Supporting Information To:

Dual emissive analogue of deoxyuridine as a sensitive hydration-reporting probe for discriminating mismatched from matched DNAs and DNA/DNA and DNA/RNA duplexes

Nicolas P. F. Barthes,[a‡] Krishna Gavvala,[b‡] Dmytro Dziuba,[a] Dominique Bonhomme,[a] Julie Karpenko,[a] Anne Sophie Dabert-Gay,[c] Delphine Debayle,[c] Alexander P. Demchenko,[d] Rachid Benhida,[a] Benoît Y. Michel,[a] Yves Mély,[*b] Alain Burger[*a]

a) Institut de Chimie de Nice, UMR 7272, Université de Nice Sophia Antipolis, CNRS, Parc Valrose, 06108 Nice Cedex 2, France.
b) Laboratoire de Biophotonique et Pharmacologie, UMR 7213, Faculté de Pharmacie, Université de Strasbourg, CNRS, 74 Route du Rhin, 67401 Illkirch, France
c) Institut de Pharmacologie Moléculaire et Cellulaire, UMR 6097, Université de Nice Sophia Antipolis, 660 Route des Lu-cioles, 06560 Valbonne, France.
d) A.V. Palladin Institute of Biochemistry, 9 Leontovicha Street, Kiev 01030, Ukraine.

Corresponding authors: burger@unice.fr; yves.mely@unistras.fr

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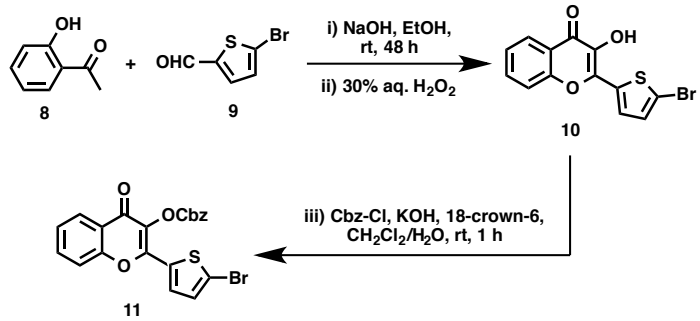
Experimental section

1. Synthetic Procedures

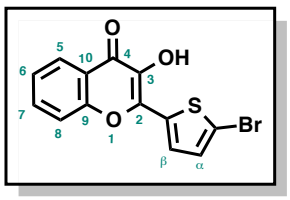
General methods:

All reactions involving water-sensitive reagents were performed in oven-dried glassware under argon using dry solvents. The synthetic intermediates were co-evaporated twice with toluene beforehand and dried in *vacuo* before use. All chemical reagents were obtained from commercial sources and were used as supplied. Anhydrous solvents were obtained according to standard procedures.[1] The reactions were monitored by thin-layer chromatography (TLC, Merck silica gel 60 F254 plates) and visualized both by UV radiation (254 & 360 nm) and by spraying with phosphomolybdic acid in ethanol followed by a subsequent warming with a heat gun. Column chromatography[2] was performed with flash silica gel (40–63 mm). All NMR spectra (^{1}H, ^{13}C, ^{2}D) were recorded on 200 or 500 Bruker Advance Spectrometers (200 or 500 MHz). ^{1}H NMR (200 and 500 MHz), ^{13}C NMR (50 and 125 MHz, recorded with complete proton decoupling) spectra were obtained with samples dissolved in $CDCl_3$, CD_2Cl_2, CD_3OD, DMSO-d^6, acetone-d^6, CD_3CN with the solvent signals used as internal references: 7.26 ppm for C*H*Cl_3, 5.32 ppm for $CDHCl_2$, 3.31 ppm for CD_2HOD, 2.50 ppm for $(CD_3)(CD_2H)S(O)$, 2.05 ppm for $(CD_3)(CD_2H)C(O)$, 1.94 ppm for CD_2HCN concerning ^{1}H NMR experiments, and 77.0 ppm for $CDCl_3$, 53.8 ppm for CD_2Cl_2, 49.0 ppm for CD_3OD, 39.4 ppm for $(CD_3)_2S(O)$, 30.8 ppm for $(CD_3)_2C(O)$ concerning ^{13}C NMR experiments.[3] Chemical shifts (δ) are given in ppm to the nearest 0.01 (1H) or 0.1 ppm (^{13}C). The coupling constants (J) are given in Hertz (Hz). The signals are reported as follows: (s=singlet, d=doublet, t=triplet, m=multiplet, br=broad). Assignments of ^{1}H and ^{13}C NMR signals were achieved with the help of D/H exchange, COSY, DEPT, APT, HMQC, HSQC, HMBC experiments. Regular mass spectra (MS) were recorded on an Esquire 3000 Plus apparatus with ESI in both positive and negative mode. High-resolution mass spectrometry was conducted with a FINIGAN MAT 95 spectrometer with EI or ESI ionization techniques. Supplementary data associated with this article: the experimental protocols for the synthesis of intermediates **3-7** and **10-11**, the ^{1}H NMR, ^{13}C NMR and (in part) ^{1}H–^{13}C COSY, ^{1}H–^{13}C HMQC and ^{1}H–^{13}C HMBC spectra of all compounds are described herein. Systematic nomenclatures are used below for the assignments of the flavone and nucleoside.

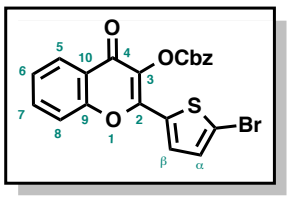
1.1 Preparation of the 3HC fluorophores



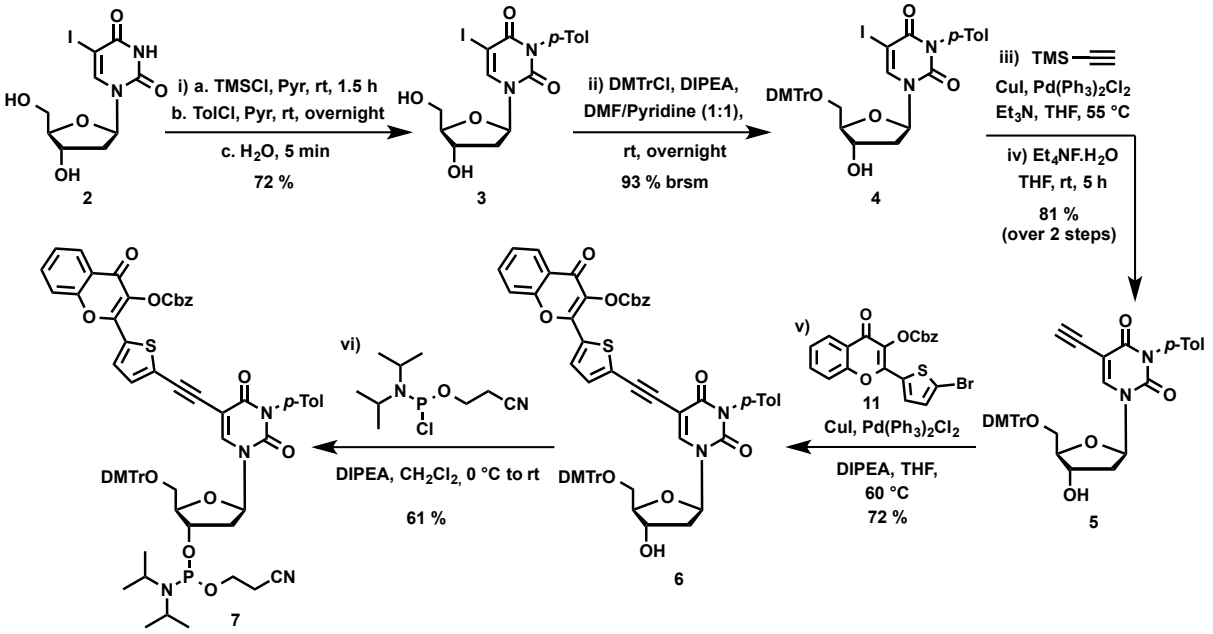
2-(5-Bromothien-2-yl)-3-hydroxy-chromen-4-one (10): To a stirred solution of *o*-hydroxyacetophenone **8** (1.16 mL, 9.65 mmol) and 5-bromo-2-thiophenecarbaldehyde **9** (1.15 mL, 9.65 mmol) in ethanol (50 mL) was dropwise added a 5 N NaOH solution (6 mL). The reaction mixture was stirred 48 h at rt before a dropwise addition of 30 % aq. hydrogen peroxide solution (2 mL). The resulting mixture was stirred 5 h at rt, and then poured into cold water (300 mL) and acidified with acetic acid to pH 4. The resulting precipitate was filtered and thoroughly washed with water and cyclohexane to provide as a yellow solid the chromone **10** (1.79 g, 57 %). $C_{13}H_7BrO_3S$ (323.16). R_f=0.39 (cyclohexane/EtOAc=7:3); ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 8.22 (1H, dd, ^{3}J=8.0 Hz, ^{4}J=1.4 Hz, H5), 7.73 (1H, d, ^{3}J=4.0 Hz, Hβ), 7.71 (1H, ddd, ^{3}J=8.4 Hz, ^{3}J=8.0 Hz, ^{4}J=1.4 Hz, H7), 7.54 (1H, d, ^{3}J=8.4 Hz, H8), 7.41 (1H, td, ^{3}J=8.0 Hz, ^{4}J=0.8 Hz, H6), 7.19 (1H, d, ^{3}J=4.0 Hz, Hα); ^{13}C-NMR (DMSO-d^6, 50 MHz): δ = 176.0 (C4), 153.3 (C9), 146.5 (C2), 141.6 (C3), 136.1 (C2-*Th*), 131.6 (C7), 129.3 (Cα), 124.7 (C5), 122.9 (C6), 122.0 (C10), 121.6 (Cβ), 117.8 (C8), 112.2 (*Th*-Br); MS (ESI$^+$, MeOH): *m/z*: 345.0, 347.0 $[M+Na]^+$.

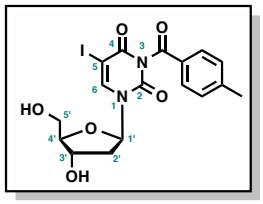


3-Benzyloxycarbonyloxy-2-(5-bromothien-2-yl)-chromen-4-one (11): To a stirred suspension of **10** (1.05 g, 3.25 mmol) in CH_2Cl_2 (13 mL) were added a 4.5 M aq. KOH solution (9 mL), 18-crown-6 (5 mol %, 43 mg) and benzyl chloroformate (700 µL, 4.88 mmol). The reaction mixture became homogenous in 5 min and was stirred at rt for 1 h. After quenching by addition of H_2O (10 mL), the organic layer was extracted with CH_2Cl_2 (3x), dried over $MgSO_4$, filtered and the volatiles were removed *in vacuo*. The residue was purified by flash chromatography on silica gel eluted with cyclohexane/EtOAc mixture (9:1 → 7:3, v/v) to provide the desired compound **11** as white crystals (1.20 g, 81 %). $C_{21}H_{13}BrO_5S$ (457.29). R_f=0.53 (cyclohexane/EtOAc=7:3); ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 8.25 (1H, dd, ^{3}J=8.0 Hz, ^{4}J=1.6 Hz, H5), 7.72 (1H, td, ^{3}J=8.4 Hz, ^{4}J=1.6 Hz, H7), 7.65 (1H, d, ^{3}J=4.0 Hz, Hβ), 7.52 (1H, dd, ^{3}J=8.4 Hz, ^{4}J=0.6 Hz, H8), 7.34–7.50 (6H, m, H6 & Cbz), 7.16 (1H, d, ^{3}J=4.0 Hz, Hα), 5.37 (2H, s, CH_2); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ = 171.3 (C4), 155.0 (C9), 151.8 (O*C*(O)CH_2Ph), 150.3 (C2), 134.5 (*i*-C-Ph), 134.1 (C7), 132.0 (C3), 131.5 (C2-*Th*), 131.0 (Cβ), 131.0 (Cα), 128.8 (*p*-C-Ph), 128.7 (*m*-C-Ph), 128.4 (*o*-C-Ph), 126.1 (C5), 125.4 (C6), 123.6 (C10), 120.4 (*Th*-Br), 117.8 (C8), 71.3 (OC(O)*C*H_2Ph); MS (ESI$^+$, MeOH/CH_2Cl_2): *m/z*: 479.5, 481.5 $[M+Na]^+$, 495.4, 497.4 $[M+K]^+$. HRMS (ESI$^+$): *m/z* calcd for $C_{21}H_{14}BrO_5S$: 456.9740 $[M+H]^+$; found 456.9737.

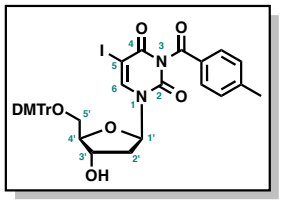


1.2 Preparation of the amidite 7

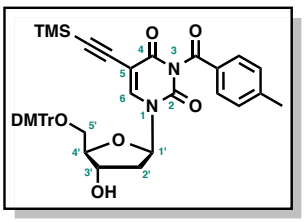




5-Iodo-3-*N*-(4-methylbenzoyl)-2′-deoxyuridine (3): To a stirred solution of 5-iodo-2'-deoxyuridine **2** (4.00 g, 11.3 mmol), in dry pyridine (14 mL), previously cooled down to 0 °C, was dropwise added Me_3SiCl (5.9 mL, 45.2 mmol, 4 eq). The reaction mixture was stirred at rt for 1.5 h. After completion by monitoring with TLC, the reaction mixture was cooled down to 0 °C and Et_3N (7.6 mL, 54.2 mmol, 4.8 eq) and *p*-toluoyl chloride (3.6 mL, 27.1 mmol, 2.4 eq) were sequentially added. The reaction mixture was stirred at rt overnight. H_2O was added and the mixture was stirred for 15 min. The organic phase was extracted with CH_2Cl_2 (2x) and EA (2x), dried over $MgSO_4$, filtered and reduced *in vacuo*. The residue was purified by flash chromatography on silica gel eluted with toluene/acetone (9:1 → 3:2, v/v) to provide the desired compound **3** as a white foam (3.85 g, 72 %). $C_{17}H_{17}IN_2O_6$ (472.2). R_f=0.55 (toluene/acetone=1:1). ^{1}H-NMR (acetone-d^6, 200 MHz): δ = 8.80 (1H, s, H6), 7.94 (2H, d, ^3J=8.2 Hz, *o*-*H*-Tol), 7.39 (2H, d, ^3J=8.2 Hz, *m*-*H*-Tol), 6.24 (1H, t, ^3J=6.3 Hz, H1′), 4.55 (1H, m, H3′), 4.01 (1H, m, H4′), 3.87 (2H, m, H5′), 2.44 (3H, s, *p*-*Me*-Tol), 2.41–2.34 (2H, m, H2′); ^{13}C-NMR (acetone-d^6, 50 MHz): δ = 168.9 (*C*(O)-Tol), 159.8 (C4), 149.9 (C2), 147.4 (*p*-*C*-Tol), 146.7 (C6), 131.4 (*o*-*C*-Tol), 130.7 (*m*-*C*-Tol), 129.7 (*i*-*C*-Tol), 88.9 (C4′), 86.8 (C1′), 71.4 (C3′), 67.3 (C5), 62.0 (C5′), 41.8 (C2′), 21.7 (*p*-*Me*-Tol); HRMS (ESI$^+$): *m/z* calcd for $C_{17}H_{17}IN_2NaO_6$: 495.0029 $[M+Na]^+$; found 495.0028.

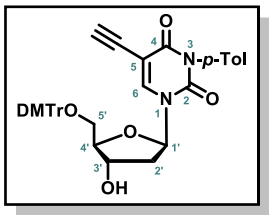


5′-*O*-(4,4′-Dimethoxytrityl)-5-iodo-3-*N*-(4-methylbenzoyl)-2′-deoxyuridine (4): To a stirred solution of **3** (3.85 g, 8.2 mmol), in a mixture of dry DMF (15 mL) and pyridine (15 mL) previously cooled down to 0 °C were added sequentially DIPEA (3.7 mL, 40.8 mmol, 5 eq) and DMTrCl (8.29 g, 24.5 mmol, 3 eq). The reaction mixture was stirred at rt overnight. The volatiles were evaporated. The residue was purified by flash chromatography on silica gel eluted with toluene/acetone (93:7 → 82:18, v/v) to provide the recovery of starting material **3** as a white foam (2.01 g, 52 %) as well as the desired derivative **4** as a beige foam (3.02 g, 93 % brsm). $C_{38}H_{35}IN_2O_8$ (774.6). R_f=0.66 (toluene/acetone=7:3). ^{1}H-NMR (CD_2Cl_2, 200 MHz): δ = 8.22 (1H, s, H6), 7.79 (2H, d, ^3J=7.3 Hz, *o*-*H*-Tol), 7.45 (2H, d, ^3J=7.3 Hz, *m*-*H*-Tol), 7.36 (4H, d, ^3J=8.6 Hz, *o*-*H*-PhOMe), 7.33–7.23 (5H, m, *H*-Ph), 6.87 (4H, d, ^3J=8.6 Hz, *m*-*H*-PhOMe), 6.25 (1H, d, ^3J=7.6, 5.9 Hz, H1′), 4.57 (1H, m, H3'), 4.07 (1H, dd, ^3J=5.9, 3.1 Hz, H4′), 3.78 (6H, s, *Me*O), 3.37 (2H, d, ^3J=3.2 Hz, H5′), 2.55–2.35 (5H, m, *p*-*Me*-Tol, H2′); HRMS (ESI$^+$): *m/z* calcd for $C_{38}H_{35}IN_2NaO_8$: 797.1336 $[M+Na]^+$; found 797.1311.

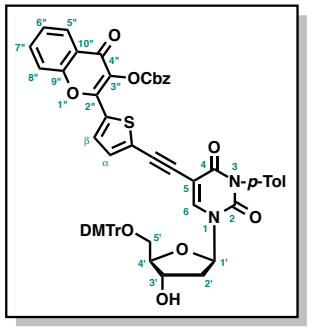


5′-*O*-(4,4′-Dimethoxytrityl)-5-trimethylsilylethynyl-3-*N*-(4-methylbenzoyl)-2′-deoxyuridine (4′): To a stirred solution of **4** (870 mg, 1 mmol) in dry THF (11 mL) under argon, were sequentially added TMS-acetylene (216 µL, 1.5 mmol, 1.5 eq), triethylamine (710 µL, 5 mmol, 5 eq) and CuI (8 mol %, 16 mg)/$PdCl_2(PPh_3)_2$ (8 mol %, 57 mg). The reaction mixture was warmed to 50 °C and stirred for 2 h. The mixture was filtered over a 545 Celite® (basic-washed) pad and the volatiles were removed *in vacuo*. The residue was purified by flash chromatography on silica gel eluted with toluene/EtOAc (93:7 → 55:45, v/v) to provide the desired compound **4′** as a beige foam (642 mg, 85 %). $C_{43}H_{44}N_2O_8Si$ (744.9). R_f=0.53 (toluene/EtOAc=7:3). ^{1}H-NMR (CD_2Cl_2, 500 MHz): δ = 8.13 (1H, s, H6), 7.85 (2H, d, ^3J=8.0 Hz, *o*-*H*-Tol), 7.52 (2H, d, ^3J=7.8 Hz, *o*-*H*-Ph), 7.42 (5H, d, ^3J=8.5 Hz, *o*-*H*-PhOMe), 7.39 (2H, t, ^3J=7.8 Hz, *m*-*H*-Ph), 7.38 (2H, d, ^3J=8.0 Hz, *m*-*H*-Tol), 7.29 (1H, t, ^3J=7.8 Hz, *p*-*H*-Ph), 6.93 (4H, d, ^3J=8.5 Hz, *m*-*H*-PhOMe), 6.27 (1H, dd, ^3J=7.6 Hz, 5.8 Hz, H1′), 4.53 (1H, dd, ^3J=6.0 Hz, 3.2 Hz, H3′), 4.12 (1H, dd, ^3J=6.0 Hz, 3.2 Hz, H4′), 3.84 (6H, s, *Me*O), 3.43 (1H, dd, ^2J=10.7 Hz, ^3J=3.2 Hz, H5′$_B$), 3.38 (1H, dd, ^2J=10.7 Hz, ^3J=3.9 Hz, H5′$_A$), 2.52–2.49 (4H, m, H2′$_A$, *p*-*Me*-Tol), 2.31 (1H, m, H2′$_B$), 0.09

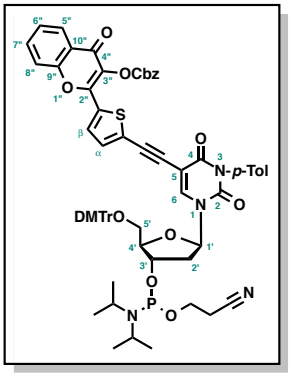
(9H, s, Si(CH_3)$_3$); ^{13}C-NMR (CD_2Cl_2, 125 MHz): δ = 168.2 (*C*(O)-Tol), 160.9 (C4), 159.1 (*p-C*-PhOMe), 148.6 (C2), 147.5 (*p-C*-Tol), 145.0 (*i-C*-Ph), 142.8 (C6), 135.9 (*i-C*-PhOMe), 130.9 (*o-C*-Tol), 130.4 (*o-C*-PhOMe), 130.4 (*m-C*-Tol), 129.3 (*i-C*-Tol), 128.5 (*m-C*-Ph), 128.3 (*o-C*-Ph), 127.3 (*p-C*-Ph), 113.7 (*m-C*-PhOMe), 100.6 (C5), 100.2 (C≡CSi), 95.1 (C≡CSi), 87.3 (C_{IV}-O-5′), 87.0 (C4′), 86.5 (C1′), 72.6 (C3′), 63.8 (C5′), 55.6 (O*Me*), 41.8 (C2′), 22.0 (*p-Me*-Tol), 0.3 (Si(CH_3)$_3$); HRMS (ESI$^+$): *m/z* calcd for $C_{43}H_{44}N_2NaO_8Si$: 767.2765 [M+Na]$^+$; found 767.2742.



5′-*O*-(4,4′-Dimethoxytrityl)-3-*N*-(4-methylbenzoyl)-5-ethynyl-2′-deoxyuridine (5): To a stirred solution at 0 °C of the protected ethynyl derivative **4′** (0.725 mmol, 540 mg) in THF (7.5 mL) was portionwise added $Et_4NF{\cdot}H_2O$ (4.35 mmol, 660 mg, 6 eq). The reaction mixture was stirred at rt for 1.5 h and then quenched with water. The organic layer was extracted with CH_2Cl_2 (3 x), dried over $MgSO_4$, filtered and reduced *in vacuo* to provide the desired compound **5** as a beige foam (468 mg, 96 %). The compound was sufficiently pure to be used in the next step without further purification. $C_{40}H_{36}N_2O_8$ (672.72). R_f=0.22 (toluene/Et_2O=7:2, v/v). ^{1}H-NMR (CD_2Cl_2, 200 MHz): δ = 8.21 (1H, s, H6), 7.81 (2H, d, ^3J=8.1 Hz, *o-H*-Tol), 7.48 (2H, dd, ^3J=8.2 Hz, ^4J=1.4 Hz, *o-H*-Ph), 7.39 (4H, d, ^3J=8.6 Hz, *o-H*-PhOMe), 7.33 (2H, d, ^3J=8.1 Hz, *m-H*-Tol), 7.31–7.20 (3H, m, *m-H*-Ph, *p-H*-Ph), 6.89 (4H, d, ^3J=8.6 Hz, *m-H*-PhOMe), 6.24 (1H, dd, ^3J=7.2, 6.0 Hz, H1′), 4.62–4.52 (1H, m, H3′), 4.12–4.03 (1H, m, H4′), 3.80 (3H, s, O*Me*), 3.41 (1H, dd, ^2J=10.8 Hz, ^3J=3.4 Hz, H5′$_B$), 3.32 (1H, dd, ^2J=10.8 Hz, ^3J=3.0 Hz, H5′$_A$), 2.97 (1H, s, C≡C*H*), 2.55–2.44 (1H, m, H2′$_B$), 2.44 (3H, s, *p-Me*-Tol), 2.43–2.25 (1H, m, H2′$_A$); ^{13}C-NMR (CD_2Cl_2, 50 MHz): δ = 168.1 (*C*(O)-Tol), 161.0 (C4), 159.2 (*p-C*-PhOMe), 148.6 (C2), 147.7 (*p-C*-Tol), 145.0 (*i-C*-Ph), 144.0 (C6), 136.0 (*i-C*-PhOMe), 135.7 (*i-C*-PhOMe), 130.9 (*o-C*-Tol), 130.4 (*o-C*-PhOMe), 130.4 (*m-C*-Tol), 128.8 (*i-C*-Tol), 128.5 (*m-C*-Ph), 128.3 (*o-C*-Ph), 127.3 (*p-C*-Ph), 113.7 (*m-C*-PhOMe), 99.3 (C5), 87.5 (C_{IV}-O-5′), 87.1 (C4′), 86.6 (C1′), 82.4 (C≡CH), 74.4 (C≡CH), 72.4 (C3′), 63.8 (C5′), 55.6 (O*Me*), 41.9 (C2′), 22.0 (*p-Me*-Tol). MS (ESI$^+$, MeOH) *m/z*: 695.5 [M+Na]$^+$ 711.5 [M+K]$^+$. HRMS (ESI$^+$): *m/z* calcd for $C_{40}H_{36}N_2NaO_8$: 695.2364 [M+Na]$^+$; found 695.2357.



5′-*O*-(4,4′-Dimethoxytrityl)-3-*N*-(4-methylbenzoyl)-5-(5-(3-benzylcarbonate-4-oxo-chromen-2-yl)thien-2-yl)ethynyl-2′-deoxyuridine (6): To a stirred solution of **5** (300 mg, 0,45 mmol, previously azeotropically coevaporated with dry toluene) and **11** (279 mg, 0.58 mmol, 1.3 eq) in THF (10 mL) under argon, were sequentially added triethylamine (311 µL, 2.23 mmol, 5 eq), and CuI (7 mol %, 0.031 mmol, 6 mg,)/$PdCl_2(PPh_3)_2$ (7 mol %, 0.031 mmol, 22 mg) all together. The reaction mixture was warmed to 60 °C and stirred for 2 h. The volatiles were removed *in vacuo* and the residue was purified by flash chromatography on silica gel eluted with toluene/EtOAc (93:7 → 3:2, v/v) to provide the desired compound **6** as a yellow foam (339 mg, 72 %). $C_{61}H_{48}N_2O_{13}S$ (1049.1). R_f=0.39 (toluene/EtOAc=7:3). ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 8.41 (1H, s, H6), 8.22 (1H, dd, ^3J=8.0 Hz, ^4J=1.6 Hz, H5″), 7.85 (2H, m, ^3J=8.2 Hz, *o-H*-Tol), 7.71 (1H, ddd, ^3J=8.3, 7.0 Hz, ^4J=1.6 Hz, H7″), 7.62 (1H, d, ^3J=4.1 Hz, Hβ), 7.51 (1H, d, ^3J=8.3 Hz, H8″), 7.46–7.16 (17H, m, *H*-Ph-Cbz, *H*-Ph, *o-H*-PhOMe, H6″, *m-H*-Tol), 6.81 (4H, d, ^3J=8.8 Hz, *m-H*-PhOMe), 6.74 (1H, d, ^3J=4.1 Hz, Hα), 6.34 (1H, dd, ^3J=7.0 Hz, 6.0 Hz, H1′), 5.31 (2H, s, CH_2-Cbz), 4.67–4.58 (1H, m, H3′), 4.18–4.11 (1H, m, H4′), 3.72 (3H, s, O*Me*), 3.71 (3H, s, O*Me*), 3.49 (1H, dd, ^2J=10.6 Hz, ^3J=2.4 Hz, H5′$_B$), 3.37 (1H, dd, ^2J=10.6 Hz, ^3J=2.8 Hz, H5′$_A$), 2.64–2.39 (5H, m, H$_2$′, *p-Me*-Tol); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ = 171.5 (C4″), 167.6 (*C*(O)-Tol), 160.2 (C4), 158.8 (*p-C*-PhOMe), 155.1 (C9″), 151.9 (*C*(O)-Cbz), 150.8 (C2″), 148.3 (C2), 147.0 (*p-C*-Tol), 144.4 (*i-C*-Ph), 142.8 (C6), 135.5 (*i-C*-PhOMe), 135.4 (*i*-C-PhOMe), 134.5 (C7″), 134.3 (*i-C*-Cbz), 133.4 (Cβ), 132.1 (C2″-*Th*), 131.8, 130.9 (*o-C*-Tol), 130.6 (Cα), 130.1, 130.0, 129.2, 128.9, 128.8, 128.7, 128.4, 128.3, 128.2, 128.0, 127.3, 126.2 (C5″), 125.5 (C6″), 123.7 (C10″), 118.0 (C8″), 113.5 (*m-C*-PhOMe), 99.7 (C5), 87.6 (Th-C≡C-), 87.3 (C_{IV}-O-5′), 87.1 (C4′), 86.6 (Th-C≡C-), 86.5 (C1′), 72.4 (C3′), 71.4 (CH_2-Cbz), 63.5 (C5′), 55.3 (*Me*O), 42.1 (C2′), 22.1 (*p-Me*-Tol); MS (ESI$^+$, MeOH) *m/z*: 1070.7 [M+Na]$^+$, 1086.8 [M+K]$^+$. HRMS (ESI$^+$): *m/z* calcd for $C_{61}H_{49}N_2O_{13}S$: 1049.2955 [M+H]$^+$; found 1049.2920.



5′-*O*-(4,4′-Dimethoxytrityl)-3-*N*-(4-methylbenzoyl)-5-(5-(3-benzylcarbonate-4-oxo-chromen-2-yl)thien-2-yl)ethynyl-2′-deoxyuridine, 3′-[(2-cyanoethyl)-*N*,*N*-diisopropyl]-phosphoramidite (7): To a stirred solution of **6** (0.32 mmol, 339 mg, previously dried azeotropically by coevaporation with dry toluene) in DCM (2.3 mL) under argon and cooled down at 0 °C, were sequentially added DIPEA (1.1 mmol, 193 µL, 3.4 eq) and 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (0.614 mmol, 138 µL, 2 eq). The reaction mixture was stirred at rt for 2.5 h. The volatiles were removed *in vacuo* and the residue was purified by flash chromatography on silica gel eluted with toluene/EtOAc (95:5 → 60:40, v/v) to provide the desired compound **7** as a yellow foam (246 mg, 61 %). $C_{70}H_{65}N_4O_{14}PS$ (1249.34). R_f=0.48 (toluene/EtOAc=8:2). ^{1}H-NMR (CD_3CN, 200 MHz): δ = 8.42 (1H, s, H6), 8.12 (1H, dd, ^3J=8.0 Hz, ^4J=1.7 Hz, H5″), 7.95 (2H, d, ^3J=8.1 Hz, *o-H*-Tol), 7.82 (1H, ddd, ^3J=8.8 Hz, ^4J=1.7 Hz, H7″), 7.76 (1H, d, ^3J=4.1 Hz, Hβ), 7.66 (1H, d, ^3J=8.0 Hz, H8″), 7.53–7.32 (10H, m, H6″, *H*-Ph-Cbz, *o-H*-PhOMe), 7.29–7.14 (7H, m, *H*-Ph, *m-H*-Tol), 6.90–6.81 (5H, m, Hα, *m-H*-PhOMe), 6.14 (1H, dd, ^3J=13.0 Hz, 6.0 Hz, H1′H1′), 5.28 (2H, s, CH_2-Cbz), 4.86–4.68 (1H, m, H3′), 4.19 (1H, m, H4′), 3.82–3.73 (2H, m, CH_2-O), 3.68 (6H, s, *Me*O), 3.64–3.52 (2H, m, N-CH(CH_3)$_2$), 3.38 (2H, m, H5′), 2.69–2.52 (4H, m, H2′, CH_2-CN), 2.45 (3H, s, *p-Me*-Tol), 1.19–1.06 (11H, m, N-CH(CH_3)$_2$); ^{31}P-NMR (CD_3CN, 81 MHz): δ=148.1, 148.0; HRMS (ESI$^+$): *m/z* calcd for $C_{70}H_{66}N_4O_{14}PS$: 1249.4034 [M+H]$^+$; found 1149.4014.

2. ODNs synthesis, purification & physical characterization

2.1 ODNs synthesis and purification:

The ODN synthesis was performed on an Expedite 8900 DNA synthesizer (Applied Biosystem) using the "trityl off" mode and mild phosphoramidite chemistry on a 0.2 µmol scale. Reagents and solvents, as well as dT, Ac-dC, Pac-dA, and dmf-dG phosphoramidites were purchased from Link Technologies. The standard DNA assembly protocol "DMT-off" was used except for the following modifications: 5-Ethylthio-1H-tetrazole (ETT) was used as activating agent; Pac-anhydride was used for capping; a longer coupling time (1200 s) was applied to the 3HC phosphoramidite. Non-labelled ODNs were purchased from Microsynth AG. The ODNs were cleaved from the solid support and deprotected with concentrated aqueous ammonia at room temperature for 12 h. The ODNs were analyzed (0.5 mL/min) and purified (2.5 mL/min) by RP HPLC (HPLC apparatus: Waters™ 600 Controller with Waters™ 996 Photodiode Array Detector and Jasco LC-Net II / ADC apparatus. Columns: analytical, 300 × 4.60 mm, 5 µm particle size, Clarity® 100Å, Phenomenex®; semi-preparative, Clarity® 5u Oligo-RP column 250 x 10 mm Phenomenex®). The following gradient system was used: 100 % A –(30 min)→ 60 % A / 40 % B –(5 min)→ 100 % B –(5 min)→ 100 % A with A=Buffer pH 7.0 (1.9 L of MilliQ® water, 160 mL acetonitrile, 28 mL triethylamine, 12 mL of acetic acid) and B=0.2 CH_3CN:0.8 Buffer.

Fig S1. HPLC profile of TMT single strand ODN (254 nm left – 390 nm right):

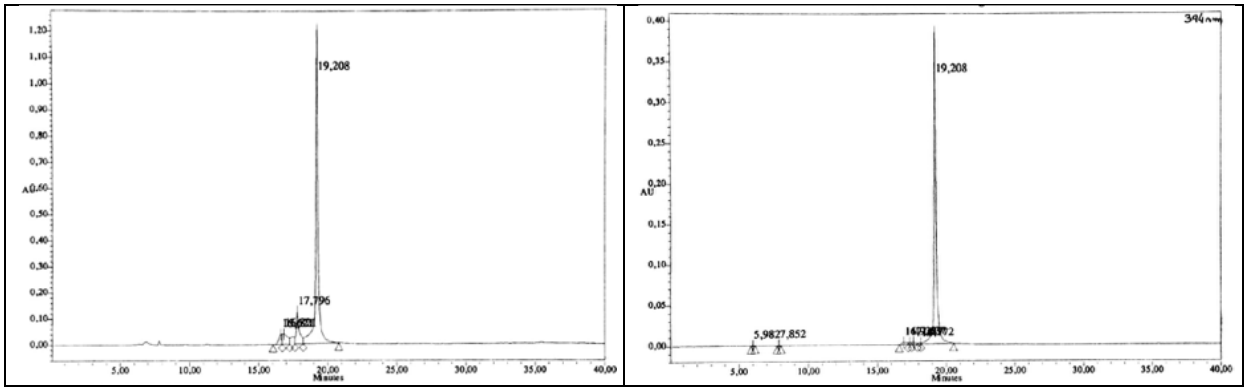


Fig S2. HPLC profile of AMA single strand ODN (254 nm left – 390 nm right):

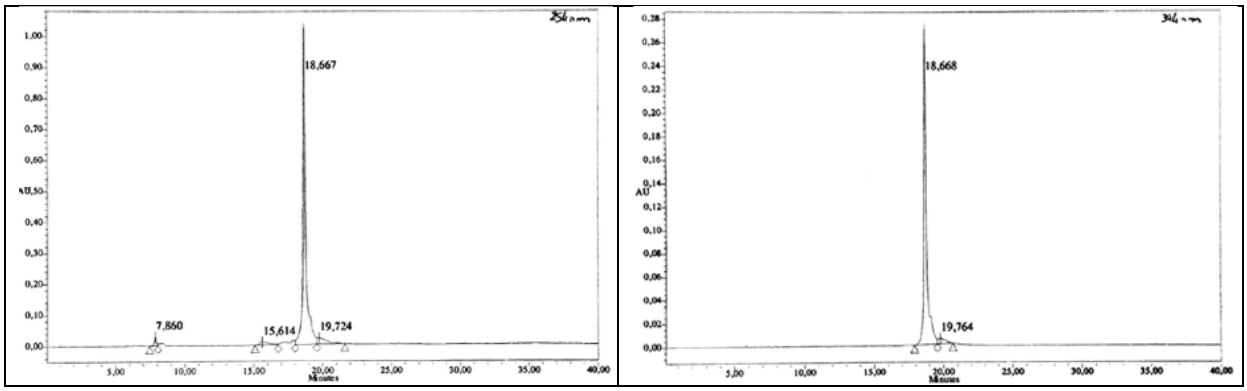


Fig S3. HPLC profile of CMC single strand ODN (254 nm left – 390 nm right):

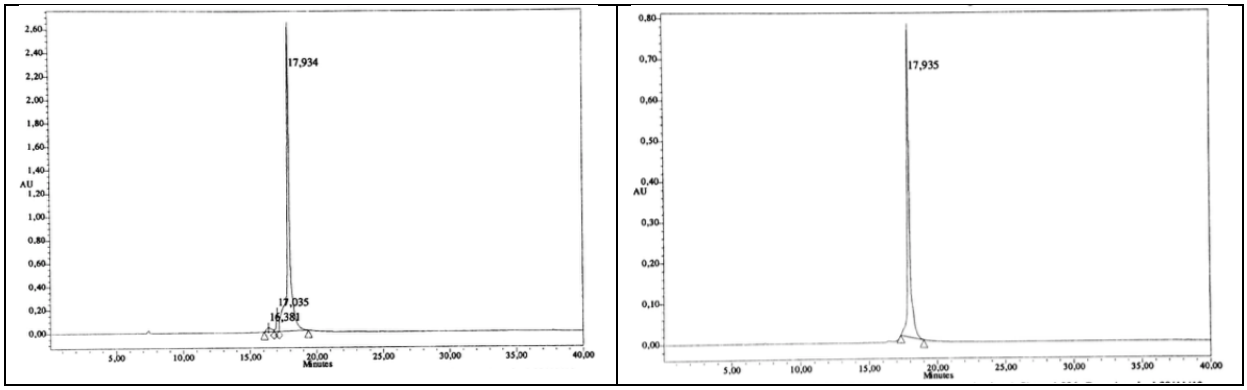
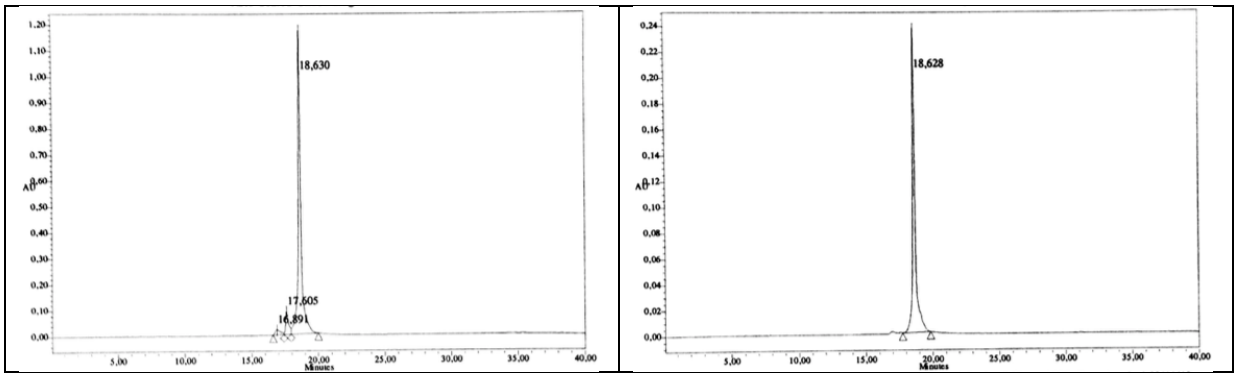


Fig S4. HPLC profile of GMG single strand ODN (254 nm left – 390 nm right):

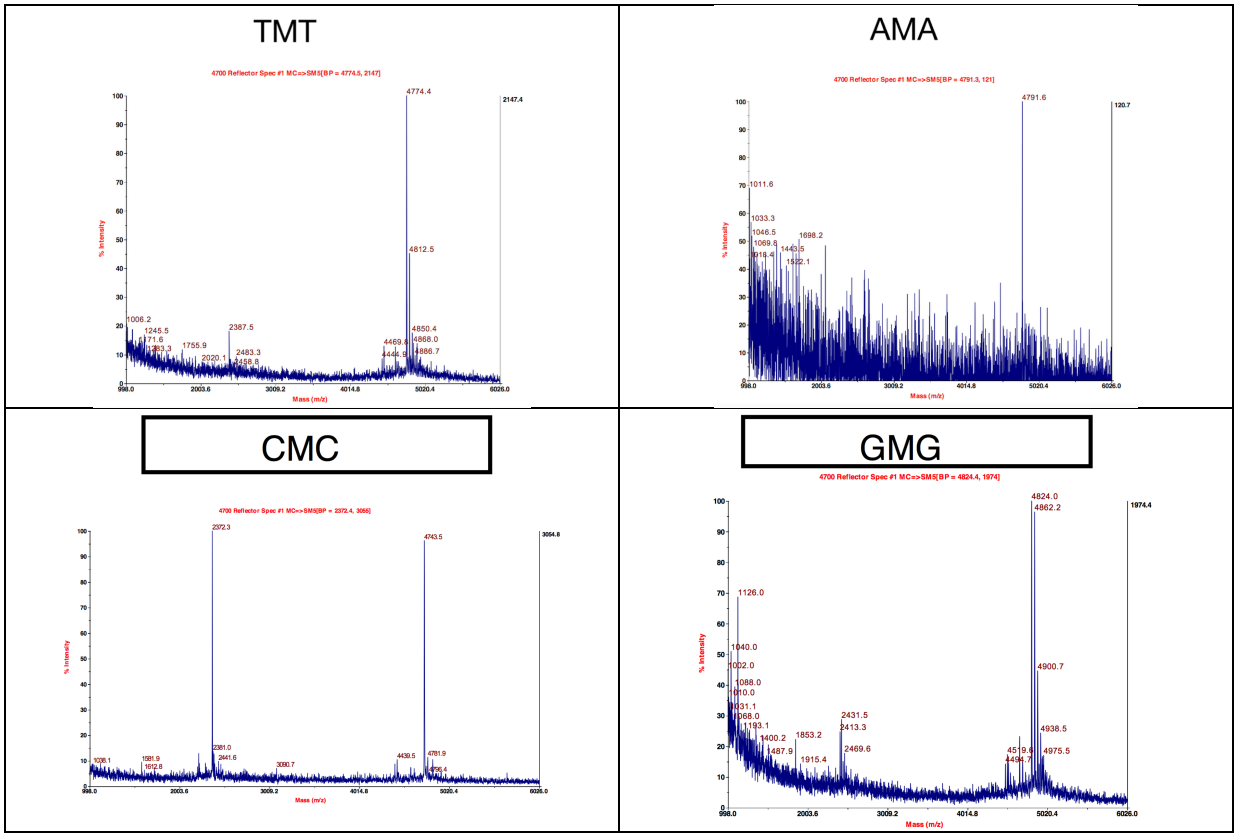


2.2 MALDI TOF/TOF analysis of ODNs:

Dibasic Ammonium Citrate (DAC) (98% capillary GC) was obtained from Sigma. Acetonitrile of HPLC grade was purchased from VWR chemical. Ultrapure 3-Hydroxypicolinic Acid (3-HPA) MALDI matrix was purchase to Protea Biosciences. C4 pipette tips (Zip-Tip) were from Millipore.
The samples (500 pmol) were diluted to 10 µL of water and were desalted with a C4 pipette Tips (Zip-tip). The Zip-tip was activated before use with 2 x 5 µL of water: CH_3CN (50:50) and 2 x 5µL of DAC (50 mg/ml diluted in water). The 10 µL of the ODN solution was loaded on Zip-tip by drawing and expelling ten times. Next the zip-tip was washed with 3 x 5 µL of DAC (50 mg/mL) and 3 x 5 µL of water. Elution was performed with 1.5 µL of 3-HPA matrix (80 mg/mL, 50:50 CH_3CN:DAC) directly on MALDI plate. The ODN profile obtained in a ABSciex MALDI-TOF/TOF mass spectrometer in reflector mode with external calibration mixture (cal Mix 1+2 distributed by ABSciex). MALDI-TOF/TOF-MS analysis: MS spectra were recorded manually in a mass range of 500-6000 Da resulting from 400 laser shots of constant intensity fixed at 6200. Data were collected using 4000 series Explorer (AB SCIEX) experiments.

Table S1. Mass of the synthesized oligonucleotides.

ODN	Sequence	MALDI-TOF found (calcd) [M^+]
1	5′d-CGTTTTT**M**TTTTTGC-3′	4774.1 (4773.2)
2	5′d-CGTTTTA**M**ATTTTGC-3′	4791.6 (4791.3)
3	5′d-CGTTTTC**M**CTTTTGC-3′	4743.5 (4743.2)
4	5′d-CGTTTTG**M**GTTTTGC-3′	4824.0 (4823.3)



3. Spectroscopic characterizations

3.1 Denaturation studies and melting temperatures

- ***Preparation of the samples:***

The ODNs were analyzed in triplicate cacodylate buffer pH 7.0 (10 mM cacodylate, 150 mM NaCl, 1 mM EDTA).

Preparation of the single strand solution: the solution of the sample was prepared by mixing 400 µL of a stock solution of 20 mM Cacodylate buffer solution pH 7.0, 80 µL of 1.5 M NaCl solution, 10 µL of 80 mM EDTA solution, 25 µL of 64 µM ssODN and 285 µL of MilliQ® water.

Preparation of the double strand solution: 400 µL of a stock solution of 20 mM Cacodylate buffer solution pH 7.0, 80 µL of 1.5 M NaCl solution, 10 µL of 80 mM EDTA solution, 25 µL of 64 µM ODN 1, 25 µL of 64 µM ODN 2 and 260 µL of MilliQ® water.

Melting curves were recorded by following the temperature-dependence of the absorbance changes at 260 nm of the sample (2 µM concentration of each strand). Absorption spectra were recorded in a Peltier thermostated cell holder on a Cary 4 spectrophotometer (Varian). The pathlength of cell was 1 cm. The temperature range for denaturation measurement was 5 – 80 °C. Speed of heating was 0.3 °C/min. The melting curves were converted into a plot of α versus temperature, where α represents the fraction of single-strands in the duplex state. The melting temperatures were extracted from these curves after differentiation as described elsewhere.[5]

Table S2. Melting temperatures of the duplexes.

Duplexes	T_m (°C)		
	M	*Wild Type* [b]	ΔT_m (°C) [c]
TMT·***AAA***	45.8	49.5 [50.2]	-3.7
TMT·***ATA***	37.9	40.5 [42.2]	-2.6
TMT·***ACA***	39.1	39.0 [42]	0.1
TMT·***AGA***	38.1	40.7 [42.9]	-2.6
TMT·***AAbA***	39.2	34.5	4.7
AMA·***TAT***	42.4	47.3 [47.4]	-4.9
AMA·***TTT***	37.6	39.8 [40.6]	-2.2
AMA·***TCT***	39.2	38.5 [39.3]	0.7
AMA·***TGT***	38.1	42.7 [42.8]	-4.6
AMA·***TAbT***	36.3	30.5	5.8
CMC·***GAG***	48.1	51.3 [53.7]	-3.2
CMC·***GTG***	37.9	41.1 [46.7]	-3.2
CMC·***GCG***	42.6	42.1 [45.3]	0.5
CMC·***GGG***	42.5	46.5 [49.5]	-4.0
CMC·***GAbG***	43.2	37.2	6.0
GMG·***CAC***	52.4	54.8 [55.1]	-2.4
GMG·***CTC***	45.6	46.6 [47.5]	-1.0
GMG·***CCC***	47.1	43.5 [44.8]	3.6
GMG·***CGC***	47.3	49.7 [51.0]	-2.4
GMG·***CAbC***	44.5	37.5	7.0

Duplexes	T_m (°C)		
	M	*Wild Type* [b]	ΔT_m (°C) [c]
TMT·***rAAA***	40.6	43.7	-3.1
TMT·***r_MAAA***	38.8	41.4	-2.6
TMT·***r_MAUA***	29.2	32.0	-2.8
AMA·***rUAU***	37.1	40.9	-3.8

[a] T_m of the corresponding duplexe formed from unmodified ODNs and its theorical values given in square brackets. [b] ΔT_m refers to the difference of T_m between the labelled and wild type ODNs.

Fig S5. Melting temperature curves of the wild type double strands:

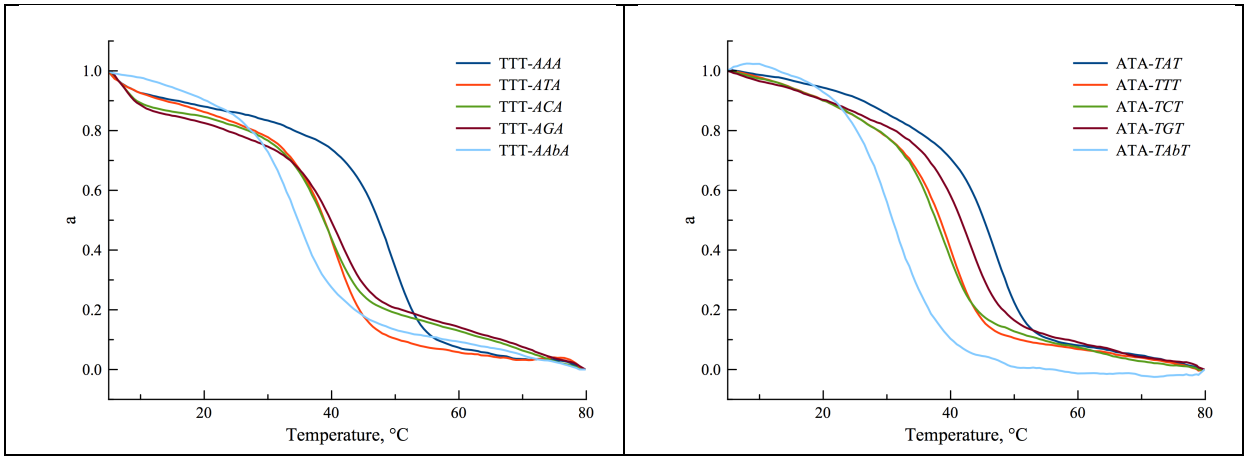
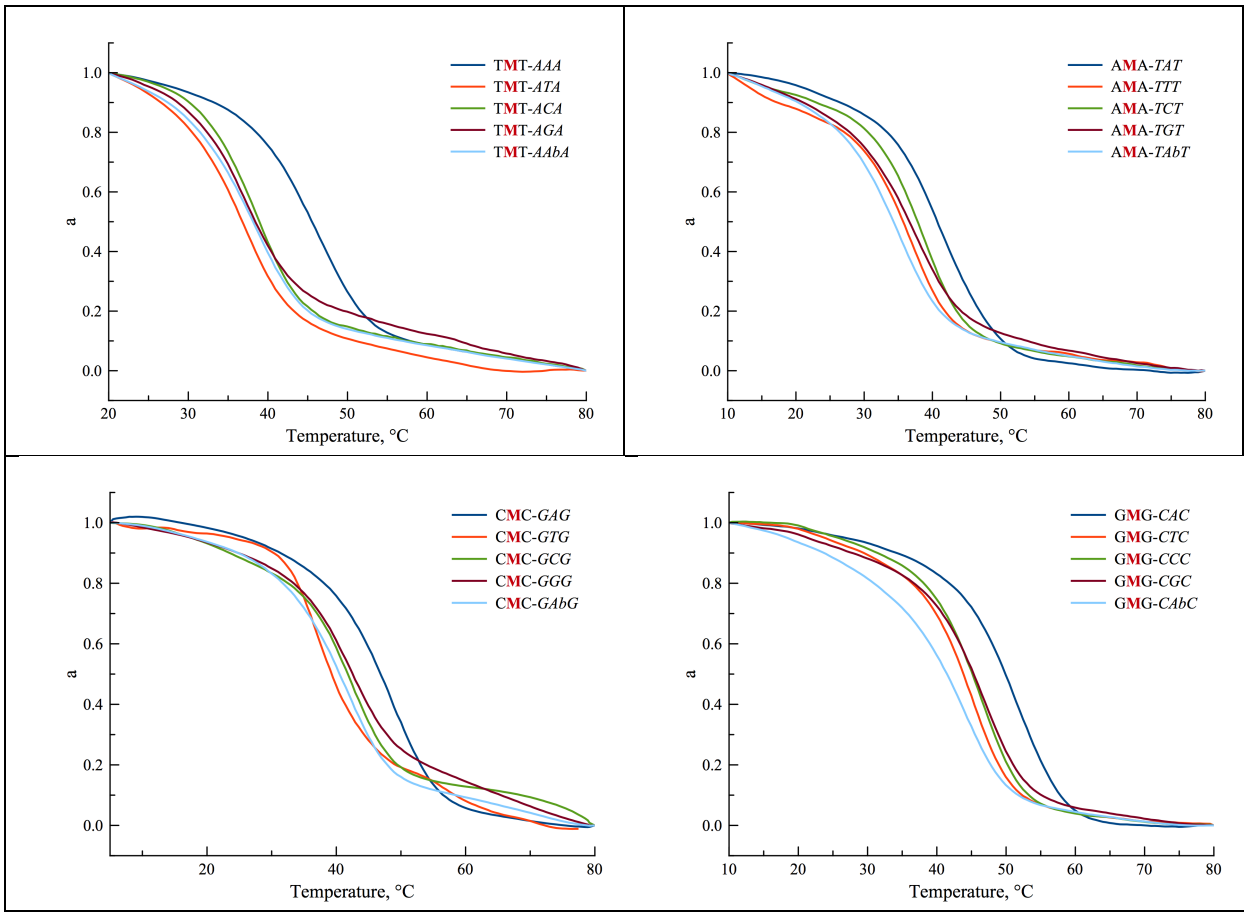


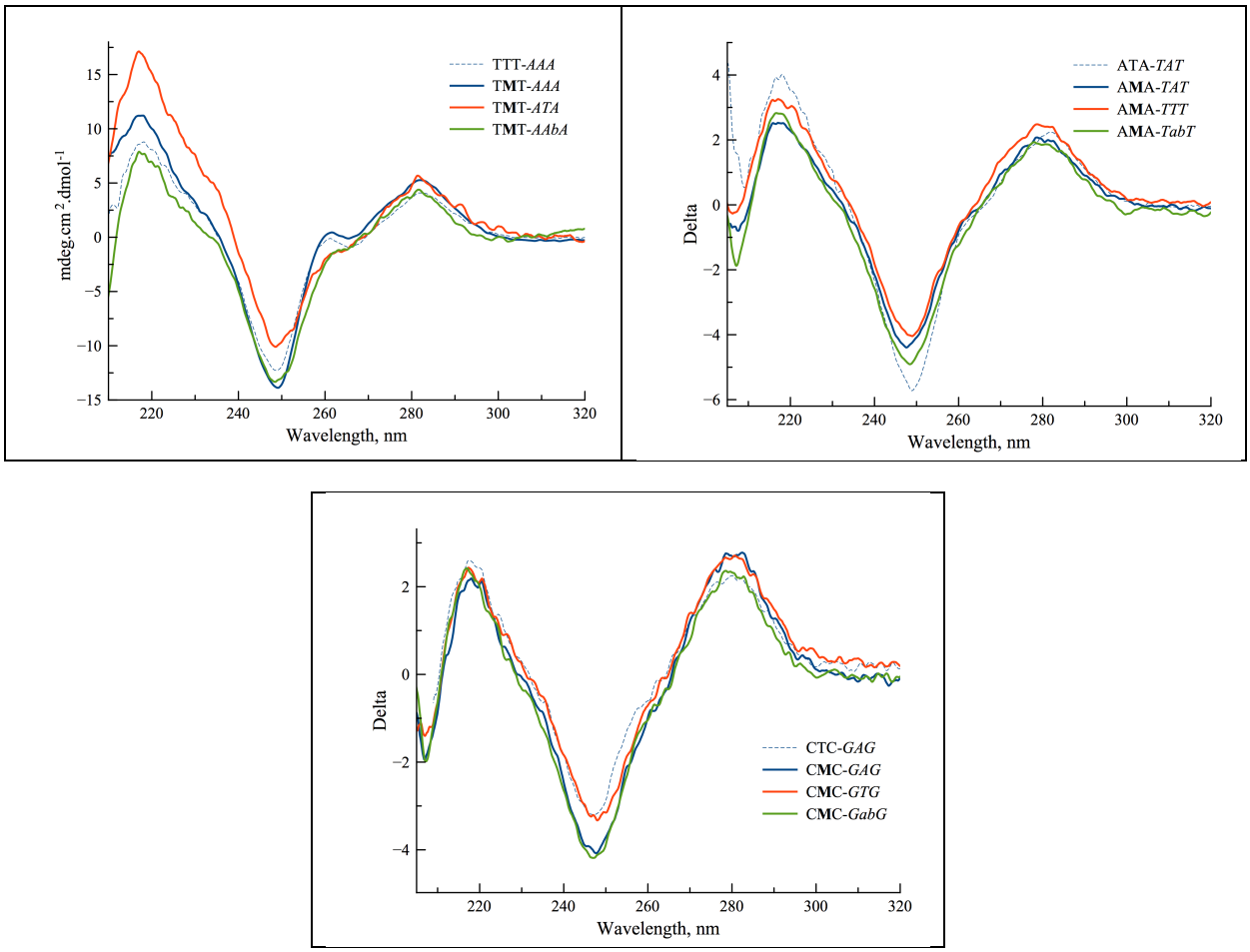
Fig S6. Melting temperature curves of labelled double strands:



3.3. Circular dichroism

Circular dichroism spectra were recorded in duplicate with 2 µM solution of the canonical dsDNA and labelled dsDNA (3HC (**M**) opposite **A**, **T** or **Ab**) in buffer pH 7.0 (20 mM Sodium phosphate, 150 mM NaCl, 1 mM EDTA) at 20 °C on a Jasco J-810 spectropolarimeter. Two maxima were observed in CD spectra: one negative at 249 nm and the other positive at 282 nm. Similar results were obtained by using an alternative buffer: 10 mM cacodylate buffer, 150 mM NaCl, 1 mM EDTA.

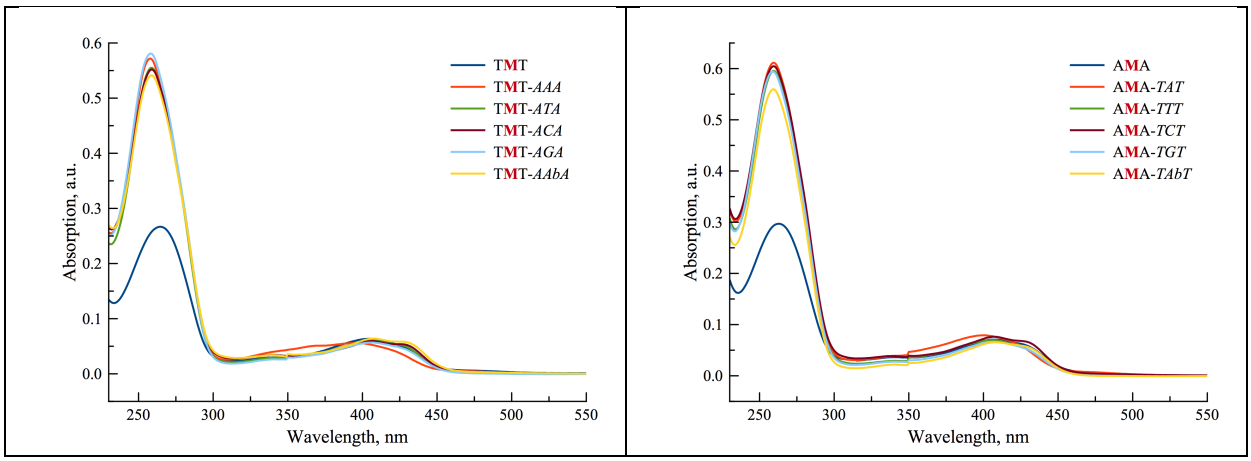
Fig S7. Representative CD spectra of wild type and labelled duplexes:



3.4. Absorption and Fluorescence spectra

The absorption and fluorescence experiments were realized in triplicate in pH 7.0 phosphate buffer (20 mM sodium phosphate, 150 mM NaCl, 1 mM EDTA). The absorption spectra were recorded on a Cary 300 Scan spectrophotometer (Varian) using 1cm quartz cells at 20 °C. The fluorescence spectra were recorded on a FluoroMax 4.0 spectrofluorometer (Jobin Yvon, Horiba) by using excitation and emission slits of 2 nm and were corrected at excitation and emission. They were taken with absorbance of about 0.05 at 20 °C at the excitation wavelength mentioned in the corresponding experiments. The quantum yields were corrected according to the variation of the refractive index of the different solvents. Quantum yields were determined by using quinine sulfate (QS) in 0.1 M HCl solution (λ_{ex} = 350 nm, Φ = 0.54) and *p*-dimethylaminoflavone (dMAF) in EtOH (λ_{ex} = 404 nm, Φ = 0.27) as standard references.[6,7] Similar results were obtained by using an alternative buffer: 10 mM cacodylate buffer, 150 mM NaCl, 1 mM EDTA.

Fig S8. UV spectra of labelled single and double strands:



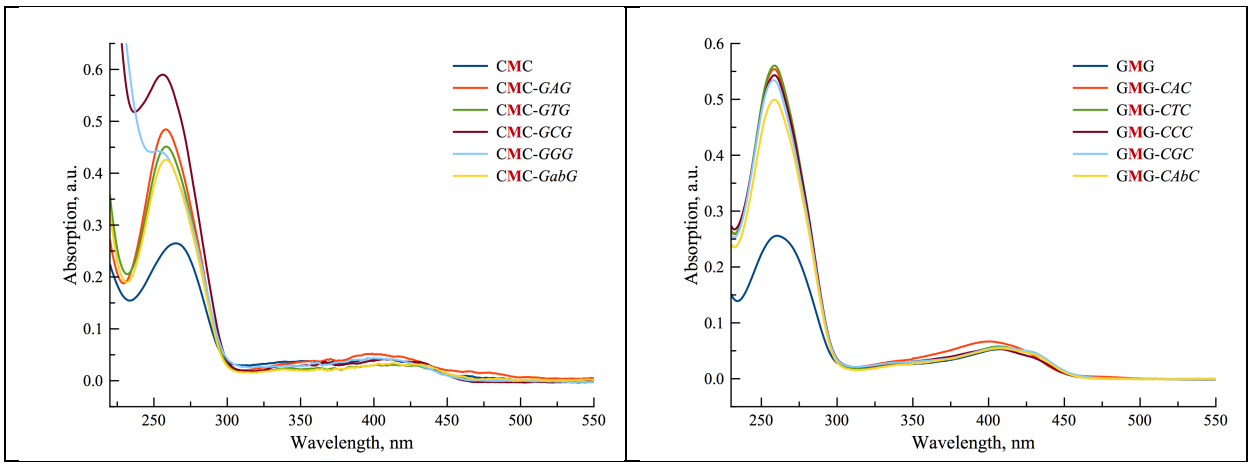


Fig S9. Fluorescence spectra of labelled single and double strands with TMT:

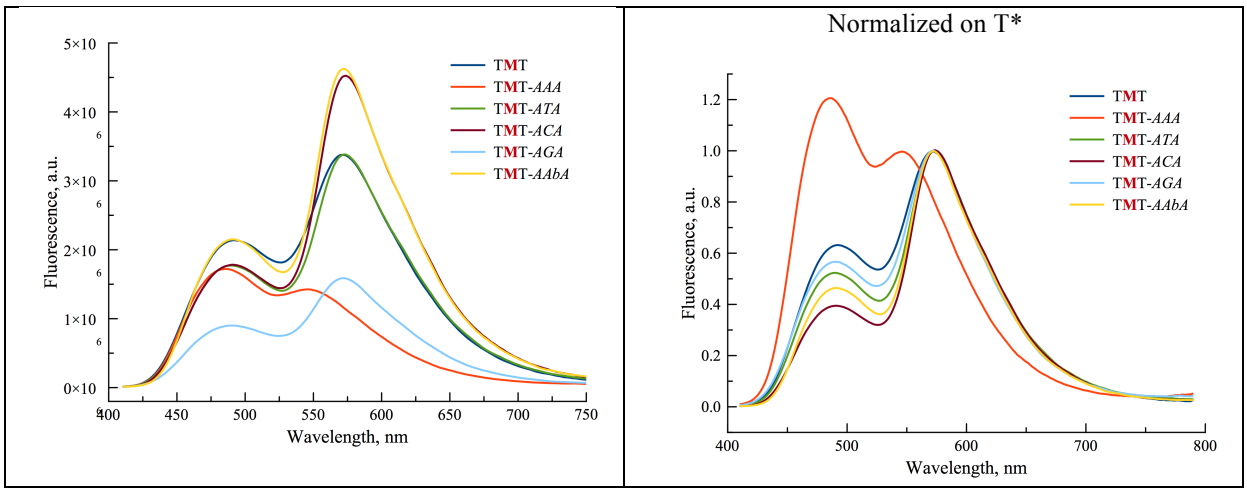


Fig S10. Fluorescence spectra of labelled single and double strands with AMA:

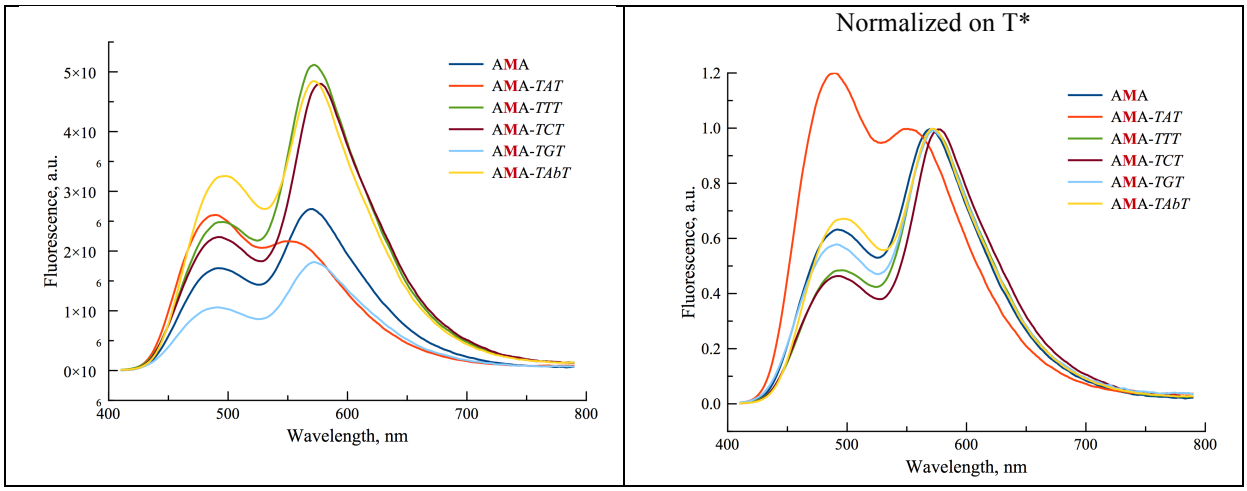


Fig S11. Fluorescence spectra of labelled single and double strands with CMC:

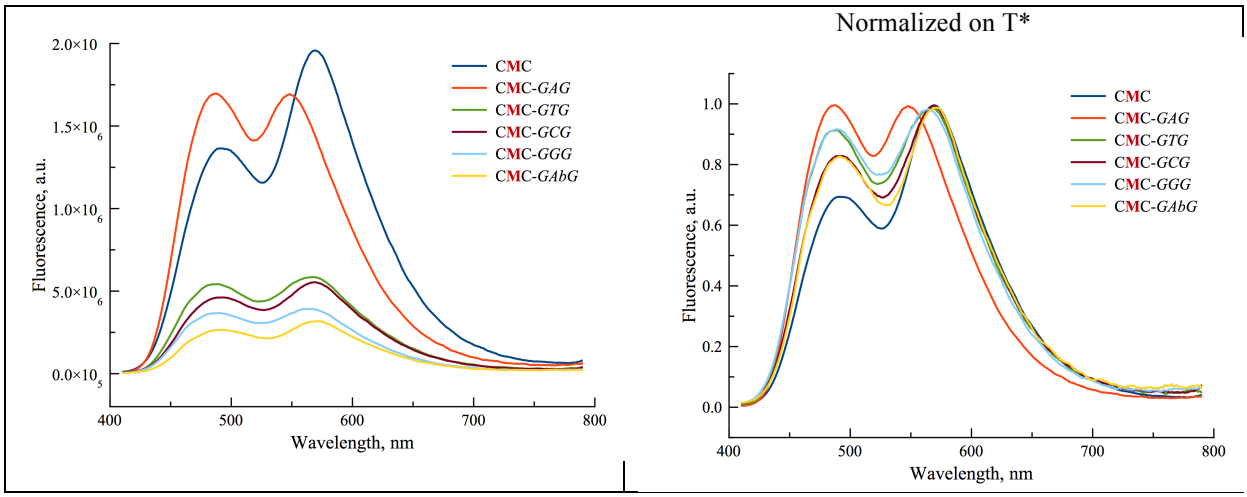


Fig S12. Fluorescence spectra of labelled single and double strands with GMG:

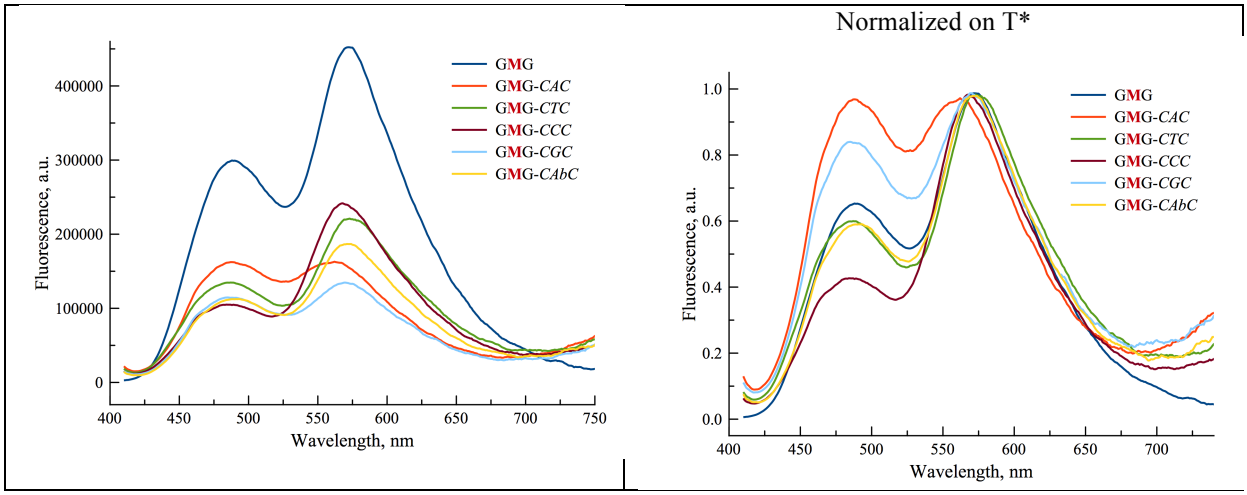
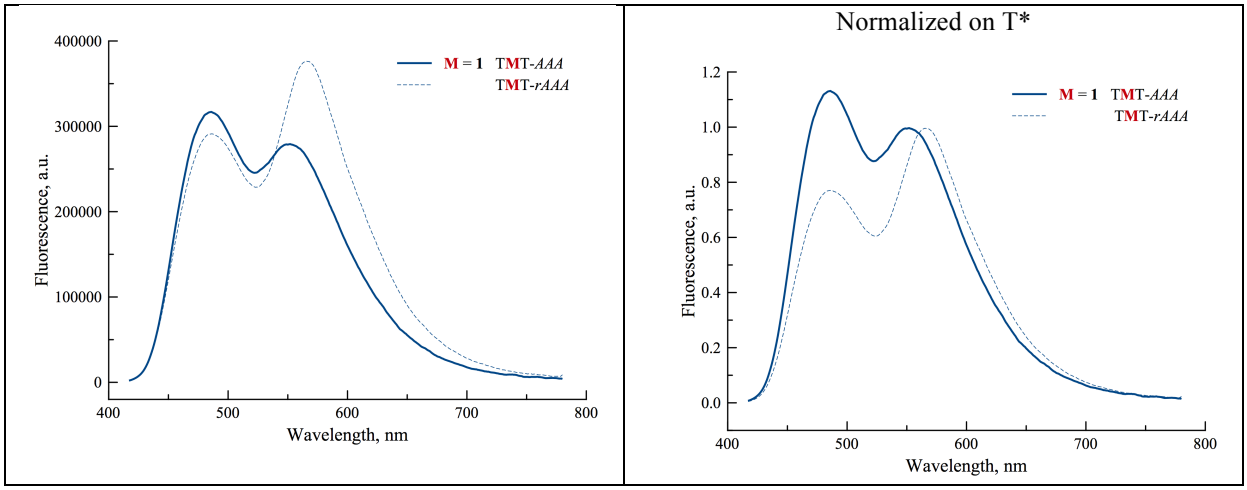


Fig S13. Fluorescence spectra of B DNA/DNA and A DNA/RNA:



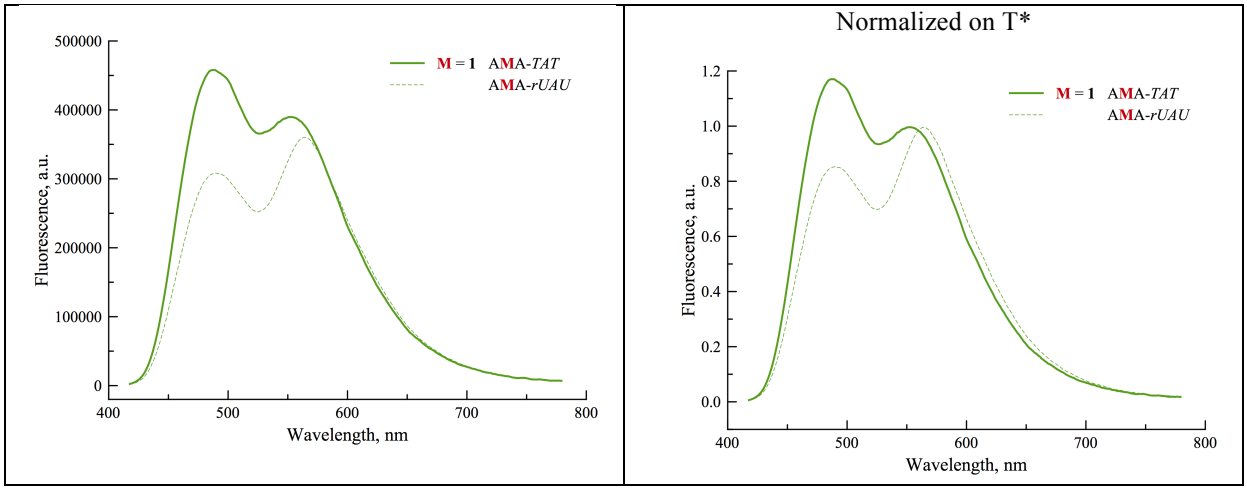
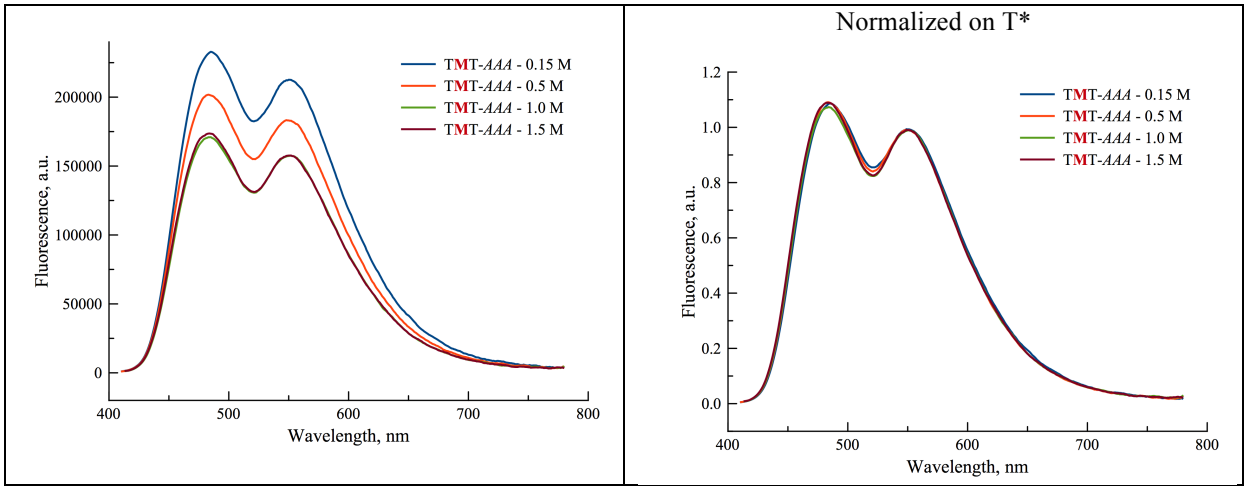


Fig S14. Fluorescence spectra of the TMT-AAA duplex at different NaCl concentrations:



References:

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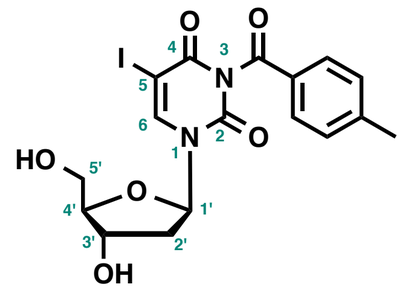
[4] N. P. F. Barthes, I. A. Karpenko, D. Dziuba, M. Spadafora, J. Auffret, A. P. Demchenko, Y. Mély, R. Benhida, B. Y. Michel, A. Burger, *RSC Adv.* **2015**, *5*, 33536-33545.
[5] K. J. Breslauer, *Methods Enzymol.* **1995**, *259*, 221-242.
[6] S. M. Ormson, R. G. Brown, F. Vollmer, W. Rettig, *J. Photochem. Photobiol. A* **1994**, *81*, 65-72.
[7] W. H. Melhuish, *J. Phys. Chem.* **1961**, *65*, 229-235.

4. NMR spectra

8.784
7.964
7.922
7.408
7.368
6.275
6.244
6.212
4.582
4.562
4.559
4.541
4.538
4.516
4.036
4.022
4.006
3.992
3.946
3.945
3.933
3.887
3.872
3.867
3.852
3.805
3.792
2.436
2.412
2.404
2.396
2.384
2.380
2.374
2.364
2.352
2.342

^{1}H NMR



Me **-Tol**

H6

o-C **-Toluoyl**

m-H **-Toluoyl**

H1'

H3'

H4'

H5'$_{A\ \&\ B}$

H2'$_{A\ \&\ B}$

MeOD

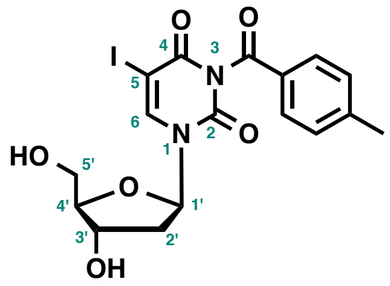
H_2O

$(CD_3)_2CO$

2.06
1
0.96
3
0.942
1.95
1.29
1.95
2.06

ppm 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5

^{13}C NMR



168.936
159.821
149.905
147.397
146.749
131.408
130.705
129.712
129.647
128.927
88.897
86.849
71.402
67.292
62.062
41.801
21.737

$(CD_3)_2CO$

o-C -Toluoyl

m-C -Toluoyl

p-C -Toluoyl

C6

i-C -Toluoyl

C=O Toluoyl

C4

C2

Toluene

Toluene

C4'

C1'

C3'

C5

C5'

C2'

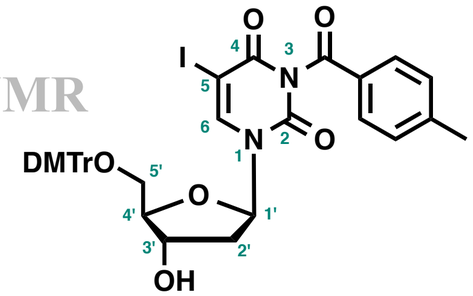
$(CD_3)_2CO$

Me -Toluoyl

Toluene

ppm 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10

8.221
7.811
7.770
7.471
7.446
7.438
7.431
7.397
7.380
7.371
7.361
7.348
7.338
7.319
7.308
7.298
7.286
7.281
6.896
6.852
6.285
6.255
6.246
6.217
4.582
4.577
4.576
4.566
4.098
4.082
4.068
4.053
3.375
3.359
2.496
2.482
2.457
2.437
2.413
2.384



^{1}H NMR

o-H **(PhOMe)-DMT**

m-H **-Toluoyl**

m-H **(PhOMe)-DMT**

Me **O-DMT**

Me **-Tol**

H6

o-H **-Toluoyl**

DMF

H -Ph **-DMT**

H1'

$CDHCl_2$

H3'

H4'

H5'$_{A\ \&\ B}$

DMF

H2'$_{A\ \&\ B}$

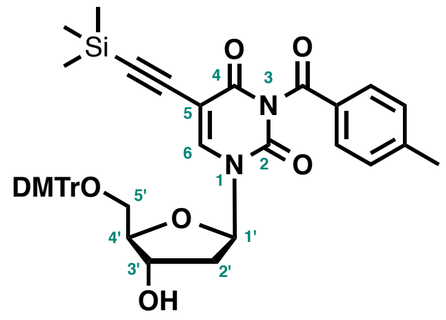
Toluene

H_2O

1.95
9.05
1
1.03
1.81
0.871
1.9
4.07
1.01
6.02
5.18

ppm 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5

^{1}H NMR



8.134
7.854
7.838
7.533
7.517
7.433
7.416
7.407
7.392
7.382
7.377
7.366
7.311
7.294
7.279
7.233
7.218
7.207
7.192
6.939
6.922
6.279
6.268
6.264
6.252
4.540
4.534
4.529
4.523
4.130
4.123
4.117
4.111
3.839
3.440
3.433
3.418
3.412
3.390
3.382
3.368
3.361
2.523
2.518
2.511
2.506
2.486
2.338
2.325
2.310
2.297
2.297
2.283
0.091

m-H -Ph **-DMT**

p-H -Ph **-DMT**

m-H **-Toluoyl**

o-H **(PhOMe)-DMT**

o-H -Ph **-DMT**

m-H **(PhOMe)-DMT**

o-H **-Toluoyl**

Me **-Tol**

Me **O-DMT**

Si-(*CH*$_3$)$_3$

H6

H1'

H3'

H4'

H5'$_{A\ \&\ B}$

H2'$_A$

H2'$_B$

X Toluene

X $CDHCl_2$

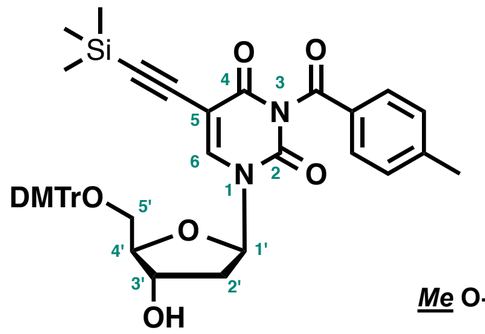
X Toluene

1.99
4.73
2.05
4.11
1
6.43
1.02
3.21
9.35
1.01
2.1
1.93
2.84
1
1.01
1.03
0.877
1.06

ppm 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

168.174
160.876
159.110
148.595
147.493
145.004
142.806
135.944
130.873
130.380
130.361
130.334
129.324
128.519
128.449
128.283
127.315
113.696
100.565
100.231
95.051
87.325
87.042
86.532
72.575
63.796
55.581
41.833
22.030
0.302

^{13}C NMR



Me O-DMT

o-C (PhOMe)-DMT

m-C -Toluoyl

m-C -Ph -DMT

o-C -Toluoyl

o-C -Ph -DMT

m-C (PhOMe)-DMT

CD_2Cl_2

Si-($\underline{C}H_3$)$_3$

i-C -Toluoyl

Me -Toluoyl

i-C -(PhOMe)-DMT

p-C -Ph -DMT

p-C -(OMe)-DMT

i-C -Ph -DMT

Si—*C*≡

C$_{IV}$ -O-5'

C4'

C3'

C5'

C2'

p-C -Tol

C6

Si—≡*C*—

C1'

C=O Toluoyl

C2

C4

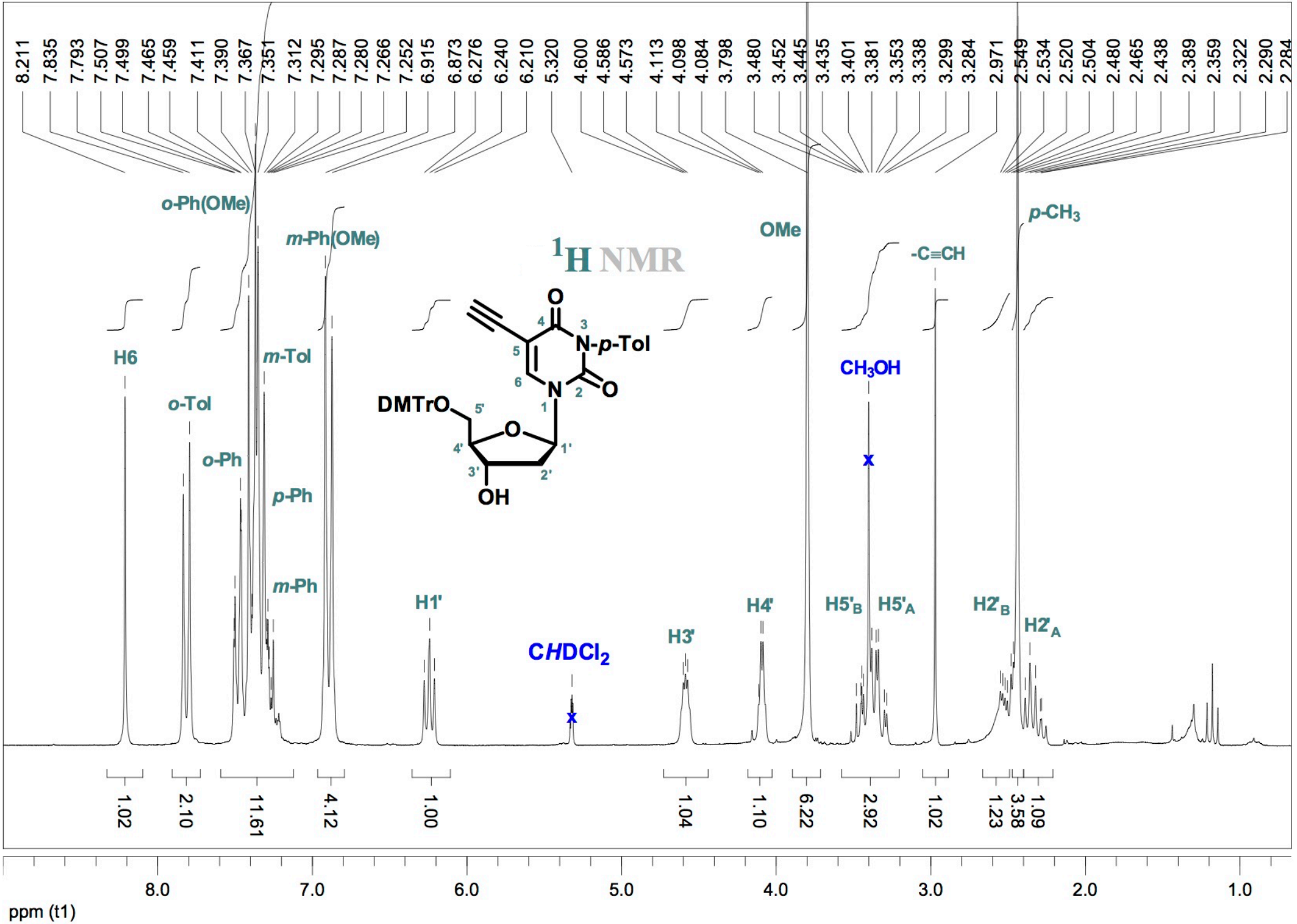
C5

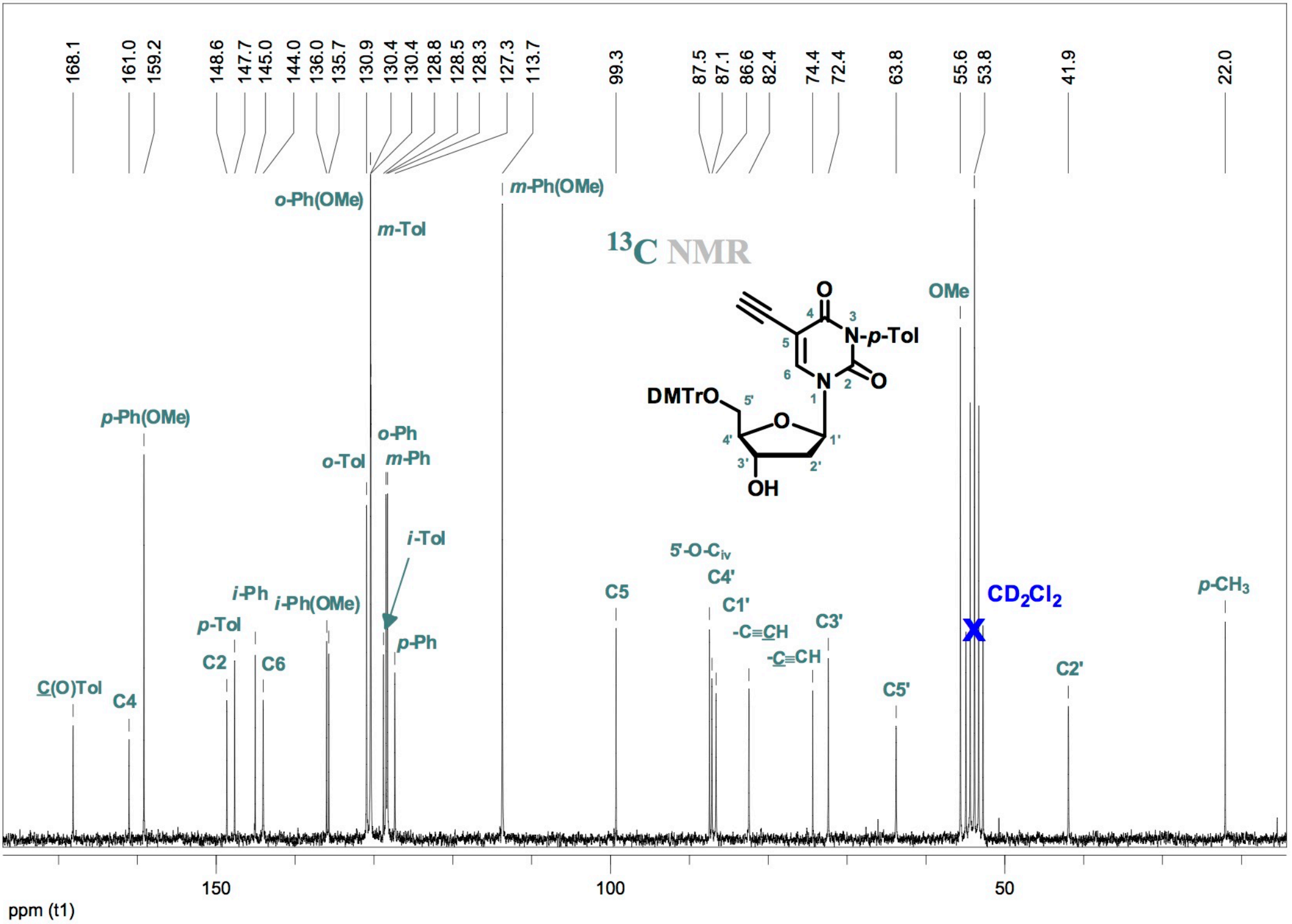
Toluene

Toluene

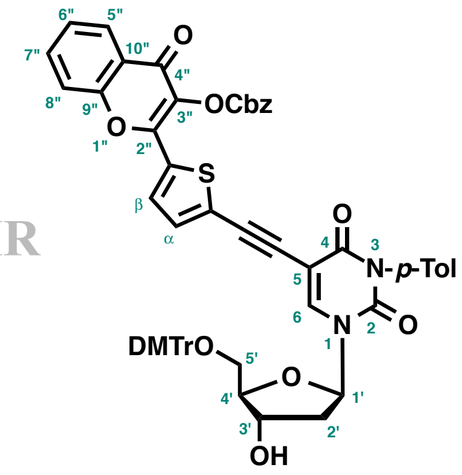
Toluene

ppm 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0


8.211
7.835
7.793
7.507
7.499
7.465
7.459
7.411
7.390
7.367
7.351
7.312
7.295
7.287
7.280
7.266
7.252
6.915
6.873
6.276
6.240
6.210
5.320
4.600
4.586
4.573
4.113
4.098
4.084
3.798
3.480
3.452
3.445
3.435
3.401
3.381
3.353
3.338
3.299
3.284
2.971
2.549
2.534
2.520
2.504
2.480
2.465
2.438
2.389
2.359
2.322
2.290
2.284
1H NMR
o-Ph(OMe)
m-Ph(OMe)
OMe
-C≡CH
p-CH3
H6
m-Tol
o-Tol
o-Ph
p-Ph
m-Ph
CH3OH
H1'
CHDCl2
H3'
H4'
H5'B
H5'A
H2'B
H2'A
N-p-Tol
DMTrO
OH
1.02
2.10
11.61
4.12
1.00
1.04
1.10
6.22
2.92
1.02
1.23
3.58
1.09
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)


168.1
161.0
159.2
148.6
147.7
145.0
144.0
136.0
135.7
130.9
130.4
130.4
128.8
128.5
128.3
127.3
113.7
99.3
87.5
87.1
86.6
82.4
74.4
72.4
63.8
55.6
53.8
41.9
22.0
o-Ph(OMe)
m-Tol
m-Ph(OMe)
13C NMR
OMe
N-p-Tol
DMTrO
OH
p-Ph(OMe)
o-Tol
o-Ph
m-Ph
i-Tol
5'-O-Civ
C4'
C1'
C5
i-Ph
i-Ph(OMe)
p-Tol
C2
C6
p-Ph
-C≡CH
-C≡CH
C3'
CD2Cl2
p-CH3
C(O)Tol
C4
C5'
C2'
150
100
50
ppm (t1)

8.424 8.266 8.259 8.259 8.258 8.227 8.226 8.225 8.218 7.887 7.846 7.770 7.762 7.735 7.728 7.727 7.720 7.692 7.684 7.646 7.626 7.551 7.549 7.547 7.508 7.437 7.430 7.387 7.382 7.345 7.279 7.215 7.214 7.212 7.211 7.210 7.209 7.180 7.180 6.857 6.849 6.849 6.812 6.805 6.766 6.745 6.389 6.359 6.355 6.325 5.327 4.648 4.648 4.167 4.165 4.155 3.730 3.722 3.524 3.511 3.468 3.458 3.413 3.399 3.360 3.345 2.644 2.629 2.616 2.600 2.574 2.562 2.545 2.532 2.491 2.446



^{1}H NMR

o-H (PhOMe)-DMT

-OC(O)CH$_2$*Ph*

H -Ph -DMT

o-H -Toluoyl

H6"

X CHCl$_3$

m-H (PhOMe)-DMT

H6

H β

H8"

m-H -Toluoyl

H5"

H7"

H α

H1'

-OC(O)C*H$_2$*Ph

X CH$_2$Cl$_2$

H3'

H4'

*Me*O-DMT

Me -Tol

H5'$_{A\ \&\ B}$

H2'$_A$

H2'$_B$

H$_2$O X

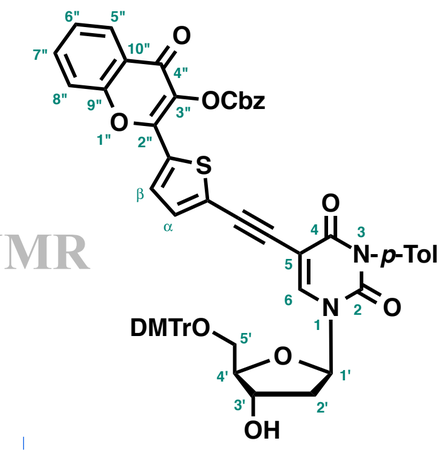
X Cyclohexane

1.09 1.19 1.21 2.16 0.997 1.83 0.984 2.17

1.07 2.15 1.06 15.3 3.94 1 1 5.93 5.5

ppm 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5

171.529
167.640
160.230
158.804
155.144
151.937
150.766
148.262
146.998
144.385
142.772
135.533
135.432
134.529
134.326
133.328
132.047
131.762
130.873
130.609
130.131
130.069
130.008
129.146
128.915
128.888
128.801
128.650
128.422
128.337
128.239
127.995
127.261
126.151
125.513
123.675
117.953
113.510
99.727
87.587
87.347
87.073
86.646
86.481
72.353
71.419
63.480
55.339
42.119
22.075



^{13}C NMR

*Me*O-DMT

i-C -(PhOMe)-DMT

C α

o-C -Toluoyl

p-C -(OMe)-DMT

C2"-*Th*-

m-C (PhOMe)-DMT

p-C -Toluoyl

i-C -Cbz

CDCl$_3$

C=O Cbz

C7"

i-C -Ph-DMT

C9"

C$_{IV}$ -O-5'

C10"

C2

Th—≡*C*—

Th—*C*=

C2"

C4

C5

C4'

-OC(O)C*H*$_2$Ph

C3'

C=O Toluoyl

C5"

C6"

C β

C8"

Me -Tol

C4"

C6

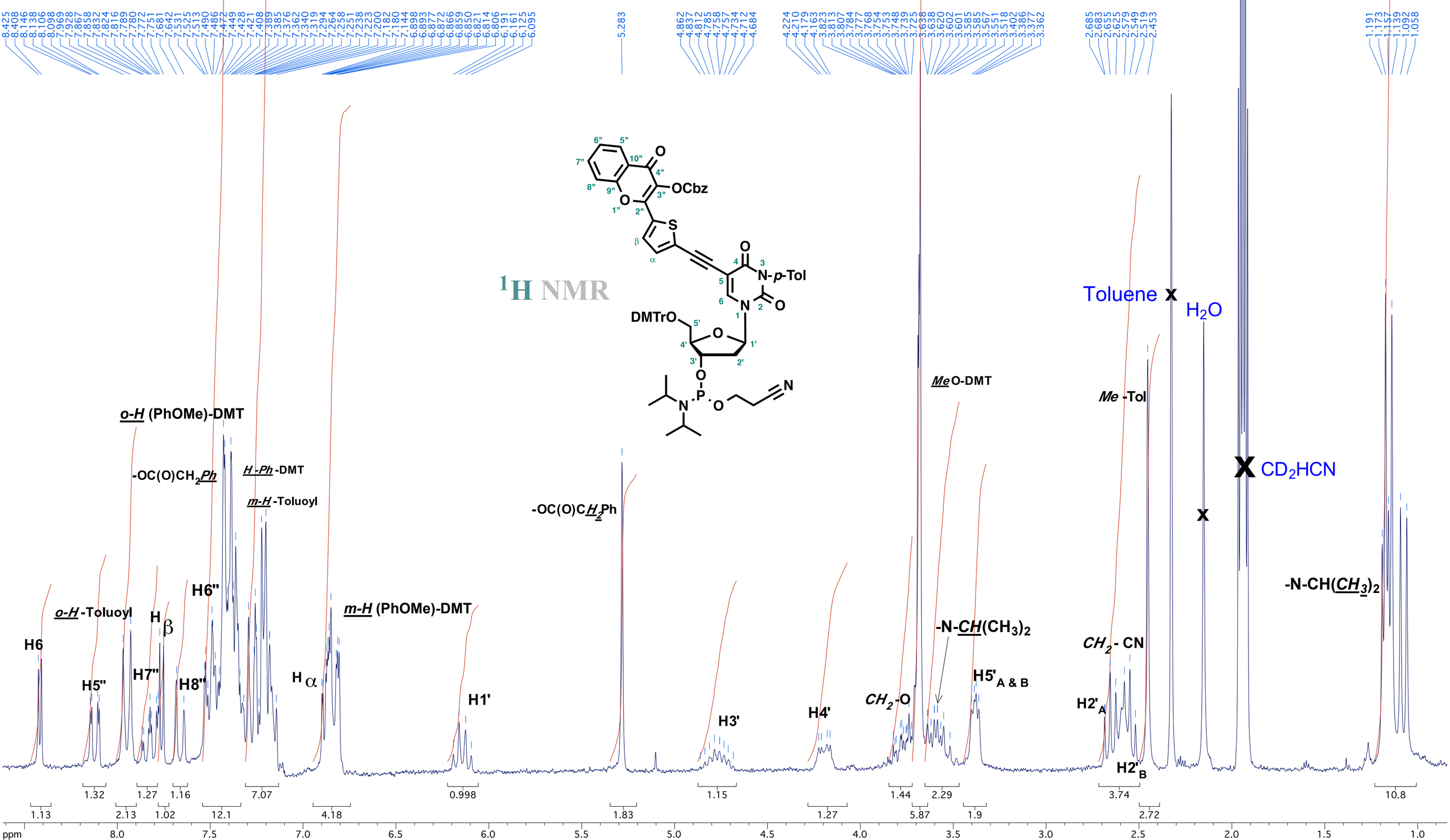
C1'

C5'

C2'

CH$_2$Cl$_2$

ppm 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20

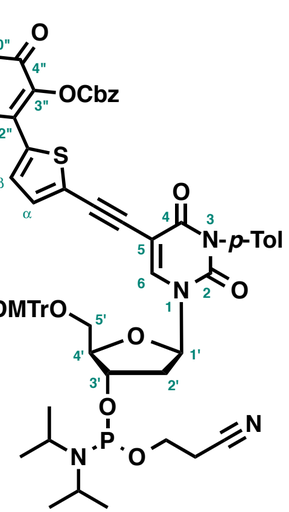

8.425 8.408 8.146 8.138 8.106 8.098 7.969 7.928 7.867 7.858 7.832 7.824 7.816 7.789 7.780 7.772 7.751 7.681 7.642 7.531 7.525 7.513 7.490 7.486 7.472 7.449 7.428 7.421 7.408 7.389 7.385 7.376 7.362 7.340 7.319 7.294 7.264 7.258 7.251 7.238 7.223 7.200 7.182 7.180 7.144 6.898 6.893 6.877 6.872 6.866 6.859 6.850 6.821 6.814 6.806 6.191 6.161 6.125 6.095
5.283
4.862 4.837 4.812 4.785 4.759 4.757 4.734 4.712 4.684
4.224 4.210 4.179 4.163 3.823 3.813 3.807 3.784 3.777 3.768 3.754 3.753 3.748 3.739 3.725 3.638 3.620 3.602 3.601 3.585 3.585 3.567 3.551 3.518 3.402 3.386 3.377 3.362
2.685 2.683 2.655 2.625 2.579 2.549 2.519 2.453
1.191 1.173 1.157 1.139 1.092 1.058
1H NMR
N-p-Tol
DMTrO
OCbz
Toluene
H2O
CD2HCN
MeO-DMT
Me -Tol
o-H (PhOMe)-DMT
-OC(O)CH2Ph
H-Ph -DMT
m-H -Toluoyl
-OC(O)CH2Ph
m-H (PhOMe)-DMT
o-H -Toluoyl
H6
H5"
H7"
H β
H8"
H6"
H α
H1'
H3'
H4'
CH2-O
-N-CH(CH3)2
H5'A & B
CH2 - CN
H2'A
H2''B
-N-CH(CH3)2
1.32 1.27 1.16 7.07 0.998 1.15 1.44 2.29 3.74 10.8
1.13 2.13 1.02 12.1 4.18 1.83 1.27 5.87 1.9 2.72
ppm
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0

148.105
147.985

147.96 ppm

148.105
147.985

^{31}P NMR

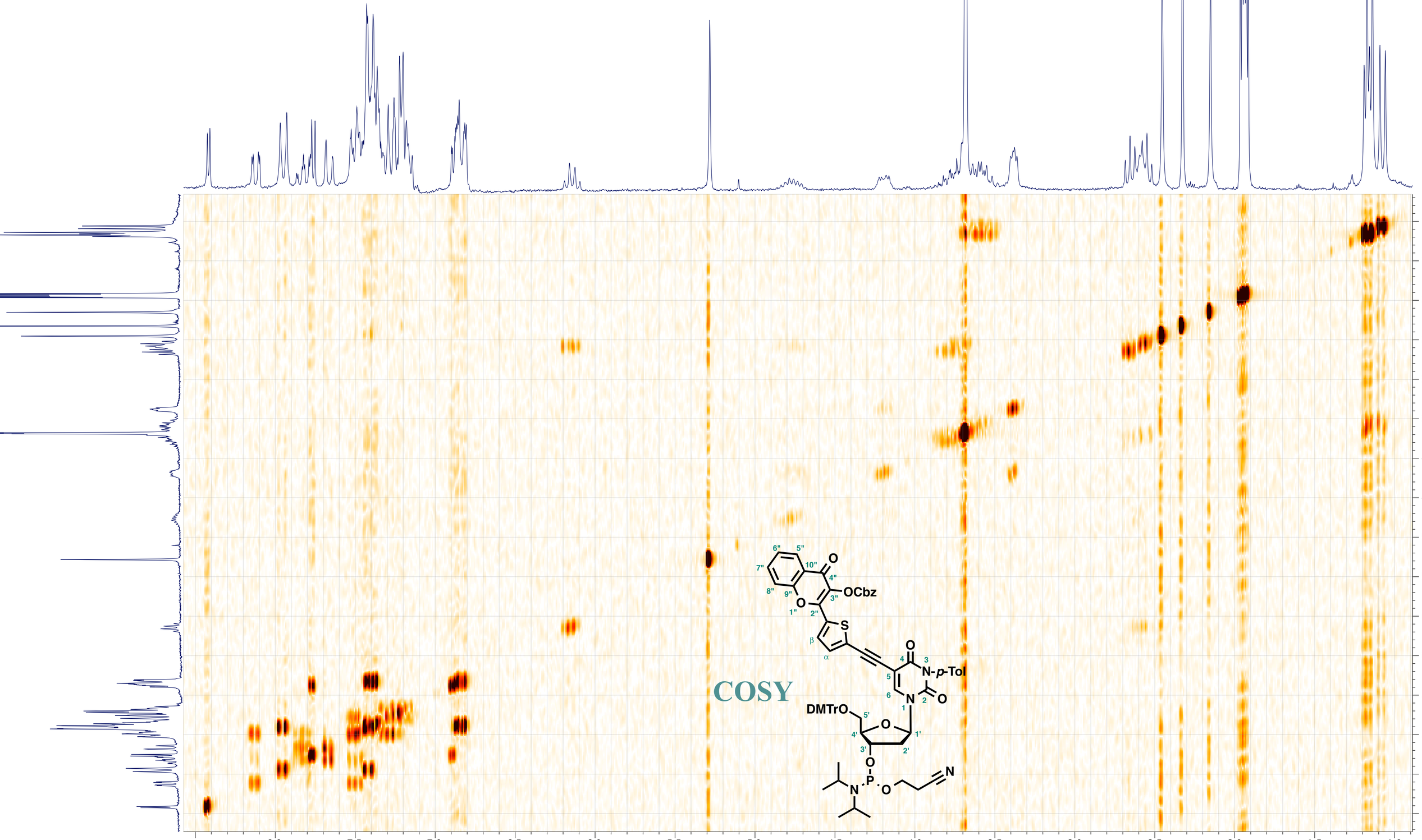


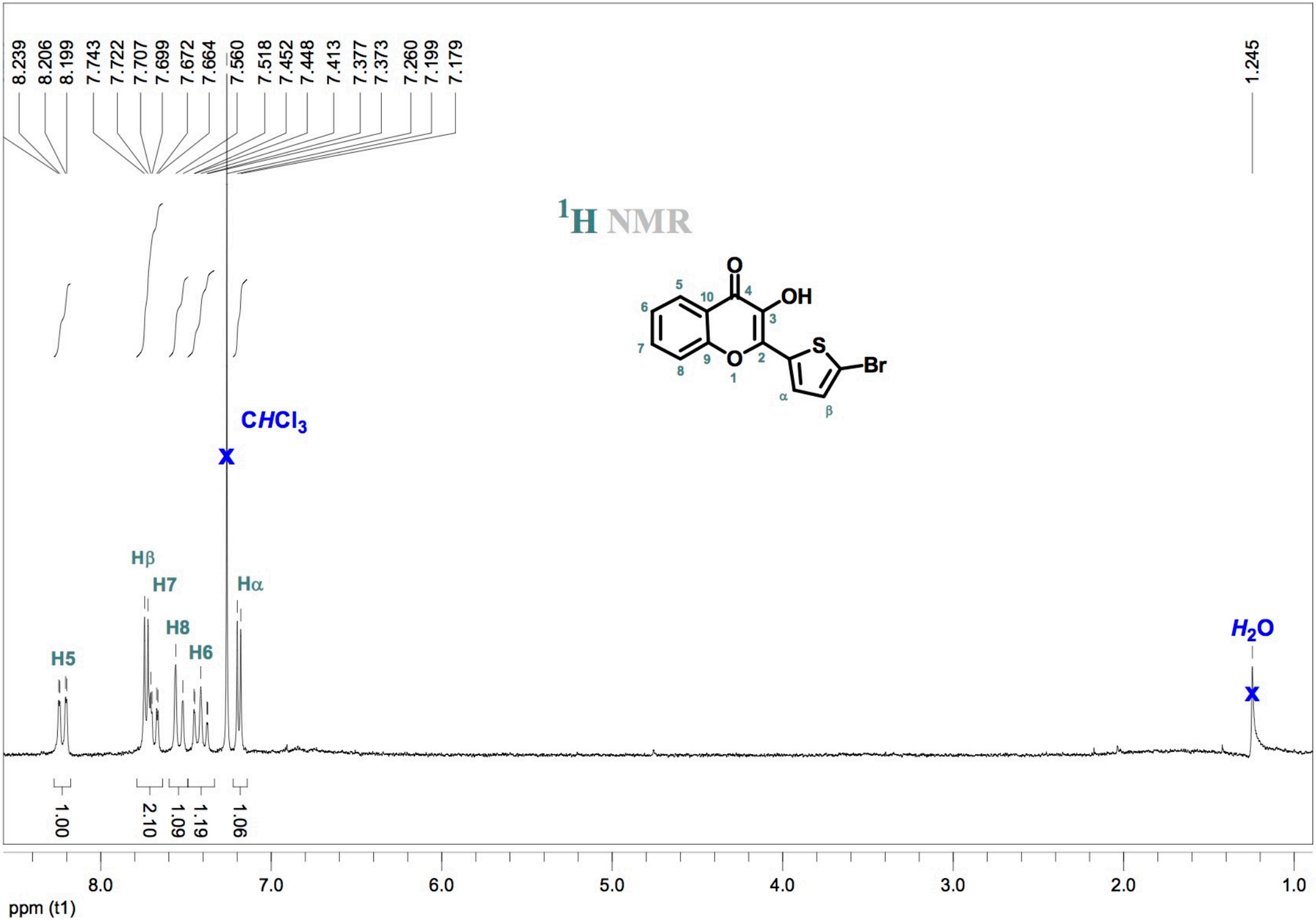
1

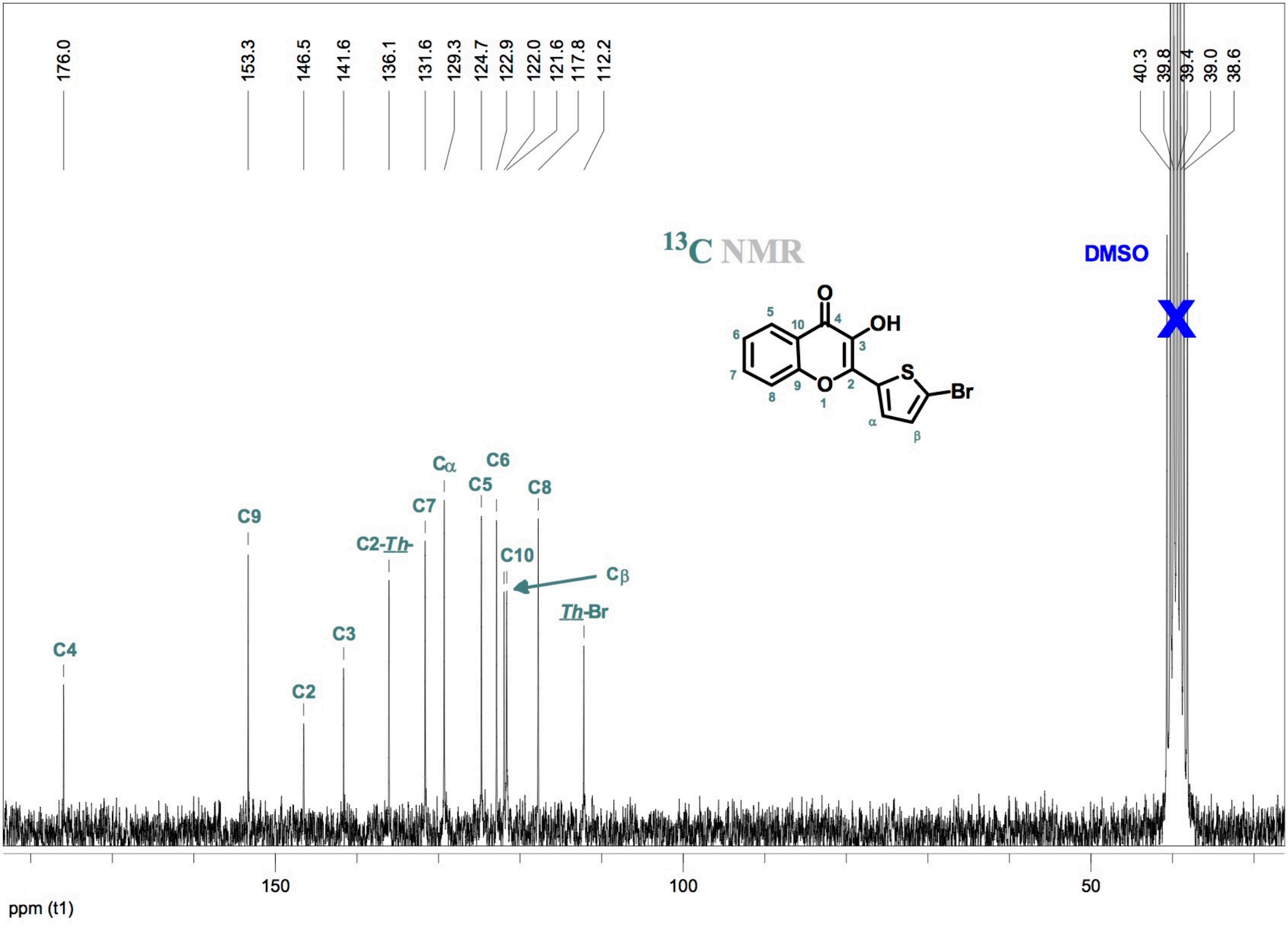
148

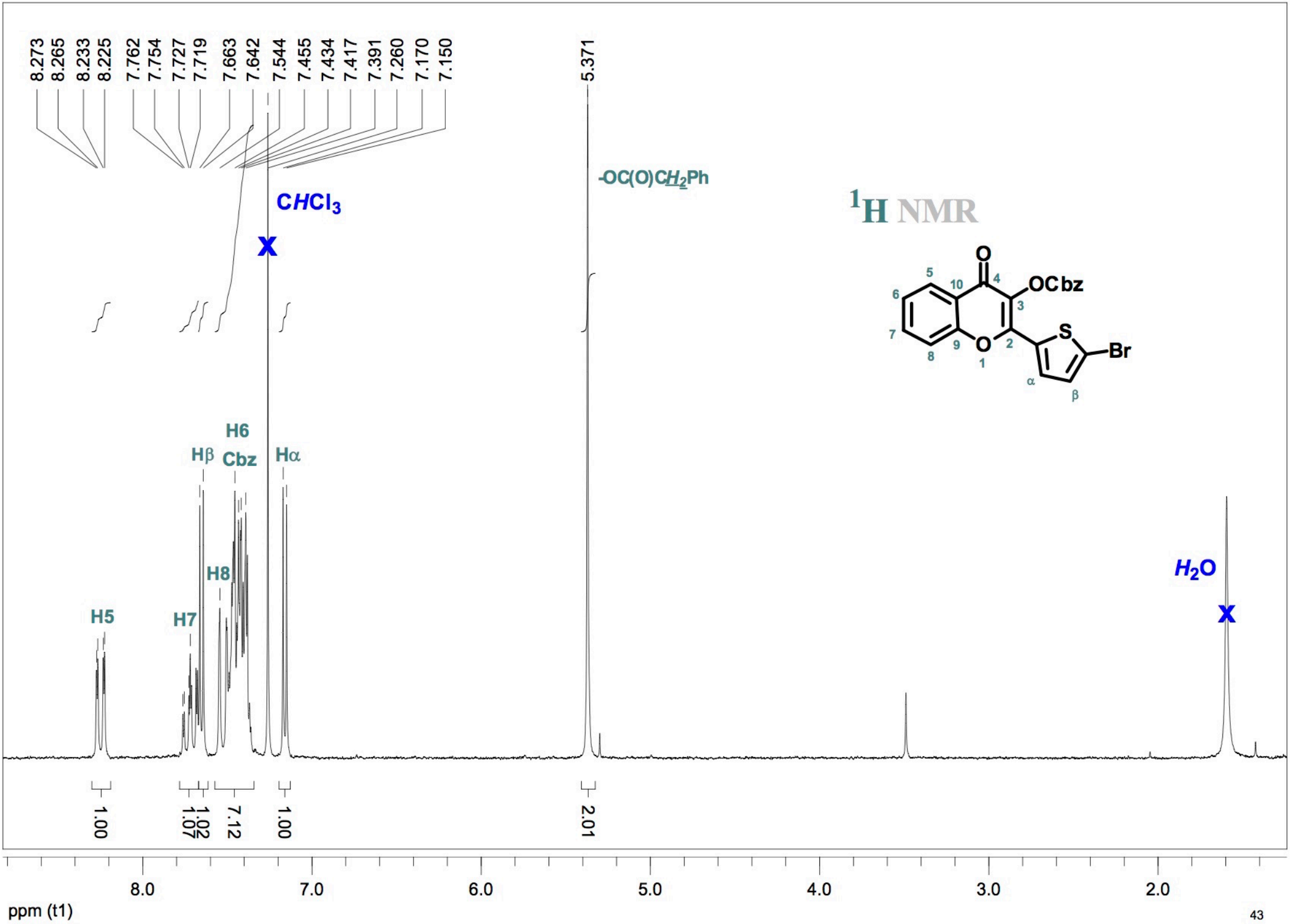
0.724

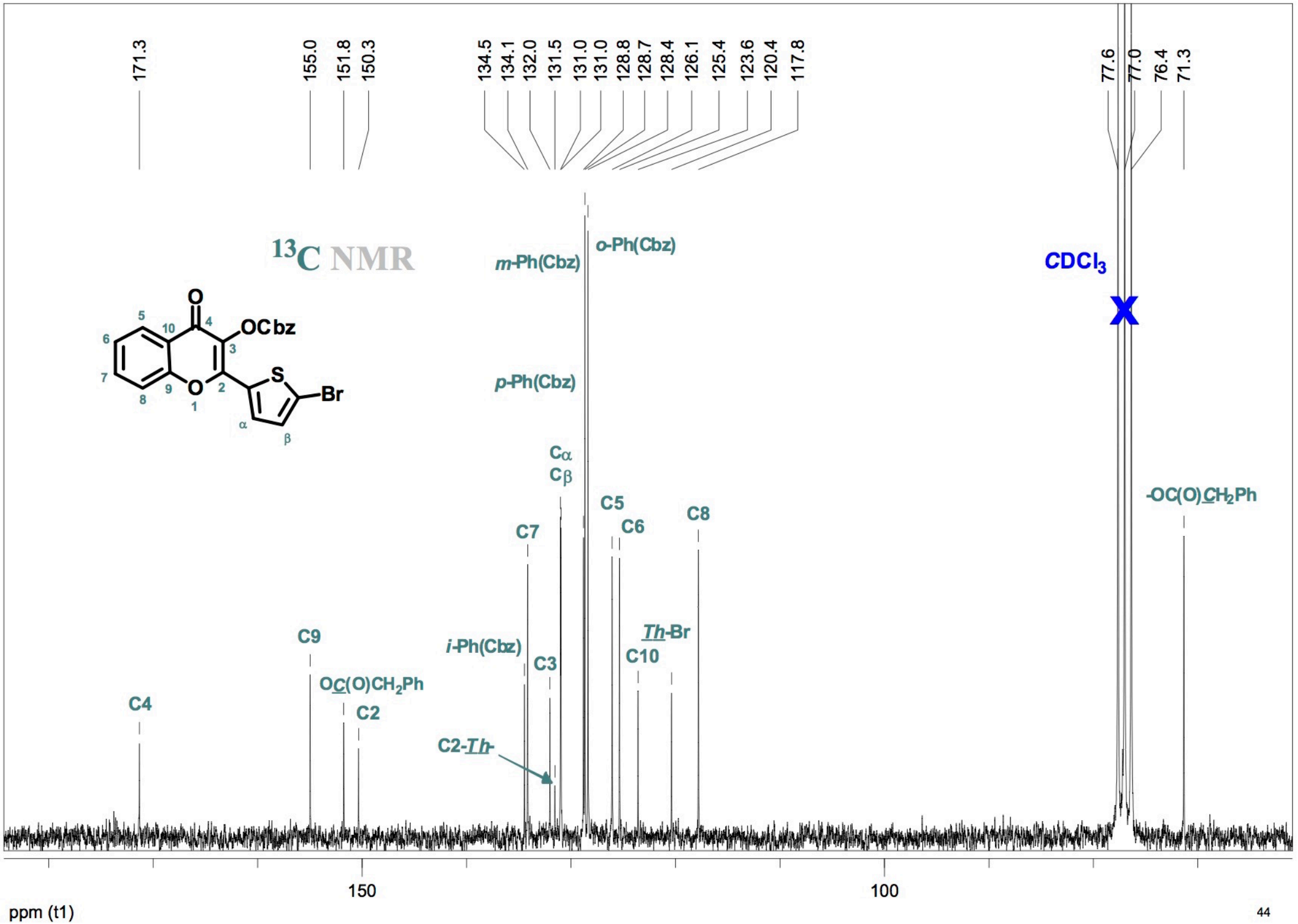
ppm 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0


COSY
OCbz
N-p-Tol
DMTrO
ppm
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0


8.239
8.206
8.199
7.743
7.722
7.707
7.699
7.672
7.664
7.560
7.518
7.452
7.448
7.413
7.377
7.373
7.260
7.199
7.179
1.245
1H NMR
O
OH
S
Br
CHCl3
Hβ
H7
Hα
H8
H6
H5
H2O
1.00
2.10
1.09
1.19
1.06
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)


176.0
153.3
146.5
141.6
136.1
131.6
129.3
124.7
122.9
122.0
121.6
117.8
112.2
40.3
39.8
39.4
39.0
38.6
13C NMR
DMSO
O
OH
S
Br
C4
C9
C2
C3
C2-Th-
C7
Cα
C5
C6
C8
C10
Cβ
Th-Br
150
100
50
ppm (t1)


8.273
8.265
8.233
8.225
7.762
7.754
7.727
7.719
7.663
7.642
7.544
7.455
7.434
7.417
7.391
7.260
7.170
7.150
5.371
CHCl3
-OC(O)CH2Ph
1H NMR
OCbz
Br
H6
Cbz
Hβ
Hα
H8
H7
H5
H2O
1.00
1.02
1.07
7.12
1.00
2.01
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t1)


171.3
155.0
151.8
150.3
134.5
134.1
132.0
131.5
131.0
131.0
128.8
128.7
128.4
126.1
125.4
123.6
120.4
117.8
77.6
77.0
76.4
71.3
13C NMR
m-Ph(Cbz)
o-Ph(Cbz)
CDCl3
p-Ph(Cbz)
Cα
Cβ
C5
C6
C8
C7
-OC(O)CH2Ph
Th-Br
C10
C9
i-Ph(Cbz)
C3
OC(O)CH2Ph
C2
C4
C2-Th-
150
100
ppm (t1)
44