Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Detection of methylcytosine by DNA photoligation via hydrophobic interaction of the alkyl group

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General method and materials.

Pyridine, dioxane, NEt_3 and DMTrCl were purchased from Kanto Chemical. 5-Iodo-2'-deoxyuridine was purchased from Tokyo Kasei. $Pd(OAc)_2$, PPh_3, 2-Cyanoethyl *N,N,N',N'*-tetra-isopropylphosphoroamidite and Methyl acrylate were purchased from Aldrich. DMAP was purchased from ACROS ORGANICS. Tetrazole was purchased from GLEN RESEARCH. The reagents for the DNA synthesizer such as I_2 solution (I_2/H_2O/pyridine/tetrahydrofuran, 3:2:19:76), A-, G-, C-, and T-β-cyanoethyl phosphoroamidites were purchased from GLEN RESEARCH. Other regents were purchased at highest commercial quality and used without further purification unless otherwise stated. Microwave reaction were conducted using the CEM Discover as a focused microwave unit. Calf intestine alkaline phosphstase (AP) (1500 units) was purchased from Promega and Roch. Nuclease P1 (500 units) was purchased from Yamasa. Reactions were monitored TLC plates precoated with Merck silica gel 60 F_{254}. Kanto Chemical Silica Gel 60 N was used for silica gel column chromatography. ^{1}H-NMR spectrum was recorded on Varian Gemini-300C (300 MHz). Coupling constants (*J* value) are reported in hertz. The chemical shift are reported in δ (ppm) relative to residual chloroform (δ = 7.24) and DMSO (δ = 2.49) as internal standards. ODNs were synthesized on an Aplid Biosystems 3400 DNA Synthesizer. Reverse phase HPLC was performed on a Cosmosil 5C_{18}AR-II (nacalai tesque) column (4.6 x 150 mm) or a CHEMCOBOND 5-ODS-H (Chemco) column (4.6 x 150 mm) with a JASCO PU-980, HG-980-31, DG-980-50 system equipped with a JASCO UV 970 spectrometer at 260 nm. Irradiation was performed by UV-LED (OMURON, 366 nm) and transilluminator (Funakoshi TR-312R/J, 312 nm). Mass spectra were recorded on a Voyager-DE PRO-SF, Applied Biosystems.

Preparation of 5-vinyl-2´-deoxyuridine (2a)

5-Iodo-2´- deoxyuridine (1.0 g, 2.82 mmol) was dissolved in DMF (2.5 ml) in a microwave tube with a stirring bar. To this was added Palladium (II) acetate (65 mg, 0.29 mmol), tri-*n*-butylamine (680 μl, 2.82 mmol) and vinyl acetate monomer (5.5 ml, 59.4 mmol). The reaction tube sealed and reacted in a microwave reactor for 20 min at 80 °C with a continuous stirring. The reaction mixture was filtered to remove the resulting precipitate, and the filtrate was evaporated to dryness in vacuo, then extracted with EtOAc (20 ml x 3) and water (30 ml). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness under reduce pressure. The crude product was purified by silica gel column chromatography ($CHCl_3$ /MeOH 9:1) to afford **2a** (405 mg, 1.59 mmol, 56%).

R_f ($CHCl_3$ / MeOH= 9/1 v/v) 0.21

^{1}H NMR (300 MHz, D_2O): δ 7.87 (s, 1H, H H-C(6)); 6.33 (dd, 1H, J=11.4, 11.4, vinylic H); 6.16 (t, 1H, J=6.6, H-C(1')); 5.70 (d, 1H, J=18.0, vinylic H); 5.17 (d, 1H, J=11.4, vinylic); 4.37 (dd, 1H,

J=5.0, 9.9, C-H(3')); 3.93 (dd, 1H, J=4.1, 8.0, H-C(4')); 3.70 (ddd, J=3.9, 12.0, 27.0, 2H, H-C(5')); 2.29 (t, J=6.6, 2H, H-C(2')).

Preparation of 5-vinyl-2´-deoxy-5´-*O*-(4,4 -dimethoxytrityl)uridine (3a)

5-Vinyl-2´-deoxyuridine (2a) (380 mg, 1.49 mmol) was dissolved in dry pyridine and coevaporated three times. 4, 4'-dimethoxytrityl chloride (600 mg, 1.77 mmol), *N, N*-dimethylamino pyridine (70 mg, 0.57 mmol) and triethylamine (250 μl, 1.77 mmol) was added to a solution of **2a** in dry pyridine (10 ml). The solution was stirred at ambient temperature under nitrogen atmosphere for 16 h. The reaction mixture was extracted with EtOAc (100 ml x 3) and water (150 ml). The organic layer was collected, dried over anhydrous Mg_2SO_4, filtered, and evaporated to dryness under reduce pressure. The crude product was purified by silica gel column chromatography ($CHCl_3$ /EtOH 97:3) to afford **3a** (638 mg, 1.15 mmol, 77%).

R_f ($CHCl_3$ / MeOH= 9/1 v/v) 0.62

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.60 (d, J=4.1, 1H, 3-H); 7.71-7.64 (m, 1H, 6-H); 7.41-7.36 (m, 1H, vinylic. H); 7.31-7.22 (m, 8H, arom.); 6.85-6.80(m, 5H, arom. H); 6.37 (dd, 1H, J=6.0, 7.2, H-C(1')); 5.81-5.67 (m, 1H, vinylic. H); 4.56 (dd, 1H, J=4.1, 9.0, vinylic. H); 4.54 (t, 1H, J=3.0, 3'-OH); 4.06(dd, 1H, J=3.3, 7.5, H-C(3')); 3.78 (d, 6H, J=1.9, OCH_3)); 3.47 (dd, 1H, J=3.3, H-C(4')); 3.41 (dd, 1H, J=3.3, 15.0, H-C(5')); 2.44 (ddd, 1H, J=3.2, 6.0, 15.0, C-H(2', 5')); 2.28 (dd, 1H, J=6.6, 10.9, C-H(2')).

Preparation of 5-vinyl-2´-deoxy-5´-*O*-(4,4 -dimethoxytrityl)uridine phosphoramidite (4a)

5-Vinyl-2´-deoxy-5´-*O*-(4,4-dimethoxytrityl)uridine (3a) (210 mg, 0.38 mmol) in dry CH_3CN (1.5 ml) in a sealed bottle with septum was dissolved in dry acetonitrile and coevaporated three times in *vacuo*. After substitution with nitrogen, 2-cyanoethyl *N, N, N', N'*-tetraisopropyl phosphorodiamidite (120 μl, 0.38 mmol) in dry acetonitrile (1.5 ml), 0.5 M tetrazole (0.95 ml, 0.42 mmol) were added, and the reaction mixture was stirred at ambient temperature for 2 h. Then the reaction mixture was extracted with AcOEt (10 ml x 3) and water (15ml). The organic layer was collected, dried over anhydrous Mg_2SO_4, filtered, and evaporated to dryness under reduced pressure. Then, the crude product cyanoethylphosphoramidite of **4a** (278 mg, 0.37 mmol, 97.) in a sealed bottle with septum was dissolved in dry acetonitrile and coevaporated three times, and was used for automated DNA synthsizer without further purification.

R_f ($CHCl_3$ / MeOH= 9/1 v/v) 0.72

Preparation of 5-heptenyl-2´-deoxyuridine (2b)

5-Iodo-2´- deoxyuridine (500 mg, 1.41 mmol) was dissolved in DMF (2.5 ml)in a microwave tube with a stirring bar. To this was added Palladium (II) acetate (33 mg, 0.15 mmol), tri-*n*-butylamine (340 μl, 1.41 mmol) and 1-heptene (5 ml, 3.53 mmol). The reaction tube sealed and reacted in a microwave reactor for 20 min at 100 °C with a continuous stirring. The reaction mixture was filtered to remove the resulting precipitate, and the filtrate was evaporated to dryness in vacuo, then extracted with EtOAc (20 ml x 3) and water (30 ml). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness under reduce pressure. The crude product was purified by silica gel column chromatography ($CHCl_3$ /MeOH 9:1) to afford **2b** (326 mg, 1.36 mmol, 97%).

R_f ($CHCl_3$ / MeOH= 9/1 v/v) 0.21

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.58 (d, 1H, J=6.0, H-C(6)); 6.21-6.13 (m, 1H, H-C(1')); 6.04 (d, 1H, J=15.9, vinylic H); 5.54-5.38 (m, 1H, vinylic H); 4.58 (br. s, 1H, H-C(3')); 4.03 (br, d, 1H, J= 3.3, H-C(4')); 3.95-3.80 (m, 2H, H-C(5')); 2.45–2.32 (m, 2H, H-C(2')); 2.16-0.84 (m, 11H, H of heptene).

MALDI-TOF MS; calcd. for 325.70 $[M + H]^+$; found 325.80.

Preparation of 5-heptenyl-2´-deoxy-5´-*O*-(4,4 -dimethoxytrityl)uridine (3b)

5-heptenyl-2´-deoxyuridine (2b) (600 mg, 1.85 mmol) was dissolved in dry pyridine and coevaporated three times. 4, 4'-dimethoxytrityl chloride (752 mg, 2.22 mmol), *N, N*-dimethylamino pyridine (68 mg, 0.56 mmol) and triethylamine (310 μl, 2.22 mmol) was added to a solution of **2b** in dry pyridine (10 ml). The solution was stirred at ambient temperature under nitrogen atmosphere for 16 h. The reaction mixture was extracted with EtOAc (100 ml x 3) and water (150 ml). The organic layer was collected, dried over anhydrous Mg_2SO_4, filtered, and evaporated to dryness under reduce pressure. The crude product was purified by silica gel column chromatography ($CHCl_3$ /EtOH 97:3) to afford **3b** (863 mg, 1.38 mmol, 75%).

R_f ($CHCl_3$ / MeOH= 9/1 v/v) 0.44

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.40 (br. s, 1H, H-C(6)); 7.45-7.20 (m, 8H, arom.H) ; 6.83-6.80 (m, 5H, arom. H); 6.39 (t, 1H, J=6.9, H-C(1')); 6.27-6.17(m, 1H, vinylic H); 5.57-5.52 (d, 1H, J=15.9, vinylic H); 4.54 (br, s, 1H, H-C(3')); 4.05-4.01 (m, 1H, H-C(4')); 3.78 (s, 6H, H of methoxyl); 3.53– 3.31 (m, 2H, H-C(5')); 2.43– 2.18 (m, 2H, H-C(2')); 1.98-0.73 (m, 11H, H of heptene).

MALDI-TOF MS; calcd. for 649.29 $[M + Na]^+$; found 649.29

Preparation of 5-heptenyl-2´-deoxy-5´-*O*-(4,4 -dimethoxytrityl)uridine phosphoramidite (4b)

5-heptenyl-2´-deoxy-5´-*O*-(4,4-dimethoxytrityl)uridine (3b) (235 mg, 0.38 mmol) in dry CH_3CN (1.5 ml) in a sealed bottle with septum was dissolved in dry acetonitrile and coevaporated three times in *vacuo*. After substitution with nitrogen, 2-cyanoethyl *N, N, N', N'*-tetraisopropyl phosphorodiamidite (120 μl, 0.38 mmol) in dry acetonitrile (1.5 ml), 0.5 M tetrazole (0.95 ml) were added, and the reaction mixture was stirred at ambient temperature for 2 h. Then the reaction mixture was extracted with AcOEt (10 ml x 3) and water (15ml). The organic layer was collected, dried over anhydrous Mg_2SO_4, filtered, and evaporated to dryness under reduced pressure. Then, the crude product cyanoethylphosphoramidite of **4b** (316 mg, 0.38 mmol, quant.) in a sealed bottle with septum was dissolved in dry acetonitrile and coevaporated three times, and was used for automated DNA synthsizer without further purification.

R_f ($CHCl_3$ / MeOH= 9/1 v/v) 0.72

Preparation of 5-cyclohexylvinyl-2´-deoxyuridine (2c)

5-Iodo-2´- deoxyuridine (505 mg, 1.42 mmol) was dissolved in DMF in a microwave tube with a stirring bar. To this was added Palladium (II) acetate (33 mg, 0.15 mmol), tri-*n*-butylamine (340 μl, 1.41 mmol) and 1-heptene (5 ml, 3.53 mmol). The reaction tube sealed and reacted in a microwave reactor for 20 min at 100 °C with a continuous stirring. The reaction mixture was filtered to remove the resulting precipitate, and the filtrate was evaporated to dryness in vacuo, then extracted with EtOAc (20 ml x 3) and water (30 ml). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness under reduce pressure. The crude product was purified by silica gel column chromatography ($CHCl_3$ /MeOH 9:1) to afford **2c** (400 mg, 1.19 mmol, 84%).

R_f ($CHCl_3$ / MeOH= 9/1 v/v) 0.31

^{1}H NMR (300 MHz, $CDCl_3$): δ 6.32 (dd, 1H, J=7.1, 15.9, vinylic H); 6.21-6.13 (m, 1H, H-C(1')); 6.03 (d, 1H, J=16.2, vinylic H); 4.63-4.59 (m, 1H, H-C(3')); 4.05 (t, 1H, J= 3.0, H-C(4')); 3.97-3.81 (m, 2H, H-C(5')); 2.51– 2.33 (m, 2H, H-C(2')); 2.14-0.89 (m, 11H, H of Cyclohexane)

MALDI-TOF MS; calcd. for 337.18 $[M + H]^+$; found 337.16.

Preparation of 5- cyclohexylvinyl -2´-deoxy-5´-*O*-(4,4-dimethoxytrityl)uridine (3c)

5-heptenyl-2´-deoxyuridine (2c) (695 mg, 2.07 mmol) was dissolved in dry pyridine and coevaporated three times. 4, 4'-dimethoxytrityl chloride (842 mg, 2.48 mmol), *N, N*-dimethylamino pyridine (76 mg, 0.62 mmol) and triethylamine (350 μl, 2.48 mmol) was added to a solution of **2c** in dry pyridine (10 ml). The solution was stirred at ambient temperature under nitrogen atmosphere for 16 h. The reaction mixture was extracted with EtOAc (100 ml x 3) and water (150 ml). The organic layer was collected, dried over

anhydrous Mg_2SO_4, filtered, and evaporated to dryness under reduce pressure. The crude product was purified by silica gel column chromatography ($CHCl_3$ /EtOH 97:3) to afford **3c** (792 mg, 1.24 mmol, 60%).

R_f ($CHCl_3$ / MeOH= 9/1 v/v) 0.45

^{1}H NMR (300 MHz, $CDCl_3$); 8.48 (br. s, 1H, NH(3)); 7.67 (s, 1H, H-C(6)); 7.43-7.20 (m, 8H, arom.H) ; 6.83-6.79 (m, 5H, arom. H); 6.41-6.39 (m, 2H, vinylic H, H-C(1')); 6.21 (dd, 1H, J=6.9, 15.9, vinylic H); 4.52 (br. S, 1H, H-C(3')); 4.06-4.02 (m, 1H, H-C(4')); 3.77(dd, 6H, J=1.1, H of methoxyl); 3.48 (s, 2H, H-C(5')); 2.39– 2.30 (m, 2H, H-C(2')); 1.96-0.72 (m, 11H, H of cyclohexane).

MALDI-TOF MS; calcd. for 661.29 $[M + Na]^+$; found 661.29.

Preparation of 5- cyclohexylvinyl -2´-deoxy-5´-*O*-(4,4-dimethoxytrityl)uridine phosphoramidite (4c)

5-Cyclohexylvinyl-2´-deoxy-5´-*O*-(4,4-dimethoxytrityl)uridine (3c) (171 mg, 0.27 mmol) in dry CH_3CN (1.5 ml) in a sealed bottle with septum was dissolved in dry acetonitrile and coevaporated three times in *vacuo*. After substitution with nitrogen, 2-cyanoethyl *N, N, N', N'*-tetraisopropyl phosphorodiamidite (85 μl, 0.27 mmol) in dry acetonitrile (1.5 ml), 0.5 M tetrazole (0.66 ml) were added, and the reaction mixture was stirred at ambient temperature for 2 h. Then the reaction mixture was extracted with AcOEt (10 ml x 3) and water (15ml). The organic layer was collected, dried over anhydrous Mg_2SO_4, filtered, and evaporated to dryness under reduced pressure. Then, the crude product cyanoethylphosphoramidite of **4c** (216 mg, 0.25 mmol, 93%.) in a sealed bottle with septum was dissolved in dry acetonitrile and coevaporated three times, and was used for automated DNA synthsizer without further purification.

Preparation of 5-*tert*-butylvinyl-2´-deoxyuridine (2d)

5-Iodo-2´- deoxyuridine (500 mg, 1.41 mmol) was dissolved in DMF in a microwave tube with a stirring bar. To this was added Palladium (II) acetate (32 mg, 0.15 mmol), tri-*n*-butylamine (340 μl, 1.41 mmol) and 3,3-dimethyl-1-butene (5.5 ml, 42.3 mmol). The reaction tube sealed and reacted in a microwave reactor for 20 min at 100 °C with a continuous stirring. The reaction mixture was filtered to remove the resulting precipitate, and the filtrate was evaporated to dryness in vacuo, then extracted with EtOAc (20 ml x 3) and water (30 ml). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness under reduce pressure. The crude product was purified by silica gel column chromatography ($CHCl_3$ /MeOH 9:1) to afford **2d** (272 mg, 0.88 mmol, 62%).

R_f ($CHCl_3$ / MeOH= 9/1 v/v) 0.31

^{1}H NMR (300 MHz, DMSO); δ 8.00 (dd, 1H, H-C(6)); 6.48-6.38 (m, 1H, vinylic H); 6.15 (t, 1H, J= 6.6, H-C(1')); 6.00 (t, 1H, J= 17.0, vinylic H); 5.23-5.20 (m, 1H, 3'-OH); 5.12 (br. s, 1H, 5'-OH); 4.24 (br, s, 1H, H-C(3')); 3.77 (d, 1H, J= 3.0, H-C(4')); 3.61– 3.52 (m, 2H, H-C(5')); 2.19– 2.04 (m, 2H, H-C(2')); 1.06-0.84 (m, 9H, H of butyl).

MALDI-TOF MS; calcd. for 311.16 $[M + H]^+$; found 311.17.

Preparation of 5- *tert*-butylvinyl -2´-deoxy-5´-*O*-(4,4-dimethoxytrityl)uridine (3d)

5- *tert*-butylvinyl -2´-deoxyuridine (2d) (497 mg, 1.60 mmol) was dissolved in dry pyridine and coevaporated three times. 4, 4'-dimethoxytrityl chloride (651 mg, 1.92 mmol), *N, N*-dimethylamino pyridine (59 mg, 0.48 mmol) and triethylamine (270 μl, 1.92 mmol) was added to a solution of **2d** in dry pyridine (10 ml). The solution was stirred at ambient temperature under nitrogen atmosphere for 16 h. The reaction mixture was extracted with EtOAc (100 ml x 3) and water (150 ml). The organic layer was collected, dried over anhydrous Mg_2SO_4, filtered, and evaporated to dryness under reduce pressure. The crude product was purified by silica gel column chromatography ($CHCl_3$/EtOH 97:3) to afford **3d** (601 mg, 0.98 mmol, 61%).

R_f ($CHCl_3$ / MeOH= 9/1 v/v) 0.46

^{1}H NMR (300 MHz, DMSO): δ 11.4 (d, 1H, J=6.6, NH); 8.58 (d, 1H, J=4.2, H-C(6)); 7.43-7.10 (m, 8H, arom.H) ; 6.87-6.85 (m, 5H, arom. H); 6.40 (d, 1H, J= 16.2, vinylic H); 6.22 (t, 1H, J= 6.6, H-C(1')); 5.69 (d, 1H, J= 16.2, vinylic H); 5.32(t, 1H, J=4.9, 3'-OH); 4.25 (br, s, 1H, H-C(3')); 3.89 (br. d, J=3.0, H-C(4')); 3.72(t, 6H, J=2.0, methoxyl H); 3.26– 3.10 (m, 2H, H-C(5')); 2.34– 2.50 (m, 2H, H-C(2')); 0.89-0.75 (m, 9H, H of butyl).

MALDI-TOF MS; calcd. for 635.27 $[M + Na]^+$; found 635.27.

Preparation of 5-*tert*-butylvinyl-2´-deoxy-5´-*O*-(4,4-dimethoxytrityl)uridine phosphoramidite (4d)

5- *tert*-butylvinyl-2´-deoxy-5´-*O*-(4,4-dimethoxytrityl)uridine (3d) (235 mg, 0.38 mmol) in dry CH_3CN (1.5 ml) in a sealed bottle with septum was dissolved in dry acetonitrile and coevaporated three times in *vacuo*. After substitution with nitrogen, 2-cyanoethyl *N, N, N', N'*-tetraisopropyl phosphorodiamidite (120 μl, 0.38 mmol) in dry acetonitrile (1.5 ml), 0.5 M tetrazole (0.95 ml) were added, and the reaction mixture was stirred at ambient temperature for 2 h. Then the reaction mixture was extracted with AcOEt (10 ml x 3) and water (15ml). The organic layer was collected, dried over anhydrous Mg_2SO_4, filtered, and evaporated to dryness under reduced pressure. Then, the crude product cyanoethylphosphoramidite of **4d** (316 mg, 0.38 mmol, quant.) in a sealed bottle with septum was dissolved in dry acetonitrile and coevaporated three times, and was used for automated DNA synthsizer without further purification.

Synthesis and characterization of ^{V}U derivatives-containing ODN.

^{V}U derivatives-containing ODN was synthesized by automated solid-phase phosphoramidite method as reported. [S1] After automated synthesis, the oligomer was deprotected by incubation with 28% ammonia for 4 h at 65 °C and was purified on a Chemcobond 5-ODS-H column (4.6 x 150 mm) by reverse phase HPLC; elution was with 0.05 M ammonium formate containing 3-20% acetonitrile, linear gradient (30 min) at a flow rate of 1.0 mL/min, 30 ˚C. Preparation of oligonucleotides was confirmed by MALDI-TOF-MS analysis.

MALDI-TOF MS

calcd. for ODN **1** (5'-^{V}UGCGTG-3') : $[(M+H)^{+}]$ 1836.24, found 1836.61. (1100 μM)

calcd. for ODN **2** (5'-^{H}UGCGTG-3') : $[(M+H)^{+}]$ 1906.37, found 1906.20. (350 μM)

calcd. for ODN **3** (5'-^{HV}UGCGTG-3') : $[(M+H)^{+}]$ 1918.38, found 1918.27. (370 μM)

calcd. for ODN **4** (5'-^{HM}UGCGTG-3') : $[(M+H)^{+}]$ 1906.37, found 1906.46. (430 μM)

calcd. for ODN **5** (5'-^{BuV}UGCGTG-3') : $[(M+H)^{+}]$ 1892.35, found 1892.42. (460 μM)

calcd. for ^{V}UGACGTGTATCGCATTGGSSSS-NH_2 : $[(M+H)^{+}]$ 7114.95, found 7114.20.

calcd. for ^{H}UGACGTGTATCGCATTGGSSSS-NH_2 : $[(M+H)^{+}]$ 7185.08, found 7184.36.

calcd. for^{HV}UGACGTGTATCGCATTGGSSSS-NH_2 :$[(M+H)^{+}]$ 7197.09, found 7197.59.

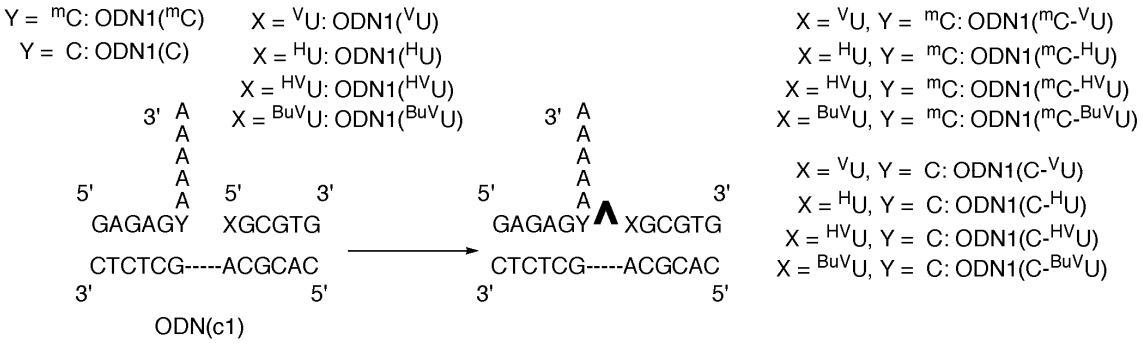
calcd. for^{Buv}UGACGTGTATCGCATTGGSSSS-NH_2:$[(M+H)^{+}]$ 7171.05, found 7171.20.

General methods for DNA photoligation

Photoligation of DNA oligomer as monitored by HPLC.

A reaction mixture (total volume 100 μl) containing 5'-d(XGCGTG)-3' (X = photosensitive pyrimidine) (20 μM, strand conc.), 5'-d(GAGAGY)-3' (Y = C or ^{m}C) (20 μM, strand conc.), and 5'-d(CACGCAGCTCTC)-3' (template ODN) (22 μM, strand conc.) in 50 mM sodium cacodylate buffer (pH 7.0) in a quartz capillary cell was irradiated at 0 °C with transilluminator (366 nm) under otherwise identical condition (Figure S1–S8). The photoligated Reaction mixture was taken up and subjected to HPLC analysis. Analysis was carried out on a COSMOSIL 5C_{18}-AR-II column (4.6 x 150 mm), detected at 260 nm; elution was with 0.05 M ammonium formate containing 3–20% acetonitrile, linear gradient (30 min) at a flow rate of 1.0 ml/ min, 30 °C.

Scheme S1



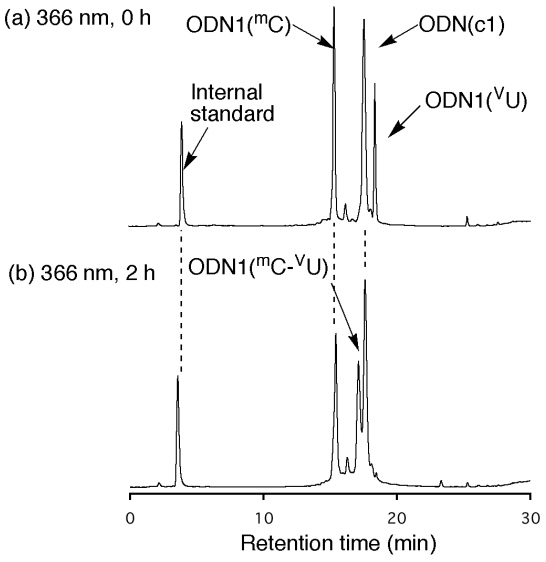


Figure S1

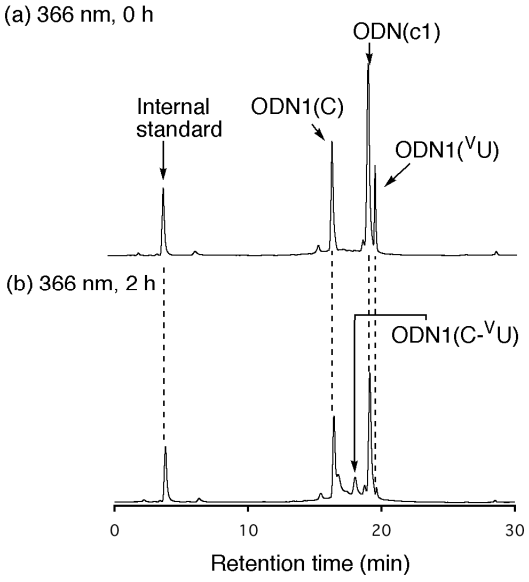


Figure S2

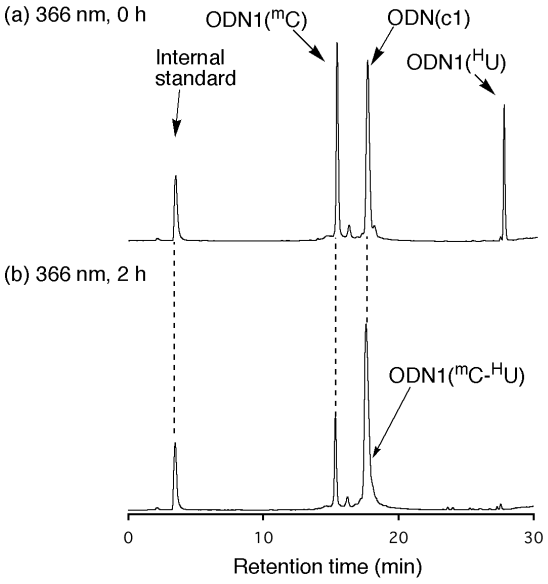


Figure S3

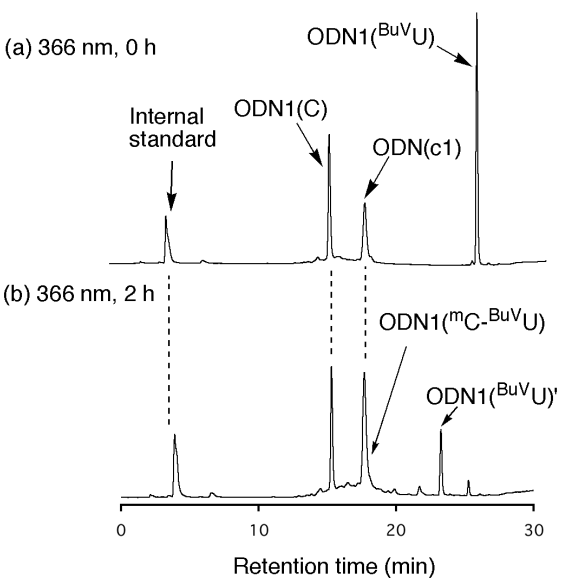


Figure S4

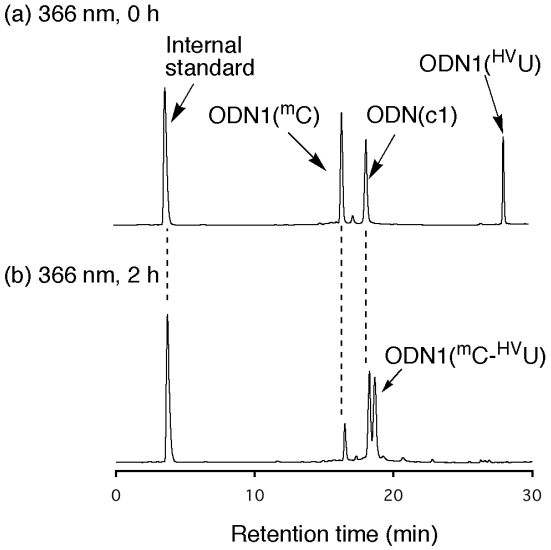


Figure S5

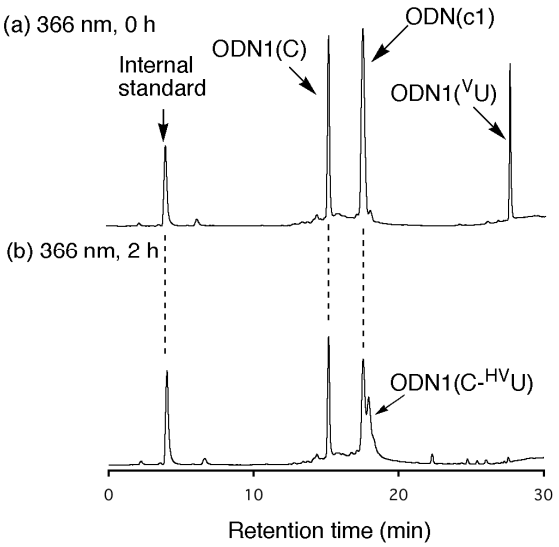


Figure S6

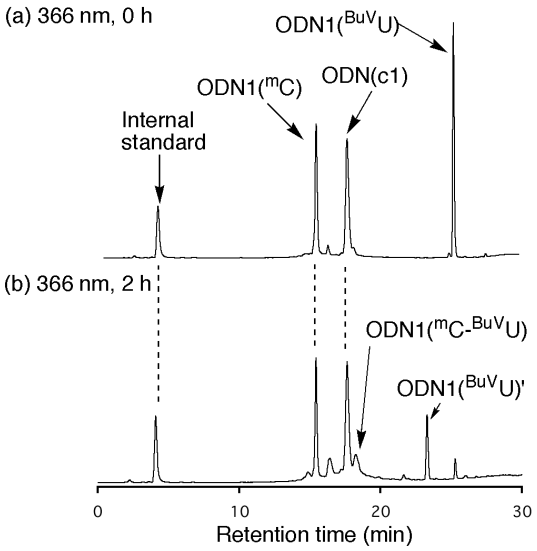


Figure S7

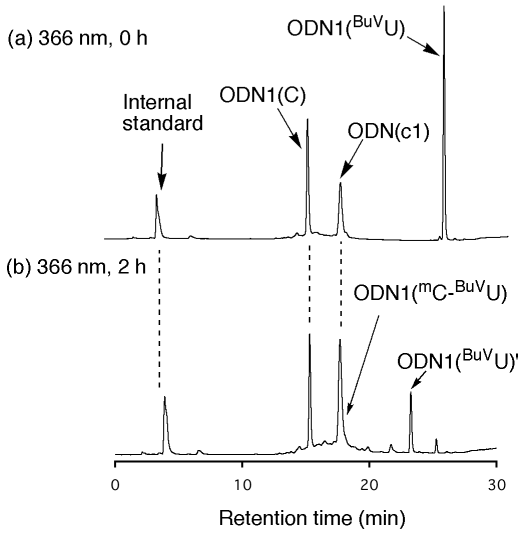


Figure S8

Table S1

	X=C	X=^{m}C
^{V}UGCGTG	23 %	18 %
^{H}UGCGTG	32 %	47 %
^{HV}UGCGTG	33 %	66%
^{BuV}UGCGTG	28 %	39 %

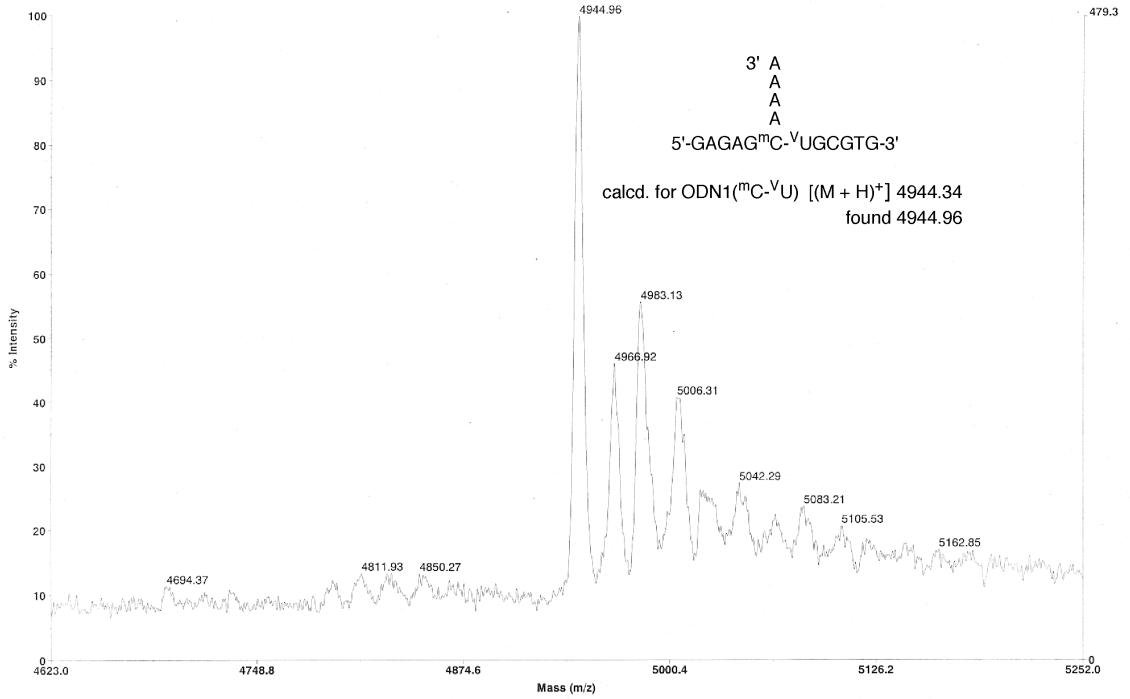


Figure S9

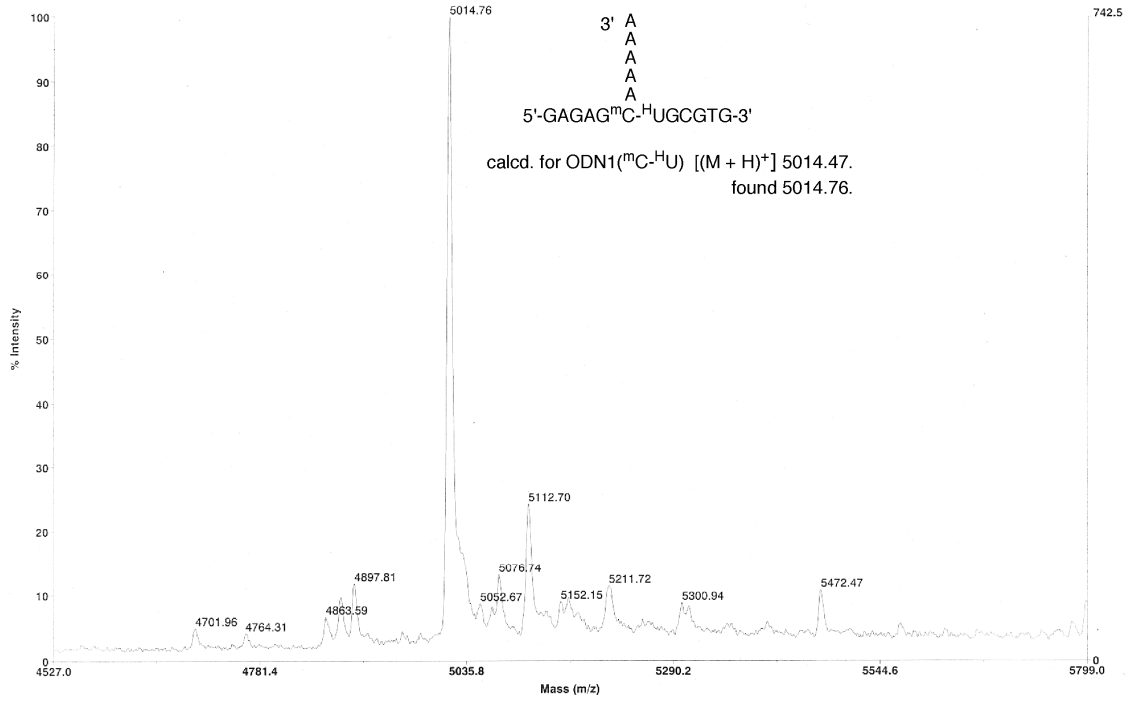


Figure S10

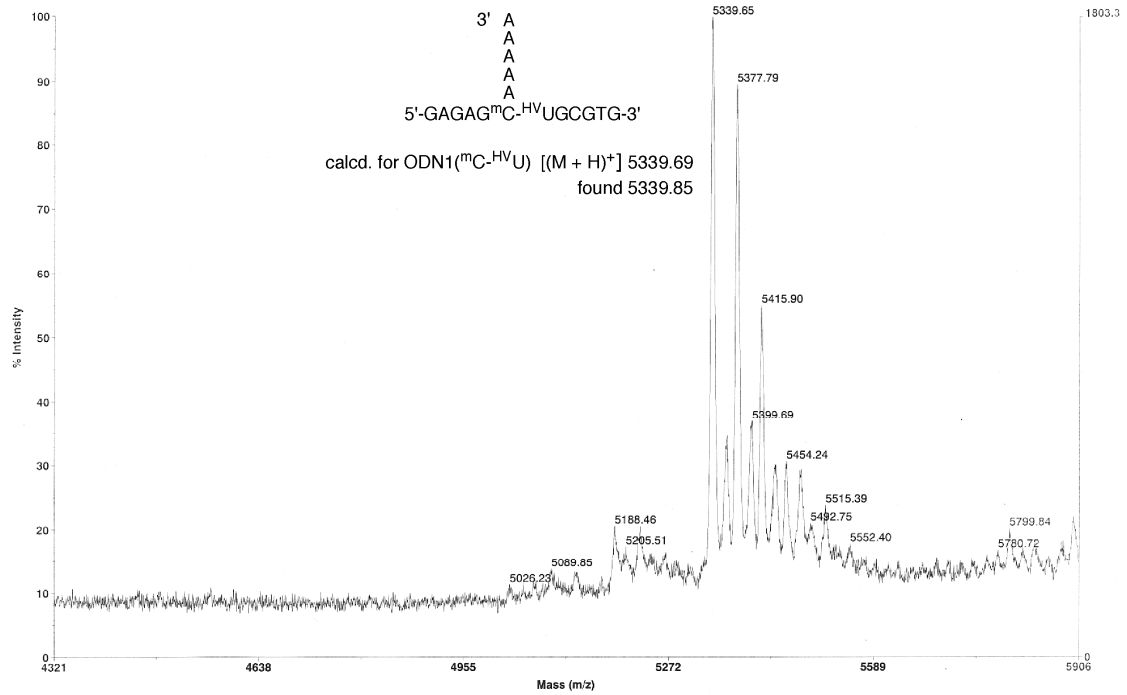


Figure S11

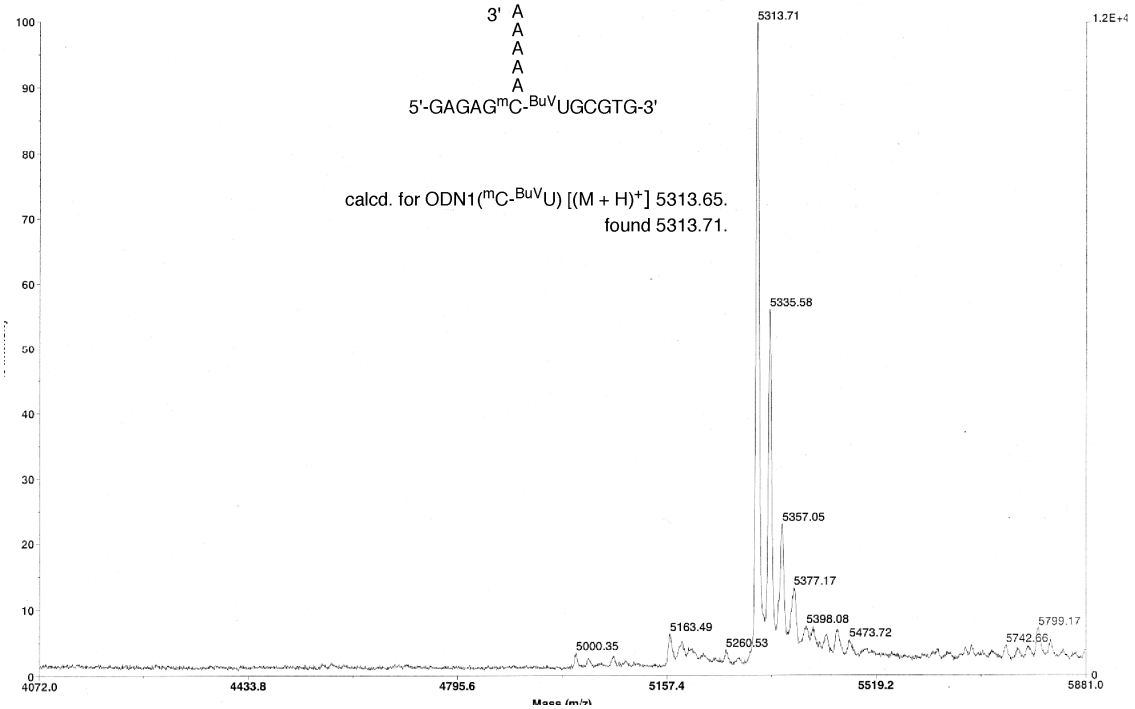


Figure S12

HPLC analysis of the enzymatic digestion of isolated photoligated ODNs.

The photoligated ODN1(^{m}C-^{V}U), ODN1(^{m}C-^{H}U), ODN1(^{m}C-^{HV}U), and ODN1(^{m}C-^{BuV}U) were purified by HPLC and subjected enzymatic digestion with s. v. PDE (0.25 unit/mL), P-1 nuclease (2.5 unit/mL) and AP (10 unit/mL) for 4 h to decompose to mononucleosides, respectively. Reaction mixture was taken up and subjected to HPLC analysis (Figure S13–16). Analysis was carried out on a COSMOSIL 5C_{18}-AR-II column (4.6 x 150 mm), detected at 260 nm; elution was with 0.05 M ammonium formate containing 3–20% acetonitrile, linear gradient (30 min) at a flow rate of 1.0 ml/ min, 30 °C. Photoadduct d(^{m}C-^{V}U), d(^{m}C-^{H}U), d(^{m}C-^{HV}U), and d(^{m}C-^{BuV}U) were confirmed by MALDI-TOF-MS analysis (Figure S17–20).

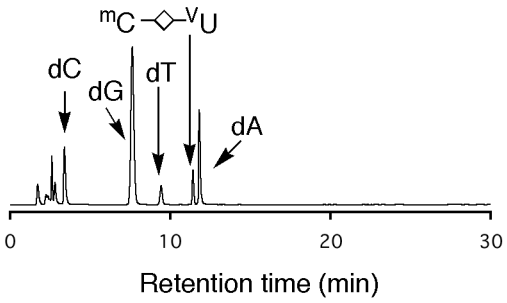


Figure S13

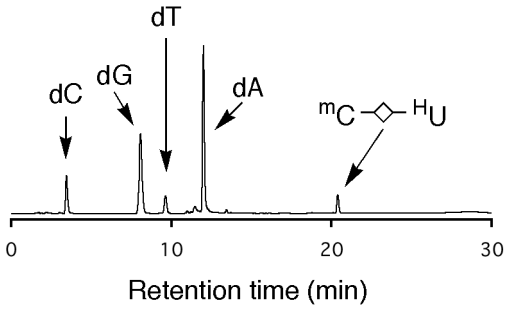


Figure S14

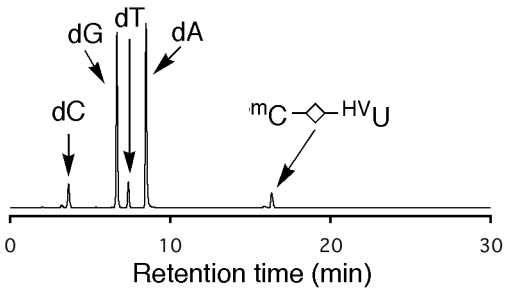


Figure S15

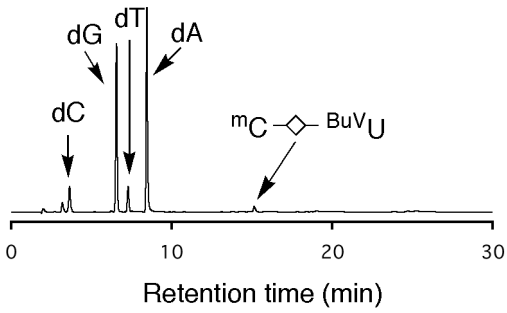


Figure S16

calcd. for ^{m}C-^{V}U : [(M+H)$^{+}$] 496.20, found 496.30.

calcd. for ^{m}C-^{H}U : [(M+H)$^{+}$] 566.28, found 566.31.

calcd. for ^{m}C-^{HV}U : [(M+H)$^{+}$] 578.28, found 578.32.

calcd. for ^{m}C-^{BuV}U : [(M+H)$^{+}$] 552.27, found 552.28.

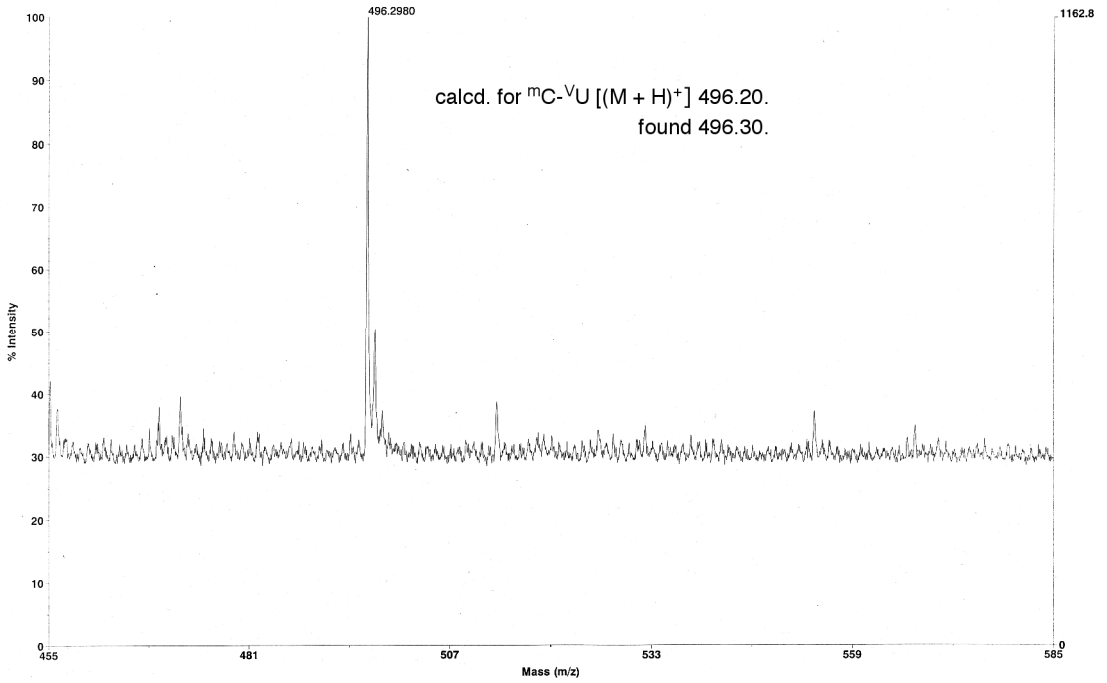


Figure S17

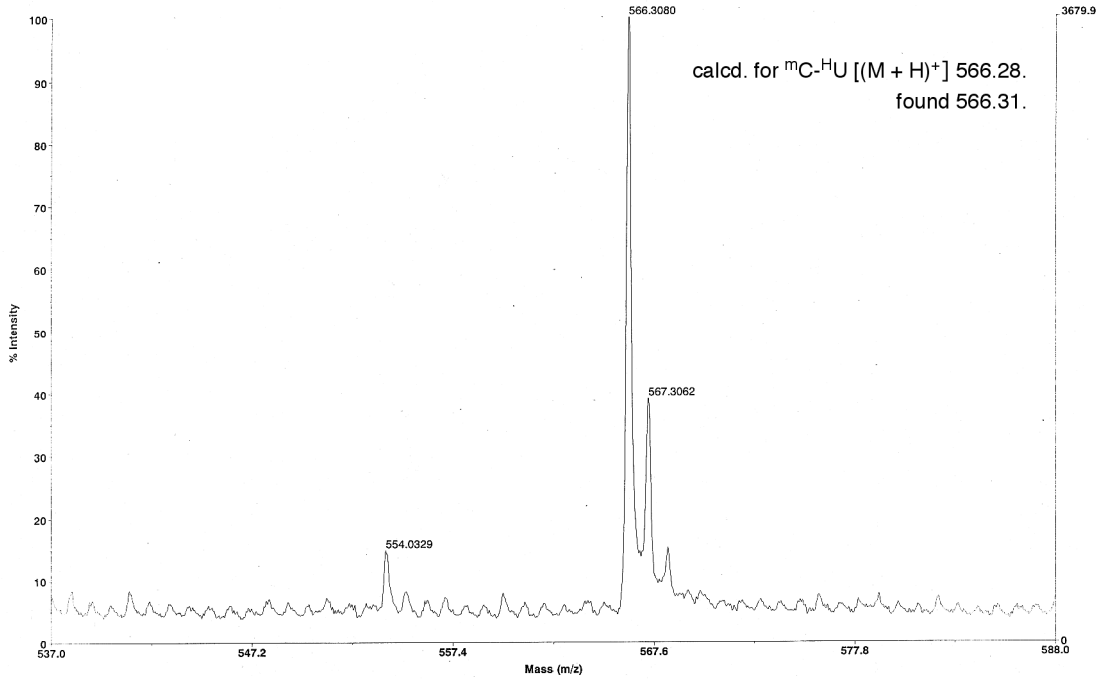


Figure S18

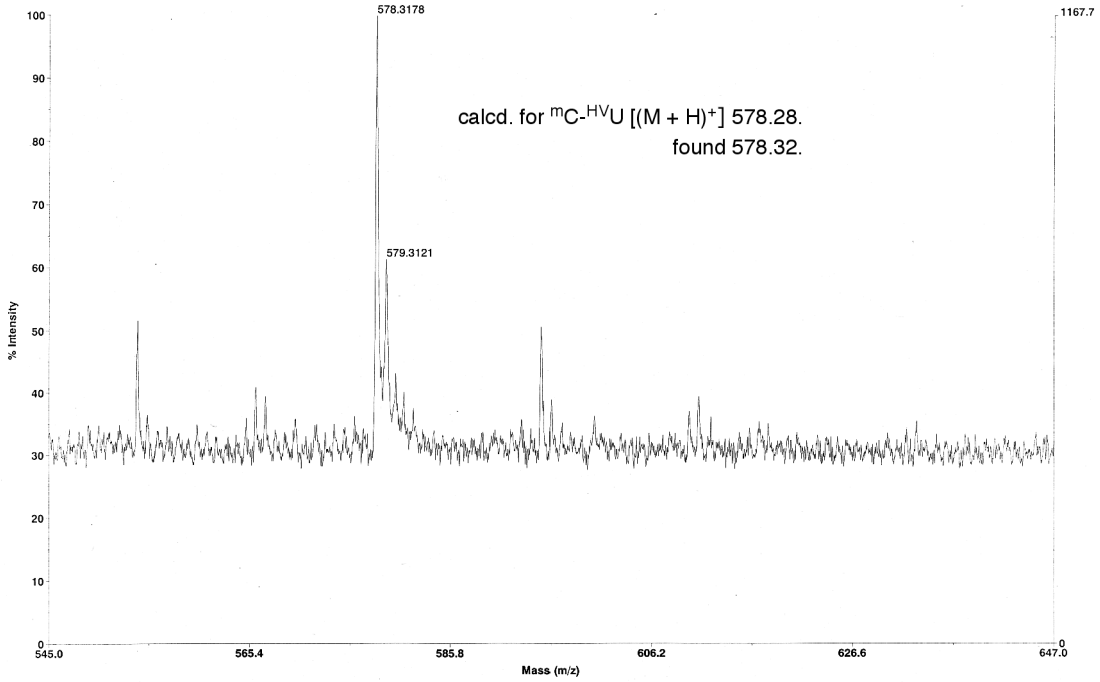


Figure S19

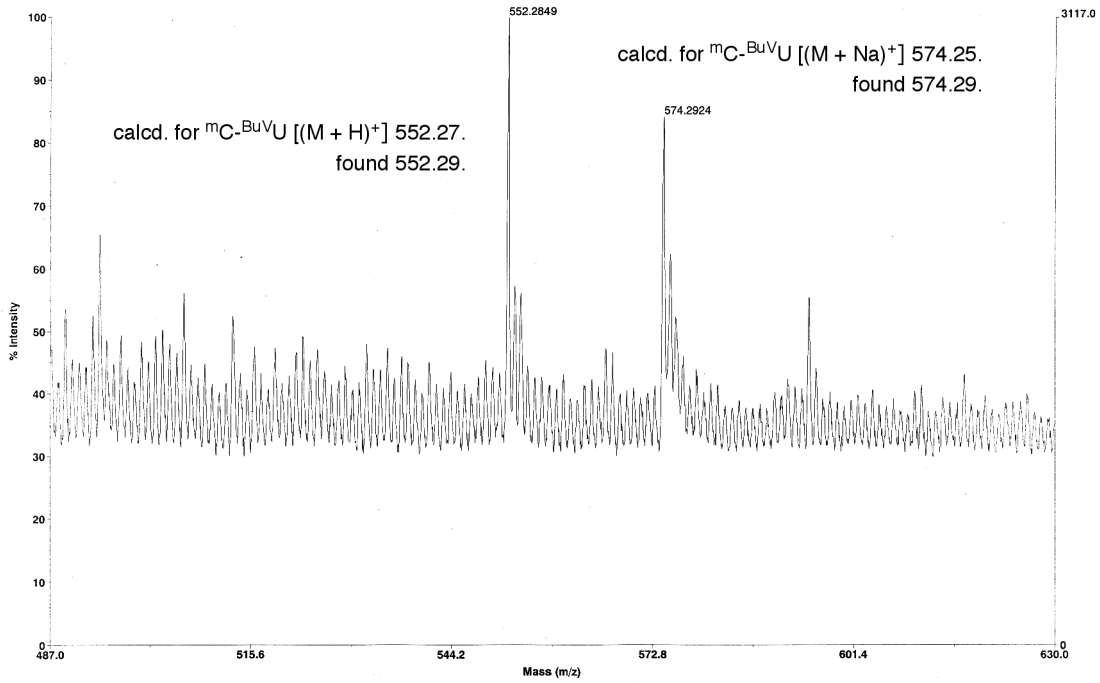


Figure S20

The thermodynamic parameters

Before photoligation, the absorbance of the sample (1.0–2.5 μM strand concentration, 50 mM sodiume cacodylate buffer (pH 7.0) and 100 mM NaCl aq.) were monitored at 260 nm from 5 °C to 70 °C with heating rate of 0.5 °C/ min using JASCO V-550 UV/ VIS spectrophotometer. The change in enthalpy, ΔH, was determined from the temperature dependence of the equilibrium constant, according to the van' t Hoff plots, lnK = ΔH/RT + ΔS/R, where ΔS is the change in entropy. Figure S22, S24, S26, and S28 show the van't Hoff plots for various ODNs combinations. The change in free energy (ΔG) at 25 °C was calculated from the standard Gibbs's equation, ΔG = ΔH–TΔS. Table S4 contains the calculated thermodynamic parameters.

Table S2

Oligonucleotide	Sequence
ODN **1**	^{V}**U**GACGTGTA
ODN **2**	^{H}**U**GACGTGTA
ODN **3**	^{HV}**U**GACGTGTA
ODN **4**	^{BuV}**U**GACGTGTA
ODN **5**	GGGGGCAG^{m}**C**GCCTCA
ODN **6**	TACACGTCAGCTGCCCCC

(^{v}U_^{m}C)

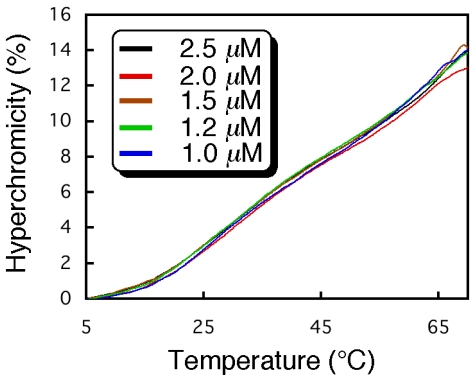


Figure S21

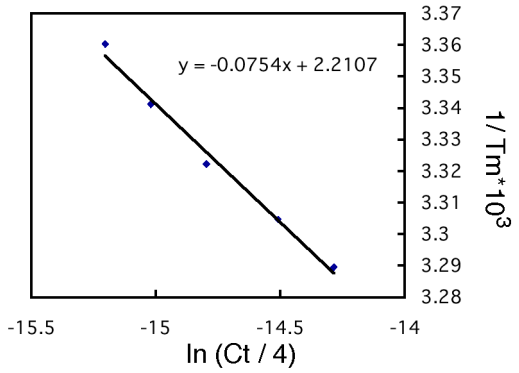


Figure S22

($^{H}U\_^{m}C$)

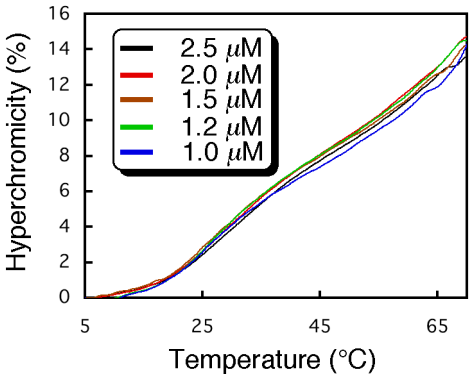


Figure S23

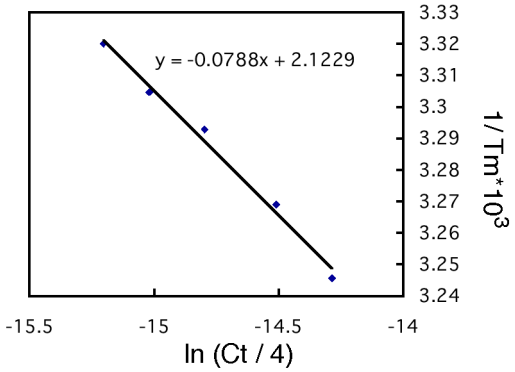


Figure S24

($^{HV}U\_^{m}C$)

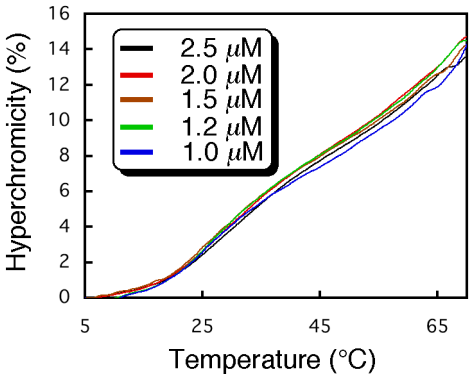


Figure S25

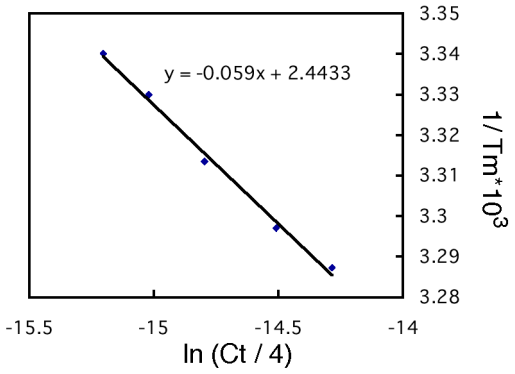


Figure S26

$^{BuV}U\_^{m}C$

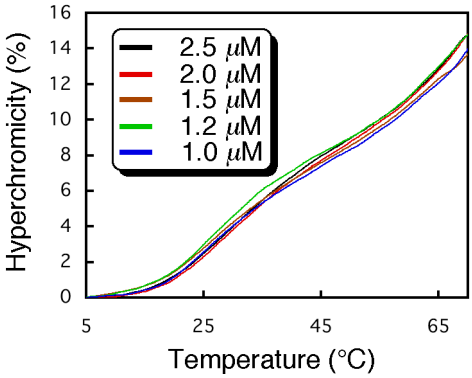


Figure S27

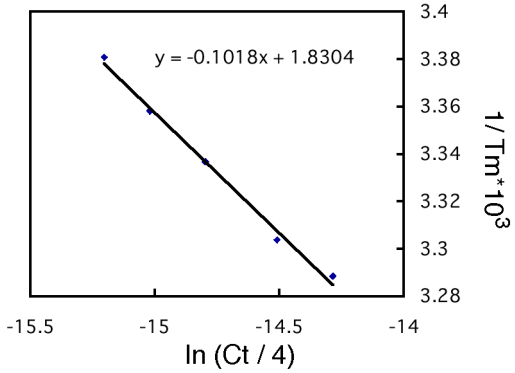


Figure S28

Table S3

	2.5 μM	2.0 μM	1.5 μM	1.2 μM	1.0 μM
^{V}U_C	32.4	30.9	28.9	26.8	25.7
^{V}U_^{m}C	31.0	29.6	28.8	26.3	24.6
^{H}U_C	32.8	31.4	29.6	26.8	24.8
^{H}U_^{m}C	35.1	32.9	30.7	29.6	28.2
^{HV}U_C	30.9	29.4	26.8	25.7	24.6
^{HV}U_^{m}C	31.2	30.3	28.8	27.3	26.4
^{BuV}U_C	31.0	29.8	27.8	25.5	24.2
^{BuV}U_^{m}C	31.1	29.7	26.7	24.8	22.8

Table S4

	ΔH (kcal mol^{-1})	ΔS (cal mol^{-1} K^{-1})	ΔG_{273} (kcal mol^{-1})
^{V}U, C	-24.3	-51.0	-10.3
^{V}U, mC	-26.4	-58.3	-10.4
^{H}U, C	-20.8	-39.5	-10.0
^{H}U, mC	-25.2	-53.5	-10.6
^{HV}U, C	-25.7	-56.1	-10.4
^{HV}U, mC	-33.7	-82.3	-11.2
^{BuV}U, C	-23.6	-49.1	-10.2
^{BuV}U, mC	-19.5	-35.7	- 9.8

Table S5

	mC/C	$\Delta\Delta H$ (kcal mol^{-1})	$\Delta\Delta S$ (cal mol^{-1} K^{-1})	Tm (2.0μM)
^{V}U ODN	2.4	- 2.1	- 7.3	30.9 (C) 29.6 (mC)
^{H}U ODN	1.6	- 4.4	-14.0	31.4 (C) 32.9 (mC)
^{HV}U ODN	2.7	- 8.0	-26.2	29.4 (C) 30.3 (mC)
^{BuV}U ODN	1.4	4.1	13.4	29.8 (C) 29.7 (mC)

ΔΔH, *ΔΔS* and *ΔΔG* values are defined by deducting these values (*ΔH*, *ΔS* and *ΔG*) of the cytosine case from the methylcytosine case, respectively.

Photosplitting of isolated photoligated ODNs as monitored by HPLC

A solution (total volume 100 μl) containing isolated photoligated ODN in 50 mM sodiume cacodylate buffer (pH 7.0) and 100 mM NaCl aq. in a quarts capillary cell was irradiated at 25 °C with a transilluminator (312 nm). After irradiation, 100 μl of aliquot was taken up and subjected to HPLC analysis. Analysis was carried out on a COSMOSIL 5C_{18}-AR-II column (4.6 x 150 mm), detected at 260 nm; elution was with 0.05 M ammonium formate containing 3–20% acetonitrile, linear gradient (30 min) at a flow rate of 1.0 ml/ min, 30 °C. The results were shown in the Figure S29-33.

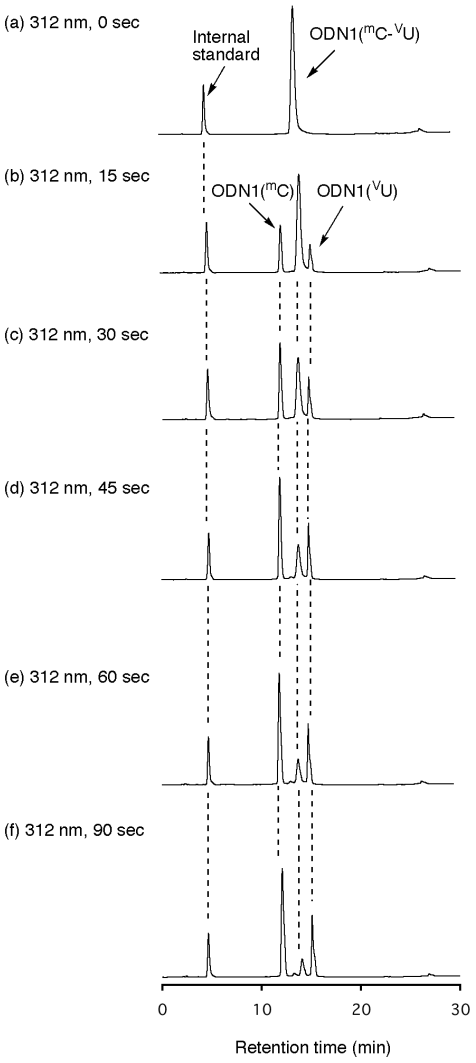


Figure S29

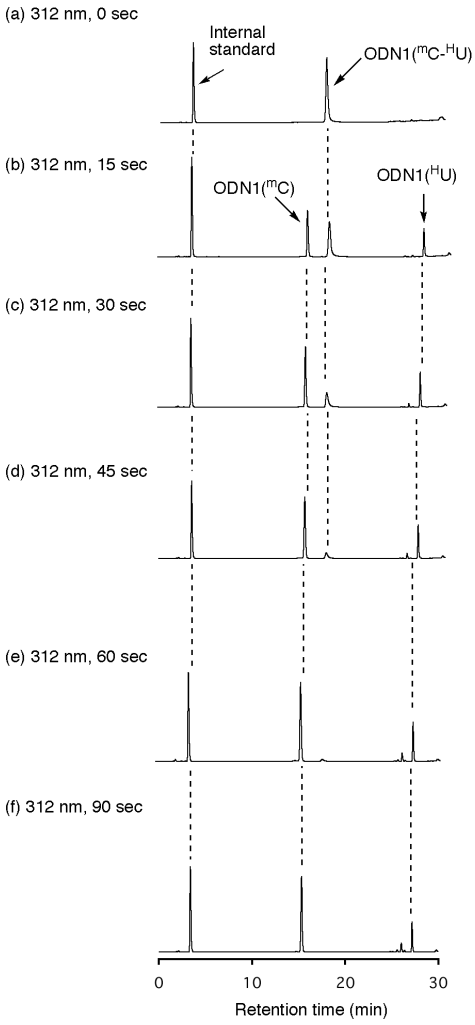


Figure S30

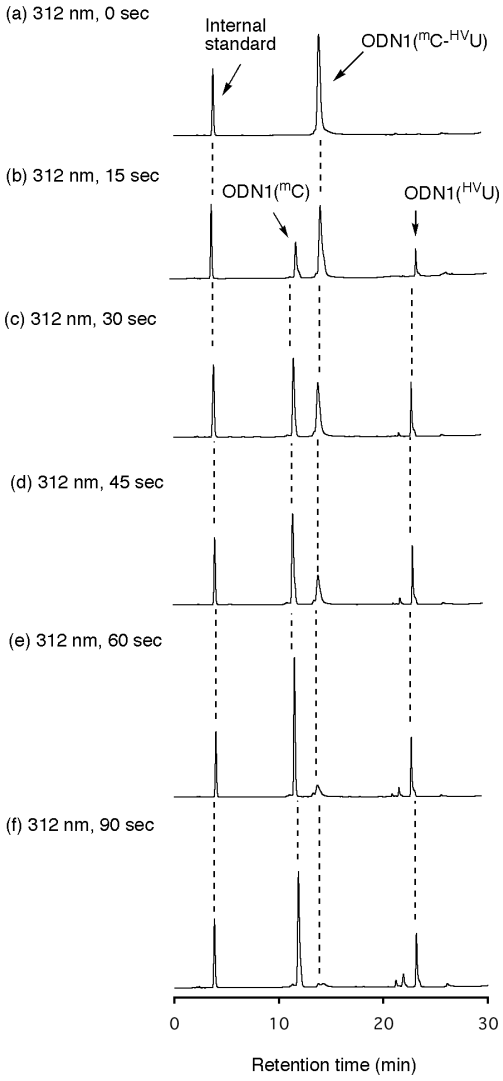


Figure S29

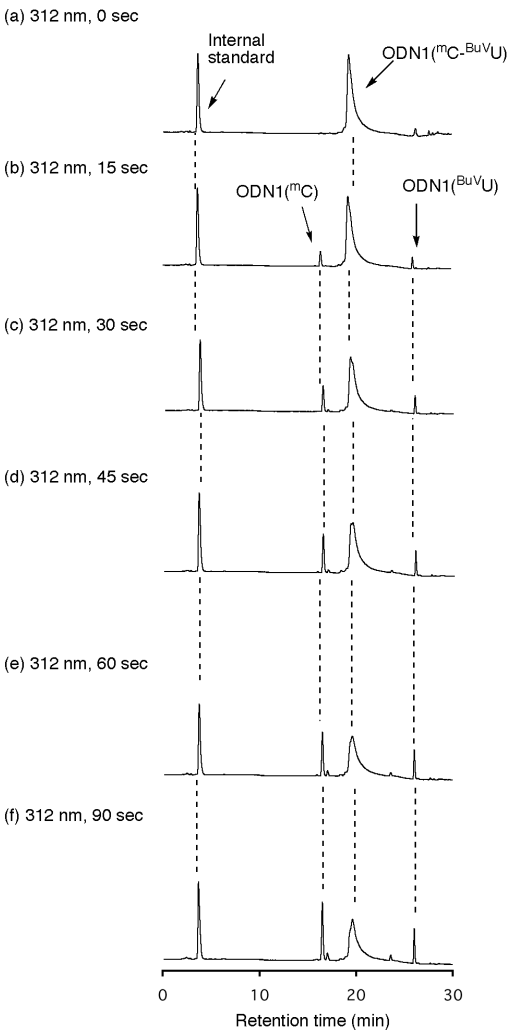


Figure S30

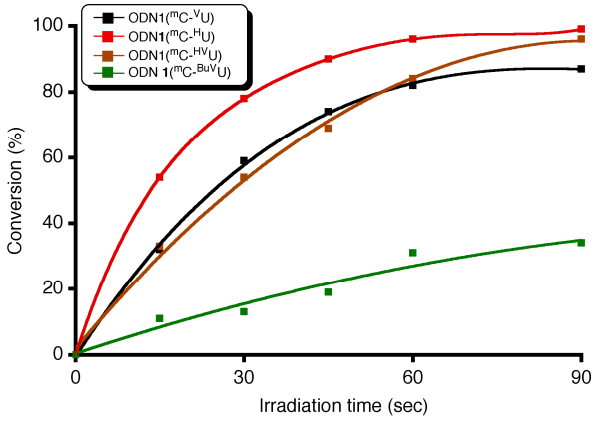
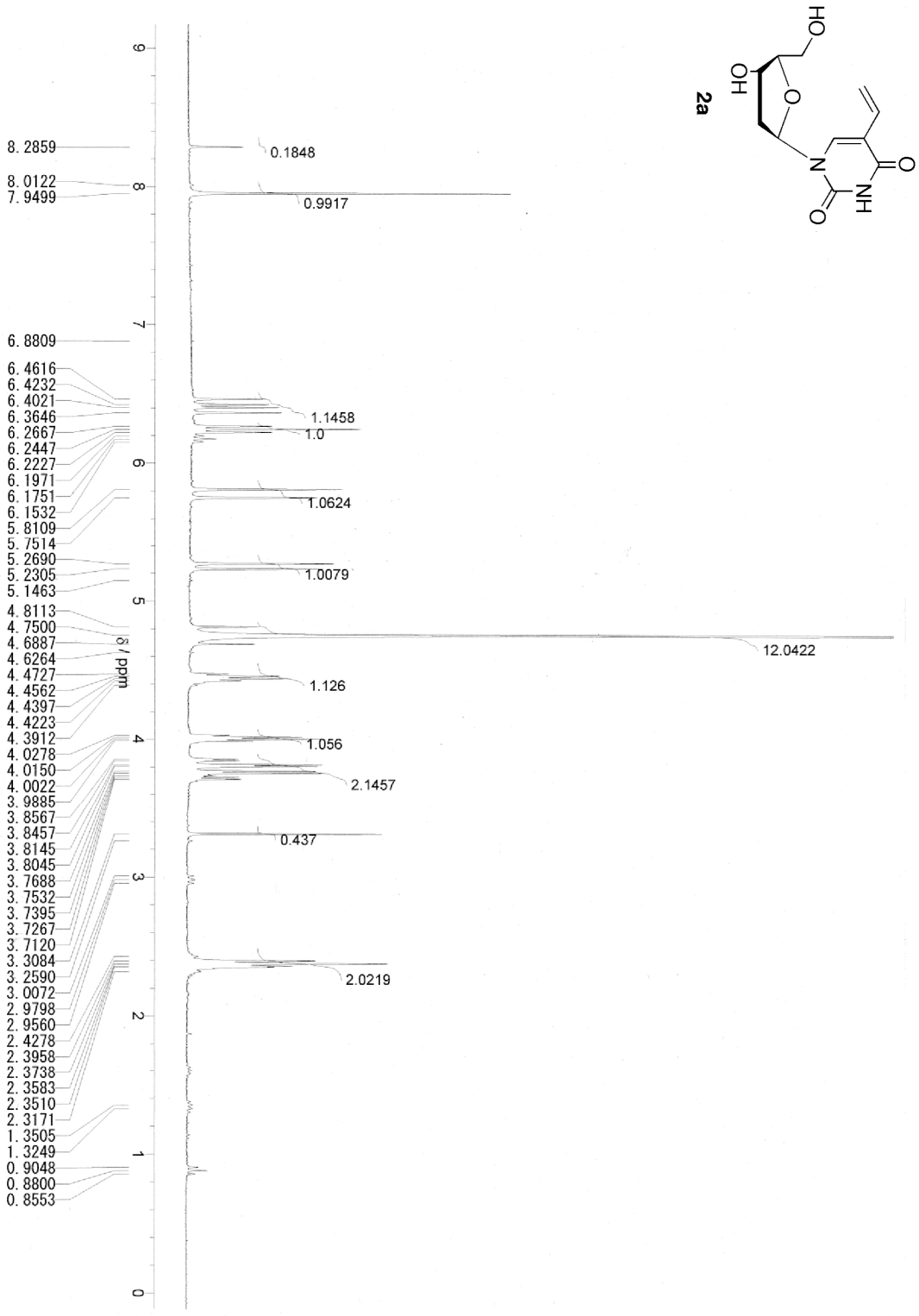


Figure S31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4
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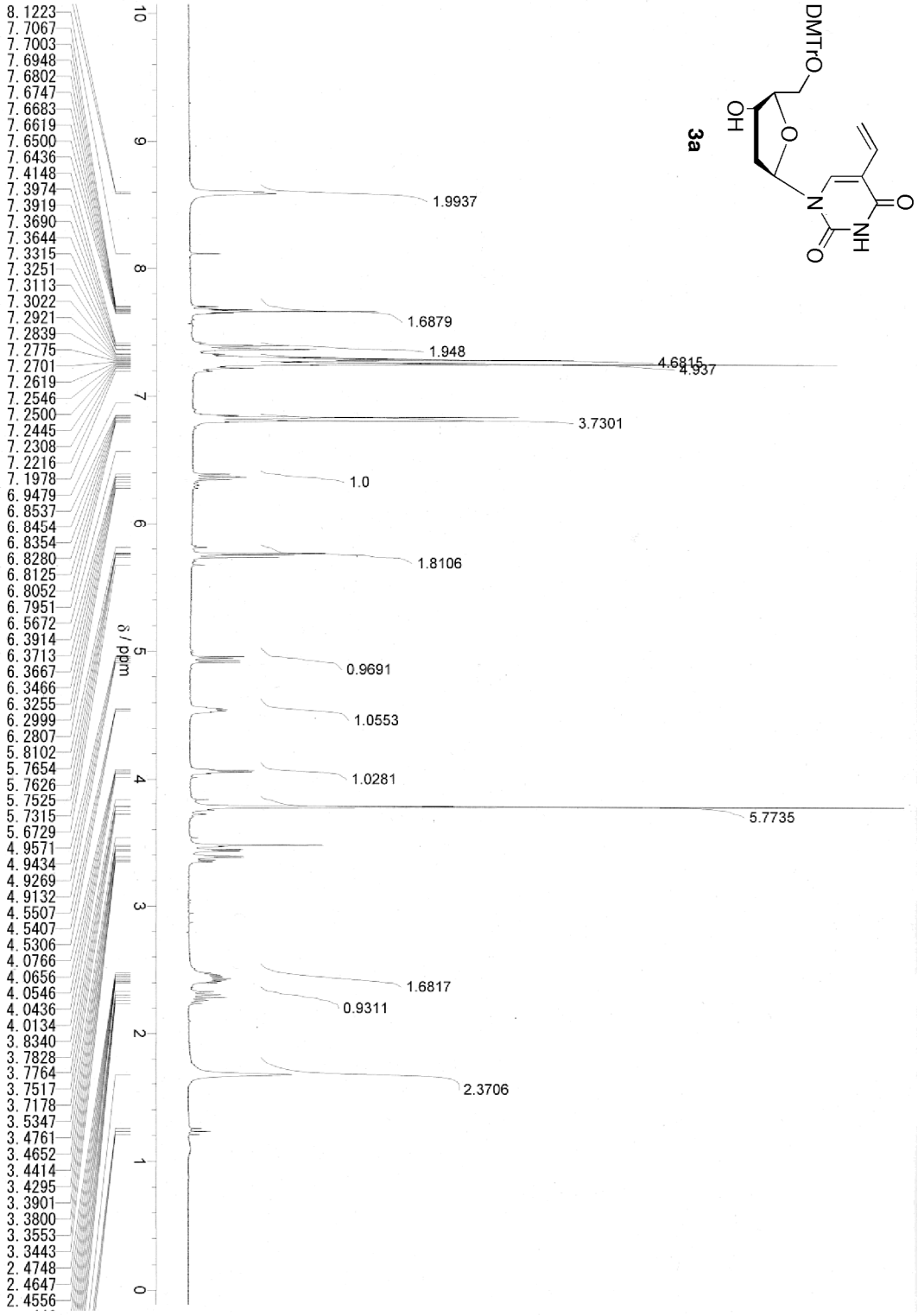
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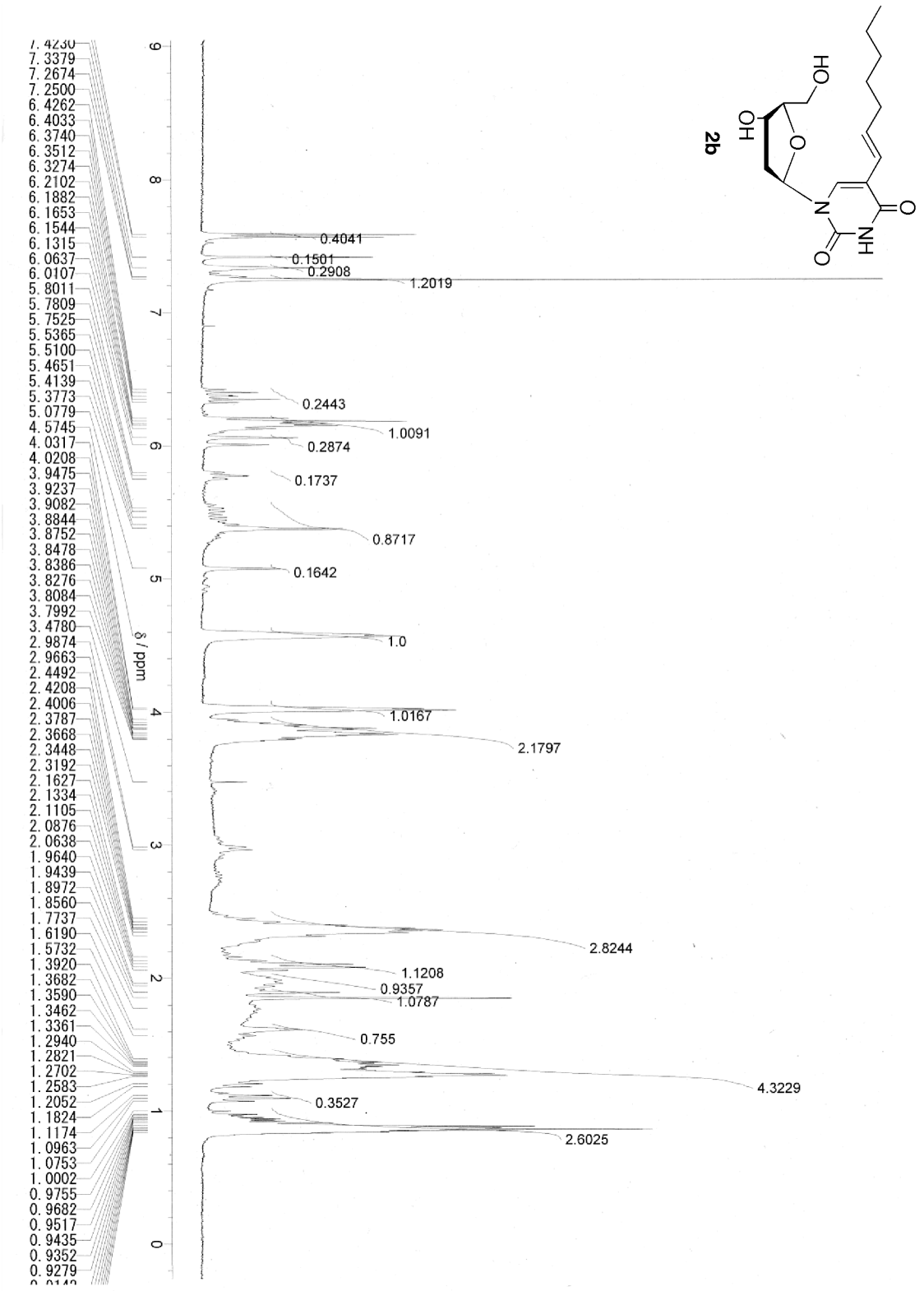
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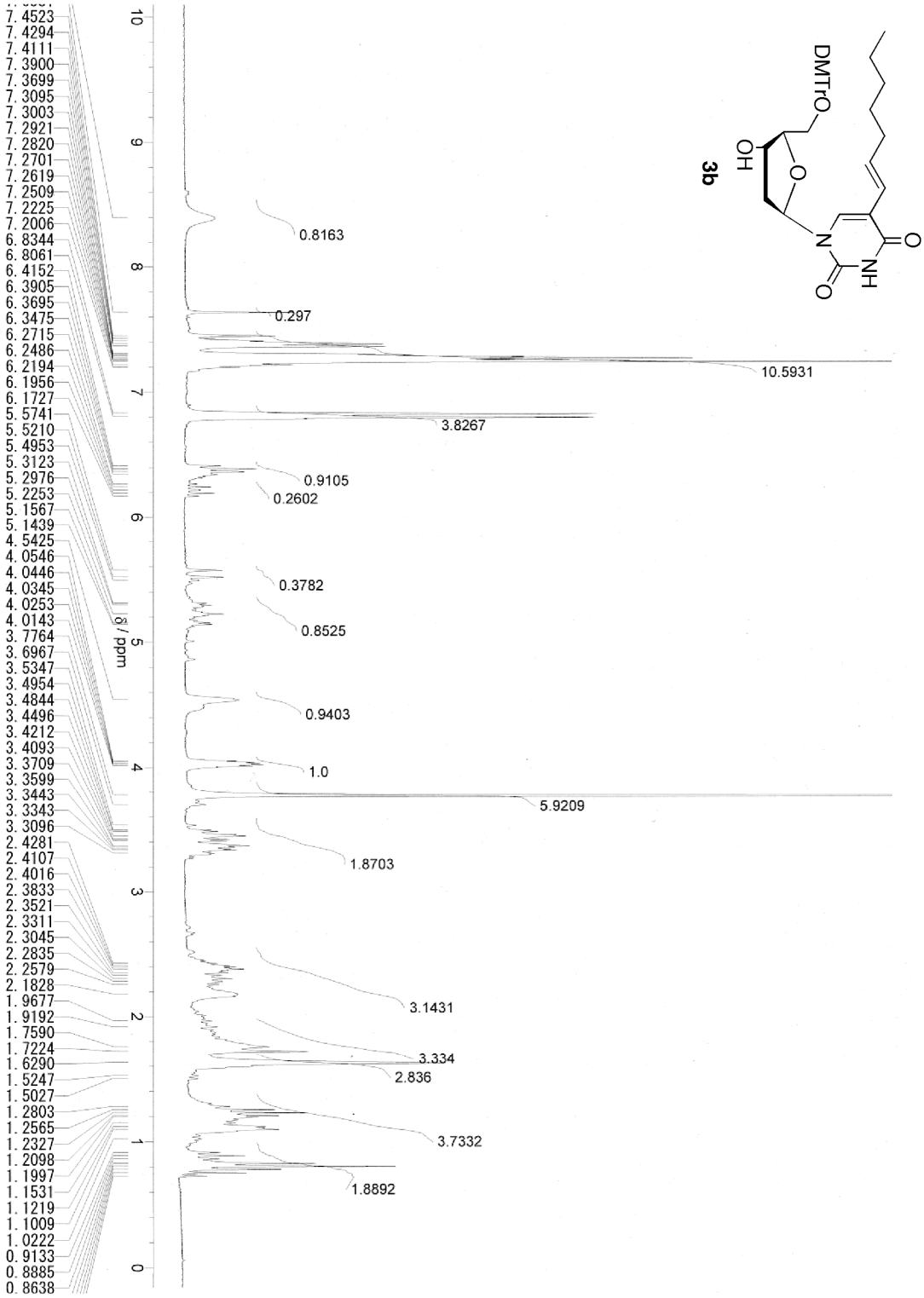
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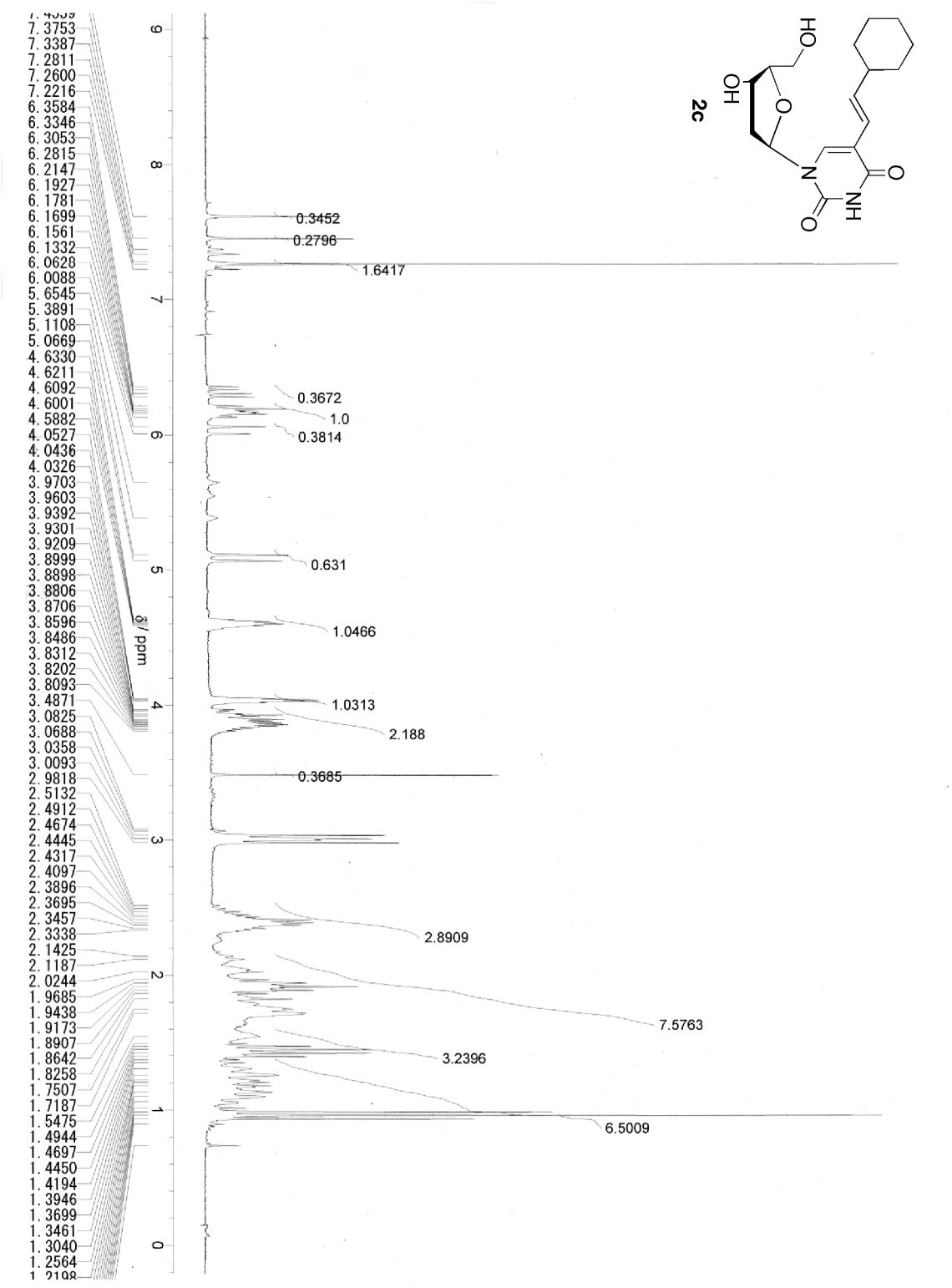
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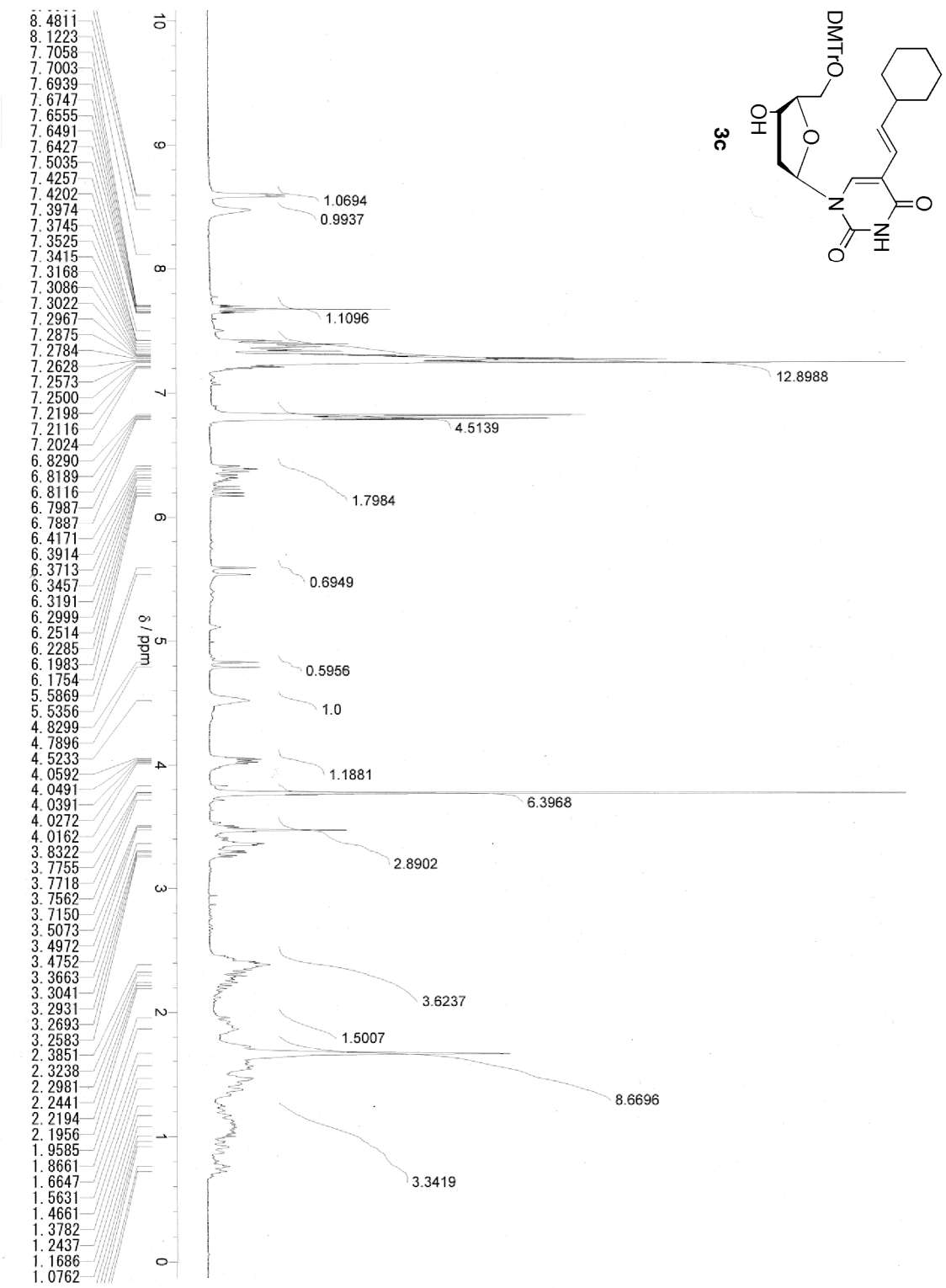
6.5009
δ / ppm


DMTrO
OH
O
N
O
NH
O
3c
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0.9937
1.1096
12.8988
4.5139
1.7984
0.6949
0.5956
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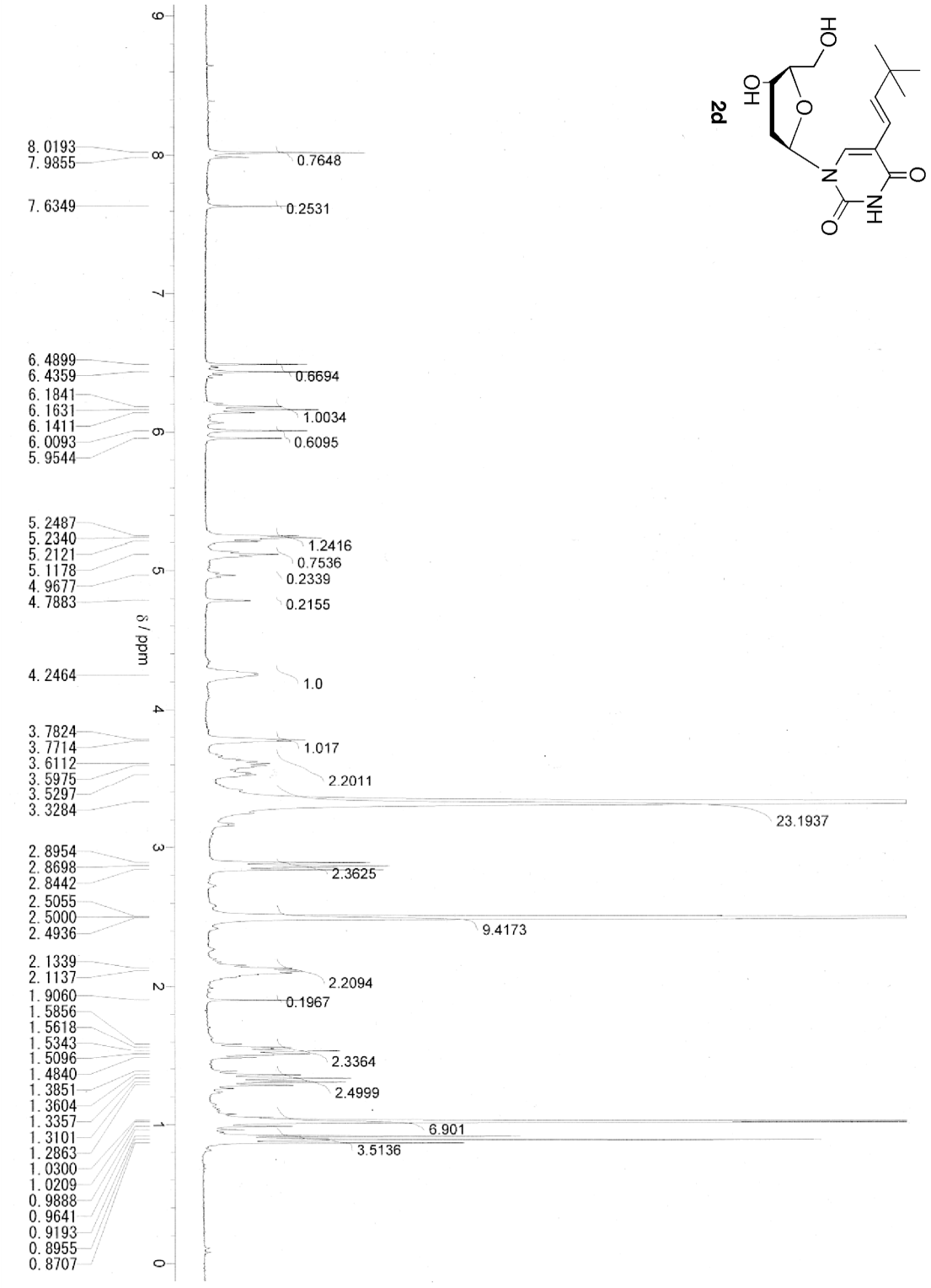
1.1881
6.3968
2.8902
3.6237
1.5007
8.6696
3.3419
δ / ppm


2d
OH
OH
O
N
NH
O
O
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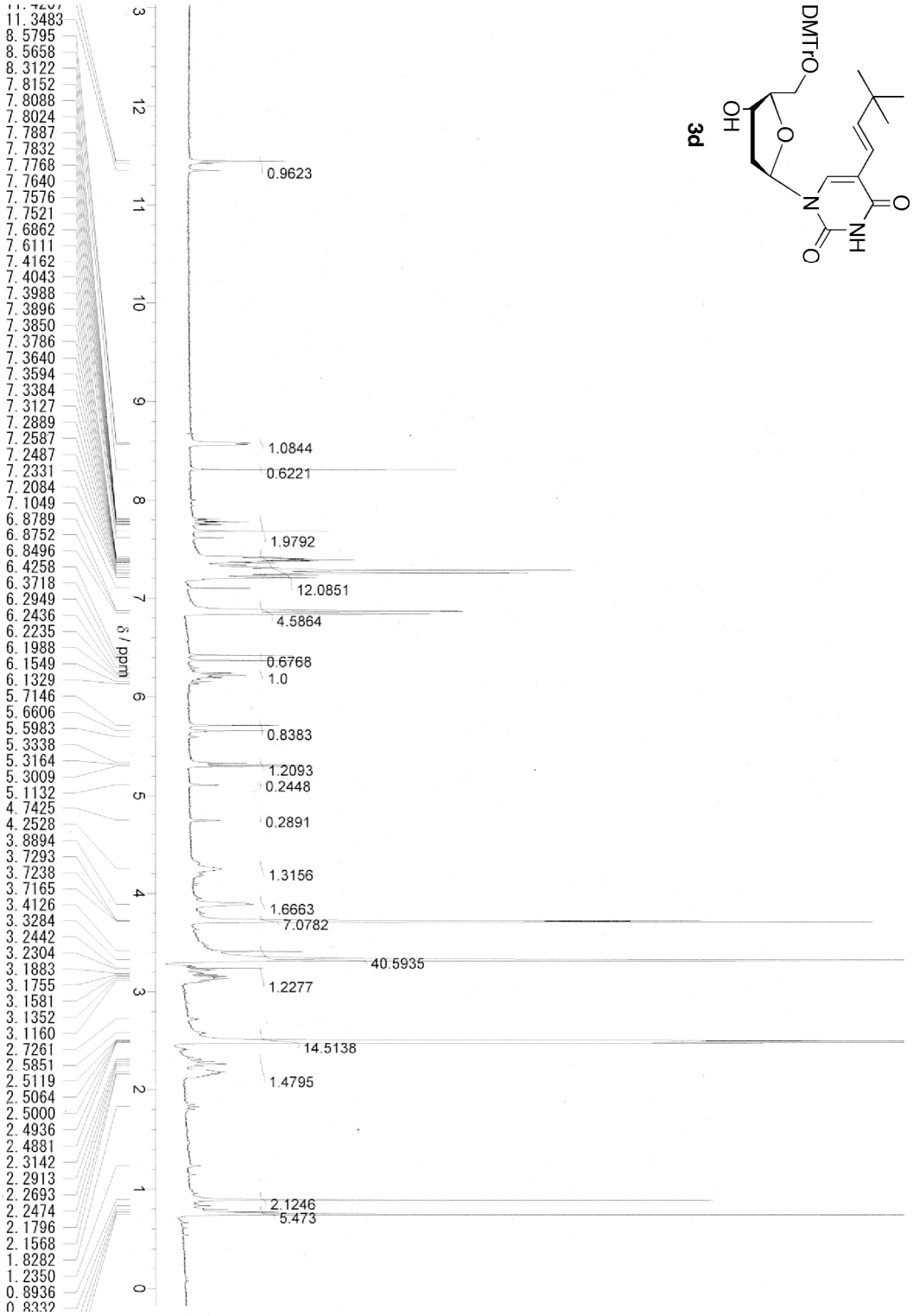
6.4359
6.1841
6.1631
6.1411
6.0093
5.9544
5.2487
5.2340
5.2121
5.1178
4.9677
4.7883
4.2464
3.7824
3.7714
3.6112
3.5975
3.5297
3.3284
2.8954
2.8698
2.8442
2.5055
2.5000
2.4936
2.1339
2.1137
1.9060
1.5856
1.5618
1.5343
1.5096
1.4840
1.3851
1.3604
1.3357
1.3101
1.2863
1.0300
1.0209
0.9888
0.9641
0.9193
0.8955
0.8707
0.7648
0.2531
0.6694
1.0034
0.6095
1.2416
0.7536
0.2339
0.2155
1.0
1.017
2.2011
23.1937
2.3625
9.4173
2.2094
0.1967
2.3364
2.4999
6.901
3.5136
δ / ppm
0
1
2
3
4
5
6
7
8
9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8.5658
8.3122
7.8152
7.8088
7.8024
7.7887
7.7832
7.7768
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7.7576
7.7521
7.6862
7.6111
7.4162
7.4043
7.3988
7.3896
7.3850
7.3786
7.3640
7.3594
7.3384
7.3127
7.2889
7.2587
7.2487
7.2331
7.2084
7.1049
6.8789
6.8752
6.8496
6.4258
6.3718
6.2949
6.2436
6.2235
6.1988
6.1549
6.1329
5.7146
5.6606
5.5983
5.3338
5.3164
5.3009
5.1132
4.7425
4.2528
3.8894
3.7293
3.7238
3.7165
3.4126
3.3284
3.2442
3.2304
3.1883
3.1755
3.1581
3.1352
3.1160
2.7261
2.5851
2.5119
2.5064
2.5000
2.4936
2.4881
2.3142
2.2913
2.2693
2.2474
2.1796
2.1568
1.8282
1.2350
0.8936
0.8332
δ / ppm
0.9623
1.0844
0.6221
1.9792
12.0851
4.5864
0.6768
1.0
0.8383
1.2093
0.2448
0.2891
1.3156
1.6663
7.0782
40.5935
1.2277
14.5138
1.4795
2.1246
5.473
DMTrO
OH
O
N
O
NH
O
3d