Polymerase-mediated synthesis of $p$-vinylaniline–coupled fluorescent DNA for the sensing of nucleolin protein–c-myc G-quadruplex interactions

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1. General information
All reagents were obtained from commercial sources and used without further purification. $^1$H and $^{13}$C NMR spectra were recorded using a Bruker AV-400 spectrometer with CDCl$_3$ or DMSO-$d_6$ as the solvent and tetramethylsilane as the internal standard. UV–Vis spectra were recorded at room temperature using a Cary Series UV–Vis spectrophotometer (Agilent Technologies) and a quartz cuvette (path length: 1 cm); absorbance changes were measured immediately after UV irradiation of the sample solution in the cuvette. Fluorescence emission spectra were recorded at room temperature using a PF–65000 spectrofluorometer. Confocal microscopy images were recorded with all-natural oligonucleotides purchased from Bioneer (Seoul, Republic of Korea). Deoxyribonucleotide triphosphates mixture 2 mM each (dNTPs), $nPfu$-special enzyme, and buffer were purchased from Enzynomics (Bioneer, Republic of Korea). Recombinant Human Nucleolin Protein was purchased from Prospec Protein specialist, Israel.

2. Gel electrophoresis
Native polyacrylamide gel electrophoresis (nPAGE, 18%) was adopted to characterize the DNA products. The reaction mixture (10 mL) was mixed with 6x loading buffer (2.5 mL) and loaded into the well. Gel electrophoresis was performed in 1x TBE buffer (89 mM Tris, 89 mM borate, 2 mM EDTA, pH 8.3) at a constant potential of 95/80 V for 90–180 min and a current of 3 mA, followed by scanning on a gel image system. Similarly, RCA products (15 μL) were mixed with the loading buffer (6x, 2.5 μL) and run with 2% agarose gel for 40/60 min (100/50 V) in 1x TBE buffer. The gel was stained with ethidium bromide (EB) and then photographed using a ChemiDoc MP imaging system (Bio-Rad).

3. Primer extension reactions and lambda exonuclease cleavage to single-strand DNA ($dU_{C2}DNA$ and $dU_{Py}DNA$)

The template (0.3 μM) and primer (0.3 μM) were annealed at 95 °C and then left to cool to room temperature, thereby forming duplexes, and then mixed with dNTPs (2 mM each; dTTP was replaced by $dU_{C2}TP$ and $dU_{Py}TP$ for the syntheses of $dU_{C2}DNA$ and $dU_{Py}DNA$, respectively) and $nPfu$-special enzyme (2U) and incubated at 37 ºC for 16 h. The obtained fraction was heated at 65 ºC (2 min) for deactivation of the DNA polymerase; lambda exonuclease (2U) was added and then the fraction was incubated at 37 ºC for 1 h. The obtained sample was purified using a Qiagen quick oligonucleotide purification kit to obtain the single-stranded DNA ($dU_{C2}DNA$ or $dU_{Py}DNA$).
<table>
<thead>
<tr>
<th>Primer 1</th>
<th>5’-CTC ACT ATA GGG AGC-3’</th>
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<tr>
<td>Template 1</td>
<td>5’-[Phosphate]-CGC TAG GAC GGT ACT GGA TCA GCT CCC TAT AGT GAG-3’</td>
</tr>
<tr>
<td>Primer 2</td>
<td>5’ – TCC AAC TAT GTA TAC – 3’</td>
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<tr>
<td>Template 2</td>
<td>5’[Phosphate] – TAA TAC GAC TCA CTA TAG CAA TTG CGT GCC GCT AGC CTT CCC CAC CCT CCC CAC CCT CCC CAG TAT ACA TAG TTG GA -3’</td>
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<td>Primer 3</td>
<td>5’ – TAA TAC GAC TCA CTA TAG CAA TTG CGT G – 3’</td>
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<tr>
<td>Template 3</td>
<td>5’-TCC AAC TAT GTA TAC TGG GGA GGG TGG GGA GGG TGG GGA AGG CTA GCG GCA CGC AAT TGC TAT AGT GAG TCG TAT TA-3’</td>
</tr>
<tr>
<td>dU&lt;sub&gt;Py&lt;/sub&gt;-mycDNA</td>
<td>5’-TCC AAC TAT GTA TAC dU&lt;sub&gt;2&lt;/sub&gt; GG GGA GGG dU&lt;sub&gt;2&lt;/sub&gt; GG GGA GGG dU&lt;sub&gt;2&lt;/sub&gt; GG GGA AGG CTA GCG GCA CGC AAT TGC TAT AGT GAG TCG TAT TA-3’</td>
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Figure S1: Photophysical properties of the nucleosides. UV–Vis and fluorescence spectra of a, b) $d\text{U}_{\text{CN}_2}$ and c, d) $d\text{U}_{\text{Py}}$ in various solvents (16 μM).
Figure S2: a, c) UV–Vis and b, d) fluorescence spectra of the primer extension products from \( dU_{CN2}TP \) and \( dU_{Py}TP \). The obtained products were purified through an oligonucleotide purification kit (Qiagen).
Figure S3: PAGE analysis of primer extension and lambda exonuclease cleavage of dU_{CN2}TP and dU_{Py}TP.

Lane 1: Primer 1; lane 2: Template 1; lane 3: primer extension with dU_{CN2}TP; lane 4: primer extension with dU_{CN2}TP and then lambda exonuclease; lane 5: primer extension with dU_{Py}TP; lane 6: primer extension with dU_{Py}TP and then lambda exonuclease.

4. Stepwise approach to synthesize the dU_{Py}c-mycDNA

\[
\text{5'}\text{[Phosphate]} - \text{TAA TAC GAC TCA CTA TAG CAA TTG GTG GCC GCT ACG CTT CCC CAC CCT CCC CAC CCT CCC CAC CCT CAG TAT ACA TAG TTG GA -3'} \quad \text{C ATA TGT ATC AAC CT -5'}
\]

\[
\text{5'}\text{[Phosphate]} - \text{TAA TAC GAC TCA CTA TAG CAA TTG GTG GCC GCT ACG CTT CCC CAC CCT CCC CAC CCT CCC CAC CCT CAG TAT ACA TAG TTG GA -3'} \quad \text{G GAA GGG GdU_{Py}G GGA GGG GdU_{Py}G GGA GGG GdU_{Py}C ATA TGT ATC AAC CT -5'}
\]

\[
\text{5'}\text{[Phosphate]} - \text{TAA TAC GAC TCA CTA TAG CAA TTG GTG GCC GCT ACG CTT CCC CAC CCT CCC CAC CCT CCC CAC CCT CAG TAT ACA TAG TTG GA -3'} \quad \text{ATT ATG CTC AGT GAT ATC GTT AAC GCA CGG CGA TCG GAA GGG GdU_{Py}G GGA GGG GdU_{Py}G GGA GGG GdU_{Py}C ATA TGT ATC AAC CT -5'}
\]

3'- ATT ATG CTC AGT GAT ATC GTT AAC GCA CGG CGA TCG GAA GGG GdU_{Py}G GGA GGG GdU_{Py}G GGA GGG GdU_{Py}C ATA TGT ATC AAC CT -5'

The template 2 (0.3 μM) and primer 2 (0.3 μM) were annealed at 95 °C and then left to cool to room temperature, thereby forming duplexes, and then mixed with dGTP, dATP and dTTP (2 mM each; dTTP
was replaced by dUrTP for the syntheses of dUr-c-mycDNA) and nPfu-special enzyme (2U) and incubated at 37 °C for 16 h. The obtained sample was purified using a Qiagen quick oligonucleotide purification kit, again then mixed with dNTPs (2 mM each) and nPfu-special enzyme (2U) and incubated at 37 °C for 1h. The obtained fraction was heated at 65 °C (2 min) for deactivation of the DNA polymerase; lambda exonuclease (2U) was added and then the fraction was incubated at 37 °C for 1 h. The obtained sample was purified using a Qiagen quick oligonucleotide purification kit to obtain the dUr-c-mycDNA.

5. Binding study using SSB protein

SSB protein from E. coli was purchased from Enzynomics (Bioneer, Republic of Korea). The single-stranded DNA (1 μM) was mixed with 0.5 equivalents of SSB protein (0.5 μM) in a 1-mL quartz cuvette at room temperature in 50 mM phosphate buffer (pH 7.4). Three parallel samples were prepared for exact comparison (ssDNA mixed with SSB protein, ssDNA diluted with phosphate buffer, and ssDNA mixed with BSA protein).

6. Protein–DNA interactions of single-stranded dUcn2DNA and dUrDNA

Figure S4. a) Schematic representation of FMR-containing oligonucleotides binding with SSB protein. b, c) Fluorescence spectra of b) dUcn2DNA (1 μM) and c) dUrDNA (1 μM) in the absence and presence of SSB protein (0.5 μM) with excitation at 425 and 474 nm, respectively (see the Supporting Information for details).
7. **32P Labeling experiment for testing primer extension efficiency**

Primer 1 (0.2 μM, 2 μL), T4-PNK buffer (1x), 32P-ATP (2 mM), dd H2O, and T4-PNK enzyme (3U) were incubated at 37 °C for 1 h. The sample was purified using a Qi-quick oligonucleotide purification kit and finally eluted with elution buffer (200 μL) to obtain the stock of 32P-primer 1. The 32P-primer 1–template (0.2 μM, 2 μL) duplex was annealed at 95 °C and then slowly cooled to room temperature.

8. **32P Labeling experiment for monitoring the Nucleolin activity**

Primer 3 (0.2 μM, 2 μL), T4-PNK buffer (1x), 32P-ATP (2 mM), dd H2O, and T4-PNK enzyme (3U) were incubated at 37 °C for 1 h. The sample was purified using a Qi-quick oligonucleotide purification kit and finally eluted with elution buffer (200 μL) to obtain the stock of 32P-primer 3. The 32P-primer 3–template (0.2 μM, 2 μL) duplex was annealed at 95 °C and then slowly cooled to room temperature.

9. **Binding study using Nucleolin protein**

Nucleolin protein Human Recombinant was purchased from Prospec Protein Specialists (Israel). The dU₆mcDNA (0.3 μM) was annealed with the primer3 (0.3 μM) at 95°C to get A. To this 25 mM KCl was added and mixed with 0.5 μg of Nucleolin protein in a 1-mL quartz cuvette at room temperature in 50 mM phosphate buffer (pH 7.4). Four parallel samples were prepared for exact comparison (Only A, A with 25 mM KCl (C), A with 25 mM KCl and Nucleolin (E), and only Nucleolin).

The annealed duplex (A), duplex with 25 mM KCl (C), duplex with 25 mM KCl and 0.5 μg of Nucleolin (E) were all subjected to primer extension with 2mM dNTPs and nPfu special enzyme (2U) at 37°C for 60 min to obtain their corresponding stop and extended products (B, D, F). Finally to the stop product form duplex with 25 mM KCl and 0.5 μg of Nucleolin (F) was added with 1 μM of c-myc (pu27) in 25 mM KCl at 37°C for 10 minutes to create competition for Nucleolin binding.
**Figure S5.** d) Fluorescence spectra of dU_pyc-mycDNA in the absence and presence of Nucleolin at various temperature conditions. The fluorescence of the duplex with with 25 mM KCl and Nucleolin (E) incubated at 37°C and at 65°C for 10 minutes were measures (black and red); the red line was further cooled to 37°C (green), to the green 0.5 µg of Nucleolin was added; The Nucleolin was incubated at 65°C for 10 minutes and then the duplex of dU_pyc-mycDNA: primer3 was added (pink); Excitation wavelength: 474 nm.
Figure S6: a) and b) 1H NMR; c) and d) Fluorescence spectrum of dUCN2 and dUPy, before and after long range UV (314 nm – 400 nm) irradiation using the Rayonet photochemical reactor to verify the photostability of the nucleosides.
Figure S7: Melting point comparison of the Natural duplex with \( \text{dU}_{\text{chu2}} \) and \( \text{dU}_{\text{Py}} \) incorporated duplex DNAs.

Figure S8: 5% nondenaturing EMSA PAGE after GreenStar™ (Bioneer) staining for Interaction of nucleolin with the Natural c-myc DNA and \( \text{dUpy c-myc DNA} \) (0.1 µM). Nucleolin concentration in the reactions ranged from 0 to 10 equivalent (0 to 1 µM).
10. Synthesis of dU\textsubscript{CN2}TP and dU\textsubscript{Py}TP

**Synthesis of dU\textsubscript{CN2}TP and dU\textsubscript{Py}TP**

\[
\begin{align*}
\text{NH} & \quad \xrightarrow{\text{K}_2\text{CO}_3, \text{CH}_2\text{CN, rt, 16h}} \quad \xrightarrow{\text{POCl}_3 (1 \text{ equiv})} \\
n & \quad \text{DMF, 0}^\circ\text{C} \text{ to rt} \quad \xrightarrow{\text{82\%}} \\
\text{1a} & \quad \xrightarrow{\text{DMF, 80}^\circ\text{C}, 6h} \\
\text{2a} & \quad \text{N} & \quad \xrightarrow{\text{Cat. Piperidine, MeOH}} \\
& \quad \text{Reflux, 80}^\circ\text{C}, 1h \quad \xrightarrow{\text{55\%}} \\
\text{dU}\text{Py} & \quad \text{OH} & \quad \xrightarrow{\text{OH}} \\
& \quad \xrightarrow{\text{HO}} \\
& \quad \text{dU}\text{CN2} & \quad \text{OH}
\end{align*}
\]

**Scheme S1.** Synthesis of the FMRs dU\textsubscript{CN2}TP and dU\textsubscript{Py}TP.
*N*-Methyl-*N*-(prop-2-yn-1-yl)aniline (1a).33 Propargyl bromide (80% in toluene, 2.54 mL, 22.8 mmol) was added dropwise at 0 °C over 10 min to a suspension of *N*-methylaniline (1.66 mL, 15.2 mmol) and K$_2$CO$_3$ (3.15 g, 22.8 mmol) in MeCN (30 mL). The mixture was stirred overnight at room temperature under a N$_2$ atmosphere. The solids were filtered off and the solution was evaporated to dryness under reduced pressure. The residue was purified through column chromatography (SiO$_2$; 5% EtOAc in hexane) to afford a pale-yellow oil (2.10 g, 95%).  $^1$H NMR (400 MHz, CDCl$_3$): δ 7.29–7.25 (m, 2H), 6.88–6.80 (m, 3H), 4.05 (d, $J$ = 2.3 Hz, 2H), 2.98 (s, 3H), 2.17 (t, $J$ = 2.3 Hz, 1H); $^{13}$C NMR (CDCl$_3$, 100 MHz): δ 149.0, 129.2, 118.4, 114.3; 79.4, 72.1, 42.5, 38.6.

4-[*N*-Methyl-*N*-(prop-2-yn-1-yl) amino] benzaldehyde (2a).33 Freshly distilled POCl$_3$ (1.25 mL, 11.0 mmol) was added dropwise to dry DMF under a N$_2$ atmosphere at 0 °C. The solution was stirred at same temperature for 30 minutes and then warmed to room temperature and stirred for an additional 20 min. The oily compound 1a (1.60 g, 11.0 mmol) was added dropwise at 0 °C. The reaction mixture was stirred at room temperature for 1 h and then at 90 °C for 4 h. The mixture was neutralized with saturated NaHCO$_3$ while cooling in an ice bath. The resultant solid was filtered off and recrystallized from hexanes to yield 2a (6.26 g, 82%). $^1$H NMR (400 MHz, CDCl$_3$): δ 9.76 (s, 1H), 7.77–7.74 (m, 2H), 6.82–6.80 (m, 2H), 4.12 (d, $J$ = 2.3 Hz, 2H), 3.08 (s, 3H), 2.24 (t, $J$ = 2.3 Hz, 1H); $^{13}$C NMR (CDCl$_3$, 100 MHz): δ 190.5, 153.2, 131.9, 126.5, 112.3, 78.3, 72.6, 41.8, 38.43.

4-((3-(1-(4-Hydroxy-5-(hydroxymethyl)tetrahydrofur-2-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)prop-2-yn-1-yl)(methyl)amino)benzaldehyde (3a). A solution of 2a (0.783 g, 4.50 mmol), iododeoxyuridine (0.800 g, 2.25 mmol), Pd(PPh$_3$)$_4$ (0.130 g, 0.112 mmol), CuI (0.0860 g, 0.450 mmol), and DIPEA (0.736 mL, 4.50 mmol) in dry DMF (6 mL) was heated at 80 °C for 6 h. After cooling to room temperature, the mixture was evaporated to dryness and the residue purified through column chromatography (5% MeOH in CH$_2$Cl$_2$) to yield 3a (0.584 g, 65%). $^1$H NMR (400 MHz, CDCl$_3$): δ 11.62 (s, 1H), 9.73 (s, 1H), 8.19 (s, 1H), 7.73 (d, $J$ = 8.79 Hz, 2H), 6.95 (d, $J$ = 8.79 Hz, 2H), 6.09 (t, $J$ = 6.46 Hz, 1H), 5.25 (d, $J$ = 4.26 Hz, 1H), 5.11 (t, $J$ = 4.96 Hz, 1H), 4.49 (s, 2H), 4.24–4.20 (m, 1H), 3.79 (q, $J$ = 3.33 Hz, 1H), 3.61–3.56 (m, 2H), 3.09 (s, 3H), 2.13–2.10 (m, 2H); $^{13}$C NMR (CDCl$_3$, 100 MHz): δ (ppm) 190.71, 161.99, 153.65, 149.86, 144.39, 131.93, 126.15, 112.85, 98.31, 88.40, 88.06, 85.26, 76.73, 70.47, 61.33, 42.22, 38.42. HRMS (FAB): calcd for C$_{20}$H$_{21}$N$_3$O$_6$ [M$^+$] m/z 399.1430; found 399.1428.

2-((3-(1-(4-Hydroxy-5-(hydroxymethyl)tetrahydrofur-2-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)prop-2-yn-1-yl)(methyl)amino)benzoyldiene)malononitrile (dUCN$_2$). Piperidine (1 drop) was added to a solution of 3a (0.200 g, 0.500 mmol) and malononitrile (0.0330 g, 0.500 mmol) in dry MeOH and then the
mixture was heated under reflux for 1 h. After cooling to room temperature, the mixture was evaporated to dryness and the residue purified through column chromatography (3% MeOH in CH$_2$Cl$_2$) to yield dU$_{CN2}$ (0.179 g, 80%). \textsuperscript{1}H NMR (400 MHz, CDCl$_3$): $\delta$ 11.62 (d, $J = 6.99$ Hz, 1H), 8.20 (d, $J = 6.91$ Hz, 1H), 8.15 (d, $J = 6.89$ Hz, 1H), 7.89 (d, $J = 7.99$ Hz, 2H), 7.01 (d, $J = 8.23$ Hz, 2H), 6.08 (q, $J_1 = 6.69$ Hz, $J_2 = 7.0$ Hz, 1H), 5.26–5.24 (m, 1H), 5.12–5.09 (m, 1H), 4.55 (d, $J = 7.43$ Hz, 2H), 4.22 (bs, 1H), 3.79–3.78 (m, 1H), 3.59–3.58 (m, 2H), 3.34 (d, $J = 8.11$ Hz, 3H), 2.12–2.11 (m, 2H).

\textsuperscript{13}C NMR (CDCl$_3$, 100 MHz): $\delta$ (ppm) 161.99, 159.79, 153.81, 149.85, 144.54, 133.92, 120.33, 116.42, 115.63, 113.30, 98.16, 88.06, 87.95, 85.28, 77.03, 71.04, 70.45, 61.32, 42.17, 38.38. HRMS (FAB): calcd for C$_{23}$H$_{21}$N$_5$O$_5$[M$^+$]/m/z 447.1543; found 447.1544.

(E)-4-(4-((3-(1-(4-Hydroxy-5-(hydroxymethyl)tetrahydrofur-2-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)prop-2-yn-1-yl)(methyl)amino)styryl)-1-methylpyridin-1-iium iodide (dU$_{Py}$).

Piperidine (0.2 mL) was added to a solution of 3a (0.200 g, 0.500 mmol) and N-methyl 4-methyl pyridinium iodide (0.117 g, 0.500 mmol) in dry MeOH and then the mixture was heated under reflux for 1 h. After cooling to room temperature, the mixture was evaporated to dryness and the residue purified through column chromatography (3% MeOH in CH$_2$Cl$_2$) to yield dUP$_y$ (0.169 g, 55%). \textsuperscript{1}H NMR (400 MHz, CDCl$_3$): $\delta$ 8.69–8.66 (m, 3H), 8.03 (d, $J = 7.16$ Hz, 2H), 7.87 (d, $J = 16.04$ Hz, 1H), 7.53 (d, $J = 8.88$ Hz, 2H), 7.10 (d, $J = 16.04$ Hz, 1H), 6.64–6.60 (m, 3H), 6.12 (t, $J = 5.73$ Hz, 1H), 5.30–5.29 (m, 1H), 5.07 (bs, 1H), 4.25–4.23 (m, 1H), 4.18 (s, 3H), 3.88–3.85 (m, 1H), 3.60–3.58 (m, 2H), 2.76 (d, $J = 4.58$ Hz, 3H), 3.24 (d, 1H), 2.23–2.13 (m, 2H).

\textsuperscript{13}C NMR (CDCl$_3$, 100 MHz): $\delta$ (ppm) 193.75, 161.71, 153.92, 152.79, 150.17, 147.10, 144.73, 142.75, 130.92, 122.81, 122.42, 116.82, 112.50, 112.17, 88.37, 86.03, 70.70, 61.45, 46.77, 44.21, 30.69. HRMS (FAB): calcd for C$_{27}$H$_{29}$N$_4$O$_5$I [M$^+$]/m/z 489.2132; found 489.2135.

11. General procedure for the preparation of triphosphates

Proton sponge (1.5 equiv) and the free nucleoside (dU$_{CN2}$ or dU$_{Py}$, 1 equiv) were dissolved in trimethylphosphate (0.3 M) and cooled to –20 °C. POCl$_3$ (1.5 equiv) was added dropwise and then the purple slurry was stirred at –20 °C for 2 h. Tributylamine (6.2 equiv) was added, followed by a solution of tributylammonium pyrophosphate (5.0 equiv) in DMF (0.5 M). After 5 min, the reaction was quenched through the addition of 0.5 M aqueous Et$_3$NH$_2$CO$_3$ (20 vol. equiv) and the resulting solution was lyophilized. Purification through reversed-phase (C18) HPLC (4–35% MeCN in 0.1 M Et$_3$NH$_2$CO$_3$, pH 7.5), followed by lyophilization, afforded the triphosphate as a solid.

dU$_{CN2}$TP:
$^{31}\text{P NMR (400 MHz, D}_2\text{O)}$: $\delta$ –24.17 to –23.48 (m, $\beta$-P), –12.7 to –11.28 (m, $\alpha$-P), –11.28 to –10.96 (m, $\gamma$-P).

**MS (LC/MS, LRMS):** calcd for C$_{23}$H$_{22}$N$_5$O$_{14}$P$_3^{2-}$ ([M]): $m/z$ 685.0242; found: 685.3575.

![Figure S9: $^{31}$P NMR spectrum of dU$_{CN2}$TP.](image)

**dU$_{Py}$TP:**

$^{31}\text{P NMR (400 MHz, D}_2\text{O)}$: $\delta$ –22.13 to –22.00 (m, $\beta$-P), –11.14 to –10.73 (m, $\alpha$-P), –6.74 to –5.98 (m, $\gamma$-P).

**MS (LC/MS, LRMS):** calcd for C$_{27}$H$_{30}$N$_4$O$_{14}$P$_3^{2-}$ ([M]): $m/z$ 727.0831; found: 727.4579.
12. Photophysical properties of functional nucleotides

\[ A = \varepsilon c l \]

\( A \) is the absorbance of the compounds measured at the wavelength maximum (\( \lambda_{\text{max}} \)).
\( \varepsilon \) is the extinction coefficient [M\(^{-1}\) cm\(^{-1}\)].
\( c \) is the concentration [M].
\( l \) is the path length of the cuvette [cm]

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<th>Compound</th>
<th>Absorbance at ( \lambda_{\text{max}} )</th>
<th>Concentration (M)</th>
<th>Path length of cuvette (cm)</th>
<th>( \lambda_{\text{max}} ) (nm)</th>
<th>Extinction coefficient (( \varepsilon )) (M(^{-1}) cm(^{-1}))</th>
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<td>( \text{dU}_{\text{CN2}} )</td>
<td>1.0219</td>
<td>( 10 \times 10^{-6} )</td>
<td>1</td>
<td>423</td>
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<td>( \text{dU}_{\text{Py}} )</td>
<td>0.3216</td>
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**Figure S11:** a, c) UV–Vis and b, d) fluorescence spectra of dU_{CN2} in a, b) MeOH and c, d) glycerol.

**Figure S12:** a, c) UV–Vis and b, d) fluorescence spectra of dU_{Py} in a, b) MeOH and c, d) glycerol.
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</tbody>
</table>
Figure S13: $^1$H and $^{13}$C NMR spectra of 1a in CDCl$_3$. 
Figure S14: $^1$H and $^{13}$C NMR spectra of 2a in CDCl$_3$. 
Figure S15: $^1$H and $^{13}$C NMR spectra of 3a in DMSO-$d_6$. 
Figure S16: $^1$H and $^{13}$C NMR spectra of dU$_{CN2}$ in DMSO-$d_6$. 
Figure S17: $^1$H and $^{13}$C NMR spectra of dU$_{py}$ in DMSO-$d_6$ (contains piperidine).