Supporting Information

A Highly Fluorescent Nucleobase Molecular Rotor

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1. Computations

The molecular geometries and thermal corrections were optimized with B3LYP DFT functional theory\textsuperscript{1} paired with the 6-31+G(d) basis set.\textsuperscript{2} Calculations were performed in the water phase (C-PCM algorithm)\textsuperscript{3} at 298.15 K. The first frequency was utilized to assess whether structures were in their true optimized form. Conformers distributions were executed using Spartan,\textsuperscript{4} and Gaussian 09\textsuperscript{5} was used to calculate orbital energies. In order to simplify calculations, deoxyribose (dR) was replaced with a methyl group.

Table S1. Calculated [b3lyp/6-31+G(d)] HOMO and LUMO energy values (kcal mol\textsuperscript{-1}) in water.

<table>
<thead>
<tr>
<th></th>
<th>t\textsuperscript{T}</th>
<th>t\textsuperscript{C\textdegree}</th>
<th>MD\textsuperscript{A}</th>
<th>2AP</th>
<th>G</th>
<th>A</th>
<th>C</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>LUMO</td>
<td>-45.9</td>
<td>-35.9</td>
<td>-30.4</td>
<td>-33.0</td>
<td>-18.0</td>
<td>-24.9</td>
<td>-27.0</td>
<td>-30.7</td>
</tr>
<tr>
<td>HOMO</td>
<td>-134.8</td>
<td>-129.9</td>
<td>-133.0</td>
<td>-143.4</td>
<td>-142.1</td>
<td>-147.2</td>
<td>-151.8</td>
<td>-152.9</td>
</tr>
</tbody>
</table>

Figure S1. Calculated [b3lyp/6-31+G(d)] HOMO and LUMO energy values (kcal mol\textsuperscript{-1}) in water.
2. Photophysical Properties of $^{15}$T

DMSO stock solutions of the $^{15}$T nucleoside 2 were prepared and stored at -20°C, and later thawed and diluted to an $OD = 0.10 \pm 0.01$ at the most red-shifted absorbance maxima. All measurements were collected on a Molecular Devices SpectraMax M5 in a 1 cm path-length quartz cuvette. Quantum yields were calculated using the most red-shifted absorbance maxima of samples. Quinine hemisulfate ($\Phi_R = 0.546$) in 0.5 M H$_2$SO$_4$ ($n_R = 1.346$) was used as a fluorescent standard. Quantum yields were calculated using the equation shown below$^5$:

$$\Phi = \Phi_R \frac{F}{F_R} \frac{A_R}{A} \frac{n^2}{n_R^2}$$

(Eq. S1)

where $\Phi_R$ is the quantum yield of the fluorescent standard, $F$ and $F_R$ are the integrated emissions of the sample and reference respectively. $A$ and $A_R$ are the optical densities of the sample and reference respectively (both set to 0.10 $\pm$ 0.01). $n$ and $n_R$ are the refractive indexes of the sample and reference respectively.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$\varepsilon_{max}$ (cm$^{-1}$ M$^{-1}$)</th>
<th>$\lambda_{abs}$ max (nm)</th>
<th>$\lambda_{em}$ max (nm)</th>
<th>Stokes shift (10$^3$ cm$^{-1}$)</th>
<th>$\Phi$</th>
<th>$E_T^{30}$ (kcal mol$^{-1}$)$^7$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toluene</td>
<td>39,600</td>
<td>315</td>
<td>405</td>
<td>7.1</td>
<td>0.75</td>
<td>33.9</td>
</tr>
<tr>
<td>Dioxane</td>
<td>32,300</td>
<td>310</td>
<td>405</td>
<td>7.6</td>
<td>0.74</td>
<td>36</td>
</tr>
<tr>
<td>DCM</td>
<td>33,600</td>
<td>310</td>
<td>415</td>
<td>8.2</td>
<td>0.57</td>
<td>40.7</td>
</tr>
<tr>
<td>DMF</td>
<td>42,800</td>
<td>310</td>
<td>415</td>
<td>8.2</td>
<td>0.69</td>
<td>43.2</td>
</tr>
<tr>
<td>MeCN</td>
<td>44,400</td>
<td>310</td>
<td>415</td>
<td>8.2</td>
<td>0.51</td>
<td>45.6</td>
</tr>
<tr>
<td>MeOH</td>
<td>32,700</td>
<td>310</td>
<td>435</td>
<td>9.3</td>
<td>0.30</td>
<td>55.4</td>
</tr>
<tr>
<td>Water</td>
<td>38,000</td>
<td>310</td>
<td>455</td>
<td>10.3</td>
<td>0.11</td>
<td>63.1</td>
</tr>
<tr>
<td>aq. PBS</td>
<td>28,500</td>
<td>310</td>
<td>455</td>
<td>10.3</td>
<td>0.12</td>
<td></td>
</tr>
</tbody>
</table>

$^a$All data were collected at 25 °C, and the concentration of $^{15}$T nucleoside was fixed at 3 μM. In order to maximize the solubility, all samples had a final DMSO content of 0.04%.

$^b$Trans stilbene itself exhibits an $\varepsilon = 29,100$ cm$^{-1}$M$^{-1}$ and $\Phi = 0.04$ in hexane,$^8,9$ and an $\varepsilon = 44,000$ cm$^{-1}$M$^{-1}$ and $\Phi = 0.02$ measured in methanol (determined here).
Figure S2. (A) Plot between Stokes shift of \(^{13}\)T nucleoside 2 and \(E_T^{30}\) value of various solvents (\(R^2 = 0.97\)). (B) Plot between quantum yield of \(^{13}\)T nucleoside 2 and \(E_T^{30}\) value of various solvents (\(R^2 = 0.95\)).

Figure S3. (A) Absorption (dashed lines) and fluorescence (solid lines) of \(^{13}\)T nucleoside 2 in a mixture of methanol and glycerol. (B) Quantum yield and extinction coefficient of \(^{13}\)T nucleoside 2 versus glycerol content. Extinction coefficients were calculated at 310 nm and emission spectra collected using Ex = 310 nm.
3. Oligomer Synthesis, Purification and Folding

Unmodified oligonucleotides were purchased from Sigma-Aldrich as HPLC-purified products. Standard DNA phosphoramidites, solid supports, and all necessary reagents were purchased from LinkTech and Sigma-Aldrich. Modified oligonucleotides were synthesized on a 1.0 μmol scale using a Bioautomation Co. Mermade 4 DNA synthesizer according to the Trityl-on procedure. Three coupling reactions were performed for the site-specific introduction of the modified nucleoside into oligonucleotides. The freshly made phosphoramidite 3 was dissolved in dry acetonitrile (>20 mg/mL) immediately prior to use. The synthesis of the oligonucleotides was monitored by DMT deprotection. Upon completion of the sequences, the oligonucleotides were cleaved from the solid support and deprotected by treatment with 1.0 mL of 33% aqueous ammonium hydroxide at 55 °C overnight in a 1.5 mL screw-top cap tube. The resulting products were lyophilized and filtered through Glen-Pak DNA purification cartridges to remove incomplete synthesis products and to deprotect the 5’-DMT during elution. The obtained solutions were lyophilized to dryness and purified by HPLC column chromatography using a semi-prep C-18 reverse-phase column (YMCbasic B-22-10P 150 x 10 mm) using a Varian 140 Pro Star HPLC system. The gradient conditions were typically acetonitrile: 0.1 M triethylammonium acetate (TEAA, pH 7.4), 2:98 to 10:90 over 35 minutes and with rate of 3.00 mL/min. Elution was monitored by UV absorption at 260 and 310 nm. Peaks were collected and twice lyophilized to dryness from water. The purities of 15T-containing oligonucleotides ODN 1 – 3 were found to be >90% (260 nm) according to analytical, reverse-phase chromatography using a Waters XBridge C8, 5 μm 4.6 x 150 mm. A gradient of 5 – 40% of acetonitrile in 0.1 M triethylammonium acetate (TEAA, pH 7.4), was applied over 35 minutes at 0.4 mL/min.

**Figure S4.** HPLC chromatograms of purified (A) ODN1, (B) ODN2 and (C) ODN3 recorded at 260 nm.

ODN1-3 were analyzed by LC-MS using a Dionex Ultimate 3000 UHPLC coupled to a Bruker Maxis Impact QTOF in negative ESI mode. Samples were run through a Phenomenex Luna C18(2)-HST column (2.5 μM 120A 2.1 x 100 mm) using a gradient of 90% mobile phase A (100 mM HFIP and 5 mM TEA in H2O) and 10% mobile phase B (MeOH) to 40% mobile phase A and 60% mobile phase B in 20 minutes. The data was processed and spectra deconvoluted using the Bruker DataAnalysis software version 4.2.

**Table S3.** Synthesized oligomers with calculated and observed masses.

<table>
<thead>
<tr>
<th>Oligonucleotides</th>
<th>Sequences (5’ → 3’)</th>
<th>Calc. (m/z)</th>
<th>Found (m/z)</th>
<th>δ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ODN1</td>
<td>GCGTA 15T CGTATA CAC</td>
<td>4687.8488</td>
<td>4687.9219</td>
<td>15.6</td>
</tr>
<tr>
<td>ODN2</td>
<td>GCGTA 15T GCGTATA CAC</td>
<td>5016.9014</td>
<td>5016.9844</td>
<td>16.6</td>
</tr>
<tr>
<td>ODN3</td>
<td>GCGTA 15T ATGTATA CAC</td>
<td>5015.9061</td>
<td>5016.0078</td>
<td>20.3</td>
</tr>
</tbody>
</table>
Figure S5. ESI-MS analysis of (A) ODN1, (B) ODN2 and (C) ODN3. Inset shows an expansion of the deconvoluted spectra and the presence of some Na⁺ and K⁺ adducts in the gas phase. Data were collected and analyzed by Dr. Alexander Wahba (McGill University).

Oligonucleotide stock solutions were prepared in deionized water and their concentrations were determined by absorbance at 260 nm using the molar extinction coefficient calculated using a nearest-neighbor model. For calculated extinction coefficients see Table S4. Double stranded oligonucleotides were prepared by diluting the complementary sequences (1.0 : 1.2 equiv. ratio) in the PBS buffer (pH = 7.4, Na⁺ concentration of ≈137 mM) and heating to 95 °C for 5 min, followed by slow cooling to room temperature over 4 h.
4. CD Spectroscopy

Circular dichroism spectra of annealed duplex DNA (2.0 μM) were measured from 220 nm to 350 nm at 25 °C with a 2 nm band width with 0.1 nm steps at a scanning rate of 20 nm min$^{-1}$ in 1 cm path length thermo-controlled strain-free quartz cuvette on a JASCO J-715 spectrometer.

**Figure S6.** CD spectra of (A) DUP1, DUP2 and DUP3 prepared from OND1, ODN2, and ODN3, respectively. (B) Matched, mismatched and unmodified duplexes of DUP1/ODN1.
5. Extinction Coefficients and Quantum Yields of Oligonucleotides

Oligonucleotide stock solutions were diluted into PBS buffer (pH = 7.4, Na+ concentration of ≈137 mM) to a final concentration of 2.0 μM using their extinction coefficient at 260 nm (Table S4). All measurements were collected on a Molecular Devices SpectraMax M5 in a 1 cm path-length quartz cuvette. Quantum yields were calculated using the most red-shifted absorbance maxima of samples. Quinine hemisulfate ($\phi_R = 0.546$) in 0.5 M $\text{H}_2\text{SO}_4$ ($n_R = 1.346$) was used as a fluorescent standard and quantum yields were calculated using the equation S1.

Table S4. Photophysical properties of DNA containing $^{\text{ts}}\text{T}$.\(^b\)

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>$\varepsilon_{260}$ (cm$^{-1}$ M$^{-1}$)</th>
<th>$\lambda_{\text{abs}}$ (nm)</th>
<th>$\lambda_{\Phi}$ (nm)</th>
<th>$\Phi$</th>
<th>$\varepsilon_{310}\times\Phi$ (cm$^{-1}$ M$^{-1}$)</th>
<th>$T_m$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ODN1(^c)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{\text{ts}}\text{T}:A$</td>
<td>257,000</td>
<td>310</td>
<td>440</td>
<td>0.139</td>
<td>4,250</td>
<td>55.0(^d)</td>
</tr>
<tr>
<td>$^{\text{ts}}\text{T}:G$</td>
<td>255,000</td>
<td>310</td>
<td>445</td>
<td>0.017</td>
<td>520</td>
<td>53.0</td>
</tr>
<tr>
<td>$^{\text{ts}}\text{T}:C$</td>
<td>251,000</td>
<td>310</td>
<td>440</td>
<td>0.005</td>
<td>150</td>
<td>51.0</td>
</tr>
<tr>
<td>$^{\text{ts}}\text{T}:T$</td>
<td>253,000</td>
<td>310</td>
<td>440</td>
<td>0.005</td>
<td>150</td>
<td>50.8</td>
</tr>
<tr>
<td>ss-$^{\text{ts}}\text{T}$</td>
<td>155,000</td>
<td>315</td>
<td>445</td>
<td>0.028</td>
<td>860</td>
<td>-</td>
</tr>
<tr>
<td>ODN2(^e)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{\text{ts}}\text{T}:A$</td>
<td>273,000</td>
<td>310</td>
<td>440</td>
<td>0.098</td>
<td>3,000</td>
<td>60.2(^f)</td>
</tr>
<tr>
<td>$^{\text{ts}}\text{T}:T$</td>
<td>269,000</td>
<td>310</td>
<td>440</td>
<td>0.012</td>
<td>370</td>
<td>56.1</td>
</tr>
<tr>
<td>ss-$^{\text{ts}}\text{T}$</td>
<td>164,000</td>
<td>315</td>
<td>440</td>
<td>0.020</td>
<td>610</td>
<td>-</td>
</tr>
<tr>
<td>ODN3(^g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{\text{ts}}\text{T}:A$</td>
<td>271,000</td>
<td>310</td>
<td>440</td>
<td>0.134</td>
<td>4,100</td>
<td>54.1(^h)</td>
</tr>
<tr>
<td>ss-$^{\text{ts}}\text{T}$</td>
<td>169,000</td>
<td>310</td>
<td>445</td>
<td>0.026</td>
<td>800</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^a\)The average extinction coefficient ($\varepsilon$) of $^{\text{ts}}\text{T}$ in DNA at 310 nm is 30,600 ± 700 cm$^{-1}$ M$^{-1}$ (Fig. S7A). \(^b\)All data were collected at 25 °C, PBS buffer and pH = 7.4. \(^c\)Sequence: 5'-GCGTA $^{\text{ts}}\text{T}$ CGTATACAC-3'. \(^d\) $T_m$ of corresponding unmodified DNA is 49.3 °C. \(^e\)Sequence: 5'-GCGTA $^{\text{ts}}\text{T}$ CGTATACAC-3'. \(^f\) $T_m$ of corresponding unmodified DNA is 56.3 °C. \(^g\)Sequence: 5'-GCGTA $^{\text{ts}}\text{T}$ ATGTATACAC-3'. \(^h\) $T_m$ of corresponding unmodified DNA is 49.7 °C.
Figure S7. (A) Absorbance spectra of $^{15}$T-modified and unmodified ODN1. The ratio of absorbance at 260 nm and 310 nm was used to calculate an extinction coefficient of $^{15}$T in DNA = 30,600 ± 700 cm$^{-1}$ M$^{-1}$. (B) Absorption (dashed line) and fluorescence (solid line) of single-stranded and duplex ODN1 containing $^{15}$T. Data were recorded at 25 °C in PBS buffer (pH = 7.4). DNA concentrations were kept at 2.0 μM.

6. Melting Temperature Analysis ($T_m$)

UV thermal denaturation data were obtained by measuring the absorbance at 260 nm as a function of temperature in a 1 cm path length thermo-controlled strain-free quartz cuvette on a Varian CARY 100 UV-visible spectrophotometer equipped with a Peltier temperature controller. Solutions of pre-folded duplex DNA (0.2 μM) in aqueous buffer (PBS buffer, pH = 7.4) were equilibrated at 20 °C for a minimum of 10 min and slowly ramped to 90 °C with 0.2 °C steps at a rate of 12 °C h$^{-1}$. The melting temperatures were determined from the maximal slope of the curve (maximal first derivative). $T_m$ values were calculated as the average from the heating and cooling curves that showed little or no hysteresis.

Figure S8. Normalized thermal melting data of (A) DUP1, DUP2 and DUP3 containing ODN1-3, respectively. (B) Matched, mismatched and unmodified duplexes of DUP1 containing ODN1 or an unmodified complement strand.
7. Fluorescence Lifetime and Dynamic Anisotropy of Oligonucleotides

TCSPC experiments employed a picosecond laser source (DD-310L, Horiba Jobin Yvon GmbH) at a repetition rate of 10 MHz in combination with a double-grating emission monochromator and a PPD-900 detection module (Fluorolog3 FL3-222, Horiba). Samples were kept at 25 °C by means of a Varian Cary PCB-150 Peltier water bath (Agilent). The fluorescence lifetime \( \tau_F \) was collected with a channel width of 27 ps and retrieved from one exponential decay by iterative reconvolution with the instrument response function (IRF) using a custom-written MATLAB routine (R2019b).\(^{10}\)

\[
I(t) = IRF * e^{-t/\tau_p}
\]  
(Eq. S2)

The time-resolved anisotropy was computed by sequentially recording the parallel \( I_{VV} \) and perpendicular \( I_{VH} \) polarization components with respect to the field vector of the exciting light pulse

\[
r(t) = \frac{I_{VV}-GI_{VH}}{I_{VV}-2GI_{VH}}
\]  
(Eq. S3)

where the G-factor is defined as \( G = I_{HV}/I_{HH} \).\(^{6}\) The dynamic anisotropy of the dye-labeled DNA molecules is best described by a single-rotor model

\[
r(t) = (r_0 - r_\infty)e^{-t/\theta} + r_\infty
\]  
(Eq. S4)

where \( r_0 \) is the fundamental anisotropy, \( r_\infty \) represents the value to which the local anisotropy decays and \( \theta \) is the rotational decay time.\(^{6}\)

Based on the rotational decay time, the hydrodynamic radius of the construct assuming a globular shape of the molecule and a strong coupling of \( ^{14}T \) and the host-molecule can be calculated according to

\[
R_H = \sqrt{\frac{3k_B T \cdot \theta}{4\pi \eta}}
\]  
(Eq. S5)

and the angle between the absorption and emission dipole is calculated

\[
\beta = \cos^{-1} \left( \frac{5 \cdot r_0 + 1}{3} \right)
\]  
(Eq. S6)

The steady-state anisotropy \( \langle r \rangle \) is calculated with

\[
\langle r \rangle = r_0 / (1 + \tau_F / \theta)
\]  
(Eq. S7)
Table S5. Fluorescence lifetime (τ), anisotropy decay (θ), steady-state anisotropy (<r>), angle between excitation and emission dipole (β) and hydrodynamic radius (R_H) of 15T in ODN1.  

<table>
<thead>
<tr>
<th></th>
<th>T (°C)</th>
<th>τ (ns)</th>
<th>θ (ns)</th>
<th>&lt;r&gt;</th>
<th>β (°)</th>
<th>R_H (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ds-15T:A</td>
<td>25</td>
<td>11.1 ± 0.1</td>
<td>20 ± 4.2</td>
<td>0.07 ± 0.01</td>
<td>44 ± 2</td>
<td>2.70 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>10.0 ± 0.1</td>
<td>19 ± 3.8</td>
<td>0.08 ± 0.01</td>
<td>43 ± 2</td>
<td>2.68 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>6.6 ± 0.1</td>
<td>8.5 ± 2.8</td>
<td>0.06 ± 0.02</td>
<td>45 ± 3</td>
<td>2.09 ± 0.22</td>
</tr>
<tr>
<td>ss-15T</td>
<td>25</td>
<td>4.5 ± 0.1</td>
<td>7.9 ± 1.7</td>
<td>0.04 ± 0.01</td>
<td>47 ± 2</td>
<td>1.98 ± 0.14</td>
</tr>
<tr>
<td>ds-15T:T</td>
<td>25</td>
<td>5.4 ± 0.1</td>
<td>5.3 ± 1.6</td>
<td>0.02 ± 0.02</td>
<td>51 ± 2</td>
<td>1.74 ± 0.17d</td>
</tr>
</tbody>
</table>

a All measurements were performed at 25 °C, PBS buffer and pH = 7.4. DNA concentrations = 2.0 μM. 
bCalculated from fundamental anisotropy, r_o (Eq. S6). c Calculated from r (Eq. S5). d Value reflects local dynamics, not R_H.

Figure S9. Fluorescence lifetime data for ODN1 constructs (A) ds-15T:A, (B) ds-15T:T and (C) ss-15T at different temperatures reaching the melting temperature of the ds-DNA at 55 °C.
Figure S10. Dynamic fluorescence anisotropy of ODN1 ds-\textsuperscript{T}:A at three different temperatures.
8. Synthesis

All reagents were obtained from commercial sources and used without further purification. NMR data were collected on either a Bruker AVIII-400 or 500 MHz. Chemical shifts (δ) are given in parts per million (ppm) and are reported relative to residual solvent peaks: CDCl$_3$ (δH 7.26, δC 77.16 ppm), DMSO-d$_6$ (δH 2.50, δC 39.52 ppm), acetone-d$_6$ (δH 2.05, δC 29.84 ppm). Coupling constants (J) are given in Hertz (Hz). $^{13}$C-spectra were recorded broadband proton decoupled. Mass spectra were recorded on an Advion expression CMS and High-resolution mass spectra were obtained on a Bruker MaXis high-resolution QTOF or a Thermo QExactive high-resolution Orbitrap. Masses are given as m/z.

Scheme S1. Synthesis of $^{13}$T phosphoramidite.

**p-Tolyl-3,5-di-O-acetyl-1-thio-2-deoxy-α,β-D-ribofuranoside [4]**

**Procedure:** To a stirring solution of 1,3,5-tri-O-acetyl-2-deoxy-α,β-D-ribose (24.0 g, 92.3 mmol, 1.0 equiv.) at -78 °C in CH$_2$Cl$_2$ (310 mL) was added p-TolSH (14.7 g, 119 mmol, 1.3 equiv.). After 5 min, BF$_3$-OEt$_2$ (43.0 mL, 349 mmol, 3.8 equiv.) was added dropwise. The reaction mixture was stirred for 20 min and then quenched with aq. sat. NaHCO$_3$. The resulting solution was extracted with CH$_2$Cl$_2$ and combined organic
layer was washed with sat. sol. NaCl, dried over MgSO$_4$, filtered and evaporated in vacuo. The yellow crude product was used directly for the next reaction without purification.

**Characterization:** $R_f = (\text{Hexane/EtOAc}, 7:3): 0.36$, $\text{MS (ESI)} = 346.87 ([\text{M+Na}]^+ \text{ calculated } 347.09)$. $R_f$ and ESI MS data are consistent with those previously reported.$^{11}$

$p$-Tolyl-1-thio-2-deoxy-$\alpha,\beta$-D-ribofuranoside [5]

![Structure of p-Tolyl-1-thio-2-deoxy-$\alpha,\beta$-D-ribofuranoside]

**Procedure:** To a stirring solution of unpurified di-acetate thioglycoside 4 (29.9 g) in a mixture of MeOH/CH$_2$Cl$_2$ (340 mL, 4:1) was added $K_2$CO$_3$ (31.5 g, 228 mmoles). The reaction mixture was stirred at room temperature for 2h and then quenched with a solution of HCl (500 mL, 1.00M). The mixture was three times extracted with CHCl$_3$, dried, filtered and evaporated in vacuo. TLC showed full conversion of 4 to 5. The crude 5 was used for the next reaction without purification.

**Characterization:** $R_f = (\text{CH}_2\text{Cl}_2:\text{MeOH}, 94:6): 0.25$, $\text{MS (ESI)} = 262.98 ([\text{M+Na}]^+ \text{ calculated } 263.07)$. $R_f$ and ESI MS data are consistent with those previously reported.$^{11}$

$p$-Tolyl-1-thio-5-O-triisopropylsilyl-2-deoxy- $\alpha,\beta$-D-ribofuranoside [6]

![Structure of p-Tolyl-1-thio-5-O-triisopropylsilyl-2-deoxy- $\alpha,\beta$-D-ribofuranoside]

**Procedure:** To a stirring solution of unpurified diol thioglycoside 5 (26.4 g) in CH$_2$Cl$_2$ (400 mL) were added TIPSCI (21.2 g, 110 mmoles) and imidazole (7.49 g, 110 mmoles). The reaction mixture was stirred at room temperature for 16h and then quenched with H$_2$O. The resulting solution was extracted with CH$_2$Cl$_2$ and the combined organic layer was dried, filtered and evaporated in vacuo. TLC showed a full conversion of 5 to 6. The crude product 6 was used for the next reaction without purification.

**Characterization:** $R_f = (\text{CH}_2\text{Cl}_2:\text{MeOH}, 94:6): 0.48$, $\text{MS (ESI)} = 419.07 ([\text{M+Na}]^+ \text{ calculated } 419.20)$. $R_f$ and ESI MS data are consistent with those previously reported.$^{11}$
**p-Tolyl-3-O-acetyl-1-thio-5-O-triisopropylsilyl-2-deoxy-α,β-D-ribofuranoside (7)**

**Procedure:** To a stirring solution of unpurified thioglycosides 6 (47.6 g) in MeCN (400 mL), were added Et₃N (24.0 mL, 172 mmoles), Ac₂O (17.0 mL, 180 mmoles) and DMAP (1.60 g, 13.1 mmoles). The reaction mixture was stirred at room temperature for 15 h. The reaction color after 30 min stirring changed from yellowish to brownish. The reaction quenched with sat. sol. NaCl and the resulting solution was extracted with CH₂Cl₂. The combined organic layer was dried by MgSO₄, filtered and evaporated under the reduced pressure. The crude product 7 (49.0 g) was filtered three times through silica (height= 4-5 cm) in a large Büchner funnel with EtOAc/hexane gradient from 0% to 20%. After three times filtration, pure thioglycoside 7 (α/β: 1.0:2.1, 35.9 g, 81.8 mmol, 89% over 4 steps) was obtained.

**Characterization:** Rᵣ = (hexane:EtOAc, 9:1): 0.38, ¹H NMR (400 MHz, CDCl₃) δ: α: 7.41 (dd, J = 8.2, 2.0 Hz, 2H), 7.10 (dd, J = 7.9, 4.5 Hz, 2H), 5.47 (dd, J = 9.3, 5.8 Hz, 1H), 5.29 (dt, J = 5.7, 1.8 Hz, 1H), 4.33 (dt, J = 5.7, 1.8 Hz, 1H), 4.08 (dd, J = 4.4, 1.5 Hz, 1H), 3.81 (dt, J = 10.5, 3.9 Hz, 1H), 3.57 (dd, J = 10.5, 6.6 Hz, 1H), 2.32 (s, 3H), 2.26 (dd, J = 9.2, 5.6 Hz, 1H), 2.09 (s, 3H), 1.10 – 1.03 (m, 21H) β: 7.41 (dd, J = 8.2, 2.0 Hz, 2H), 7.10 (dd, J = 7.9, 4.5 Hz, 2H), 5.68 (dd, J = 7.7, 3.1 Hz, 1H), 5.23 (dt, J = 7.4, 2.8 Hz, 1H), 4.33 (q, J = 3.4 Hz, 1H), 3.94 (dd, J = 10.8, 3.4 Hz, 1H), 3.84 (dd, J = 7.6, 3.2 Hz, 1H), 2.79 (dt, J = 14.9, 7.6 Hz, 1H), 2.32 (s, 3H), 2.13 (t, J = 2.8 Hz, 1H), 2.09 (s, 3H), 1.28 – 1.24 (m, 21H). MS (ESI) = 460.86 ([M+Na]⁺ calculated 461.22). The spectroscopic data are consistent with those previously reported.¹¹

**1'-(3'-O-acetyl-5'-O-triisopropylsilyl-2'-deoxy-β-D-ribofuranoside)-6-bromo-quinazoline-2,4-(3H)-dione (8)**

**Procedure:** To a suspension of 6-bromo-quinazoline-2, 4-(1H, 3H)-dione (5.70 g, 23.7 mmol, 1.25 equiv.) in dry CH₂Cl₂ (300 mL) with activated molecular sieves (MS 4Å, 30.0 g), BSA (12.0 mL, 49.3 mmol, 2.6 equiv.) was added dropwise over 5 min under Ar atmosphere. The solution was stirred at room temperature for 180 min. This solution was then cooled to 0 °C and the solution of thioglycoside 7 (α/β = 1.0:2.5, 8.31 g, 19.0 mmol, 1.0 equiv.) was added to the reaction mixture. After 20 min, NIS (4.70 g, 20.9 mmol, 1.1 equiv.) and TMSOTf (2.10 mL, 11.6 mmol, and 0.61 equiv.) were added and the reaction mixture color changed from light brown to a red. The reaction mixture was stirred for 5 min at 0 °C, then quenched with aq. sat. Na₂S₂O₃ and diluted with CH₂Cl₂. To this green color solution, aq. sat. NaHCO₃ was added and the organic phase was washed for three times. The aqueous phase was also washed with CH₂Cl₂ for three times. The combined organic phase was dried over MgSO₄, filtered and concentrated under reduced pressure to result crude 8 as yellow oil. The crude product was subjected to the flash column.
chromatography on silica gel EtOAc/hexane, gradient from 0% to 40%, to isolate β isomer (5.82 g, 10.5 mmol, 55%) and α isomer (3.42 g, 6.16 mmol, 33%) as white foams.

Characterization: *Rf* (hexane/EtOAc, 6:4): 0.85; *1H NMR* (400 MHz, CDCl$_3$) δ: 8.52 (br, 1H), 8.32 (d, *J* = 2.5 Hz, 1H), 7.87 (d, *J* = 9.1 Hz, 1H), 7.64 (dd, *J* = 9.1, 2.5 Hz, 1H), 6.79 (dd, *J* = 9.6, 6.2 Hz, 1H), 5.52 (dt, *J* = 8.0, 2.9 Hz, 1H), 4.09-4.00 (m, 3H), 2.88-2.80 (m, 1H), 2.15 (dd, *J* = 6.3, 2.1 Hz, 1H), 2.11 (s, 3H), 1.29-1.24 (m, 21H). The spectroscopic data are consistent with those previously reported.

**1’-(3’-O-tertbutyldimethylsilyl-5’-O-triisopropylsilyl-2’-deoxy-β-D ribofuranoside)-6-bromoquinazoline-2,4-(3H)-dione (1)**

![Diagram](image)

**Procedure:** To a stirred solution of β nucleoside 8 (1.70 g, 3.06 mmol, 1.0 equiv.) in a mixture of MeOH/CH$_2$Cl$_2$ (80.0 mL, 4:1), was added K$_2$CO$_3$ (710 mg, 5.14 mmoles, 1.7 equiv.). The reaction mixture was stirred at room temperature for 36 h and then concentrated under the reduced pressure. The crude material was taken up in EtOAc and washed with sat. sol. NaCl. The aqueous phase was back extracted with EtOAc and the combined organic layer was dried, filtered and evaporated to give unprotected nucleoside as an off-white foam. The product was used in the next step without further purification. To a stirred solution of unprotected nucleoside in DMF (65.0 mL), TBDMSCl (730 mg, 4.84 mmoles, 1.6 equiv.) and imidazole (340 mg, 4.99 mmoles, 1.6 equiv.) were added. The reaction mixture was stirred at room temperature for 40 h and then quenched with sat. sol. NaCl. The resulting solution was extracted with EtOAc and the combined organic layer was dried, filtered and concentrated under reduced pressure. The crude product (1.88 g) was subjected to flash column chromatography on silica gel EtOAc/hexane, gradient from 0% to 40%, to give protected nucleoside 1 (1.78 g, 2.90 mmoles, 95% over two steps) as a white foam.

Characterization: *Rf* (hexane/EtOAc, 9:1): 0.35; *1H NMR* (400 MHz, CDCl$_3$) δ: 8.97 (br, 1H), 8.32 (d, *J* = 2.4 Hz, 1H), 7.69 (d, *J* = 9.2 Hz, 1H), 7.63 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.73 (t, *J* = 7.7 Hz, 1H), 4.73 (dt, *J* = 8.7, 4.4 Hz, 1H), 4.04 (dd, *J* = 11.5, 2.8 Hz, 1H), 3.92 (dd, *J* = 11.5, 3.2 Hz, 1H), 3.84 (dt, *J* = 5.7, 2.9 Hz, 1H), 2.72 (dt, *J* = 13.3, 8.1 Hz, 1H), 2.08-2.02 (m, 1H), 1.16-1.08 (m, 21H), 0.90 (d, *J* = 1.1 Hz, 9H), 0.10 (d, *J* = 1.0 Hz, 3H), 0.08 (d, *J* = 1.0 Hz, 3H). The spectroscopic data are consistent with those previously reported.
1’-(3’-O-tertbutyldimethylsilyl-5’-O-trisopropylsilyl-2’-deoxy-β-D ribofuranoside)-6-styryl-quinazoline-2,4-(3H)-dione (9)

**Procedure:** Cs₂CO₃ (1.90 g, 5.83 mmol, 3.2 equiv.) was added to a dry round flask contained nucleoside 1 (1.14 g, 1.85 mmol, 1.0 equiv.). Then, trans-styryl boronic acid pinacol ester (894 mg, 3.89 mmol, 2.1 equiv.), Pd(dppf)Cl₂ (213 mg, 0.291 mmol, 0.16 equiv.) and freshly degassed dry 1,4-dioxane (15 mL, 0.12M) were added to the mixture. The reaction was stirred at 100 °C for 24 h under an Ar atmosphere. Then, the reaction was quenched with sat. sol. NaCl, extracted with EtOAc, dried over MgSO₄ and filtered through a pad of celite. The crude product was subjected to flash column chromatography on silica gel EtOAc/Hexane, gradient from 0 % to 40 %, to give protected Tₜ nucleoside 9 (1.17 g, 1.80 mmol, 98 %) as a white foam.

**Characterization:** Rₚ(hexane/EtOAc, 8:2): 0.42; ᵃH NMR (400 MHz, CDCl₃) δ: 8.32 (d, J = 2.1 Hz, 1H), 8.18 (br, 1H), 7.76 (d, J = 8.7 Hz, 1H), 7.70 (dd, J = 9.0, 1.9 Hz, 1H), 7.53 (d, J = 7.7 Hz, 2H), 7.38 (t, J = 7.5 Hz, 2H), 7.29 (t, J = 7.5 Hz, 1H), 7.19 – 7.05 (m, 2H), 6.74 (t, J = 7.6 Hz, 1H), 4.76 (dt, J = 8.7, 4.5 Hz, 1H), 4.06 (dd, J = 11.2, 3.0 Hz, 1H), 3.94 (dd, J = 11.3, 3.5 Hz, 1H), 3.87 – 3.85 (m, 1H), 2.81 (dt, J = 13.2, 8.0 Hz, 1H), 2.07 (ddd, J = 12.5, 7.6, 3.8 Hz, 1H), 1.20 – 1.05 (m, 21H), 0.91 (9H, s), 0.11 (s, 3H), 0.10 (s, 3H). ᵃC NMR (126 MHz, CDCl₃) δ 162.2, 150.1, 138.9, 136.8, 133.1, 132.6, 129.8, 128.8, 128.0, 126.6, 126.3, 125.9, 117.2, 117.1, 86.5, 84.0, 70.2, 61.1, 37.1, 25.8, 18.1, 18.0, 12.0, -4.5, -4.8. HRMS (ESI): m/z 651.36445 ([M+H]+C₃₆H₅₅O₅N₂Si₂ requires 651.36440).

1’-(2’-deoxy-β-D ribofuranoside)-6-styryl-quinazoline-2,4-(3H)-dione (2)

**Procedure:** To a stirred solution of nucleoside 9 (870 mg, 1.34 mmol, 1.0 equiv.) in THF (15 mL), was added TBAF (1M solution in THF, 6.6 mL, 4.9 equiv.). The mixture was stirred at room temperature for 22 h and then directly loaded onto a silica gel column chromatography. Purification using 100 % MeCN as eluent afforded pure deprotected nucleoside 2 (480 mg, 1.26 mmol, 94 %) as a white solid.

**Characterization:** Rₚ(CH₂Cl₂/MeOH, 95:5): 0.15; ᵃH NMR (500 MHz, DMSO-d₆) δ 11.67 (br, 1H), 8.18 (d, J = 2.1 Hz, 1H), 7.93 (dd, J = 9.0, 2.2 Hz, 1H), 7.89 (d, J = 8.9 Hz, 1H), 7.64 (d, J = 7.3 Hz, 2H), 7.39 (t, J = 7.7
Hz, 2H), 7.33 (d, J = 6.7 Hz, 1H), 7.30 – 7.27 (m, 2H), 6.69 (t, J = 7.8 Hz, 1H), 5.28 (d, J = 5.1 Hz, 1H), 5.00 (t, J = 5.2 Hz, 1H), 4.42 (dq, J = 8.6, 4.4 Hz, 1H), 3.74 – 3.63 (m, 3H), 2.65 (dt, J = 13.3, 8.2 Hz, 1H), 1.95 (ddd, J = 13.5, 7.4, 3.6 Hz, 1H). 

$^{13}$C NMR (101 MHz, acetone-d$_6$) δ 162.4, 151.0, 140.2, 138.1, 133.5, 132.9, 130.0, 129.6, 128.6, 127.5, 127.5, 126.3, 118.5, 118.1, 87.9, 85.3, 71.2, 62.3, 37.6.

HRMS (ESI): m/z 379.13001 ([M-H] - C$_{21}$H$_{19}$O$_5$N$_2$ requires 379.12995).

1’-[5’-O-{4,4-dimethoxytrityl}-2’-deoxy-β-d-ribofuranoside]-6-styryl-quinazoline- 2,4-(3H)-dione (10)

![Chemical Structure](image)

**Procedure:** Nucleoside 2 (548 mg, 1.44 mmol, 1.0 equiv.) was co-evaporated with pyridine (3 x 3.00 mL) and then suspended in pyridine (5.00 mL). To the stirring solution was added dropwise DMTCl (690 mg, 2.03 mmol, 1.4 equiv.) dissolved in 3.00 mL pyridine. A clear red reaction mixture was observed which was stirred at room temperature for 165 min. The reaction was then quenched with a sat. sol. NaHCO$_3$. The resulting mixture was extracted with CH$_2$Cl$_2$ and the combined organic layer was dried over MgSO$_4$, filtered, and co-evaporated in vacuo with pyridine. The crude product was purified by column chromatography on silica gel (CH$_2$Cl$_2$/MeOH/Et$_3$N, 99.5:0.5 → 89.5:10:0.5) to obtain nucleoside 10 (666 mg, 0.980 mmol, 68%) as a white foam.

**Characterization:** Rf (CH$_2$Cl$_2$/MeOH, 94:6): 0.25; $^1$H NMR (400 MHz, CDCl$_3$) δ 8.18 (s, 1H), 8.06 (d, J = 8.9 Hz, 1H), 7.46 (ddt, J = 7.5, 5.9, 3.0 Hz, 4H), 7.39 – 7.21 (m, 10H), 6.95 – 6.78 (m, 8H), 5.53 (d, J = 5.1 Hz, 1H), 4.89 – 4.92 (m, 1H), 4.05 (s, 1H), 3.73 (s, 6H), 2.96 – 2.89 (m, 2H), 2.27 – 2.22 (m, 1H). $^{13}$C NMR (126 MHz, CDCl$_3$) δ 162.7, 158.6, 150.9, 144.5, 138.6, 136.9, 135.8, 135.6, 132.6, 132.1, 130.4, 130.3, 129.2, 128.7, 128.6, 127.9, 127.8, 127.0, 126.5, 126.4, 126.1, 118.0, 117.2, 113.2, 113.1, 86.5, 85.2, 83.9, 70.2, 62.3, 55.1, 37.1. HRMS (ESI): m/z 705.25641 ([M+Na]$^+$ C$_{42}$H$_{38}$O$_5$N$_2$Na$^+$ requires 705.25712).
1’-[3’-O-[2-cyanoethoxy-(N,N-diisopropylamino)-phosphino]-5’-O-(4,4-dimethoxytrityl)-2’-deoxy-β-d-ribofuranoside]-6-styryl-quinazoline-2,4-(3H)-dione (3)

**Procedure:** To a stirred solution of nucleoside 10 (474 mg, 0.694 mmol, 1.0 equiv.) in CH$_2$Cl$_2$ (10.0 mL) at 0 ºC, was added freshly distilled diisopropylethylamine (DIPEA, 607 μL, 3.49 mmol, 5.0 equiv.) and the reaction was stirred 10 min at 0 ºC under an atmosphere of Ar. To this solution was added 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (607 μL, 2.72 mmol, 4.0 equiv) and the reaction was stirred at 25 ºC for 150 min while it was monitored by TLC every 30 min. The reaction mixture was then loaded directly to a silica gel column, without any quenching or work-up steps. The residue was purified by flash column chromatography (elution time of 10 min) on silica gel (hexane/EtOAc/Et$_3$N, 60:40:0.5 → 40:60:0.5) to obtain nucleoside 3 (552 mg, 0.625 mmol, 90%, diastereomeric mixture) as a white foam. It is worth noting that chromatography solvents were distillated prior to use.

**Characterization:** R$_f$ (hexane/EtOAc, 4:6): 0.45 and 0.55; $^1$H NMR (400 MHz, CDCl$_3$) δ 8.20 (s, 2H), 8.09 (d, J = 2.7 Hz, 1H), 8.07 (d, J = 2.8 Hz, 1H), 7.49 – 7.44 (m, 8H), 7.40 – 7.28 (m, 20H), 6.93 – 6.80 (m, 16H), 5.07 – 4.96 (m, 2H), 4.22 – 4.09 (m, 8H), 3.77 – 3.76 (m, 12H), 2.93 (dd, J = 13.8, 8.9 Hz, 2H), 2.76 (td, J = 6.2, 2.0 Hz, 4H), 2.60 (t, J = 6.3 Hz, 2H), 2.38 (t, J = 6.5 Hz, 2H), 1.29 – 1.26 (m, 24H), 1.02 (d, J = 6.7 Hz, 6H). $^{31}$P NMR (162 MHz, CDCl$_3$) δ 150.4, 149.9. HRMS (ESI): m/z 905.36436 ([M+Na]$^+$ requires 905.36497).
9. NMR Spectra

1H NMR (CDCl₃, 400 MHz)
1H NMR (CDCl3, 400 MHz)
DEPT 135 NMR (CDCl3, 125.7 MHz)
1H NMR (DMSO-d6, 500 MHz)
$^{13}$C(H) NMR (Acetone-d$_6$, 101 MHz)
1H NMR (CDCl3, 400 MHz)
$^{13}$C NMR (CDCl$_3$, 125.7 MHz)
1H NMR (CDCl₃, 400 MHz)
$^{31}$P($^1$H) NMR (CDCl$_3$, 162 MHz)

![Chemical Structure Image]
10. References


5. Frisch, M. J., Gaussian, Inc. *Wallingford, CT* **2010**.


