyl-5′-(1-ethoxyethyl)-8,5′ (R)-cyclo-2′-deoxyadenosine (12R)

Tetra-n-butylammonium fluoride (1.0 M solution in THF, 0.50 mL) was added to a solution of **11*S*** (0.132 g, 0.227 mmol) in THF (1.5 mL) at ambient temperature and stirred for 1 hour. The solvent was removed under

reduced pressure and the crude product was purified by flash chromatography (DCM/MeOH/Et_3N 96.5:2.5:1) to yield the **12*S*** as a white powder in quantitative yield (95 mg). TLC $R_f = 0.22$ (DCM/MeOH 97:3). ^1H NMR (400 MHz; $CDCl_3$): δ 1.07–1.27 (dt, 3H), 1.25–1.48 (dd, 3H), 2.32 (m, 1H), 2.59–2.66 (bm, 3H), 3.62 (m, 1H), 3.81 (m, 1H), 4.84 (d, 1H), 4.93 (q, 1H), 5.28 (d, 1H), 5.33 (q, 1H), 6.50 (d, 1H), 7.52 (dt, 2H), 7.60 (dt, 1H), 7.97 (dd, 2H), 8.74 (s, 1H). ESI-MS (pos.): 426.1780 $[M+H]^+$ (HSMS calc. 426.1777). **12*R*** was prepared using the same procedure as the *S* diastereomer to yield the product as a white powder in quantitative yield (92 mg). TLC $R_f = 0.17$ (DCM/MeOH 97.5:2.5). ^1H NMR (400 MHz; $CDCl_3$): δ 1.19–1.30 (bm, 3H), 1.41 (dd, 3H, $J = 47.6$, 5.6 Hz), 2.28 (m, 1H), 2.49 (m, 1H), 2.96 (q, 2H), 3.59–3.78 (bm, 2H), 4.42 (m, 1H), 4.86 (s, 1H), 4.87 (s, 1H), 5.14–5.36 (dq, 1H, $J = 82.8$, 5.6 Hz), 6.59 (d, 1H), 7.50 (dt, 2H), 7.59 (t, 1H), 7.97 (d, 2H), 8.73 (s, 1H). ESI-MS (pos.): 426.1772 $[M+H]^+$ (HSMS calc. 426. 1777).

N-Benzoyl-3′-(2-cyanoethyl N,N-diisopropyl phosphorodiamidite)-5′-(1-ethoxyethyl)-8,5′ (S)-cyclo-2′-deoxyadenosine (13S) and N-Benzoyl-3′-(2-cyanoethyl N,N-diisopropyl phosphorodiamidite) -5′-(1-ethoxyethyl)-8,5′ (R)-cyclo-2′-deoxyadenosine (13R)

Compound **12*S*** (66.0 mg, 0.155 mmol) was dissolved in acetonitrile (6.2 mL). 1H-tetrazole in acetonitrile (0.45 M solution in MeCN, 0.21 mL) was added followed by 2-Cyanoethyl N,N,N′,N′-tetraisopropyl phosphorodiamidite (61.0 μL, 0.186 mmol) and the mixture was stirred overnight at ambient temperature. The reaction was quenched with 5% triethylamine in methanol and the solvents were removed under vacuum. The residue was dissolved in DCM and washed with saturated sodium bicarbonate three times and once with brine. The organic layer was dried over sodium sulfate. After removing the sodium sulfate, hexanes were added to effect precipitation. The precipitate was collected by filtration and washed with hexanes several times to yield **13*S*** as a white powder (60 mg, 60%). TLC $R_f = 0.39$ (DCM/MeOH 95:5). ^{31}P NMR (162 MHz; $CDCl_3$): δ 146.7, 146.8, 147.0, 147.2. ESI-MS (pos.): 648.2671 $[M+Na]^+$ (HSMS calc. 648.2675). Compound **13*R*** was obtained by a similar procedure as **13*S*** to yield a white powder (22 mg, 50%). TLC $R_f = 0.42$ (DCM/MeOH 95:5). ^{31}P NMR (162 MHz; $CDCl_3$): δ 147.4, 147.6, 147.7, 147.9. ESI-MS (pos.): 648.2665 $[M+Na]^+$ (HSMS calc. 648.2675).

5-Chloro-3′,5′-diacetyl-2′-deoxyuridine (15)

Compound **14** (34.2 g, 109 mmol) was dissolved in a acetonitrile acetic acid mixture (1:1, 600 mL total) and cerium (IV) ammonium nitrate (120 g, 220 mmol) and lithium chloride (5.64 g, 133 mmol) were added at ambient temperature, then slowly heated to 80°C and kept for 6 hours. When TLC

monitoring showed the reaction was complete the reaction was quenched with water and the solvent was removed under reduced pressure. The orange residue was taken up in ethyl acetate (400 mL), washed with water (3 × 150 mL) and brine (150 mL). After drying over anhydrous Na_2SO_4, the organic layer was concentrated under reduced pressure and purified by flash chromatography (DCM/Acetone 90:10), to yield **15** (37.6 g, 87.9%) as a white foam: $R_f = 0.34$ (DCM/Acetone 90:10). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.10 (s, 3H), 2.15 (s, 3H), 2.19 (s, 1H), 2.51 (m, 1H), 4.28 (m, 1H), 4.39 (m, 2H), 5.22 (d, J = 4.0 Hz, 1H), 6.28 (t, J = 6.0 Hz 1H), 7.78 (s, 1H), 8.86 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$), δ 21.0, 21.1, 38.1, 64.0, 74.3, 82.6, 85.5, 103.2, 139.0, 150.2, 162.8, 170.4, 170.6. HRMS (ESI) Calcd for $C_{13}H_{16}ClN_2O_7$, 347.06460; Found, 347.06459.

5-Chloro -2′-deoxyuridine (16)

15 (19.6 g, 56.6 mmol) was dissolved in methanol (80 mL) and 7 N ammonia in methanol (80 mL) was added at room temperature. After stirring for four hours the solvent was removed under reduced pressure and the resulting brown oil was purified by flash chromatography (DCM/MeOH 90:10) to yield **16** (14.1 g, 94.8%): R_f = 0.33 (DCM/MeOH 90:10). The spectral data obtained for this compound was consistent with that reported by Suzuki.[18]

5-Chloro-3′-acetyl-5′-tosyl-2′-deoxyuridine (17)

16 (19.6 g, 74.7 mmol) was dissolved in pyridine (500 mL) and cooled to 0°C. A solution of tosyl chloride (2.53 M in dry pyridine, 40 mL, 101 mmol) was added drop wise over 45 minutes. The reaction mixture was slowly warmed to ambient temperature and then stirred overnight. To the reaction mixture was added acetic anhydride (17.5 mL, 186 mmol), and the mixture stirred at ambient temperature for 4 hours. The reaction was quenched with water and the volatiles were removed under reduced pressure and the brown oil-like residue was purified by flash chromatography (DCM/Acetone 90:10) to yield **17** as a white powder (20.9 g, 61.2%): R_f = 0.42 (DCM/Acetone 90:10). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.08 (s, 3H), 2.10–2.19 (m, 1H), 2.44 (s, 3H), 2.47–2.49 (m, 1H), 4.19 (d, J = 2.0 Hz, 1H), 4.23–4.39 (m, 2H), 5.21 (d, J = 6.8 Hz, 1H), 6.29 (q, J = 4.8 Hz, 1H), 7.37 (d, J = 8.4 Hz, 2H), 7.79 (s, 1H), 7.81 (d, J = 8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 21.0, 21.9, 37.7, 69.0, 74.8, 82.8, 85.5, 110.2, 128.2, 130.5, 132.3, 136.3, 145.9, 149.2, 158.4, 170.7,. HRMS (ESI) Calcd for $C_{18}H_{20}ClN_2O_8S$, 459.06289; Found, 459.06250.

5-Chloro-3′-acetyl-5′-iodo-2′-deoxyuridine (18)

A mixture of **17** (1.30 g, 2.84 mmol) and sodium iodide (2.13 g, 14.2 mmol) was dissolved in anhydrous acetone and heated to reflux for

6 hours. After quenching with water, volatiles were removed under reduced pressure. The crude residue was dissolved in ethyl acetate (30 mL) and washed with 2M sodium thiosulfate (25 mL) and brine (25 cmL). The organic layer was dried over sodium sulfate and concentrated under vacuum to yield **18** as a white foam. This crude material was of sufficient purity to be carried on to the next step. $R_f = 0.50$ (DCM/Acetone 90:10).

6,5′-cyclo-2′,5′-Dideoxyuridine (20)

18 (11.3 g, 27.3 mmol) was dissolved in benzene (300 mL) and heated to reflux. A mixture of Bu_3SnH (6.9 mL, 26.0 mmol) and AIBN (225 mg, 1.37 mmol) in benzene (30 mL) was added dropwise over a period of 4 hours. After addition the mixture was refluxed for an extra 45 minutes. After TLC indicated full conversion of starting material, the volatiles were removed under reduced pressure to yield crude **19**. The resulting yellow solid was dissolved in dry ethanol (160 mL), and sodium ethoxide (2.70 M in ethanol) was added slowly. The reaction mixture was heated to reflux and stirred for 1 hour. After being cooled to room temperature, the remaining sodium ethoxide was neutralized with HCl (1 M aqueous solution). TLC monitoring demonstrated full conversion of starting materials. Some of the product precipitated out of solution and was filtered. The remaining liquid was concentrated in a rotary evaporator and purified by flash chromatography (DCM/MeOH 90:10) to yield **20** (3.84 g, 66.7%) as a white powder: $R_f = 0.23$ (DCM/MeOH 95:5). The spectral data obtained for this compound was consistent with that reported by Suzuki.[18]

3′-Triisopropylsilyl-6,5′-cyclo-2′,5′-dideoxyuridine (21)

The 3′-OH cyclized intermediate (2.02 g, 9.60 mmol), $AgNO_3$ (2.55 g, 15.0 mmol), and 1H-imidazole (1.31 g, 19.3 mmol) were dissolved in pyr (100 mL) and *N*,*N*-diisopropylethylamine (2.48 mL, 15.0 mmol). The stirring reaction mixture was heated to 60°C and triisopropylsilyl chloride (3.18 mL 15.1 mmol) was added. The temperature was maintained at 60°C and the reaction was stirred for 12 hours. When TLC had indicated the full conversion of starting materials, the reaction was quenched with saturated ammonium chloride. Insoluble solids were filtered out, and the filtrate was concentrated under reduced pressure. The residue was taken by ethyl acetate (80 mL) and washed with saturated $NaHCO_3$ (60 mL) and brine (60 mL). The organic layer was dried over sodium sulfate before being evaporated under vacuum and purified by flash chromatography (DCM/Acetone 5–10% Acetone gradient) to yield **21** as a white powder (2.62 g, 76.1%): $R_f = 0.21$ (silica gel, 5% acetone in DCM); ^{1}H NMR ($CDCl_3$, 400 M Hz) δ 8.05 (s, 1H), 6.40 (d, $J = 5.6$ Hz, 1H), 5.47 (s, 1H), 4.52 (d, $J = 6.4$ Hz, 1H), 4.41 (q, $J = 3.6$ Hz, 1H), 3.21(ddd, $J1 = 1.8$ Hz, $J2 = 6.8$ Hz, $J3 = 18$ Hz), 2.54(m, 2H), 2.26(m, 1H), 1.01–1.10 (m, 3H), 1.03 (d, $J = 4.8$ Hz, 1H) ppm; ^{13}C NMR

($CDCl_3$, 100 M Hz), δ 162.1, 149.3, 149.0, 101.5, 84.5, 82.6, 74.9, 46.7, 30.7, 18.1, 12.1 ppm; HRMS (ESI-TOF) Calc. Mass for $C_{18}H_{31}N_2O_4Si^+$ $[M+H]^+$ 367.20531, found: 367.20467.

3′-Triisopropylsilyl-6,5′ (S)-cyclo-2′-deoxyuridine (22S) and 3′-Triisopropylsilyl-6,5′ (R)-cyclo-2′-deoxyuridine (22R)

21 (903 mg, 2.46 mmol) and SeO_2 (136 mg, 1.23 mmol) were dissolved in 1,4-dioxane (30 mL) and tertbutyl peroxide (480 μL, 3.48 mmol). The reaction mixture was heated to 80°C and stirred for 3.5 hours. When TLC monitoring showed that the starting material was fully consumed, the reaction mixture was cooled to ambient temperature and the insoluble solid was filtered. The filtrate was concentrated under reduced pressure to yield a red foam. The residue was purified by flash chromatography (DCM/Acetone/Et_3N 92:5:3) to yield crude **22*S*** and crude **22*R***. Separately, each crude diastereomer was further purified by flash chromatography (DCM/Acetone 90:10) to yield white powder **22*R*** (390 mg, 41.4%), $R_f = 0.46$ (DCM/Acetone/Et_3N 87:10:3) and white powder **22*S*** (375 mg, 39.8%), $R_f = 0.30$ (DCM/Acetone/Et_3N 87:10:3). Compound **22*S*** ^1H NMR (DMSO-d_6, 400 M Hz) δ 11.18 (bs, 1H), 6.49 (d, $J = 6.4$ Hz, 1H), 6.18 (d, $J = 5.2$ Hz, 1H), 5.65 (s, 1H), 4.71 (q, $J = 3.6$ Hz, 1H), 4.67(t, $J = 6$ Hz, 1H), 4.31 (d, $J = 6$ Hz, 1H), 2.58 (dd, $J1 = 7.6$ Hz, $J2 = 16$ Hz, 1H), 2.00 (dd, $J1 = 4$ Hz, $J2 = 8.4$ Hz, 1H), 1.01–1.08 (m, 3H), 1.03 (d, $J = 3.2$ Hz, 18H) ppm; ^{13}C NMR (DMSO-d_6, 100 M Hz), δ 163.3, 154.6, 149.6, 100.5, 86.3, 84.7, 69.3, 63.2, 45.7, 18.2, 11.8 ppm; HRMS (ESI-TOF) Calc. Mass for $C_{18}H_{31}N_2O_5Si^+$ $[M+H]^+$ 383.20022, found: 383.19989. Compound **22*R*** ^1H NMR (DMSO-d_6, 400 M Hz) δ 11.249(bs, 1H), 6.28 (d, $J = 6.4$ Hz, 1H), 6.22 (d, $J = 5.2$ Hz, 1H), 5.62 (s, 1H), 4.46 (dd, $J1 = 3.2$ Hz, $J2 = 7.2$ Hz, 1H), 4.23 (d, $J = 8.0$ Hz, 1H), 4.22 (s, 1H), 2.44–2.47 (m, 1H), 1.92–1.98 (m, 1H), 1.05–1.14 (m, 3H), 1.02 (d, $J = 6$ Hz, 1H) ppm; ^{13}C NMR (DMSO-d_6, 100 M Hz), δ 163.0, 151.6, 149.4 102.7, 89.2, 83.4, 71.1, 64.2, 43.99, 17.7, 11.2 ppm; HRMS (ESI-TOF) Calc. Mass for $C_{18}H_{31}N_2O_5Si^+$ $[M+H]^+$ 383.20022, found: 383.20056.

3′-Triisopropylsilyl-5′-(1-ethoxyethyl)-6,5′ (S)-cyclo-2′-deoxyuridine (23S) and 3′-Triisopropylsilyl-5′-(1-ethoxyethyl)-6,5′ (R)-cyclo-2′-deoxyuridine (23R)

22*S* (138 mg, 0.36 mmol) was dissolved in dry DCM/ethyl vinyl ether (2:1, 21 mL total volume). Pyridinium para-toluenesulfonate (36.4 mg, 0.14 mmol) was added and the reaction mixture was stirred at room temperature for 4 hours, resulting in a clear solution. Volatiles were removed under reduced pressure and the residue was purified by flash chromatography (silica gel pre-washed by three column volumes of DCM/Et_3N 99:1 followed by three column volumes of 100% DCM. Column eluent was DCM/Acetone 90:10) to yield **23*S*** as a white foam (136 mg, 83.1%), $R_f = 0.33$

(DCM/Acetone 90:10). Compound **23*R*** was obtained by the same procedure as **23*S*** to yield a white foam (130 mg, 79.7%), $R_f = 0.33$ (DCM/Acetone 90:10). Compound **23*S*** ^{1}H NMR ($CDCl_3$, 400 M Hz) δ 8.08 (br, 1H), 6.29 (t, $J = 6$ Hz, 1H), 6.01 (d, $J = 1.6$ Hz, 0.4H), 5.74 (d, $J = 2.0$ Hz, 0.6H), 4.47–4.86 (m, 5H), 3.52–3.62 (m, 2H), 2.45–2.51 (m, 1H), 2.20 (dt, $J1 = 4.4$ Hz, $J2 = 13.2$ Hz), 1.34–1.38(m, 3H), 1.19–1.23 (m, 3H), 1.03–1.07 (m, 21H) ppm; ^{13}C NMR ($CDCl_3$, 100 M Hz), δ 162.6, 162.5, 152.9, 152.5, 149.0, 147.9, 103.2, 102.1, 101.2, 100.1, 86.2, 85.7, 85.6, 84.8, 69.7, 69.7, 69.1, 66.7, 62.3, 62.1, 46.5, 46.5, 20.2, 20.2, 18.2, 18.1, 15.5, 15.5, 12.2, 12.2 ppm; HRMS (ESI-TOF) Calc. Mass for $C_{21}H_{39}N_2O_6Si^+$ $[M+H]^+$ 455.25774, found: 455.25732 Compound **23*R*** ^{1}H NMR ($CDCl_3$, 400 M Hz) δ 8.29 (br, 1H), 6.36 (t, $J = 4$ Hz, 1H), 5.76 (s, 0.4H), 5.69 (s, 0.6H), 4.21–4.99 (m, 5H), 3.47–3.64 (m, 2H), 2.38–2.50 (m, 1H), 2.17–2.22 (m, 1H), 1.35–1.40 (m, 3H), 1.18–1.23 (m, 3H), 1.07–1.11 (m, 3H), 1.04 (d, $J = 3.6$ Hz, 18H) ppm; ^{13}C NMR ($CDCl_3$, 100 M Hz), δ 162.5, 162.5, 152.9, 149.4, 149.2, 149.1, 148.6, 104.9, 104.5, 99.4, 98.7, 88.2, 86.5, 85.1, 85.0, 71.5, 71.1, 68.1, 67.7, 60.5, 59.6, 45.4, 45.1, 20.3, 19.8, 18.1, 18.0, 15.4, 15.3, 12.2, 12.1 ppm; HRMS (ESI-TOF) Calc. Mass for $C_{21}H_{39}N_2O_6Si^+$ $[M+H]^+$ 455.25774, found: 455.25664.

5′-(1-Ethoxyethyl)-6,5′ (*S*)-cyclo-2′-deoxyuridine (24*S*) and 5′-(1-Ethoxyethyl)-6,5′ (*R*)-cyclo-2′-deoxyuridine (24*R*)

23*S* (184 mg, 0.41 mmol) was dissolved in THF (4 mL). TBAF (1 M in THF, 2 mL) was added and the reaction mixture was stirred at ambient temperature for 45 minutes. The reaction was quenched with water and the solvents were removed under reduced pressure. The resulting brown oil residue was purified by flash chromatography (silica gel pre-washed by three column volumes of DCM/Et_3N 99:1 followed by three column volumes of 100% DCM. Column eluent was DCM/MeOH 95:5) to afford **24*S*** as a white solid (120 mg, 0.40 mmol), $R_f = 0.24$, 0.20 (DCM/MeOH 95:5). **24*R*** was obtained by the same procedure and yielded a white solid in nearly quantitative yield, $R_f = 0.13$ (DCM/MeOH 95:5). **24*S***: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.14 (dt, $J = 7.2$, 1.6 Hz, 3H), 1.33 (m, 3H), 1.95 (m, 1H), 2.45–2.49 (m, 1H), 3.52–3.55 (m, 1H), 3.56–3.69 (m, 1H), 4.43 (m, 2H), 4.73 (dd, $J = 24$, 6.0 Hz, 1H), 5.00 (m, 1H), 5.42 (m, 1H), 5.60 (d, $J = 19.6$ Hz, 1H), 6.14 (d, $J = 5.2$ Hz, 1H), 11.25 (s, 1H). HRMS (ESI) Calcd for $C_{13}H_{19}N_2O_6$, 299.12431; Found, 299.12320. ***24R***: ^{1}H NMR (Methanol-d_4, 500 MHz) δ 1.24 (m, 3H), 1.38 (m, 3H), 2.03–2.08 (m, 1H), 2.42–2.47 (m, 1H), 3.57–3.72 (m, 2H), 4.35 (m, 1H), 4.42 (dt, J1 = 21, 1.0 Hz, 1H), 4.51 (d, $J = 19.6$ Hz, 1H), 5.01 (m, 1H), 5.75 (d, $J = 25.5$ Hz, 1H), 6.30 (d, $J = 5.0$ Hz, 1H). HRMS (ESI) Calcd for $C_{13}H_{19}N_2O_6$, 299.12431; Found, 299.12434.

3′-(2-Cyanoethyl N,N-diisopropyl phosphorodiamidite)-5′-(1-ethoxyethyl)-6,5′ (S)-cyclo-2′-deoxyuridine (25S) and 3′-(2-Cyanoethyl N,N-diisopropyl phosphorodiamidite)-5′-(1-ethoxyethyl)-6,5′ (R)-cyclo-2′-deoxyuridine (25R)

24*S* (184 mg, 0.62 mmol) was co-evaporated with acetonitrile (5 mL) three times, and dissolved in acetonitrile (6.5 mL). The stirring reaction mixture was cooled to 0°C and 2-Cyanoethyl N,N,N′,N′-tetraisopropyl phosphorodiamidite (245 μL, 0.74 mmol), was added followed by tetrazole (0.45 M in THF, 830 μL, 0.37 mmol). The reaction was allowed to warm at ambient temperature and stirred for 4 hours before the solvent was removed under reduced pressure. The crude residue was subjected to flash chromatography (silica gel pre-washed by three column volumes of Hexanes/Et_3N 99:1 followed by three column volumes of 100% Hexanes. Column eluent was Ethyl Acetate/Hexane 60:40). This crude material dissolved in dry DCM and precipitated from solution upon slow addition of hexanes to yield **25*S*** as a white foam (185.5 mg, 60.2%), $R_f = 0.51$ (Ethyl Acetate/Hexanes 75:25). Compound **25*R*** was obtained by the same procedure and isolated as a white foam $R_f = 0.49$ (Ethyl Acetate/Hexanes 75:25). Compound **25*S*** ^{31}P NMR ($CDCl_3$, 200 M Hz) δ 146.5, 146.5, 146.2; HRMS (ESI-TOF) Calc. Mass for $C_{22}H_{36}N_4O_7P^+$ $[M+H]^+$ 499.23216, found: 499.23142. Compound **25*R*** ^{31}P NMR ($CDCl_3$, 200 M Hz) δ 149.9, 149.5 149.2, 149.0; HRMS (ESI-TOF) Calc. Mass for $C_{22}H_{36}N_4O_7P^+$ $[M+H]^+$ 499.23216, found: 499.23101.

Enzymatic Digestion

A mixture of 3 μL of alkaline phosphatase, 3 μL of phosphodiesterase I, 4 μL of nuclease P1, 10 μL of 10X alkaline phoaphatase buffer, 10 μL of 1.0 M $MgCl_2$, and 70 μL of water was added to a eppendorf tube containing 2 mmol of the DNA oligomer. The mixture was left at 37°C overnight, then cooled to room temperature. The digested nucleoside mixture was analyzed by reverse-phase analytical HPLC with a self-packed C18 column [0.46 cm 25 cm, 5 μm 100 Å, resins from Varian Analytical Instrument (Walnut Creek, CA, USA)]. Mobile phase buffer A: 100 mM triethylammonium acetate buffer (pH 7.0). Mobile phase buffer B: acetonitrile. Flow rate: 1 mL/min. Gradient of the elution: 0–25% in 30 minutes and 25%–100% in 10 minutes. The mixtures were monitored by UV at 260 nm.

Thermal Denaturation Studies

All UV melting experiments were carried out in 20 mM pH 7.0 sodium phosphate buffer with 100 mM or 1 M sodium chloride using AVIV Biomedical Inc. (Lakewood, NJ, USA) spectrophotometer model 14DS UV-Vis with a temperature controller. Self-complimentary sequence had a concentration of 1 μM while non-self-complementary sequences had concentrations of 0.5 μM for each strand. The samples were heated to 95°C for 5 minutes,

cooled to room temperature briefly, then further cooled to 4°C. Samples were placed in a 1 cm path length quartz cell with a stopper. The denaturation experiments were carried out between 4°C and 95°C. Absorption data was collected every degree at 260 nm. The T_m values were determined by the first derivative of the melting profile using Origin and Microsoft Excel software.

REFERENCES

1. Halliwell, B.; Gutteridge, J.M.C. *Free Radicals in Biology and Medicine*, Oxford University Press, Oxford, New York, 2007.
2. Friedberg, E.C. *DNA Repair and Mutagenesis*, ASM Press, Washington, DC, 2006.
3. Sonntag, C.V. *Free-Radical-Induced DNA Damage and its Repair: A Chemical Perspective*, Springer, Berlin, 2006.
4. Dizdaroglu, M. Oxidative damage to DNA in mammalian chromatin. *Mutat. Res.* **1992**, 275, 331–342.
5. Breen, A.P.; Murphy, J.A. Reactions of oxyl radicals with DNA. *Free Radical Bio. Med.* **1995**, 18, 1033–1077.
6. Evans, M.D.; Dizdaroglu, M.; Cooke, M.S. Oxidative DNA damage and disease: Induction, repair and significance. *Mutat. Res.-Rev. Mutat.* **2004**, 567, 1–61.
7. Jaruga, P.; Dizdaroglu, M. 8,5′-Cyclopurine-2′-deoxynucleosides in DNA: Mechanisms of formation, measurement, repair and biological effects. *DNA Repair* **2008**, 7, 1413–1425.
8. Marietta, C.; Gulam, H.; Brooks, P.J. A single 8,5′-cyclo-2′-deoxyadenosine lesion in a TATA box prevents binding of the TATA binding protein and strongly reduces transcription in vivo. *DNA Repair* **2002**, 1, 967–975.
9. Pullman, B.; Pullman, A. Submolecular structure of nucleic acids. *Nature* **1961**, 189, 725–727.
10. Haromy, T.P.; Raleigh, J.; Sundaralingam, M. Enzyme-bound conformations of nucleotide substrates-X-ray structure and absolute-configuration of 8,5′-cycloadenosine monohydrate. *Biochemistry* **1980**, 19, 1718–1722.
11. Birnbaum, G.I.; Cygler, M.; Dudycz, L.; Stolarski, R.; Shugar, D. Comparison of solid-state and solution conformations of R-Epimer and S-Epimer of 8,5′-cycloadenosine and their relevance to some enzymatic-reactions. *Biochemistry* **1981**, 20, 3294–3301.
12. Miaskiewicz, K.; Miller, J.H.; Fuciarelli, A.F. Theoretical-analysis of DNA intrastrand cross-linking by formation of 8,5′-cyclodeoxyadenosine. *Nucleic Acids Res.* **1995**, 23, 515–521.
13. Theruvathu, J.A.; Jaruga, P.; Dizdaroglu, M.; Brooks, P.J. The oxidatively induced DNA lesions 8,5′-cyclo-2′-deoxyadenosine and 8-hydroxy-2′-deoxyadenosine are strongly resistant to acid-induced hydrolysis of the glycosidic bond. *Mech. Ageing Dev.* **2007**, 128, 494–502.
14. Dirksen, M.L.; Blakely, W.F.; Holwitt, E.; Dizdaroglu, M. Effect of DNA conformation on the hydroxyl radical-induced formation of 8,5′-cyclopurine 2′-deoxyribonucleoside residues in DNA. *Int. J. Radiat. Biol.* **1988**, 54, 195–204.
15. Brooks, P.J.; Wise, D.S.; Berry, D.A.; Kosmoski, J.V.; Smerdon, M.J.; Somers, R.L.; Mackie, H.; Spoonde, A.Y.; Ackerman, E.J.; Coleman, K.; Tarone, R.E.; Robbins, J.H. The oxidative DNA lesion 8,5′-(S)-cyclo-2′-deoxyadenosine is repaired by the nucleotide excision repair pathway and blocks gene expression in mammalian cells. *J. Biol. Chem.* **2000**, 275, 22355–22362.
16. Kuraoka, I.; Bender, C.; Romieu, A.; Cadet, J.; Wood, R.D.; Lindahl, T. Removal of oxygen free-radical-induced 5′,8-purine cyclodeoxynucleosides from DNA by the nucleotide excision-repair pathway in human cells. *P. Natl. Acad. Sci. USA* **2000**, 97, 3832–3837.
17. Juo, Z.S.; Chiu, T.K.; Leiberman, P.M.; Baikalov, I.; Berk, A.J.; Dickerson, R.E. How proteins recognize the TATA box. *J. Mol. Biol.* **1996**, 261, 239–254.
18. Suzuki, Y.; Matsuda, A.; Ueda, T. Synthesis of 6,5′-cyclo-2′,5′-dideoxypyrimidine nucleosides. *Chem. Pharm. Bull.* **1987**, 35, 1085–1092.
19. Theile, C.S.; McLaughlin, L.W. An efficient synthetic approach to 6,5′-(S)- and 6,5′-(R)-cyclouridine. *Chem. Commun.* **2012**, 48, 5587–5589.

20. Drew, H.R.; Wing, R.M.; Takano, T.; Broka, C.; Tanaka, S.; Itakura, K.; Dickerson, R.E. Structure of a B-DNA dodecamer - Conformation and dynamics. *P. Natl. Acad. Sci. USA-Biological Sciences* **1981**, 78, 2179–2183.
21. Partridge, B.L.; Salisbury, S.A. Structural studies on nucleic acids. *NDB ID: BDL075*, **1996**.
22. Romieu, A.; Gasparutto, D.; Molko, D.; Cadet, J. Site-specific introduction of (5′ S)-5′,8-cyclo-2′-deoxyadenosine into oligodeoxyribonucleotides. *J. Org. Chem.* **1998**, 63, 5245–5249.