Supporting Information for

Transformation of cytosine to uracil in signle-stranded DNA via their oxime sulfonates.

Yoshimi Oka,[†,‡] Fumie Takei,[†] and Kazuhiko Nakatani[*,†]

[†] Institute of Scientific and Industrial Research, Osaka University, 8-1 Mihogaoka, Ibaraki, Osaka 567-0047, Japan, and [‡] Career Design Laboratory for Women, Osaka University, 8-1 Mihogaoka, Ibaraki, Osaka 567-0047, Japan

The contents of supporting information include:

Materials and Methods

Sodium hydrogensulfite, hydroxylamine hydrochloride, *O*-methylhydroxylamine hydrochloride and diethylamine were purchased from Wako Chemical Co., Inc. and used without further purification. DNA oligomers were purchased from Hokkaido System Science Co., Inc. and Japan Bio Service Co., Inc.

Preparation of ODN1C* and ODN2C*

To the aqueous solution of sodium hydrogensulfite, NaOH solution was added and the resulted solution was adjusted to pH 6.0 and 2 M. The hydroxylamine solution adjusted to pH 6.0 and 4 M was prepared from aqueous solution of hydroxylamine hydrochloride and diethylamine. To the aqueous solution of DNA oligomers and NaCl, bisulfite solution (pH 6.0, 2 M) and hydroxylamine solution (pH 6.0, 4 M) were added. The final concentrations were adjusted to 25 μM ODN (ODN**1C** or ODN**2C**), 100 mM NaCl, 1 M bisulfite and 1 M hydroxylamine. After the reaction at 0 °C for 1 d, products were separated by HPLC equipped with a CHEMCOBOND 5-ODS-H column (4.6 × 150 mm) by a gradient elution of 0.1 M TEAA (pH 7.0) with increasing acetonitrile (5-25% over 45 min). With UV detection at 260 nm of UV, the ODN products (ODN**1C***, ODN**2C*** or ODN**2C*′**) were isolated. The ODN solutions were dried in vacuo.

Preparation of ODN1C**

As for the synthesis of ODN**1C****, to the aqueous solution of ODN**1C** and NaCl, bisulfite solution (pH 6.0, 2 M) and O-methylhydroxylamine solution (pH 6.0, 4 M) were added. *O*-methylhydroxylamine solution adjusted to pH 6.0 and 4 M was prepared from aqueous solution of *O*-hydroxylamine hydrochloride and diethylamine. The final concentration was adjusted to 25 μM ODN (ODN**1C**), 100 mM NaCl, 1 M bisulfite and 1 M *O*-methylhydroxylamine. After the reaction at 0 °C for 1 d, ODN**1C**** was isolated.

BPO reaction of ODN1C* and ODN2C*

To the aqueous solution of oxime slufonate (ODN**1C*** and ODN**2C***), NaCl and cacodylate buffer (pH 7.0), benzoyl peroxide and cobalt(II) chloride were added. The final concentrations were adjusted to 25 μM ODN, 100 mM NaCl, 10 mM cacodylate buffer, 250 μM benzoyl peroxide and 1 mM cobalt(II) chloride. After the reaction at 40 °C for 10 min or 1 h, products (ODN**1U*** and ODN**2U***) were separated by HPLC as described above. As for the reference, ODN**1C**** and ODN**1C** was treated as well as ODN**1C***.

Conversion to ODN1U* and **ODN2U***

To the aqueous solution of the mixture of the ODN reactant (ODN**1C*** or ODN**2C***) and product (ODN**1U*** or ODN**2U***), sodium hydroxide (final concentration: 20 mM) was added. After the reaction at 25 °C for 10 min, products (ODN**1U** or ODN**2U**) were separated by HPLC as described above.

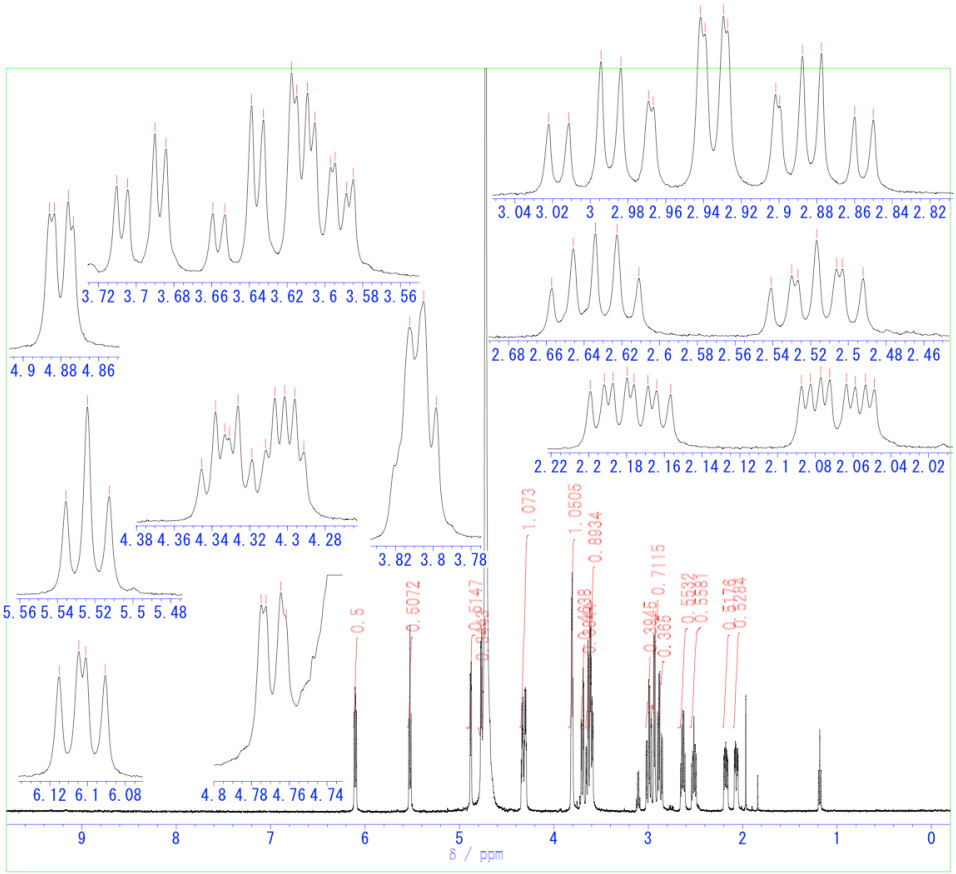
Enzymatic digestion of ODN2U

The ODN**2U** was treated with snake venom phosphodiesterase, nuclease P1, and alkaline phosphatase. After the reaction at 37 °C for 2 h, the nucleoside mixtures were analyzed by HPLC equipped with a Cosmosil 5C18-AR column (4.6 × 150 mm) by a gradient elution of 0.1 M TEAA (pH 7.0) with increasing acetonitrile (0-10% over 20 min). The peaks were identified by comparing with the authentic samples.

Synthesis of Sodium 2′-deoxy-*N*^{4}-hydroxy-5,6-dihydrocytidine-6-sulfonate (dC*)

The dC* was synthesized according to the method reported by Hayatsu (*J. Mol. Biol.* **1977**, *115*, 19-31.). Hydroxylamine hydrochloride (0.556 g) and Na_2SO_3 (0.966 g) were added to a solution of 2′-deoxycytidine (113 mg in 7.5 ml water). The resulted solution was pH 6.3 and left in a refrigerator for 3 d. After the reaction, the major product was isolated by RP-HPLC using CHEMCOBOND 5-ODS-H column (4.6 × 150 mm) with 260 nm of UV detector (95% water: 5% acetonitrile, v/v). The product was dried in vacuo, giving a pure sample of dC* (108 mg; yield: 63%). The dC* was obtained as a mixture of diastereoisomers. ^{1}H NMR (600 MHz, D_2O, 293 K): δ = 5.52 and 6.10 (total 1 H, dd, J = 6.8, 6.9 Hz and J = 6.2, 8.4 Hz), 4.77 and 4.88 (total 1 H, dd, J = 1.6, 6.3 Hz, and J = 1.6, 6.1 Hz), 4.29-4.35 (1 H, m), 3.79-3.82 (1 H, m), 3.65 and 3.70 (total 1 H, dd, J = 3.8, 12.3 Hz and J = 3.5 and 12.2 Hz), 3.60 and 3.61 (total 1 H, dd, J = 2.2, 12.3 and J = 1.5, 12.3), 2.91 and 2.95 (total 1 H, dd, J = 1.3, 16.6 and J = 1.5, 16.5), 2.87 and 3.00 (total 1 H, dd, J = 6.0, 16.5 and J = 6.4, 16.6), 2.52 and 2.63 (total 1 H, ddd, J = 6.3, 8.2, 14.2 Hz and J = 6.9, 7.0, 13.9 Hz), 2.07 and 2.18 (total 1 H, ddd, J = 2.8 6.0, 14.2 Hz and J = 4.5, 7.2, 13.8 Hz). ^{13}C NMR (150.8 MHz, D_2O, acetonitrile as a reference (δ = 1.47 and 119.7),[a] 299 K): δ = 152.2 and 153.4, 144.9 and 145.1, 87.3 and 91.3, 85.9 and 86.4, 71.5 and 71.8, 64.6 and 68.8, 62.5, 37.5 and 38.4, 26.2 and 26.9. ESI-MS: *m/z* calcd for $[M + Na]^+$ $C_9H_{14}N_3Na_2O_8S$ 370.3, found *m/z* 369.9. Ultraviolet absorption spectrum (in 10mM cacodylate buffer (pH 7.0)): λ_{max} 217 nm.

[a] Chemical shifts were reported in the literature: Gottlieb, H. E.; Kotlyar, V.; Nudelman, A. *J. Org. Chem.* **1997**, *62*, 7512-7515.



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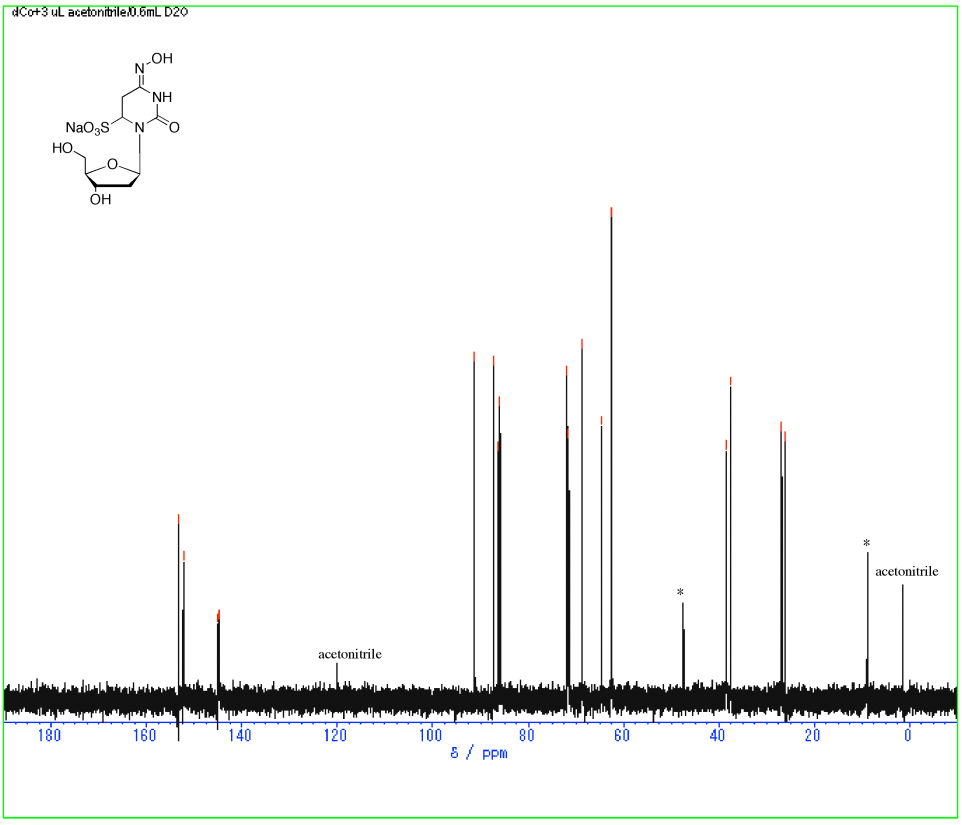
RGain 18

Operator_____



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Pulse1 5.5 μs
IrrNuc ^{1}H
ProbeHeaD TH5
Instrument JEOL LAMBDA
Pulse Program
Gradient Program
Temperature 26.2 °C
Solvent D_2O
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Window Exponential
RGain 28

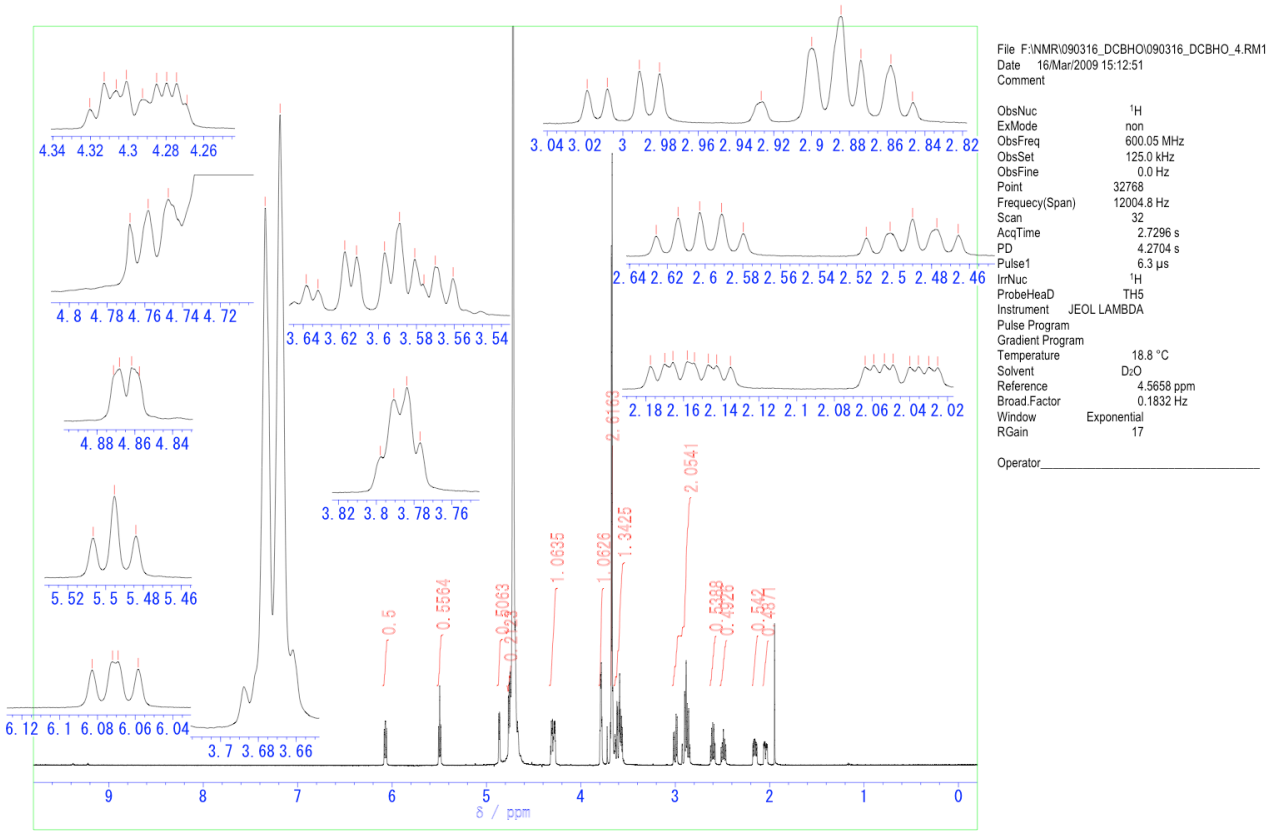
Operator_____

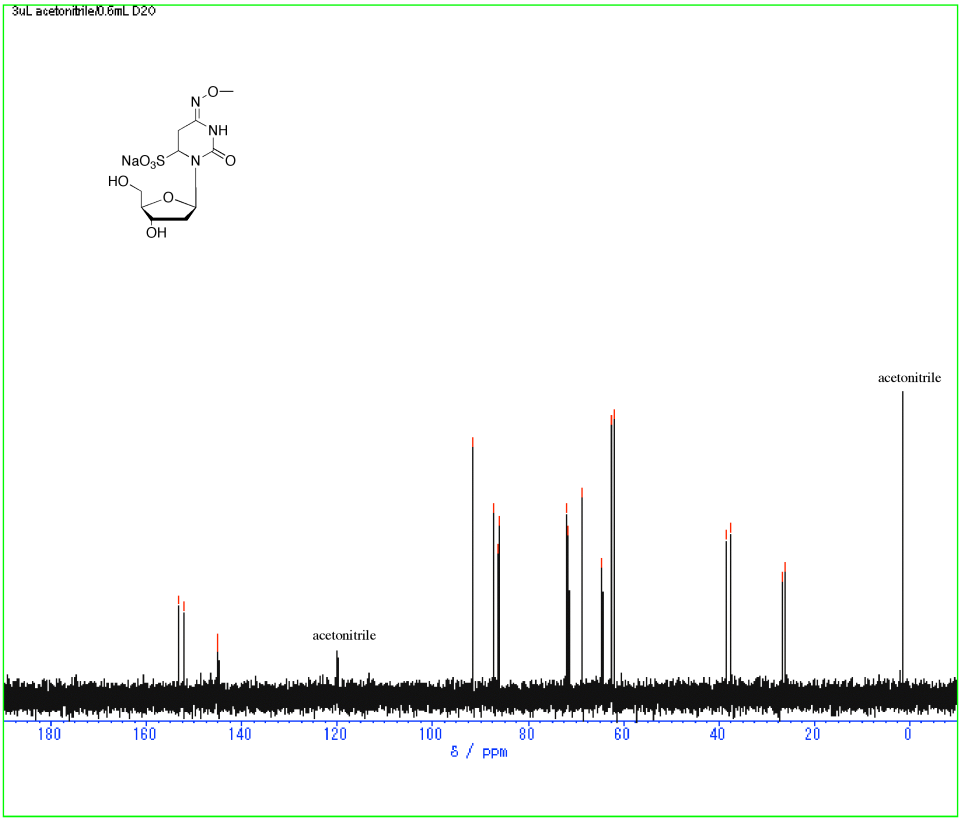
*: triethylamine as an impurity

Synthesis of Sodium 2′-deoxy-*N*^{4}-methoxy-5,6-dihydrocytidine-6-sulfonate (dC)**

Hydroxylamine hydrochloride (0.795 g) and Na_2SO_3 (0.981 g) were added to a solution of 2′-deoxycytidine (107 mg in 7.5 ml water). The resulted solution was pH 5.3 and left in a refrigerator for 3 d. After the reaction, the major product was isolated by RP-HPLC using CHEMCOBOND 5-ODS-H column (4.6 × 150 mm) with 260 nm of UV detector (95% water: 5% acetonitrile, v/v). The product was dried in vacuo, giving a pure sample of dC** (93 mg; yield: 55%). ^{1}H NMR (600 MHz, D_2O, 292 K): δ = 5.50 and 6.07 (total 1 H, dd, J = 6.8, 6.8 Hz and J = 6.4, 8.2 Hz), 4.76 and 4.86 (total 1 H, dd, J = 6.1, 6.1 Hz, and J = 2.2, 6.0 Hz), 4.27-4.33 (1 H, m), 3.77-3.80 (1 H, m), 3.67 and 3.68 (total 3 H, s), 3.56-3.64 (2 H, m), 2.84-3.03 (2 H, m), 2.49 and 2.60 (total 1 H, ddd, J = 7.3, 7.3, 14.8 Hz and J = 6.9, 6.9, 13.8 Hz), 2.04 and 2.16 (total 1 H, ddd, J = 2.7, 6.1, 14.1 Hz and J = 4.4, 7.1, 13.9 Hz). ^{13}C NMR (150.8 MHz, D_2O, acetonitrile as a reference (δ = 1.47 and 119.68),[a] 299 K): δ = 152.1 and 153.3, 144.9 and 145.2, 87.3 and 91.5, 86.0 and 86.4, 71.5 and 71.8, 64.5 and 68.8, 62.5, 61.9, 37.6 and 38.4, 26.0 and 26.8. ESI-MS: *m/z* calcd for $[M + Na]^+$ $C_9H_{14}N_3Na_2O_8S$ 384.3, found *m/z* 383.9. Ultraviolet absorption spectrum (in 10mM cacodylate buffer (pH 7.0)): λ_{max} 217 nm.

[a] Chemical shifts were reported in the literature: Gottlieb, H. E.; Kotlyar, V.; Nudelman, A. *J. Org. Chem.* **1997**, *62*, 7512-7515.





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InrNuc ^{1}H
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Instrument JEOL LAMBDA
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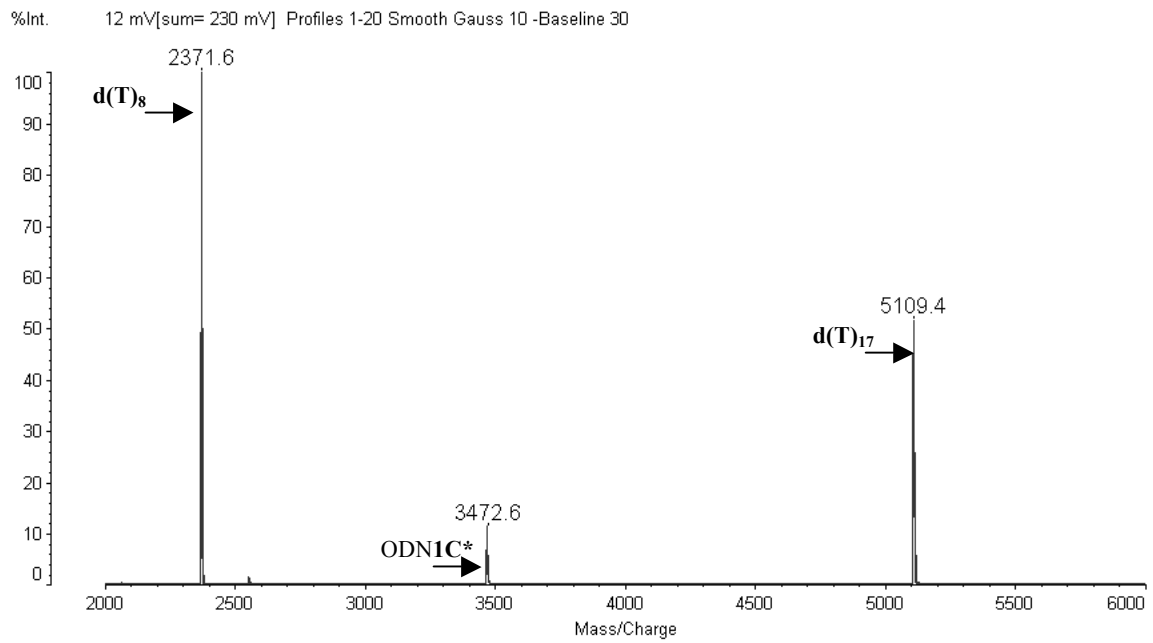
Operator_____

A



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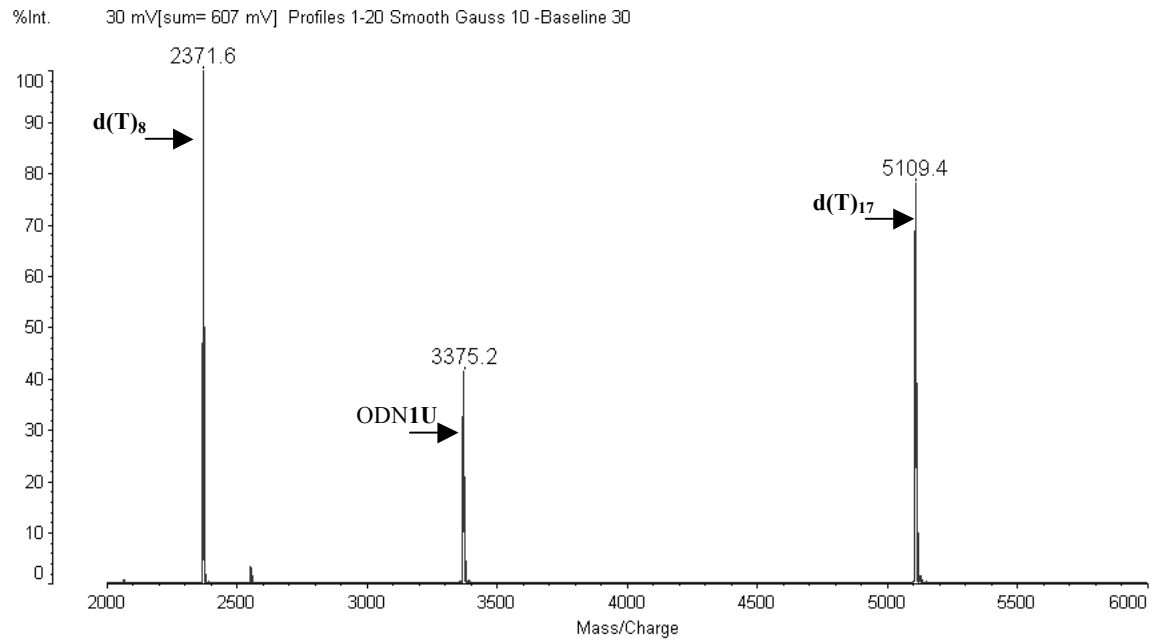


Figure S1. MALDI-TOF MS for the reactions of ODN**1C*** to Co^{II}-BPO and subsequent sodium hydroxide. (A) The reactant ODN**1C*** and (B) the final product ODN**1U** on the HPLC profile of Figure 2C were isolated and identified by MALDI-TOF MS. As internal standards, $d(T)_8$ ($[M-H]^-$ = 2371.6) and $d(T)_{17}$ ($[M-H]^-$ = 5109.4) were added.

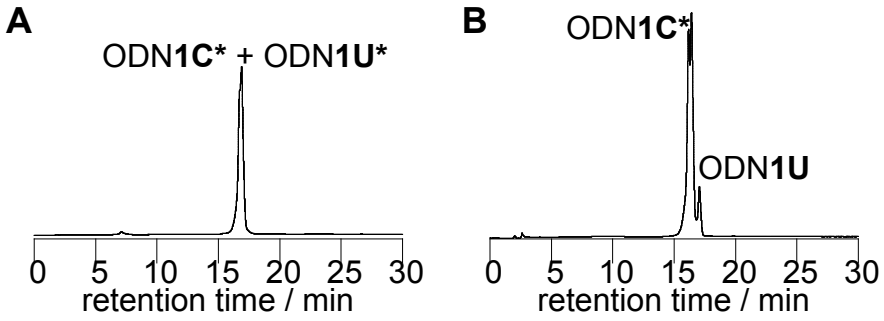


Figure S2. HPLC profiles of ODN**1C*** with BPO in the absence of Co(II) and subsequent sodium hydroxide treatments. (A) The 11-mer single-stranded 5′-d(TAA AC*G GAA AT)-3′ (ODN**1C***, 25 μM) in NaCl (100 mM) and cacodylate buffer (10 mM, pH 7.0) was treated with BPO (250 μM) without cobalt(II) chloride at 40 °C for 10 min; (B) Subsequent treatment with sodium hydroxide (20 mM) at 25 °C for 10 min.

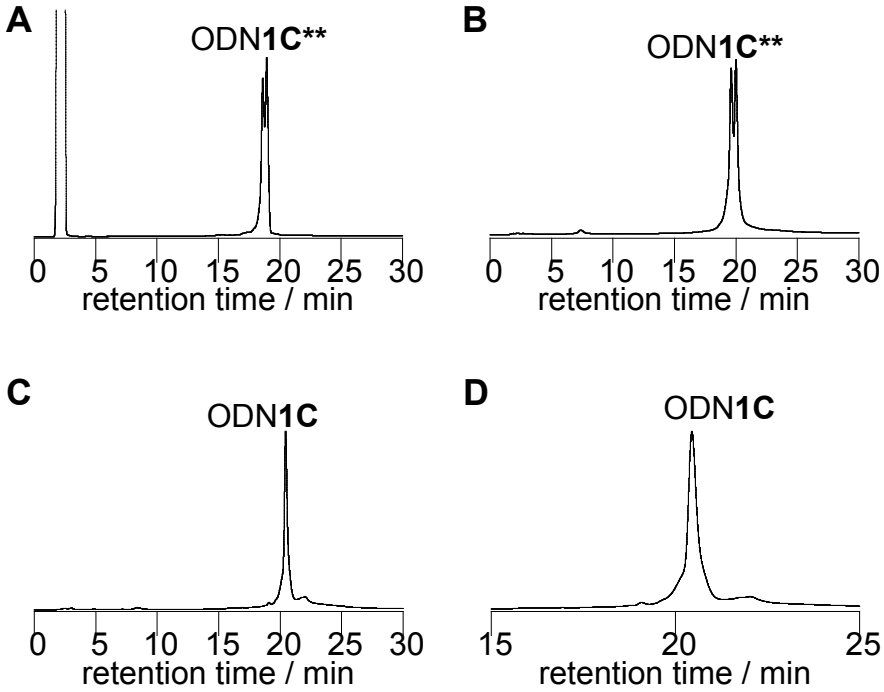
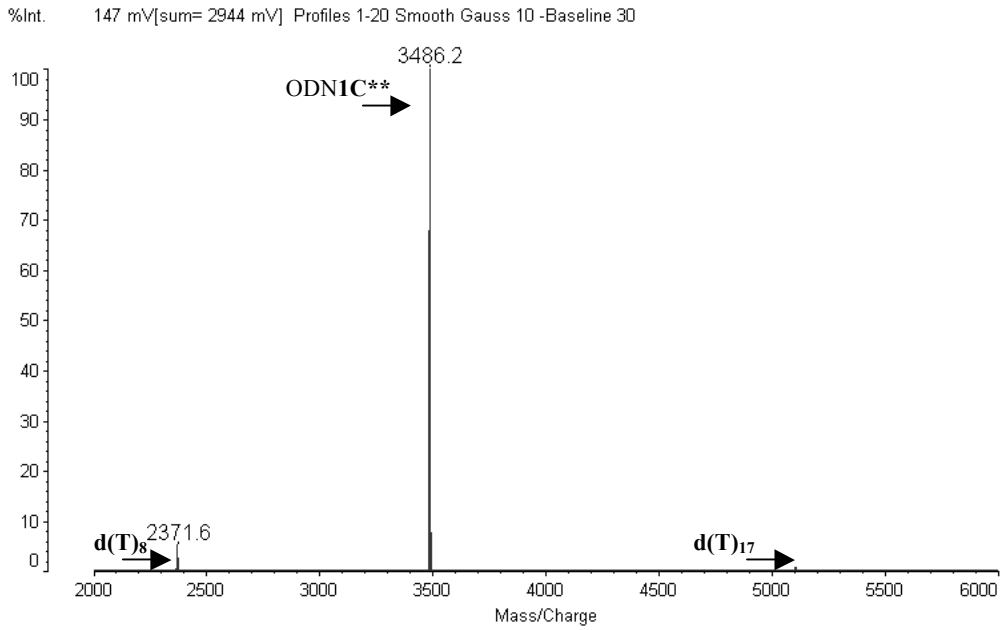


Figure S3. HPLC profiles of ODN**1C**** and ODN**1C** under Co^{II}-BPO treatment. (A) The 11-mer single-stranded 5′-d(TAA ACG GAA AT)-3′, ODN**1C** (25 μM) in NaCl (100 mM) was treated with bisulfite and O-methylhydroxylamine (1.0 M, pH 6.0) at 0 °C for 1 d. The product ODN**1C**** was isolated from reactant, but contained both diastereomers. (B) ODN**1C**** (25 μM) in NaCl (100 mM) and cacodylate buffer (10 mM, pH 7.0) was treated with BPO (250 μM) with Co^{II} (1 mM) at 40 °C for 10 min. (C) ODN**1C** was treated with Co^{II}-BPO under the same condition as B. (D) Zoom of the peak in Figure C. No apparent reactions of ODN**1C**** and ODN**1C** were detected under the conditions, showing that the reaction of ODN**1C*** at C* is much more efficient than the possible guanine oxidation.

A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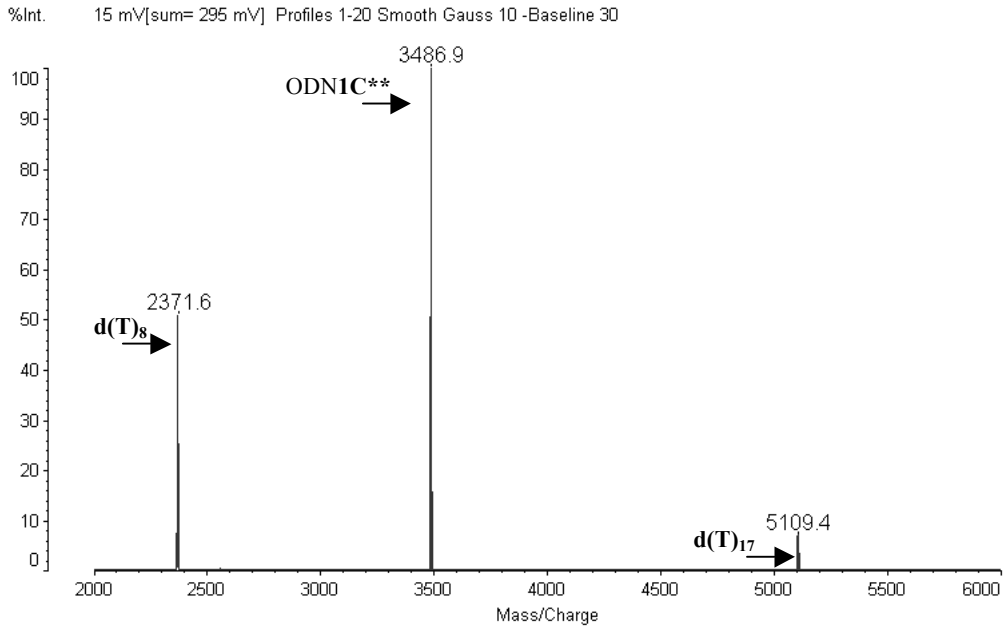
d(T)17
Mass/Charge

B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Mass/Charge

C

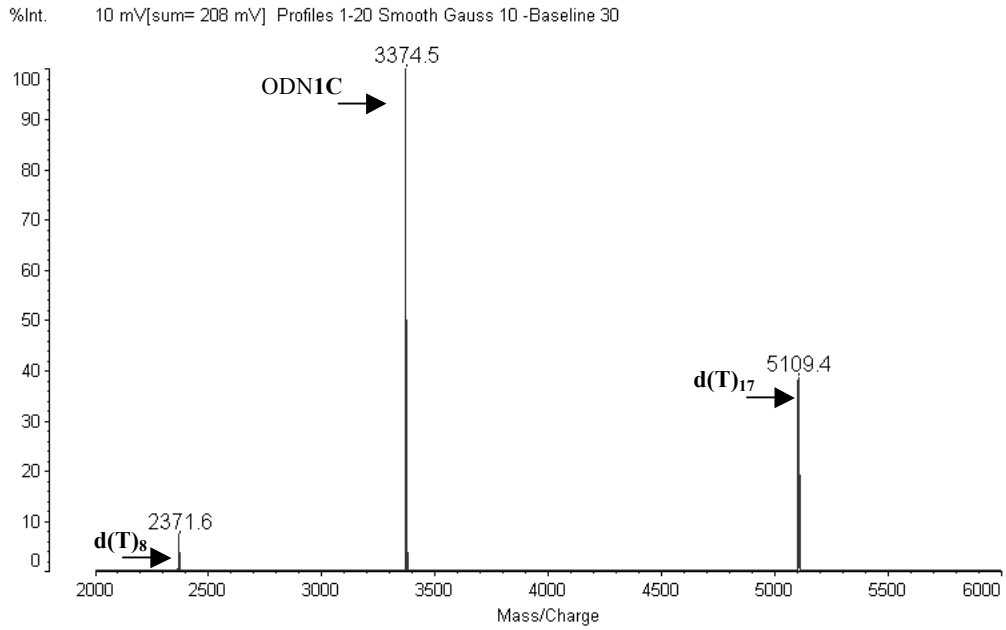
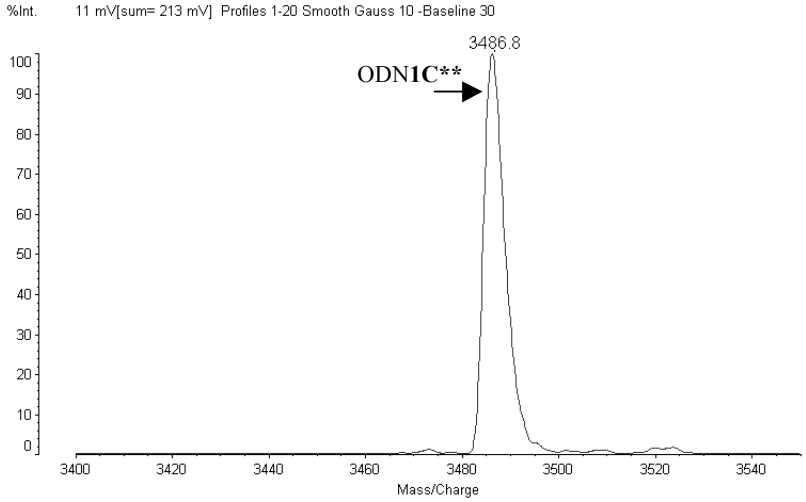


Figure S4. MALDI-TOF MS for the reaction of ODN**1C** toward bisulfite with O-methylhydroxylamine. The product ODN**1C**** containing diastereoisomers (A and B) on the HPLC profile of Figure S3A were isolated and identified by MALDI-TOF MS. (C) MALDI-TOF MS for the reactant ODN**1C**. As internal standards, $d(T)_8$ ($[M-H]^-$ = 2371.6) and $d(T)_{17}$ ($[M-H]^-$ = 5109.4) were added.

A



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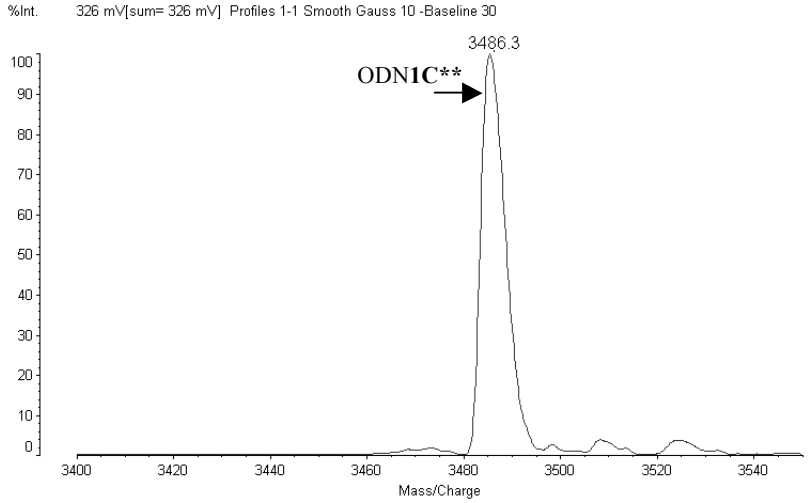

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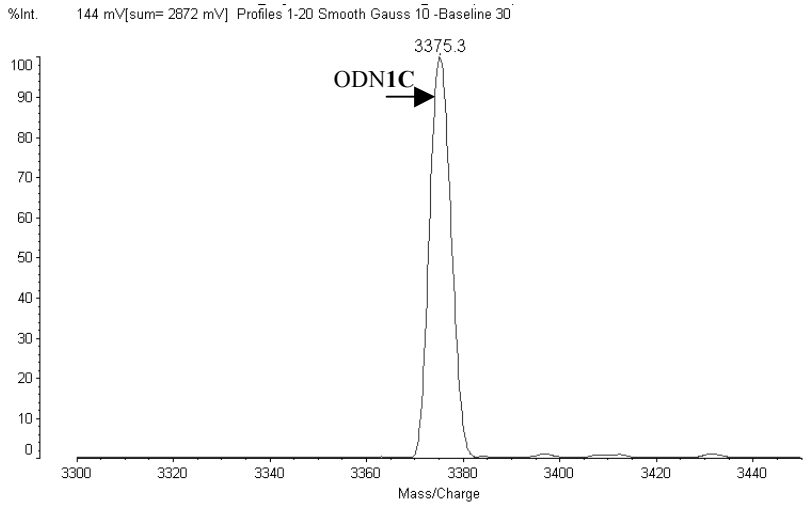


Figure S5. MALDI-TOF MS for the treatment with Co^{II}-BPO of ODN**1C**** (A and B) and ODN**1C** (C). For the both cases, the reactants remained unchanged. As internal standards, $d(T)_8$ ($[M-H]^-$ = 2371.6) and $d(T)_{17}$ ($[M-H]^-$ = 5109.4) were added.

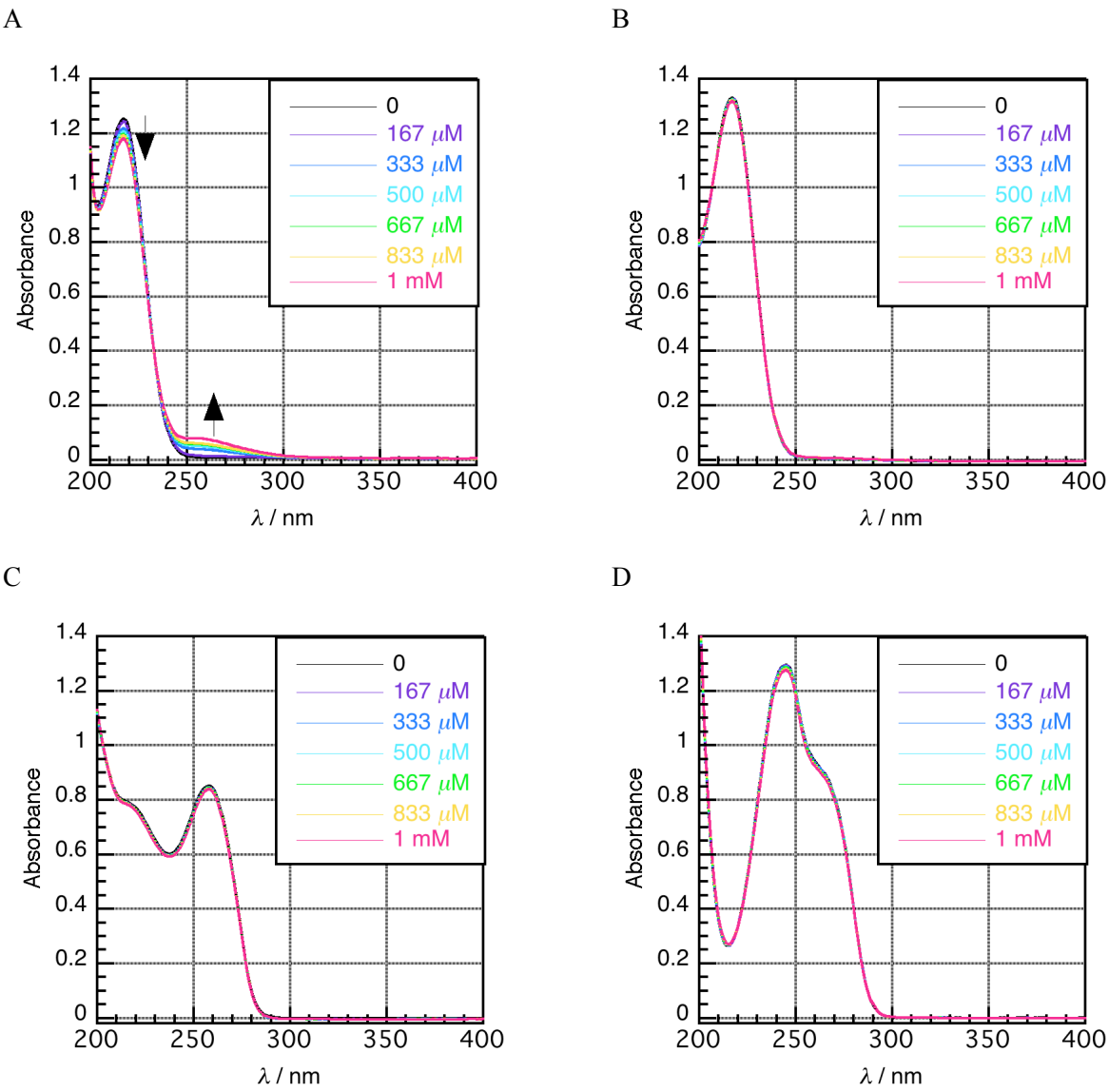
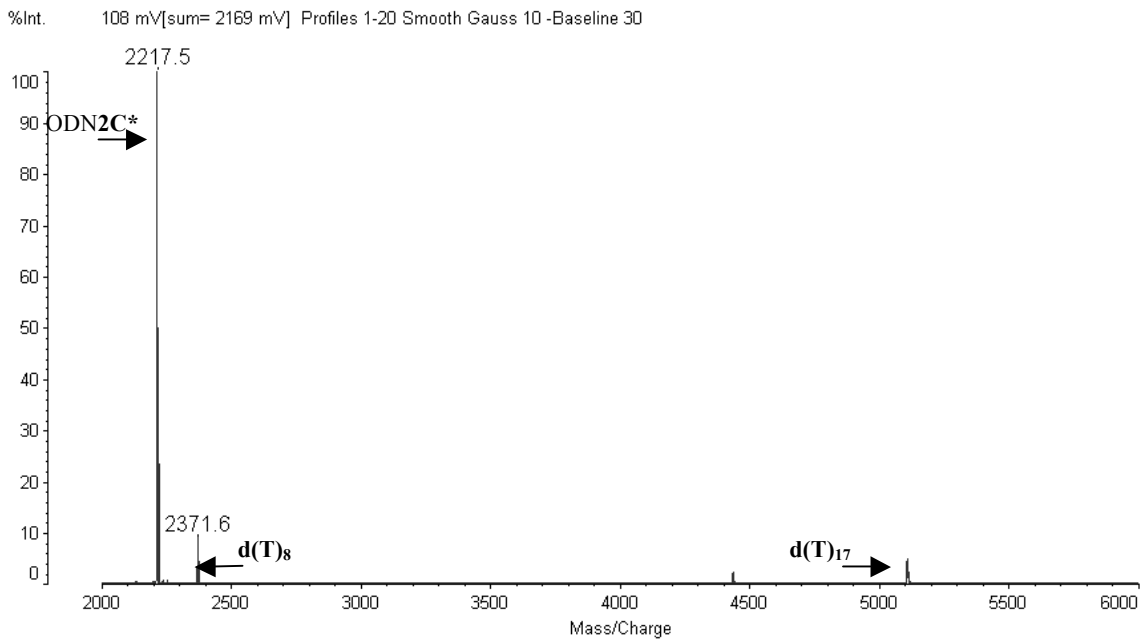


Figure S6. Absorbance spectra for titration of (A) dC*, (B) dC**, (C) dC and (D) dG (100 μM) with cobalt(II) chloride (0, 167, 333, 500, 667, 833, 1000 μM) in cacodylate buffer (10 mM, pH7.0).

A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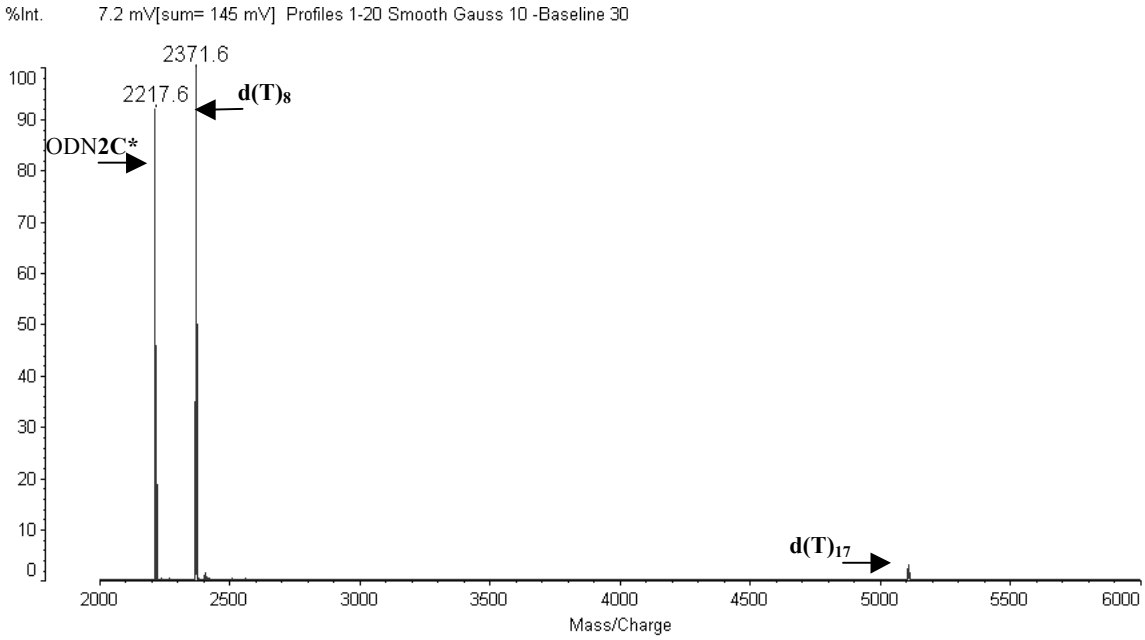
d(T)17
Mass/Charge

B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Mass/Charge

C

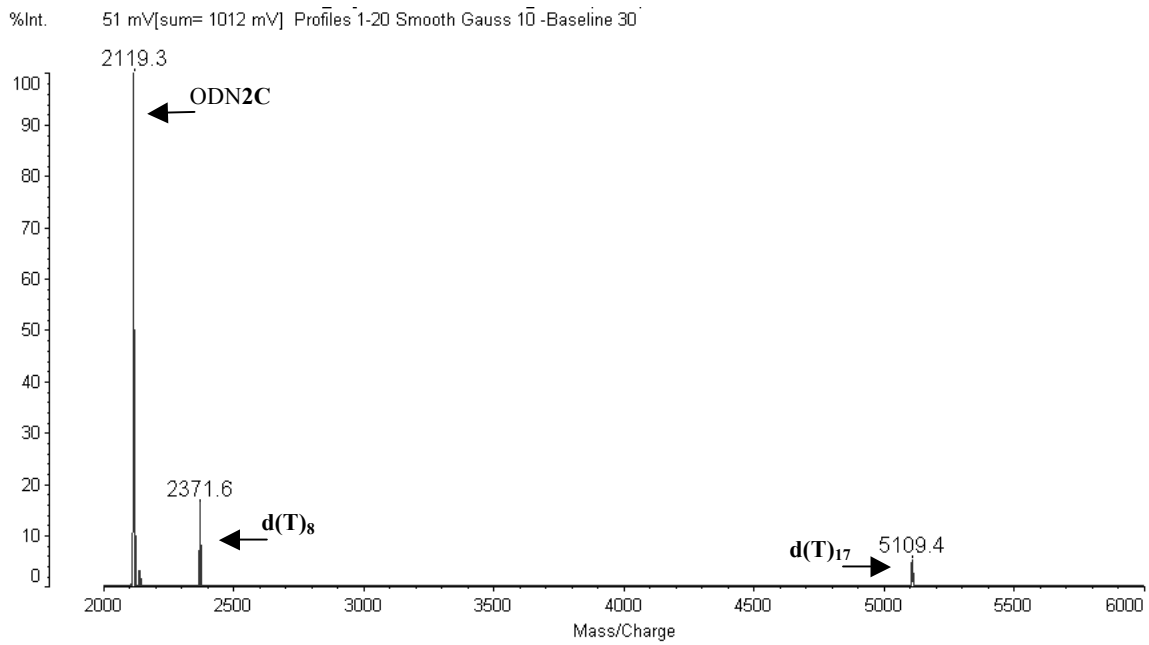


Figure S7. MALDI-TOF MS for the reaction of ODN**2C** toward bisulfite-hydroxylamine. The product (A) ODN**2C*** (with a shorter retention time), (B) ODN**2C*** (with a longer retention time) and (C) the reactant ODN**2C** on the HPLC profile of Figure 3A were isolated and identified by MALDI-TOF MS. As internal standards, $d(T)_8$ ($[M-H]^-$ = 2371.6) and $d(T)_{17}$ ($[M-H]^-$ = 5109.4) were added.

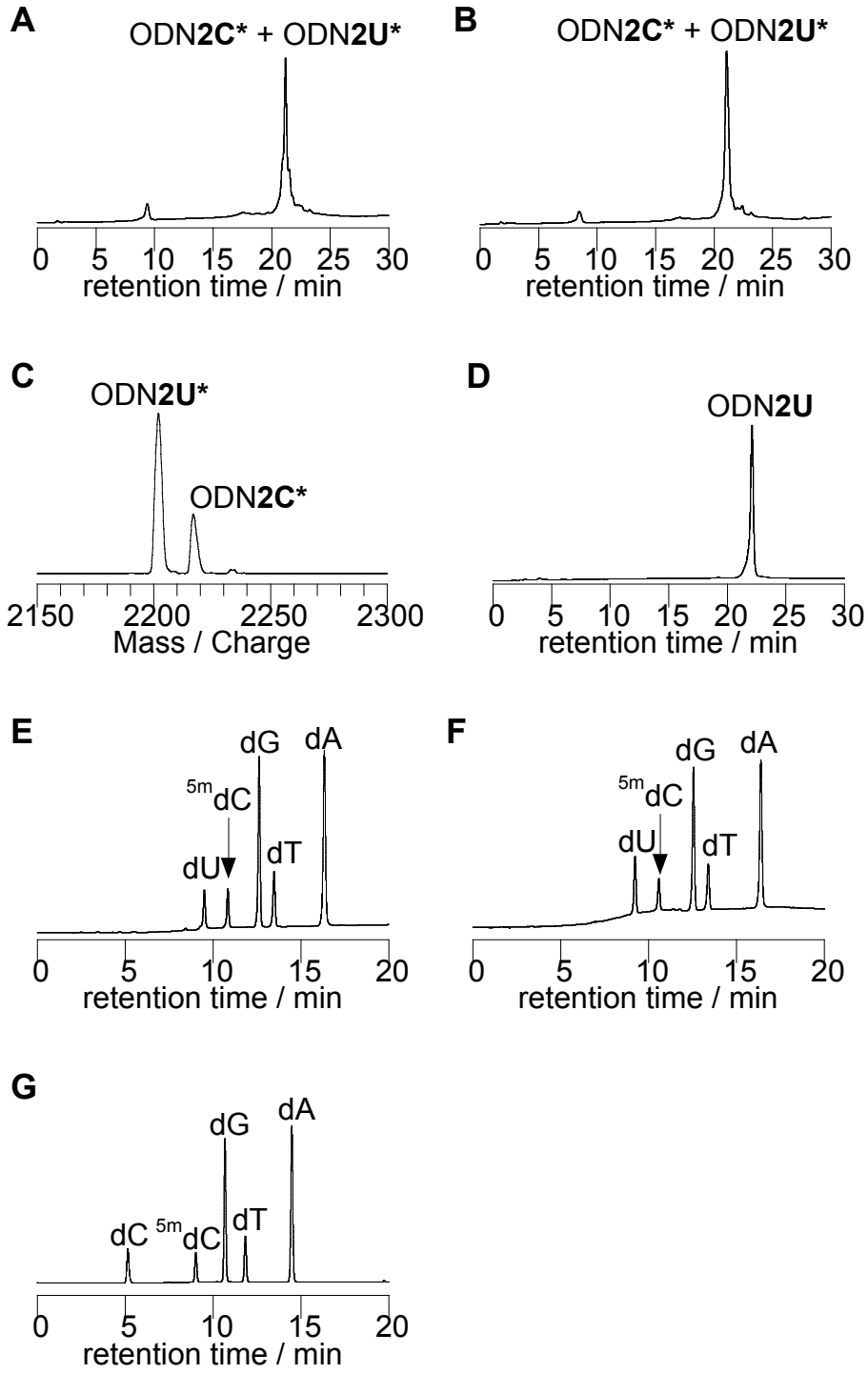
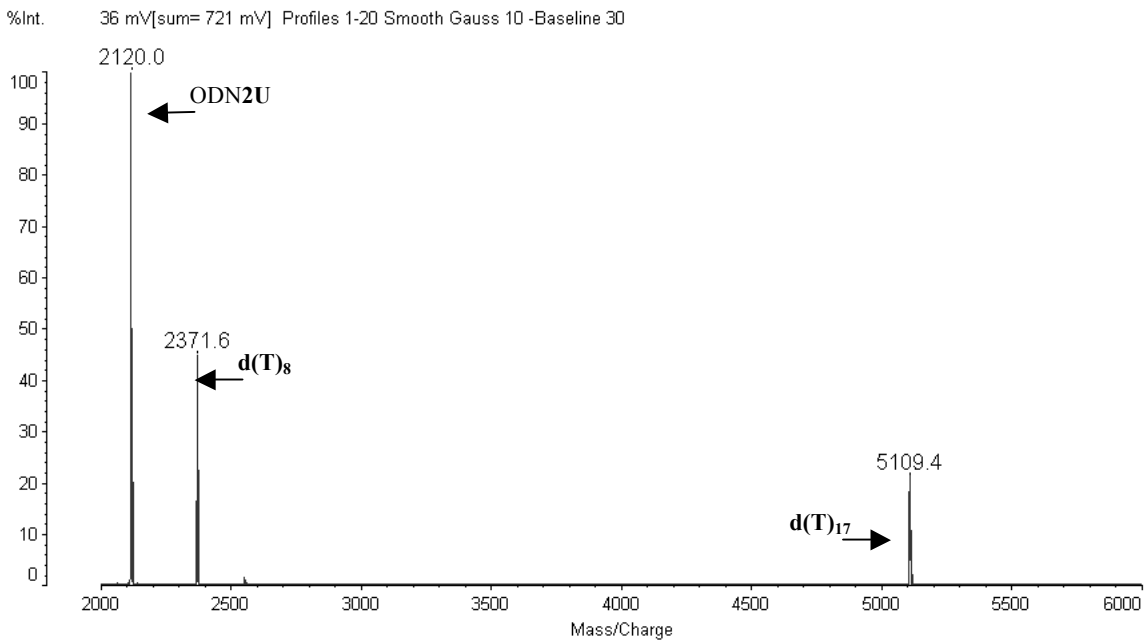


Figure S8. HPLC profiles and mass spectra of ODN**2C*** containing each diastereomer of C* under Co^{II}-BPO and subsequent sodium hydroxide treatments. Two diastereoisomers of ODN**2C*** (with a (A) shorter and (B) longer retention time) in cacodylate buffer (10 mM, pH 7.0) was treated with BPO (250 μM) in the presence of Co^{II} (1 mM) at 40 °C for 1 h, respectively. (C) MALDI-TOF MS of the product. (D) Subsequent treatment with sodium hydroxide (20 mM) at 25 °C for 10 min from the other isomer of ODN**2C***. Nucleoside analysis of (E) ODN**2U**, (F) authentic 5′-d(A ^{5m}CG A UG T)-3′, and (G) authentic 5′-d(A ^{5m}CG A CG T)-3′ with snake venom phosphodiesterase, nuclease P1, and alkaline phosphatase.

A



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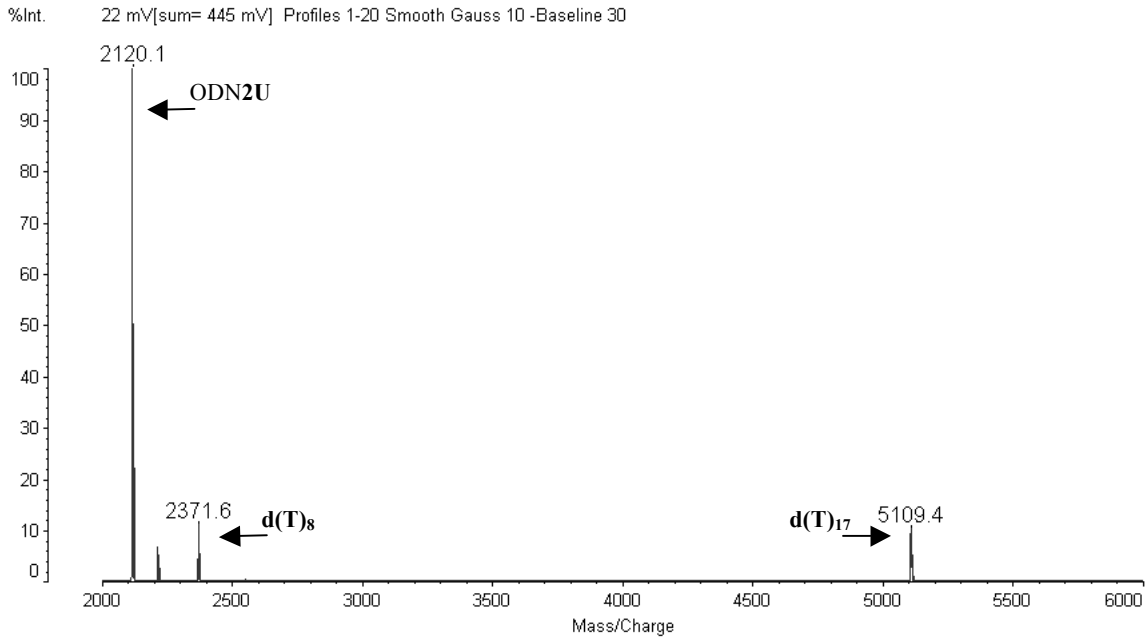

Figure S9. MALDI-TOF MS of the reaction product of ODN**2C*** (with a (A) shorter and (B) longer retention time) toward Co^{II}-BPO and subsequent sodium hydroxide treatment.