Supporting Information

Photo-cross-linking of DNA using 4-methylpyranocarbazole with thymine-base selectivity

† Junichi Mihara, † Kenzo Fujimoto *
† School of Advanced Institute Science and Technology, Japan Advanced Institute of Science and Technology, Asahidai 1-1, Nomi, Ishikawa, 923-1292, Japan.

Table of Contents

1. UPLC analysis of photo-cross-linking
2. MALDI-TOF-MS analysis of photo-cross-linked dsDNA
3. UV spectra of MEPK and PCX nucleoside
4. Melting temperature measurement of duplex DNA
5. Circular Dichroism (CD) spectra of duplex DNA
6. Molecular modeling of duplex ODN
7. UPLC analysis of photo-splitting
8. MALDI-TOF-MS spectra of ODNs
9. NMR spectra and ESI-FT-ICR MS analysis of the synthesized compound
1. **UPLC analysis of photo-cross-linking**

\[
\begin{align*}
5'-\text{TGCY}^{\text{MEP}}\text{KCCGT-3'} \\
3'-\text{ACGY}^*---\text{GGGCA-5'} \\
Y/Y^*=\text{A/T or G/C}
\end{align*}
\]

\begin{align*}
\overset{\text{400 nm}}{\text{600 sec}} \\
\overset{\text{0 sec}}{\text{1 sec}} \\
\overset{\text{30 sec}}{\text{60 sec}} \\
\overset{\text{300 sec}}{\text{600 sec}} \\
\overset{\text{0 sec}}{\text{1 sec}} \\
\overset{\text{30 sec}}{\text{60 sec}} \\
\overset{\text{300 sec}}{\text{600 sec}}
\end{align*}

**Figure S1.** Photo-cross-linking using MEP. 10 μM of ODNs in 50 mM Na-cacodylate buffer containing 100 mM NaCl was annealed and photoligated at 400 nm on ice.
2. MALDI-TOF-MS analysis of photo-cross-linked dsDNA

**Figure S2.** MALDI-TOF-MS analysis of photo-cross-linked dsDNA consisting of ODN(A\textsuperscript{MET}K) and ODN (GT).
3. UV spectra of $^{\text{MEP}}$K and $^{\text{PC}}$X nucleoside

![UV spectra of MEP and PCX nucleoside](image)

**Figure S3.** UV spectra of $^{\text{MEP}}$K and $^{\text{PC}}$X nucleoside
4. Melting temperature measurement of duplex DNA

**ODN(A\textsuperscript{MEPK})/ODN(GT)**

![Graph showing hypochromicity at 260 nm vs. temperature and correlation with \(\ln(C_{4})/T_{M}\) for ODN(A\textsuperscript{MEPK})/ODN(GT).]

**ODN(A\textsuperscript{PCX})/ODN(GT)**

![Graph showing hypochromicity at 260 nm vs. temperature and correlation with \(\ln(C_{4})/T_{M}\) for ODN(A\textsuperscript{PCX})/ODN(GT).]

**ODN(A\textsuperscript{CNVK})/ODN(GT)**

![Graph showing hypochromicity at 260 nm vs. temperature and correlation with \(\ln(C_{4})/T_{M}\) for ODN(A\textsuperscript{CNVK})/ODN(GT).]

**ODN(G\textsuperscript{MEPK})/ODN(GC)**

![Graph showing hypochromicity at 260 nm vs. temperature and correlation with \(\ln(C_{4})/T_{M}\) for ODN(G\textsuperscript{MEPK})/ODN(GC).]
Figure S4. Melting curve and 1/Tm vs ln (Ct/4) plot of DNA duplexes containing. Sample solutions were DNA duplexes in 50 mM Na-cacodylate buffer containing 100 mM NaCl. Absorbance was measured at 260 nm from 5 °C to 80 °C.
5. Circular Dichroism (CD) spectra of duplex DNA

**Figure S5.** Circular dichroism spectra of duplexes DNA. [duplex]=10 μM in 50 mM Na-Cacodylate buffer (pH 7.4) containing 100 mM NaCl.
6. Molecular modeling of duplexes ODN

**Figure S6.** Molecular modeling of the ODN(A_{MEP}K)/ODN(GT) [left] and ODN(G_{MEP}K)/ODN(GC) [right] duplexes. Upper: side view of duplex; lower: top view of photo-cross-linking region.
7. UPLC analysis of photo-splitting

**Figure S7.** The photo-splitting of MEPK. The 10 µM photoadduct in 50 mM Na-cacodylate buffer containing 100 mM NaCl was photoirradiated at 330 nm at 60 °C.
8. MALDI-TOF-MS spectra of ODNs

Figure S8. MALDI-TOF-MS analysis of ODN(A\textsuperscript{MEPK})

Figure S9. MALDI-TOF-MS analysis of ODN(G\textsuperscript{MEPK})
Figure S10. MALDI-TOF-MS analysis of ODN(TMEP)K

Figure S11. MALDI-TOF-MS analysis of ODN(CMEP)K
Figure S12. MALDI-TOF-MS analysis of ODN(A^PCX)

Figure S13. MALDI-TOF-MS analysis of ODN(G^PCX)
**Figure S14.** MALDI-TOF-MS analysis of ODN(A<sup>CMP</sup>K)

**Figure S15.** MALDI-TOF-MS analysis of ODN(G<sup>CMP</sup>K)
Figure S16. MALDI-TOF-MS analysis of ODN(GT)

Figure S17. MALDI-TOF-MS analysis of ODN(GC)
9. NMR spectra and ESI-FT-ICR MS analysis of synthesized compound

**Figure S18.** $^1$H-NMR spectra of 4-methylpyrano[2,3-b]carbazol-2(10H)-one (2)

**Figure S19.** $^{13}$C-NMR spectra of 4-methylpyrano[2,3-b]carbazol-2(10H)-one (2)
Figure S20. ESI-FT-ICR MS analysis of 4-methylpyrano[2,3-b]carbazol-2(10H)-one (2)

Figure S21. $^1$H-NMR spectra of 10-(2-Deoxy-$\beta$-D-ribofuranosyl)-4-methylpyrano[2,3-b]carbazol-2(10H)-one (3): MEP{$\kappa$}
**Figure S22.** $^{13}$C-NMR spectra of 10-(2-Deoxy-β-D-ribofuranosyl)-4-methylpyrano[2,3-b]carbazol-2(10H)-one (3): MEPK

**Figure S23.** ESI-FT-ICR MS analysis of 10-(2-Deoxy-β-D-ribofuranosyl)-4-methylpyrano[2,3-b]carbazol-2(10H)-one (3): MEPK
Figure S24. $^1$H-NMR spectra of 5'-O-(Dimethoxytrityl)-10-(2-Deoxy-β-D-ribofuranosyl)-4-methylpyrano[2,3-b]carbazol-2(10H)-one (4)

Figure S25. $^{13}$C-NMR spectra of 5'-O-(Dimethoxytrityl)-10-(2-Deoxy-β-D-ribofuranosyl)-4-methylpyrano[2,3-b]carbazol-2(10H)-one (4)
**Figure S26.** ESI-FT-ICR MS analysis of 5’-O-(Dimethoxytrityl)-10-(2-Deoxy-β-D-ribofuranosyl)-4-methylpyrano[2,3-b]carbazol-2(10H)-one (4)

**Figure S27.** 1H-NMR spectra of 5’-O-(Dimethoxytrityl)-10-(2-Deoxy-β-D-ribofuranosyl)-4-methylpyrano[2,3-b]carbazol-2(10H)-one 3’-O-(2-cyanoethyl-Ν,Ν’-diisopropyl)phosphoramidite (5)
Figure S28. $^{13}\text{C}$-NMR spectra of 5'-O-(Dimethoxytrityl)-10-(2-Deoxy-$\beta$-D-ribofuranosyl)-4-methylpyrano[2,3-b]carbazol-2(10H)-one 3'-O-(2-cyanoethyl-$N,N'$-diisopropyl)phosphoramidite (5)

| Meas. m/z | Ion Formula | m/z    | err (ppm) | |err| [mDa] |
|-----------|-------------|--------|-----------|-----------|-----------|
| 868.372478 | C51H55N3O8P | 868.372129 | -0.4 | 0.3 |
| 890.354233 | C51H54N3NaO8P | 890.354073 | -0.1 | 0.1 |
| 906.32597  | C51H54KN3O8P | 906.32801 | 2.3 | 2 |

Figure S29. ESI-FT-ICR MS analysis of 5'-O-(Dimethoxytrityl)-10-(2-Deoxy-$\beta$-D-ribofuranosyl)-4-methylpyrano[2,3-b]carbazol-2(10H)-one 3'-O-(2-cyanoethyl-$N,N'$-diisopropyl)phosphoramidite (5)