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Specific Induced Circular Dichroism and Enhanced B to Z Transitions of Duplexes Stabilized by Chromophore-Linked Alkynylnucleoside Residues

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Abstract: We have developed an induced circular dichroism (ICD) probe with a chromophore-linked alkynyldeoxyribose skeleton for analyzing higher-order structures of DNA duplexes in the visible-light region. When CG-repeated oligonucleotides (ODNs) with the probe at their 5′ ends adopted Z-form duplexes at a high NaCl concentration, strong ICD signals were observed at the absorptive region of the chromophore. On the other hand, their B-form duplexes, formed at a low NaCl concentration, produced a faint ICD signal. The specific ICD for the Z-form duplexes was found to appear only when the chromophores were attached at the 5′ ends of each of the ODNs. Furthermore, the chromophoric alkynylnucleoside residues effectively promoted the B to Z transition of the ODN.

Keywords: chromophore-labeled DNA · circular dichroism · duplex transitions · nucleosides

Introduction

In nucleic acid chemistry, left-handed Z-DNAs are one of the conundrums that confront chemists and biologists because their functions are still obscured in vivo.[1] Prior to the exploration of their latent biological functions, their physiological characteristics such as intermolecular interactions with Z-DNA-binding proteins[2] should be comprehensively studied at the oligonucleotide (ODN) level in vitro. Circular dichroism (CD) spectroscopy works well for detecting Z-DNA duplexes at their absorptive region, but this strategy is inappropriate to analyze interactions of DNAs with proteins owing to the overlap between DNA and protein absorptive regions.[3] Therefore, discrimination between B- and Z-DNA duplexes should be carried out at a visible-light region where biological molecules scarcely absorb. This methodology may allow the intermolecular interactions of Z-DNAs with proteins to be easily detected.

With this in mind, several research groups have developed chromophore-based sensor molecules that can discriminate between B- and Z-DNA duplexes at the visible-light region.[4] For instance, Purrello et al. have recently reported that anionic nickel(II) porphyrins selectively bind to Z-form duplexes of poly-CG sequences in the presence of spermine such that the B- and Z-form duplexes can be discriminated by CD spectroscopy.[4a] Although this type of sensor molecules based on intermolecular interactions are favorable for detecting Z-form duplexes as they are, the sensors hardly avoid the inherent problem that their detection abilities are seriously dependent on their binding constants with DNA. This situation may disturb the quantitative assessment for the content of Z-form duplexes against B-form ones on the basis of the CD signals. In addition, these exogenous sensor molecules are likely to interfere with the interaction of Z-DNAs with proteins.

Covalent labeling of ODNs with a chromophore at their 5′ and/or 3′ ends will surmount the problems mentioned above and have the potential to assess the interaction of Z-DNAs with proteins quantitatively.[5,6] Berova et al. have used a tetraarylporphyrin derivative as an ODN-labeling chromopore and have investigated the B to Z transition of CG-repeated 8-mer ODN labeled with the chromophore by CD spectroscopy.[5] Upon self-hybridization of the labeled ODN, strong induced CD (ICD) signals appeared from the porphyrin at a high NaCl concentration condition that promotes Z-duplex formation, whereas moderate ICD signals were observed at a low NaCl concentration. The two porphyrins attached at the 5′ ends of each of ODNs might communicate with each other through the π stacking, resulting in an exciton-type ICD. They claimed that the labeled ODN

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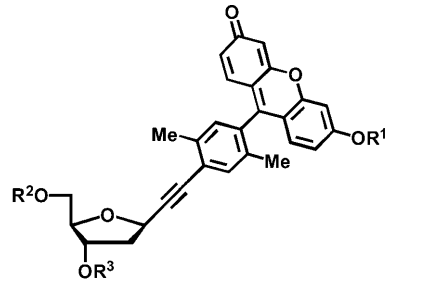
predominantly adopts the Z-form duplex at a saturated NaCl concentration on the basis of the CD spectral change. However, in this case the content of the Z-form duplex seems to be partial even at the saturated condition.[7] We thought that the tetraarylporphyrin moieties on the duplex structure may hinder the B to Z transition owing to their large π planes. Taking these results into account, we designed a chromophoric ICD probe **1** with an alkynyldeoxyribose skeleton that has previously reported by us for analyzing the B to Z transition of DNAs in the visible-light region. Here we report strong ICDs that are specific for Z-form duplexes of the chromophore-labeled ODNs and promotion of their B to Z transitions by the attached chromophores.

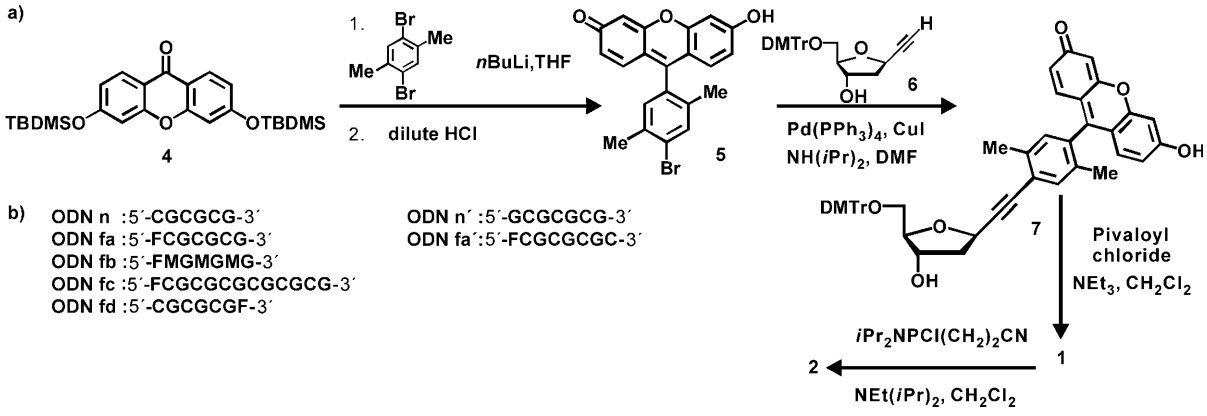
Results and Discussion

The alkynyldeoxyribose-based ICD probe **1** (Piv = pivaloyl; DMTr = 4,4′-dimethoxytrityl) with a fluorescein-like chromophore was converted into the phosphoramidite **2** and then introduced into ODNs by solid-phase DNA synthesis. The alkynyldeoxyribose skeleton has been previously developed by us and can be linked to various aromatic groups by Sonogashira coupling reactions.[8] We expected that the chromophore of **1** would be a superior reporter for duplex analysis by CD spectroscopy for several reasons. First, the molar extinction coefficient (ε) of the chromophore is high enough to give an intense ICD signal at its absorptive region. Indeed, ε of the deprotected **3** is approximately 80000 $mol^{-1}Lcm^{-1}$ in a Tris-HCl buffer (100 mM, pH 7.0), which is comparable to that of fluorescein. Second, the π-plane size of its xanthene moiety is smaller than that of porphyrin, which leads to the expectation of a smooth B to Z transition in the labeled ODNs.

Scheme 1 shows the synthesis of the probe **1** and the ODN sequences used in this study. A xanthone derivative **4**[9] was allowed to react with the mono-lithiated product of 2,5-dibromo-*p*-xylene to yield **5** as a basic chromophore skeleton.[10] The cross-coupling reaction of **5** with the published alkynyldeoxyribose **6**[8] gave **7**, which was protected on the xanthene moiety by pivaloylation to provide the probe **1**. CG-repeated ODNs were modified with the phosphoramidite **2** at their ends with a DNA synthesizer. Five labeled ODNs, **fa**, **fa′**, **fb**, **fc**, and **fd**, were used in this study in which the chromophore containing the ethynyl group is abbreviated as F.

Rich et al. have reported that a hairpin ODN, $d(CG)_3T_4(CG)_3$, can bind to a set of the two Zα domains of the human RNA editing enzyme, double-stranded RNA deaminase I (ADAR1).[2d] This research reveals the possibility that left-handed short duplexes may play a role as the binding site to ADAR1 in a gene. Therefore, we measured the CD spectra for the palindromic **ODNn** and **ODNfa** of 6-mer to assess their B to Z transitions in the presence of NaCl. The nonlabeled **ODNn** was found to be on its way to the B to Z transition at 25 °C in the presence of NaCl (5.0 M) by comparing it with the CD spectra between 25 °C and 5 °C (Figure S1 in the Supporting Information). For **ODNfa** in the presence of NaCl (0.1 M), CD bands of typical double-stranded B-form duplexes were observed at the absorptive region of nucleobases (Figure 1, left), whereas no meaningful signals existed at ≥330 nm (Figure 1, right). In the presence of 5.0 M NaCl, the characteristic positive and negative CD signals for Z-form duplexes clearly appeared at 269 and 293 nm at 25 °C, respectively, accompanied with the disappearance of the negative CD signal around 250 nm. This





Scheme 1. a) A synthetic scheme for the alkynyldeoxyribose-based ICD probe. b) The ODN sequences used in this study (F and M represent the residue of **3** and 5-methylcytosine, respectively).

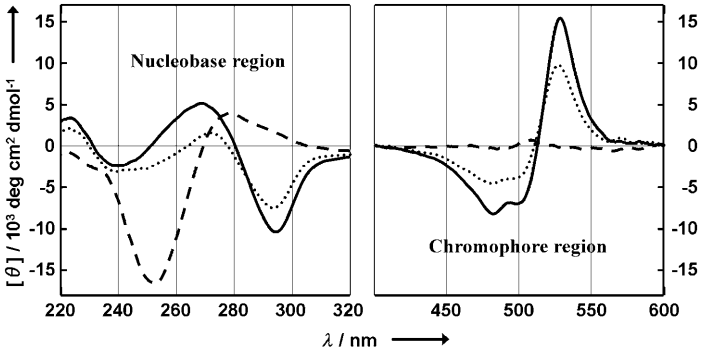


Figure 1. CD spectra of **ODNfa** (20 μM) in a Tris-HCl buffer (100 mM, pH 7.0) in the presence of NaCl (0.1–5.0 M) at 25 °C (——=5.0, •••••=2.0, and -----=0.1 M NaCl).

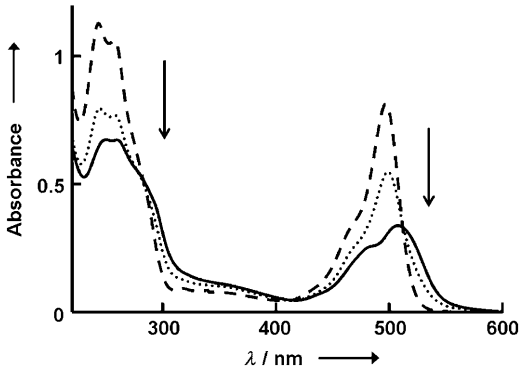


Figure 3. UV/Vis spectra of **ODNfa** (20 μM) in a Tris-HCl buffer (100 mM, pH 7.0) in the presence of NaCl (0.1–5.0 M) at 25 °C (——=5.0 M, •••••=2.0 M, and -----=0.1 M NaCl).

spectral change at the absorptive region of the nucleobases means that the Z-form duplex of **ODNfa** exclusively exists in the presence of 5.0 M NaCl. Note that under these conditions two new intense bands appeared at long wavelengths with a plus-minus pattern near 530 and 480 nm (Figure 1, right). The emerging bands are assigned to exciton-type ICD between the two chromophoric residues of **1**.[5,11] The meaningless ICD signal in the B-form duplex was switched to a strong one in the Z-form duplex. To correlate the intensity of the ICD with the content of the Z-form duplex, the spectral changes of the CD intensities at 529 nm and 293 nm were both plotted as a function of NaCl concentration (Figure 2). The two concentration-dependent transition curves coincide well with each other. Therefore, the concentration dependence of the ICD intensity was found to directly reflect the content of the Z-form duplex.

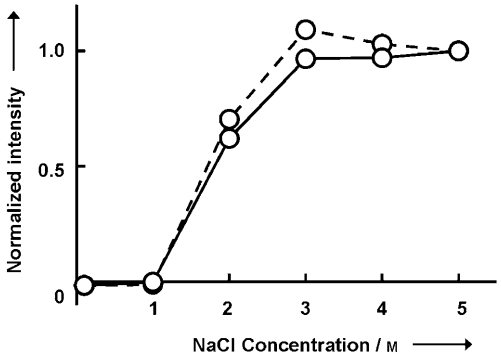


Figure 2. Normalized intensities derived from the CD spectra of **ODNfa** at 293 nm (-----) and 529 nm (——) plotted against NaCl concentration. Each CD intensity was normalized on the basis of the intensities at the NaCl concentration of 5.0 M.

To elucidate the origin of the specific ICD, we measured the UV/Vis spectra of **ODNfa**. As the concentration of NaCl was increased, large hypochromicity arose both in the nucleobase and in the chromophore absorption bands accompanied with isosbestic points at 283, 414, and 513 nm (Figure 3). These isosbestic points indicate the presence of only two chromophoric species, probably the B- and Z-form duplexes. The hypochromicity observed during the transition is attributed to the enforced π stacking between the chromophore and the nucleobase pairs in the Z-form duplex compared with the B-form duplex. A conformational search by using computational chemistry was performed for the B- and Z-duplexes of **ODNfa** for reinforcing the contribution of the π stacking to the hypochromicity. Figure 4 displays

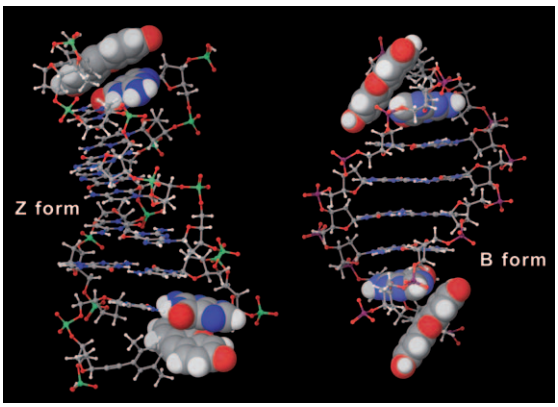


Figure 4. Plausible structures for the Z- and B-duplexes of **ODNfa**. The CPK models in these structures represent the π planes of the xanthene moieties and the guanine bases at the 5′ and 3′ ends of **ODNfa**, respectively.

plausible structures for the Z and B forms, which were optimized in water without NaCl by MacroModel 9.5 (Mestro 9.0 interface) by using an OPLS 2005 force field. In the optimized structure for the Z form, the π plane of the xanthene moiety is stacked with that of the guanine base at the 3′ end of the complementary strand. On the other hand, in the case of the B form, the xanthene π plane adopts an almost perpendicular conformation against that of the nucleobase. These findings support the fact that the two chromophores may communicate with each other through the base pairs, resulting in the observed exciton-type ICD in the Z-form duplex.

However, a large amount of NaCl could somewhat affect the UV/Vis spectral changes at the absorptive region of the chromophore in **ODNfa**. Therefore, we performed the following control experiments to rule out this ambiguity. In the UV/Vis spectra of the deprotected **3**, the influence of NaCl was negligible (Figure S2 in the Supporting Information). Subsequently, **ODNfb**, which consists of 5-methylcytosine instead of cytosine, was subjected to the same measurements because this substitution is well known to promote the formation of Z-form duplexes at a relatively low NaCl concentration (Figure S3 in the Supporting Information).[12] Indeed, the NaCl concentration of 2.0 M is enough to complete the Z-duplex formation in **ODNfb** to afford a strong ICD signal at the same level of that for **ODNfa** in the presence of 5.0 M NaCl. These experiments support the fact that the large hypochromicity mentioned above is only attributed to the tight π stacking arising between the chromophore and the nucleobase pairs and not to the presence of a large amount of NaCl.[13]

For exploration of the thermal influence of the chromophore upon Z-form duplex formation, we measured the CD spectra of **ODNfa** in the presence of NaCl (5.0 M) at various temperatures. Figure 5 shows the CD spectral change for the

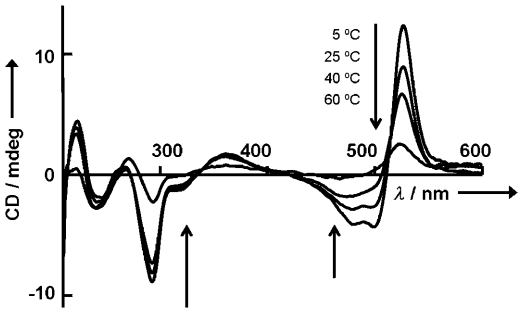


Figure 5. CD spectra of **ODNfa** (10 μM) in a Tris-HCl buffer (100 mM, pH 7.0) in the presence of NaCl (5.0 M) at temperatures ranging from 5 °C to 60 °C.

Z-duplex at temperatures ranging from 5 °C to 60 °C. As the temperature increased, both of the CD intensities from the nucleobases and from the chromophore gradually decreased. Surprisingly, the Z-form duplex partially survived at 60 °C on the basis of the negative CD band around 290 nm, whereas the CD spectra of **ODNn** lacking the chromophore showed the complete melting of its Z-form duplex at the same temperature (Figure S1 in the Supporting Information). This finding indicates that the attached chromophore provides the Z-form duplexes of the labeled ODNs with an extra thermal stability by strong π stacking. To ensure the thermal effect of the chromophore, melting points (T_m) for the duplexes of **ODNfa** were measured by using UV/Vis spectroscopy at 260 nm. In the heating and cooling processes between 20 °C and 80 °C, a hysteresis curve was observed only for the Z-form duplex of **ODNfa**. The T_ms for the B and Z forms were estimated to be 45 (45) and 62 (46) °C, respectively (the temperatures in parentheses were obtained from the cooling process). Similar hysteresis phenomena are known to be observed in DNA triplex structures.[14] In the triplexes, the slow chemical equilibrium between the triplex and duplex structures causes a hysteresis curve in the processes. In our system, the stabilization of the Z-form duplex by the two chromophores might slow down the renaturation to afford the observed hysteresis phenomenon.

The longer CG-repeated palindromic **ODNfc** that consists of 12 bases showed a similar spectral tendency to **ODNfa** (Figure S4 in the Supporting Information). The ICD intensity of the Z-form duplex in **ODNfc** was approximately 1.6 times as large as that in **ODNfa** under the same condition despite the longer distance between the two chromophores. Lewis et al. have reported that the ICD intensity of labeled ODNs with two stilbenes placed on the edges of their duplex structures is sensitive not only to the distance between the two stilbenes but also to the orientation of the dipole moments in them.[15] Because one turn in Z-DNA duplexes involves 12 base pairs, **ODNfc** is just able to form one turn duplex, whereas **ODNfa** corresponds to half of this size.[16] The orientation of the dipole moments in the two chromophores on **ODNfc** is considered to be more favorable for yielding the ICD than that in **ODNfa**. Thus, the well-fitted orientation of the two chromophores in **ODNfc** not only compensated for the distance disadvantage but it also enhanced the intensity of the ICD signal.

Several CG-sequenced ODNs with the chromophores were analyzed by CD spectroscopy to examine the influence of the number and the position of the chromophore upon the specific ICD and the structural stability in the Z-form duplexes of the labeled ODNs. Figure S5 in the Supporting Information exhibits the CD spectra of the combination of **ODNn′**/**ODNfa′** and palindromic **ODNfd**. The duplex structure of the former has only a single chromophore at the 5′ end, and the latter bears the chromophore at the 3′ end. The chromophores, independently of the NaCl concentrations, provided no ICDs in both of these cases.[17] Next, the CD signals at the absorptive region of nucleobases were compared between the four duplexes, **ODNfa**, **ODNn**, **ODNn′**/**ODNfa′**, and **ODNfd**, to evaluate the contents of their Z-form duplexes. In the positive CD signals around 270 nm, the λ_{max} in **ODNfa** was 268 nm, whereas those in the others were $\geq$273 nm. Furthermore, **ODNfa** showed a much higher absolute molar ellipticity, $|[\theta]|$, of 10400 in the negative CD signal around 290 nm than others (2300–2700). In the complexes of CG-sequenced ODNs with the Z-DNA-binding proteins, their absolute molar ellipticities were reported to be beyond 10000.[2] Therefore, our data imply that a set of two chromophores attached at the 5′ ends effectively stabilized the Z-form duplexes and produced the specific ICD.

Conclusion

We have developed an ICD probe for DNA duplexes by using an alkynyldeoxyribose skeleton. The CG-repeated

ODNs labeled with the chromophores at their 5′ ends self-hybridized to form stable Z-form duplexes at a high NaCl concentration. A strong ICD appeared from the absorptive region of the chromophore only in the case of the Z-form duplexes, and the attached chromophore was found to promote the B to Z transition effectively. A good linear relationship was observed between the contents of Z-form duplexes and the intensities of the ICD signals. Therefore, our probe is expected to be applied in accurate CD analysis for the interaction of Z-DNA with Z-DNA-binding proteins in vitro.

Experimental Section

General methods and materials: ^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively. MALDI-TOF mass spectra were recorded with 3-hydroxypicolinic acid as a matrix. High-resolution mass spectra were obtained by using an ESI-TOF method. Melting points are uncorrected. Reagents were purchased from commercial sources and used without further purification. The starting materials, 3,6-bis-*O*-(*tert*-butyldimethylsilyl)xanthone **4**,[9] 5-(4,4′-dimethoxytrityl)-1-β-ethynyl-2-deoxy-D-ribofuranoside **6**[8] have been previously reported and were synthesized according to the published procedures.

A basic chromophore skeleton (5): A solution of *n*BuLi (1.57 M) in *n*-hexane (6.37 mL, 10 mmol) was added to a solution of 2,5-dibromo-*p*-xylene (2.64 g, 10 mmol) in THF (26 mL) at −78 °C. The reaction mixture was stirred for 30 min at that temperature, and a solution of **4**[9] (4.57 g, 10 mmol) in THF (45 mL) was added to the mixture. The mixed solution was heated to room temperature and was quenched by the addition of HCl (1 N, 20 mL). The resulting yellow precipitate was collected by filtration, washed with a small quantity of *n*-hexane, and dried in vacuo to give **5** as a brown solid (yield 84%, 3.3 g). M.p. = 284–287 °C; ^{1}H NMR (CD_3OD:[D_6]DMSO = 1:2): δ = 7.71 (s, 1H), 7.24 (s, 1H), 6.98 (d, J = 9.2 Hz, 2H), 6.66 (d, J = 8.8 Hz, 2H), 6.65 (s, 2H), 2.41 (s, 3H), 2.00 ppm (s, 3H); ^{13}C NMR ([D_6]DMSO) δ = 156.8, 149.1, 135.9, 135.6, 134.0, 132.2, 131.5, 130.5, 125.5, 114.9, 103.8, 38.9, 22.0, 18.4 ppm; IR (KBr): $\tilde{\nu}$ = 3419, 1566 cm^{-1}; HRMS (ESI): *m*/*z*: calcd for $C_{21}H_{16}O_3Br$: 395.0283; found: 395.0315 $[M+H]^+$.

A nucleoside derivative (7): A solution of **6**[8] (222 mg, 0.5 mmol) in DMF (5 mL) was added to a solution of **5** (198 mg, 0.5 mmol), NH(iPr)$_2$ (3 mL), Pd(PPh_3)$_4$ (87 mg, 0.075 mmol), and CuI (4.76 mg, 0.025 mmol) in DMF (10 mL) at 60 °C under an Ar atmosphere. The reaction mixture was stirred overnight at that temperature and filtered through a pad of florisil. The filtrate was evaporated and chromatographed (silica gel; eluent: from CH_2Cl_2:MeOH = 100:1 to 10:1) to give **7** as a red foam (yield 76%, 289 mg). M.p. = 158–162 °C; ^{1}H NMR ($CDCl_3$): δ = 7.47 (d, J = 8.0 Hz, 2H), 7.37–7.34 (m, 5H), 7.26–7.21 (m, 2H), 7.16–7.13 (m, 1H), 7.04 (d, J = 8.4 Hz, 2H), 6.96 (s, 1H), 6.87 (s, 2H), 6.83 (d, J = 9.2 Hz, 2H), 6.77 (dd, J = 8.8, 2.0 Hz, 4H), 5.98 (br s, 1H), 5.12 (t, J = 6.8 Hz, 1H), 4.49 (m, 1H), 4.03 (m, 1H), 3.72 (s, 6H), 3.27 (d, J = 3.6 Hz, 2H), 2.45–2.36 (m, 5H), 1.89 ppm (s, 3H); ^{13}C NMR ($CDCl_3$): δ = 175.8, 158.4, 158.0, 154.8, 144.9, 138.0, 136.1, 135.9, 133.2, 132.4, 131.0, 130.1, 128.2, 128.0, 126.7, 124.01, 122.3, 114.7, 113.1, 113.0, 103.8, 93.7, 86.3, 86.1, 83.3, 74.3, 68.2, 64.5, 55.2, 42.5, 20.2, 18.9 ppm; IR (KBr): $\tilde{\nu}$ = 1594 cm^{-1}; HRMS (ESI): *m*/*z*: calcd for $C_{49}H_{43}O_8$: 759.2958; found: 759.2982 $[M+H]^+$.

An ICD probe (1): A solution of pivaloyl chloride (40 μL, 0.33 mmol) in CH_2Cl_2 (1 mL) was added to a solution of **7** (228 mg, 0.3 mmol) and NEt_3 (84 μL, 0.6 mmol) in CH_2Cl_2 (4 mL) at 0 °C. The reaction mixture was stirred for 1 h at room temperature, quenched by the addition of a saturated $NaHCO_3$ aqueous solution (2 mL), and extracted with CH_2Cl_2. The CH_2Cl_2 extract was evaporated and chromatographed (silica gel; eluent: CH_2Cl_2:MeOH = 50:1) to give **1** as a yellow foam (yield 82%, 208 mg). M.p. = 91–94 °C; ^{1}H NMR ($CDCl_3$): δ = 7.50 (d, J = 7.6 Hz, 2H), 7.39 (dd, J = 9.2, 3.2 Hz, 5H), 7.31–7.25 (m, 2H), 7.21–7.17 (m, 1H), 7.07–6.92 (m, 4H), 6.82 (dd, J = 9.0, 2.4 Hz, 5H), 6.60 (dd, J = 9.6, 2.0 Hz, 1H), 6.45 (d, J = 1.6 Hz, 1H), 5.14 (t, J = 7.8 Hz, 1H), 4.52–4.50 (m, 1H), 4.06–4.00 (m, 1H), 3.77 (s, 6H), 3.30 (d, J = 4.8 Hz, 2H), 2.48–2.32 (m, 5H), 1.97 (s, 3H), 1.39 ppm (s, 9H); ^{13}C NMR ($CDCl_3$): δ = 186.0, 176.3, 158.6, 158.5, 158.4, 155.0, 153.1, 144.9, 138.2, 136.0, 135.9, 134.0, 133.4, 132.1, 130.9, 130.5, 130.1, 129.9, 129.1, 128.9, 128.2, 127.79, 127.77, 127.73, 126.69, 120.1, 118.5, 118.0, 113.11, 113.08, 110.2, 106.2, 93.7, 86.2, 86.0, 83.2, 74.4, 68.2, 64.5, 55.2, 55.1, 42.5, 39.3, 27.0, 20.1, 18.9 ppm; IR (KBr): $\tilde{\nu}$ = 3420, 2930, 1759, 1640, 1601 cm^{-1}; HRMS (ESI): *m*/*z*: calcd for $C_{54}H_{51}O_9$: 843.3533; found: 843.3488 $[M+H]^+$.

Phosphoramidite (2): NEt(iPr)$_2$ (108 μL, 0.62 mmol) and NC-(CH_2)$_2$OPCl(NiPr$_2$) (87 μL, 0.39 mmol) were added to a solution of **1** (130 mg, 0.15 mmol) in CH_2Cl_2 (3 mL) at room temperature. The reaction mixture was stirred for 30 min at that temperature. After removal of the solvent by a rotary evaporator, the residue was chromatographed (silica gel pretreated with CH_2Cl_2 including 1% (*v*/*v*) Et_3N; eluent: CH_2Cl_2) to give **2** as a yellow foam (yield 97%, 156 mg). M.p. = 70–74 °C; ^{1}H NMR ($CDCl_3$); δ = 7.52–7.49 (m, 2H), 7.42–7.36 (m, 6H), 7.29–7.19 (m, 2H), 7.04 (dd, J = 7.0, 2.8 Hz, 1H), 6.99–6.90 (m, 3H), 6.82–6.78 (m, 5H), 6.58 (dd, J = 9.6, 1.6 Hz, 1H), 6.43 (d, J = 2.0 Hz, 1H), 5.13–5.09 (m, 1H), 4.31–4.22 (m, 1H), 3.79 (s, 6H), 3.77–3.68 (m, 2H), 3.62–3.56 (m, 1H), 3.40–3.28 (m, 2H), 3.21–3.15 (m, 1H), 2.67–2.57 (m, 2H), 2.54–2.38 (m, 5H), 1.96 (s, 3H), 1.38 (s, 9H), 1.24–1.09 ppm (m, 12H); ^{13}C NMR (CD_3OD): δ = 186.0, 176.3, 158.5, 158.4, 154.9, 153.1, 147.5, 144.7, 138.3, 138.2, 135.9, 134.1, 133.5, 131.1, 130.5, 130.2, 130.0, 128.9, 128.3, 128.3, 127.8, 127.8, 126.7, 120.2, 118.4, 118.0, 113.14, 113.08, 110.3, 106.3, 86.4, 86.4, 86.2, 68.6, 68.5, 55.6, 55.20, 55.18, 43.3, 39.3, 30.9, 27.0, 24.7, 24.6, 24.5, 21.0, 20.2, 18.9 ppm; IR (KBr): $\tilde{\nu}$ = 3421, 2927, 1750, 1639, 1602 cm^{-1}; HRMS (ESI): *m*/*z*: calcd for $C_{63}H_{67}N_2NaO_{10}P$: 1065.4431; found: 1065.4480 $[M+Na]^+$.

F nucleoside (3): CCl_3CO_2H (29 mg, 0.17 mmol) was added to a solution of **7** (26 mg, 0.034 mmol) in CH_2Cl_2 (2 mL) at room temperature. The reaction mixture was stirred for 30 min at that temperature and was quenched by the addition of NEt_3 (1 mL). After removal of the solvent by using a rotary evaporator, the residue was chromatographed (silica gel; eluent: CH_2Cl_2:MeOH = 10:1) to give **3** as a red solid (yield 99%, 15 mg). M.p. = 192–194 °C; ^{1}H NMR (CD_3OD): δ = 7.48 (s, 1H), 7.13 (s, 1H), 7.05 (d, J = 8.0 Hz, 2H), 6.72–6.70 (m, 4H), 5.03 (t, J = 7.6 Hz, 1H), 4.34 (dd, J = 7.1, 4.4 Hz, 1H), 3.89–3.85 (m, 1H), 2.45 (s, 3H), 2.28–2.25 (m, 2H), 1.99 ppm (s, 3H); ^{13}C NMR (CD_3OD): δ = 200.6, 154.9, 139.4, 134.9, 134.8, 134.0, 132.1, 131.1, 125.3, 115.9, 104.5, 94.6, 89.0, 84.2, 73.8, 69.2, 63.9, 43.6, 20.2, 18.9 ppm; IR (KBr): $\tilde{\nu}$ = 1592 cm^{-1}; Φ_f (Tris-HCl buffer, 100 mM, pH 7.0) 0.83; HRMS (ESI): *m*/*z*: calcd for $C_{28}H_{25}O_6$: 457.1651; found: 457.1654 $[M+H]^+$.

F-modified ODNs: F-modified ODNs were synthesized by using a conventional phosphoramidite method with a DNA synthesizer. The synthesized ODNs were purified by reverse-phase HPLC on a CHEMCOBOND 5-ODS-H column (10 × 150 mm; Chemco SCIENTIFIC; eluent: 5 mM ammonium formate with the respective CH_3CN percentage of linear gradient over 60 min at a flow rate of 2.0 mL min^{-1} (5–30% for **ODNfa**, **ODNfa′**, and **ODNfd**, 0–50% for **ODNfb**, and 5–50% for **ODNfc**)).

MALDI-TOF mass data for F-modified ODNs: **ODNfa**: MS (ESI): *m*/*z*: calcd for $C_{85}H_{97}N_{24}O_{42}P_6$: 2311.46; found: 2311.53 $[M+H]^+$; **ODNfa′**: MS (ESI): *m*/*z*: calcd for $C_{94}H_{108}N_{27}O_{48}P_7$: 2600.51; found: 2600.17 $[M+H]^+$; **ODNfb**: MS (ESI): *m*/*z*: calcd for $C_{88}H_{103}N_{24}O_{42}P_6$: 2353.51; found: 2354.00 $[M+H]^+$; **ODNfc**: MS (ESI): *m*/*z*: calcd for $C_{142}H_{169}N_{48}O_{78}P_{12}$: 4165.76; found: 4166.05 $[M+H]^+$; **ODNfd**: MS (ESI): *m*/*z*: calcd for $C_{85}H_{96}N_{24}O_{42}P_6$: 2311.46; found: 2311.17 $[M+H]^+$.

Determination of T_m: Melting curves were measured at 260 nm at temperatures ranging from 20 °C to 80 °C by using UV/Vis spectroscopy. **ODNfa** (2.0 μM) was dissolved in a solution of Tris-HCl (100 mM, pH 7.0) including NaCl (0.1 M or 5.0 M).

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[17] In the UV/Vis spectra of **ODNfd**, the absorption band from the chromophore hardly changed at various NaCl concentrations (Figure S5c in the Supporting Information).

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