Supporting Information To:

Development of environmentally sensitive fluorescent and dual emissive deoxyuridine analogues

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Experimental section

General methods:

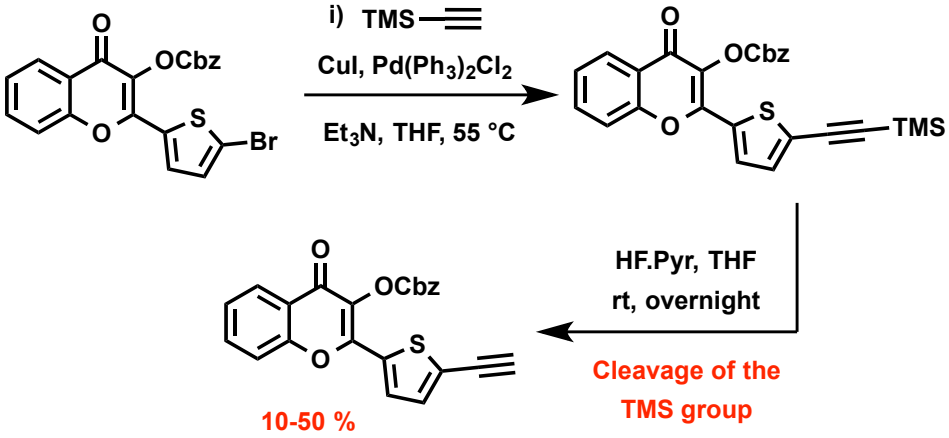
All non-aqueous reactions involving water-sensitive reagents were performed in oven dried glassware under argon using dry solvents. The synthetic intermediates were coevaporated twice with toluene beforehand and dried in vacuo before use. All chemical reagents were obtained from commercial sources and 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 & 365 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). Analytical HPLC were performed using a Waters™ 600 (600 pump, 600E Controler, 996 Photodiode Array Detector) and a Jasco LC-Net II / ADC apparatus. A RP C18 column (300 × 4.60 mm, 5 µm particle size, Luna® 100Å, Phenomenex®) was used with mobile phases: A: H_2O (0.1 % HCOOH) and B: CH_3CN (0.1 % HCOOH) at a flow rate of 0.5 mL/min (see SI page 109 for the control method). The NMR spectra (^1H, ^{13}C, ^2D: ^1H–^{13}C COSY, ^1H–^{13}C HMQC and ^1H–^{13}C HMBC) were recorded on 200 or 500 Bruker Advance Spectrometers (200 or 500 MHz). ^1H 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_3OD, or DMSO-d^6, with the solvent signals as internal references: 7.26 ppm for C*H*Cl_3, 3.31 ppm for CD_2*H*OD, 2.50 ppm for (CD_3)(CD_2*H*)S(O) for ^1H NMR experiments, and 77.0 ppm for $CDCl_3$, 49.0 ppm for CD_3OD, 39.4 ppm for (CD_3)$_2$S(O) for ^{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 ^1H 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. All solvents for absorption and fluorescence experiments were of spectroscopic grade. Stock solutions were prepared using 1,4-dioxane. The samples used for spectroscopic measurements contained ≈ 0.1% v/v of the stock solvent. 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). They were recorded using excitation and emission slits of 2 nm and were corrected at excitation and emission. They were taken with absorbance less than 0.05 at 20 °C at the excitation wavelength mentioned in the corresponding experiments. The quantum yields were determined taking into account the change of the refractive index of the solvents.

[1] W. L. F. Armarego and C. L. L. Chai, *Purification of Laboratory Chemicals*, 7th ed.; Butterworth-Heinemann: Oxford, 2013.
[2] W. C. Still, M. Kahn and A. Mitra, *J. Org. Chem.*, 1978, **43**, 2923–2925.
[3] (*a*) H. E. Gottlieb, V. Kotlyar and A. Nudelman, *J. Org. Chem.*, 1997, **62**, 7512–7515; (*b*) G. R. Fulmer, A. Miller, N. H. Sherden and H. E. Gottlieb, *Organometallics*, 2010, **29**, 2176–2179.

Supporting Information

1. Additional Data

Preparation of ethynyl 3HCs



Scheme S1. Cleavage of the TMS group, exhibiting a lack of reproducibility due to a partial and concomitant removal of Cbz as well as a decomposition of the anionic form.

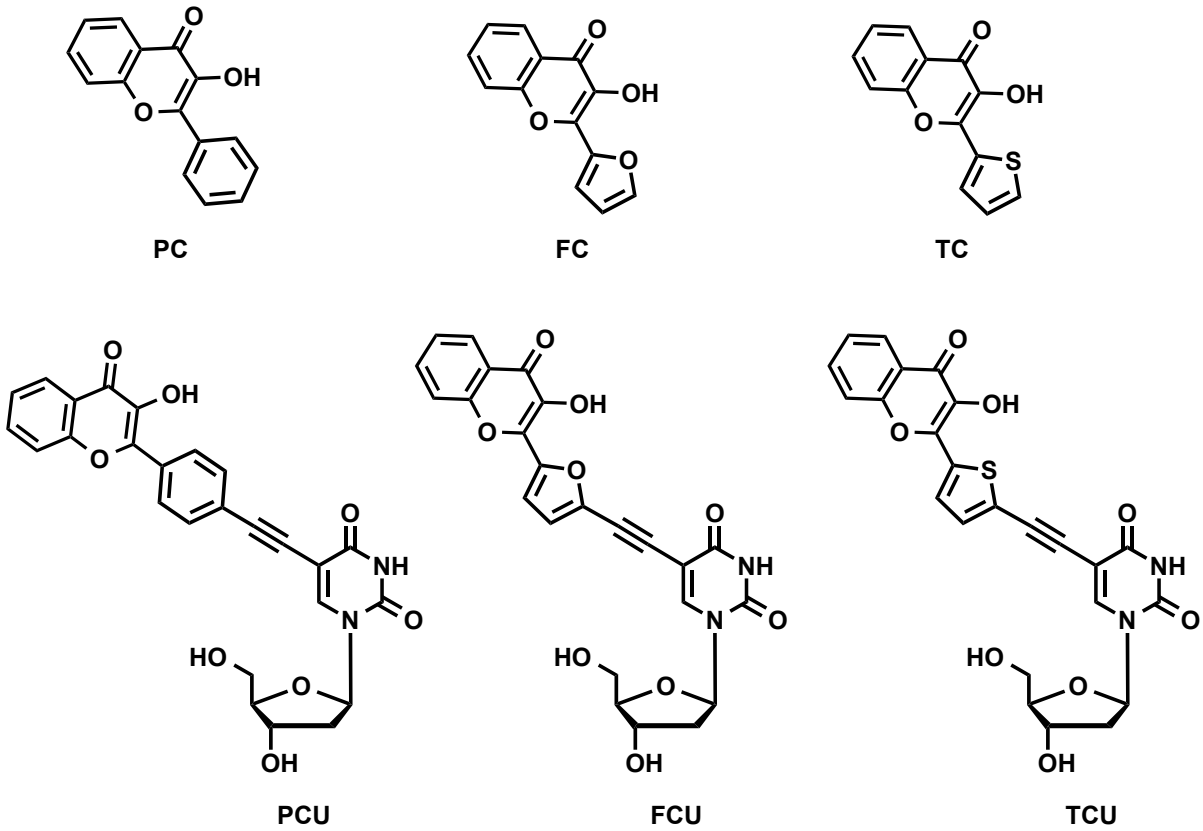


Figure S1. Considered 3-hydroxychromones for the spectroscopic comparison listed in Table 1: parent 2-aryl-3-HCs (**PC**, **TC** and **TC**) and corresponding nucleosidic conjugates (**PCU**, **FCU** and **TCU**) are depicted on the first and second rows, respectively.

2. Steady-state fluorescence measurements

PCU

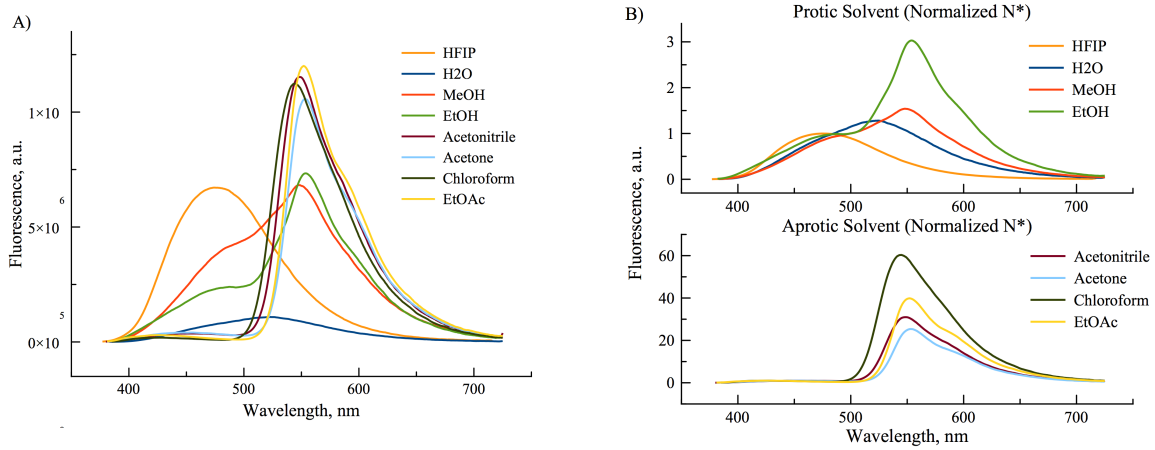


Fig. S2 A) Fluorescence spectra in the set of investigated solvents; B) Normalized emission spectra by N* band for protic (top) and aprotic (bottom) solvents.

FCU

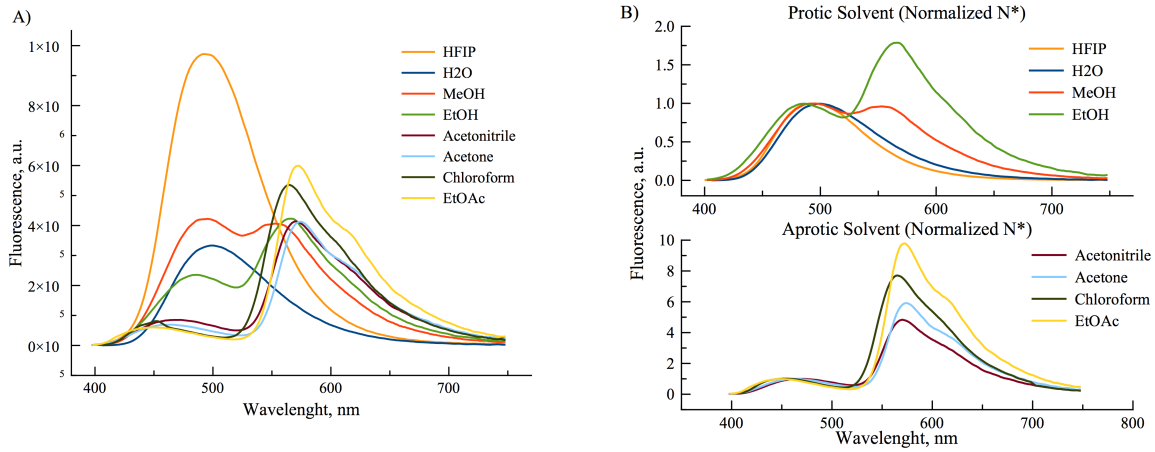


Fig. S3 A) Fluorescence spectra in the set of investigated solvents; B) Normalized emission spectra by N* band for protic (top) and aprotic (bottom) solvents.

TCU

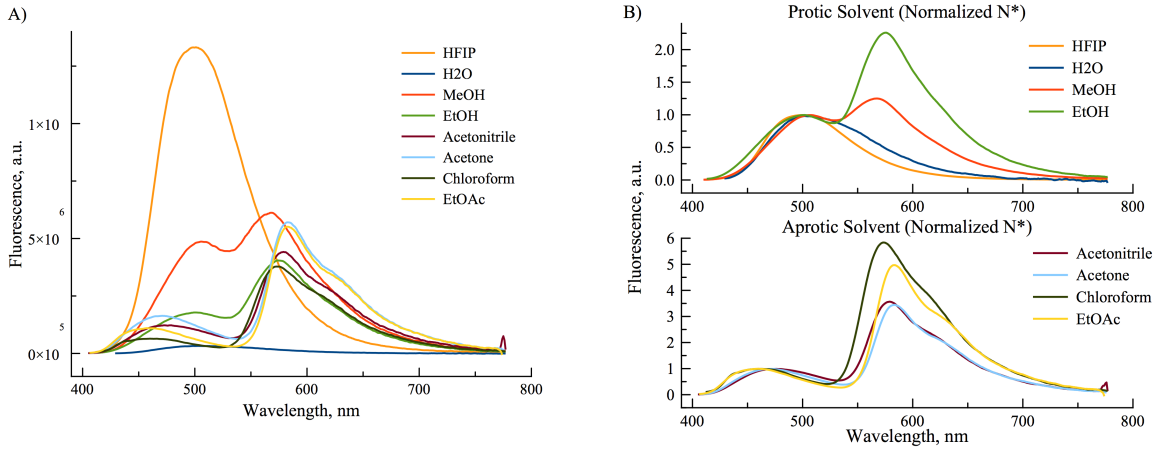


Fig. S4 A) Fluorescence spectra in the set of investigated solvents; B) Normalized emission spectra by N* band for protic (top) and aprotic (bottom) solvents.

Table S1: Lippert-Mataga data of **FCU** and **TCU**

Solvants	Δf [a]	$\Delta\nu$ (cm^{-1}) [b]	
		FCU	**TCU**
HFIP [c]	0.3118	5690	5253
H_2O	0.3201	5535	5396
MeOH	0.3087	5719	5475
EtOH	0.2888	5348	5166
CH_3CN	0.3055	4719	4544
Acetone	0.2842	4533	4188
$CHCl_3$	0.1482	3251	3307
EtOAc	0.1998	4083	3789

Footnote: [a] Lippert's Parameter: $\Delta f = \left(\frac{\varepsilon - 1}{2\varepsilon + 1} - \frac{n^2 - 1}{2n^2 + 1} \right)$. In this equation, n is the refractive index and ε is the dielectric constant (values found in: R. C. Weast., M. J. Astle,; CRC Handbook of Chemistry and Physics, 60th ed.; CRC: Boca Raton, Florida, 1980, p:2528) [b] Stock shift values, [c] Value of ε found in Hong D. P., Hoshino M., Kuboi R., Goto Y. (**1999**) *J. Am. Chem. Soc. 121*, 8427–8433.

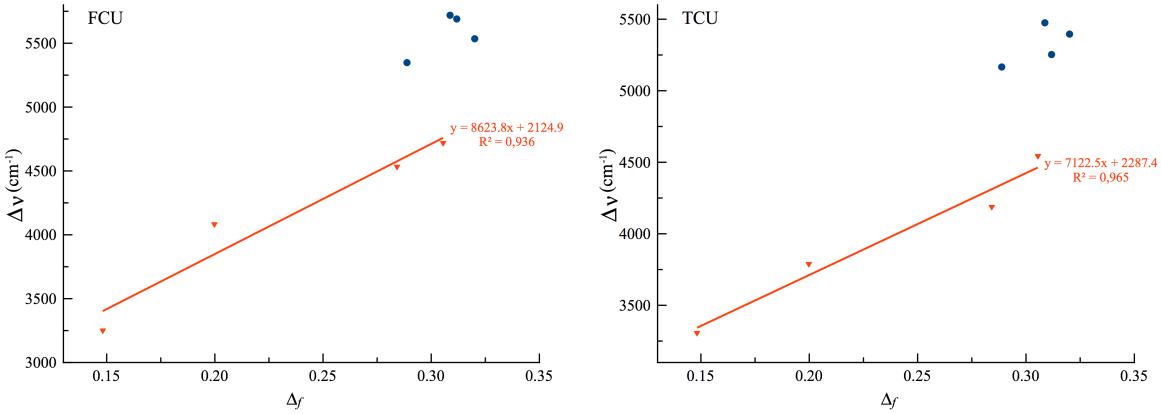


Fig. S5 & S6: Stokes shift vs. orientation polarizability function (Δf) for **FCU** and **TCU**. The linear plots shown were obtained with aprotic solvents according to the Lippert's equation (red triangles and red line). The positions of the points for protic solvents are shown in blue dots.

Lippert's Equation: $\nu_A - \nu_F = \frac{2}{hc} \Delta f \frac{(\mu_E - \mu_G)^2}{a^3}$.

In this equation, h (= 6.6256 x 10^{-27} ergs) is the Planck's constant, c (= 2.9979 x 10^{10} cm/s) is the speed of light, and a is the radius of the Onsager's cavity. ν_A and ν_F are the wavenumbers (cm^{-1}) of the absorption and emission, respectively, $\Delta\nu$ is the Stokes shift (cm^{-1}), Δf is the Lippert's parameter, and μ_e and μ_g are the dipole moments of the excited and ground states, respectively.

Remark: Exploiting the Lippert's equation to get the difference of the dipole moments ($\mu_e - \mu_g$) needs to determine the Onsager radius. In the Lippert-Mataga model the fluorophore is treated as a solute occupying a spherical volume for which the radius corresponds to the Onsager radius. Measuring the distance at the two extremes of the fluorophore and dividing the distance by two determine the Onsager radius. In general this distance corresponds to the distance between the donor and acceptor groups of the fluorophore. Applying this approximation to **FCU** and **TCU** is not satisfactory because they are extended conjugated fluorophores with the acceptor group, the carbonyl group, which is not positioned at one extreme.

3. Hydration Study

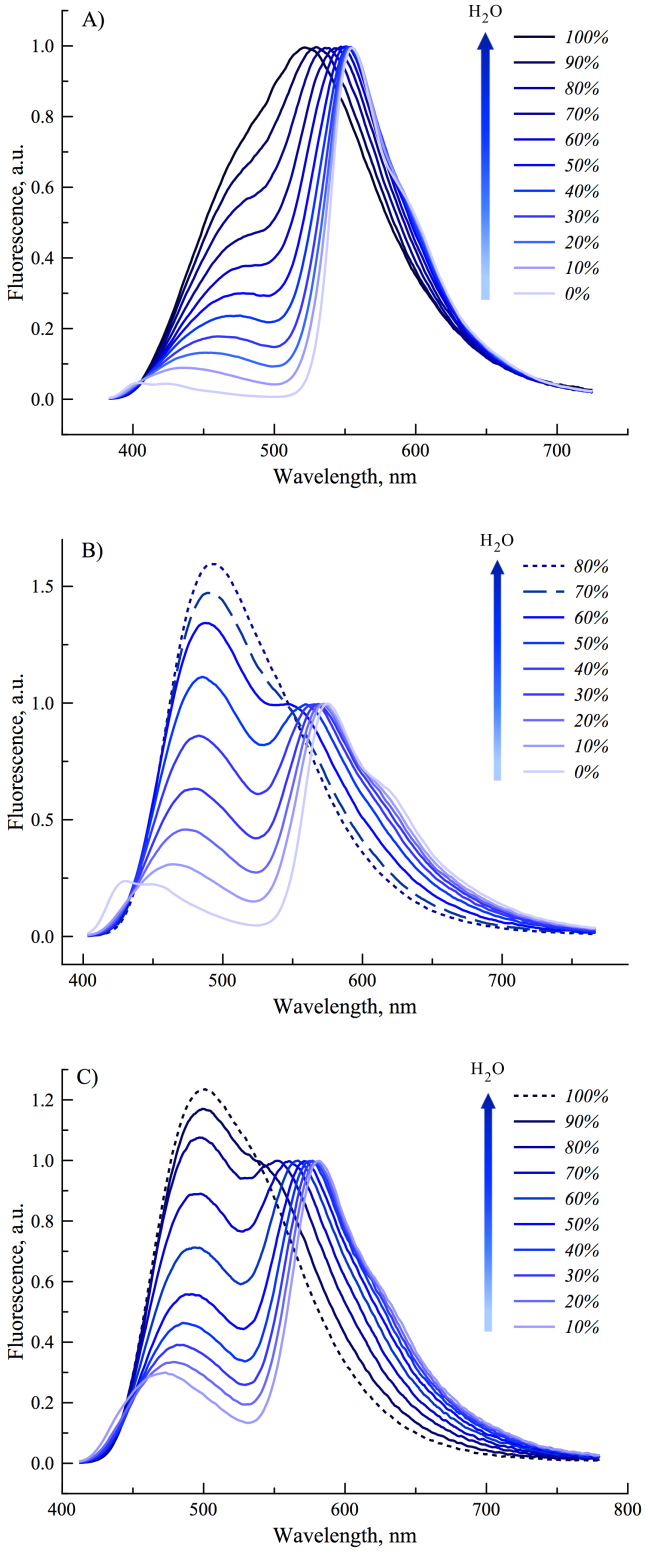


Fig. S7 Sensitivity of the emission spectra to hydration for A) **PCU**, B) **FCU** and C) **TCU**. Data were recorded in gradual mixtures of dioxane and water. Emission spectra were normalized by T* band.

Table S2. Spectroscopic properties of 3-hydroxychromone uridine analogs **PCU**, **FCU**, and **TCU** in dioxane / water and acetonitrile / water mixtures.

	Acetonitrile									**Dioxane**								
	PCU			***FCU***			***TCU***			***PCU***			***FCU***			***TCU***		
% Water	λ_{N*}[a]	λ_{T*}[b]	I_{N*}/I_{T*}[c]	λ_{N*}[a]	λ_{T*}[b]	I_{N*}/I_{T*}[c]	λ_{N*}[a]	λ_{T*}[b]	I_{N*}/I_{T*}[c]	λ_{N*}[a]	λ_{T*}[b]	I_{N*}/I_{T*}[c]	λ_{N*}[a]	λ_{T*}[b]	I_{N*}/I_{T*}[c]	λ_{N*}[a]	λ_{T*}[b]	I_{N*}/I_{T*}[c]
100	480	522	0.74	495	-[d]	-[d]	501	-[d]	-[d]	480	521	0.75	497	-[d]	-[d]	500	-[d]	-[d]
90	481	529	0.67	495	-[d]	-[d]	501	538	1.15	480	528	0.66	494	-[d]	-[d]	497	539	1.17
80	481	533	0.56	495	-[d]	-[d]	497	547	1.07	480	539	0.57	494	-[d]	-[d]	498	552	1.08
70	180	539	0.46	492	539	1.40	498	555	0.93	480	542	0.47	490	539	1.37	494	561	0.89
60	478	542	0.39	492	541	1.35	496	560	0.77	480	546	0.38	489	544	1.32	493	567	0.72
50	477	543	0.34	491	546	1.25	496	565	0.65	476	550	0.31	487	559	1.11	490	571	0.56
40	478	546	0.31	490	555	1.13	494	566	0.56	467	551	0.24	483	562	0.84	485	574	0.46
30	478	548	0.28	490	556	0.98	492	568	0.47	461	553	0.18	478	568	0.64	481	578	0.39
20	474	548	0.23	488	562	0.79	494	572	0.39	451	554	0.14	475	570	0.47	479	579	0.34
10	472	550	0.17	483	565	0.53	487	571	0.30	439	554	0.09	466	573	0.32	473	581	0.30
0	454	550	0.03	466	573	0.20	473	578	0.27	422	554	0.05	448	573	0.34	461[e]	584	-[e]

Footnotes: a) position of the N* emission band maximum; b) position of the T* emission band maximum; c) ratio of two intensity maxima for the N* and T* emission bands; d) single emission band was observed; e) unexplained additional emission band was observed at 438 nm.

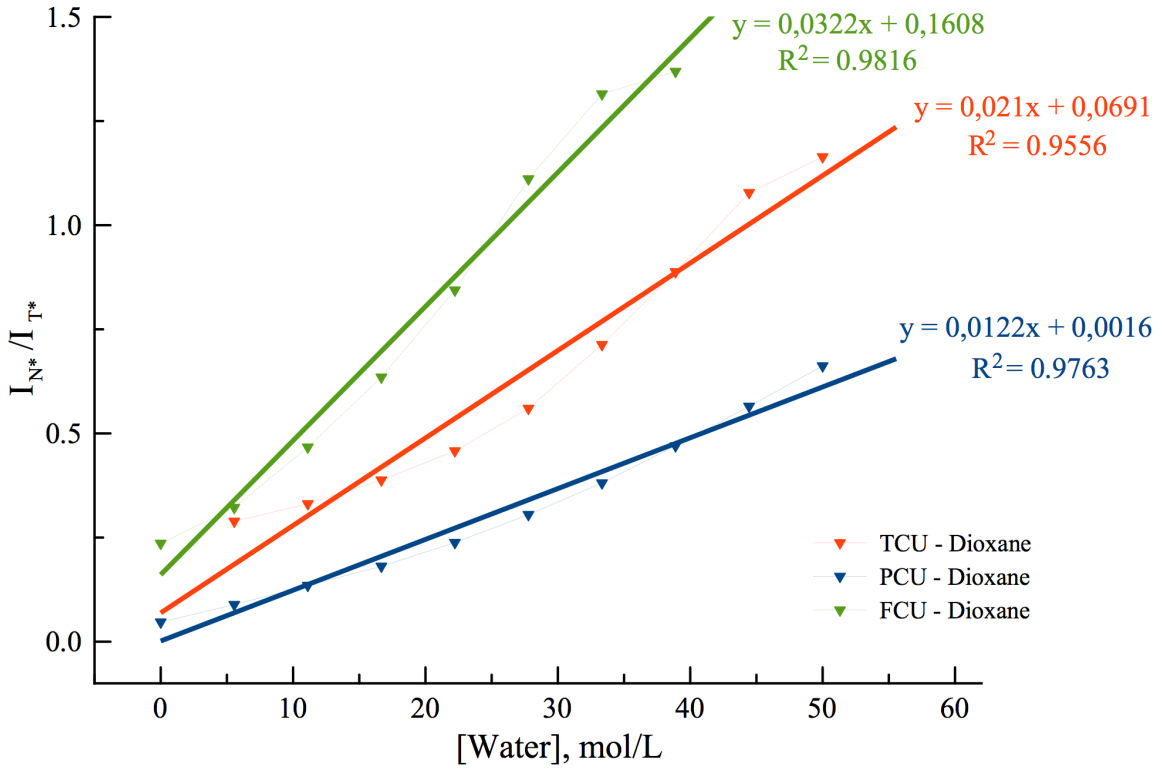


Fig. S8 Dependence of the ratiometric response (I_{N*}/I_{T*}) of **PCU** (blue), **FCU** (green) and **TCU** (orange) on the water concentration in dioxane. The ratios were extracted from the spectra in Fig. S4.

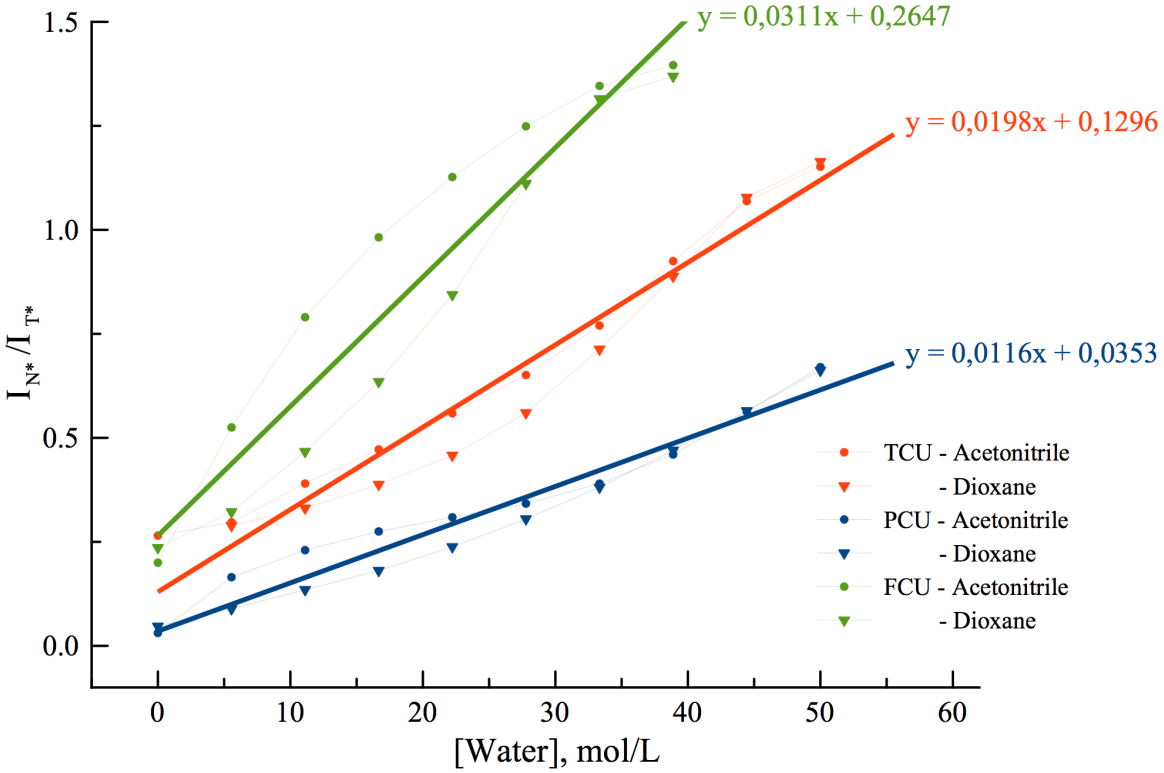
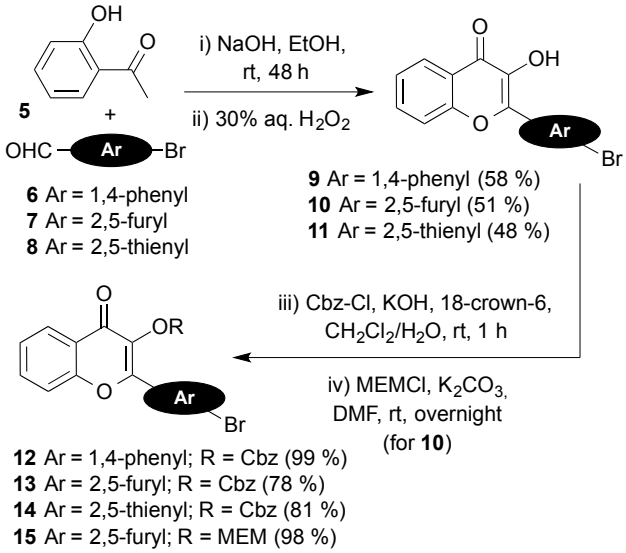
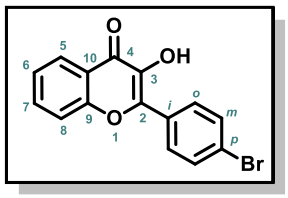


Fig. S9 Summary of Fig 5 & S5: correlation between the ratiometric response (I_{N*}/I_{T*}) and the water concentration in dioxane and acetonitrile.

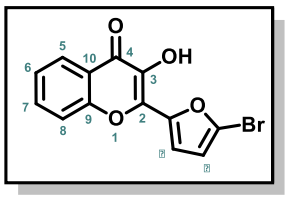
4. Synthetic procedures

Preparation of the 3HC fluorophores



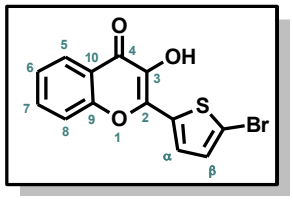


2-(4-Bromophenyl)-3-hydroxy-chromen-4-one (9): To a stirred solution of *o*-hydroxyacetophenone **5** (1.16 mL, 9.65 mmol) and 4-bromobenzaldehyde **6** (1.79 g, 9.65 mmol) in ethanol (50 mL) was dropwise added a 5 N NaOH solution (6 mL). The reaction mixture was stirred 48 h at rt before a dropwise addition of 30 % aq. hydrogen peroxide solution (2 mL). The resulting mixture was stirred 1 day at rt, and then poured into cold water (300 mL) and acidified with 1 N HCl to pH 3. The resulting precipitate was filtered and thoroughly washed with water and cyclohexane to provide as a yellow solid the target derivative **9** (1.78 g, 58 %). $C_{15}H_9BrO_3$ (317.13). R_f = 0.49 (cyclohexane/EA = 7:3 ^{1}H-NMR ($CDCl_3$, 200 MHz): 7.09 (s, 1H, OH), 7.43 (td, ^3J=7.0 Hz, ^4J=1.0 Hz, H6), 7.59 (d, ^3J=8.0 Hz, 1H, H8), 7.66 (d, ^3J=8.8 Hz, 1H, H$_m$-Ph), 7.73 (ddd, ^3J=8.0, 7.0 Hz, ^4J=1.4 Hz, 1H, H7), 8.15 (d, ^3J=8.8 Hz, 2H, H$_o$-Ph), 8.25 (dd, ^3J=8.0 Hz, ^4J=1.4 Hz, 1H, H5); ^{13}C-NMR (DMSO-d^6, 50 MHz): δ = 118.3 (C8), 121.2 (C10), 123.2 (C$_p$-Br), 124.5 (C6), 124.7 (C5), 129.4 (C$_o$-Ph), 130.5 (C$_i$-Ph), 131.5 (C$_m$-Ph), 133.7 (C7), 139.4 (C3), 143.9 (C2), 154.4 (C9), 172.9 (C4); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ = 118.2 (C8), 120.6 (C10), 124.7 (C6), 125.5 (C5), 129.2 (C$_m$-Ph), 130.0 (C$_p$-Br), 131.9 (C$_o$-Ph), 133.8 (C7), 138.5 (C3), 143.8 (C$_i$-Ph), 155.3 (C9), 157.3 (C2), 173.4 (C4); MS (ESI$^+$, MeOH): *m/z*: 339.0, 341.0 [M+Na]$^+$.

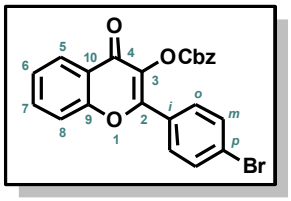


2-(5-Bromofur-2-yl)-3-hydroxy-chromen-4-one (10): To a stirred solution of *o*-hydroxyacetophenone **5** (3.0 mL, 24.9 mmol) and 5-bromo-2-furanecarbaldehyde (fufural) **7** (4.37 g, 24.9 mmol) in ethanol (45 mL) was dropwise added a 5 N NaOH solution (9 mL). The reaction mixture was stirred 48 h at rt before a dropwise addition of 30 % aq. hydrogen peroxide solution (17 mL). The resulting mixture was stirred 30 min at rt, and then poured into cold water (300 mL) and acidified with 1 N HCl to pH 3. The resulting precipitate was filtered and thoroughly washed with water and cyclohexane to provide as a yellow solid the target derivative **10** (3.90 g, 51 %). $C_{13}H_7BrO_4$ (307.10). R_f = 0.33 (CyHex/EA = 7:3); ^{1}H-NMR (DMSO-d^6, 200 MHz): δ = 6.89 (d, 1H, ^3J=2.4 Hz, Hβ-fur), 7.24 (d, 1H, ^3J=2.4 Hz, Hα-fur), 7.45 (dd, 1H, ^3J=6.8, 5.8 Hz, H6), 7.64–7.84 (m, 2H, H7, H8), 8.09 (d, 1H, ^3J=7.6 Hz, H5), 10.23 (s, 1H, 3-OH); 6.74 (d, 1H, ^3J=3.4 Hz, Hβ-fur), 7.28–7.36 (m, 1H, H6), 7.33 (d, 1H, ^3J=3.4 Hz, Hα-fur), 7.58–7.66 (m, 2H, H8, H7), 8.05 (d, 1H, ^3J=7.8 Hz, H5); ^{13}C-NMR (DMSO-d^6, 50 MHz): δ = 112.5 (Cα), 114.2 (Cβ), 117.9 (C8), 121.1 (C10), 121.8 (*Fur*-Br), 123.0 (C6), 124.8 (C5), 131.6 (C7), 137.8 (C3), 149.5 (C2-*Fur*), 149.6 (C2), 153.4 (C9), 176.2 (C4); MS (ESI$^+$, MeOH): *m/z*: 329.1, 331.0 [M+Na]$^+$.

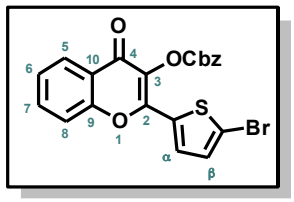
2-(5-Bromothien-2-yl)-3-hydroxy-chromen-4-one (11): To a stirred solution of *o*-hydroxyacetophenone **5** (1.16 mL, 9.65 mmol) and 5-bromo-2-thiophenecarbaldehyde **8** (1.15 mL, 9.65 mmol) in ethanol (50 mL) was dropwise added a 5 N NaOH solution (6 mL). The reaction mixture was stirred 48 h at rt 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 target derivative **11** (1.79 g, 57 %). $C_{13}H_7BrO_3S$ (323.16). R_f = 0.39 (cyclohexane/EA = 7:3); ^{1}H-NMR ($CDCl_3$, 200 MHz): 7.19 (d, ^3J=4.0 Hz, 1H, Hβ-thioph.), 7.41 (td, ^3J=8.0 Hz, ^4J=0.8 Hz, 1H, H6), 7.54 (d, ^3J=8.4 Hz, 1H, H8), 7.71 (ddd, ^3J=8.4 Hz, ^3J=8.0 Hz, ^4J=1.4 Hz, 1H, H7), 7.73 (d, ^3J=4.0 Hz, 1H, Hα-thioph.), 8.22 (dd, ^3J=8.0 Hz, ^4J=1.4 Hz, 1H, H5); ^{13}C-NMR (DMSO-d^6, 50 MHz): δ = 112.2 (Th-Br), 117.8 (C8), 121.6 (Cα), 122.0 (C10), 122.9 (C6), 124.7 (C5), 129.3 (Cβ), 131.6 (C7), 136.1 (C2-*Th*), 141.6 (C3), 146.5 (C2), 153.3 (C9), 176.0 (C4); MS (ESI$^+$, MeOH): *m/z*: 345.0, 347.0 $[M+Na]^+$.



3-Benzyloxycarbonyloxy-2-(4-bromophenyl)-chromen-4-one (12): To a stirred suspension of **9** (500 mg, 1.58 mmol) in a mixture of CH_2Cl_2 (8 mL) and toluene (8 mL) at 0 °C, were added 18-crown-6 (7 mol %, 30 mg), a 25 % w/w aq. NaOH solution (1.6 mL) and benzyl chloroformate (338 µL, 2.37 mmol). The reaction mixture was stirred overnight at rt. After carefully neutralizing by addition of 1 N aq. HCl (10 mL), the organic layer was extracted with CH_2Cl_2 (3 x), washed with brine, dried over $MgSO_4$, filtered and the volatiles were removed *in vacuo*. The residue was purified by flash chromatography on silica gel eluted with toluene/Et_2O mixture (98:2 → 85:15, v/v) to provide the desired compound **12** as a yellow solid (705 mg, 99 %). $C_{23}H_{15}BrO_5$ (451.27). R_f = 0.58 (toluene/Et_2O = 9:1); ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 5.17 (s, 2H, CH_2), 7.22–7.33 (m, 6H, H6 & Cbz), 7.40 (d, ^3J=8.4 Hz, 1H, H8), 7.45 (d, ^3J=8.8 Hz, 1H, H$_m$-Ph), 7.57 (ddd, ^3J=8.6, 7.0 Hz, ^4J=1.6 Hz, 1H, H7), 7.62 (d, ^3J=8.8 Hz, 1H, H$_o$-Ph), 8.13 (dd, ^3J=8.0 Hz, ^4J=1.4 Hz, 1H, H5); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ = 70.9 (OC(O)*C*H$_2$Ph), 118.0 (C8), 123.4 (C10), 125.3 (C6), 125.9 (C5), 126.1 (C$_p$-Br), 128.1 (*m*-C$_{Cbz}$), 128.3 (C$_i$-Ph), 128.5 (*o*-C$_{Cbz}$), 128.6 (*p*-C$_{Cbz}$), 129.7 (*o*-C$_{Ph}$), 131.9 (*m*-C$_{Ph}$), 133.8 (C3), 134.1 (C7), 134.3 (*i*-C$_{Cbz}$), 152.0 (O*C*(O)CH$_2$Ph), 154.9 (C2), 155.2 (C9), 171.8 (C4); MS (ESI$^+$, MeOH) *m/z*: 451.2, 453.2 $[M+H]^+$, 473.1, 475.2 $[M+Na]^+$, 489.2, 491.1 $[M+K]^+$. HRMS (ESI$^+$): *m/z* calcd for $C_{23}H_{16}BrO_5$: 451.0176 $[M+H]^+$; found 451.0177.

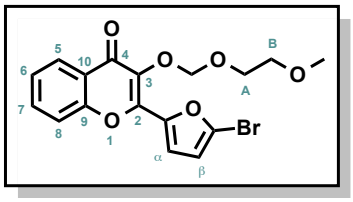


3-Benzyloxycarbonyloxy-2-(5-bromofur-2-yl)-chromen-4-one (13): To a stirred suspension of **10** (600 mg, 1.95 mmol) in CH_2Cl_2 (8 mL) were added a 4.5 M aq. KOH solution (5 mL), 18-crown-6 (5 mol %, 26 mg) and benzyl chloroformate (420 µL, 2.93 mmol). The reaction mixture became homogenous in 5 min 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 (3 x), dried over $MgSO_4$, filtered and the volatiles were removed *in vacuo*. The residue was purified by flash chromatography on silica gel eluted with with toluene/Et_2O mixture (99:1 → 92:8, v/v) to provide the desired compound **13** as a brown solid (674 mg, 78 %). $C_{21}H_{13}BrO_6$ (441.23). R_f = 0.54 (toluene/Et_2O = 9:1). ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 5.35 (s, 2H, CH_2), 6.53 (d, 1H, ^3J=3.8 Hz, Hβ-fur), 7.12 (d, 1H, ^3J=3.8 Hz, Hα-fur), 7.37–7.48 (m, 6H, H6 & Cbz), 7.57 (dd, ^3J= 8.4 Hz, ^4J=0.8 Hz, 1H, H8), 7.72 (ddd, ^3J=8.6, 7.0 Hz, ^4J=1.7 Hz, 1H, H7), 8.26 (dd, ^3J=8.0 Hz, ^4J=1.6 Hz, 1H, H5); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ = 71.2 (OC(O)*C*H$_2$Ph), 114.6 (Cβ), 118.1 (C8), 118.9 (Cα), 123.8 (C10), 125.4 (C6), 126.0 (C5), 127.6 (*Fur*-Br), 128.4 (*o*-C$_{Cbz}$), 128.6 (*m*-C$_{Cbz}$), 128.7 (*p*-C$_{Cbz}$), 131.3 (C3), 134.1 (C7), 134.5 (*i*-C$_{Cbz}$), 145.1 (C2-*Fur*), 146.8 (C2), 152.1 (O*C*(O)CH$_2$Ph), 154.9 (C9), 171.5 (C4); MS (ESI$^+$, MeOH) *m/z*: 441.2, 443.2 $[M+H]^+$, 463.2, 465.2 $[M+Na]^+$, 479.1, 481.1 $[M+K]^+$. HRMS (ESI$^+$): *m/z* calcd for $C_{21}H_{13}NaBrO_6$: 462.9788 $[M+H]^+$; found 462.9768.



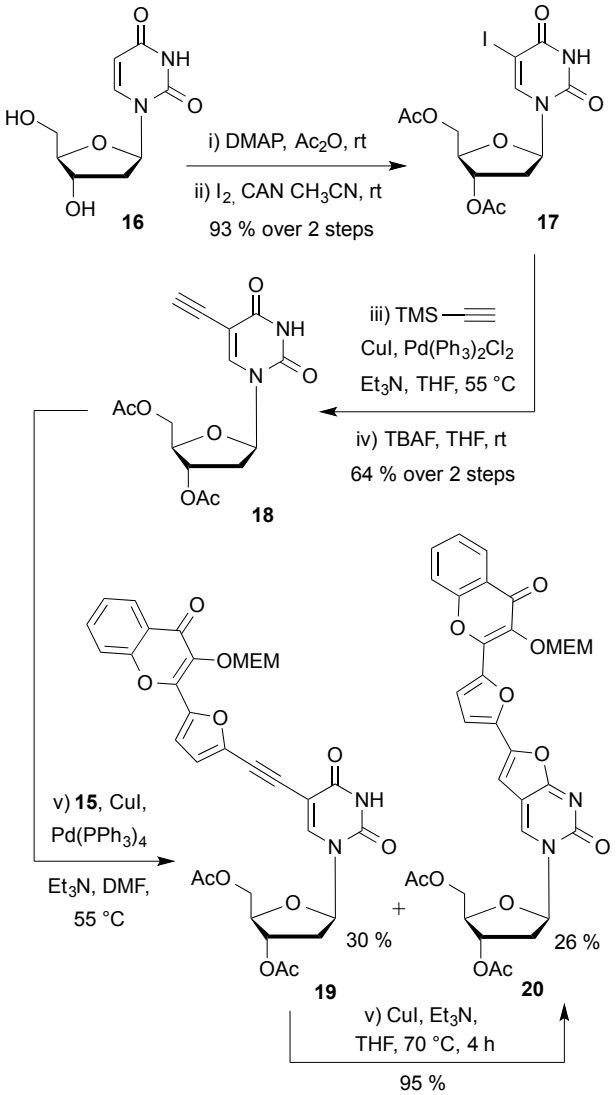
3-Benzyloxycarbonyloxy-2-(5-bromothien-2-yl)-chromen-4-one (14): To a stirred suspension of **11** (1.05 g, 3.25 mmol) in CH_2Cl_2 (13 mL) were added a 4.5 M aq. KOH solution (9 mL), 18-crown-6 (5 mol %, 43 mg) and benzyl chloroformate (700 µL, 4.88 mmol). The reaction mixture became homogenous in 5 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 (3 x), 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/EA mixture (9:1 → 7:3, v/v) to provide the desired compound **14** as white crystals (1.20 g, 81 %). $C_{21}H_{13}BrO_5S$ (457.29). R_f = 0.53 (cyclohexane/EA = 7:3); ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 5.37 (s, 2H, CH_2), 7.16 (d, ^3J=4.0 Hz, 1H, Hβ-thioph.), 7.34–7.50 (m, 6H, H6 & Cbz), 7.52 (dd, ^3J=8.4 Hz, ^4J=0.6 Hz, 1H, H8), 7.65 (d, ^3J=4.0 Hz, 1H, Hα-thioph.), 7.72 (td, ^3J=8.4 Hz, ^4J=1.6

Hz, 1H, H7), 8.25 (dd, ^{3}J=8.0 Hz, ^{4}J = 1.6 Hz, 1H, H5); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ = 71.3 (OC(O)<u>C</u>H_2Ph), 117.8 (C8), 120.4 (<u>*Th*</u>-Br), 123.6 (C10), 125.4 (C6), 126.1 (C5), 128.4 (*o*-C_{Ph}), 128.7 (*m*-C_{Ph}), 128.8 (*p*-C_{Ph}), 131.0 (Cβ), 131.0 (Cα), 131.5 (C2-<u>*Th*</u>), 132.0 (C3), 134.1 (C7), 134.5 (*i*-C_{Ph}), 150.3 (C2), 151.8 (O<u>C</u>(O)CH_2Ph), 155.0 (C9), 171.3 (C4); 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.

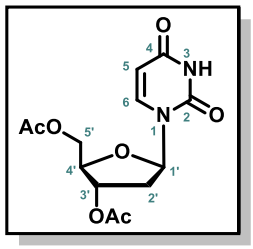


3-((2-methoxyethoxy)methoxy)-2-(5-bromofur-2-yl)-chromen-4-one (15): To a stirred solution of **10** (500 mg, 1.63 mmol) in DMF (3 mL) at 0 °C, were sequentially added K_2CO_3 (451 mg, 3.26 mmol) and 2-methoxyethoxymethyl chloride (MEMCl, 372 μL, 3.26 mmol). The reaction mixture was stirred overnight at rt. After quenching by addition of H_2O (10 mL), the organic layer was extracted with CH_2Cl_2 (3 x), 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/EA mixture (19:1 → 1:1, v/v) to provide the desired compound **15** as a brown solid (630 mg, 98 %). $C_{17}H_{15}BrO_6$ (395.20). R_f = 0.32 (cyclohexane/EA = 7:3); ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 3.28 (s, 3H, OCH_3), 3.42–3.47 (m, 2H, CH_{2B}), 3.82–3.86 (m, 2H, CH_{2A}), 5.46 (s, 2H, OCH_2O), 6.52 (d, 1H, ^{3}J=3.6 Hz, Hβ-fur.), 7.35 (d, 1H, ^{3}J=3.6 Hz, Hα-fur.), 7.30–7.38 (m, 1H, H6), 7.51 (dd, 1H, ^{4}J=0.9 Hz, ^{3}J=8.4 Hz, H8), 7.63 (ddd, 1H, ^{4}J=1.8 Hz, ^{3}J=7.0, 8.4 Hz, H7), 8.15 (dd, 1H, ^{4}J=1.6 Hz, ^{3}J=8.0 Hz, H5); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ = 59.0 (OCH_3), 69.7 (CH_{2A}), 71.6 (CH_{2B}), 96.6 (OCH_2O), 114.5 (Cβ), 118.0 (C8), 118.9 (Cα), 124.1 (C10), 124.9 (C6), 125.7 (C5), 126.2 (<u>*Fur*</u>-Br), 133.6 (C7), 135.9 (C3), 145.4 (C2-<u>*Fur*</u>), 146.1 (C2), 154.7 (C9), 173.8 (C4); MS (ESI$^+$, MeOH) *m/z*: 416.9, 418.9 [M+Na]$^+$. HRMS (ESI$^+$): *m/z* calcd for $C_{17}H_{16}BrO_6$: 395.0130 [M+H]$^+$; found 395.0127.

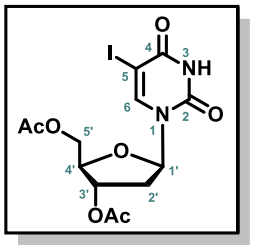
<u>Preliminary approach for the final assembly via a Sonogashira coupling</u>



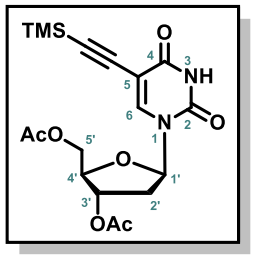
3′,5′-Di-*O*-acetyl-2′-deoxyuridine: DMAP (0.16 mmol, 20 mg) was added to a stirred suspension of 2′-deoxyuridine **16** (1 mmol, 228 mg) in acetic anhydride (7.5 mL). The reaction mixture became homogenous in 6 h, and then the volatiles were removed in vacuo. The residue was purified by flash chromatography on silica gel eluted with EA/cyclohexane (4:1 → 19:1, v/v) to provide the desired product as a white resin (296 mg, 95 %). $C_{13}H_{16}N_2O_7$ (312.28). R_f = 0.39 (EA/cyclohexane = 9:1); ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 2.10 (s, 3H, 3′-CH_3), 2.11 (s, 3H, 5′-CH_3), 2.10–2.23 (m, 1H, H2′), 2.53 (ddd, ^3J=14.3, 5.7, 2.0 Hz, 1H, H2′), 4.23–4.41 (m, 3H, H5′, H4′), 5.20 (dt, ^3J=6.6, 2.1, 1.8 Hz, 1H, H3′), 5.79 (d, ^3J=8.1 Hz, 1H, H5), 6.26 (dd, ^3J=8.4, 5.6 Hz, 1H, H1′), 7.49 (d, ^3J=8.1 Hz, 1H, H6), 9.66 (br s, 1H, NH); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ = 20.7 (CH_3), 20.8 (CH_3), 37.7 (C2′), 63.7 (C5′), 74.0 (C3′), 82.2 (C4′), 85.2 (C1′), 102.9 (C5), 138.8 (C6), 150.3 (C2), 163.4 (C4), 170.2 (5′-C=O), 170.3 (3′-C=O); MS (ESI$^+$, MeOH) *m/z*: 335.3 $[M+Na]^+$.



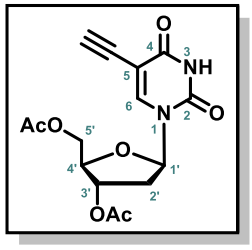
3′,5′-Di-*O*-acetyl-5-iodo-2′-deoxyuridine (17): I_2 (3.46 mmol, 880 mg) and CAN (2.88 mmol, 1.58 g) were added to a stirred solution of the diacetate (5.77 mmol, 1.8 g) in acetonitrile (60 mL). The reaction mixture was refluxed for 1 h then cooled down to rt. The volatiles were removed *in vacuo* and the residue was dissolved in EA (60 mL). The organic solution was washed with water and brine, dried over $MgSO_4$, filtered and reduced under vacuum. The residue was purified by flash chromatography on silica gel eluted with cyclohexane/EA (1:1 → 0:1, v/v) to provide the desired product **17** as a white solid (2.48 g, 98 %). $C_{13}H_{15}IN_2O_7$ (438.17). R_f = 0.61 (EA/cyclohexane = 4:1); ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 2.12 (s, 3H, 3′-CH_3), 2.13–2.24 (m, 1H, H2′$_A$), 2.21 (s, 3H, 5′-CH_3), 2.54 (ddd, ^3J=14.3, 5.6, 2.0 Hz, 1H, H2′$_B$), 4.30 (m, 1H, H4′) 4.37 (ddd, ^3J=20.0, 12.6, 1.7 Hz, 1H, H5′), 5.23 (dt, ^3J=6.4, 2.0 Hz, 1H, H3′), 6.29 (dd, ^3J=8.2, 5.6 Hz, 1H, H1′), 7.97 (s, 1H, H6), 8.81 (br s, 1H, NH); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ = 20.8 (3′-CH_3), 21.0 (5′-CH_3), 38.1 (C2′), 63.7 (C5′), 69.0 (C5), 74.0 (C3′), 82.5 (C4′), 85.4 (C1′), 143.8 (C6), 150.0 (C2), 160.0 (C4), 170.1 (5′-C=O), 170.3 (3′-C=O); MS (ESI$^+$, MeOH) *m/z*: 461.2 $[M+Na]^+$, 899.2 $[2M+Na]^+$.



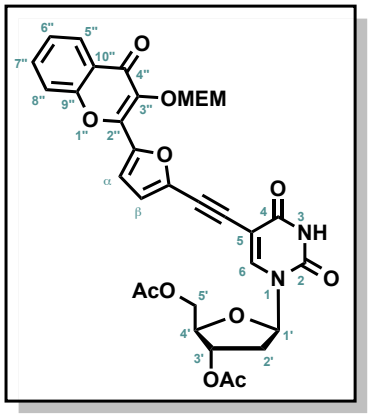
3′,5′-Di-*O*-acetyl-5-trimethylsilylethynyl-2′-deoxyuridine: To a stirred solution of **17** (4.56 mmol, 2.00 g, previously azeotropically coevaporated with dry pyridine) in THF (23 mL) under argon, TMS-acetylene (2 eq., 9.13 mmol, 1.30 mL), $Pd(PPh_3)_4$ (6 mol %, 0.27 mmol, 316 mg), CuI (5 mol %, 0.23 mmol, 43 mg) and NEt_3 (5 eq, 22.82 mmol, 3.16 mL) were added. The reaction mixture was refluxed for 30 min then cooled down to rt, filtered through a Celite® 545 pad and the volatiles were evaporated *in vacuo*. The residue was purified by flash chromatography on silica gel eluted with cyclohexane/EA (1:0 → 1:1, v/v) to provide the desired compound as a beige solid (1.75 g, 4.29 mmol, 94 %). $C_{18}H_{24}N_2O_7Si$ (408.48). R_f = 0.35 (Cyclohexane/EA = 1:1). ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 0.16 (s, 9H, $SiCH_3$), 2.08 (s, 3H, 3′-CH_3), 2.08–2.24 (m, 1H, H2′$_A$), 2.14 (s, 3H, 5′-CH_3), 2.50 (ddd, ^3J=14.2, 5.8, 2.2 Hz, 1H, H2′$_B$), 4.26 (t, ^3J =2.6 Hz, 1H, H4′), 4.32 (d, ^3J =2.6 Hz, 2H, H5′), 5.14–5.30 (m, 1H, H3′), 6.29 (dd, ^3J =7.8, 6.0 Hz, 1H, H1′), 7.80 (s, 1H, H6), 9.83 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ = -0.3 ($SiMe_3$), 20.7 (3′-CH_3), 20.8 (5′-CH_3), 38.1 (C2′), 63.7 (C5′), 73.9 (C3′), 82.4 (C4′), 85.2 (C1′), 95.1 (C≡C-TMS), 99.6 (C≡C-TMS), 100.8 (C5), 142.1 (C6), 149.3 (C2), 161.1 (C4), 170.0 (5′-C=O), 170.3, (3′-C=O); MS (ESI$^+$, MeOH) *m/z*: 430.7 $[M+Na]^+$.



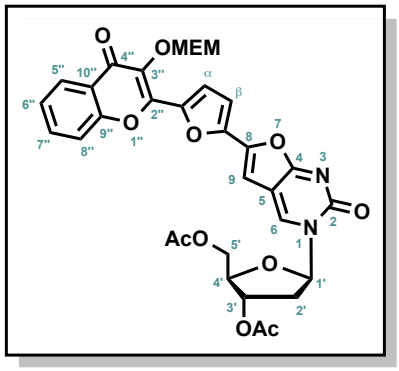
3′,5′-Di-*O*-acetyl-5-ethynyl-2′-deoxyuridine (18): TBAF (4.1 mL mL, 1.0 M in THF) was added to a stirred solution of the silylated alkyne (4.29 mmol, 1.75 g) in THF (20 mL). After 20 min, the resulting mixture was concentrated *in vacuo* and the residue was purified by flash chromatography on silica gel eluted with cyclohexane/EA (1:1 → 1:9, v/v) to provide the desired compound **18** as a white solid (1.4 g, 4.16 mmol, 97 %). $C_{15}H_{16}N_2O_7$ (336.30). R_f = 0.15 (cyclohexane/EA = 1:1); ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 2.12 (s, 3H, 3′-CH_3), 2.17 (s, 3H, 5′-CH_3), 2.17–2.27 (m, 1H, H2′$_A$), 2.56 (ddd, ^2J=14.3 Hz, ^3J=5.8, 2.4 Hz, 1H, H2′$_B$), 3.20 (s, 1H, C≡CH), 4.21–4.48 (m, 3H, H4′, H5′), 5.17–5.28 (m, 1H, H3′), 6.30 (dd, ^3J=7.8, 5.9 Hz, 1H, H1′), 7.91 (s, 1H, H6), ^{13}C-NMR ($CDCl_3$, 50 MHz): δ = 20.8 (CH_3), 20.8 (CH_3), 38.3 (C2′), 63.7 (C5′), 73.9 (C3′), 74.5 (C≡CH), 82.2 (C≡CH), 82.6 (C4′), 85.5 (C1′), 99.6 (C5), 142.7 (C6), 149.1 (C2), 161.1 (C4), 170.1 (5′-C=O), 170.4 (3′-C=O); MS (ESI$^+$, MeOH/DCM) *m/z*: 337.0 $[M+H]^+$, 359.0 $[M+Na]^+$.



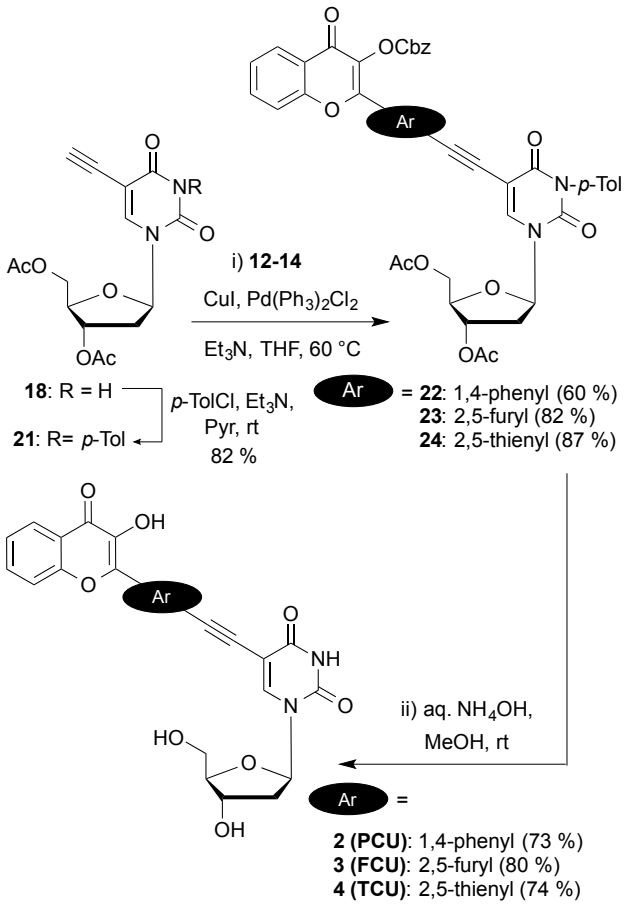
3′,5′-Di-*O*-acetyl-5-(5-(3-((2-methoxyethoxy)methoxy)-4-oxo-chromen-2-yl)fur-2-yl)ethynyl-2′-deoxyuridine (19): To a stirred solution of the chromone derivative **15** (1.0 mmol, 359 mg) and the nucleoside derivative **18** (306 mg, 0.91 mmol) in DMF (9 mL) under argon, NEt_3 (5 eq, 589 µL, 4.55 mmol), $Pd(PPh_3)_4$ (7 mol %), and CuI (7 mol %) were sequentially added. The mixture was warmed for 4 h at 60 °C and then cooled down to rt. The volatiles were removed *in vacuo* and the resulting crude was purified by flash chromatography on silica gel eluted with cyclohexane/EA (1:1 → 1:4, v/v) to give a mixture of two compounds which was further purified by reverse phase chromatography eluted with water/acetone, (2 :3, v/v) to provide the desired compound **19** as a yellow solid (178 mg, 30 %) and the 5-*endo*-dig cyclized product **20** as an orange solid (154 mg, 26 %). $C_{32}H_{30}N_2O_{13}$ (650.59). R_f = 0.4 (cyclohexane/EA = 1:4). ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 2.12 (s, 3H, CH_3), 2.21–2.35 (m, 1H, H2′$_A$), 2.28 (s, 3H, CH_3), 2.60 (ddd, ^2J=14.3 Hz, ^3J=5.8, 2.6 Hz, 1H, H2′$_B$), 3.32 (s, 3H, OCH_3), 3.46–3.51 (m, 2H, CH_{2B}), 3.85–3.89 (m, 2H, CH_{2A}), 4.29–4.36 (m, 1H, H4′), 4.36–4.44 (m, 2H, H5′), 5.24–5.29 (m, 1H, H3′), 5.52 (s, 2H, OCH_2O), 6.34 (dd, ^3J=7.3, 6.1 Hz, 1H, H1′), 6.86 (d, ^3J=3.7 Hz, 1H, Hβ-fur.), 7.39 (ddd, ^3J=7.9, 6.8 Hz, ^4J=1.4 Hz, 1H, H6″), 7.46 (d, ^3J=3.7 Hz, 1H, Hα-fur.), 7.61 (d, ^3J=8.4 Hz, 1H, H8″), 7.69 (ddd, ^3J=8.4, 6.8 Hz, ^4J=1.6 Hz, 1H, H7″), 8.06 (s, 1H, H6), 8.20 (dd, ^3J=7.9 Hz, ^4J=1.1 Hz, 1H, H5″), 9.35 (br. s, 1H, NH); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ = 20.8 (C(O)<u>C</u>H$_3$), 20.9 (C(O)<u>C</u>H$_3$), 38.4 (C2′), 58.9 (OCH_3), 63.7 (C5′), 69.7 (CH_{2A}), 71.6 (CH_{2B}), 73.7 (C3′), 82.7 (C4′), 83.2 (Fur-<u>C</u>≡C-), 85.7 (C1′), 87.0 (Fur-C≡<u>C</u>-), 96.6 (OCH_2O), 99.6 (C5), 117.8 (Cα), 118.0 (C8″), 118.4 (Cβ), 124.1 (C10″), 124.9 (C6″), 125.7 (C5″), 133.7 (C7″), 136.7 (C3″), 138.4 (<u>*Fur*</u>-C≡C-), 142.5 (C6), 145.0 (C2″-<u>*Fur*</u>), 146.8 (C2″), 148.9 (C2), 154.8 (C9″), 160.4 (C4), 170.2 (C=O), 170.3 (C=O), 173.9 (C4″); MS (ESI^+, MeOH) *m/z*: 673.1 $[M+Na]^+$. HRMS (ESI^+): *m/z* calcd for $C_{32}H_{31}N_2O_{13}$: 651.1826 $[M+H]^+$; found 651.1820.

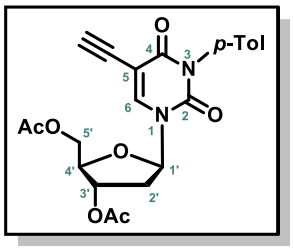


3-(2′-deoxy-3′,5′-di-*O*-acetyl-*β*-D-ribofuranosyl-1′)-6-[5-(3-((2-methoxyethoxy)methoxy)-4-oxo-chromen-2-yl)fur-2-yl]-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (20): See above procedure since **20** was obtained as a side product. **20** can be derived from **19** via a 5-*endo-dig*-cyclization according to the protocol herein. To a stirred solution of the coupled derivative (400 mg, 0.615 mmol) in a mixture of THF/NEt_3 (15:13 mL), CuI (117 mg, 0.615 mmol) was portionwise added. The reaction mixture was stirred at 70 °C for 4 h and then cooled down to rt. The volatiles were removed *in vacuo*. The residue was purified by flash chromatography on silica gel eluted with cyclohexane/EA (1:1 → 1:4, v/v) to provide the cyclized compound **20** as a yellow solid (380 mg, 95 %). $C_{32}H_{30}N_2O_{13}$ (650.59). R_f = 0.33 (cyclohexane/EA = 1:19). ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 2.09 (s, 3H, CH_3), 2.09–2.17 (m, 1H, H2′), 2.13 (s, 3H, CH_3), 3.01 (ddd, ^2J=14.3 Hz, ^3J=5.5, 2.2 Hz, 1H, H2′$_B$), 3.31 (s, 3H, OCH_3), 3.46–3.50 (m, 2H, CH_{2B}), 3.86–3.91 (m, 2H, CH_{2A}), 4.40–4.48 (m, 3H, H4′, H5′), 5.20–5.28 (m, 1H, H3′), 5.54 (s, 2H, OCH_2O), 6.32 (dd, ^3J=7.4, 5.6 Hz, 1H, H1′), 6.92 (s, 1H, H9), 7.04 (d, ^3J=3.7 Hz, 1H, Hβ-fur.), 7.41 (ddd, ^3J=8.0, 7.0 Hz, ^4J=1.1 Hz, 1H, H6″), 7.55 (d, ^3J=3.7 Hz, 1H, Hα-fur.), 7.58 (d, ^3J=8.3 Hz, 1H, H8″), 7.71 (ddd, ^3J=8.3, 7.0 Hz, ^4J=1.5 Hz, 1H, H7″), 8.22 (dd, ^3J=8.0 Hz, ^4J=1.5 Hz, 1H, H5″), 8.41 (s, 1H, H6); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ = 20.9 (C(O)<u>C</u>H$_3$), 20.9 (C(O)<u>C</u>H$_3$), 39.4 (C2′), 59.0 (OCH_3), 63.7 (C5′), 69.7 (CH_{2A}), 71.6 (CH_{2B}), 74.0 (C3′), 83.5 (C4′), 88.8 (C1′), 96.8 (OCH_2O), 99.3 (C9), 107.4 (C5), 112.3 (Cα), 117.9 (C8″), 118.5 (Cβ), 124.2 (C10″), 125.0 (C6″), 125.8 (C5″), 133.7 (C7″), 135.6 (<u>*Fur*</u>-C8), 136.9 (C3″), 145.7 (C2″-<u>*Fur*</u>), 146.0 (C6), 146.6 (C8), 146.8 (C2″), 154.2 (C2), 154.7 (C9″), 170.3 (C=O), 170.4 (C=O), 171.7 (C4), 173.8 (C4″); MS (ESI^+, MeOH) *m/z*: 673.1 $[M+Na]^+$. HRMS (ESI^+): *m/z* calcd for $C_{32}H_{31}N_2O_{13}$: 651.1826 $[M+H]^+$; found 651.1821.

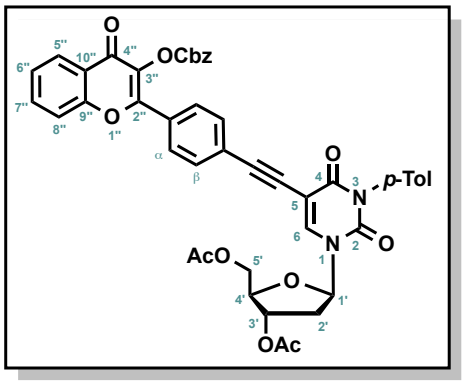


Preparation of the fluorescent emissive nucleosides 2, 3 and 4





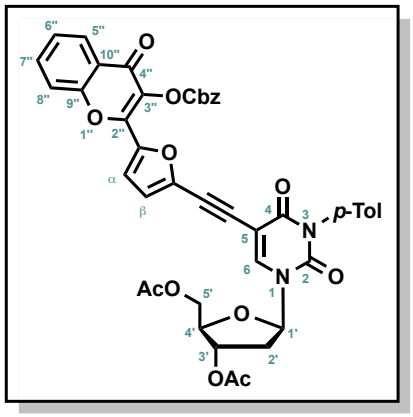
3′,5′-Di-*O*-acetyl-3-*N*-(4-methylbenzoyl)-5-ethynyl-2′-deoxyuridine (21): *p*-Toluoyl chloride (0.36 mmol, 0.05 mL) and $NEt(iPr)_2$ (0.9 mmol, 0.15 mL) were added to a stirred solution of **18** (0.3 mmol, 100 mg) in pyridine (3 mL). The reaction mixture was stirred for 5 h at rt, and then the volatiles were reduced *in vacuo*. The residue was purified by flash chromatography on silica gel eluted with cyclohexane/EA (9:1 → 1:4, v/v) to provide the desired compound **21** as a beige powder (111 mg, 82 %). $C_{23}H_{22}N_2O_8$ (454.43). R_f = 0.42 (cyclohexane/EA = 1:1); ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 2.08 (s, 3H, CH_3), 2.18 (s, 3H, CH_3), 2.28 (dd, ^2J=14.4 Hz, ^3J=7.4 Hz, 1H, H2′$_A$), 2.43 (s, 3H, *p*-CH_3), 2.56 (ddd, ^2J=14.4 Hz, ^3J=5.8, 2.6 Hz, 1H, H2′$_B$), 3.20 (s, 1H, C≡CH), 4.28–4.32 (m, 1H, H4′), 4.32–4.45 (m, 1H, H5′), 5.24 (dt, ^3J=6.6, 2.6 Hz, 1H, H3′), 6.29 (dd, ^3J=7.6, 6.0 Hz, 1H, H1′), 7.28 (d, ^3J=8.2 Hz, 2H, H$_m$-Tol), 7.80 (d, ^3J=8.2 Hz, 2H, H$_o$-Tol), 7.99 (s, 1H, H6); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ = 20.8 (C(O)<u>C</u>H$_3$), 20.8 (C(O)<u>C</u>H$_3$), 21.9 (CH_3-Tol), 38.3 (C2′), 63.6 (C5′), 73.7 (C3′), 74.1 (C≡<u>C</u>H), 82.6 (<u>C</u>≡CH), 82.7 (C4′), 85.8 (C1′), 99.6 (C5), 128.4 (C$_i$-Tol), 129.9 (C$_m$-Tol), 130.7 (C$_o$-Tol), 142.3 (C6), 146.8 (C$_p$-Tol), 148.1 (C2), 160.1 (C4), 167.1 (C=O-Tol), 170.1 (C=O), 170.3 (C=O); MS (ESI$^+$, MeOH/DCM) *m/z:* 454.9 [M+H]$^+$, 477.1 [M+Na]$^+$. HRMS (ESI$^+$): *m/z* calcd for $C_{23}H_{23}N_2O_8$: 455.1454 [M+H]$^+$; found 455.1450.



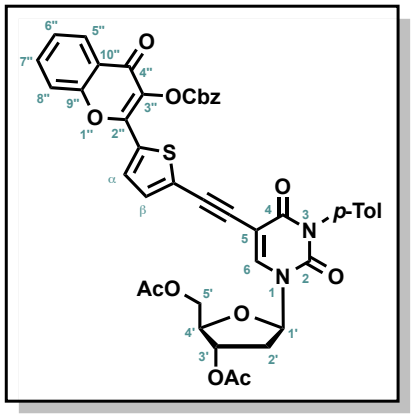
3′,5′-Di-*O*-acetyl-4-(5-(3-(benzyloxycarbonyloxy)-4-oxo-chromen-2-yl)phenyl)ethynyl-3-*N*-(4-methylbenzoyl)-2′-deoxyuridine (22): To a stirred solution of the chromone derivative **12** (0.363 mmol, 172 mg) and the nucleoside derivative **21** (0.242 mmol, 110 mg) in THF (5.5 mL) under argon, NEt_3 (5 eq, 163 µL, 1.21 mmol), $Pd(PPh_3)_2Cl_2$ (7 mol %) and CuI (7 mol %) were sequentially added. The mixture was warmed for 2 h at 60 °C then cooled down to rt. The volatiles were removed *in vacuo* and the resulting crude was purified by flash chromatography on silica gel eluted with toluene/EA (7:3 → 5:5, v/v) to provide the desired compound **22** as a yellow solid (120

mg, 60 %). $C_{46}H_{36}N_2O_{13}$ (824.78). R_f = 0.26 (toluene/EA = 4:1). 2.12 (s, 3H, CH_3), 2.22 (s, 3H, CH_3), 2.28–2.39 (dd, ^2J=14.3 Hz, ^3J=7.0 Hz, 1H, H2′$_A$), 2.44 (s, 3H, CH_3(Tol)), 2.61 (ddd, ^2J=14.3 Hz, ^3J=5.8, 2.4 Hz, 1H, H2′$_B$), 4.31–4.38 (m, 1H, H4′), 4.40–4.47 (m, 2H, H5′), 5.28 (s, 2H, CH_2), 5.28–5.33 (m, 1H, H3′), 6.34 (dd, ^3J=7.4, 5.8 Hz, 1H, H1′), 7.32 (d, ^3J=8.1 Hz, 2H, H$_m$-Tol), 7.36–7.41 (m, 5H, Cbz), 7.45 (ddd, ^3J=8.0, 7.1 Hz, ^4J=1.0 Hz, 1H, H6″), 7.56 (d, ^3J=8.5 Hz, 2H, H$_m$-Ph), 7.57 (d, ^3J=8.5 Hz, 1H, H8″), 7.74 (ddd, ^3J=8.5, 7.1 Hz, ^4J=1.6 Hz, 1H, H7″), 7.85 (d, ^3J=8.1 Hz, 2H, H$_o$-Tol), 7.88 (d, ^3J=8.5 Hz, 2H, H$_o$-Ph), 8.04 (s, 1H, H6), 8.28 (dd, ^3J=8.0 Hz, ^4J=1.6 Hz, 1H, H5″); ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 20.8 (C(O)<u>C</u>H$_3$), 20.9 (C(O)<u>C</u>H$_3$), 21.9 (CH_3-Tol), 38.4 (C2′), 63.7 (C5′), 71.1 (OC(O)<u>C</u>H$_2$Ph), 73.7 (C3′), 82.6 (Ph-C≡<u>C</u>-), 82.8 (C4′), 85.9 (C1′), 93.5 (Ph-<u>C</u>≡C-), 100.5 (C5), 118.1 (C8″), 123.6 (C10″), 125.4 (C$_p$-Ph), 125.4 (C6″), 126.1 (C5″), 128.3 (*o*-C$_{Cbz}$), 128.4 (C$_o$-Ph), 128.4 (C$_i$-Tol), 128.6 (*m*-C$_{Cbz}$), 128.8 (*p*-C$_{Cbz}$), 129.6 (C2″-<u>*i*-*Ph*</u>), 130.0 (C$_m$-Tol), 130.7 (C$_o$-Tol), 131.7 (C$_m$-Ph), 134.1 (C3″), 134.2 (*i*-C$_{Cbz}$), 134.4 (C7″), 141.3 (C6), 146.9 (C$_p$-Tol), 148.1 (C2), 152.2 (O<u>C</u>(O)CH$_2$Ph), 155.1 (C2″), 155.5

3′,5′-Di-*O*-acetyl-5-(5-(3-(benzyloxycarbonyloxy)-4-oxo-chromen-2-yl)fur-2-yl)ethynyl-3-*N*-(4-methylbenzoyl)-2′-deoxyuridine (23): To a stirred solution of the chromone derivative **13** (0.238 mmol, 110 mg) and the nucleoside derivative **21** (0.198 mmol, 90 mg) in THF (4.5 mL) under argon, NEt_3 (5 eq, 162 µL, 0.99 mmol), $Pd(PPh_3)_2Cl_2$ (7 mol %), and CuI (7 mol %) were sequentially added. The mixture was warmed for 2 h at 60 °C and then cooled down to rt. The volatiles were removed *in vacuo* and the resulting crude was purified by flash chromatography on silica gel eluted with toluene/EA (95:5 → 7:3, v/v) to provide the desired compound **23** as a yellow solid (132 mg, 82 %). $C_{44}H_{34}N_2O_{14}$ (814.75). R_f = 0.26 (toluene/EA = 4:1). ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 2.12 (s, 3H, CH_3), 2.24–2.38 (m, 1H, H2′$_A$), 2.28 (s, 3H, CH_3), 2.45 (s, 3H, CH_3(Tol)), 2.61 (ddd, ^2J=14.4 Hz, ^3J=5.9, 2.9 Hz, 1H, H2′$_B$), 4.30–4.38 (m, 1H, H4′), 4.38–4.45 (m, 2H, H5′), 5.25–5.31 (m, 1H, H3′), 5.34 (s, 2H, CH_2), 6.33 (dd, ^3J=7.2, 6.2 Hz, 1H, H1′), 6.79 (d, ^3J=3.8 Hz, 1H, Hβ-thioph.), 7.16 (d, ^3J=4.0 Hz, 1H, Hα-thioph.), 7.28 (d, ^3J=8.2 Hz, 2H, H$_m$-Tol), 7.34–7.48 (m, 6H, H6″, Cbz), 7.63 (d, ^3J=7.8 Hz, 1H, H8″), 7.74 (ddd, ^3J=8.4, 6.8 Hz, ^4J=1.4 Hz, 1H, H7″), 7.84 (d, ^3J=8.2 Hz, 2H, H$_o$-Tol), 8.13 (s, 1H, H6), 8.26 (dd, ^3J=7.9 Hz, ^4J=1.5 Hz, 1H, H5″); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ = 20.8 (C(O)<u>C</u>H$_3$), 20.9 (C(O)<u>C</u>H$_3$), 21.9 (CH_3-Tol), 38.4 (C2′), 63.6 (C5′), 71.2 (OC(O)<u>C</u>H$_2$Ph), 73.5 (C3′), 82.8 (C4′), 83.2 (Fur-<u>C</u>≡C-), 86.0 (C1′), 87.0 (Fur-C≡<u>C</u>-), 99.4 (C5), 117.9 (Cα), 118.1 (C8″), 118.4 (Cβ), 123.7 (C10″), 125.4 (C6″), 125.9 (C5″), 128.3 (*o*-C$_{Cbz}$), 128.3 (C$_i$-Tol),128.6 (*m*-C$_{Cbz}$), 128.7 (*p*-C$_{Cbz}$), 130.0 (C$_m$-Tol), 130.7 (C$_o$-Tol), 132.0 (C3″), 134.3 (C7″), 134.5 (*i*-C$_{Cbz}$), 139.3 (C2″-<u>*Fur*</u>), 142.3 (C6), 143.7 (<u>*Fur*</u>-C≡C-), 146.8 (C2″), 147.0 (C$_p$-Tol), 148.0 (C2), 151.9 (O<u>C</u>(O)CH$_2$Ph), 155.0 (C9″), 159.4 (C4), 167.0 (C=O-Tol), 170.1 (C=O), 170.3 (C=O), 171.4 (C4″); MS (ESI$^+$, MeOH) *m/z*: 815.5 [M+H]$^+$, 837.4 [M+Na]$^+$, 853.4 [M+K]$^+$. HRMS (ESI$^+$): *m/z* calcd for $C_{44}H_{34}N_2NaO_{14}$: 837.1902 [M+Na]$^+$; found 837.1895.



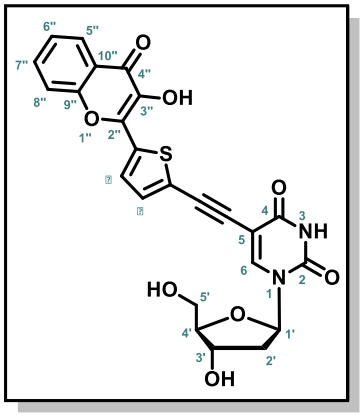
3′,5′-Di-*O*-acetyl-5-(5-(3-(benzyloxycarbonyloxy)-4-oxo-chromen-2-yl)thien-2-yl)ethynyl-3-*N*-(4-methylbenzoyl)-2′-deoxyuridine (24): To a stirred solution of the chromone derivative **14** (0.26 mmol, 120 mg) and the acetylenic nucleoside **21** (0.24 mmol, 108 mg) in THF (2 mL) under argon, NEt_3 (5 eq, 162 µL, 1.2 mmol), $Pd(PPh_3)_2Cl_2$ (7 mol %), and CuI (7 mol %) were sequentially added. The mixture was warmed for 2 h at 60 °C and then cooled down to rt. The volatiles were reduced *in vacuo* and the resulting crude was purified by flash chromatography on silica gel eluted with cyclohexane/EA (7:3 → 1:1, v/v) to provide the desired compound **24** as yellow solid (173 mg, 87 %). $C_{44}H_{34}N_2O_{13}S$ (830.81). R_f = 0.34 (cyclohexane/EA = 1:1); ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 2.11 (s, 3H, CH_3), 2.19 (s, 3H, CH_3), 2.33 (ddd, ^2J=14.4 Hz, ^3J=7.4, 7.0 Hz, 1H, H2′$_A$), 2.44 (s, 3H, CH_3(Tol)), 2.61 (ddd, ^2J=14.4 Hz, ^3J=6.0, 2.8 Hz, 1H, H2′$_B$), 4.30–4.37 (m, 1H, H4′), 4.37–4.45 (m, 2H, H5′), 5.23–5.32 (m, 1H, H3′), 5.37 (s, 2H, CH_2), 6.33 (dd, ^3J=7.4, 6.0 Hz, 1H, H1′), 7.27 (d, ^3J=4.0 Hz, 1H, Hβ-thioph.), 7.28 (d, ^3J=8.2 Hz, 2H, H$_m$-Tol), 7.32–7.51 (m, 6H, H6″, Cbz), 7.52 (d, ^3J=8.5 Hz, 1H, H8″), 7.72 (ddd, ^3J=8.5, 7.0 Hz, ^4J=1.6 Hz, 1H, H7″), 7.77 (d, ^3J=4.0 Hz, 1H, Hα-thioph.), 7.84 (d, ^3J=8.2 Hz, 2H, H$_o$-Tol), 8.05 (s, 1H, H6), 8.24 (dd, ^3J=7.9 Hz, ^4J=1.5 Hz, 1H, H5″); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ = 20.8 (C(O)<u>C</u>H$_3$), 20.9 (C(O)<u>C</u>H$_3$), 21.9 (CH_3-Tol), 38.4 (C2′), 63.6 (C5′), 71.4 (OC(O)<u>C</u>H$_2$Ph), 73.6 (C3′), 82.8 (C4′), 86.0 (C1′), 86.5 (Th-<u>C</u>≡C-), 87.4 (Th-C≡<u>C</u>-), 100.1 (C5), 117.9 (C8″), 123.6 (C10″), 125.4 (C6″), 126.0 (C5″), 128.4 (*o*-C$_{Cbz}$),



128.4 (C_i-Tol), 128.7 (*m*-C_{Cbz}), 128.8 (C2-*Th*), 128.8 (*p*-C_{Cbz}), 130.0 (C_m-Tol), 130.7 (C_o-Tol), 130.7 (Cα), 132.0 (C3″), 132.1 (*Th*-C≡C-), 133.2 (Cβ), 134.2 (C7″), 134.4 (*i*-C_{Cbz}), 141.7 (C6), 147.0 (C_p-Tol), 148.0 (C2), 150.4 (C2″), 151.8 (O*C*(O)CH_2Ph), 155.0 (C9″), 159.6 (C4), 167.1 (C=O-Tol), 170.0 (C=O), 170.3 (C=O), 171.3 (C4″); HRMS (ESI^+): *m/z* calcd for $C_{44}H_{35}N_2O_{13}S$: 831.1854 $[M+H]^+$; found 831.1854.

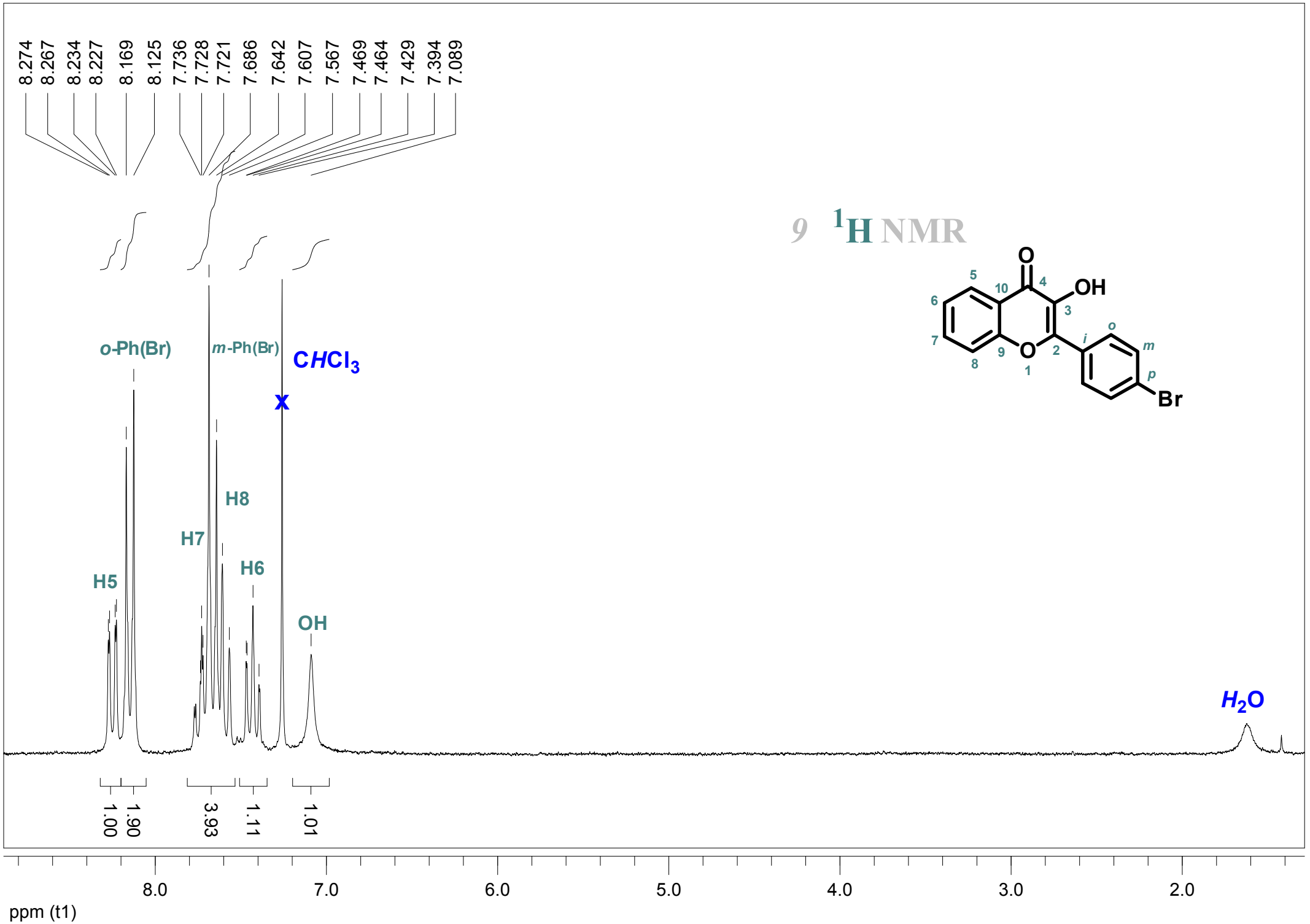
5-(5-(3-Hydroxy-4-oxo-chromen-2-yl)phenyl)ethynyl-2′-deoxyuridine (2: PCU): To a stirred solution of **22** (0.024 mmol, 20 mg) in MeOH (1.4 mL) was dropwise added a 33 % ammonium hydroxide aq. solution (900 µL). Protected from light, the reaction mixture was stirred at rt for 6 h and then acidified with AcOH (900 µL). After addition of 6.5 mL of milliQ water, the resulting solution was stored 12 h at 0 °C. The heterogeneous mixture was centrifuged for settling solid particles and the supernatant was carefully taken out with a syringe with a fine needle. The precipitate was triturated with a solution of MeOH/H_2O (7:3) and after centrifugation, the supernatant was removed by suction. The final product was partially solubilized in water (300 µL). The resulting solution was frozen and lyophilized to provide the desired compound **2 (PCU)** as an orange solid (6 mg, 50 %). $C_{26}H_{20}N_2O_8$ (488.45). R_f = 0.65 (DCM/MeOH = 9:1). ^{1}H-NMR (DMSO-d^6, 500 MHz): δ = 2.14–2.18 (m, 1H, H2′$_A$), 2.21 (dd, ^2J=13.5 Hz, ^3J=6.5 Hz, 1H, H2′$_B$), 3.60 (ddd, ^2J=12.0 Hz, ^3J=5.0, 3.5 Hz, 1H, H5′$_A$), 3.68 (ddd, ^2J=12.0 Hz, ^3J=5.0, 3.5 Hz, 1H, H5′$_B$), 3.81–3.83 (q, ^3J=3.5 Hz, 1H, H4′), 4.26–4.29 (m, 1H, H3′), 5.19 (t, ^3J=5.0 Hz, 1H, 5′-OH), 5.27 (d, ^3J=4.0 Hz, 1H, 3′-OH), 6.14 (t, ^3J=6.5 Hz, 1H, H1′), 7.48 (td, ^3J=8.0 Hz, ^4J=1.5 Hz, 1H, H6″), 7.66 (d, ^3J=8.5 Hz, 2H, $H_{m\text{-Ph}}$), 7.79 (d, ^3J=8.0 Hz, 1H, H8″), 7.83 (td, ^3J=8.0 Hz, ^4J=1.5 Hz, 1H, H7″), 8.13 (dd, ^3J=8.0 Hz, ^4J=1.5 Hz, 1H, H5″), 8.29 (d, ^3J=8.5 Hz, 2H, $H_{o\text{-Ph}}$), 8.45 (s, 1H, H6), 9.87 (s, 1H, 3″-OH), 11.73 (s, 1H, NH); ^{13}C-NMR (DMSO-d^6, 125 MHz): δ = 40.1 (C2′), 60.7 (C5′), 69.8 (C3′), 84.6 (Ph-C≡*C*-), 84.8 (C4′), 87.5 (C1′), 91.6 (Ph-*C*≡C-), 97.8 (C5), 118.4 (C8″), 121.2 (C10″), 123.5 (C6″), 124.5 ($C_{i\text{-Ph}}$), 124.7 (C5″), 127.6 ($C_{o\text{-Ph}}$), 131.1 ($C_{p\text{-Ph}}$), 131.1 ($C_{m\text{-Ph}}$), 133.8 (C7″), 139.5 (C3″), 144.0 (C2), 144.2 (C6), 149.3 (C2″), 154.5 (C9″), 161.3 (C4), 172.9 (C4″); MS (ESI^+, MeOH) *m/z*: 489.4 $[M+H]^+$, 511.3 $[M+Na]^+$. HRMS (ESI^+): *m/z* calcd for $C_{26}H_{21}N_2O_8$: 489.1292 $[M+H]^+$; found 489.1294.

5-(5-(3-Hydroxy-4-oxo-chromen-2-yl)fur-2-yl)ethynyl-2′-deoxyuridine (3: FCU): To a stirred solution of **23** (0.049 mmol, 40 mg) in MeOH (3 mL) was dropwise added a 33 % ammonium hydroxide aq. solution (1.8 mL). Protected from light, the reaction mixture was stirred at rt for 4 h and then acidified with AcOH (1.7 mL). After addition of 13 mL of milliQ water, the resulting solution was stored 12 h at 0 °C. The heterogeneous mixture was centrifuged for settling solid particles and the supernatant was carefully taken out with a syringe with a fine needle. The precipitate was triturated with a solution of MeOH/H_2O (7:3), and after centrifugation the supernatant was removed by suction. The final product was partially solubilized in water (300 µL). The resulting solution was frozen and lyophilized to provide the desired compound **3 (FCU)** as an orange solid (14 mg, 58 %). $C_{24}H_{18}N_2O_9$ (478.41). R_f = 0.42 (DCM/MeOH = 9:1). ^{1}H-NMR (DMSO-d^6, 500 MHz): δ = 2.14–2.20 (m, 1H, H2′$_A$), 2.22 (dd, ^2J=13.7 Hz, ^3J=6.3 Hz, 1H, H2′$_B$), 3.59 (ddd, ^2J=11.8 Hz, ^3J=4.5, 3.5 Hz, 1H, H5′$_A$), 3.67 (ddd, ^2J=11.8 Hz, ^3J=4.5, 3.5 Hz, 1H, H5′$_B$), 3.82 (q, ^3J=3.5 Hz, 1H, H4′), 4.24–4.28 (m, 1H, H3′), 5.18 (t, ^3J=5.0 Hz, 1H, 5′-OH), 5.27 (d, ^3J=4.5 Hz, 1H, 3′-OH), 6.13 (t, ^3J=6.3 Hz, 1H, H1′), 7.11 (d, ^3J=3.5 Hz, 1H, Hβ-thioph.), 7.33 (d, ^3J=3.5 Hz, 1H, Hα-thioph.), 7.48 (ddd, ^3J=8.0, 5.5 Hz, ^4J=2.5 Hz, 1H, H6″), 7.78–7.82 (m, 2H, H8″, H7″), 8.12 (dd, ^3J=8.0 Hz, ^4J=1.0 Hz, 1H, H5″), 8.50 (s, 1H, H6), 10.28 (s, 1H, 3″-OH), 11.80 (s, 1H, NH); ^{13}C-NMR (DMSO-d^6, 125 MHz): δ = 40.2 (C2′), 60.7 (C5′), 69.8 (C3′), 81.5 (Fur-*C*≡C-), 85.0 (C1′), 87.6 (C4′), 89.1 (Fur-C≡*C*-), 96.8 (C5), 116.2 (Cα), 118.3 (Cβ), 118.4 (C8″), 121.8 (C10″), 124.7 (C6″), 124.8 (C5″), 133.7 (C7″), 137.2 (C3″), 138.0 (*Fur*-C≡C-), 138.2 (C2″-*Fur*), 145.0 (C6), 145.0 (C2″), 149.2 (C2), 154.1 (C9″), 161.1 (C4), 171.8 (C4″); MS (ESI^+, MeOH) *m/z*: 479.1 $[M+H]^+$, 501.3 $[M+Na]^+$. HRMS (ESI^+): *m/z* calcd for $C_{24}H_{19}N_2O_9$: 479.1085 $[M+H]^+$; found 479.1084.



5-(5-(3-Hydroxy-4-oxo-chromen-2-yl)thien-2-yl)ethynyl-2′-deoxyuridine (4: TCU): To a stirred suspension of **24** (0.042 mmol, 35 mg) in MeOH (3 mL) was dropwise added a 33 % ammonium hydroxide aq. solution (1.8 mL). Protected from light, the reaction mixture was stirred at rt for 3 h and then acidified with AcOH (1.8 mL). After addition of 12 mL of milliQ water, the resulting solution was stored 12 h at 0 °C. The heterogeneous mixture was centrifuged for settling solid particles and the supernatant was carefully taken out with a syringe with a fine needle. The precipitate was triturated with a solution of MeOH/H_2O (7:3), and after centrifugation the supernatant was removed by suction. This operation was repeated 3 times. The final product was partially solubilized in water (300 µL). The resulting solution was frozen and lyophilized to provide the desired compound **4 (TCU)** as an orange solid (11 mg, 54 %). $C_{24}H_{18}N_2O_8S$ (494.47). R_f = 0.50 (DCM/MeOH = 9:1). ^{1}H-NMR (DMSO-d^6, 500 MHz): δ = 2.15 (ddd, ^2J=13.5 Hz, ^3J=6.5, 4.0 Hz, 1H, H2′$_A$), 2.21 (dd, ^2J=13.5 Hz, ^3J=6.5 Hz, 1H, H2′$_B$), 3.59 (ddd, ^2J=12.0 Hz, ^3J=4.5, 3.5 Hz, 1H, H5′$_A$), 3.66 (ddd, ^2J=12.0 Hz, ^3J=4.5, 3.5 Hz, 1H, H5′$_B$), 3.82 (q, ^3J=3.5 Hz, 1H, H4′), 4.24–4.28 (m, 1H, H3′), 5.19 (t, ^3J=5.0 Hz, 1H, 5′-OH), 5.27 (d, ^3J=4.5 Hz, 1H, 3′-OH), 6.13 (t, ^3J=6.5 Hz, 1H, H1′), 7.47 (ddd, ^3J=8.0, 7.0 Hz, ^4J=1.0 Hz, 1H, H6″), 7.48 (d, ^3J=4.3 Hz, 1H, Hβ-thioph.), 7.73 (d, ^3J=8.5 Hz, 1H, H8″), 7.81 (ddd, ^3J=8.5, 7.0 Hz, ^4J=1.5 Hz, 1H, H7″), 7.89 (d, ^3J=4.3 Hz, 1H, Hα-thioph.), 8.10 (dd, ^3J=8.0 Hz, ^4J=1.5 Hz, 1H, H5″), 8.44 (s, 1H, H6), 10.67 (s, 1H, 3″-OH), 11.77 (s, 1H, NH); ^{13}C-NMR (DMSO-d^6, 125 MHz): δ = 40.1 (C2′), 60.7 (C5′), 69.8 (C3′), 84.7 (Th-<u>C</u>≡C-), 84.9 (C1′), 87.6 (C4′), 89.1 (Th-C≡<u>C</u>-), 97.6 (C5), 118.1 (C8″), 121.8 (C10″), 124.6 (C6″), 124.8 (C5″), 125.8 (C2″-<u>Th</u>), 128.0 (Cα), 132.4 (Cβ), 133.2 (<u>Th</u>-C≡C-), 133.7 (C7″), 137.3 (C3″), 142.1 (C2″), 144.3 (C6), 149.3 (C2), 154.1 (C9″), 161.1 (C4), 171.9 (C4″); MS (ESI$^+$, MeOH) *m/z*: 495.3 [M+H]$^+$, 517.2 [M+Na]$^+$. HRMS (ESI$^-$): *m/z* calcd for $C_{24}H_{19}N_2O_8S$: 493.0700 [M-H]$^-$; found 493.0689.

1. <u>NMR spectra</u>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7.469
7.464
7.429
7.394
7.089
9 1H NMR
O
OH
Br
o-Ph(Br)
m-Ph(Br)
CHCl3
H8
H7
H6
H5
OH
H2O
1.00
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7.0
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4.0
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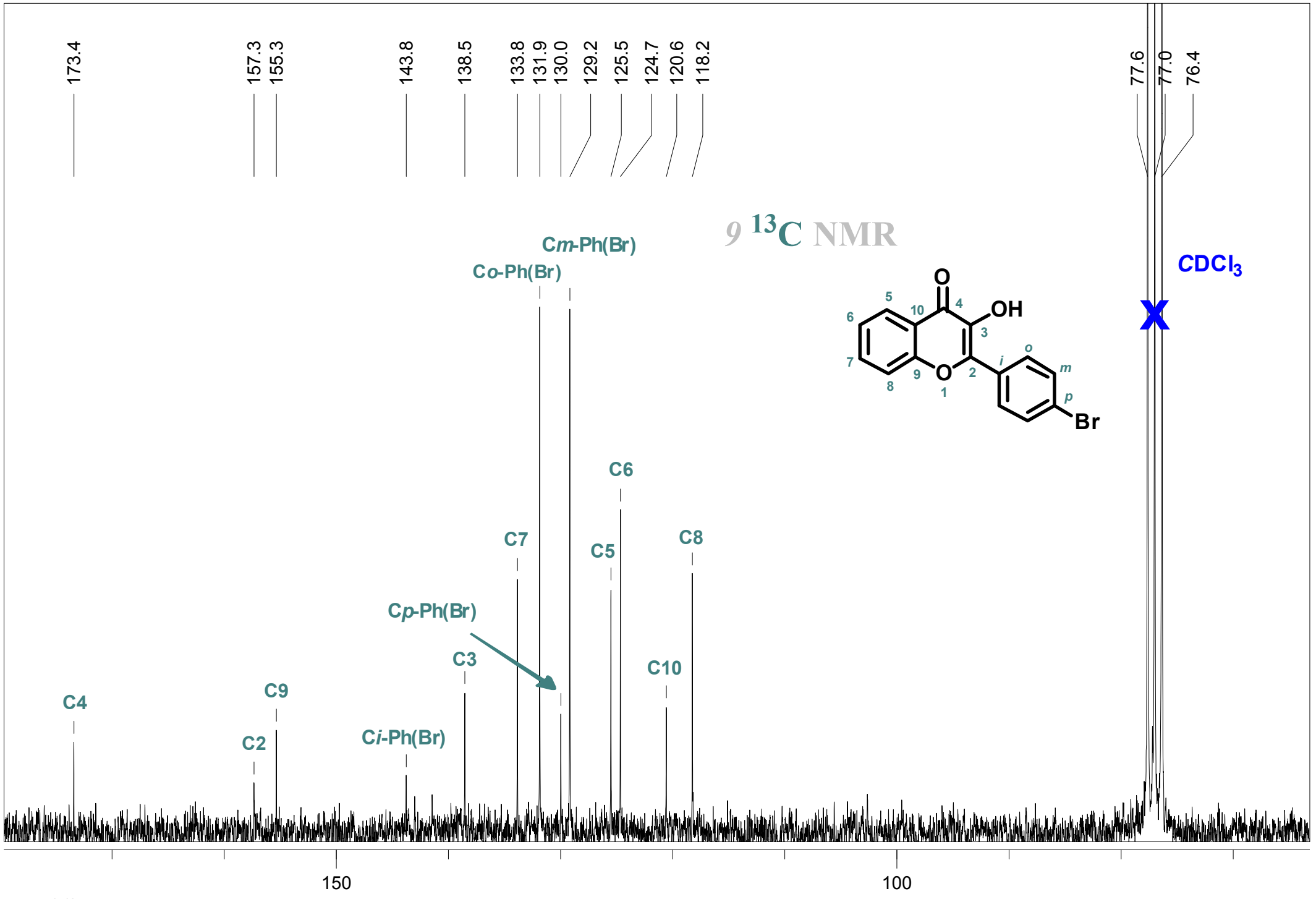
2.0
ppm (t1)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157.3
155.3
143.8
138.5
133.8
131.9
130.0
129.2
125.5
124.7
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118.2
77.6
77.0
76.4
9 13C NMR
CDCl3
Cm-Ph(Br)
Co-Ph(Br)
C6
C7
C5
C8
Cp-Ph(Br)
C3
C10
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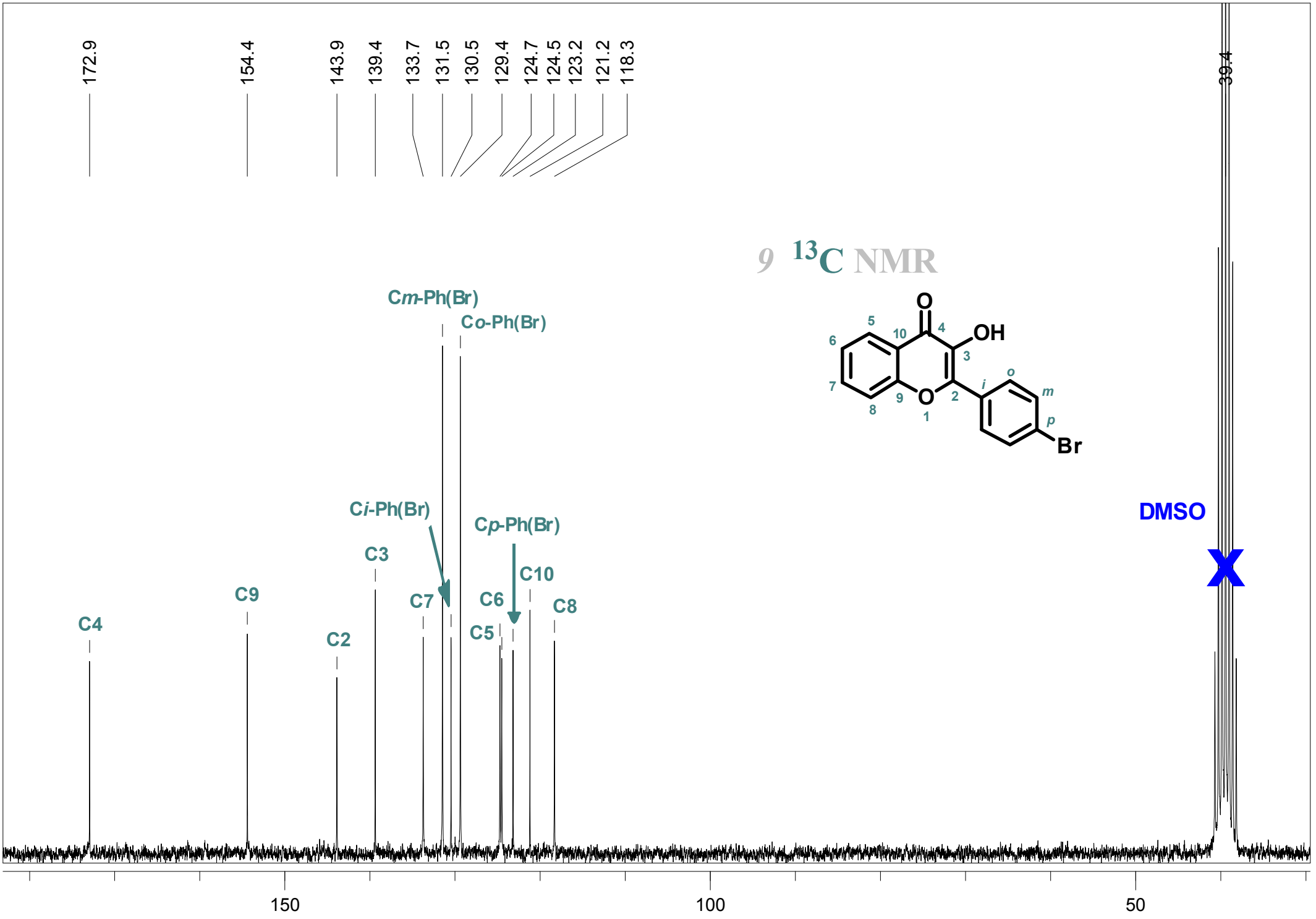
Ci-Ph(Br)
O
OH
Br
150
100
ppm (t1)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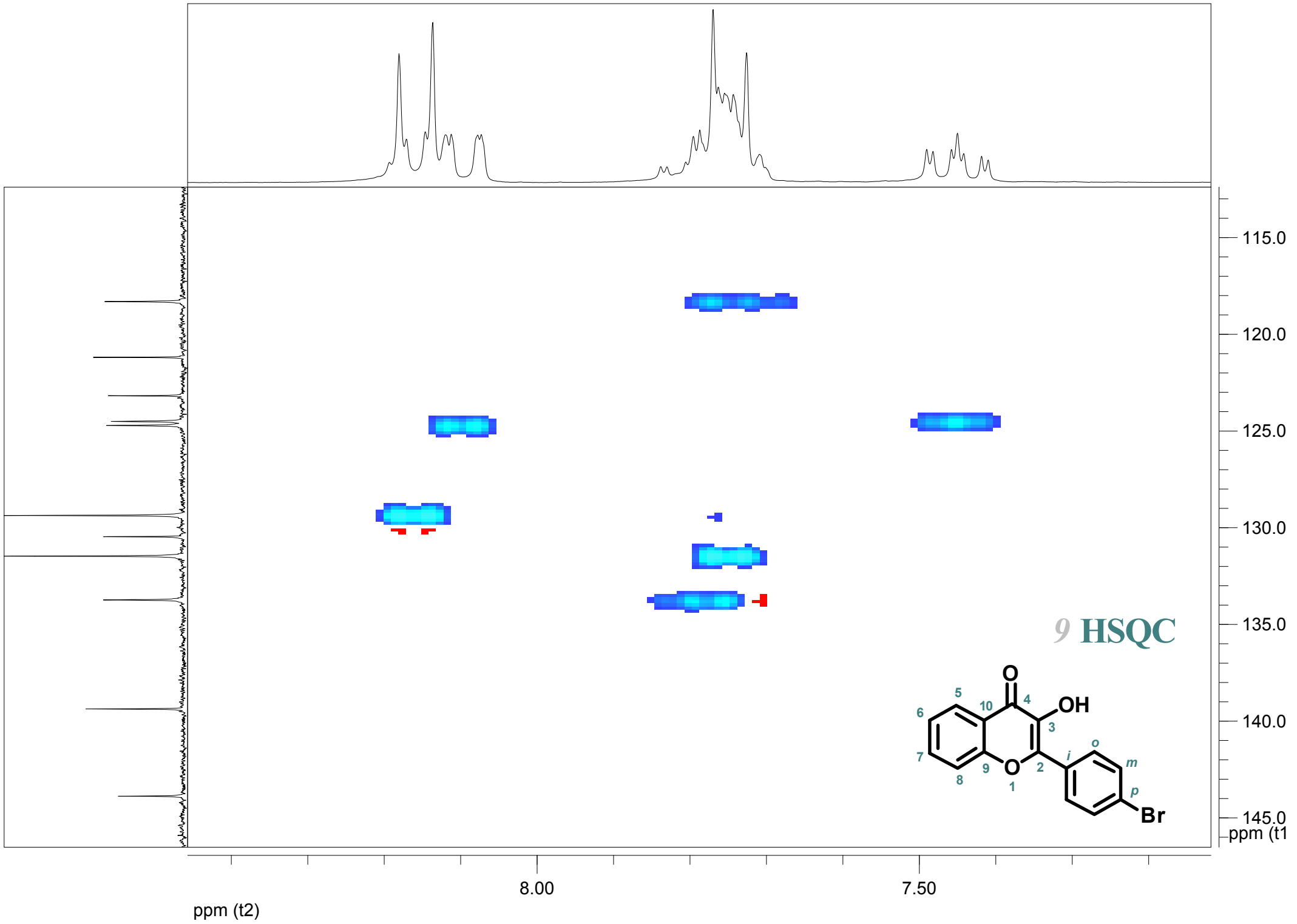
143.9
139.4
133.7
131.5
130.5
129.4
124.7
124.5
123.2
121.2
118.3
39.4
9 13C NMR
Cm-Ph(Br)
Co-Ph(Br)
Ci-Ph(Br)
Cp-Ph(Br)
C4
C9
C2
C3
C7
C6
C5
C10
C8
DMSO
OH
Br
150
100
50
ppm (t1)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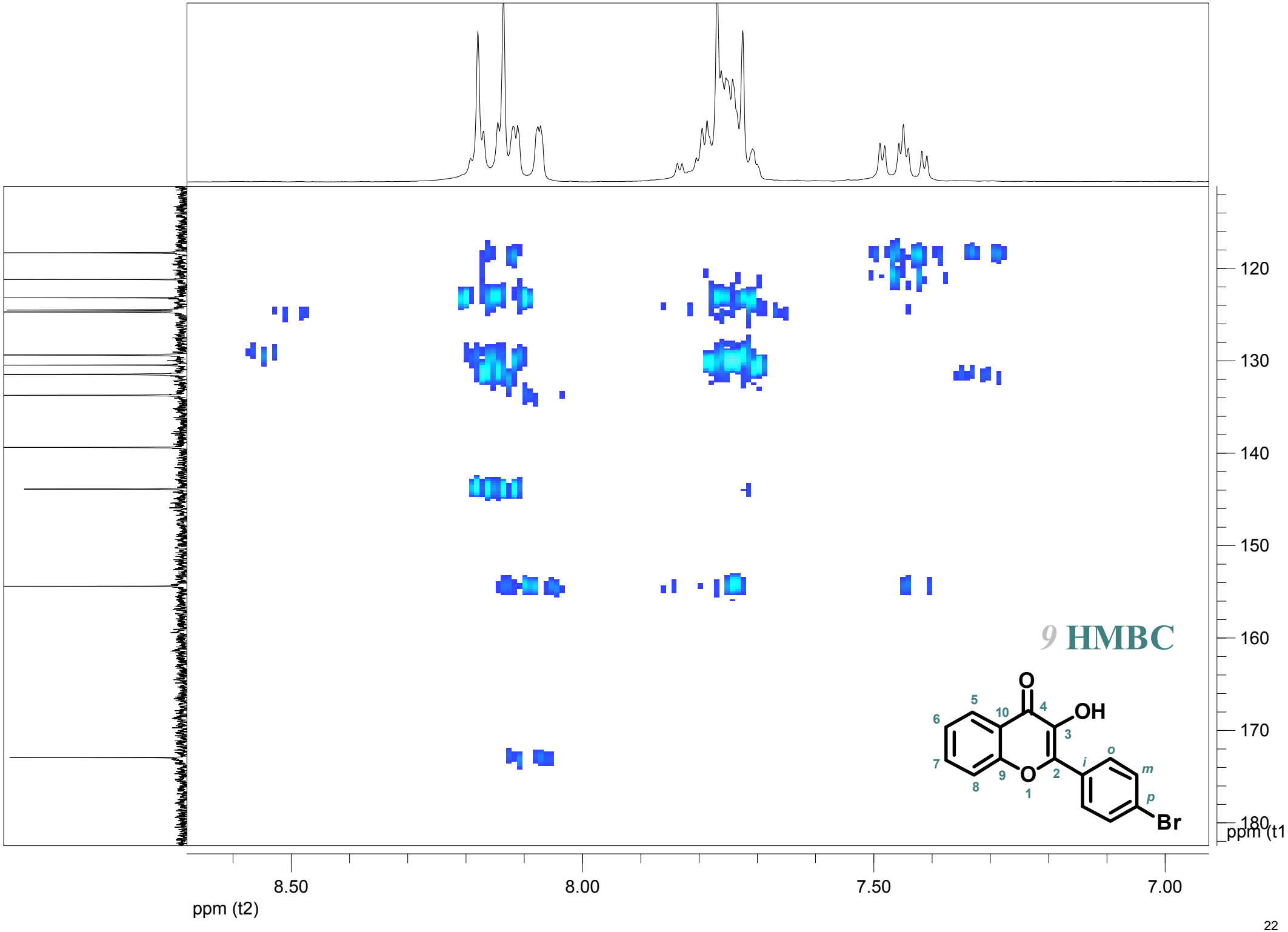
125.0
130.0
135.0
140.0
145.0
ppm (t1
8.00
7.50
ppm (t2)
O
OH
Br
1
2
3
4
5
6
7
8
9
10
i
o
m
p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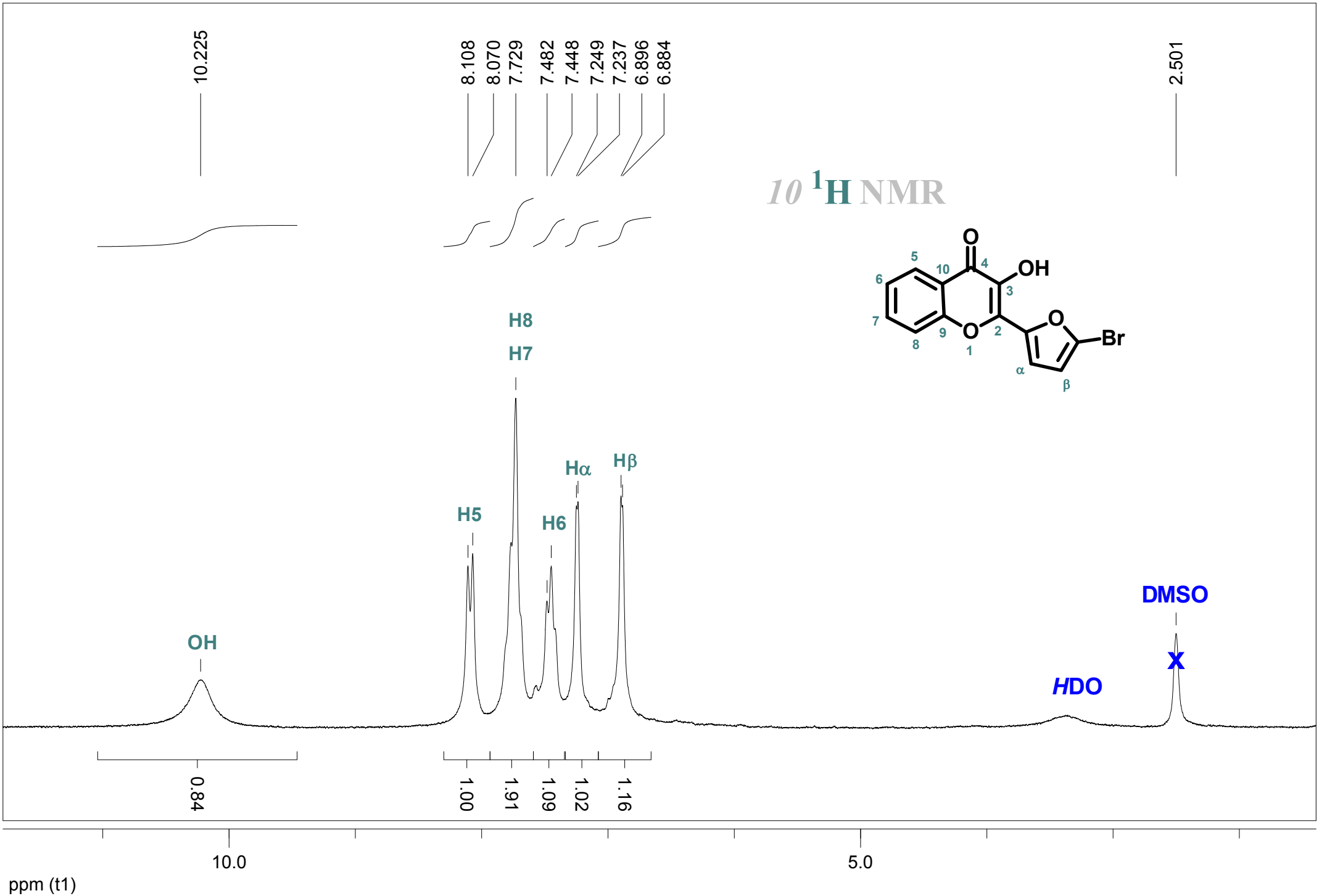
130
140
150
160
170
180
ppm (t1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OH
Br
H8
H7
H5
H6
Hα
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DMSO
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1.16
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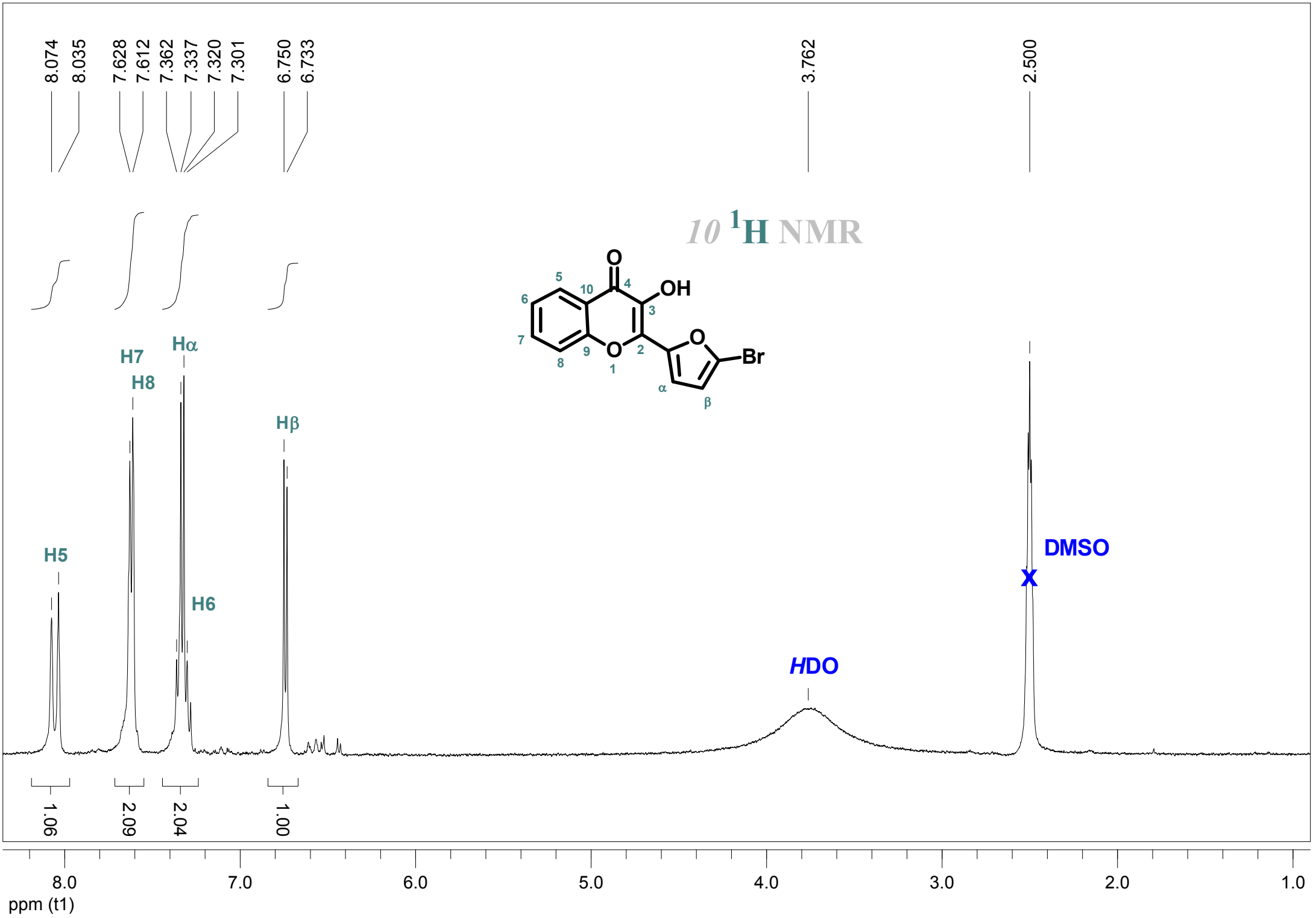
ppm (t1)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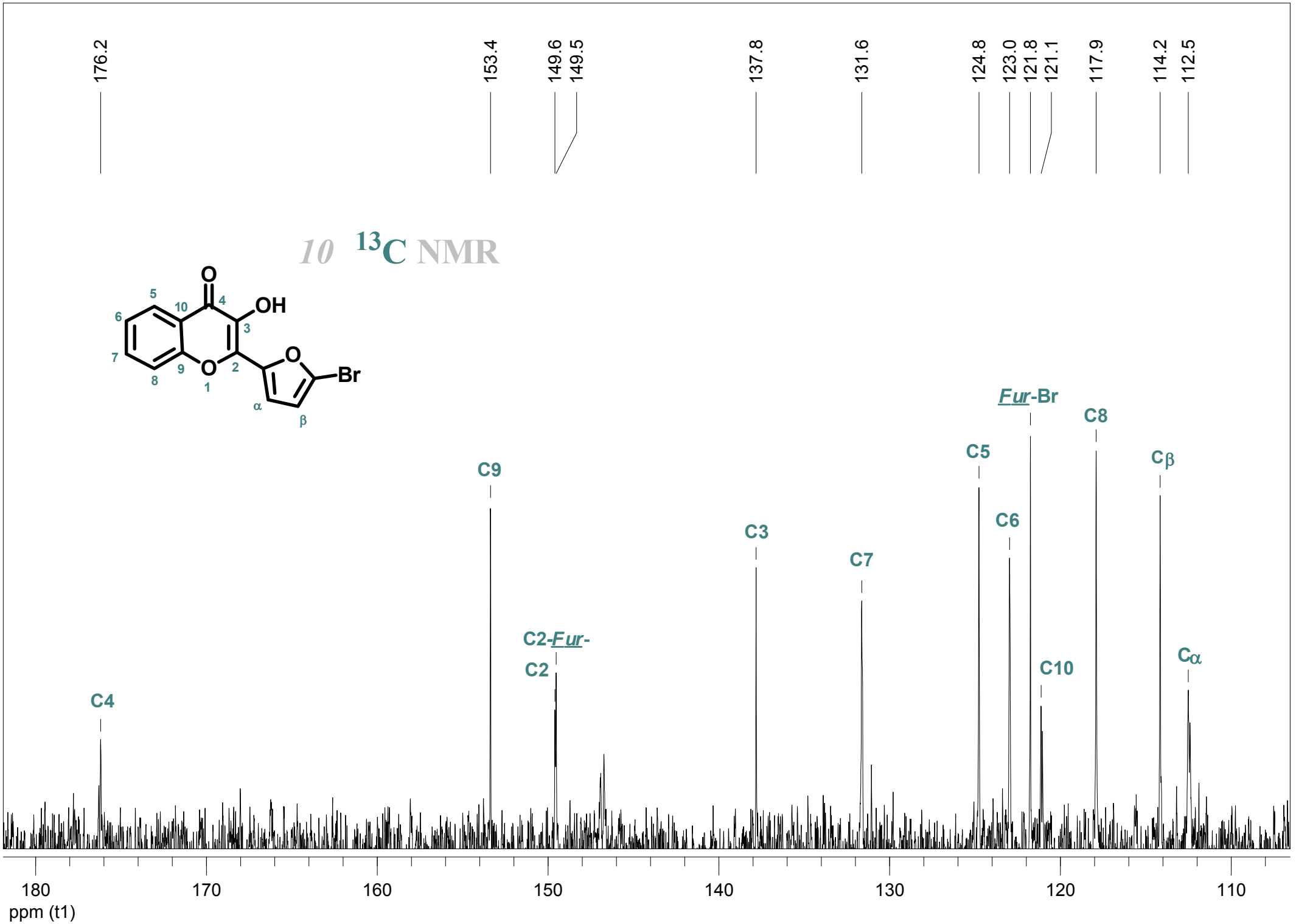
O
OH
Br
H7
H8
Hα
Hβ
H5
H6
HDO
DMSO
X
1.06
2.09
2.04
1.00
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)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112.5
10 13C NMR
O
OH
Br
5
10
4
6
3
7
8
9
O
2
1
O
α
β
C9
C2-Fur-
C2
C3
C7
C5
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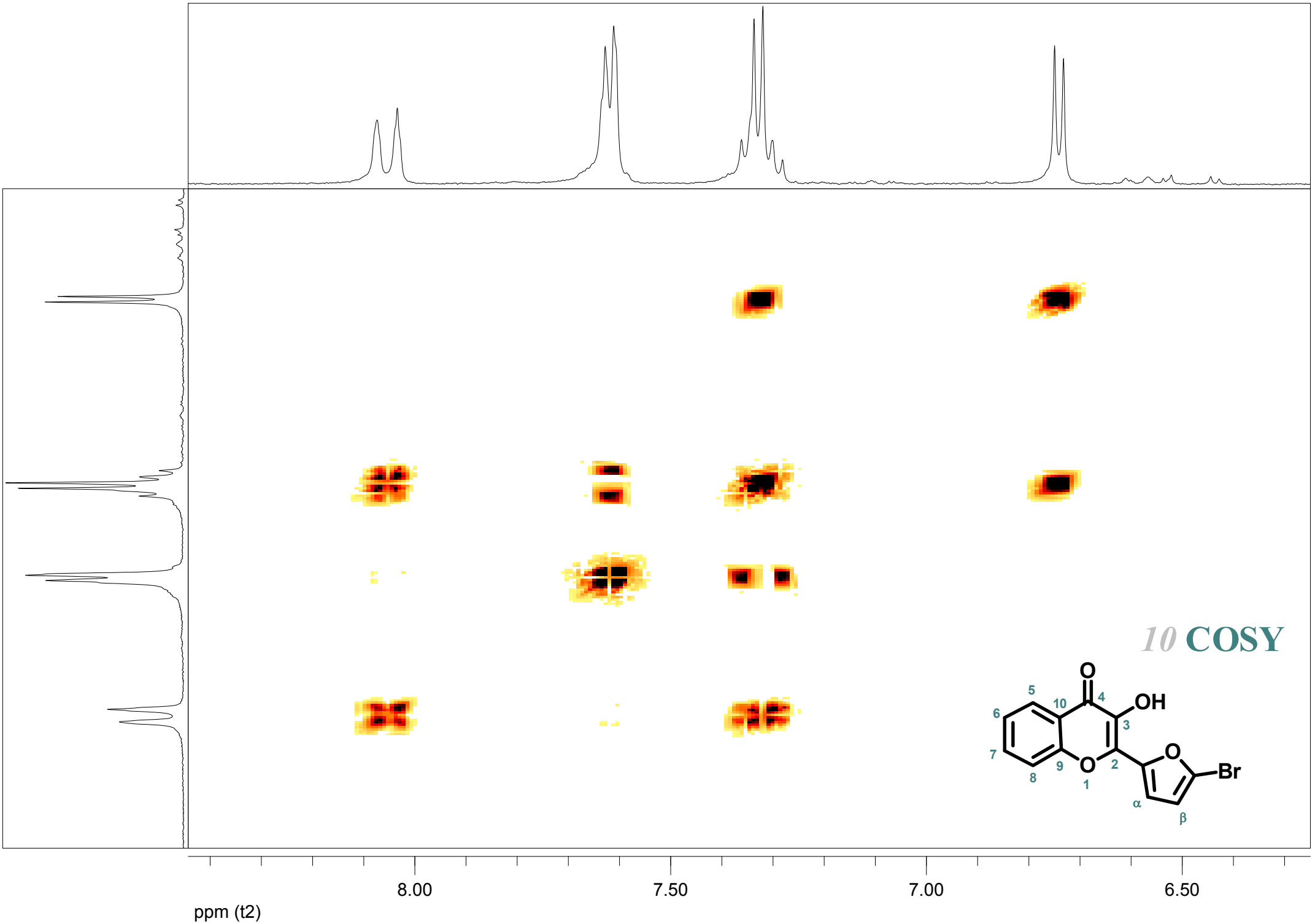
170
160
150
140
130
120
110
ppm (t1)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ppm (t2)
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OH
Br
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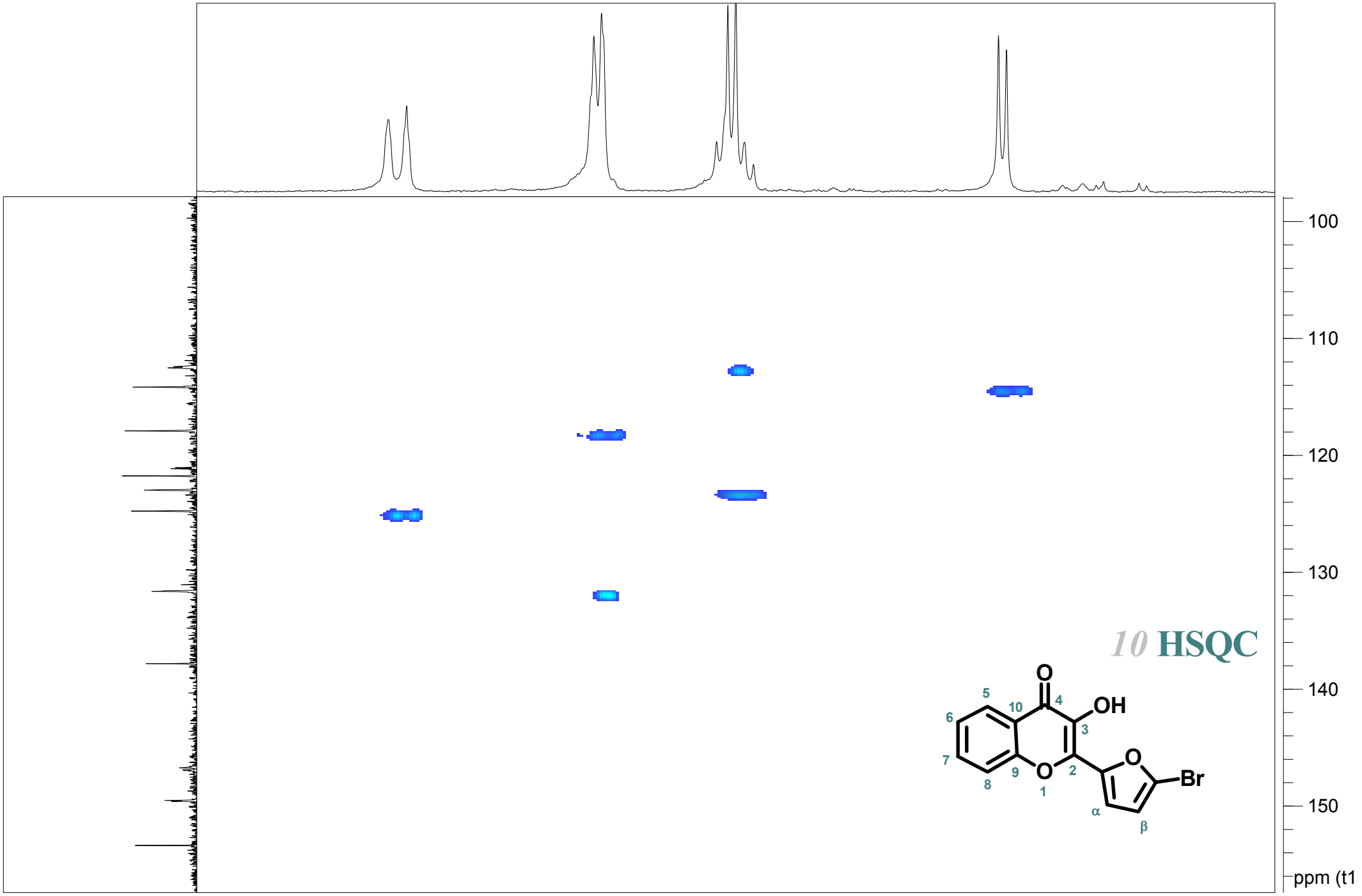
2
3
4
5
6
7
8
9
10
α
β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Br
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2
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4
5
6
7
8
9
10
α
β
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110
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140
150
ppm (t1
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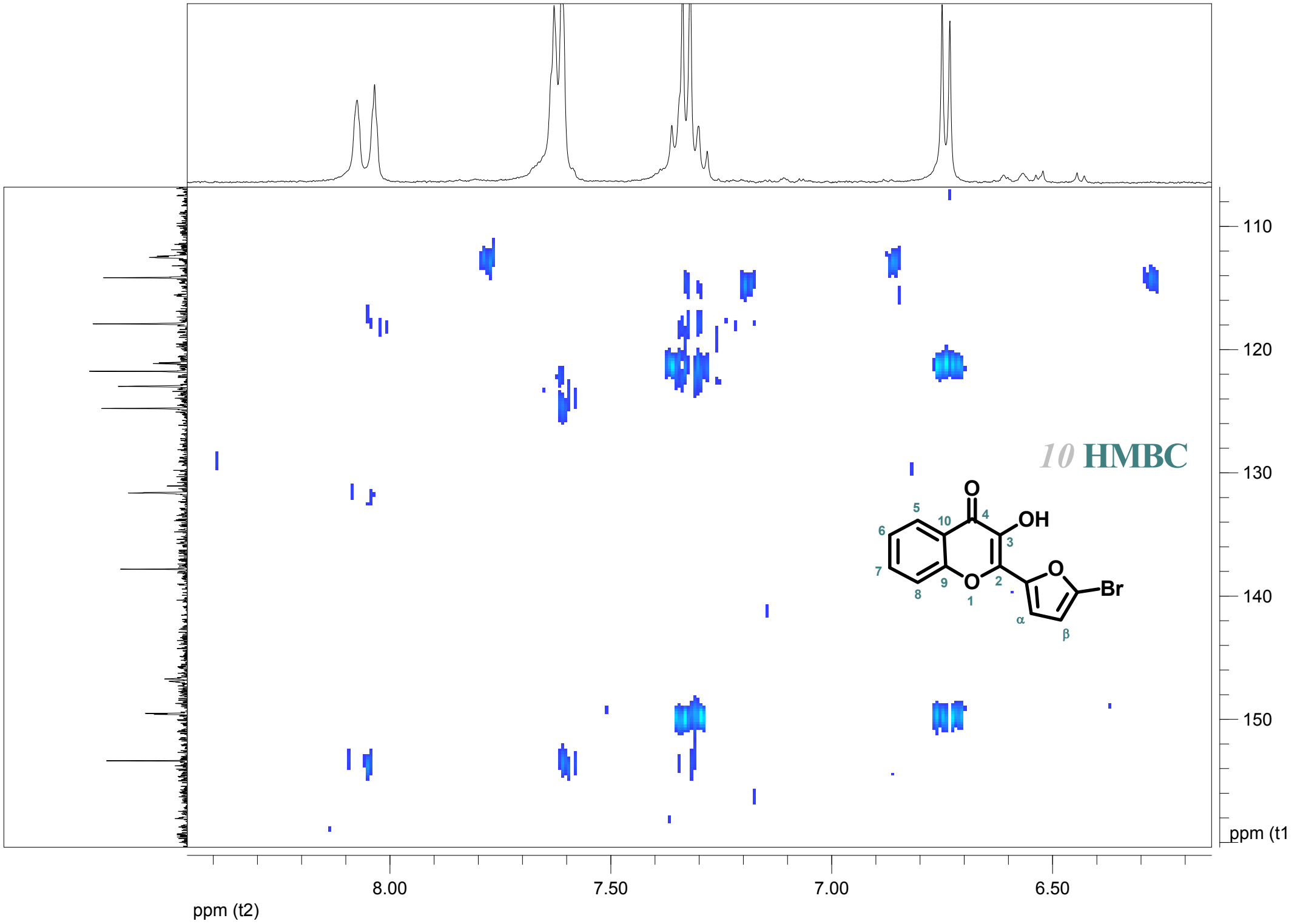
7.50
7.00
6.50
ppm (t2)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OH
Br
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120
130
140
150
ppm (t1
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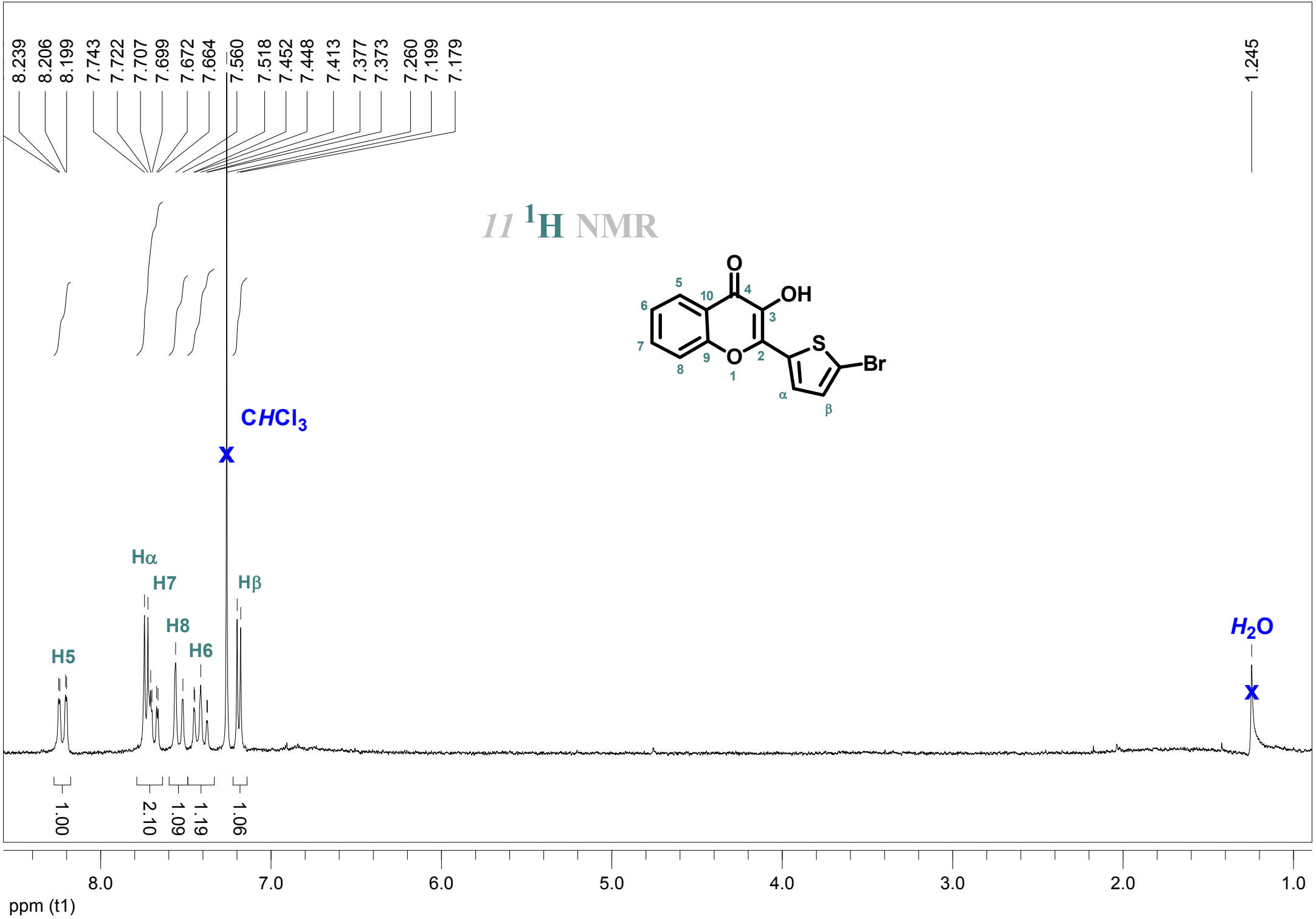
7.00
6.50
ppm (t2)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7.452
7.448
7.413
7.377
7.373
7.260
7.199
7.179
1.245
11 1H NMR
O
OH
S
Br
CHCl3
Hα
H7
Hβ
H8
H6
H5
H2O
1.00
2.10
1.09
1.19
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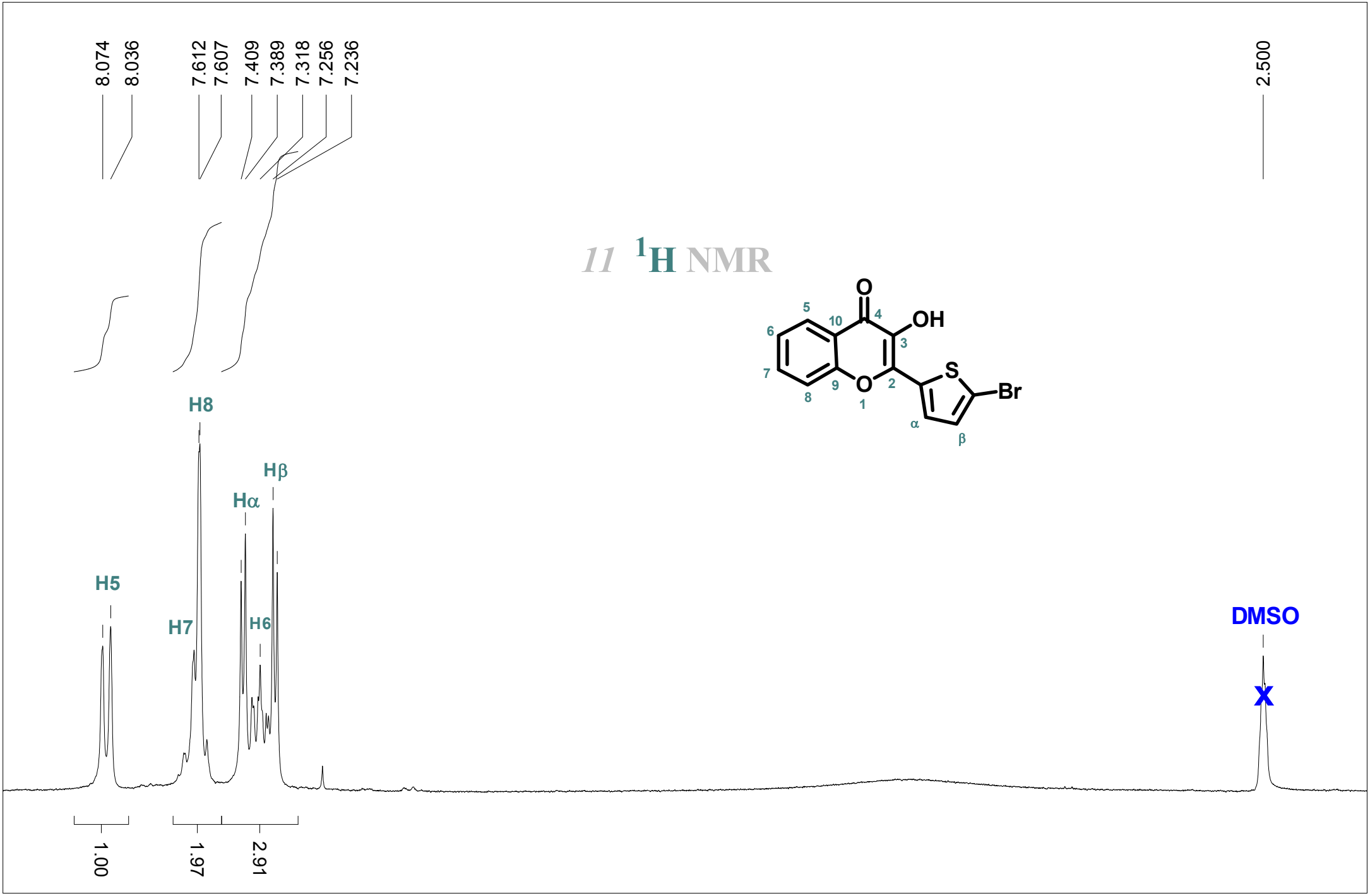
ppm (t1)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Hα
H5
H7
H6
DMSO
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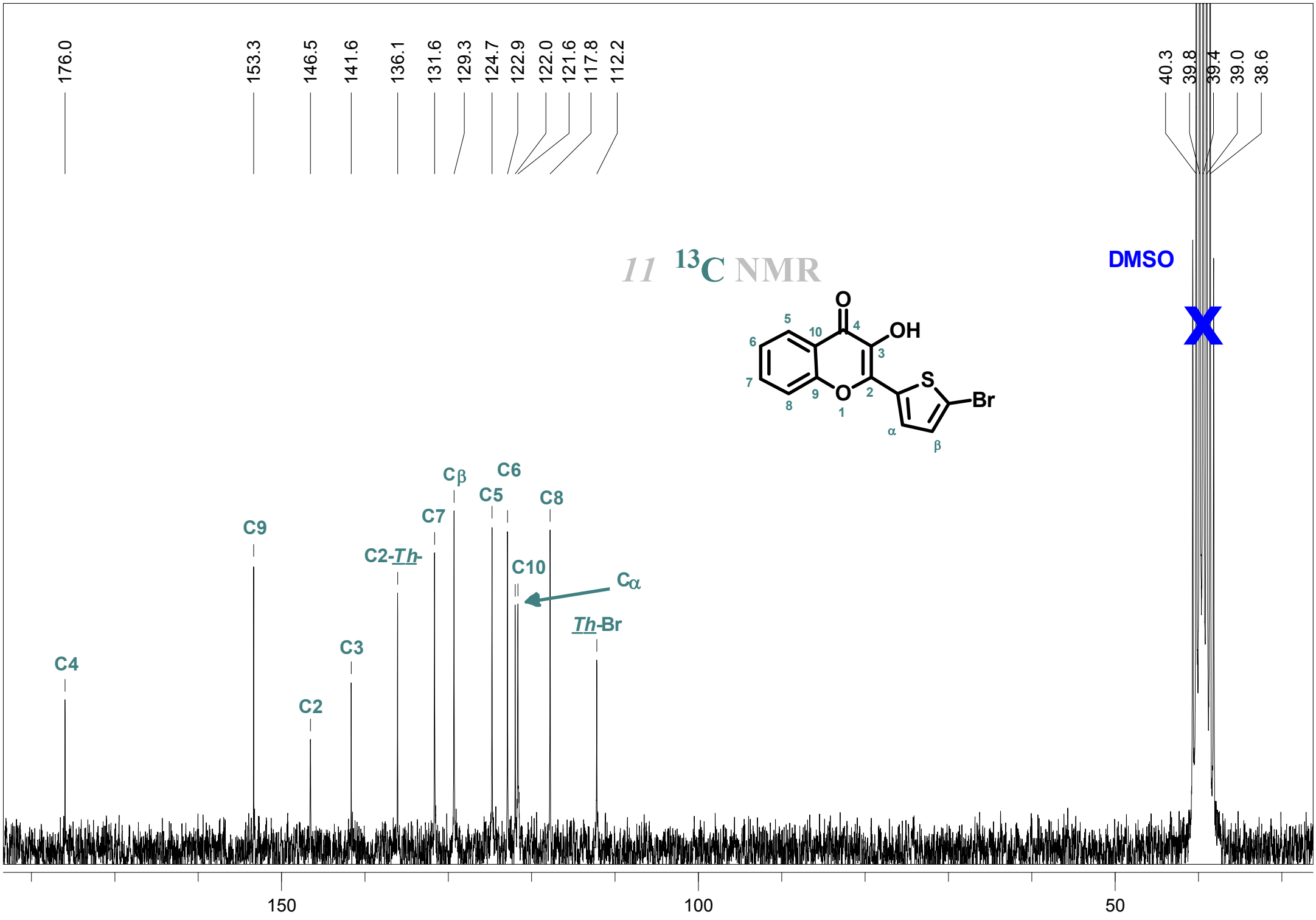
5.0
4.0
3.0
ppm (t1)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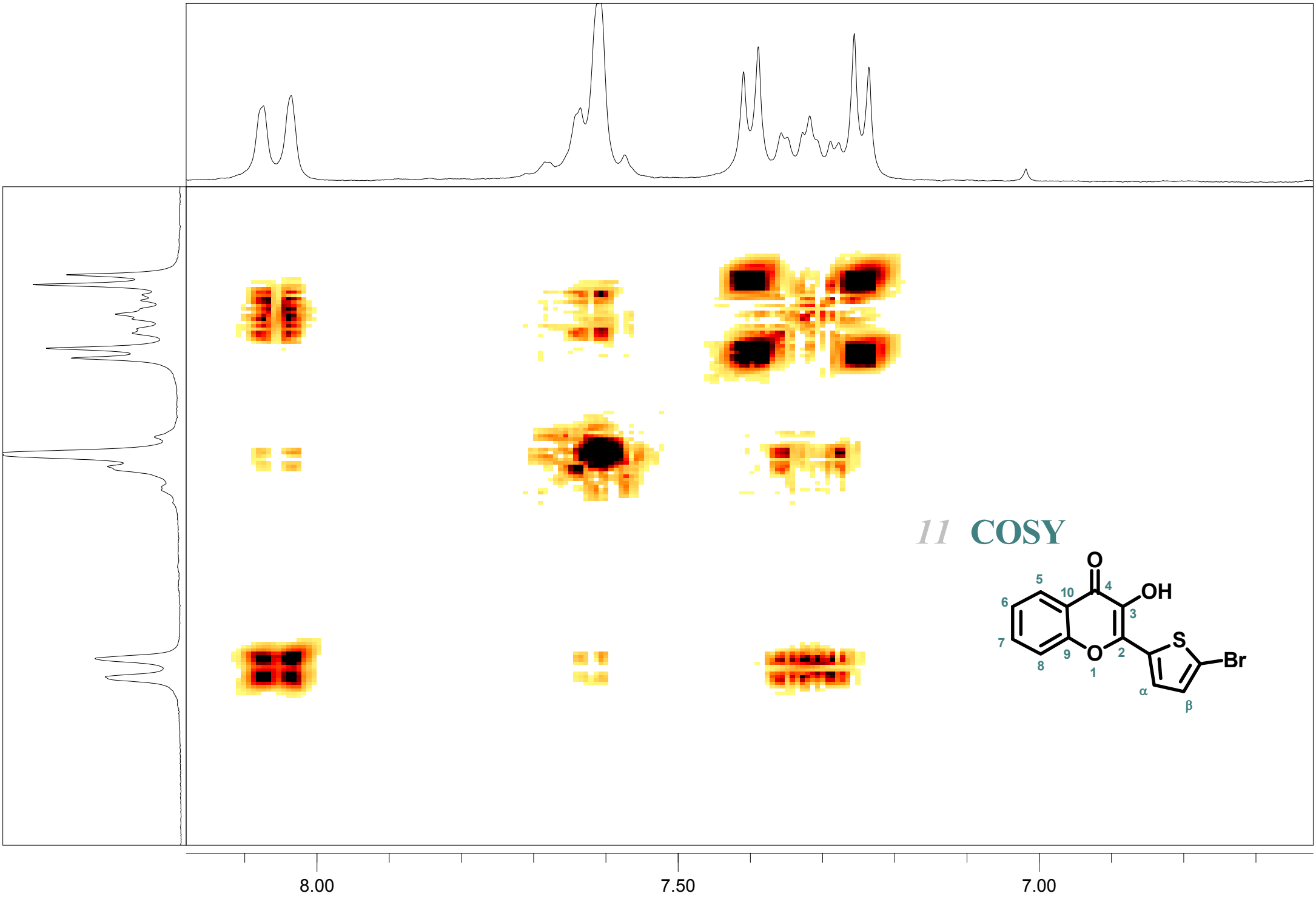
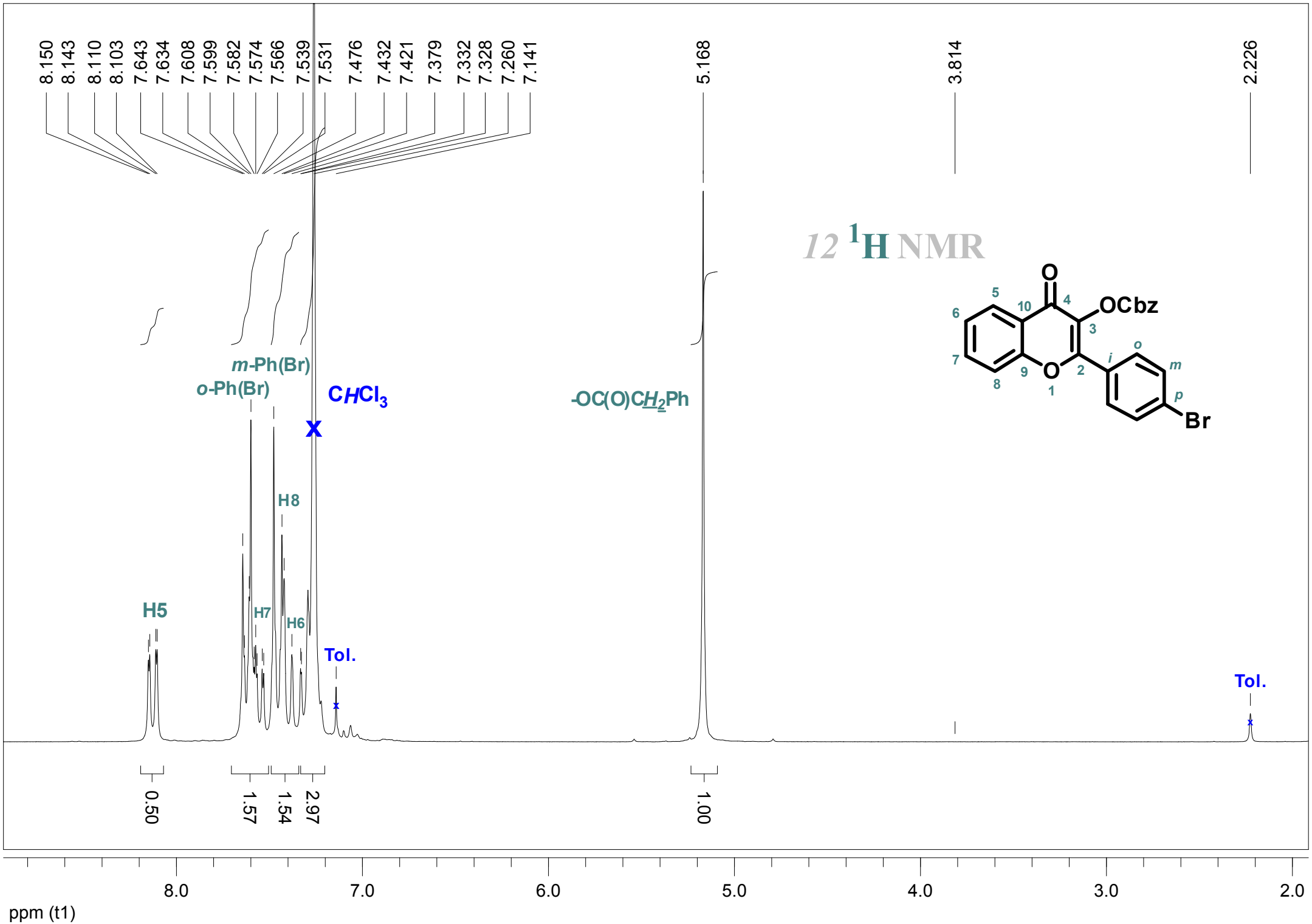
112.2
40.3
39.8
39.4
39.0
38.6
11 13C NMR
DMSO
O
OH
S
Br
1
2
3
4
5
6
7
8
9
10
α
β
Cβ
C6
C5
C8
C7
C9
C2-Th-
C10
Cα
Th-Br
C3
C4
C2
150
100
50
ppm (t1)



11 COSY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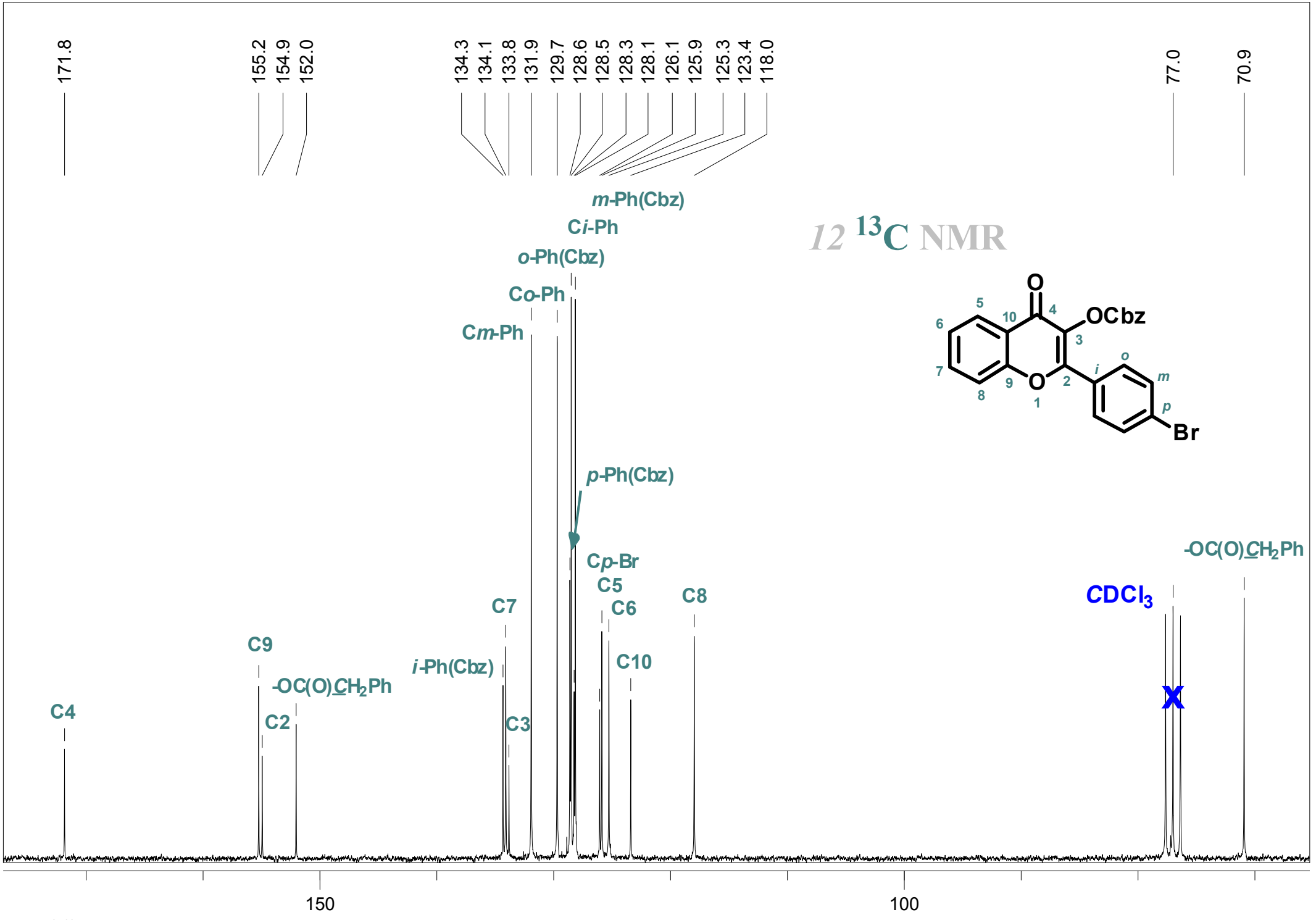
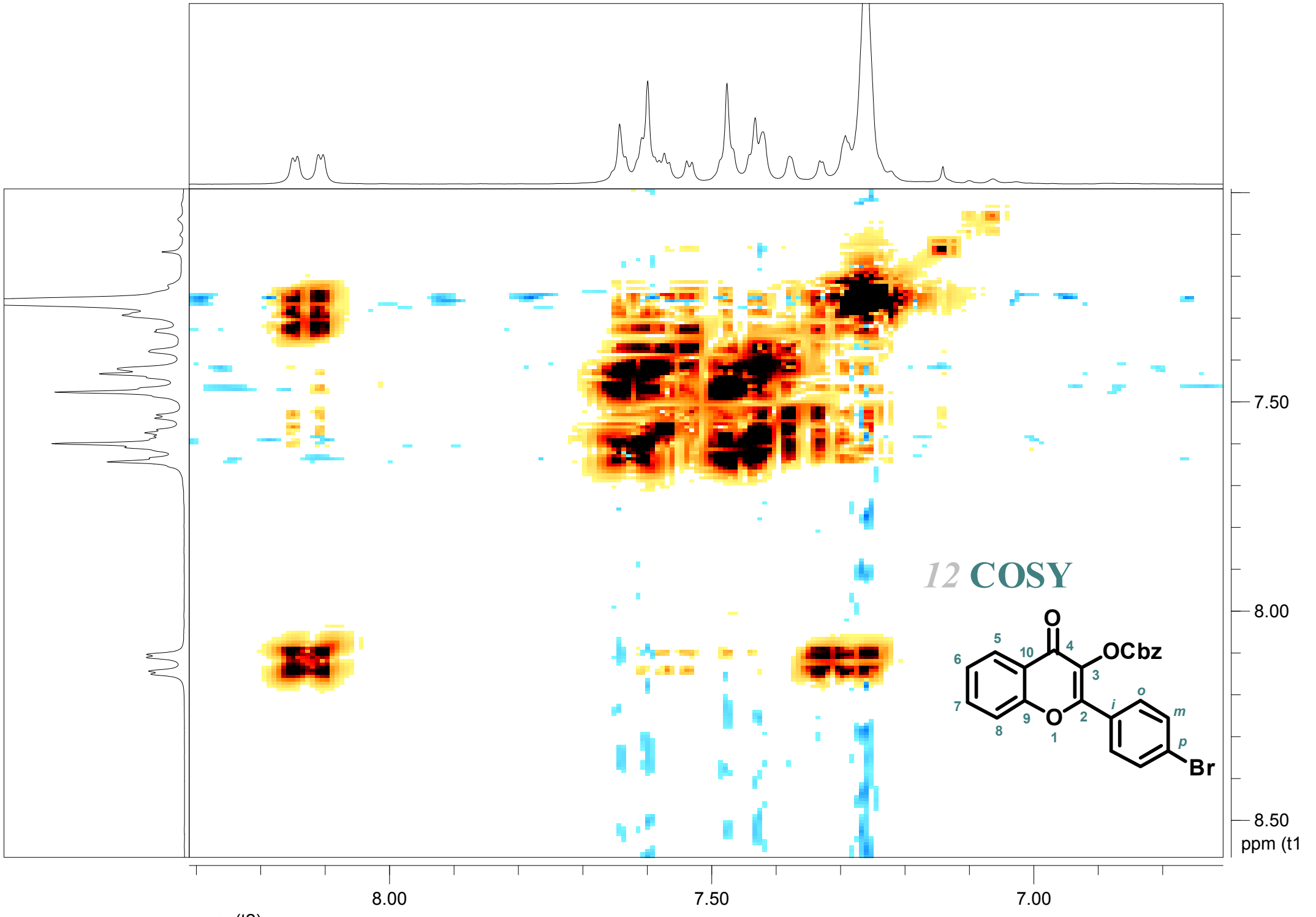
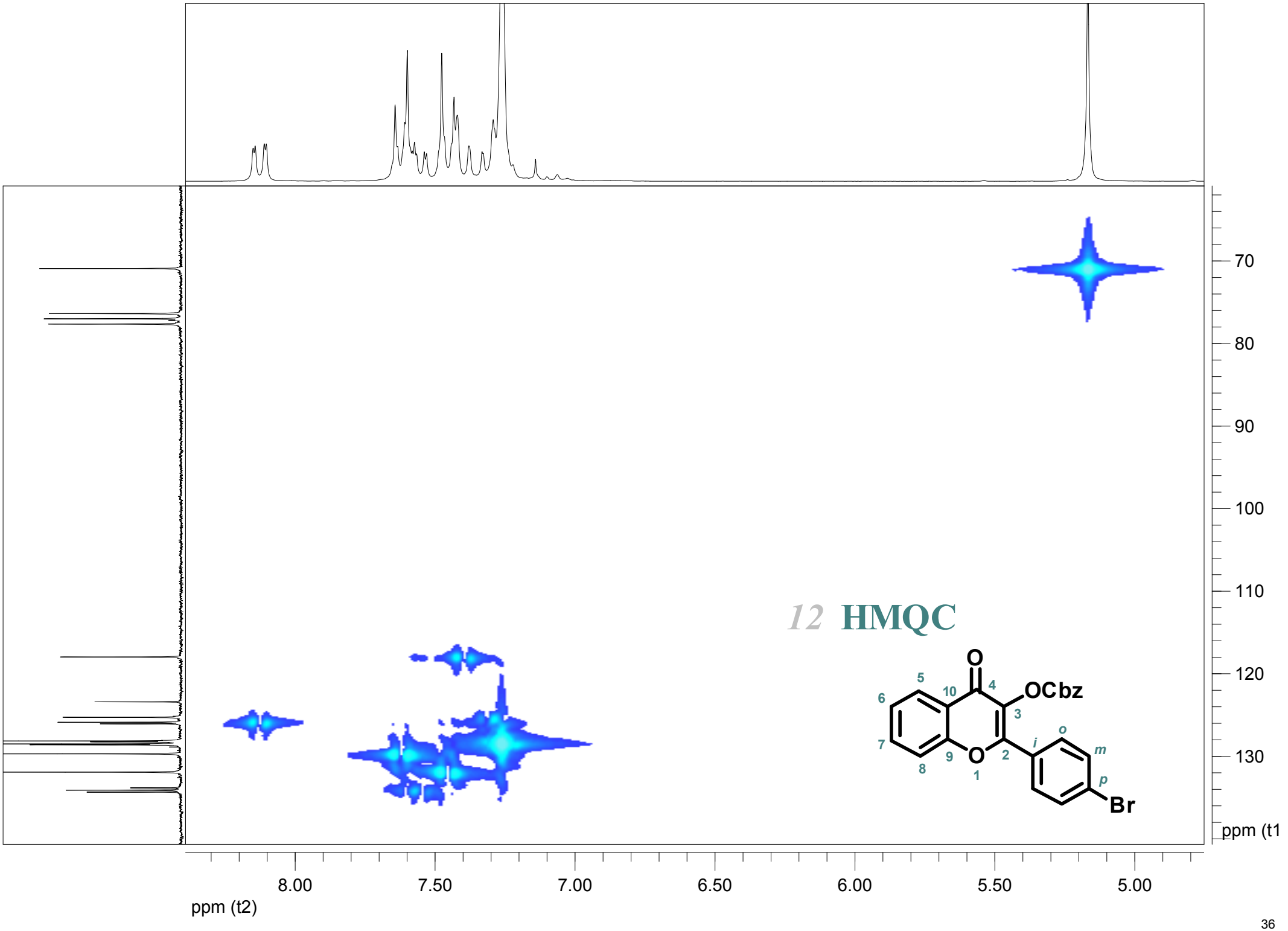
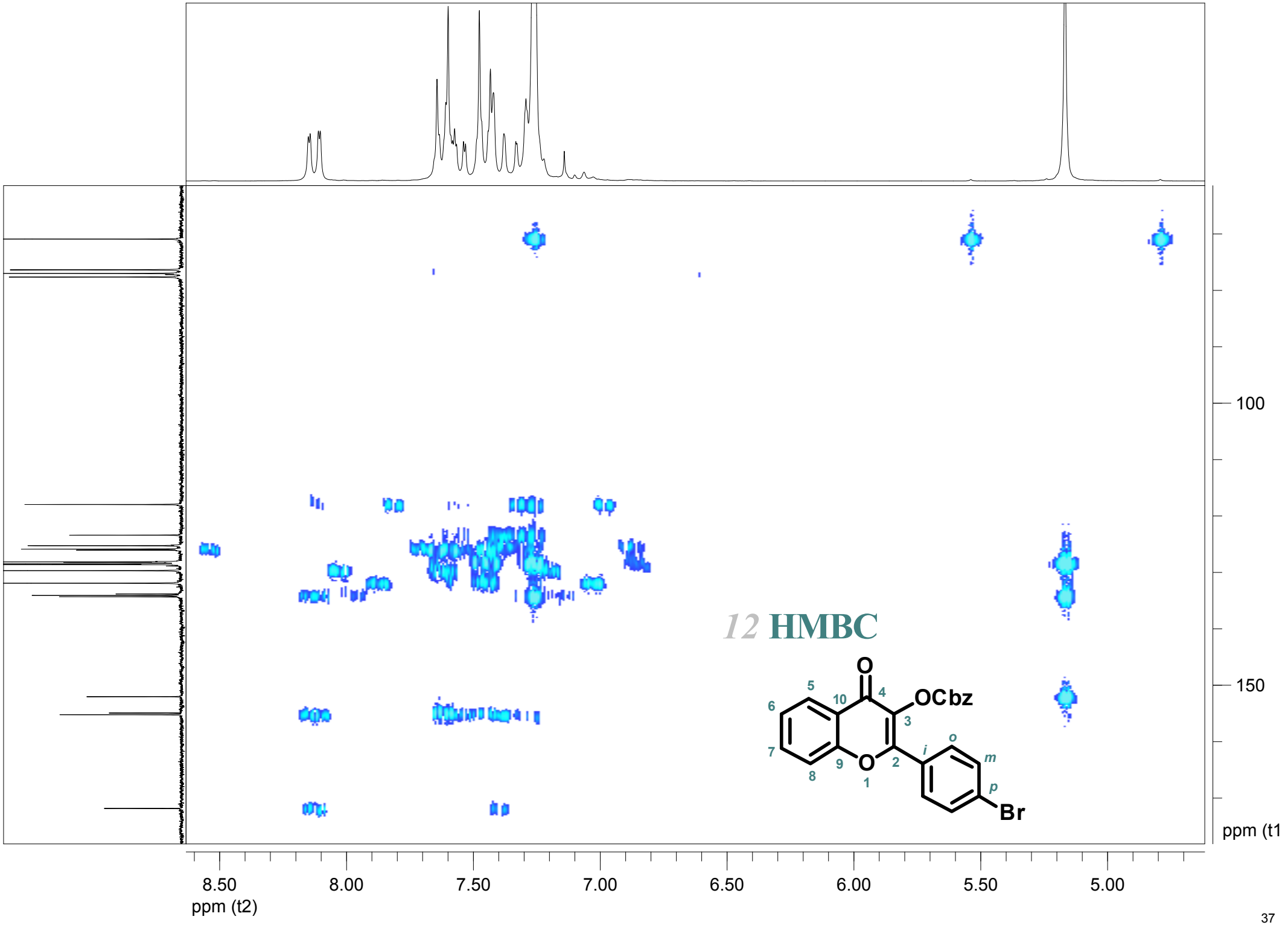
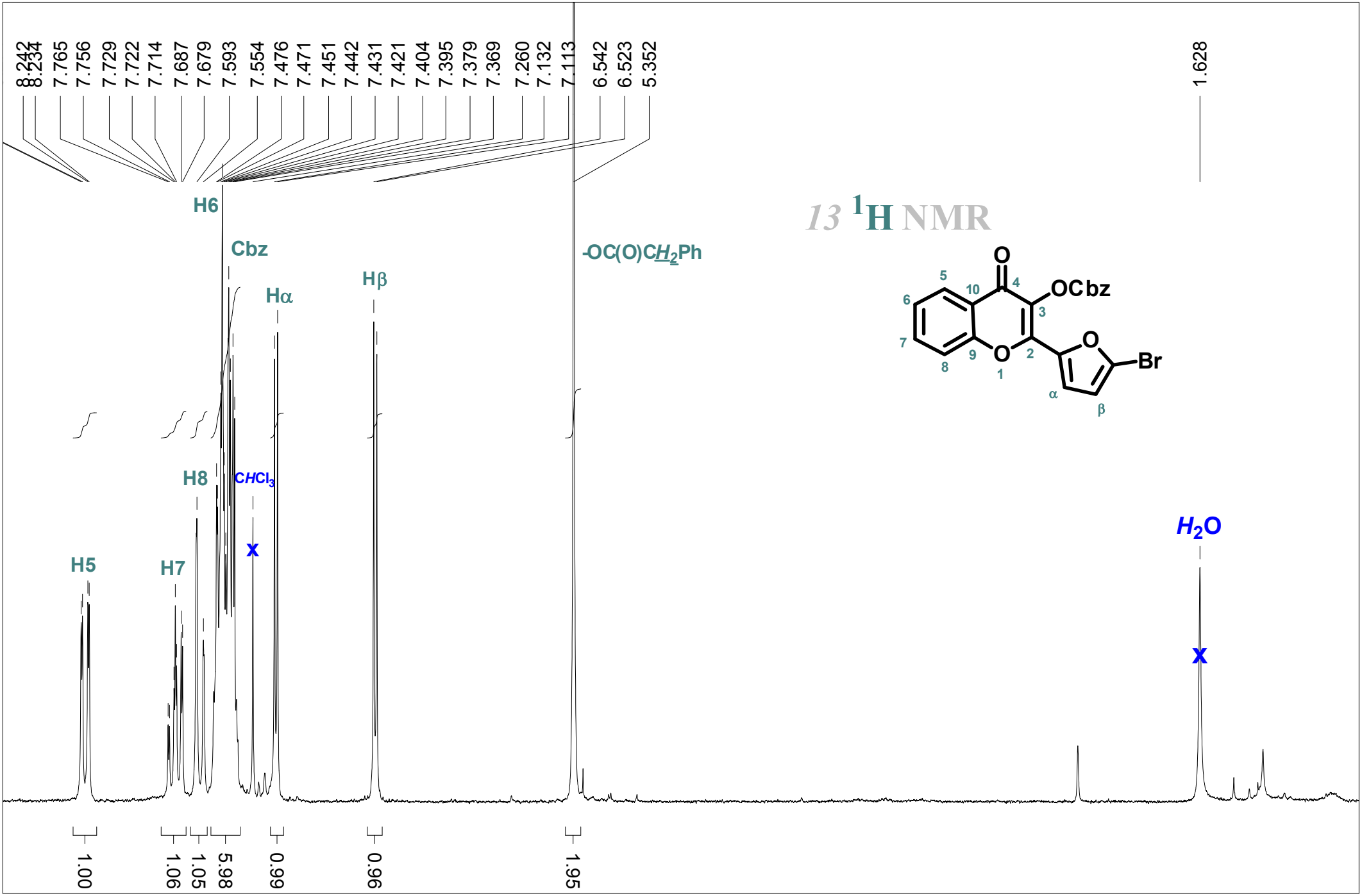
7.476
7.432
7.421
7.379
7.332
7.328
7.260
7.141
5.168
3.814
2.226
12 1H NMR
m-Ph(Br)
o-Ph(Br)
CHCl3
-OC(O)CH2Ph
H5
H7
H6
H8
Tol.
Tol.
OCbz
Br
0.50
1.57
1.54
2.67
1.00
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t1)

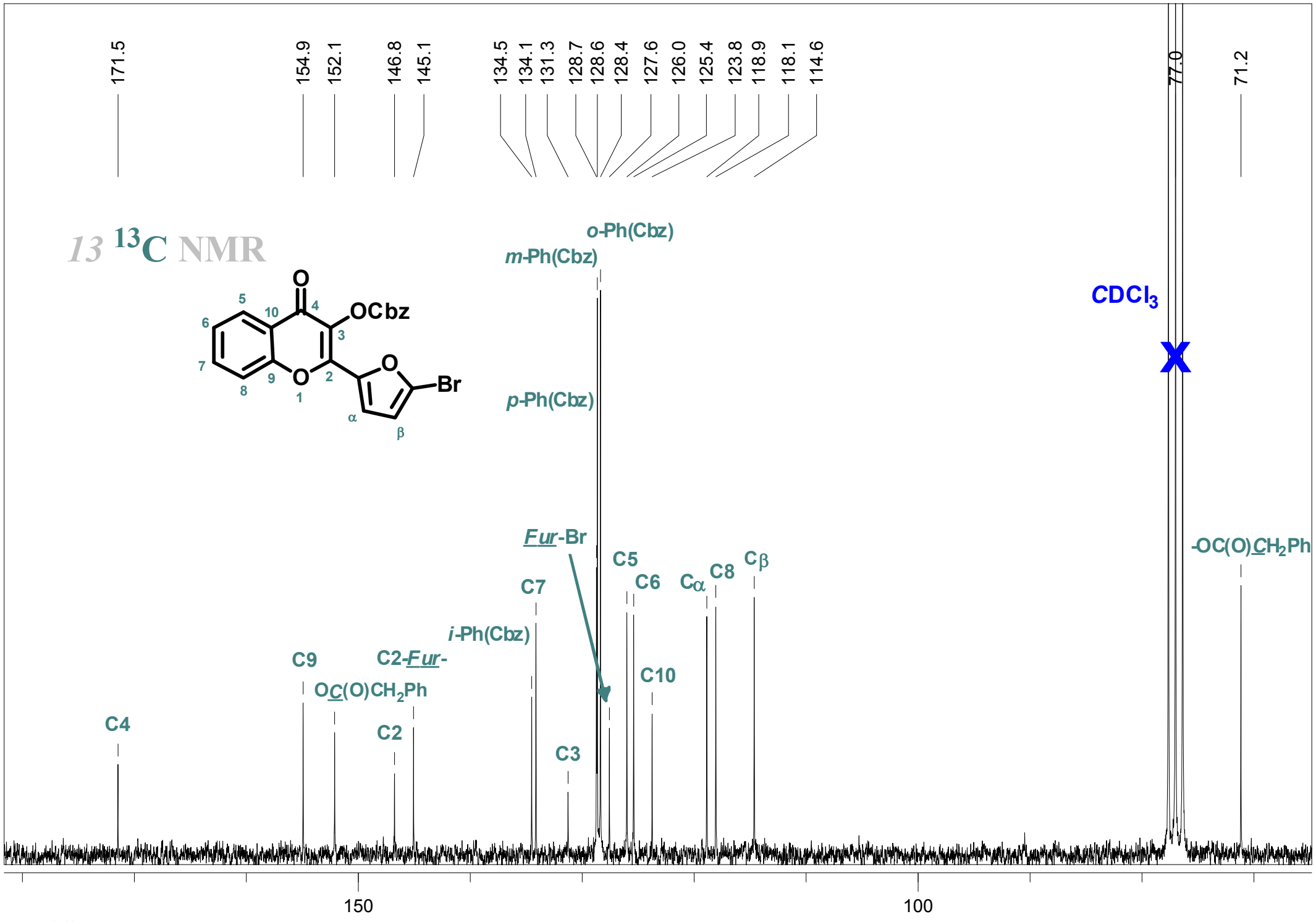

171.8
155.2
154.9
152.0
134.3
134.1
133.8
131.9
129.7
128.6
128.5
128.3
128.1
126.1
125.9
125.3
123.4
118.0
77.0
70.9
m-Ph(Cbz)
Ci-Ph
o-Ph(Cbz)
Co-Ph
Cm-Ph
p-Ph(Cbz)
Cp-Br
C5
C6
C7
C8
C9
C10
i-Ph(Cbz)
-OC(O)CH2Ph
C4
C2
C3
CDCl3
-OC(O)CH2Ph
12 13C NMR
OCbz
Br
150
100
ppm (t1)

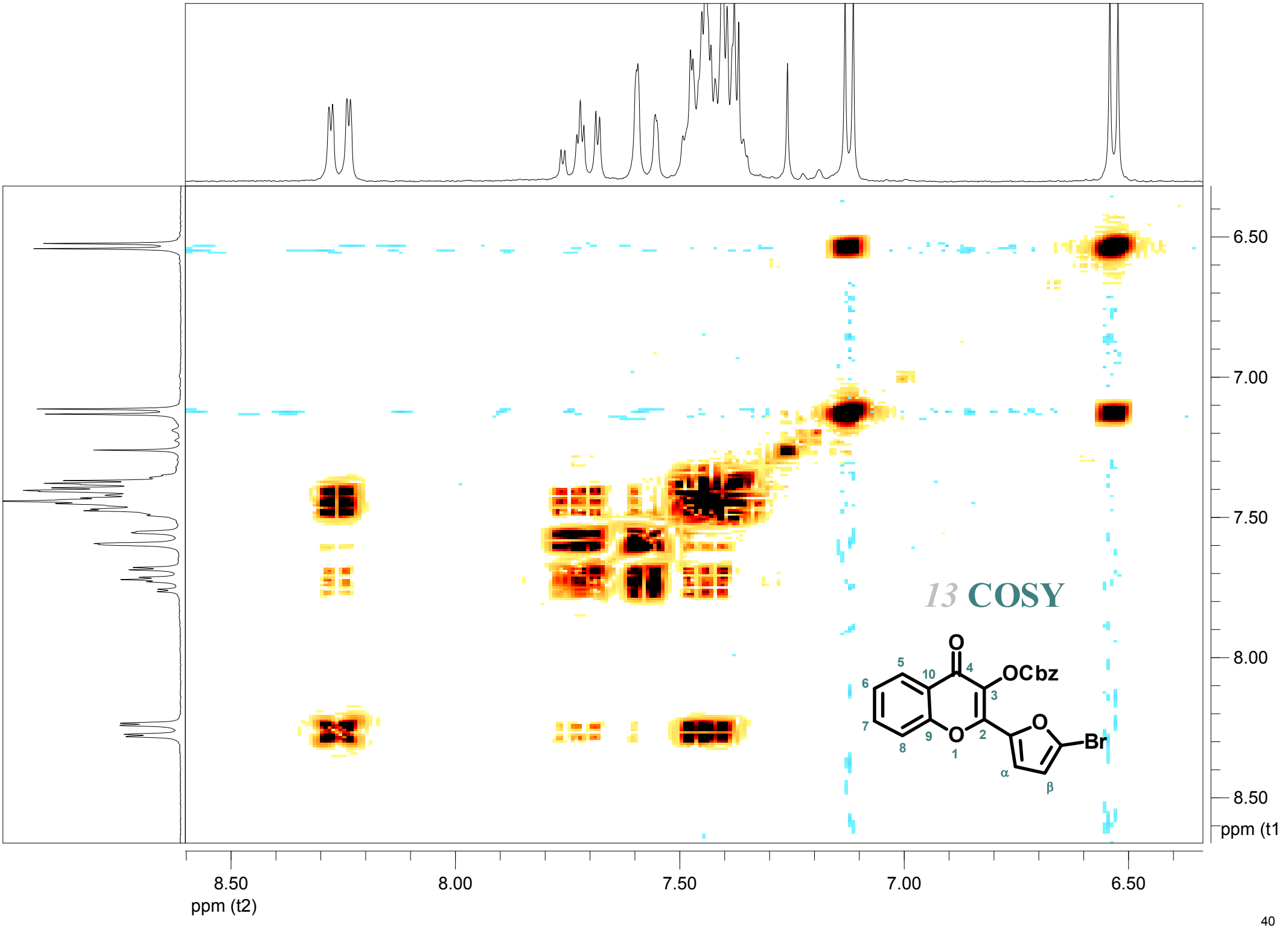

12 COSY
O
OCbz
Br
7.50
8.00
8.50
ppm (t1
8.00
7.50
7.00
ppm (t2)

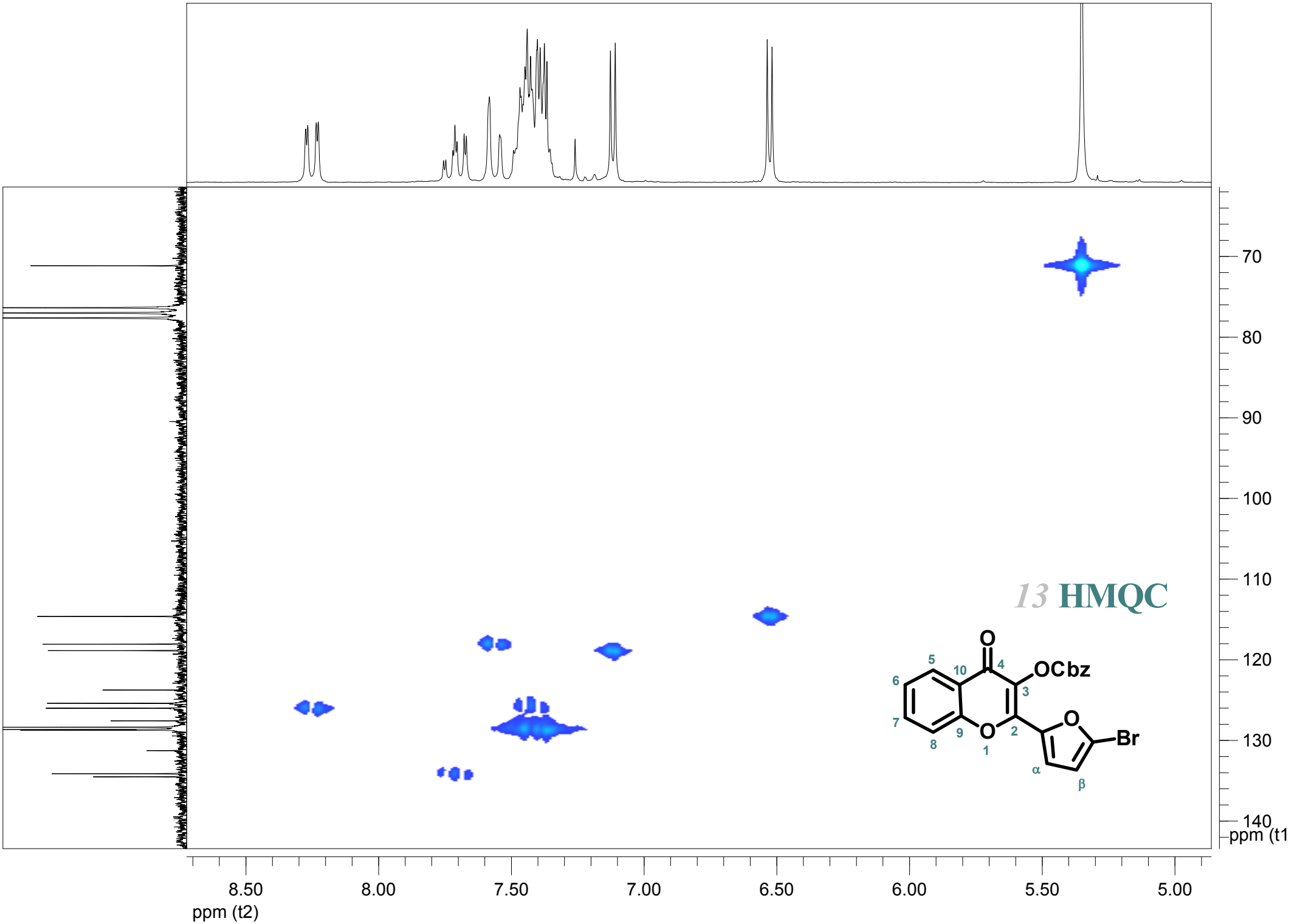

12 HMQC
OCbz
Br
ppm (t2)
ppm (t1
8.00
7.50
7.00
6.50
6.00
5.50
5.00
70
80
90
100
110
120
130

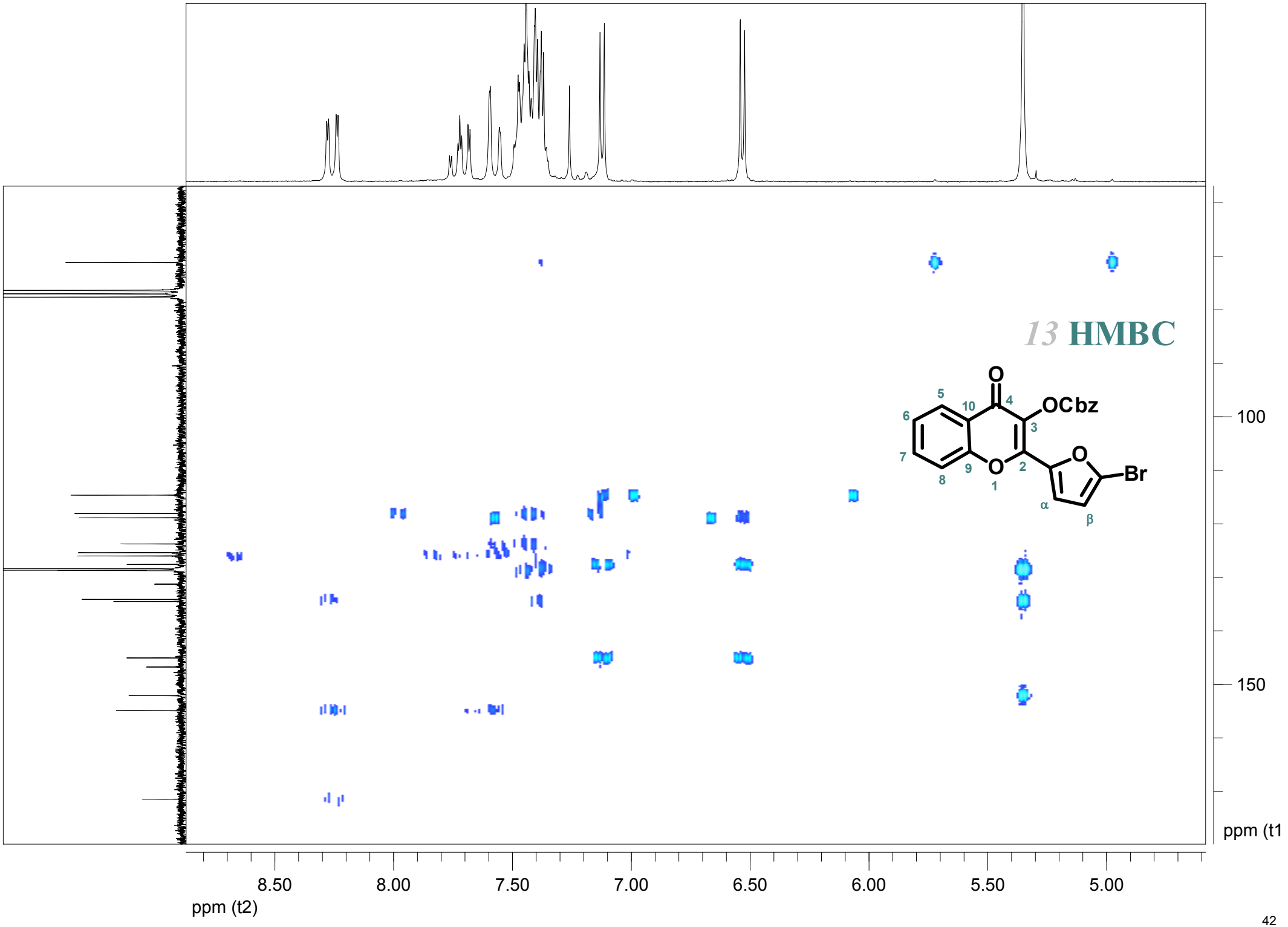

12 HMBC
O
OCbz
Br
100
150
ppm (t1
8.50
8.00
7.50
7.00
6.50
6.00
5.50
5.00
ppm (t2)

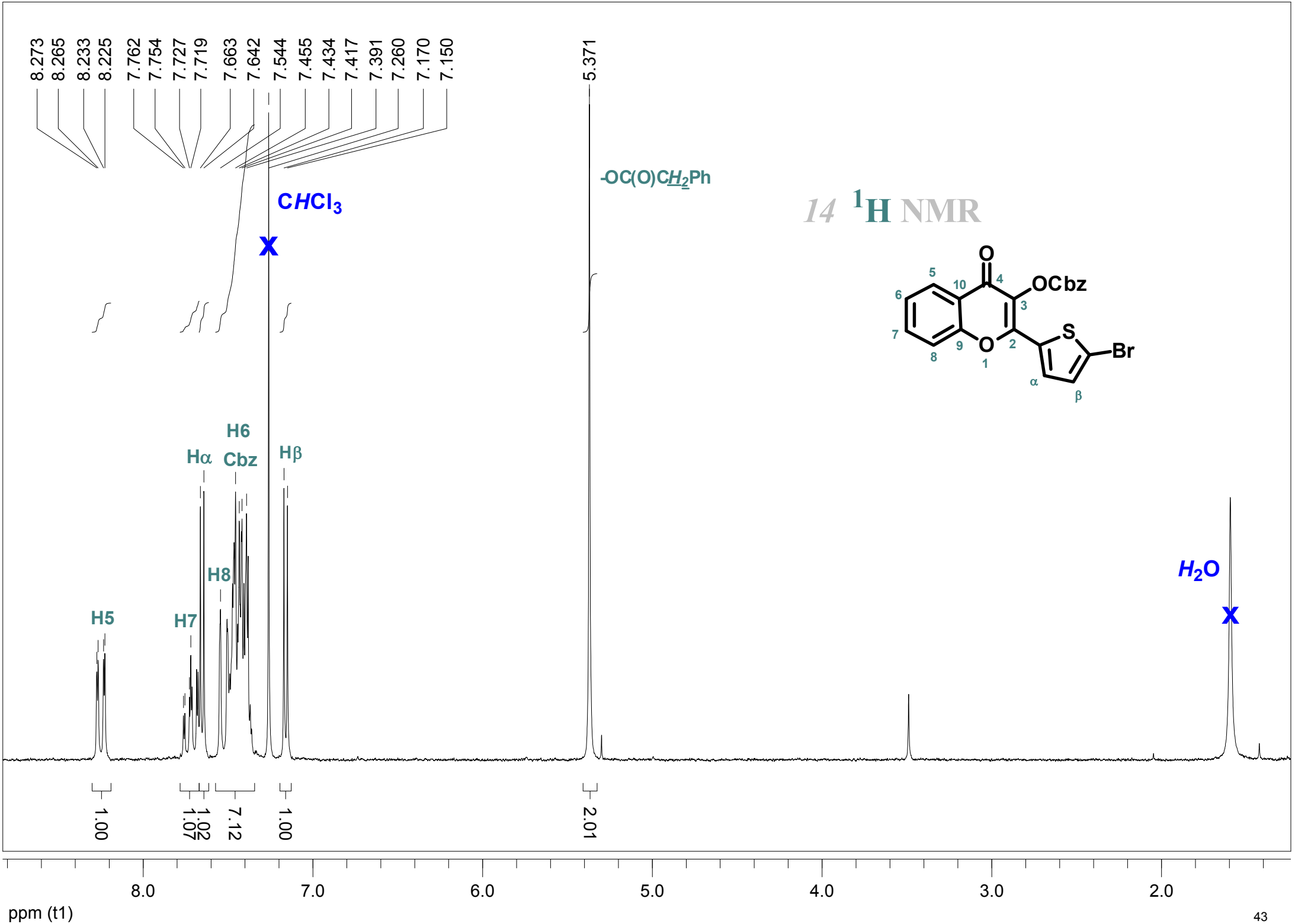

8.242
8.234
7.765
7.756
7.729
7.722
7.714
7.687
7.679
7.593
7.554
7.476
7.471
7.451
7.442
7.431
7.421
7.404
7.395
7.379
7.369
7.260
7.132
7.113
6.542
6.523
5.352
1.628
13 1H NMR
H6
Cbz
Hα
Hβ
-OC(O)CH2Ph
H8
CHCl3
H5
H7
H2O
OCbz
Br
1.00
1.09
1.05
5.98
0.99
0.96
1.95
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)

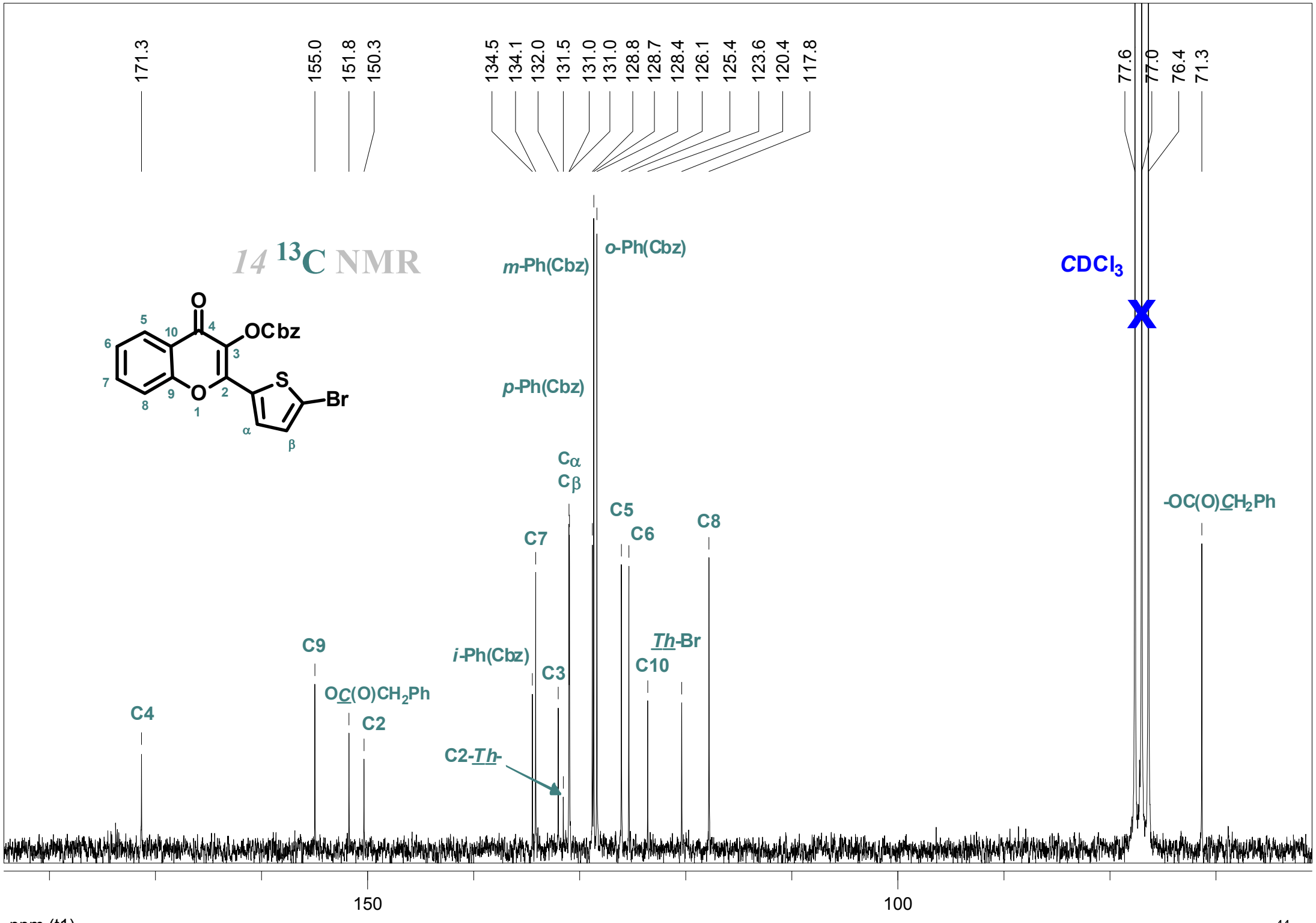

13 13C NMR
171.5
154.9
152.1
146.8
145.1
134.5
134.1
131.3
128.7
128.6
128.4
127.6
126.0
125.4
123.8
118.9
118.1
114.6
77.0
71.2
OCbz
Br
o-Ph(Cbz)
m-Ph(Cbz)
p-Ph(Cbz)
CDCl3
Fur-Br
C5
C6
Cα
C8
Cβ
C7
i-Ph(Cbz)
C9
C2-Fur-
OC(O)CH2Ph
C10
C4
C2
C3
-OC(O)CH2Ph
150
100
ppm (t1)

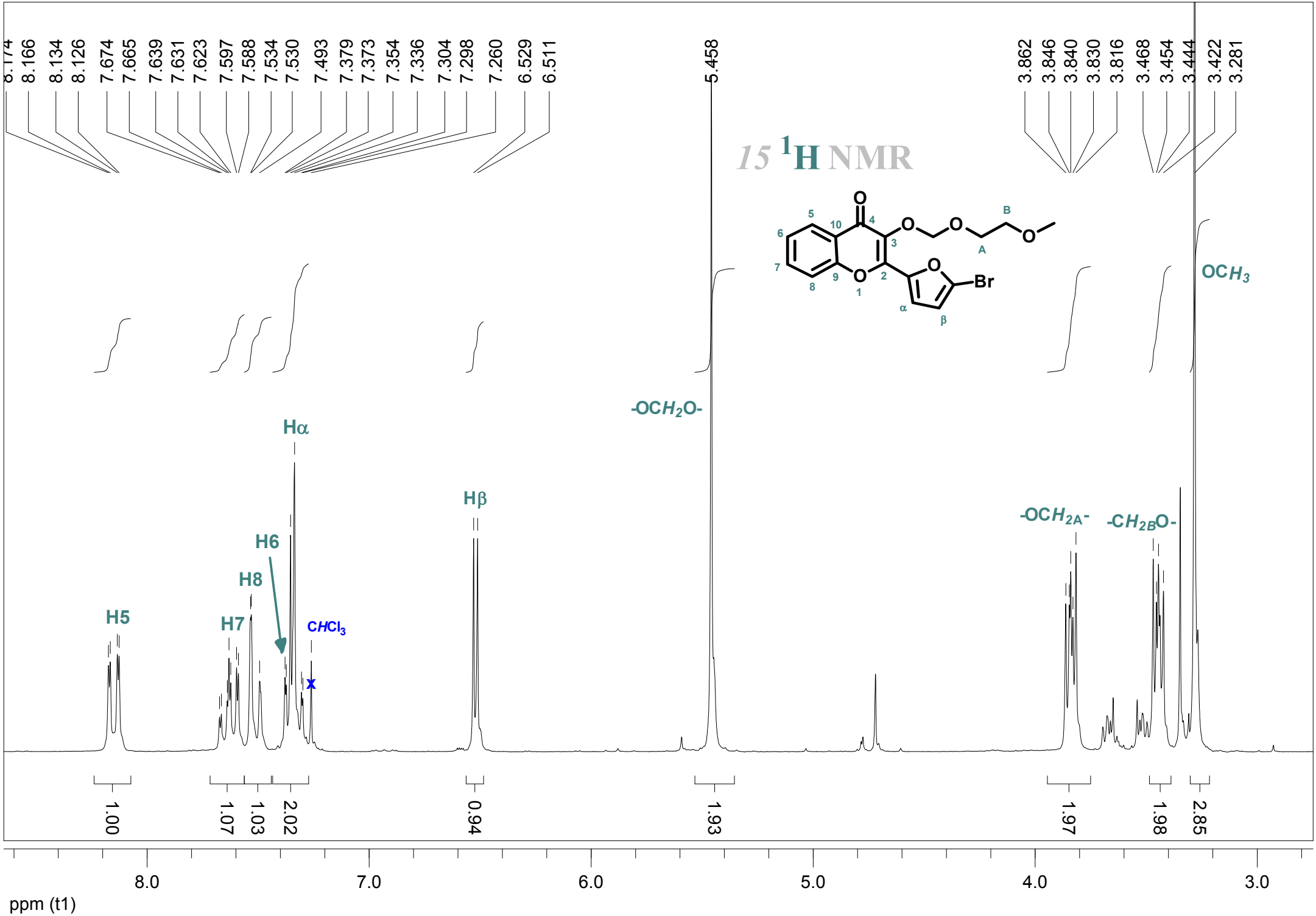

13 COSY
OCbz
Br
O
O
O
1
2
3
4
5
6
7
8
9
10
α
β
6.50
7.00
7.50
8.00
8.50
ppm (t1
8.50
8.00
7.50
7.00
6.50
ppm (t2)

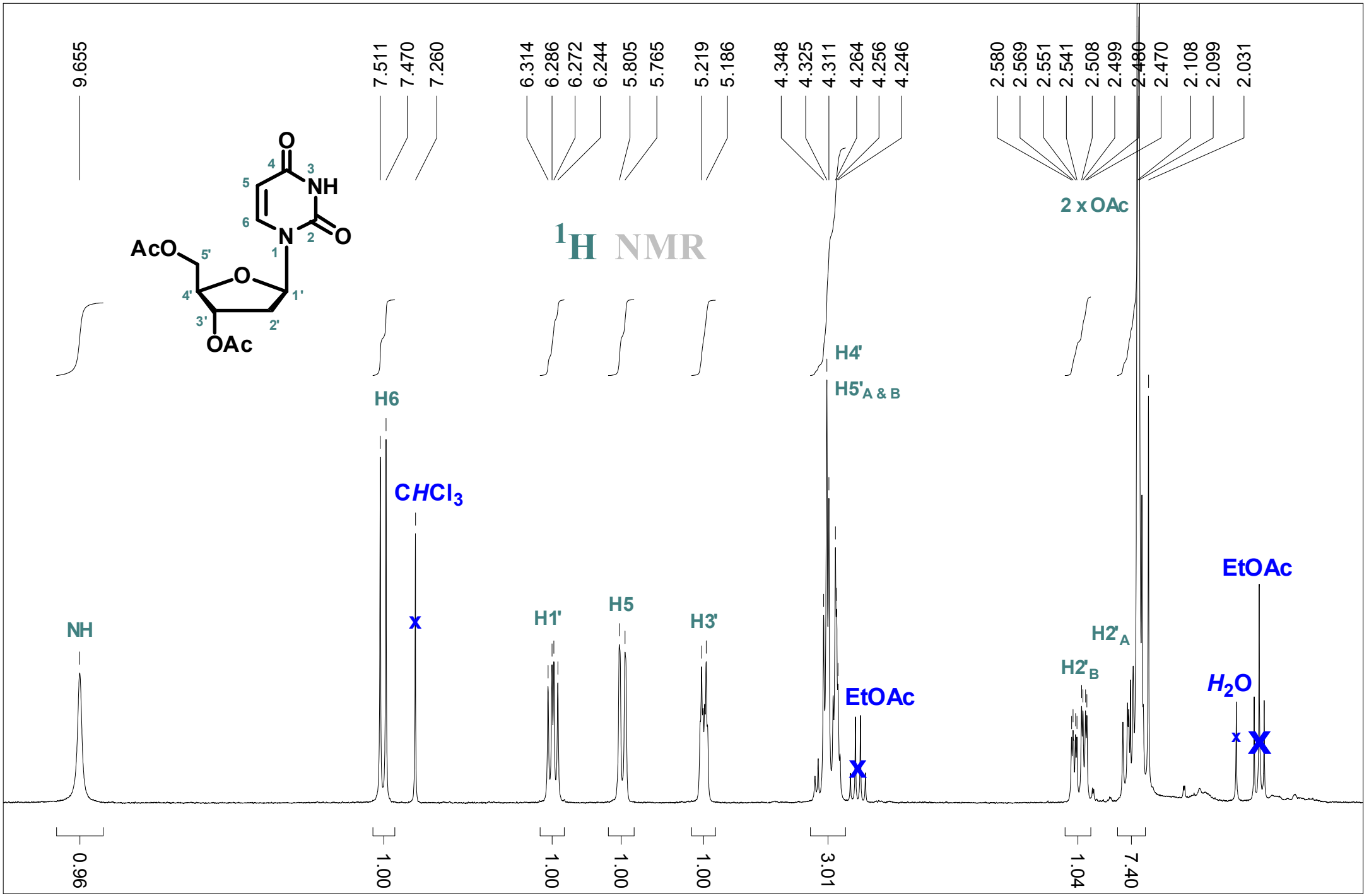

13 HMQC
OCbz
Br
8.50
8.00
7.50
7.00
6.50
6.00
5.50
5.00
ppm (t2)
70
80
90
100
110
120
130
140
ppm (t1

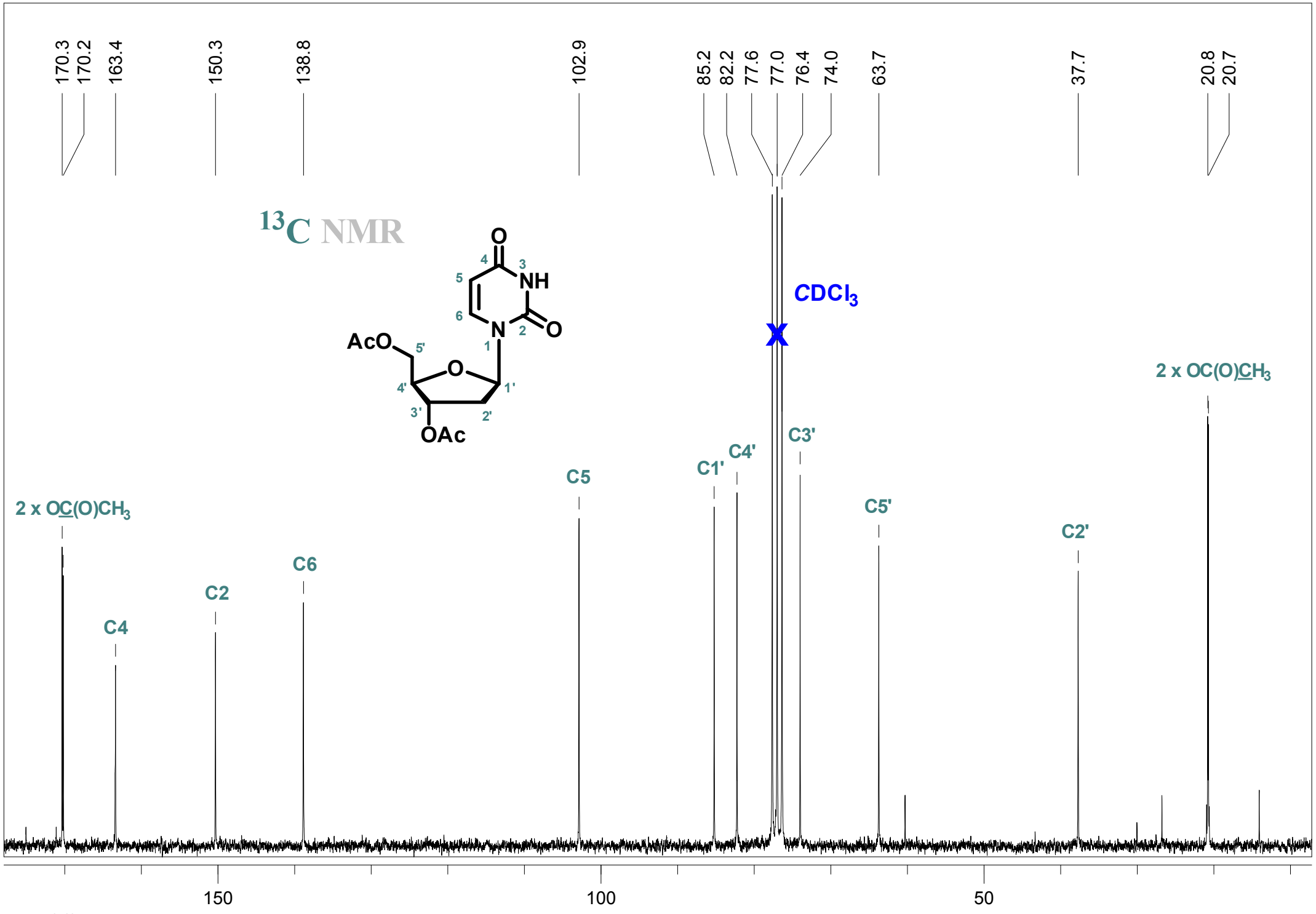

13 HMBC
O
OCbz
Br
100
150
ppm (t1
8.50
8.00
7.50
7.00
6.50
6.00
5.50
5.00
ppm (t2)

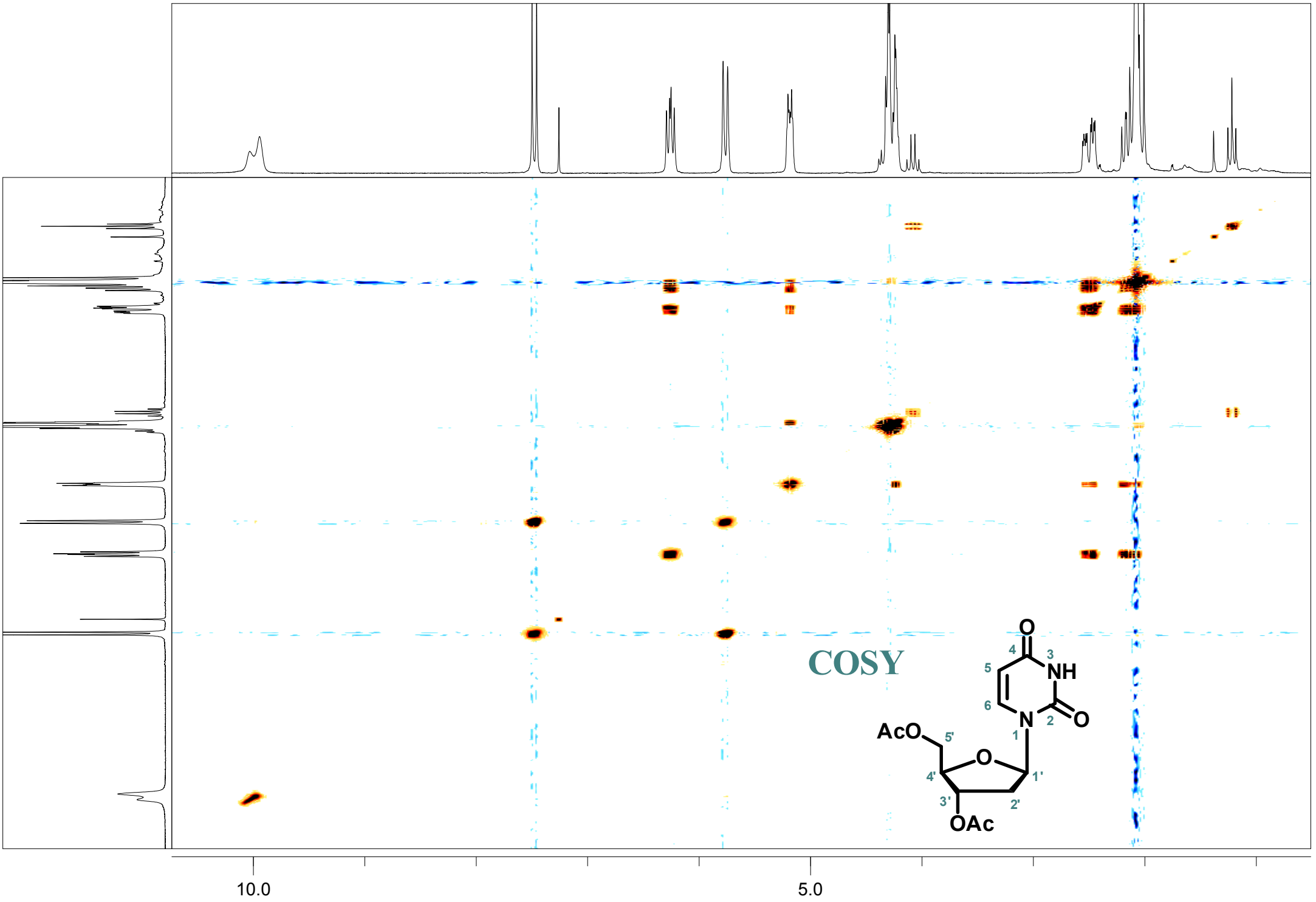

8.273
8.265
8.233
8.225
7.762
7.754
7.727
7.719
7.663
7.642
7.544
7.455
7.434
7.417
7.391
7.260
7.170
7.150
5.371
CHCl3
-OC(O)CH2Ph
14 1H NMR
OCbz
Br
H6
Cbz
Hα
Hβ
H8
H7
H5
H2O
1.00
1.02
1.07
7.12
1.00
2.01
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t1)

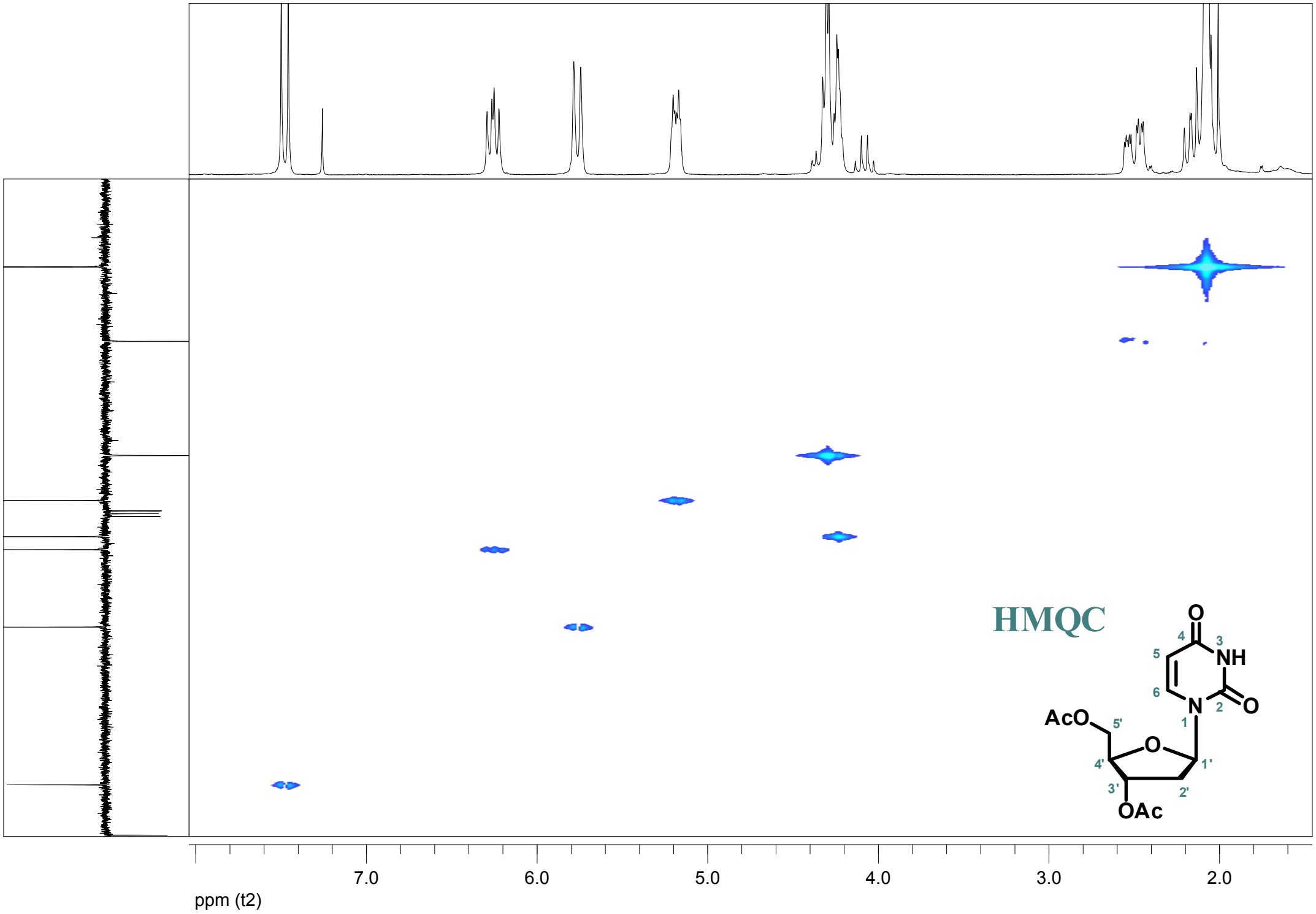

171.3
155.0
151.8
150.3
134.5
134.1
132.0
131.5
131.0
131.0
128.8
128.7
128.4
126.1
125.4
123.6
120.4
117.8
77.6
77.0
76.4
71.3
14 13C NMR
O
OCbz
S
Br
m-Ph(Cbz)
o-Ph(Cbz)
CDCl3
p-Ph(Cbz)
Cα
Cβ
C5
C6
C8
C7
-OC(O)CH2Ph
Th-Br
C10
i-Ph(Cbz)
C3
C9
OC(O)CH2Ph
C2
C4
C2-Th-
150
100
ppm (t1)

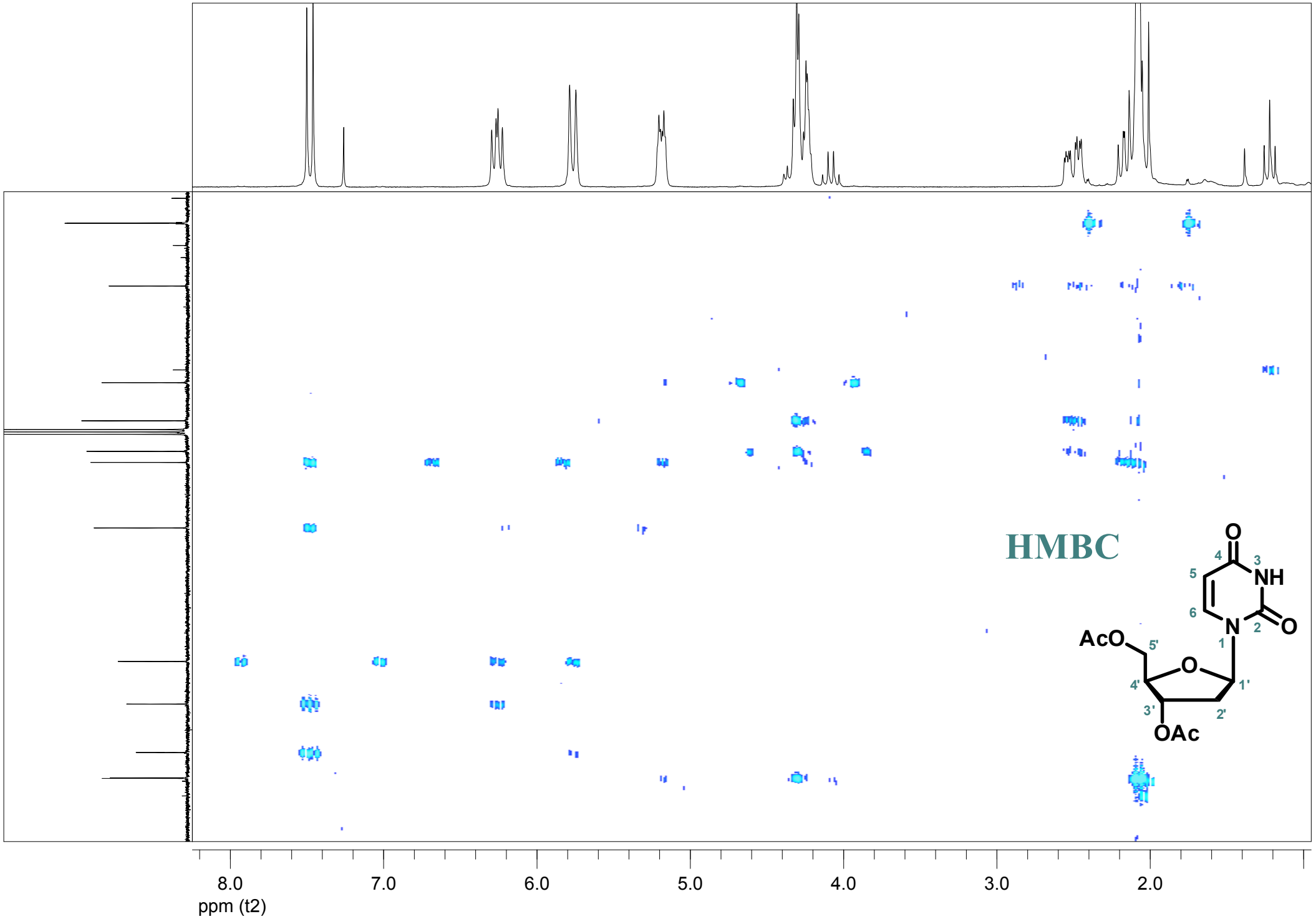

8.174
8.166
8.134
8.126
7.674
7.665
7.639
7.631
7.623
7.597
7.588
7.534
7.530
7.493
7.379
7.373
7.354
7.336
7.304
7.298
7.260
6.529
6.511
5.458
3.862
3.846
3.840
3.830
3.816
3.468
3.454
3.444
3.422
3.281
15 1H NMR
Br
-OCH2O-
Hα
Hβ
H6
H8
H7
H5
CHCl3
-OCH2A-
-CH2BO-
OCH3
1.00
1.07
1.03
2.02
0.94
1.93
1.97
1.98
2.85
8.0
7.0
6.0
5.0
4.0
3.0
ppm (t1)

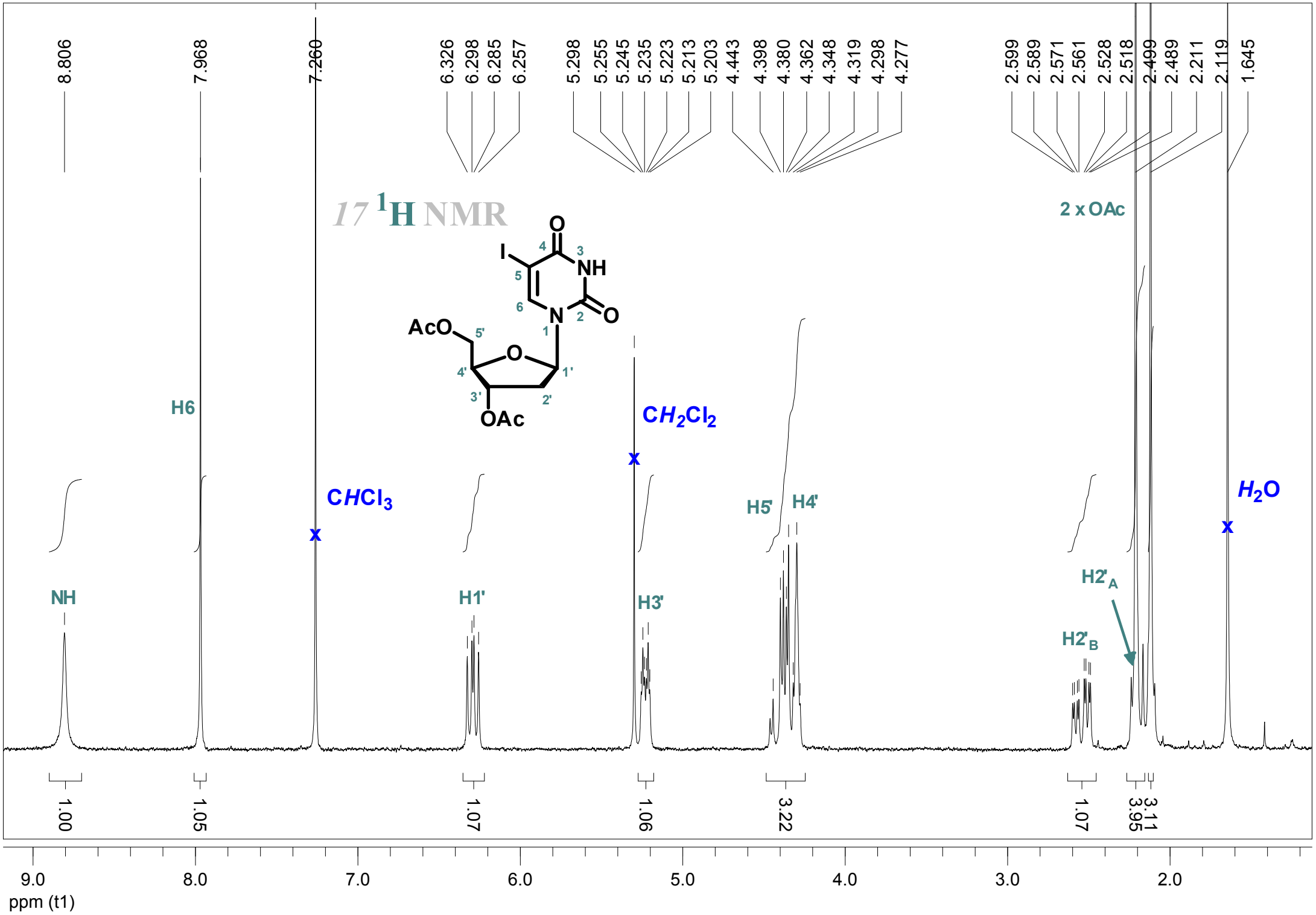

9.655
7.511
7.470
7.260
6.314
6.286
6.272
6.244
5.805
5.765
5.219
5.186
4.348
4.325
4.311
4.264
4.256
4.246
2.580
2.569
2.551
2.541
2.508
2.499
2.480
2.470
2.108
2.099
2.031
AcO
OAc
1H NMR
2 x OAc
H4'
H5'A & B
H6
CHCl3
NH
H1'
H5
H3'
EtOAc
H2'A
H2'B
H2O
EtOAc
0.96
1.00
1.00
1.00
1.00
3.01
1.04
7.40
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)

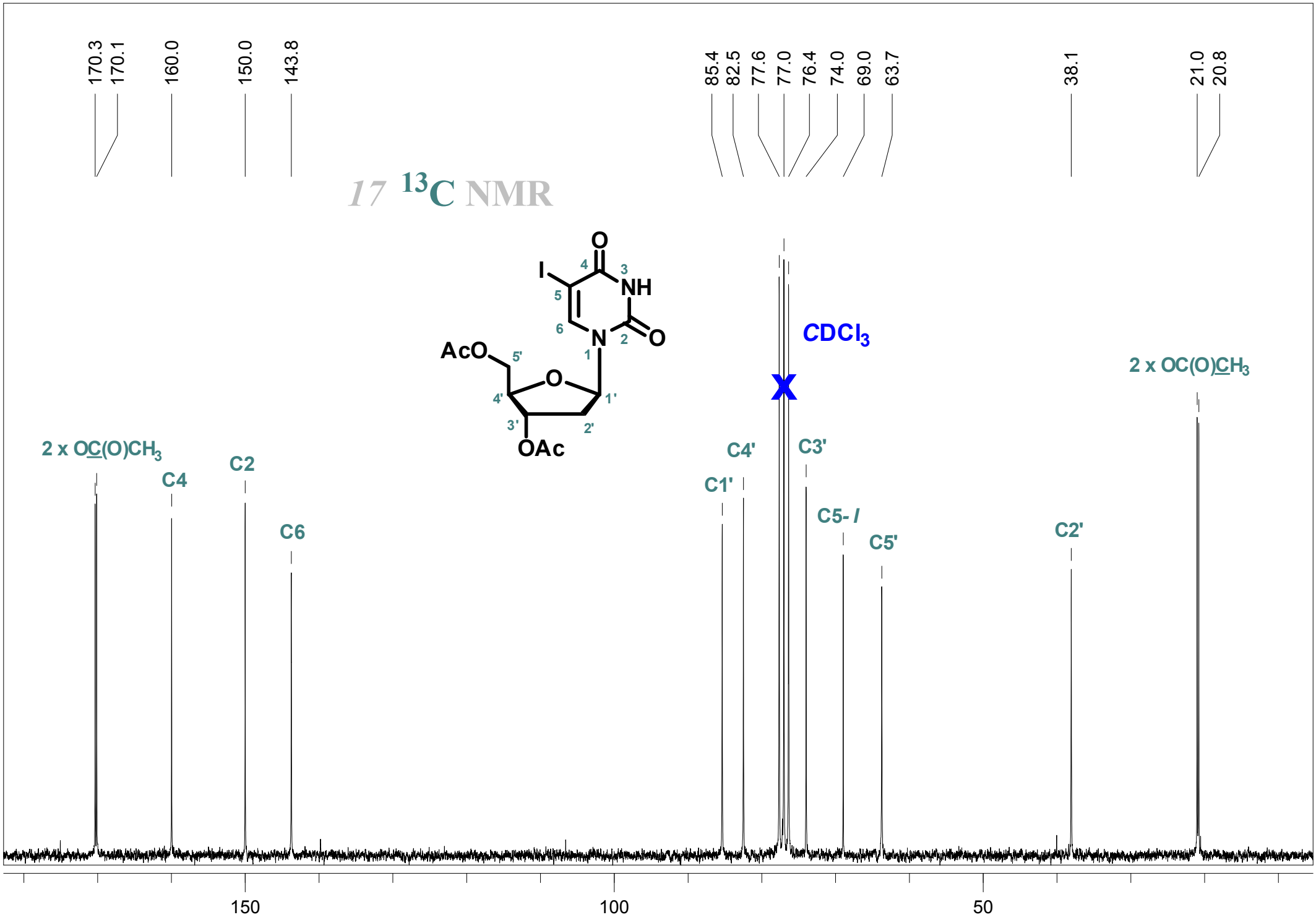

170.3
170.2
163.4
150.3
138.8
102.9
85.2
82.2
77.6
77.0
76.4
74.0
63.7
37.7
20.8
20.7
13C NMR
CDCl3
2 x OC(O)CH3
C4
C2
C6
C5
C1'
C4'
C3'
C5'
C2'
2 x OC(O)CH3
150
100
50
ppm (t1)

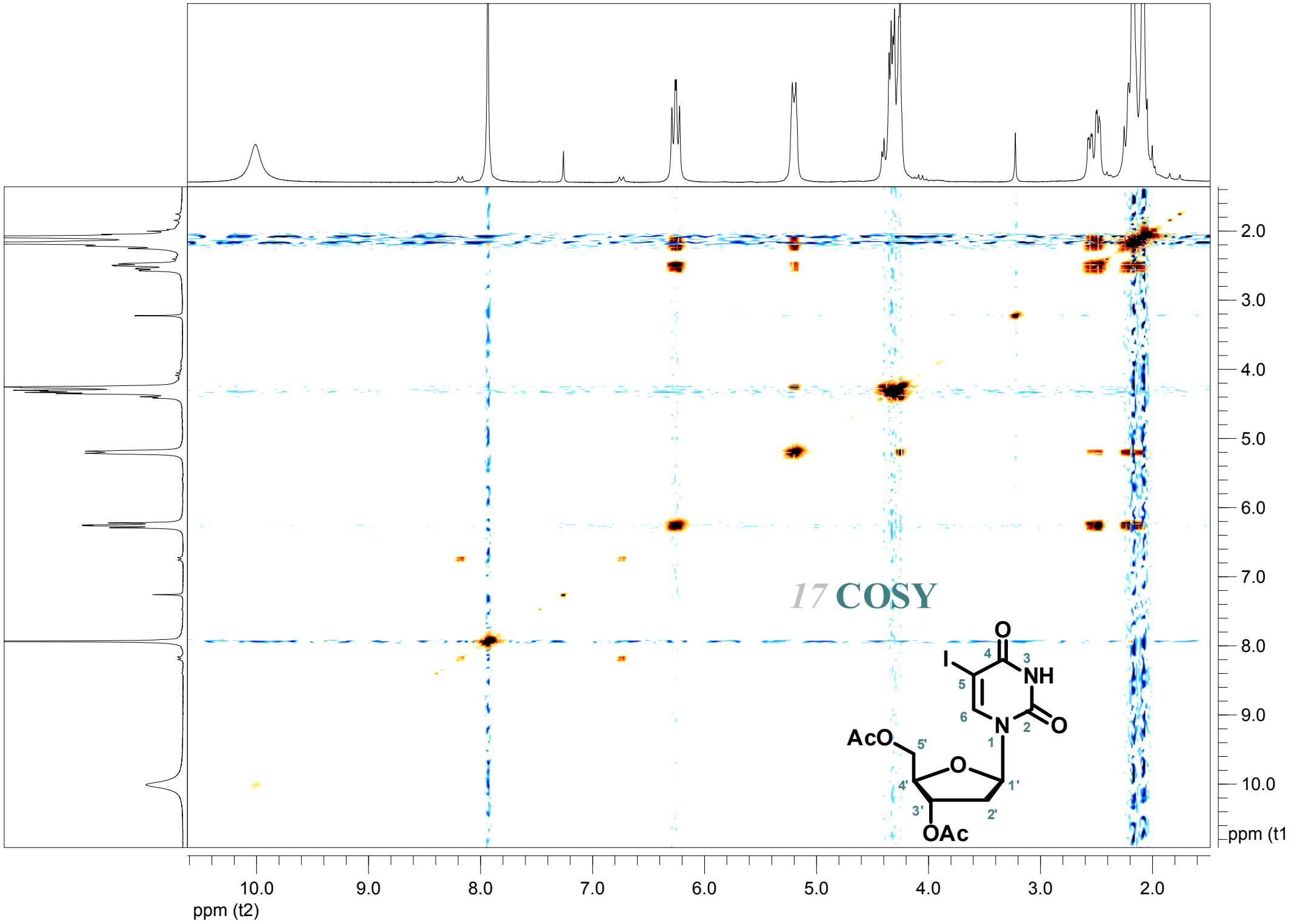

COSY
O
4
3
NH
5
6
N
2
O
1
AcO
5'
O
4'
1'
3'
2'
OAc
10.0
5.0
ppm (t2)

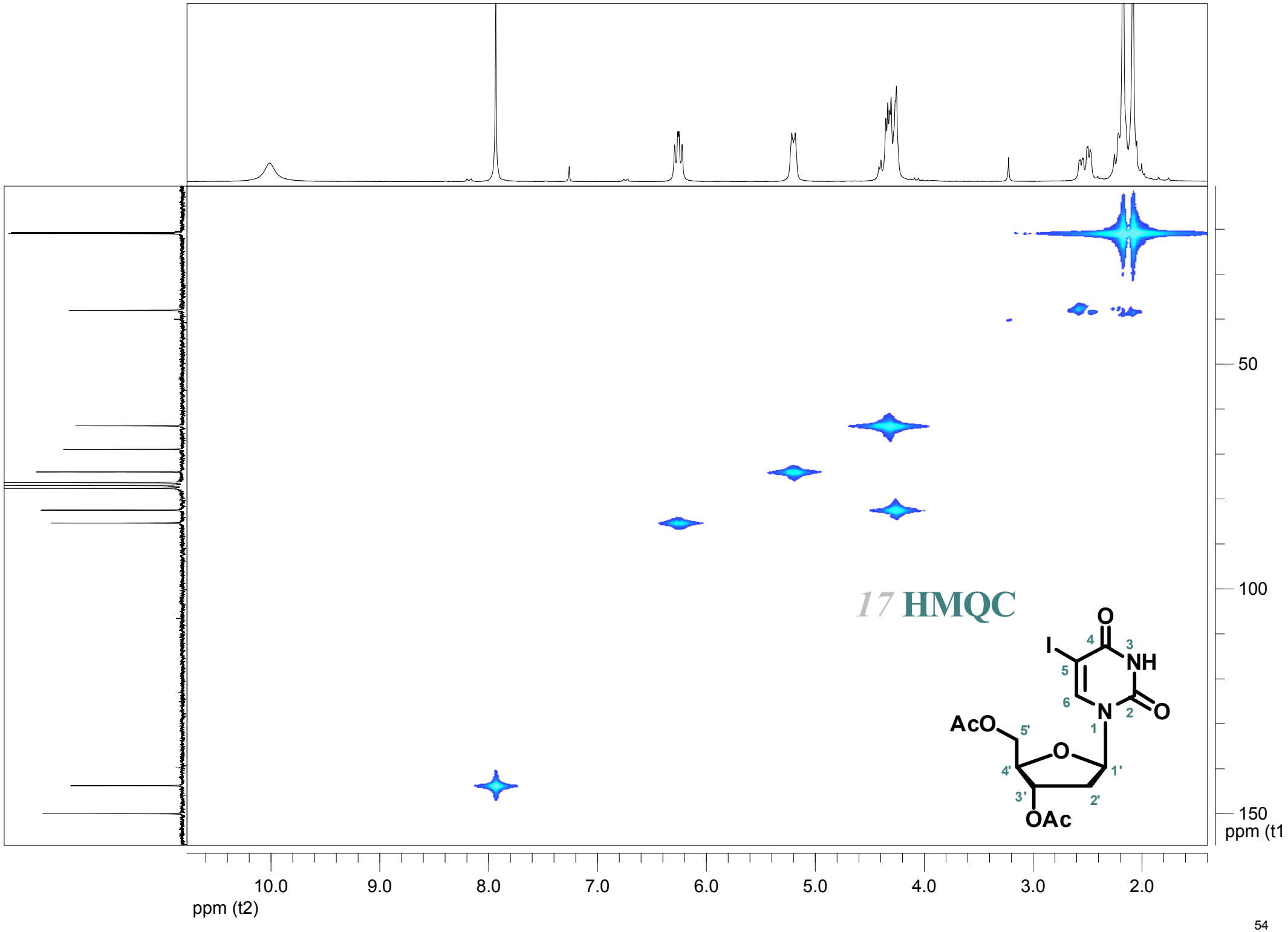

HMQC
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t2)
O
NH
N
AcO
OAc
O
1
2
3
4
5
6
1'
2'
3'
4'
5'

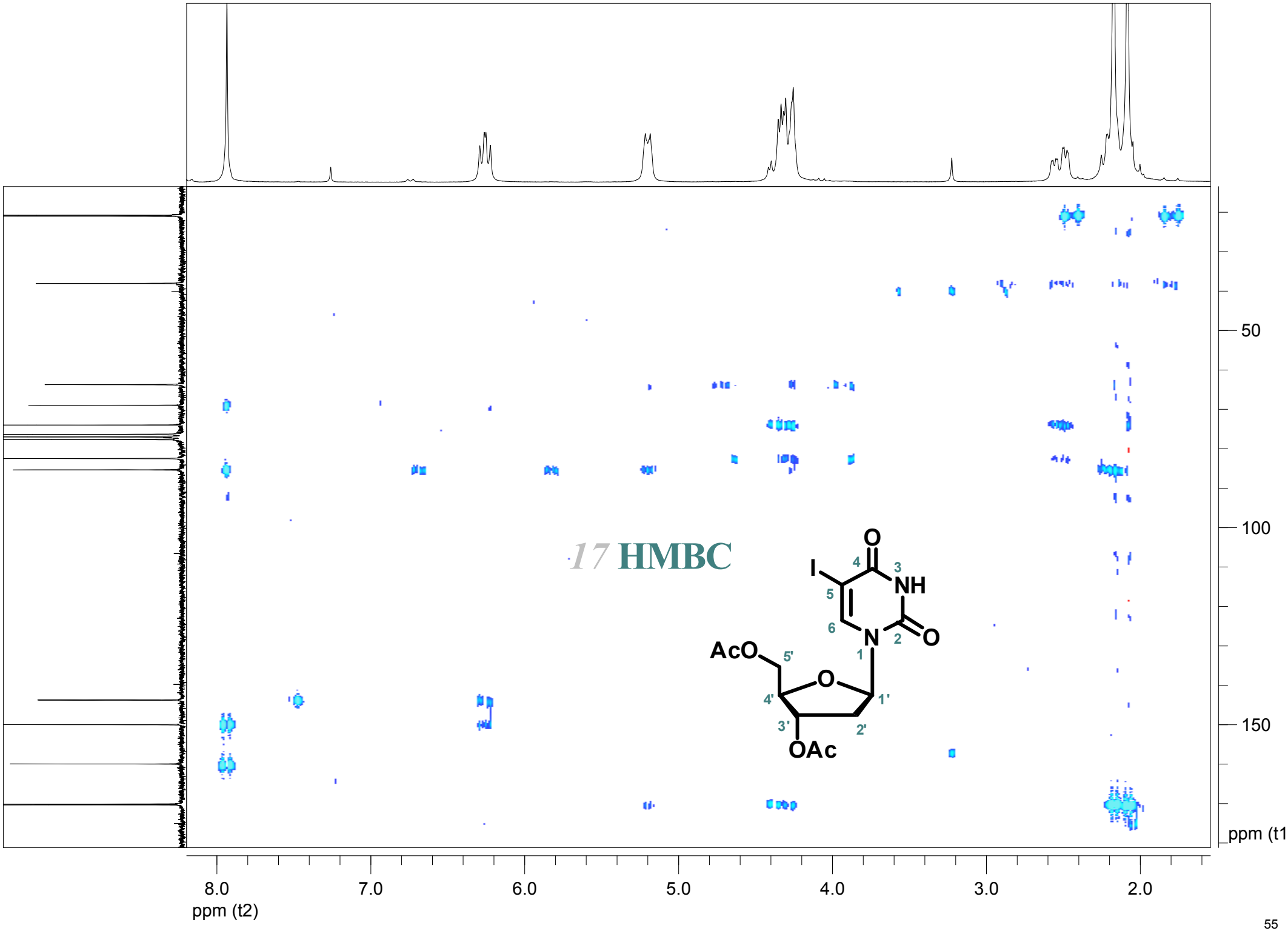

HMBC
O
4
3
NH
5
6
N
2
O
1
AcO
5'
O
4'
1'
3'
2'
OAc
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t2)

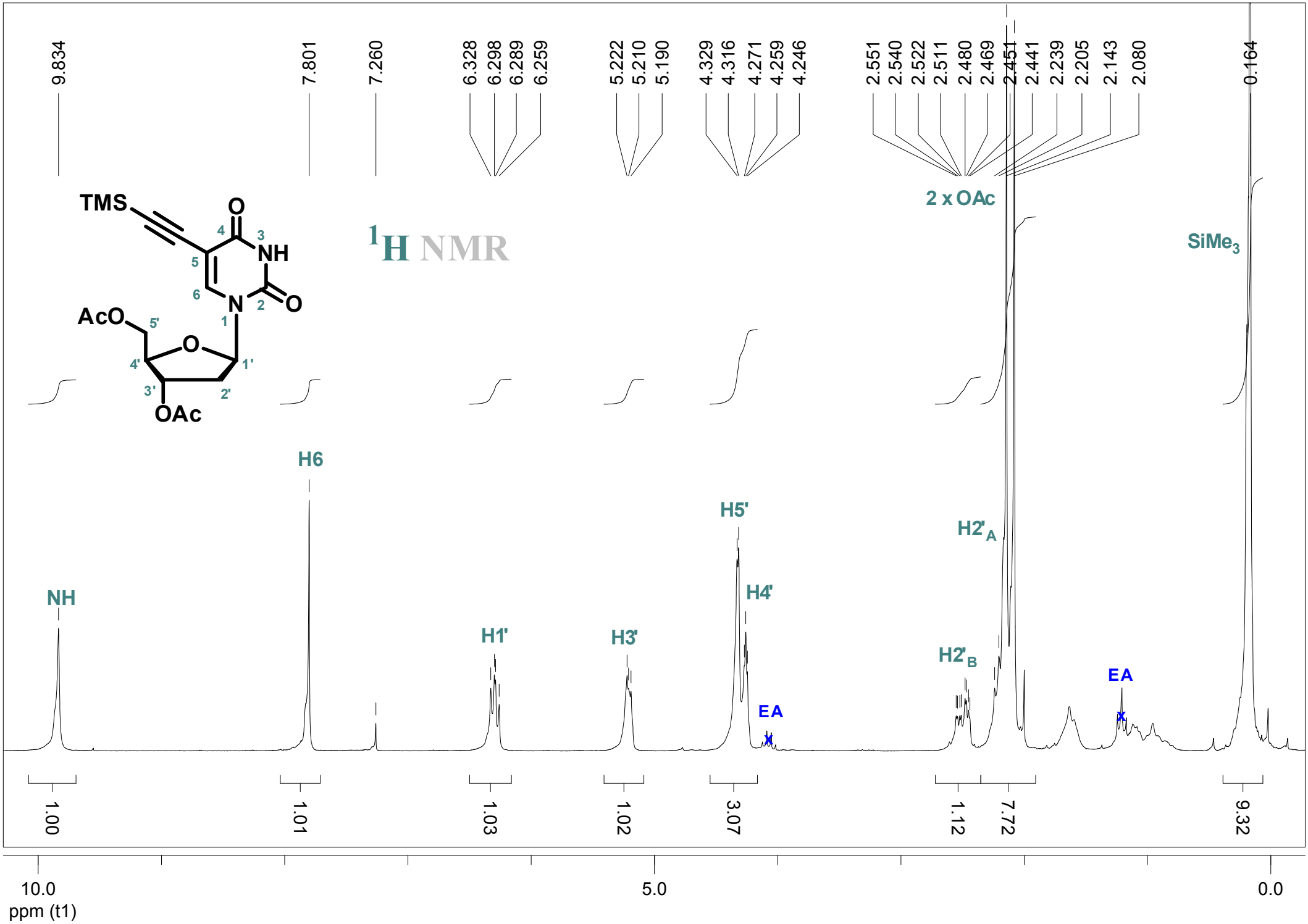

8.806
7.968
7.260
6.326
6.298
6.285
6.257
5.298
5.255
5.245
5.235
5.223
5.213
5.203
4.443
4.398
4.380
4.362
4.348
4.319
4.298
4.277
2.599
2.589
2.571
2.561
2.528
2.518
2.499
2.489
2.211
2.119
1.645
17 1H NMR
2 x OAc
H6
CHCl3
CH2Cl2
H5'
H4'
H2'A
H2'B
NH
H1'
H3'
H2O
1.00
1.05
1.07
1.06
3.22
1.07
3.95
3.11
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t1)

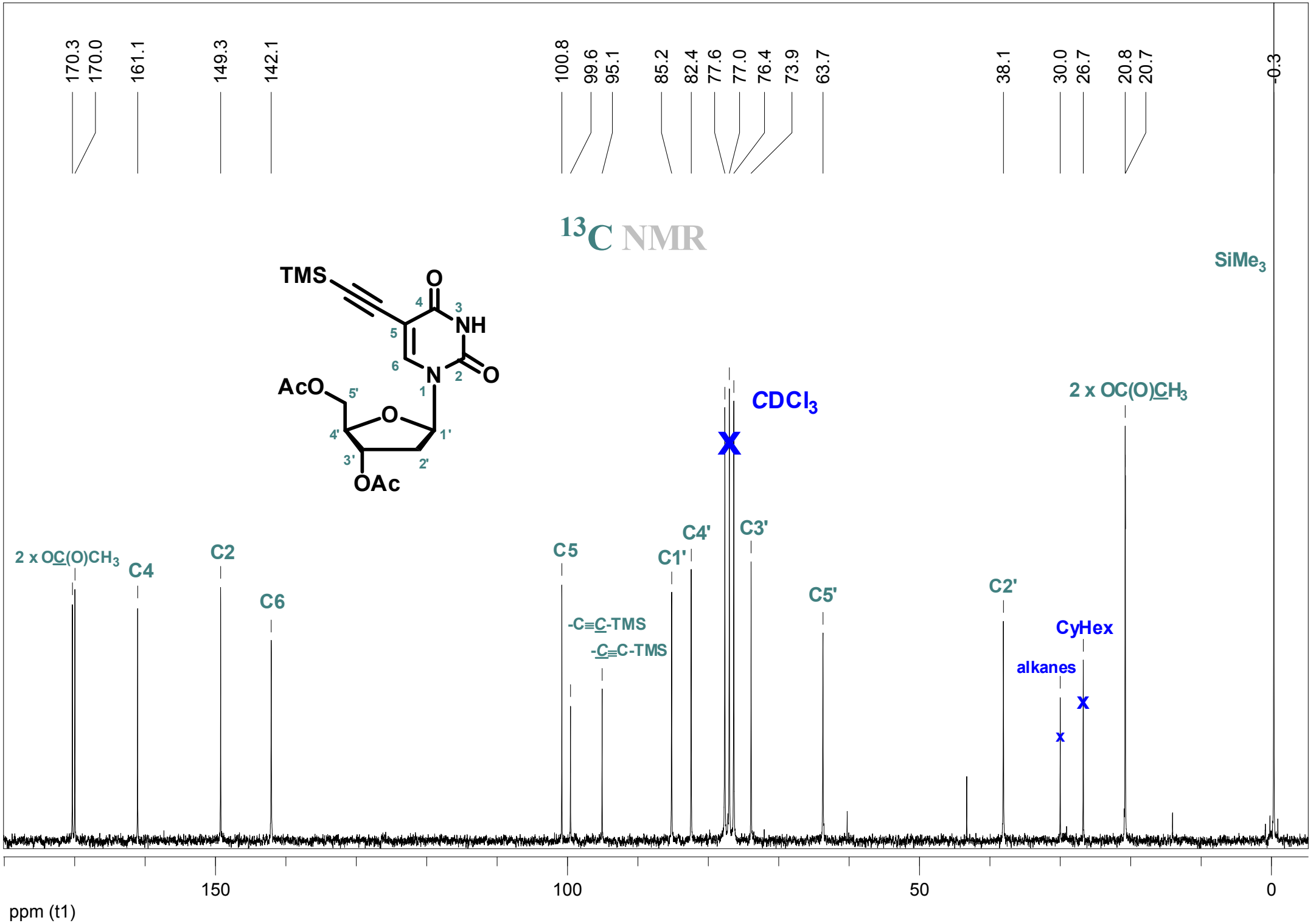

170.3
170.1
160.0
150.0
143.8
85.4
82.5
77.6
77.0
76.4
74.0
69.0
63.7
38.1
21.0
20.8
17 13C NMR
AcO
OAc
CDCl3
2 x OC(O)CH3
C4
C2
C6
C1'
C4'
C3'
C5-I
C5'
C2'
2 x OC(O)CH3
150
100
50
ppm (t1)

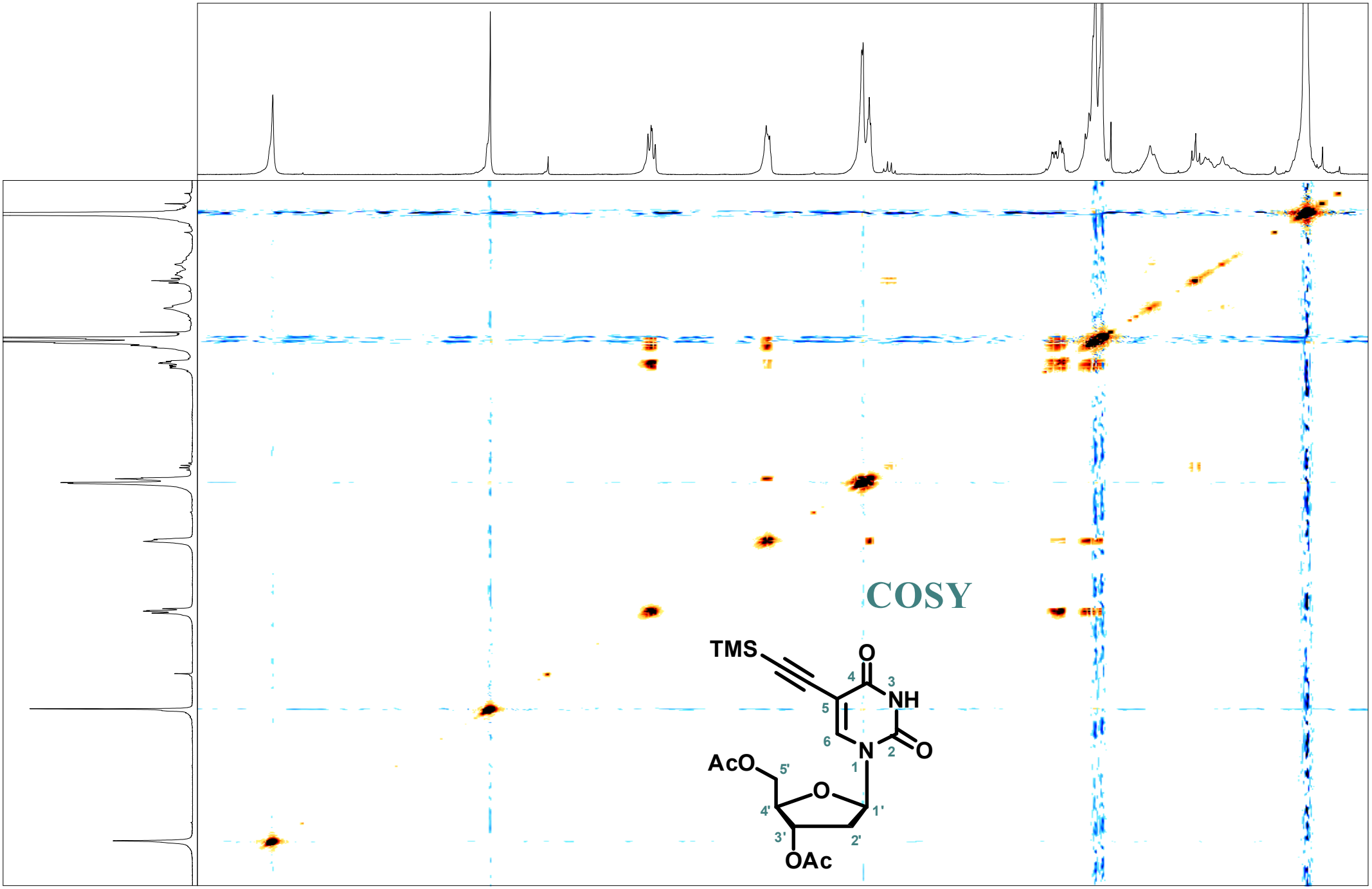

17 COSY
I
O
NH
N
AcO
O
OAc
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t2)
ppm (t1

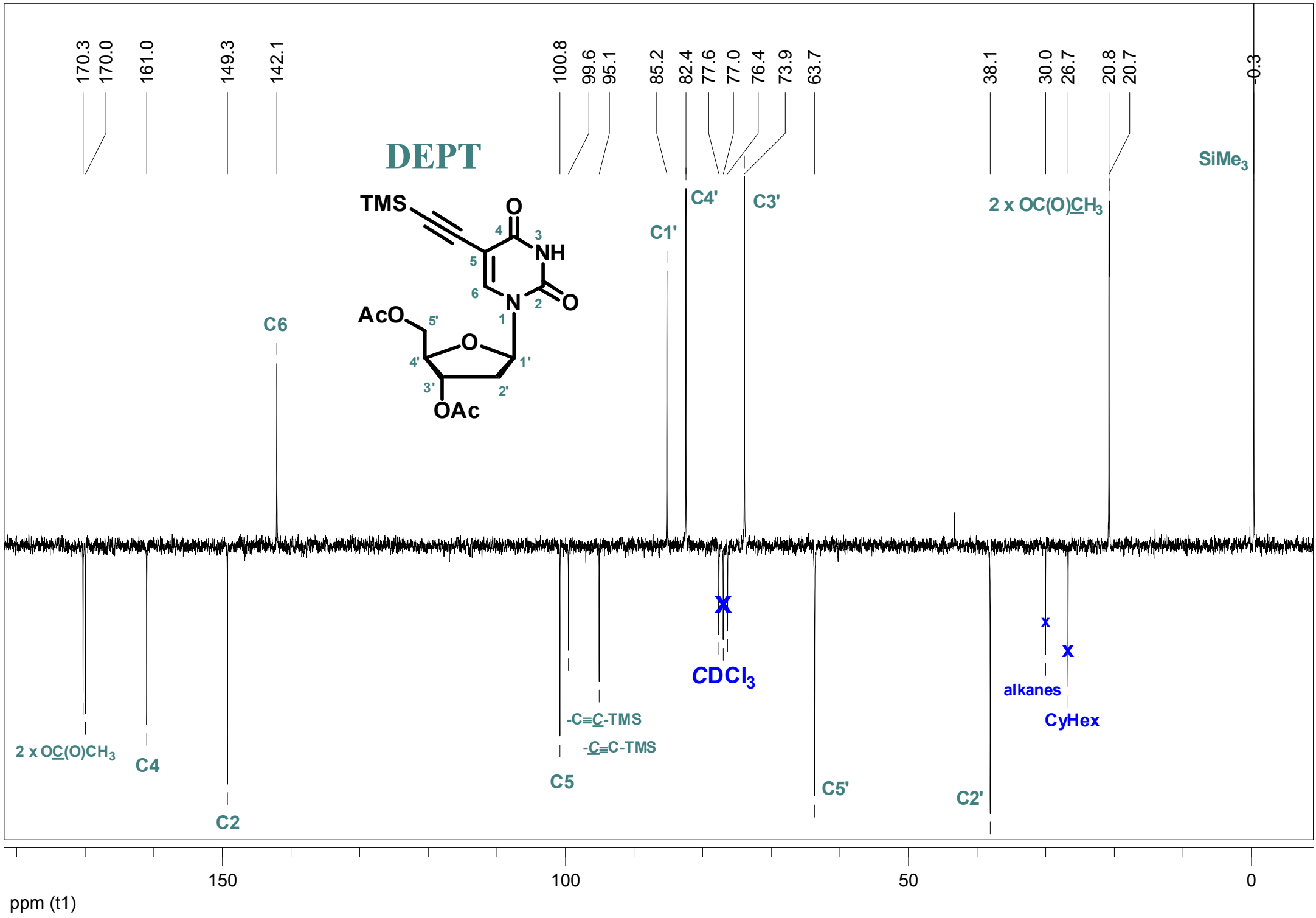

17 HMQC
50
100
150
ppm (t1
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t2)
O
I
4
3
NH
5
6
N
2
O
1
AcO
5'
O
4'
1'
3'
2'
OAc

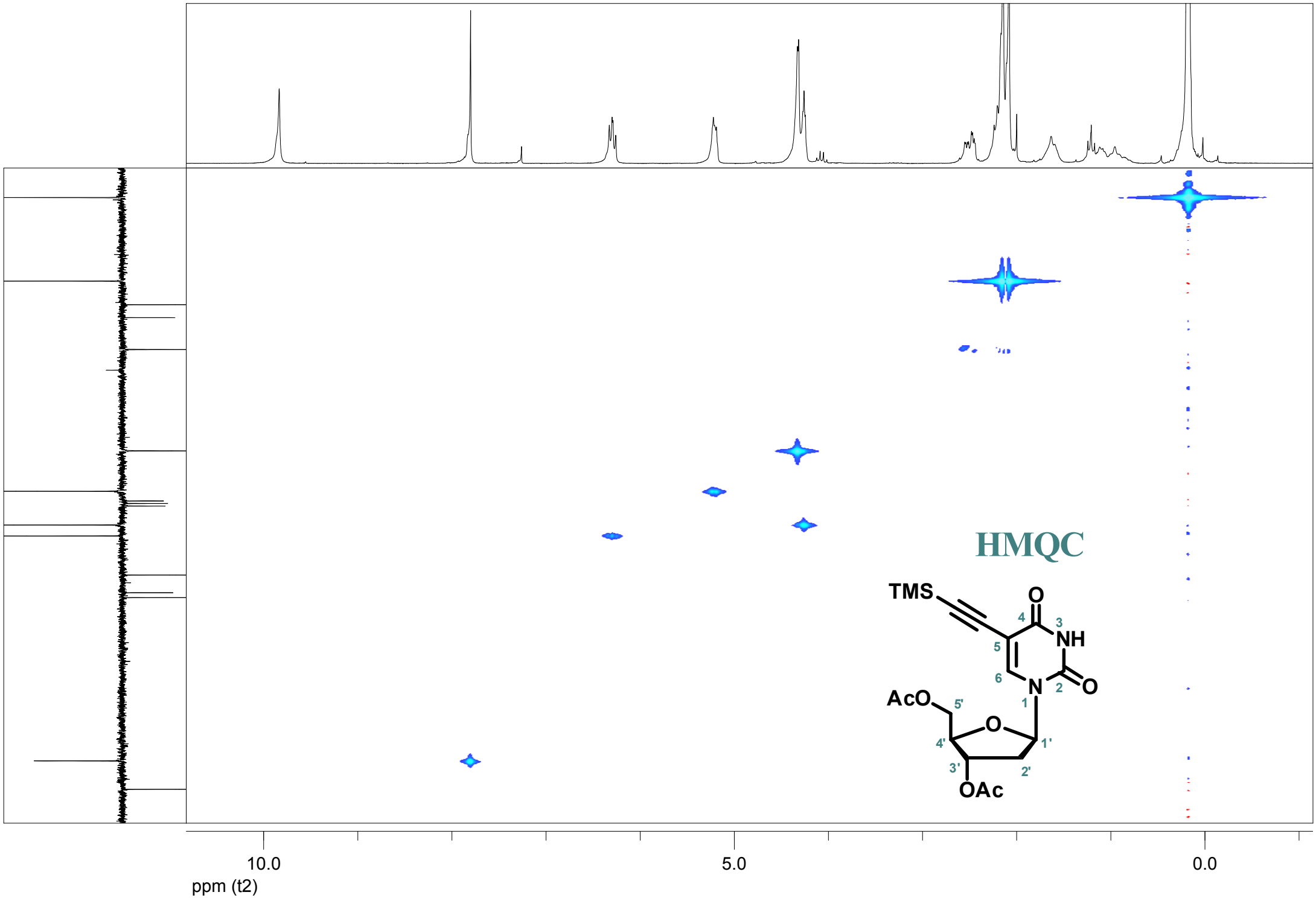

17 HMBC
I
O
4
3
NH
5
6
N
2
O
1
AcO
5'
O
4'
1'
3'
2'
OAc
50
100
150
ppm (t1
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t2)

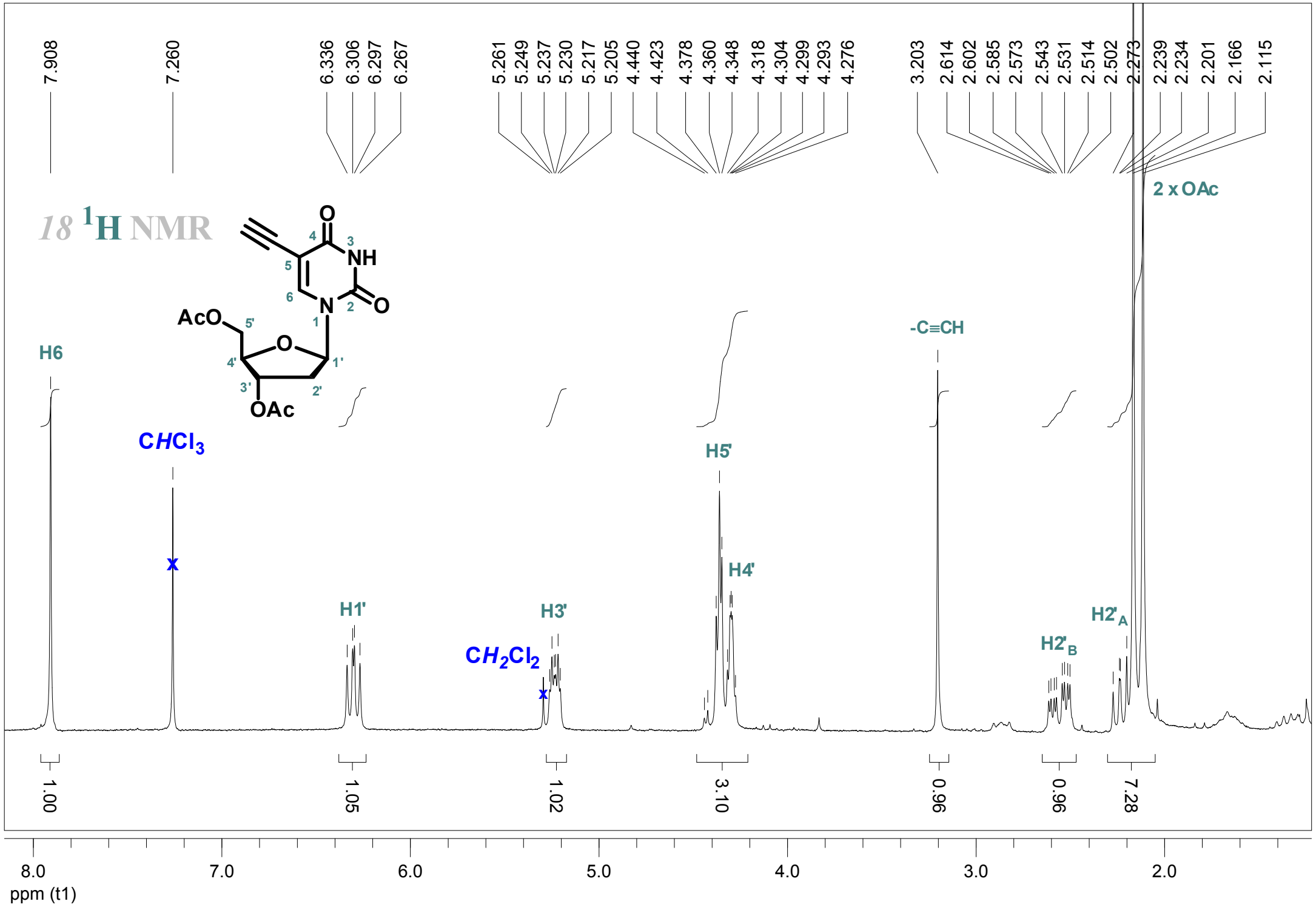

9.834
7.801
7.260
6.328
6.298
6.289
6.259
5.222
5.210
5.190
4.329
4.316
4.271
4.259
4.246
2.551
2.540
2.522
2.511
2.480
2.469
2.451
2.441
2.239
2.205
2.143
2.080
0.164
TMS
AcO
OAc
1H NMR
2 x OAc
SiMe3
H6
NH
H1'
H3'
H5'
H4'
H2'A
H2'B
EA
EA
1.00
1.01
1.03
1.02
3.07
1.12
7.72
9.32
10.0
ppm (t1)
5.0
0.0

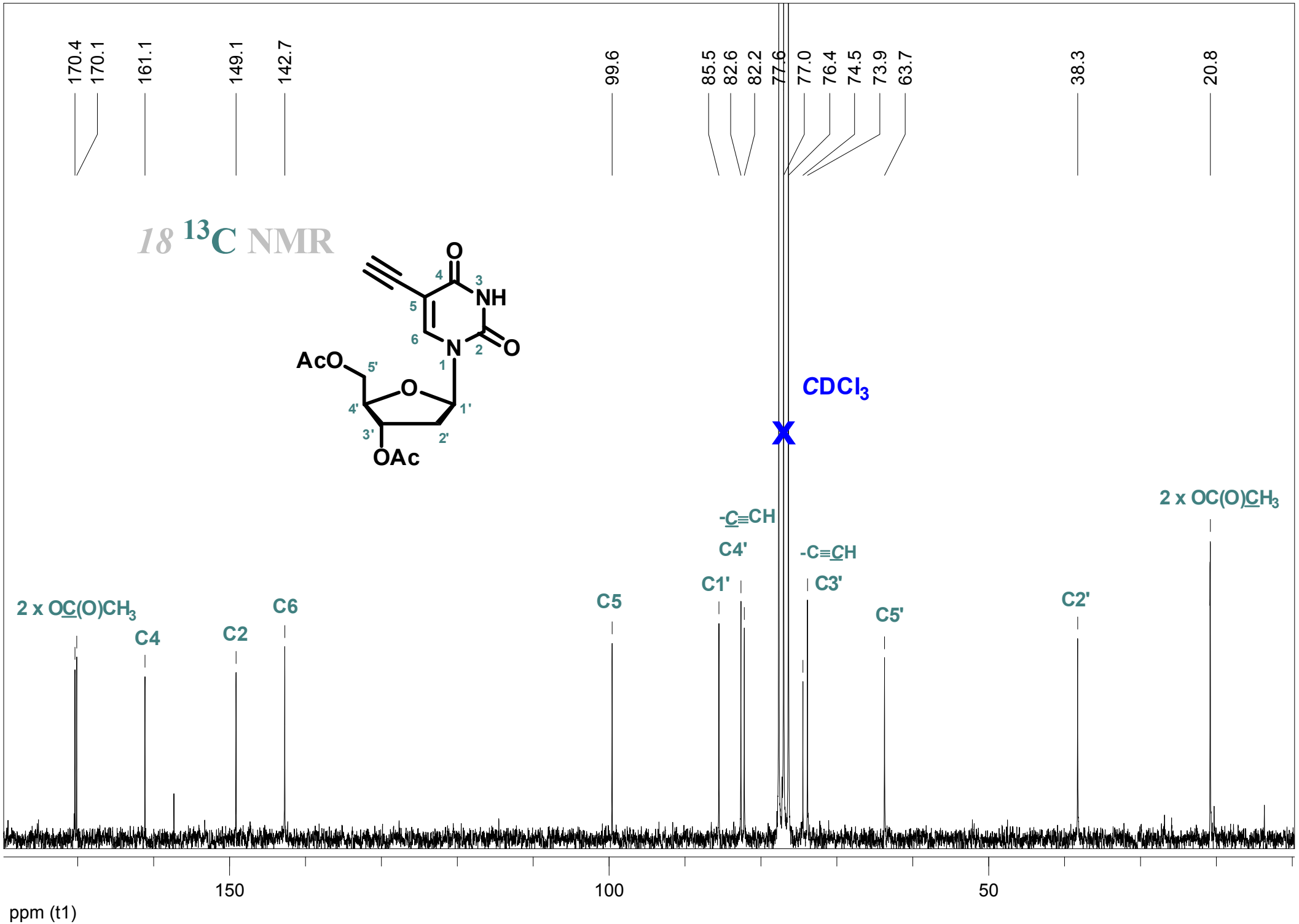

170.3
170.0
161.1
149.3
142.1
100.8
99.6
95.1
85.2
82.4
77.6
77.0
76.4
73.9
63.7
38.1
30.0
26.7
20.8
20.7
-0.3
13C NMR
SiMe3
TMS
AcO
OAc
2 x OC(O)CH3
CDCl3
C4
C2
C6
C5
C1'
C4'
C3'
C5'
C2'
-C≡C-TMS
-C≡C-TMS
CyHex
alkanes
150
100
50
0
ppm (t1)

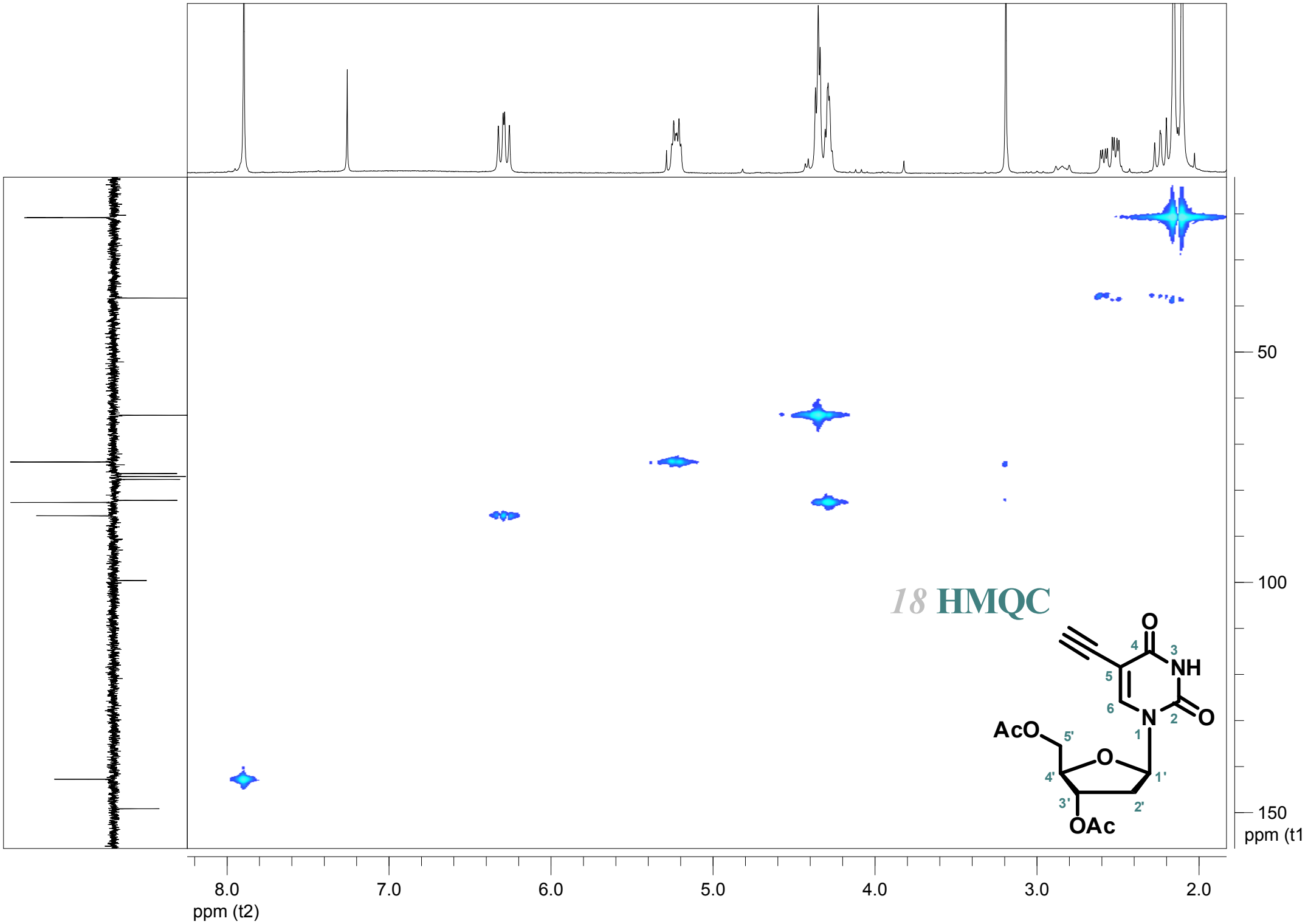

COSY
TMS
O
4
3
NH
5
6
N
2
O
1
AcO
5'
O
4'
1'
3'
2'
OAc
10.0
ppm (t2)
5.0
0.0

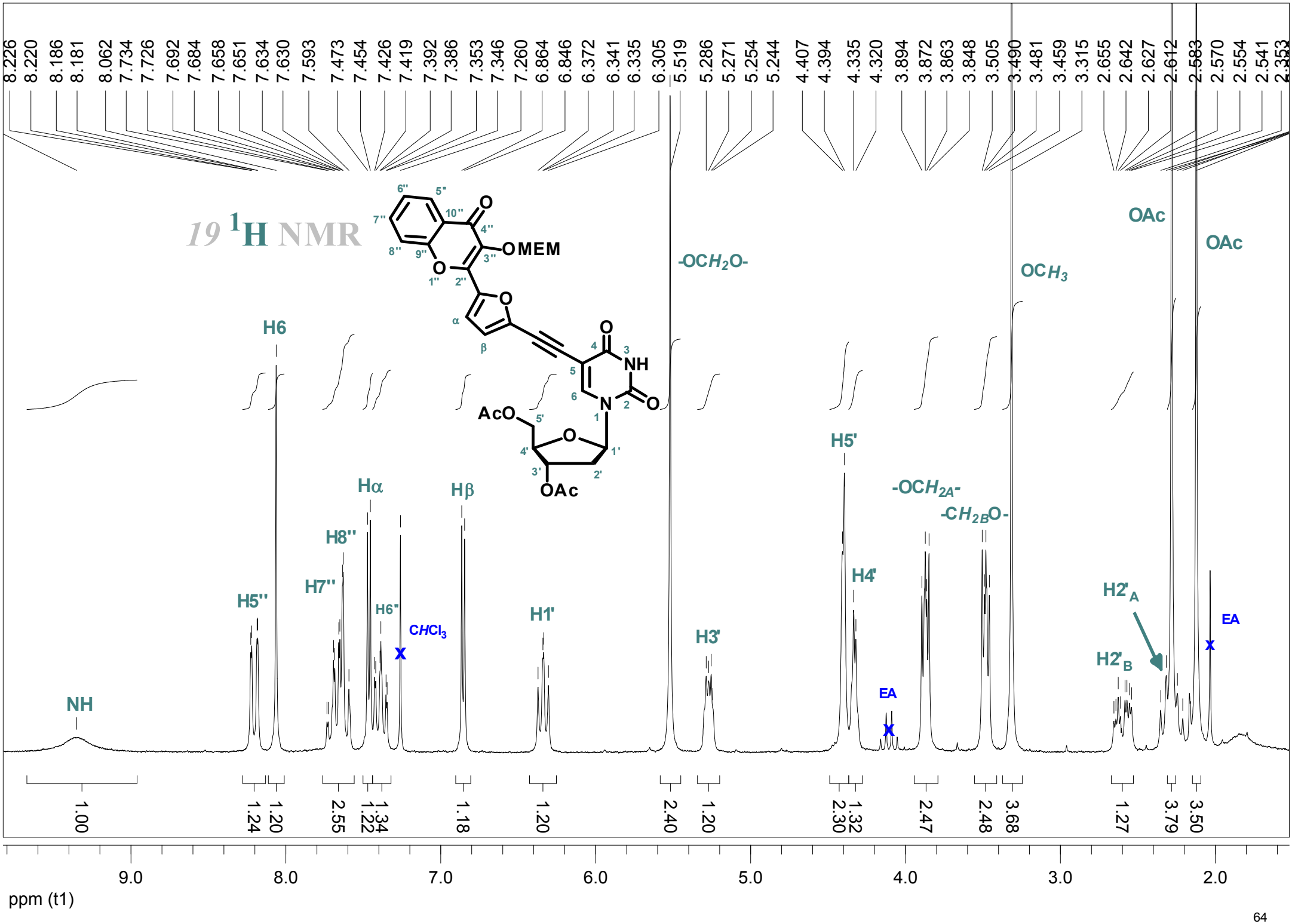

170.3
170.0
161.0
149.3
142.1
100.8
99.6
95.1
85.2
82.4
77.6
77.0
76.4
73.9
63.7
38.1
30.0
26.7
20.8
20.7
0.3
DEPT
TMS
AcO
OAc
NH
O
N
C6
C1'
C4'
C3'
2 x OC(O)CH3
SiMe3
2 x OC(O)CH3
C4
C2
-C≡C-TMS
-C≡C-TMS
C5
CDCl3
C5'
C2'
alkanes
CyHex
150
100
50
0
ppm (t1)

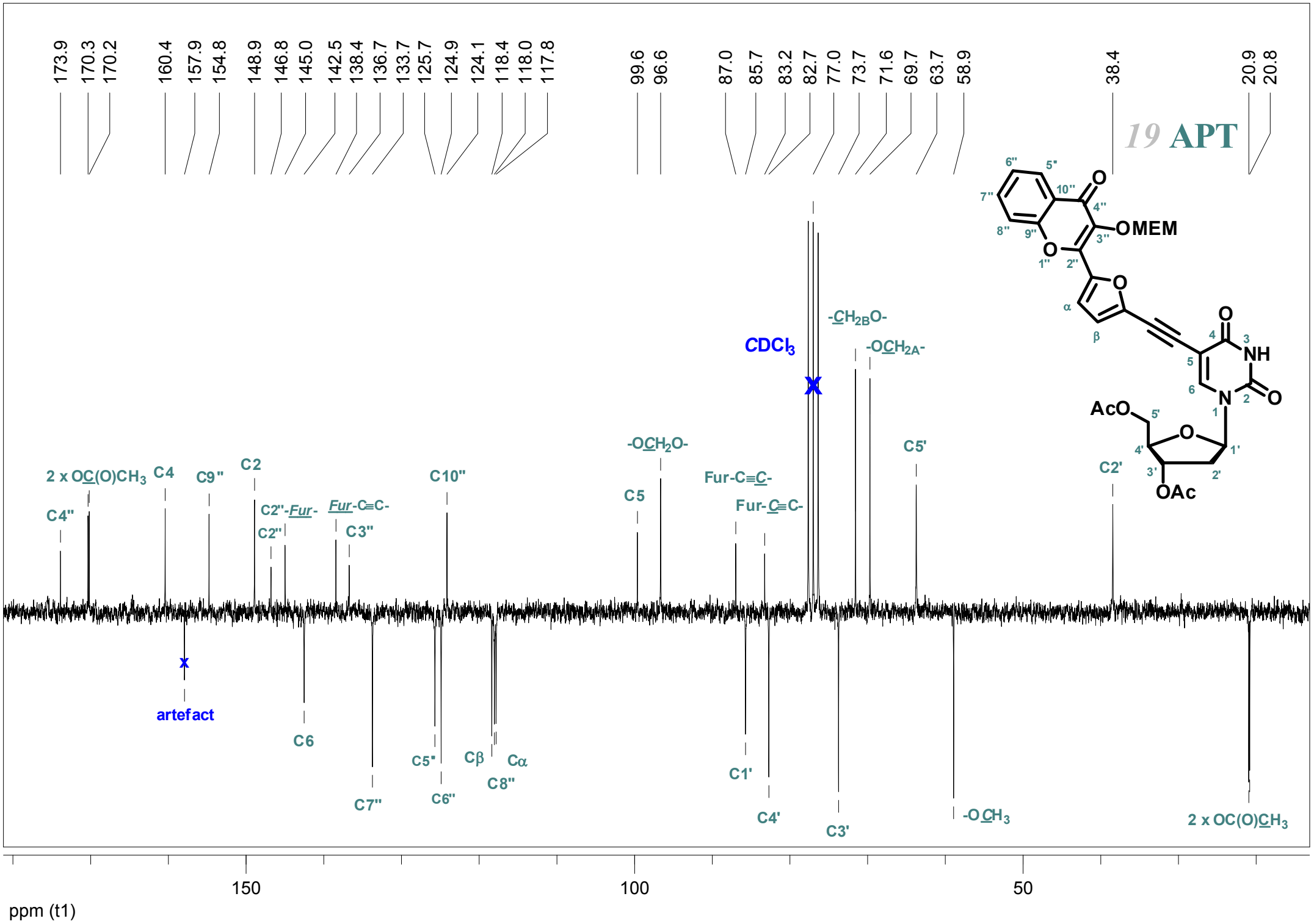

HMQC
TMS
O
4
3
NH
5
6
N
2
O
1
AcO
5'
O
4'
1'
3'
2'
OAc
10.0
5.0
0.0
ppm (t2)

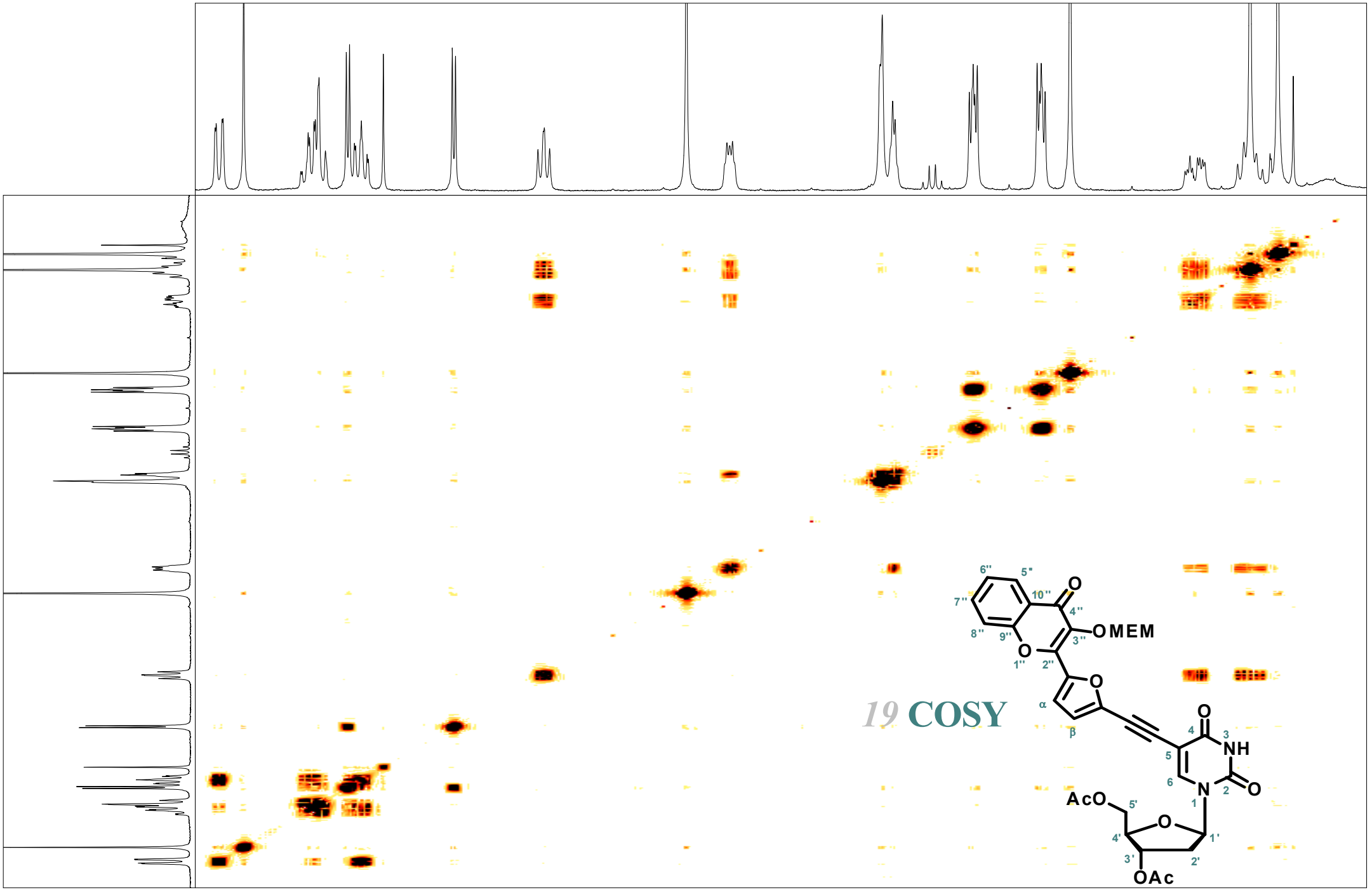

7.908
7.260
6.336
6.306
6.297
6.267
5.261
5.249
5.237
5.230
5.217
5.205
4.440
4.423
4.378
4.360
4.348
4.318
4.304
4.299
4.293
4.276
3.203
2.614
2.602
2.585
2.573
2.543
2.531
2.514
2.502
2.273
2.239
2.234
2.201
2.166
2.115
18 1H NMR
2 x OAc
H6
CHCl3
H1'
H3'
CH2Cl2
H5'
H4'
-C≡CH
H2'A
H2'B
1.00
1.05
1.02
3.10
0.96
0.96
7.28
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t1)

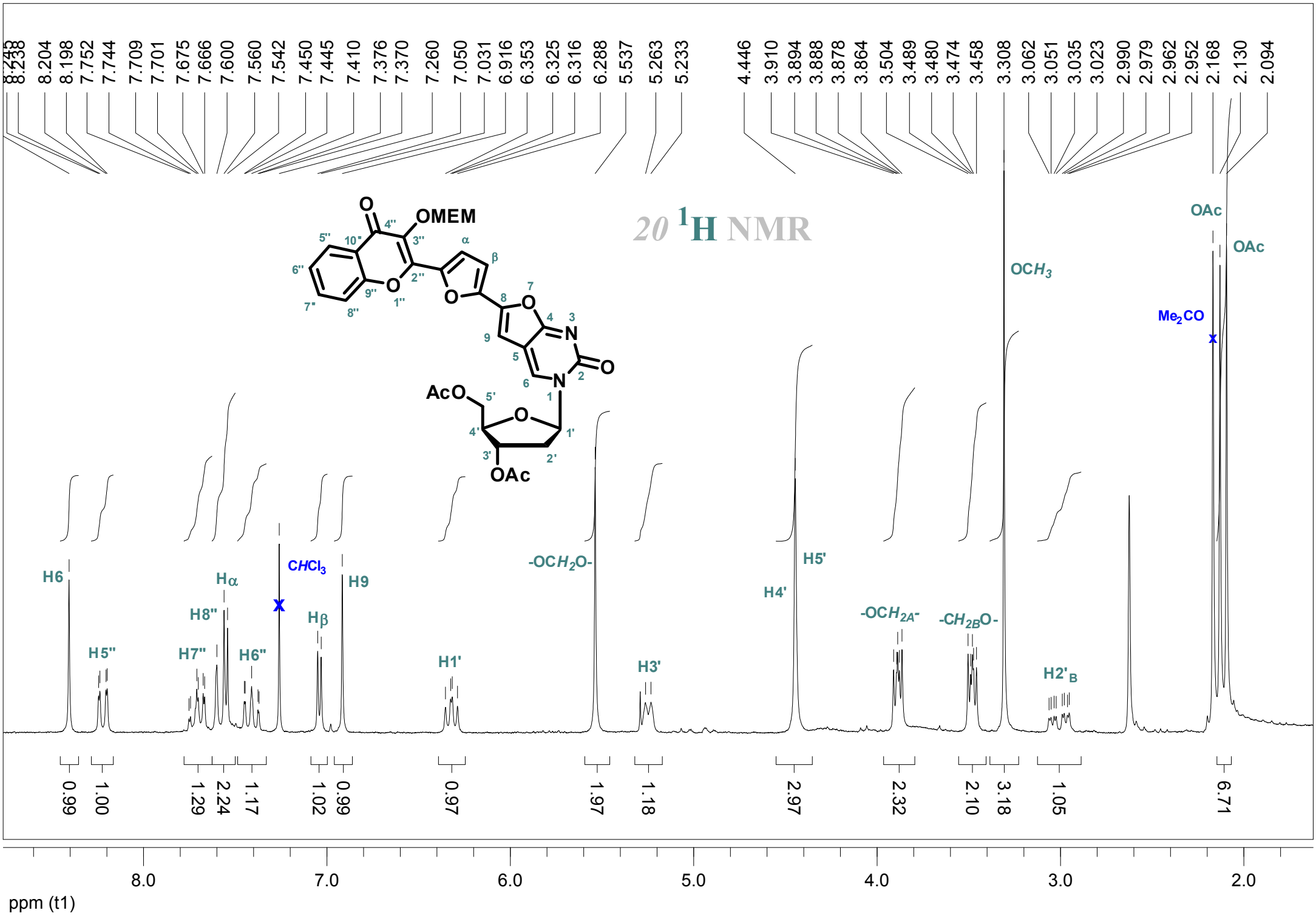

170.4
170.1
161.1
149.1
142.7
99.6
85.5
82.6
82.2
77.6
77.0
76.4
74.5
73.9
63.7
38.3
20.8
18 13C NMR
O
4
3
NH
5
6
N
2
O
1
AcO
5'
O
4'
1'
3'
2'
OAc
CDCl3
2 x OC(O)CH3
-C≡CH
C4'
-C≡CH
C1'
C3'
2 x OC(O)CH3
C5
C6
C4
C2
C5'
C2'
150
100
50
ppm (t1)

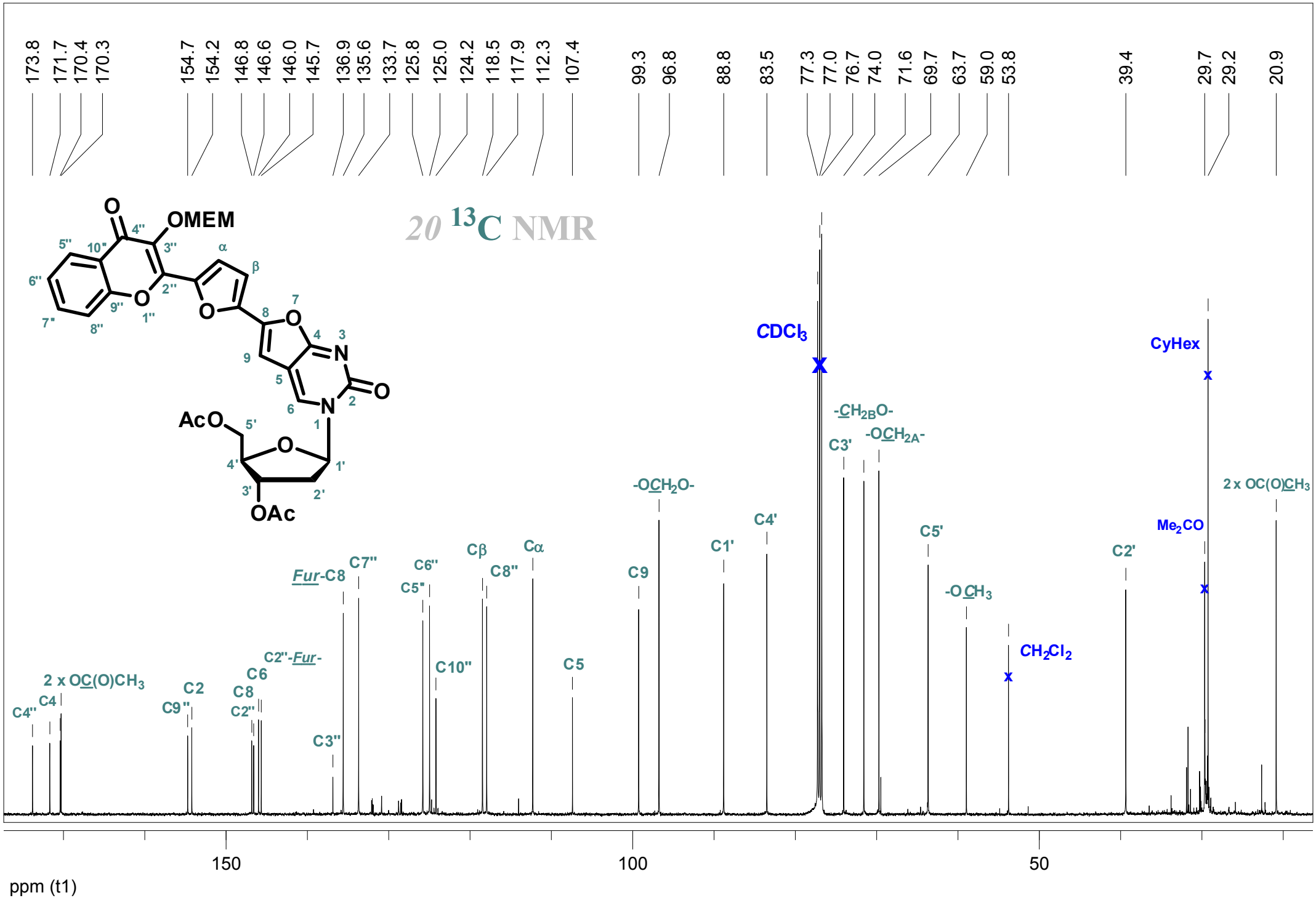

18 HMQC
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t2)
50
100
150
ppm (t1
O
NH
N
AcO
OAc
1
2
3
4
5
6
1'
2'
3'
4'
5'

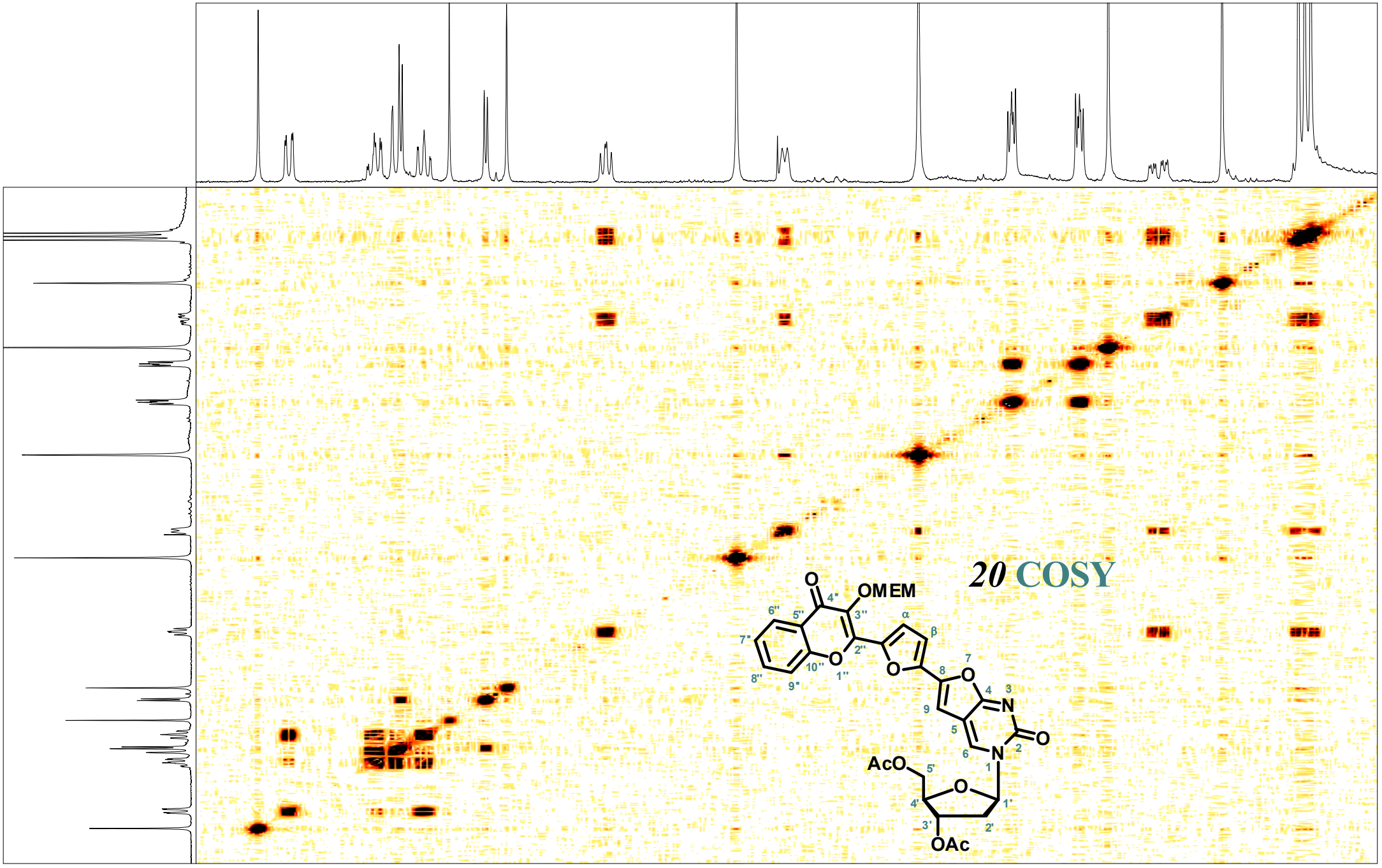

19 1H NMR
8.226 8.220 8.186 8.181 8.062 7.734 7.726 7.692 7.684 7.658 7.651 7.634 7.630 7.593 7.473 7.454 7.426 7.419 7.392 7.386 7.353 7.346 7.260 6.864 6.846 6.372 6.341 6.335 6.305 5.519 5.286 5.271 5.254 5.244 4.407 4.394 4.335 4.320 3.894 3.872 3.863 3.848 3.505 3.490 3.481 3.459 3.315 2.655 2.642 2.627 2.612 2.583 2.570 2.554 2.541 2.353
-OCH2O-
OCH3
OAc
OAc
H6
Hα
Hβ
H8''
H7''
H5''
H6''
CHCl3
H1'
H3'
H5'
H4'
-OCH2A-
-CH2BO-
H2'A
H2'B
EA
EA
NH
1.00 1.24 1.20 2.55 1.34 1.22 1.18 1.20 2.40 1.20 2.30 1.32 2.47 2.48 3.68 1.27 3.79 3.50
9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0
ppm (t1)

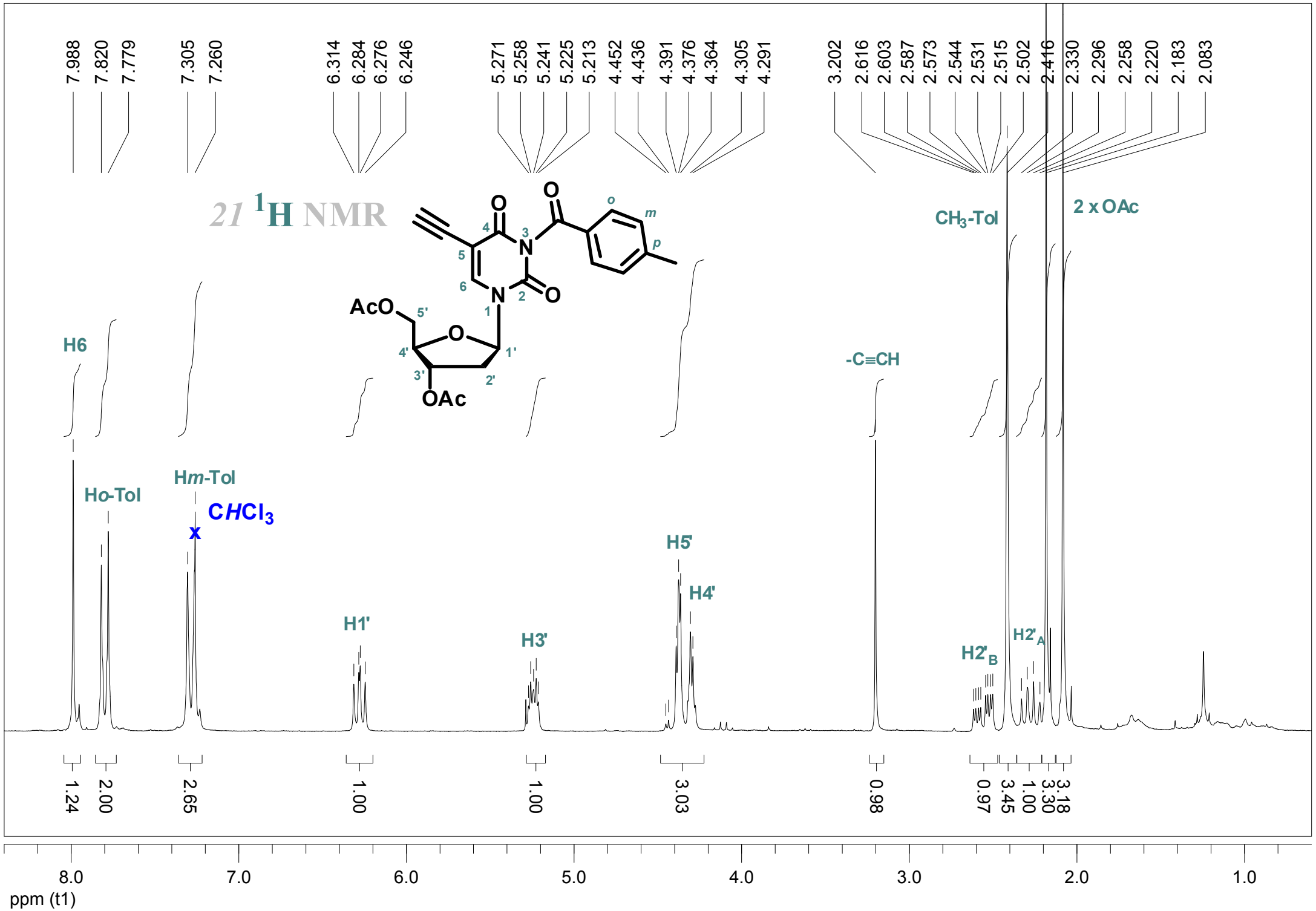

173.9
170.3
170.2
160.4
157.9
154.8
148.9
146.8
145.0
142.5
138.4
136.7
133.7
125.7
124.9
124.1
118.4
118.0
117.8
99.6
96.6
87.0
85.7
83.2
82.7
77.0
73.7
71.6
69.7
63.7
58.9
38.4
20.9
20.8
19 APT
CDCl3
-CH2BO-
-OCH2A-
-OCH2O-
C5
C5'
C2'
Fur-C≡C-
Fur-C≡C-
2 x OC(O)CH3
C4"
C4
C9"
C2
C2"-Fur-
C2"
Fur-C≡C-
C3"
C10"
artefact
C6
C7"
C5"
C6"
Cβ
Cα
C8"
C1'
C4'
C3'
-OCH3
2 x OC(O)CH3
OMEM
AcO
OAc
NH
150
100
50
ppm (t1)

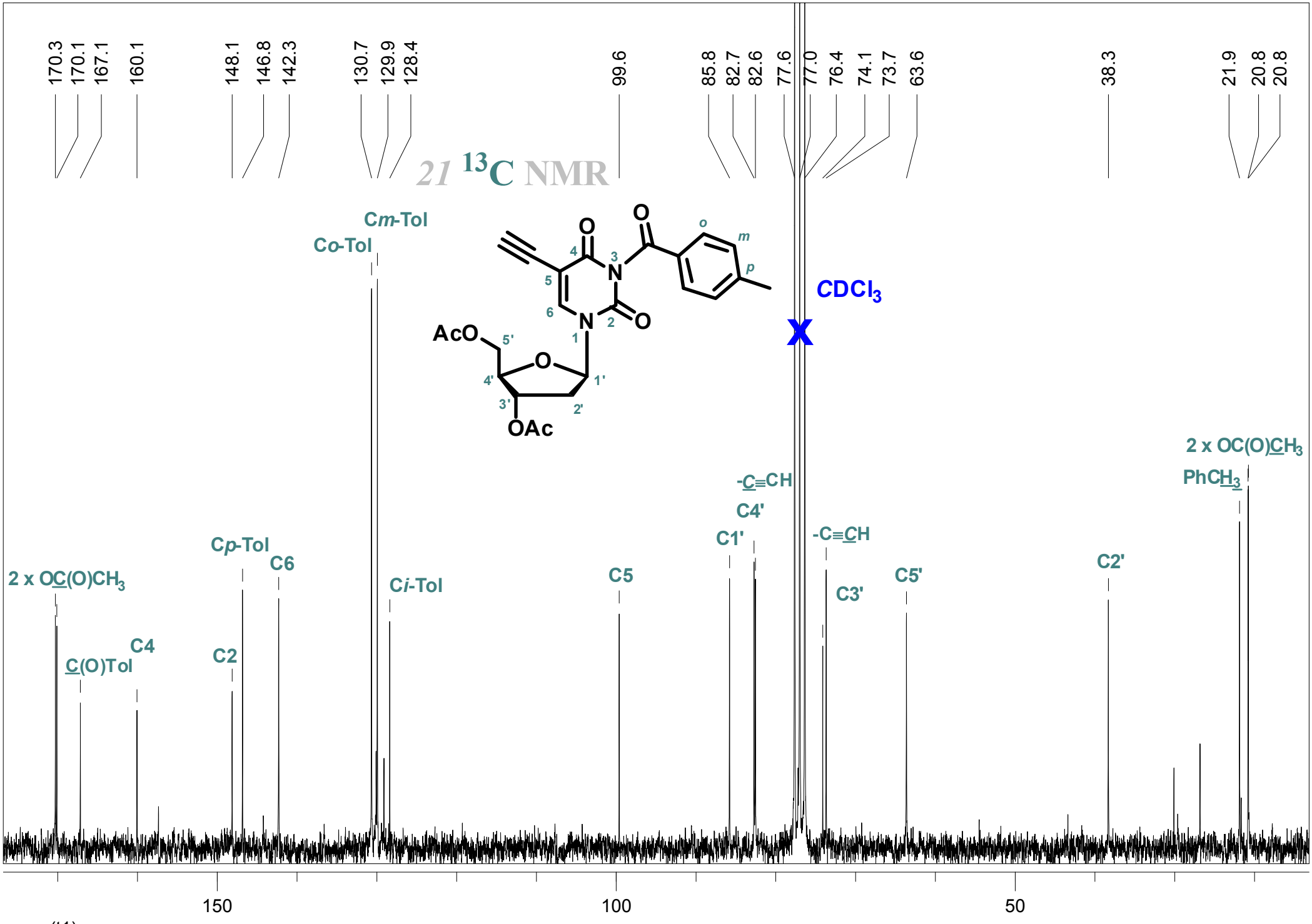

8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t2)
19 COSY
OMEM
AcO
OAc
NH

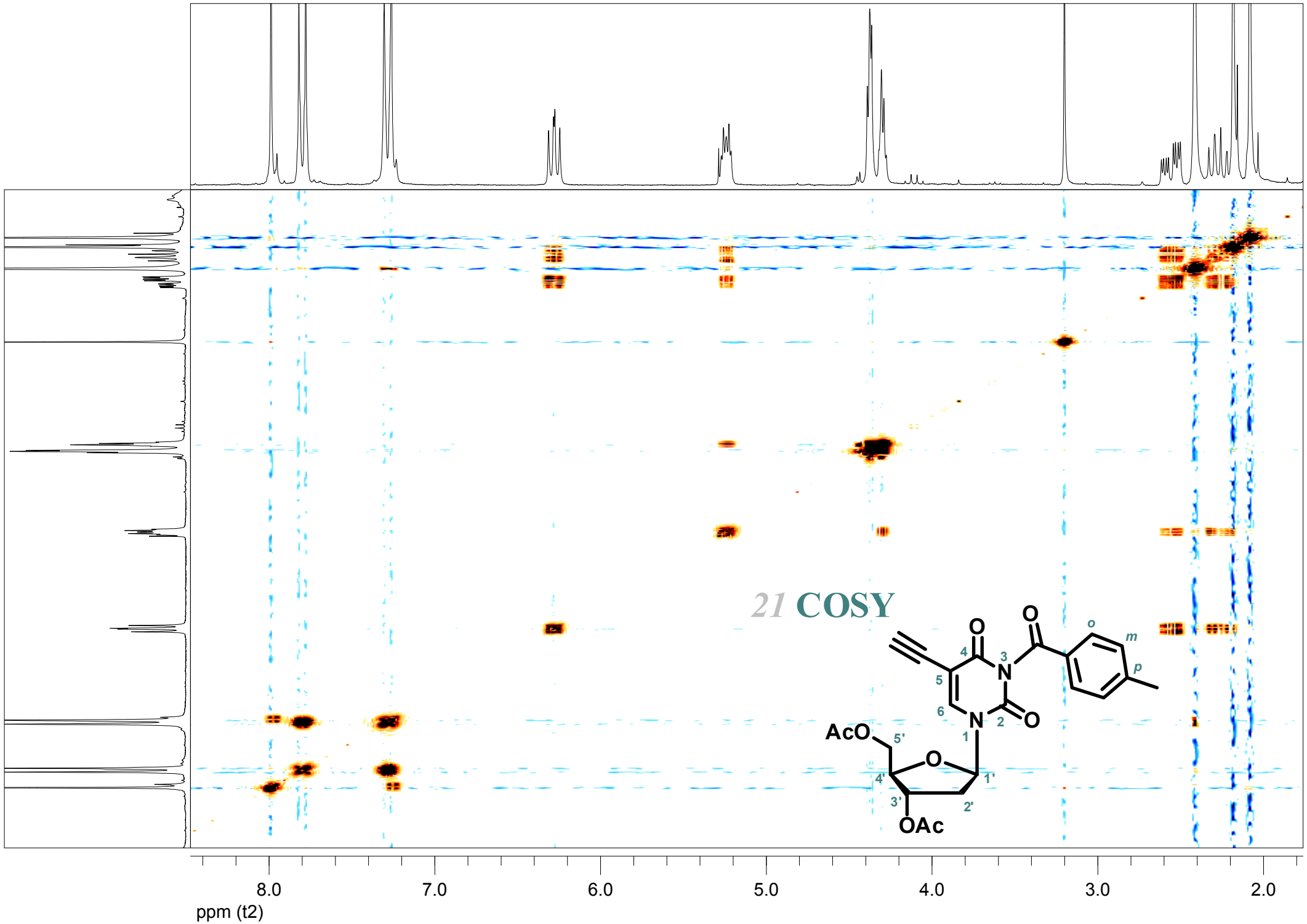

20 1H NMR
8.245
8.238
8.204
8.198
7.752
7.744
7.709
7.701
7.675
7.666
7.600
7.560
7.542
7.450
7.445
7.410
7.376
7.370
7.260
7.050
7.031
6.916
6.353
6.325
6.316
6.288
5.537
5.263
5.233
4.446
3.910
3.894
3.888
3.878
3.864
3.504
3.489
3.480
3.474
3.458
3.308
3.062
3.051
3.035
3.023
2.990
2.979
2.962
2.952
2.168
2.130
2.094
OMEM
AcO
OAc
H6
H5"
H8"
H7"
Hα
H6"
CHCl3
Hβ
H9
H1'
-OCH2O-
H3'
H4'
H5'
-OCH2A-
-CH2BO-
OCH3
H2'B
Me2CO
OAc
OAc
0.99
1.00
1.29
2.24
1.17
1.02
0.99
0.97
1.97
1.18
2.97
2.32
2.10
3.18
1.05
6.71
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t1)

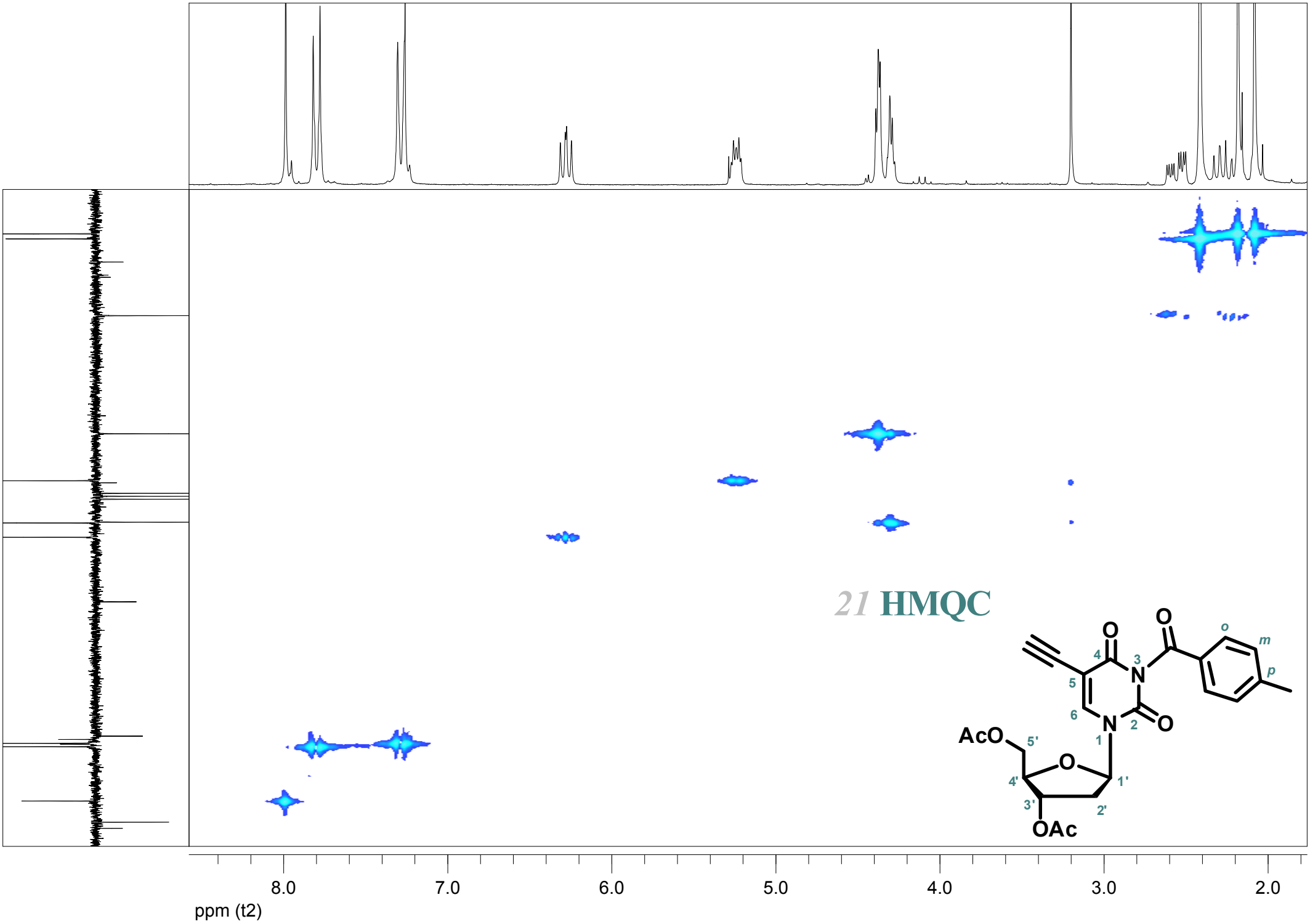

173.8
171.7
170.4
170.3
154.7
154.2
146.8
146.6
146.0
145.7
136.9
135.6
133.7
125.8
125.0
124.2
118.5
117.9
112.3
107.4
99.3
96.8
88.8
83.5
77.3
77.0
76.7
74.0
71.6
69.7
63.7
59.0
53.8
39.4
29.7
29.2
20.9
20 13C NMR
OMEM
AcO
OAc
CDCl3
CyHex
-CH2BO-
-OCH2A-
C3'
-OCH2O-
C4'
C1'
C5'
C2'
2 x OC(O)CH3
Me2CO
-OCH3
CH2Cl2
C9
Cβ
Cα
C8"
C6"
C7"
Fur-C8
C5"
C10"
C5
C2"-Fur-
C6
C8
C2"
C2
C9"
C4
C4"
C3"
150
100
50
ppm (t1)

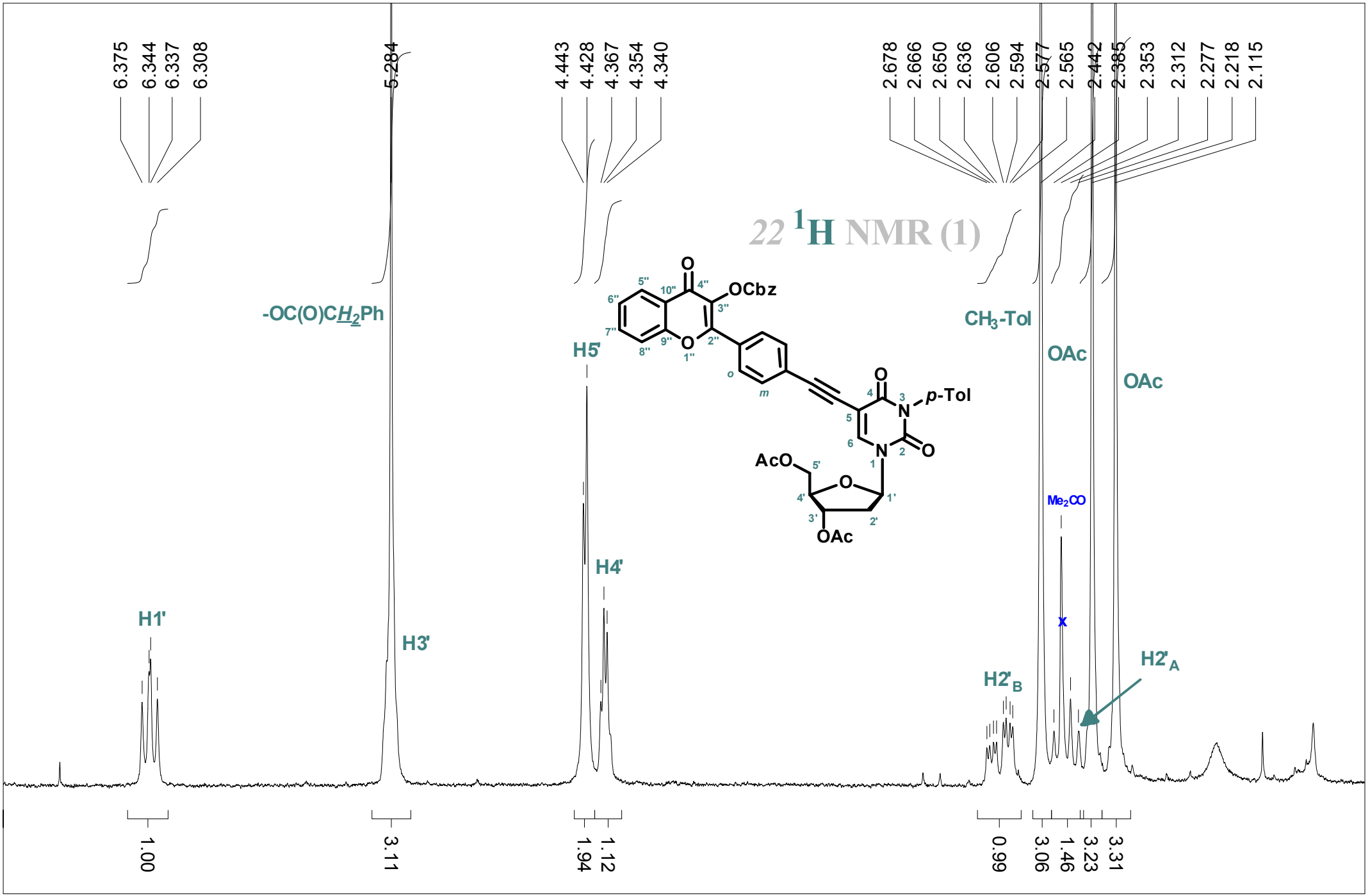

20 COSY
OMEM
AcO
OAc
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t2)

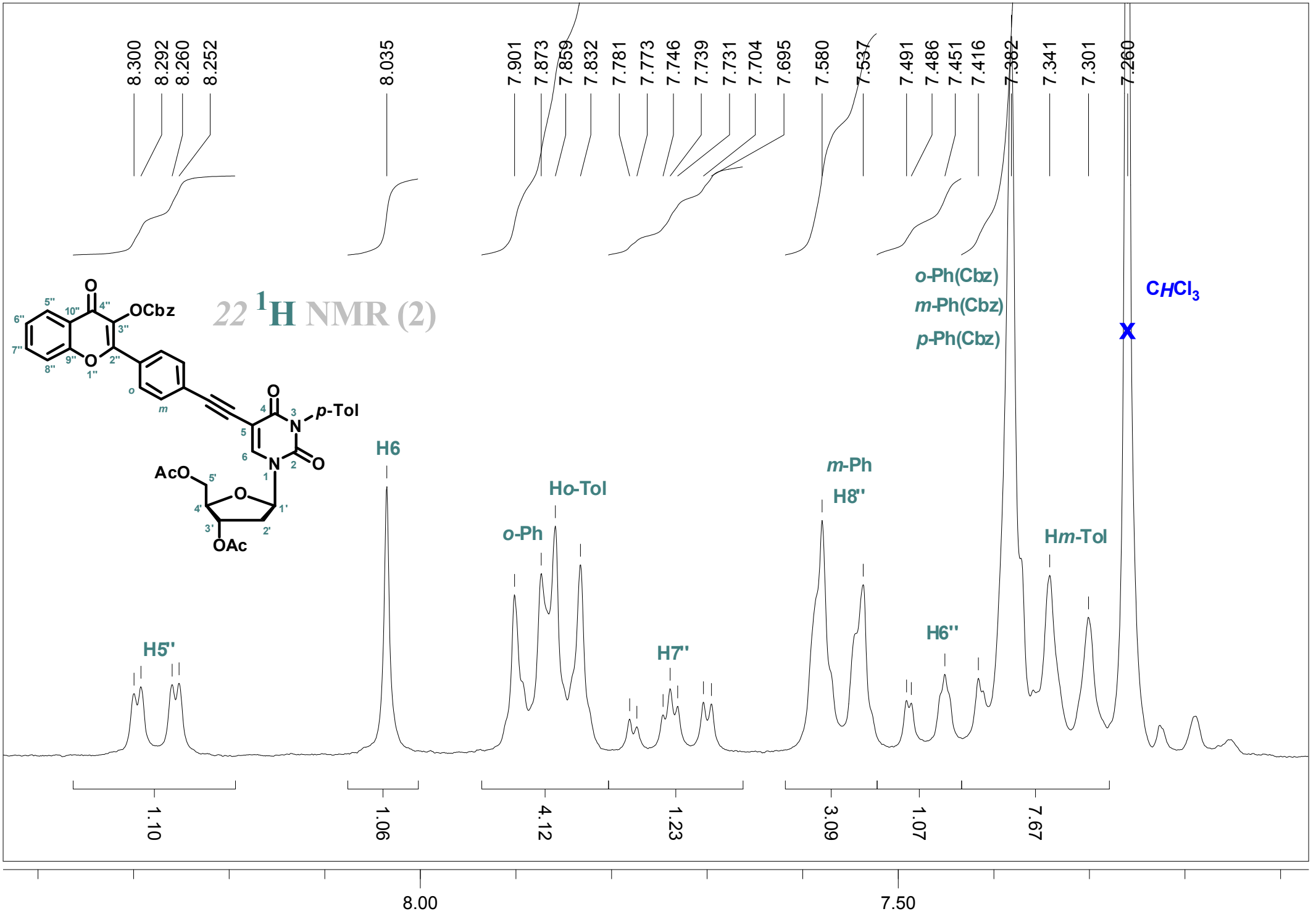

7.988
7.820
7.779
7.305
7.260
6.314
6.284
6.276
6.246
5.271
5.258
5.241
5.225
5.213
4.452
4.436
4.391
4.376
4.364
4.305
4.291
3.202
2.616
2.603
2.587
2.573
2.544
2.531
2.515
2.502
2.416
2.330
2.296
2.258
2.220
2.183
2.083
21 1H NMR
AcO
OAc
H6
Ho-Tol
Hm-Tol
CHCl3
H1'
H3'
H5'
H4'
-C≡CH
CH3-Tol
2 x OAc
H2'B
H2'A
1.24
2.00
2.65
1.00
1.00
3.03
0.98
0.97
3.45
1.00
3.30
3.18
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)

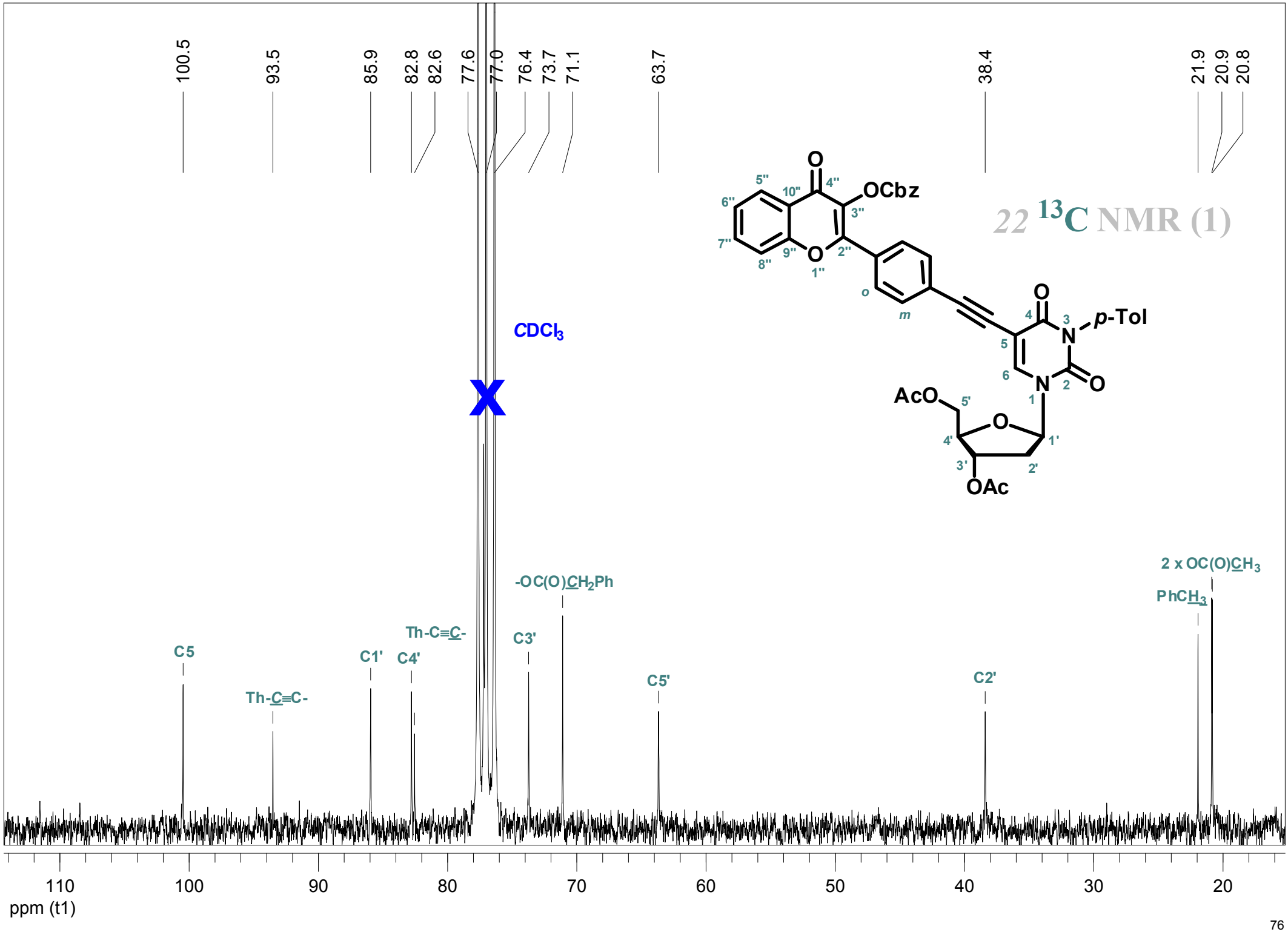

170.3
170.1
167.1
160.1
148.1
146.8
142.3
130.7
129.9
128.4
99.6
85.8
82.7
82.6
77.6
77.0
76.4
74.1
73.7
63.6
38.3
21.9
20.8
20.8
21 13C NMR
Cm-Tol
Co-Tol
CDCl3
2 x OC(O)CH3
C(O)Tol
C4
Cp-Tol
C2
C6
Ci-Tol
C5
-C≡CH
C4'
C1'
-C≡CH
C3'
C5'
C2'
2 x OC(O)CH3
PhCH3
150
100
50
ppm (t1)

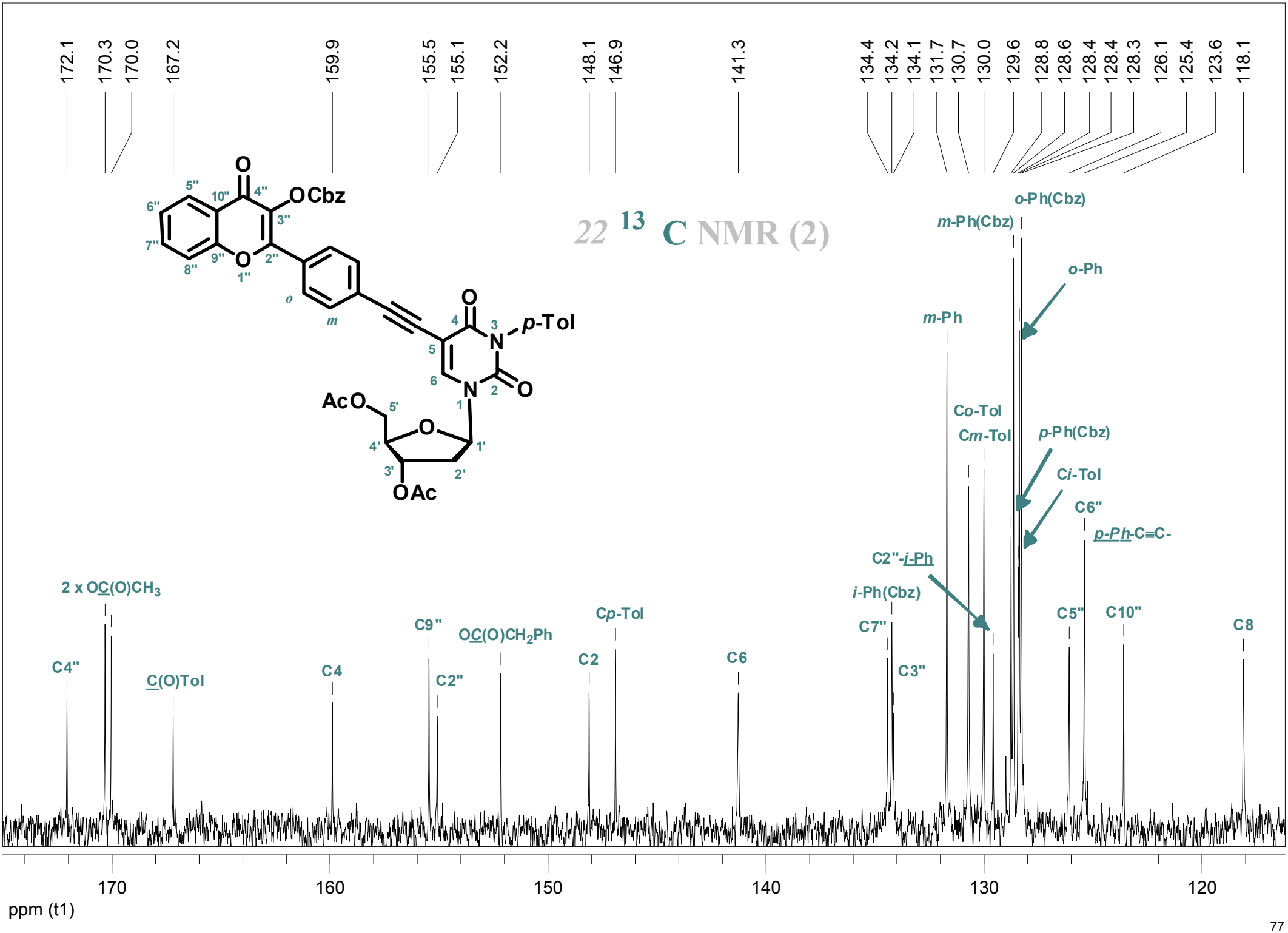

21 COSY
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t2)
AcO
OAc
O
N
1
2
3
4
5
6
1'
2'
3'
4'
5'
o
m
p

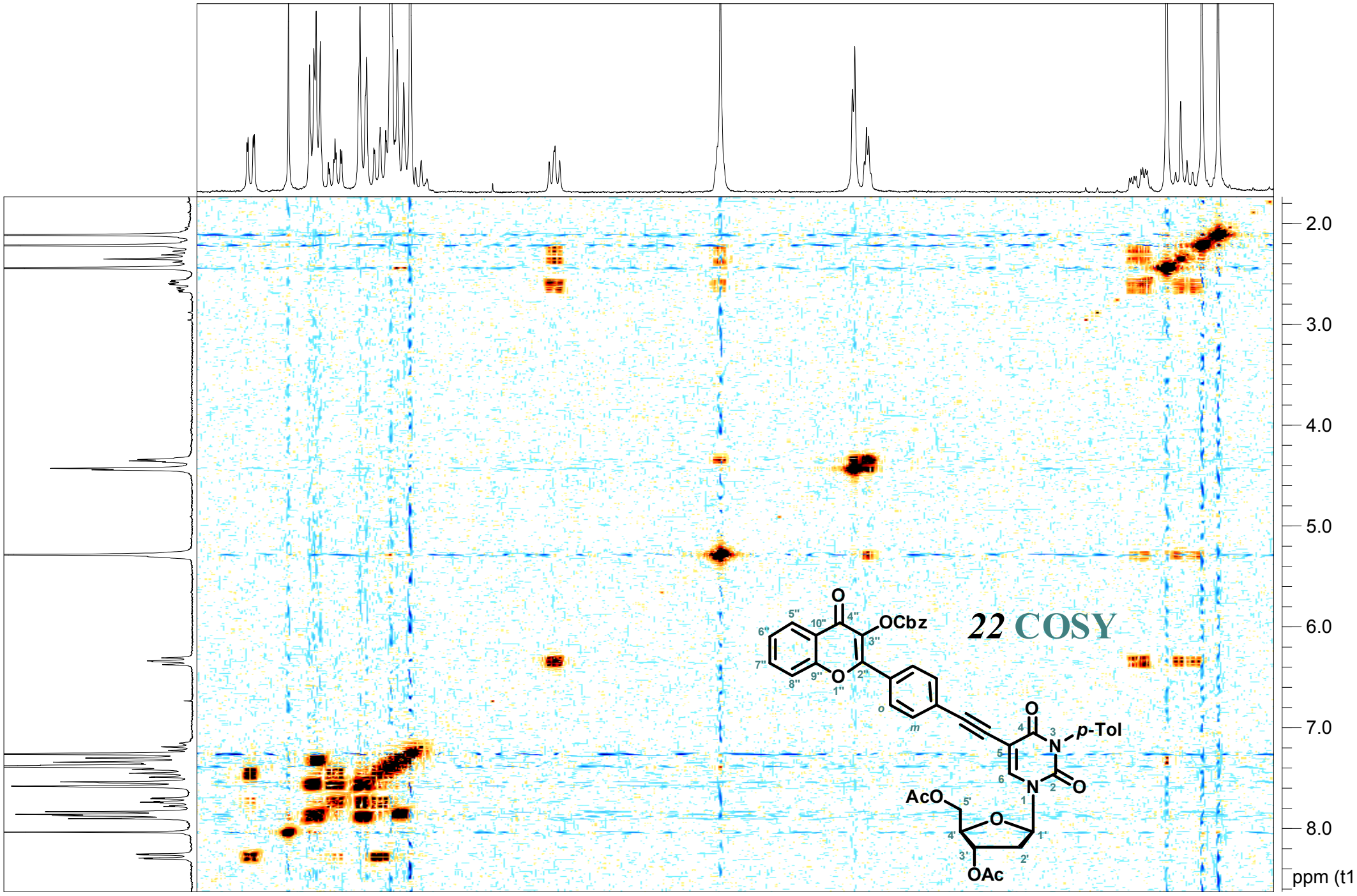

21 HMQC
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t2)
AcO
OAc
O
N
1
2
3
4
5
6
1'
2'
3'
4'
5'
o
m
p

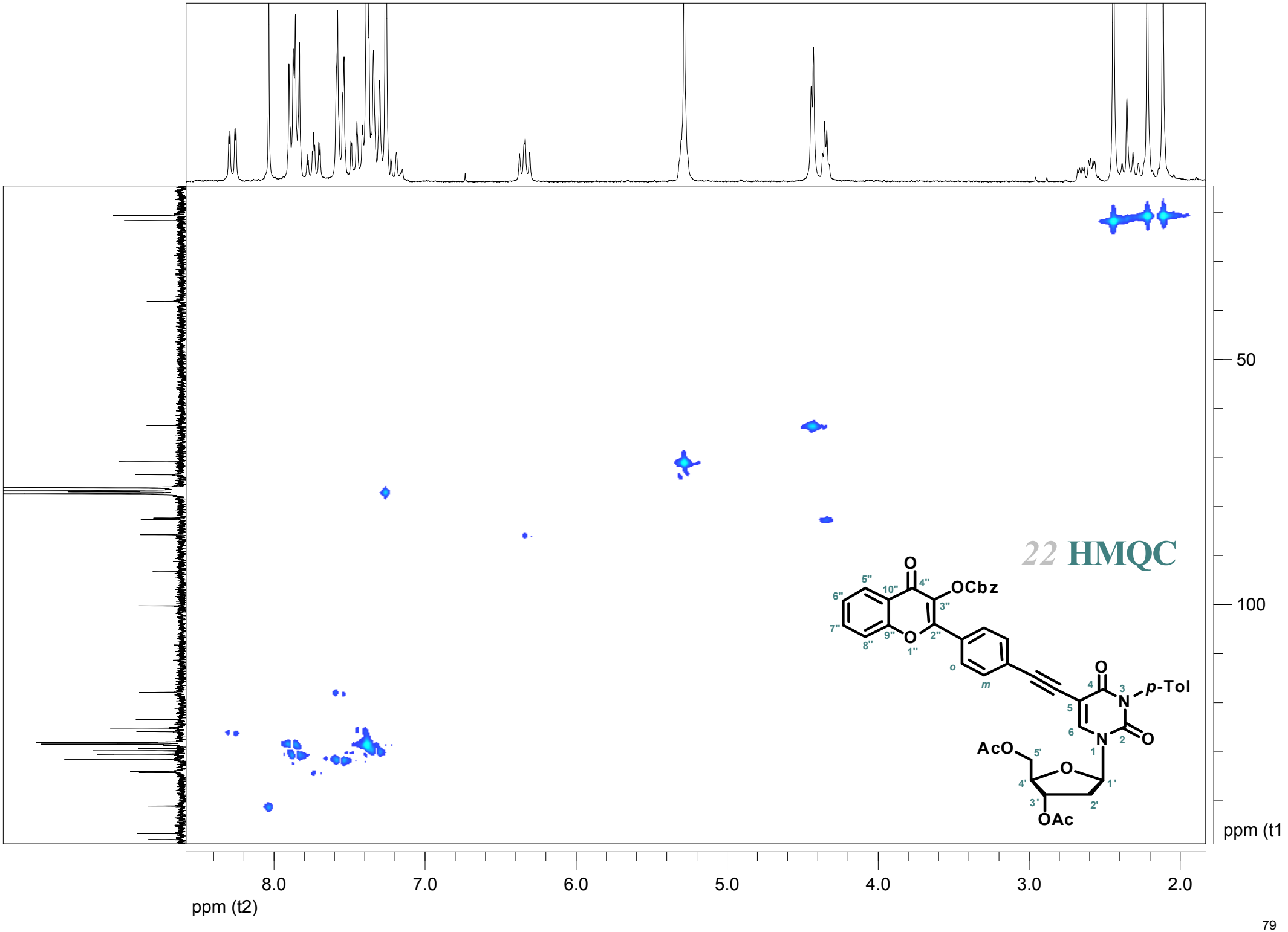

22 1H NMR (1)
6.375
6.344
6.337
6.308
5.284
4.443
4.428
4.367
4.354
4.340
2.678
2.666
2.650
2.636
2.606
2.594
2.577
2.565
2.442
2.385
2.353
2.312
2.277
2.218
2.115
-OC(O)CH2Ph
H5'
CH3-Tol
OAc
OAc
OCbz
p-Tol
AcO
OAc
Me2CO
H4'
H1'
H3'
H2'B
H2'A
1.00
3.11
1.94
1.12
0.99
3.06
1.46
3.23
3.31
6.0
5.0
4.0
3.0
2.0
ppm (t1)

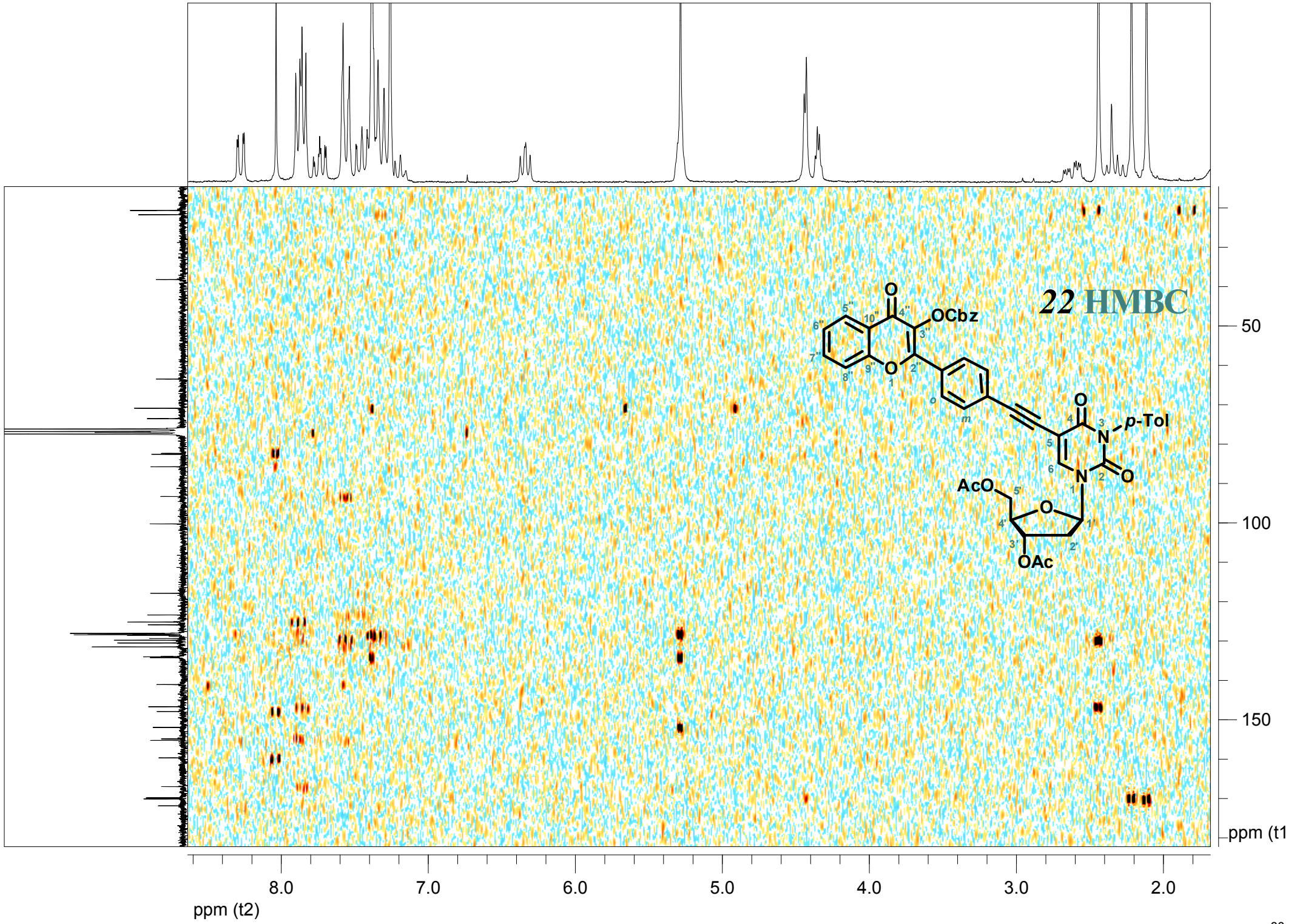

8.300
8.292
8.260
8.252
8.035
7.901
7.873
7.859
7.832
7.781
7.773
7.746
7.739
7.731
7.704
7.695
7.580
7.537
7.491
7.486
7.451
7.416
7.382
7.341
7.301
7.260
22 1H NMR (2)
OCbz
p-Tol
AcO
OAc
H6
Ho-Tol
o-Ph
m-Ph
H8''
H5''
H7''
H6''
Hm-Tol
o-Ph(Cbz)
m-Ph(Cbz)
p-Ph(Cbz)
CHCl3
1.10
1.06
4.12
1.23
3.09
1.07
7.67
8.00
7.50
ppm (t1)

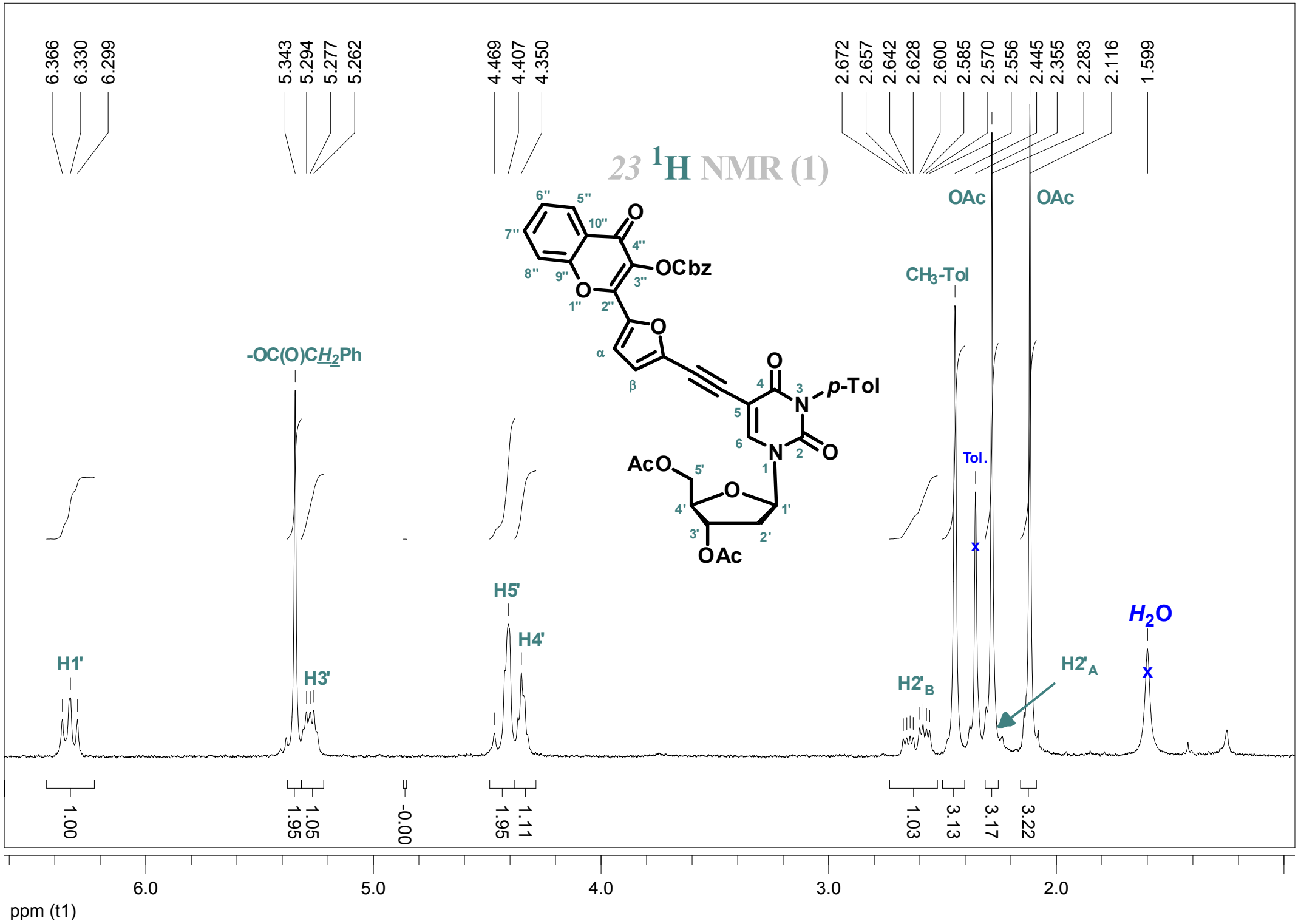

100.5
93.5
85.9
82.8
82.6
77.6
77.0
76.4
73.7
71.1
63.7
38.4
21.9
20.9
20.8
22 13C NMR (1)
CDCl3
C5
Th-C≡C-
C1'
C4'
Th-C≡C-
C3'
-OC(O)CH2Ph
C5'
C2'
2 x OC(O)CH3
PhCH3
110
100
90
80
70
60
50
40
30
20
ppm (t1)

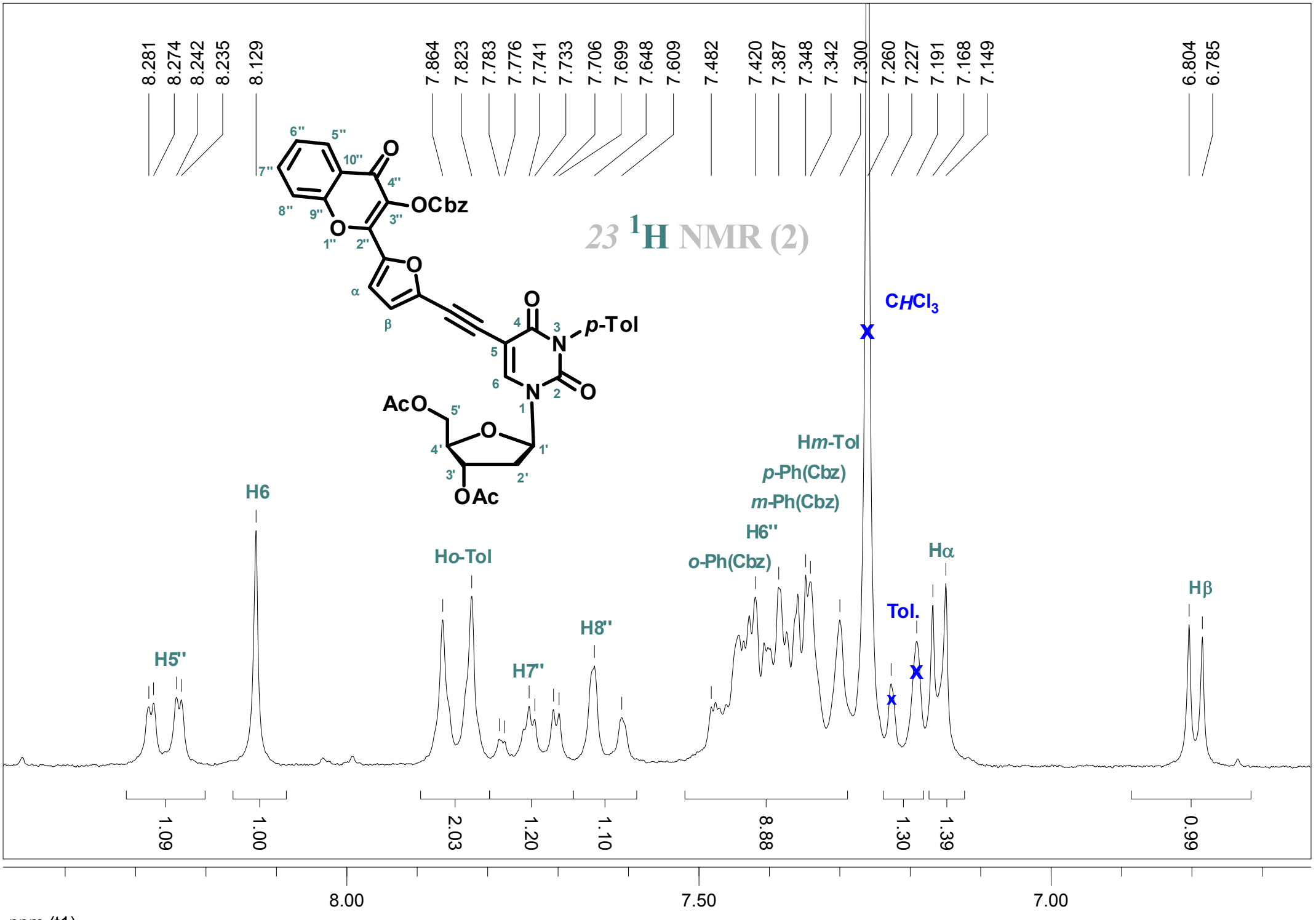

172.1
170.3
170.0
167.2
159.9
155.5
155.1
152.2
148.1
146.9
141.3
134.4
134.2
134.1
131.7
130.7
130.0
129.6
128.8
128.6
128.4
128.4
128.3
126.1
125.4
123.6
118.1
22 13 C NMR (2)
OCbz
p-Tol
AcO
OAc
o-Ph(Cbz)
m-Ph(Cbz)
o-Ph
m-Ph
Co-Tol
Cm-Tol
p-Ph(Cbz)
Ci-Tol
C6"
p-Ph-C≡C-
C2"-i-Ph
i-Ph(Cbz)
C7"
C3"
C5"
C10"
C8
2 x OC(O)CH3
C4"
C(O)Tol
C4
C9"
C2"
OC(O)CH2Ph
C2
Cp-Tol
C6
170
160
150
140
130
120
ppm (t1)

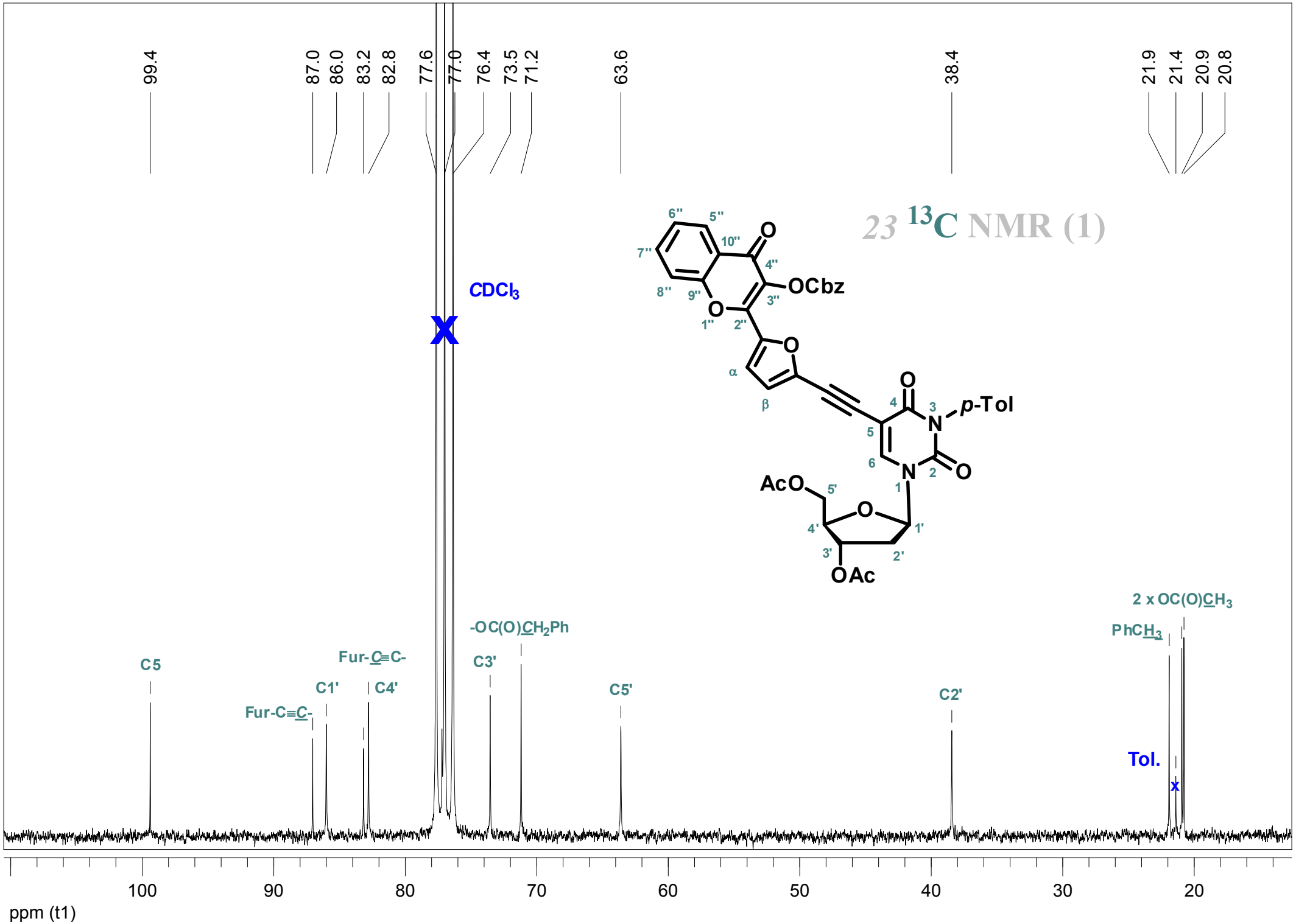

22 COSY
OCbz
p-Tol
AcO
OAc
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t2)
ppm (t1

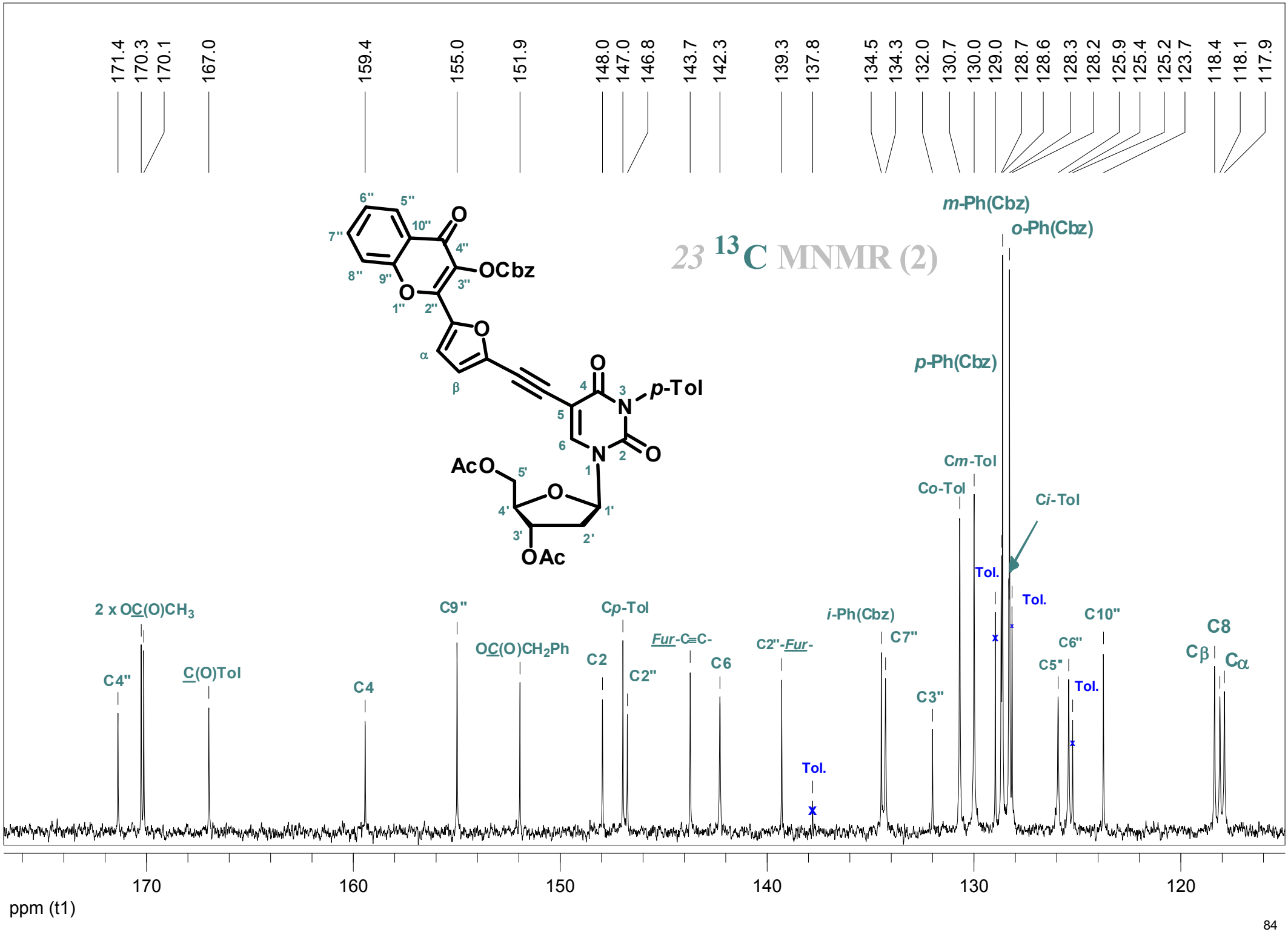

22 HMQC
OCbz
p-Tol
AcO
OAc
50
100
ppm (t1
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t2)

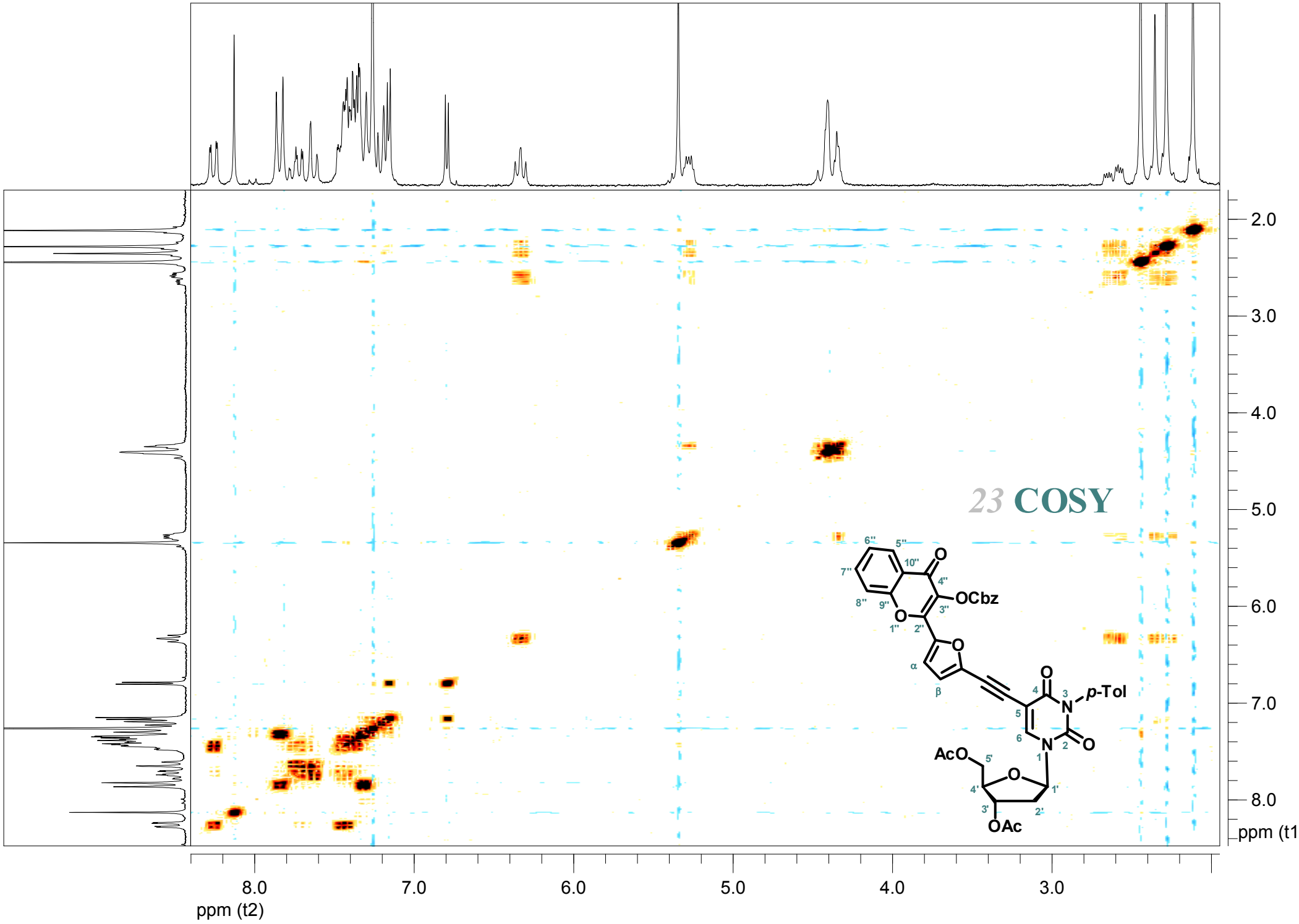

22 HMBC
OCbz
p-Tol
AcO
OAc
50
100
150
ppm (t1
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t2)

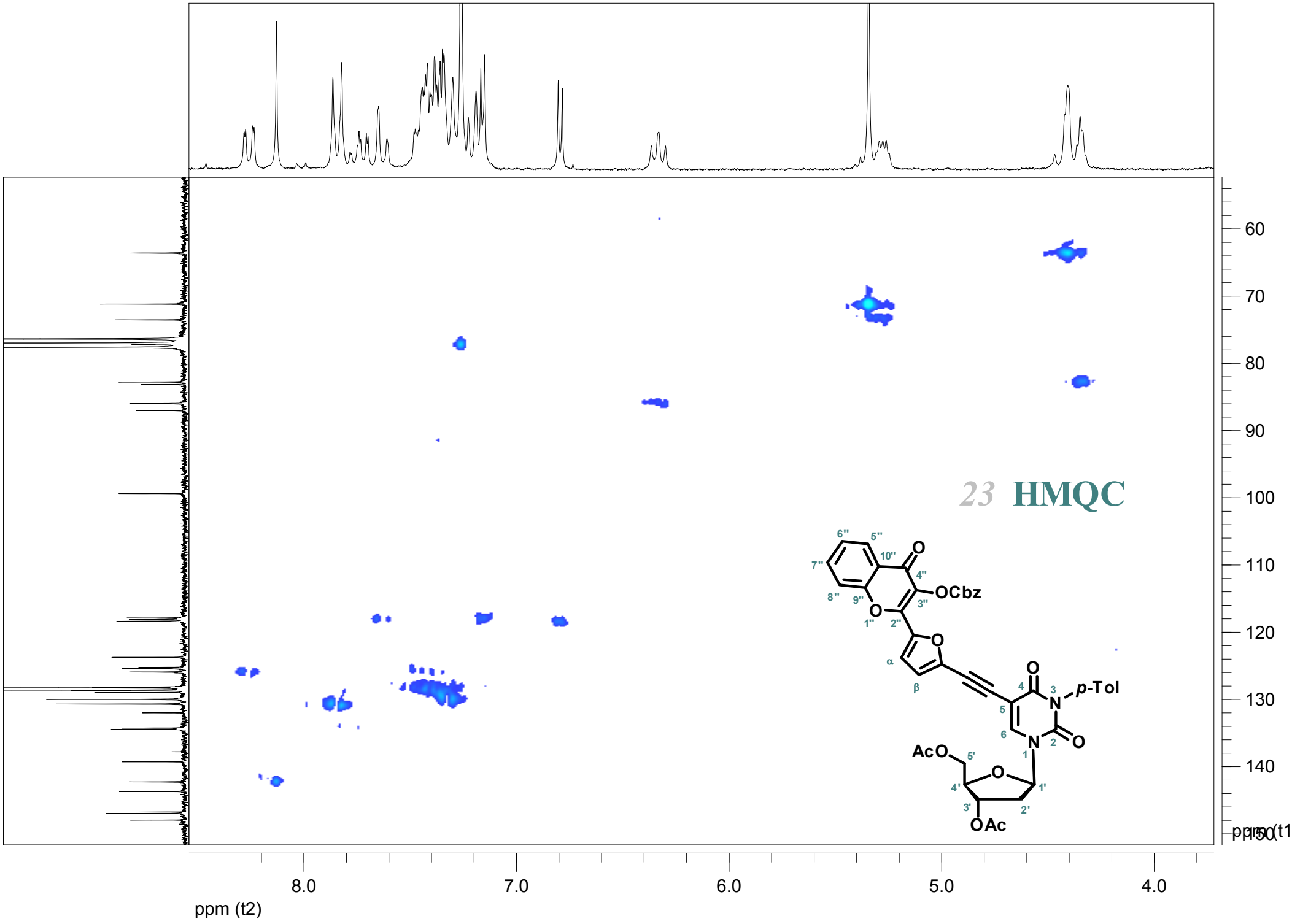

23 1H NMR (1)
6.366
6.330
6.299
5.343
5.294
5.277
5.262
4.469
4.407
4.350
2.672
2.657
2.642
2.628
2.600
2.585
2.570
2.556
2.445
2.355
2.283
2.116
1.599
OAc
OAc
CH3-Tol
Tol.
-OC(O)CH2Ph
p-Tol
OCbz
AcO
OAc
H5'
H4'
H1'
H3'
H2'B
H2'A
H2O
1.00
1.95
1.05
-0.00
1.95
1.11
1.03
3.13
3.17
3.22
6.0
5.0
4.0
3.0
2.0
ppm (t1)

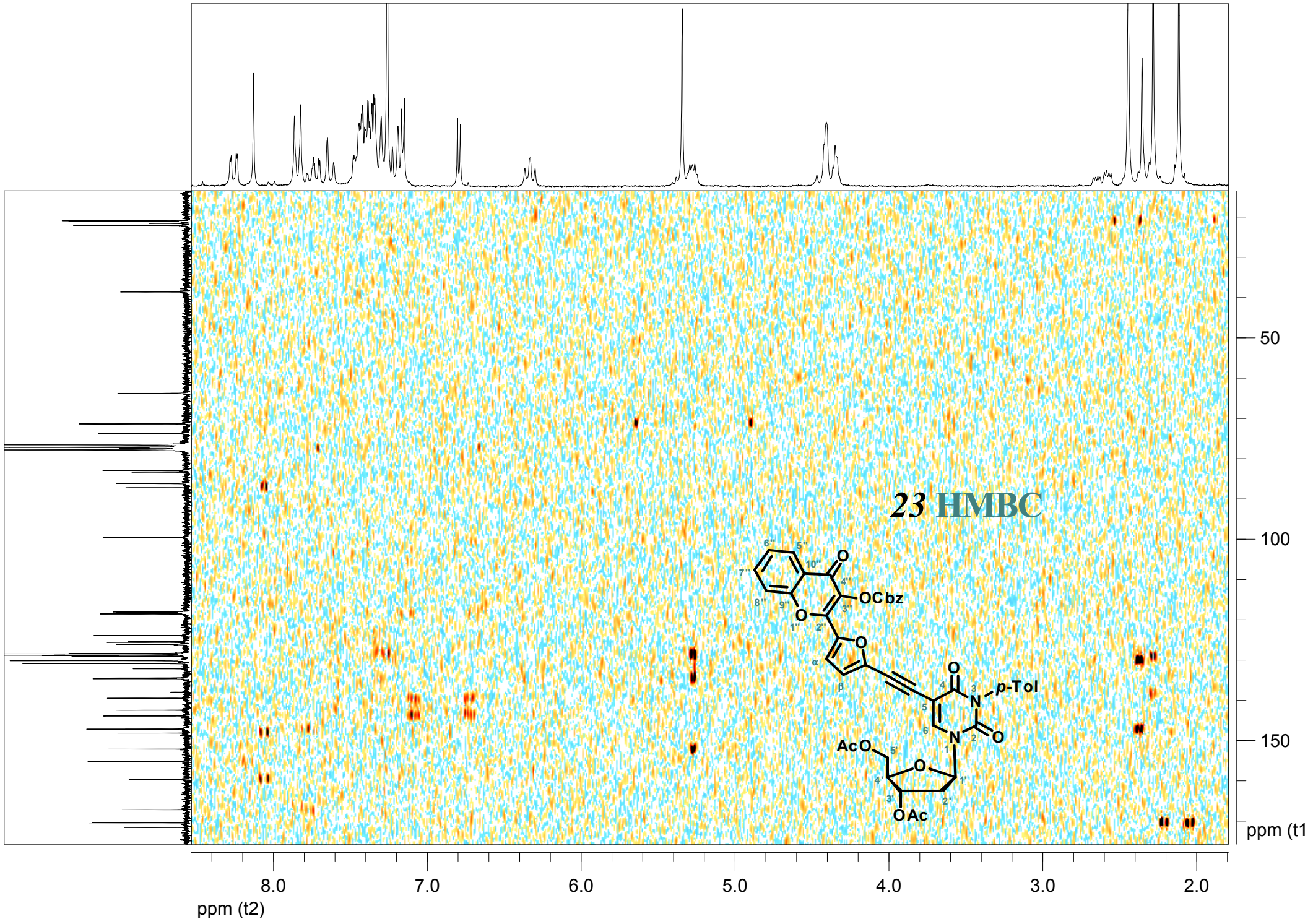

8.281
8.274
8.242
8.235
8.129
7.864
7.823
7.783
7.776
7.741
7.733
7.706
7.699
7.648
7.609
7.482
7.420
7.387
7.348
7.342
7.300
7.260
7.227
7.191
7.168
7.149
6.804
6.785
23 1H NMR (2)
OCbz
p-Tol
AcO
OAc
CHCl3
H6
Ho-Tol
H5''
H7''
H8''
Hm-Tol
p-Ph(Cbz)
m-Ph(Cbz)
H6''
o-Ph(Cbz)
Hα
Hβ
Tol.
1.09
1.00
2.03
1.20
1.10
8.88
1.30
1.39
0.99
8.00
7.50
7.00
ppm (t1)

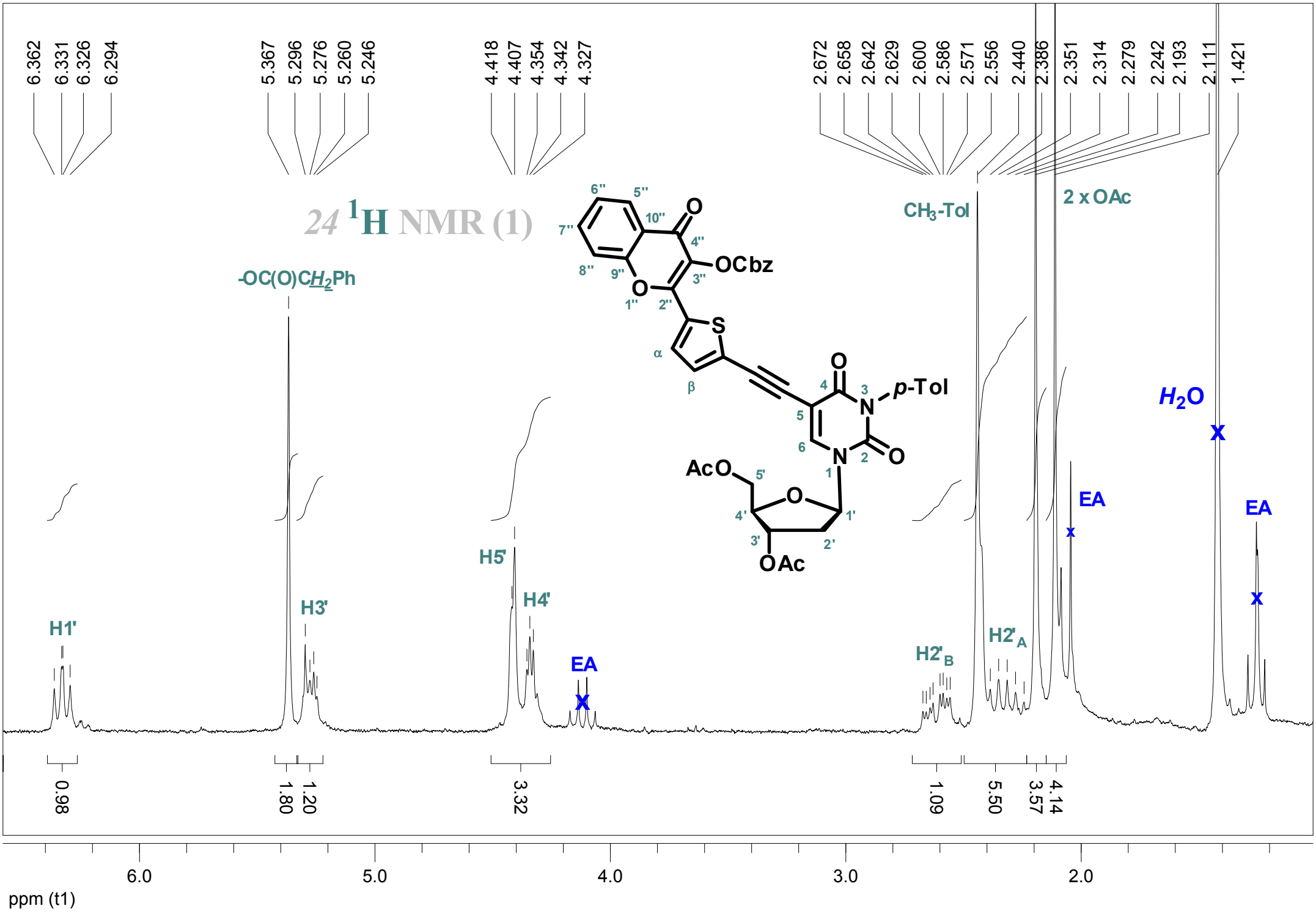

99.4
87.0
86.0
83.2
82.8
77.6
77.0
76.4
73.5
71.2
63.6
38.4
21.9
21.4
20.9
20.8
23 13C NMR (1)
CDCl3
OCbz
p-Tol
AcO
OAc
C5
Fur-C≡C-
C1'
C4'
Fur-C≡C-
C3'
-OC(O)CH2Ph
C5'
C2'
2 x OC(O)CH3
PhCH3
Tol.
100
90
80
70
60
50
40
30
20
ppm (t1)

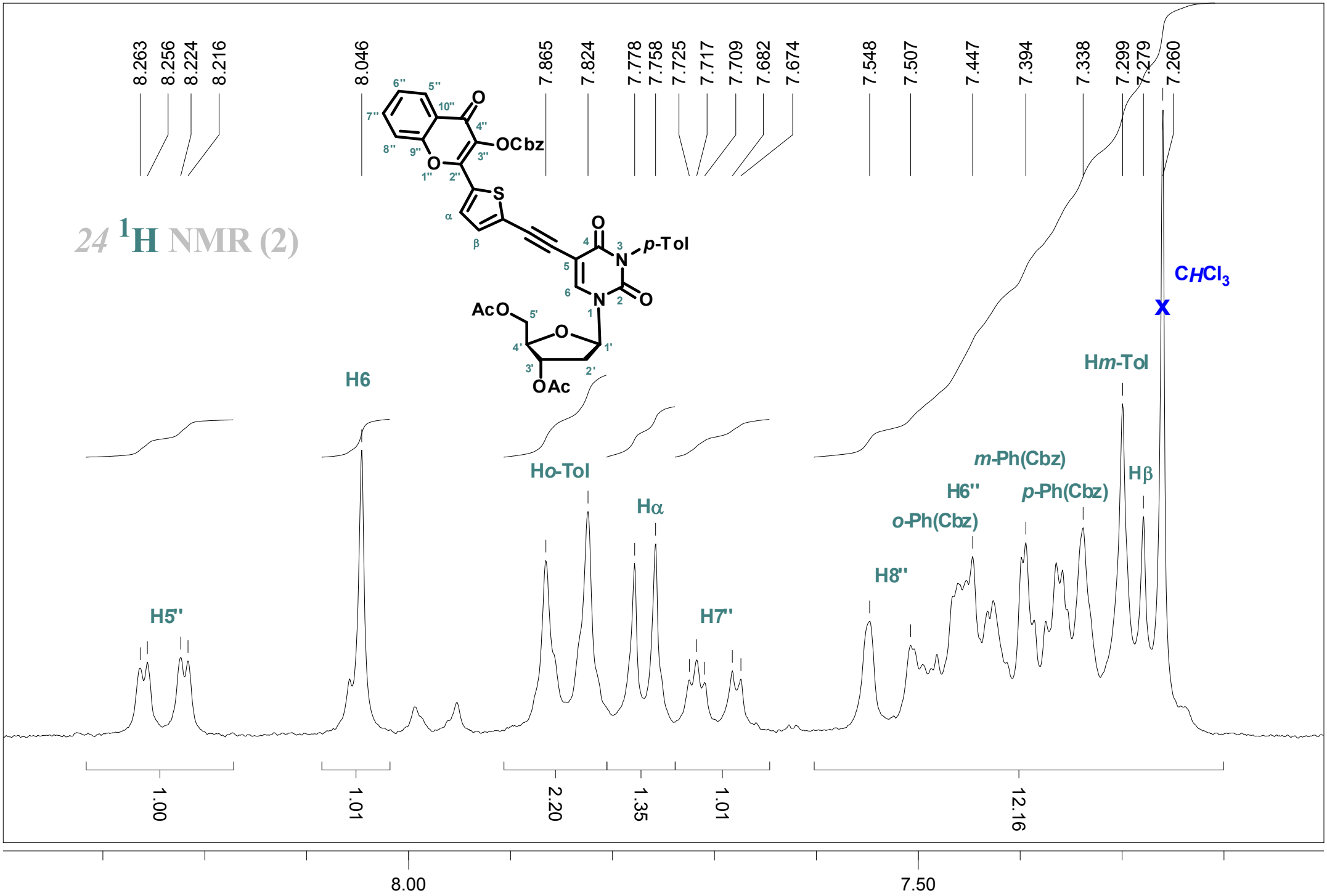

171.4
170.3
170.1
167.0
159.4
155.0
151.9
148.0
147.0
146.8
143.7
142.3
139.3
137.8
134.5
134.3
132.0
130.7
130.0
129.0
128.7
128.6
128.3
128.2
125.9
125.4
125.2
123.7
118.4
118.1
117.9
23 13C MNMR (2)
m-Ph(Cbz)
o-Ph(Cbz)
p-Ph(Cbz)
Cm-Tol
Co-Tol
Ci-Tol
Tol.
Tol.
Tol.
Tol.
C10"
C8
Cβ
Cα
C6"
C5"
C3"
i-Ph(Cbz)
C7"
C2"-Fur-
Fur-C≡C-
C6
C2"
Cp-Tol
C2
C9"
OC(O)CH2Ph
C4
C(O)Tol
C4"
2 x OC(O)CH3
170
160
150
140
130
120
ppm (t1)
p-Tol
OCbz
AcO
OAc

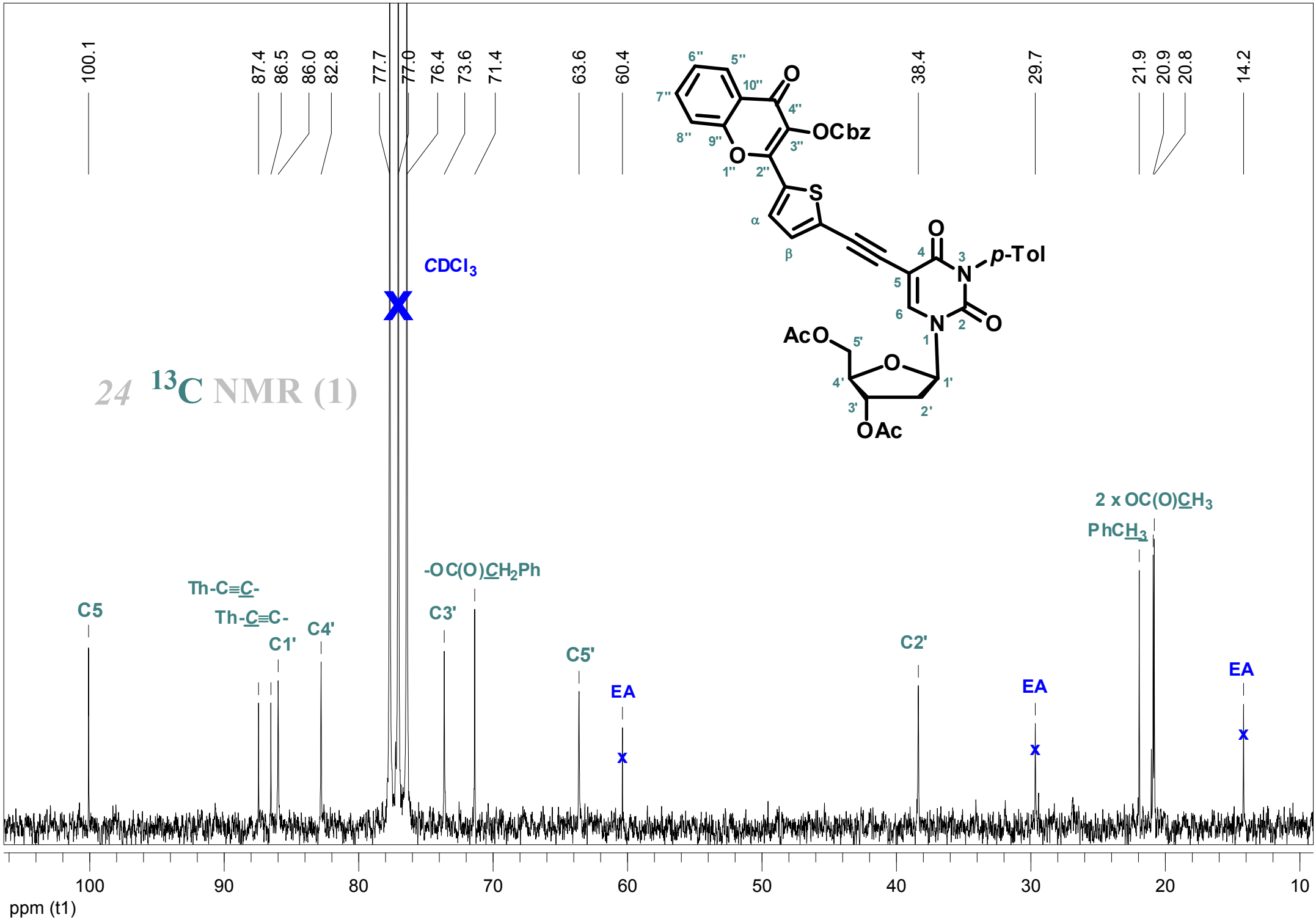

23 COSY
OCbz
p-Tol
AcO
OAc
8.0
7.0
6.0
5.0
4.0
3.0
ppm (t2)
2.0
3.0
4.0
5.0
6.0
7.0
8.0
ppm (t1

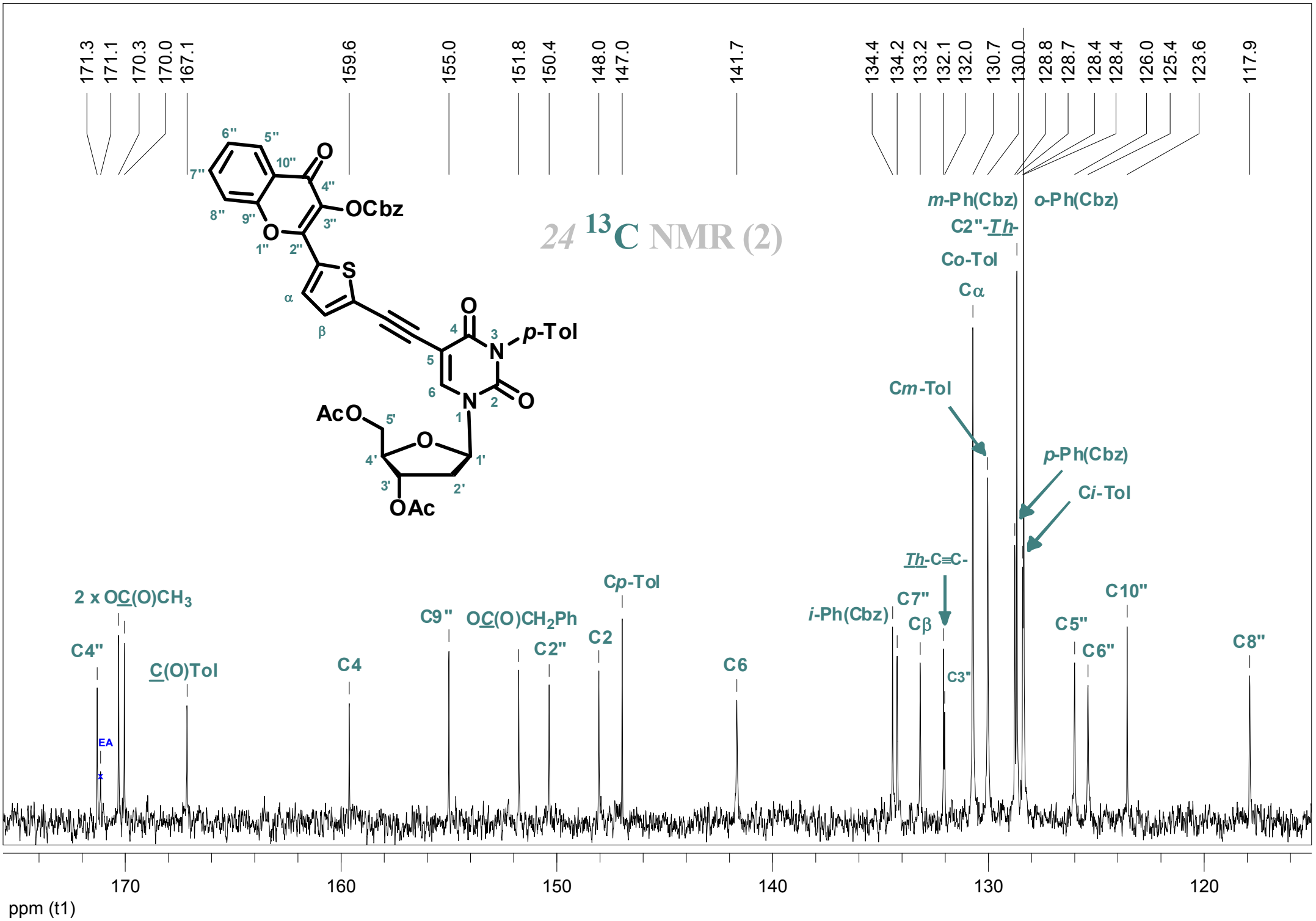

23 HMQC
8.0
7.0
6.0
5.0
4.0
ppm (t2)
60
70
80
90
100
110
120
130
140
150
ppm (t1
OCbz
p-Tol
AcO
OAc

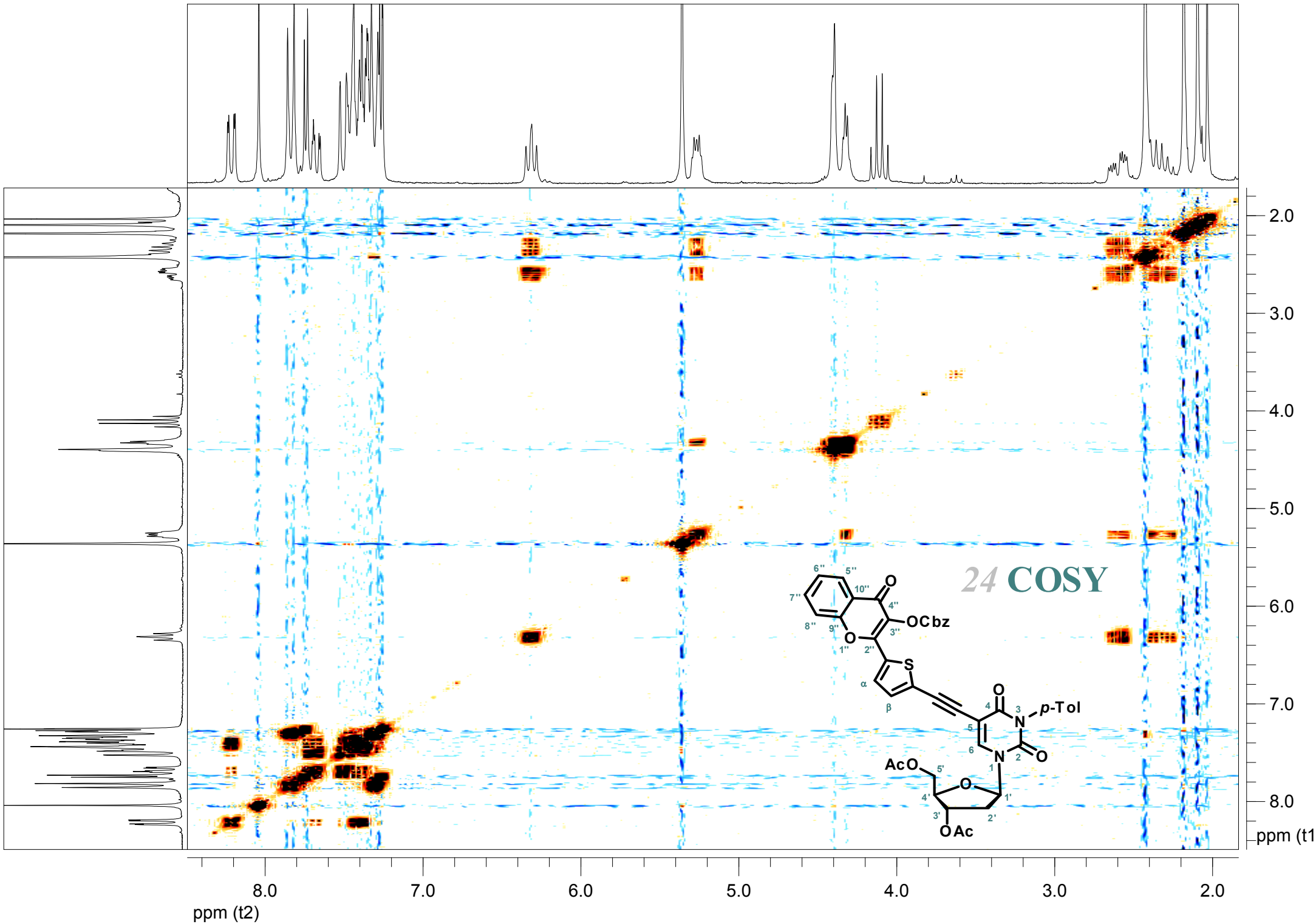

23 HMBC
OCbz
p-Tol
AcO
OAc
50
100
150
ppm (t1
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t2)

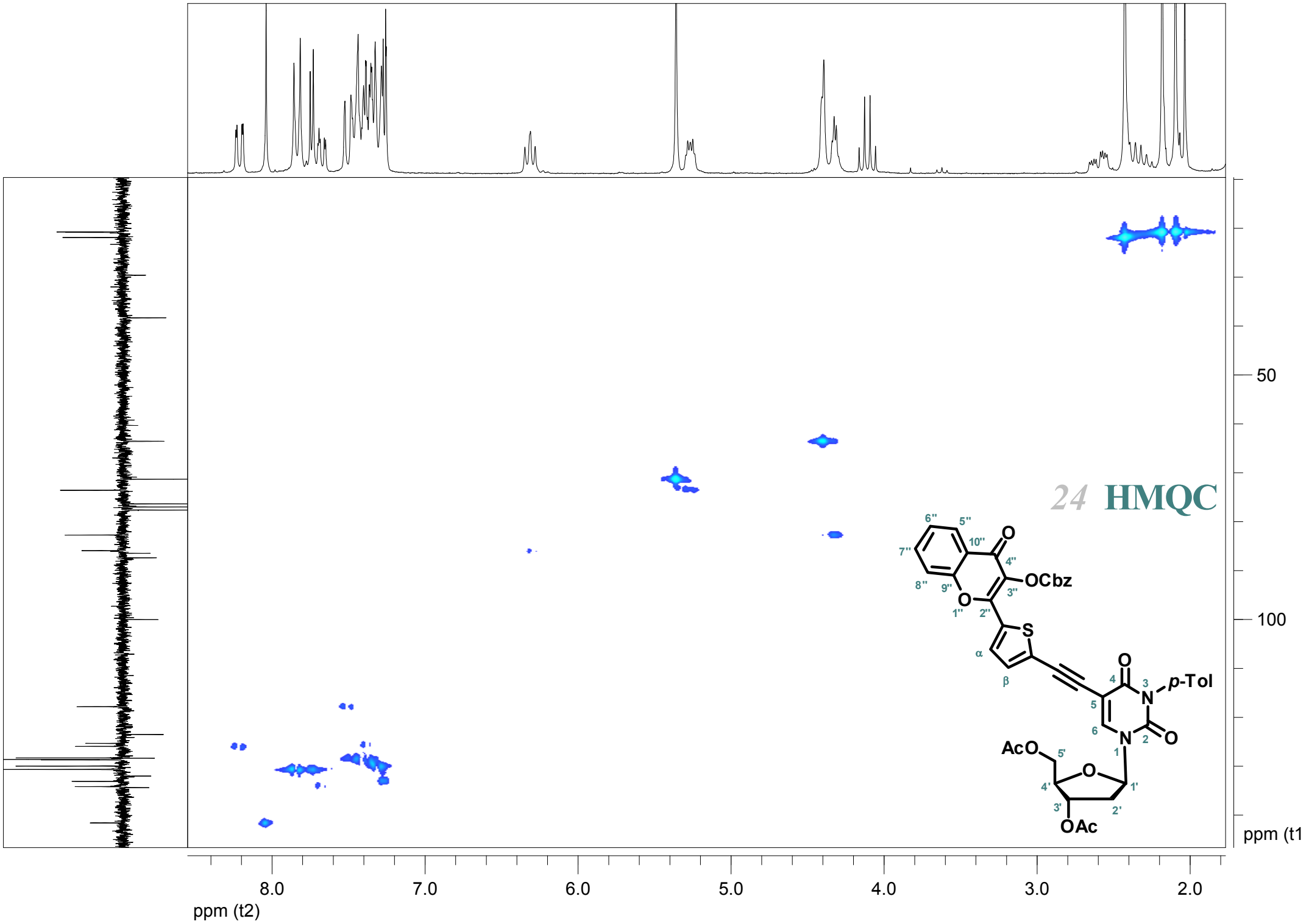

24 1H NMR (1)
6.362
6.331
6.326
6.294
5.367
5.296
5.276
5.260
5.246
4.418
4.407
4.354
4.342
4.327
2.672
2.658
2.642
2.629
2.600
2.586
2.571
2.556
2.440
2.386
2.351
2.314
2.279
2.242
2.193
2.111
1.421
-OC(O)CH2Ph
CH3-Tol
2 x OAc
H2O
EA
EA
EA
H1'
H3'
H5'
H4'
H2'B
H2'A
0.98
1.80
1.20
3.32
1.09
5.50
3.57
4.14
6.0
5.0
4.0
3.0
2.0
ppm (t1)

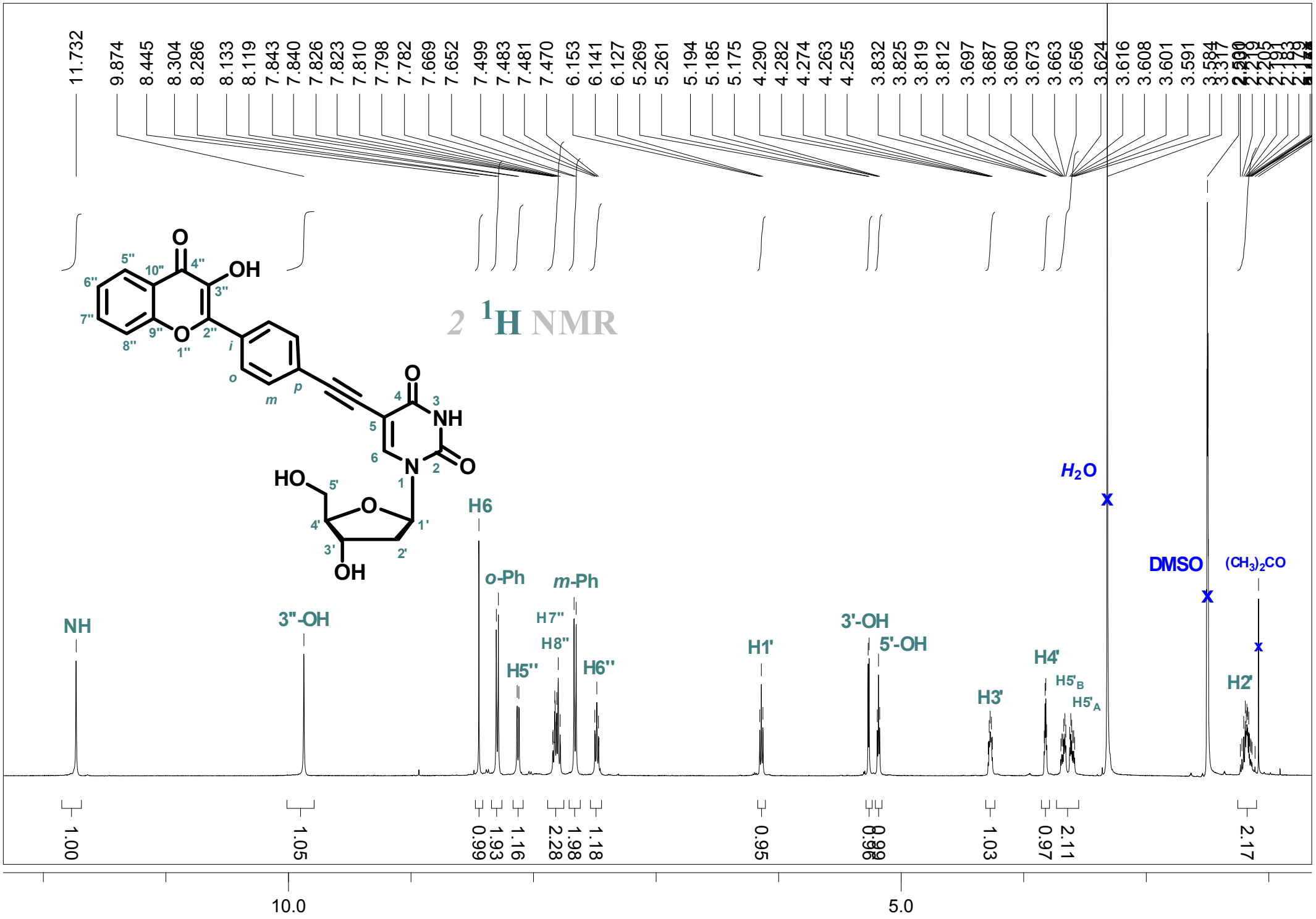

24 1H NMR (2)
8.263
8.256
8.224
8.216
8.046
7.865
7.824
7.778
7.758
7.725
7.717
7.709
7.682
7.674
7.548
7.507
7.447
7.394
7.338
7.299
7.279
7.260
p-Tol
OCbz
AcO
OAc
CHCl3
H6
Ho-Tol
Hα
H7''
H8''
o-Ph(Cbz)
H6''
m-Ph(Cbz)
p-Ph(Cbz)
Hβ
Hm-Tol
H5''
1.00
1.01
2.20
1.35
1.01
12.16
8.00
7.50
ppm (t1)

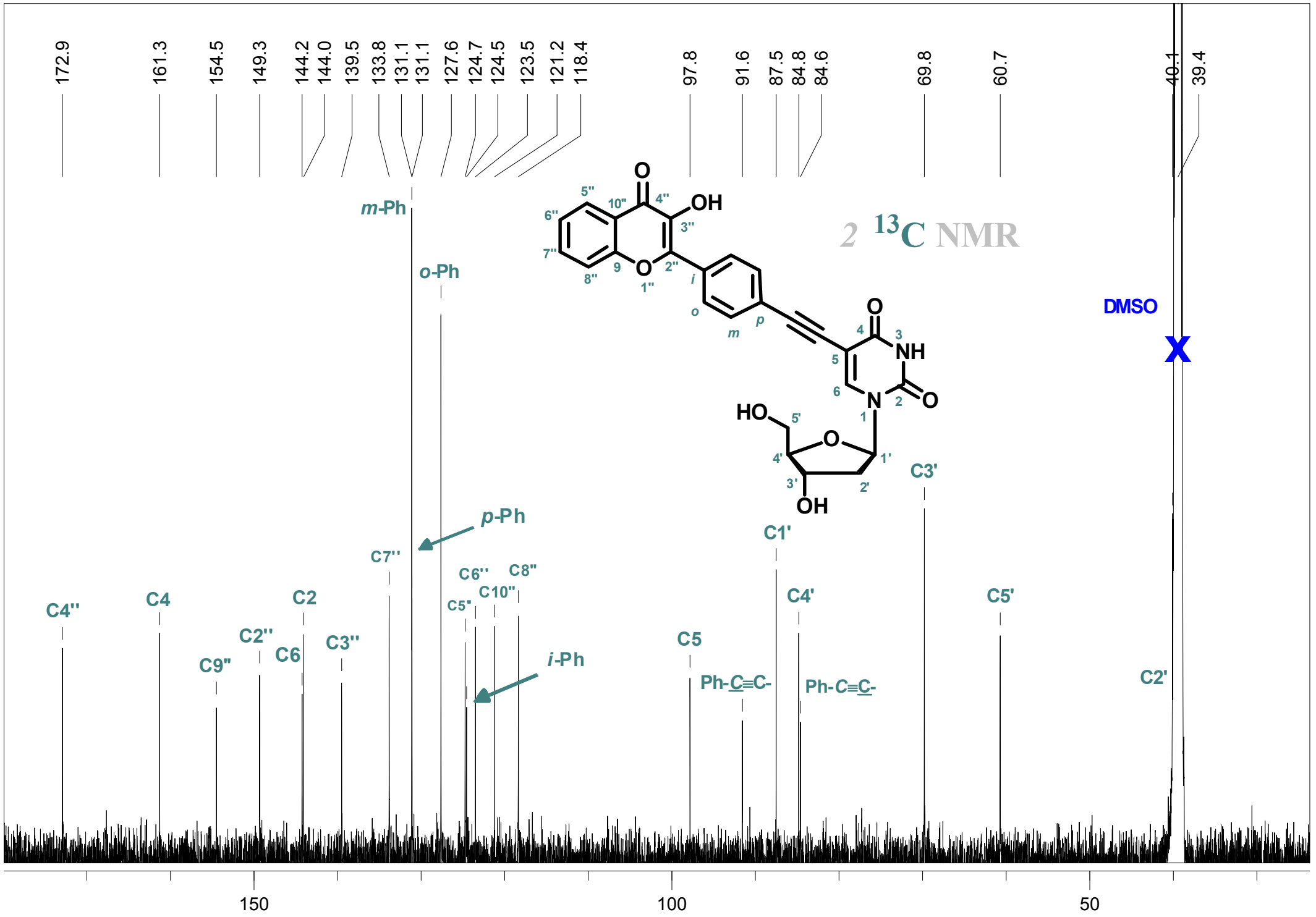

100.1
87.4
86.5
86.0
82.8
77.7
77.0
76.4
73.6
71.4
63.6
60.4
38.4
29.7
21.9
20.9
20.8
14.2
CDCl3
24 13C NMR (1)
C5
Th-C≡C-
Th-C≡C-
C1'
C4'
-OC(O)CH2Ph
C3'
C5'
EA
C2'
EA
2 x OC(O)CH3
PhCH3
EA
ppm (t1)

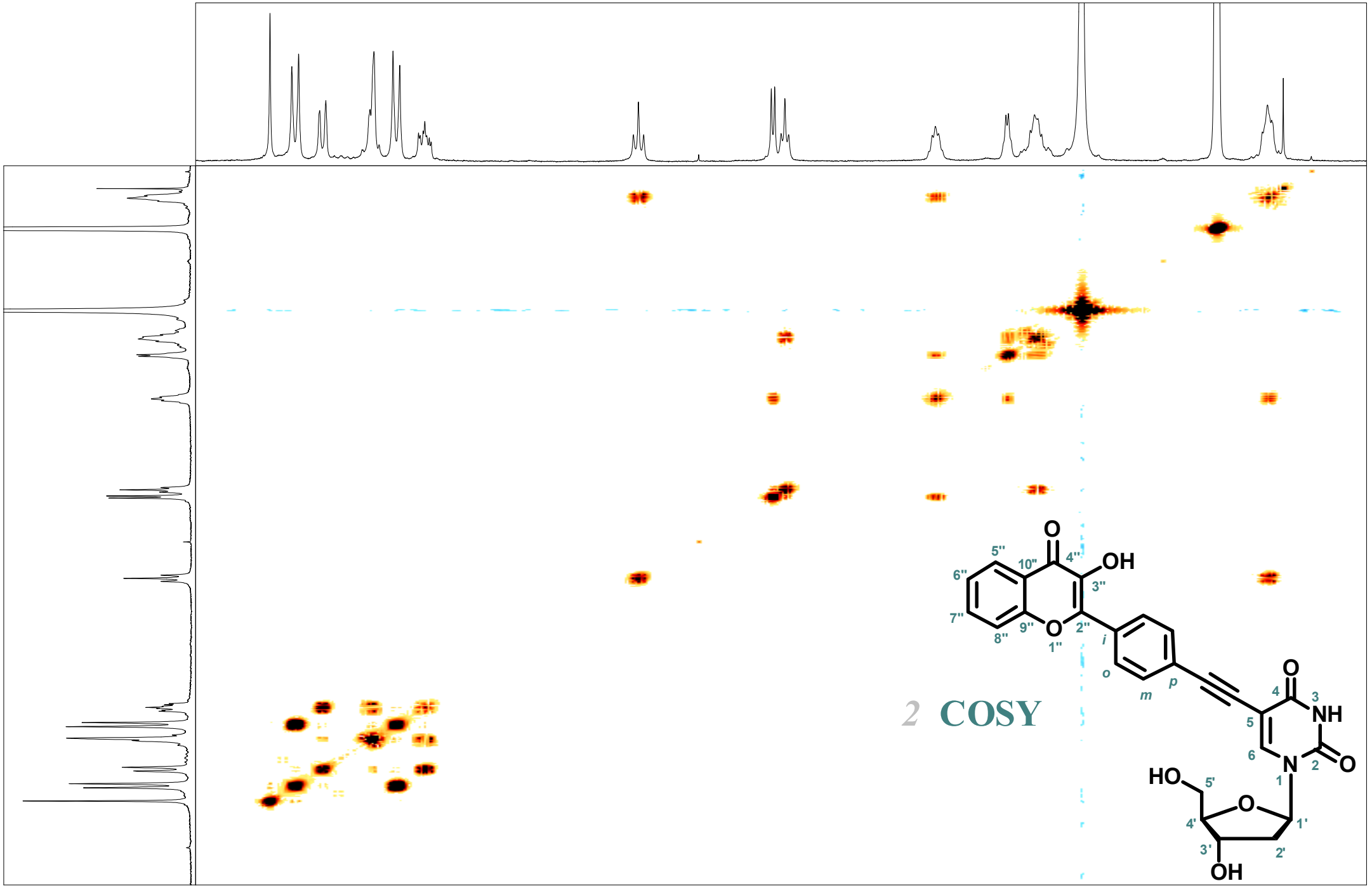

171.3
171.1
170.3
170.0
167.1
159.6
155.0
151.8
150.4
148.0
147.0
141.7
134.4
134.2
133.2
132.1
132.0
130.7
130.0
128.8
128.7
128.4
128.4
126.0
125.4
123.6
117.9
24 13C NMR (2)
m-Ph(Cbz)
o-Ph(Cbz)
C2"-Th-
Co-Tol
Cα
Cm-Tol
p-Ph(Cbz)
Ci-Tol
Th-C≡C-
C7"
Cβ
C3"
i-Ph(Cbz)
C10"
C5"
C6"
C8"
Cp-Tol
C2
C6
C9"
OC(O)CH2Ph
C2"
C4
C(O)Tol
2 x OC(O)CH3
C4"
EA
170
160
150
140
130
120
ppm (t1)
91

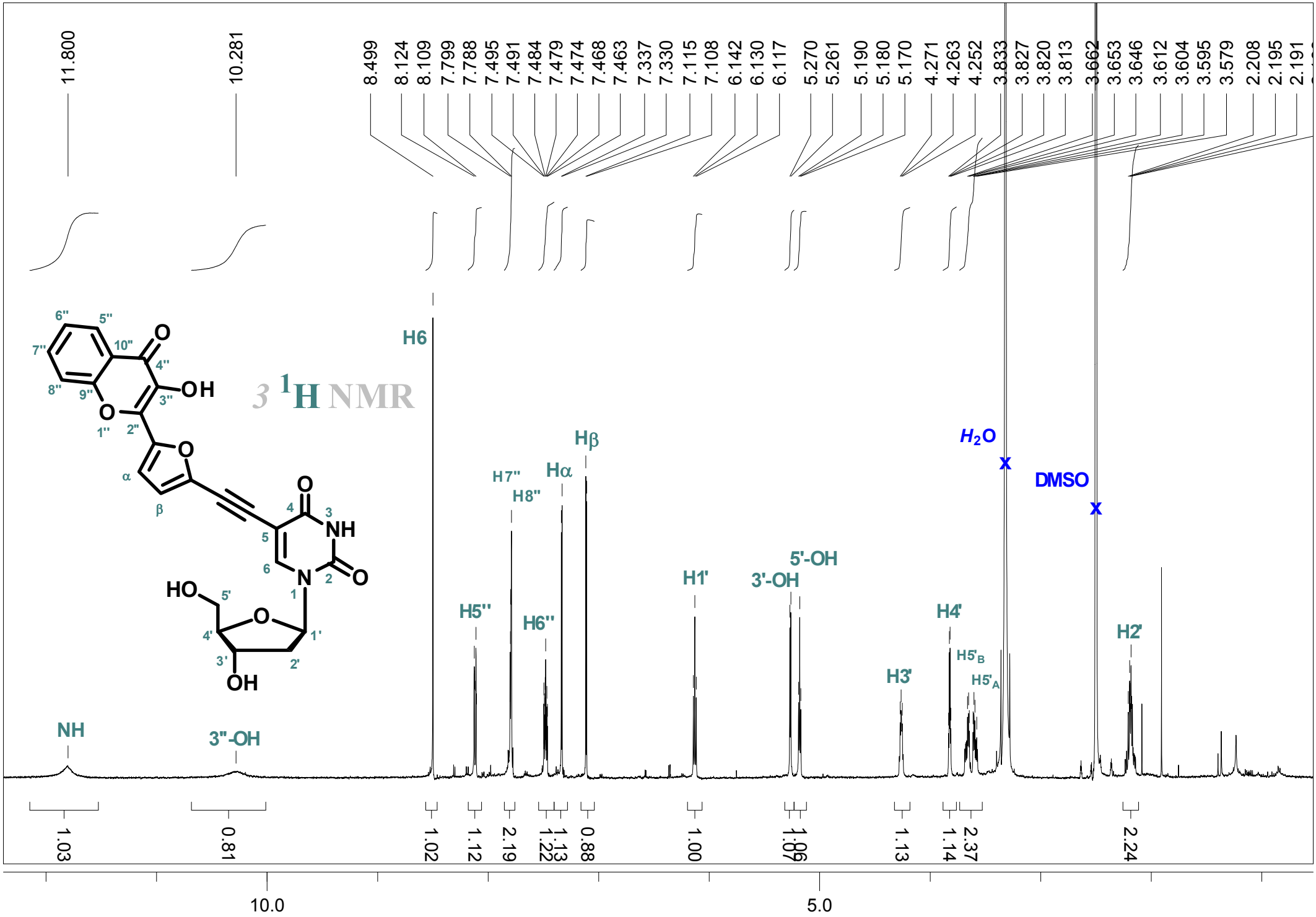

24 COSY
OCbz
p-Tol
AcO
OAc
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t2)
ppm (t1

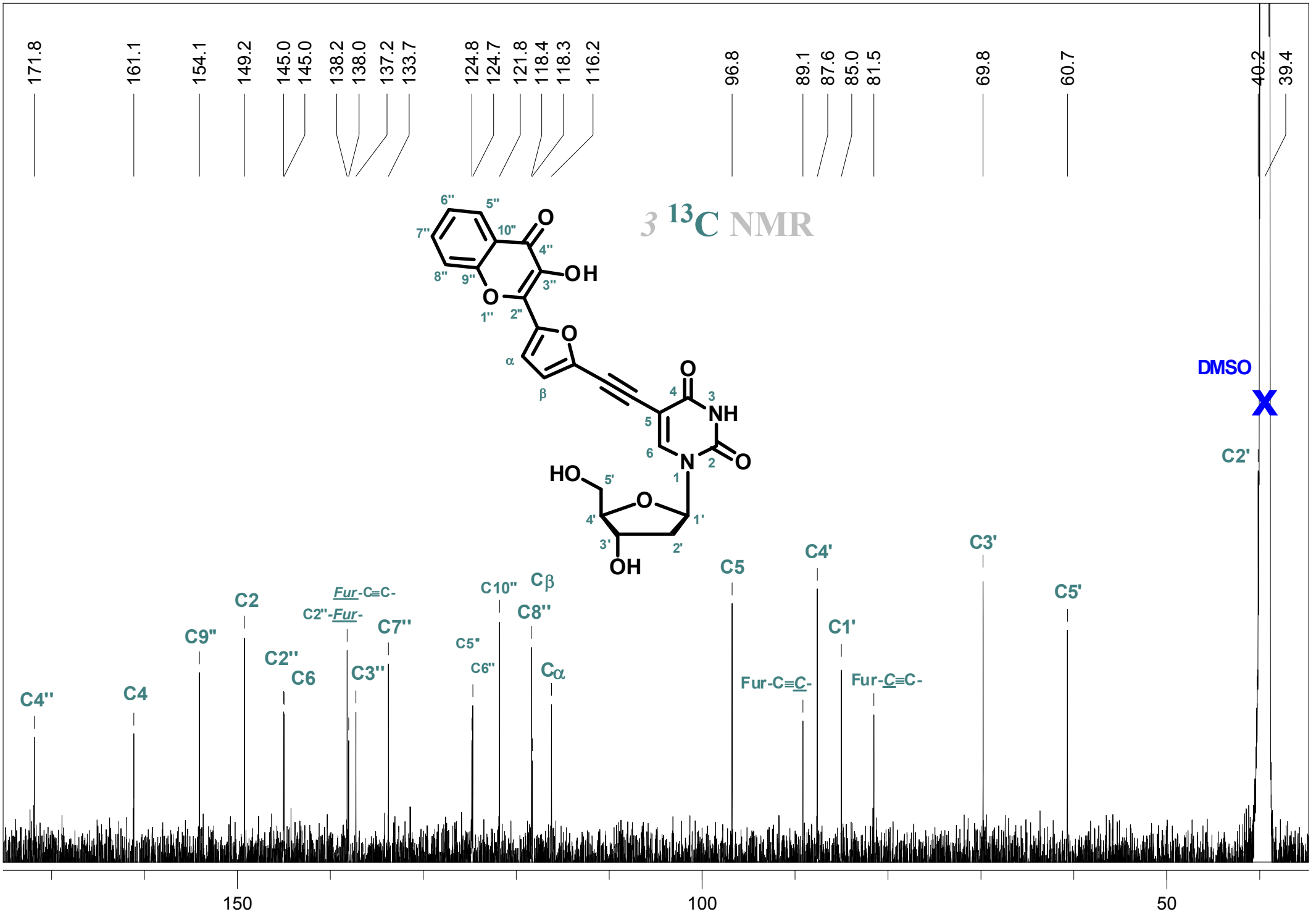

24 HMQC
OCbz
p-Tol
AcO
OAc
50
100
ppm (t1
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t2)

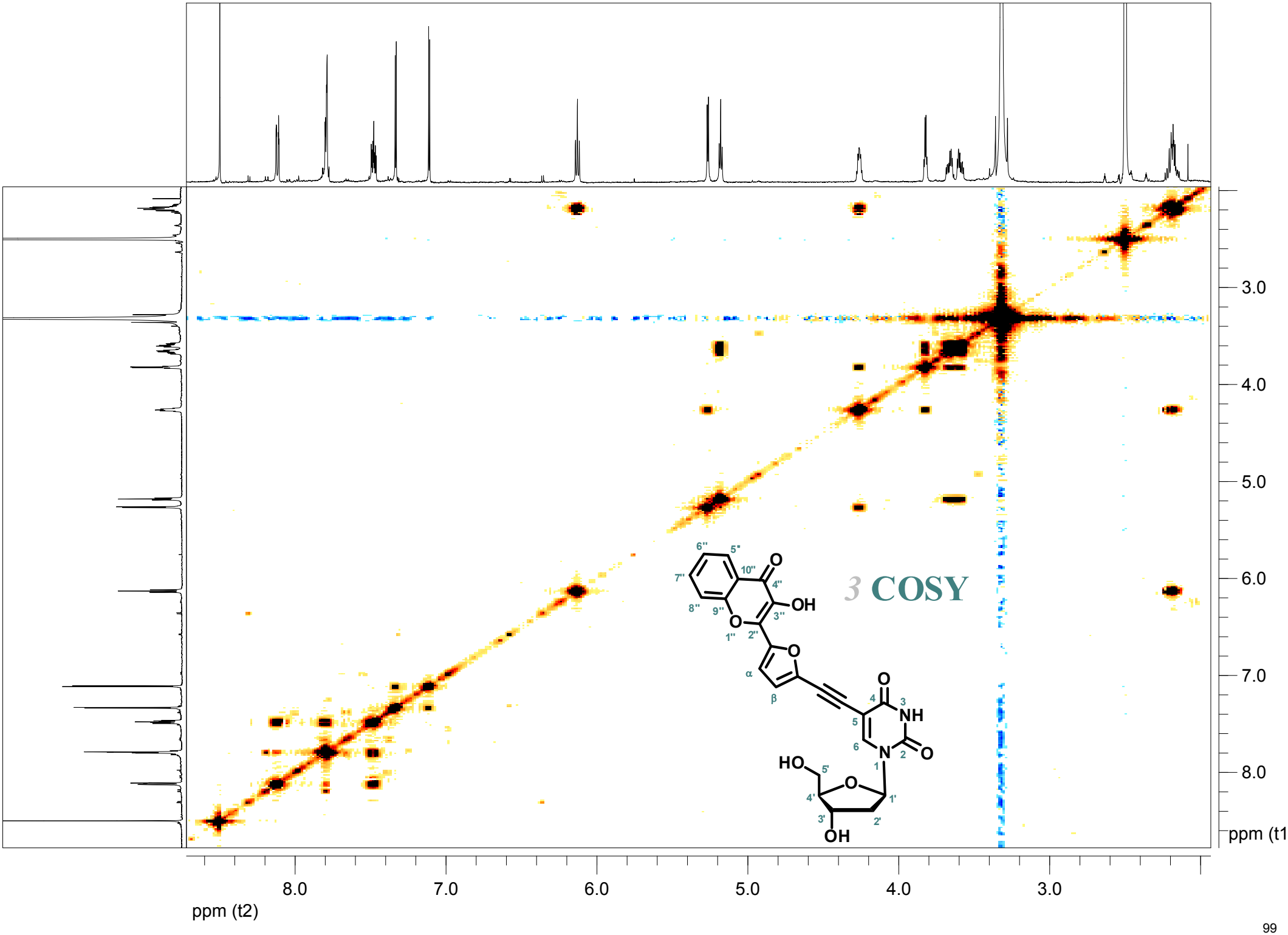

11.732
9.874
8.445
8.304
8.286
8.133
8.119
7.843
7.840
7.826
7.823
7.810
7.798
7.782
7.669
7.652
7.499
7.483
7.481
7.470
6.153
6.141
6.127
5.269
5.261
5.194
5.185
5.175
4.290
4.282
4.274
4.263
4.255
3.832
3.825
3.819
3.812
3.697
3.687
3.680
3.673
3.663
3.656
3.624
3.616
3.608
3.601
3.591
3.584
3.317
2.518
2.205
2.191
2.183
2 1H NMR
H6
o-Ph
m-Ph
H7"
H8"
H5"
H6"
H1'
3'-OH
5'-OH
H3'
H4'
H5'B
H5'A
H2'
NH
3"-OH
H2O
DMSO
(CH3)2CO
1.00
1.05
0.99
1.93
1.16
2.28
1.98
1.18
0.95
0.96
0.99
1.03
0.97
2.11
2.17
10.0
5.0
ppm (t1)

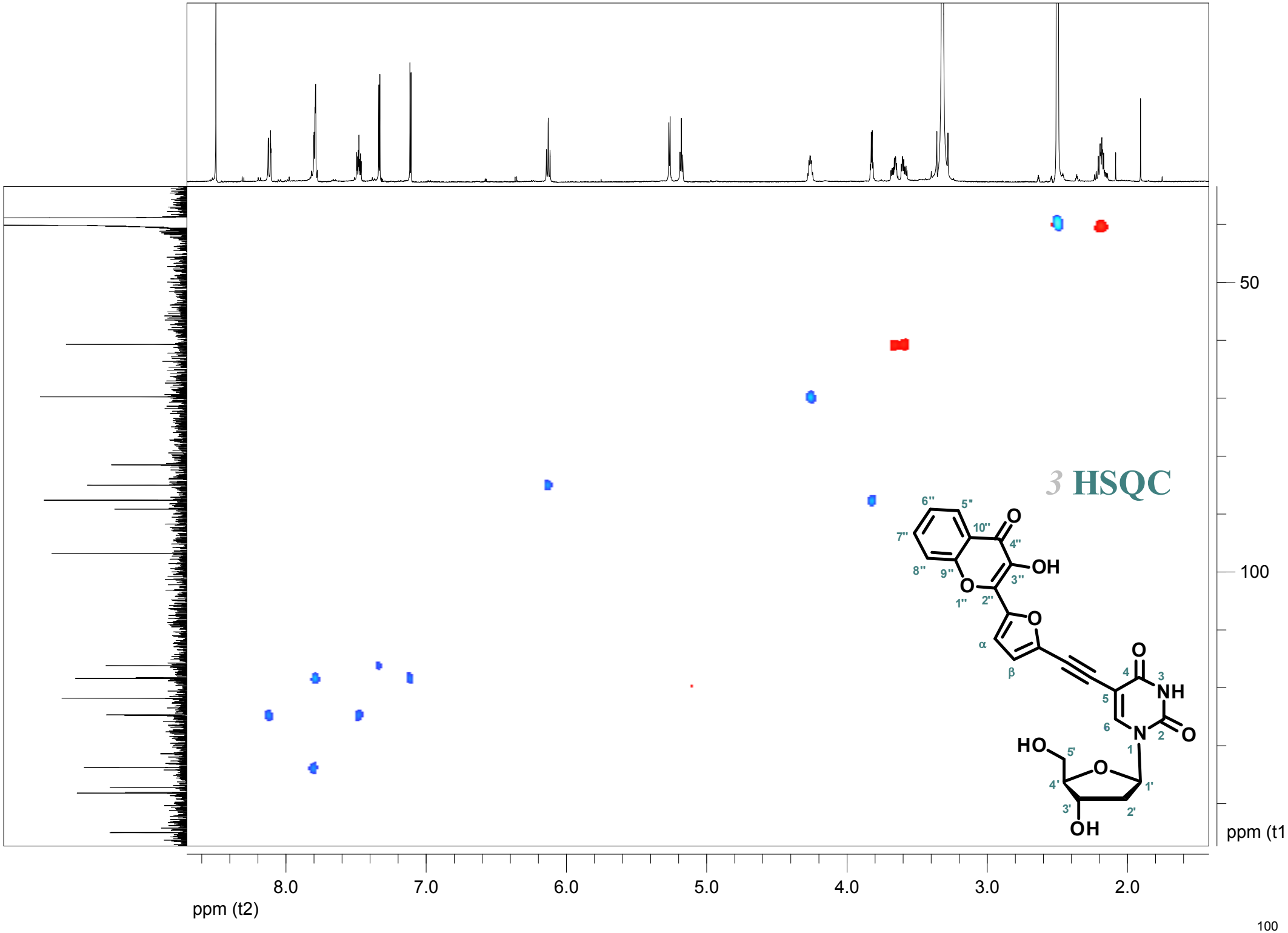

172.9
161.3
154.5
149.3
144.2
144.0
139.5
133.8
131.1
131.1
127.6
124.7
124.5
123.5
121.2
118.4
97.8
91.6
87.5
84.8
84.6
69.8
60.7
40.1
39.4
2 13C NMR
DMSO
m-Ph
o-Ph
p-Ph
i-Ph
C4''
C4
C9''
C2''
C6
C2
C3''
C7''
C6''
C8''
C10''
C5''
C5
Ph-C≡C-
C1'
C4'
Ph-C≡C-
C3'
C5'
C2'
OH
NH
HO
150
100
50
ppm (t1)

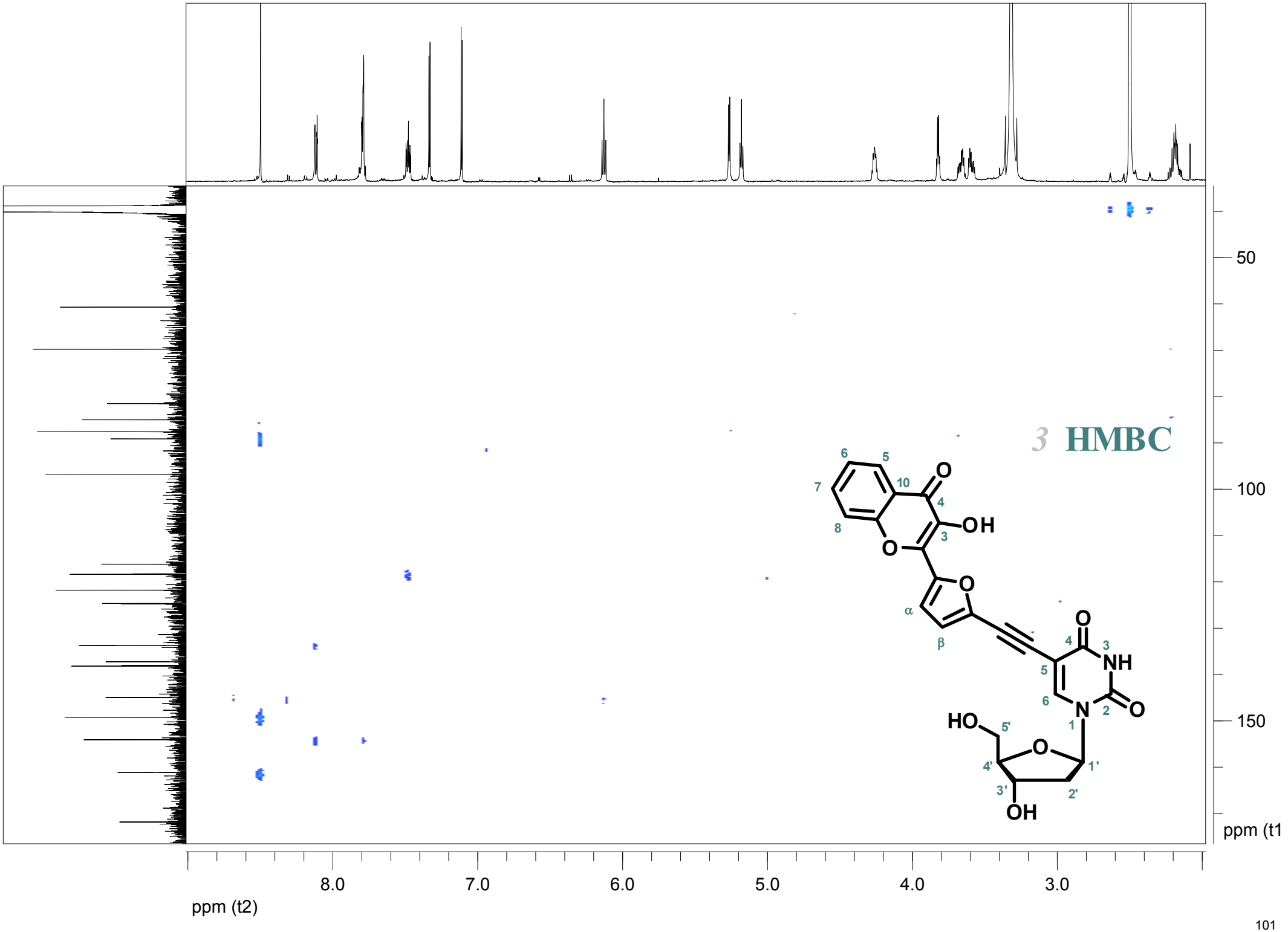

8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t2)
2 COSY
O
OH
NH
HO
N
O

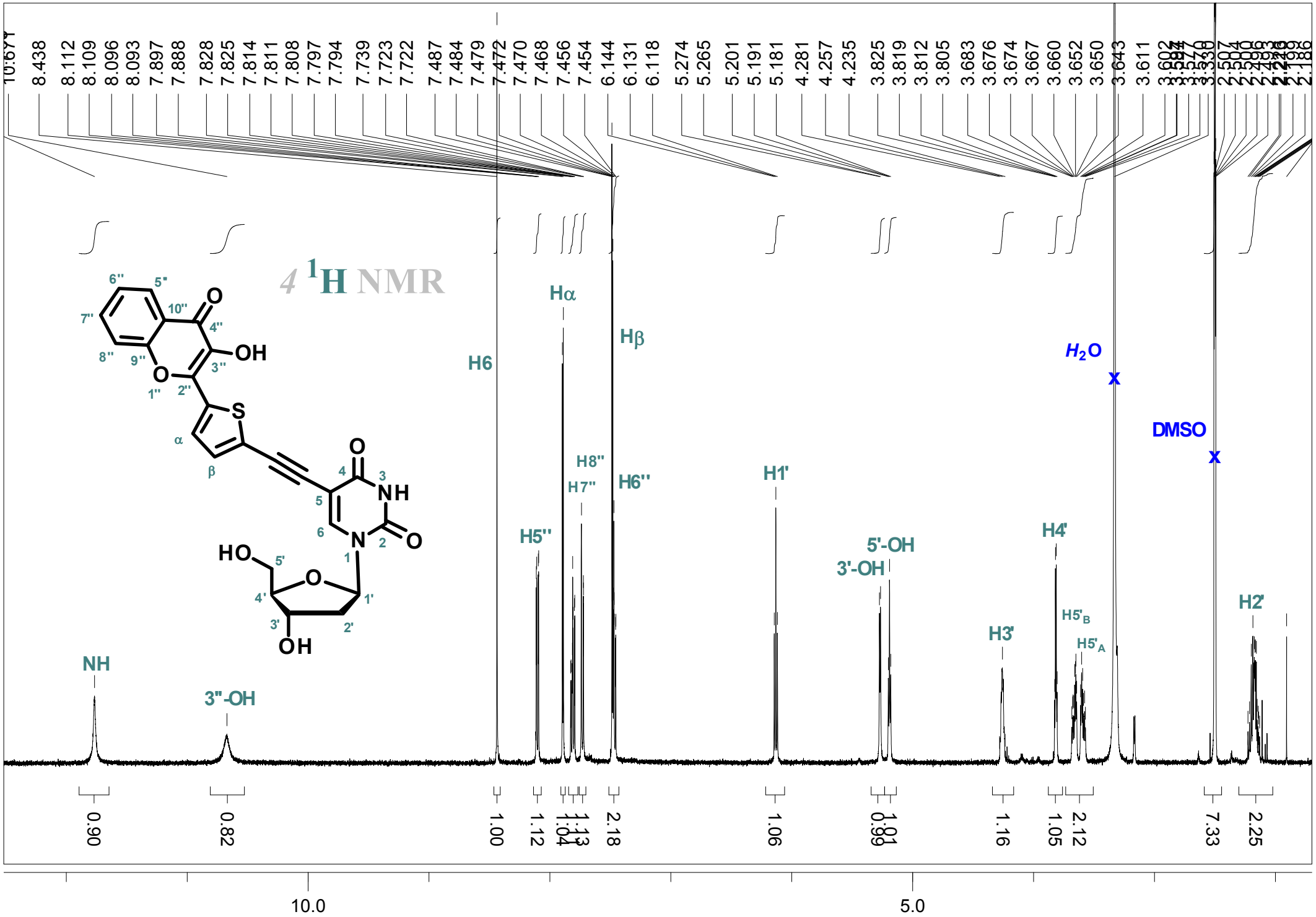

11.800
10.281
8.499
8.124
8.109
7.799
7.788
7.495
7.491
7.484
7.479
7.474
7.468
7.463
7.337
7.330
7.115
7.108
6.142
6.130
6.117
5.270
5.261
5.190
5.180
5.170
4.271
4.263
4.252
3.833
3.827
3.820
3.813
3.662
3.653
3.646
3.612
3.604
3.595
3.579
2.208
2.195
2.191
3 1H NMR
H6
Hβ
Hα
H7''
H8''
H5''
H6''
H1'
5'-OH
3'-OH
H4'
H3'
H5'B
H5'A
H2'
H2O
DMSO
NH
3''-OH
1.03
0.81
1.02
1.12
2.19
1.22
1.13
0.88
1.00
1.07
1.06
1.13
1.14
2.37
2.24
10.0
5.0
ppm (t1)

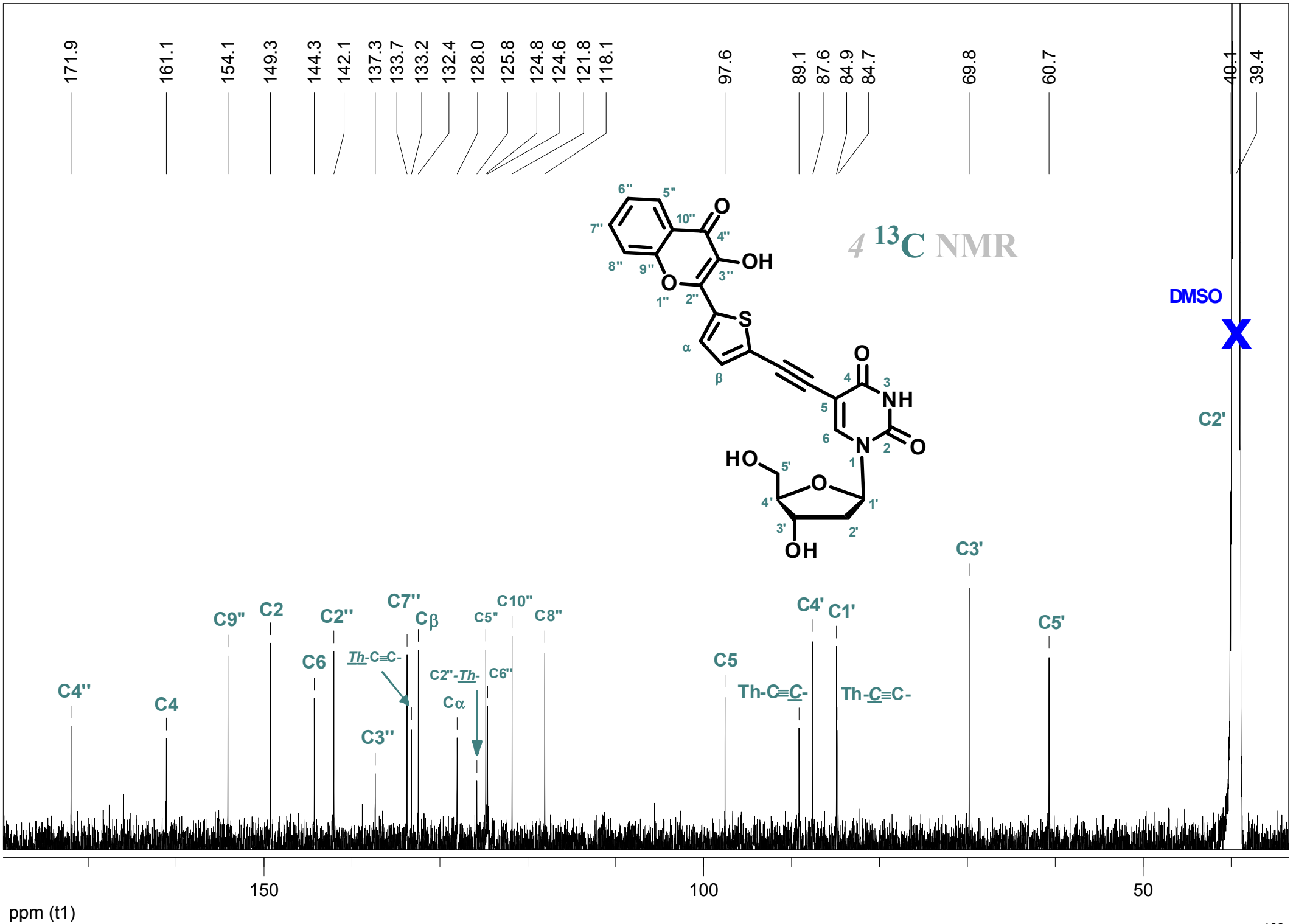

171.8
161.1
154.1
149.2
145.0
145.0
138.2
138.0
137.2
133.7
124.8
124.7
121.8
118.4
118.3
116.2
96.8
89.1
87.6
85.0
81.5
69.8
60.7
40.2
39.4
3 13C NMR
DMSO
C2'
C4''
C4
C9''
C2
C2''
C6
Fur-C≡C-
C2''-Fur-
C3''
C7''
C5''
C6''
C10''
Cβ
C8''
Cα
C5
Fur-C≡C-
C4'
C1'
Fur-C≡C-
C3'
C5'
150
100
50
ppm (t1)

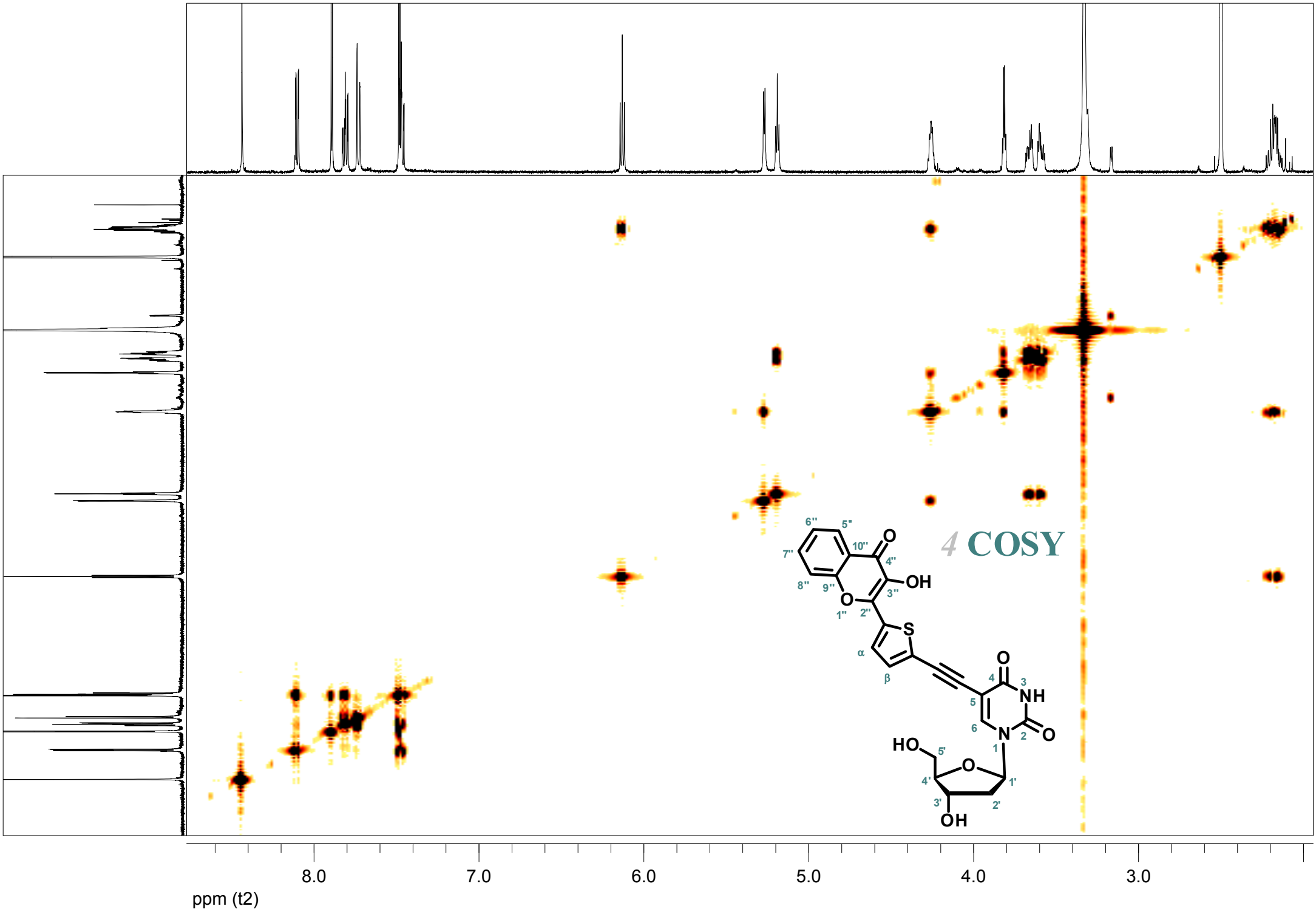

3 COSY
8.0
7.0
6.0
5.0
4.0
3.0
ppm (t2)
ppm (t1
OH
NH
HO
O
N

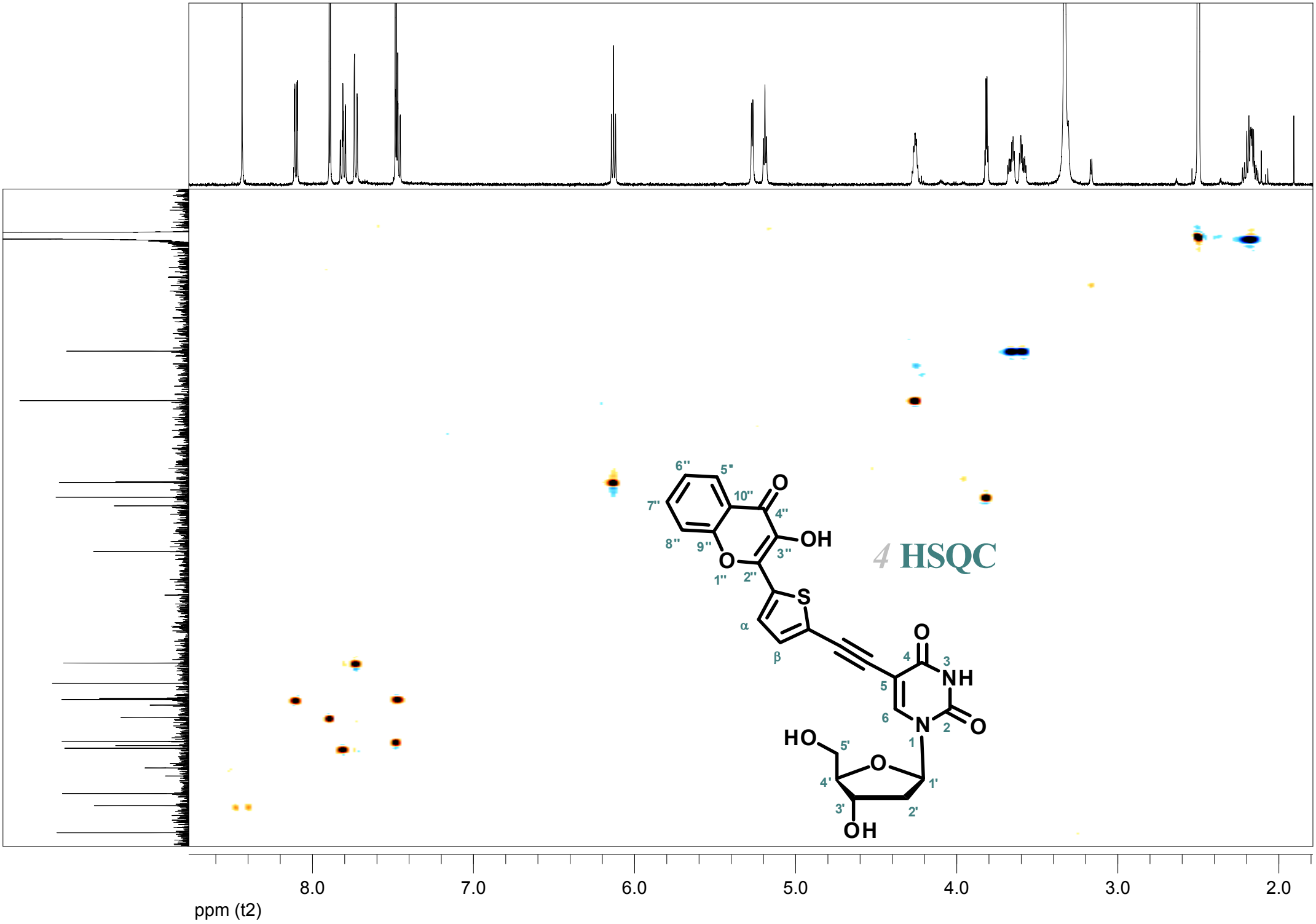

3 HSQC
50
100
ppm (t1
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t2)
HO
OH
NH
O
N

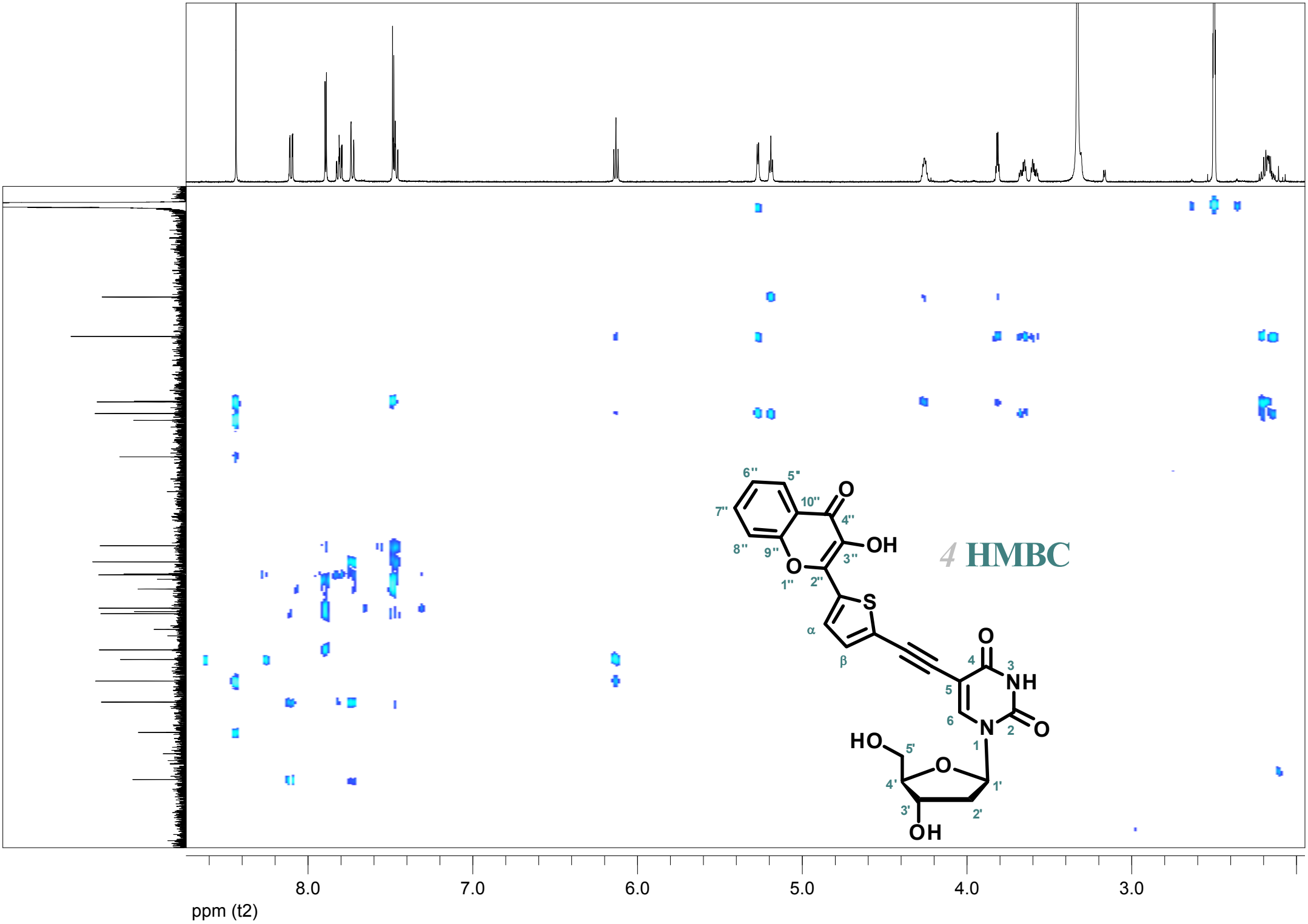

3 HMBC
50
100
150
ppm (t1
8.0
7.0
6.0
5.0
4.0
3.0
ppm (t2)
O
OH
NH
HO
N
α
β


4 1H NMR
H6
Hα
Hβ
H8''
H7''
H6''
H5''
H1'
5'-OH
3'-OH
H4'
H3'
H5'B
H5'A
H2'
H2O
DMSO
NH
3''-OH
0.90
0.82
1.00
1.12
1.04
1.13
2.18
1.06
0.99
1.01
1.16
1.05
2.12
7.33
2.25
10.0
5.0
ppm (t1)


171.9
161.1
154.1
149.3
144.3
142.1
137.3
133.7
133.2
132.4
128.0
125.8
124.8
124.6
121.8
118.1
97.6
89.1
87.6
84.9
84.7
69.8
60.7
40.1
39.4
4 13C NMR
DMSO
C2'
C3'
C4'
C1'
C5'
C5
Th-C≡C-
Th-C≡C-
C4''
C4
C9"
C2
C6
C2''
Th-C≡C-
C3''
C7''
Cβ
C2"-Th-
Cα
C6"
C5"
C10"
C8"
150
100
50
ppm (t1)


4 COSY
8.0
7.0
6.0
5.0
4.0
3.0
ppm (t2)


4 HSQC
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t2)


4 HMBC
8.0
7.0
6.0
5.0
4.0
3.0
ppm (t2)

Control Method

Control Method	
Name	Niko-060214-Free-Nucleotide-analytique
User Name	Administrator
Date Modified	10/02/2014 09:17:43
Description	
Method Time	61,0 [min]

Pump #1

Initial Condition

Pump Mode	LPG1
Flow	0,500 [mL/min]
Max. Pressure	25,0 [MPa]
Min. Pressure	0,0 [MPa]
Solvents	
A	90,0 [%] H2O + 0.1% AF
B	10,0 [%] ACN + 0.1% AF
C	0,0 [%]
D	0,0 [%]

Time Program

#	Time [min]	Function	Value #1	Value #2	Value #3	Value #4
1	5,00	Composition [%]	90,0	10,0	0,0	0,0
2	40,00	Composition [%]	0,0	100,0	0,0	0,0
3	45,00	Composition [%]	0,0	100,0	0,0	0,0
4	55,00	Composition [%]	90,0	10,0	0,0	0,0
5	60,00	Composition [%]	90,0	10,0	0,0	0,0

Pump #1 Valve / Event

Initial Condition

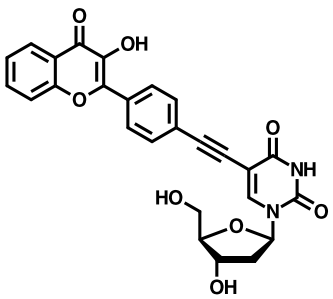
Valve #1	1
Event #1	Off
Event #2	Off
Event #3	Off

4WL-UV Detector

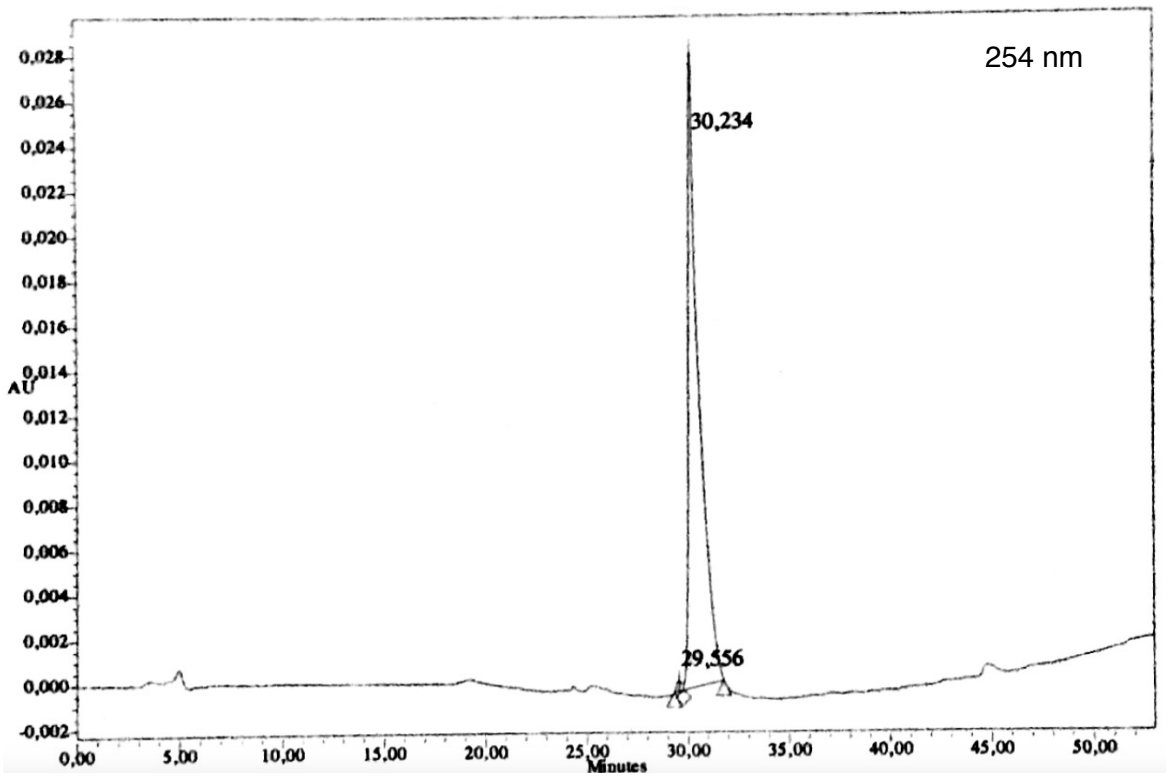
Initial Condition

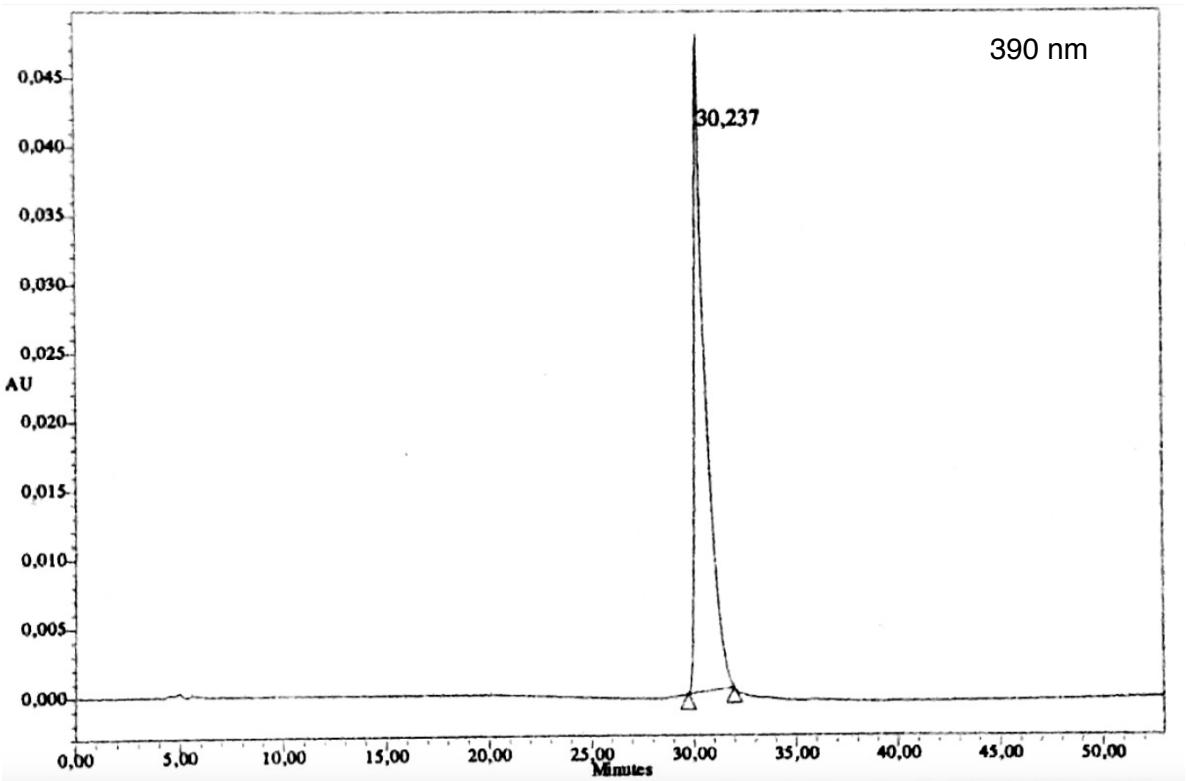
Wavelength #1	254 [nm]
Wavelength #2	280 [nm]
Wavelength #3	360 [nm]
Wavelength #4	393 [nm]
Wavelength #5 (Ratio)	0 [nm]
Response	STD
Autozero	On

PCU

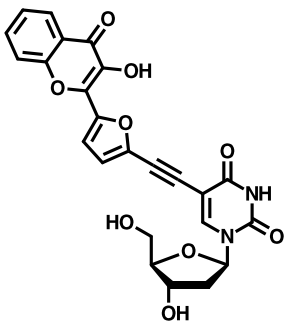

O
OH
O
O
NH
HO
N
O
O
OH

PCU

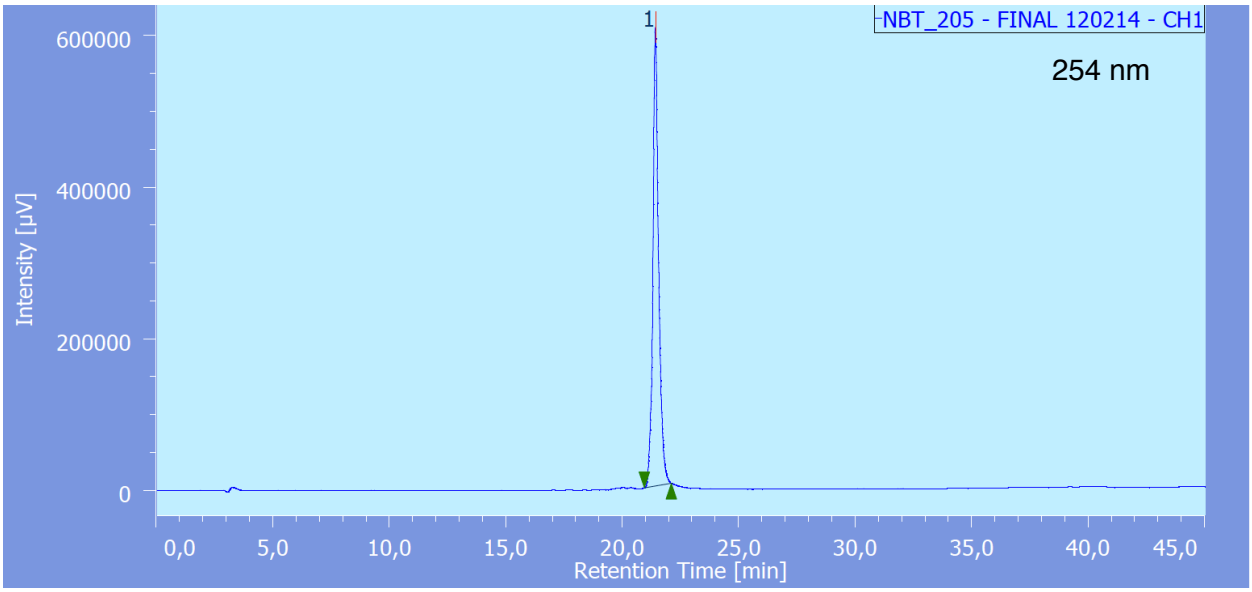

254 nm
30,234
29,556
AU
0,028
0,026
0,024
0,022
0,020
0,018
0,016
0,014
0,012
0,010
0,008
0,006
0,004
0,002
0,000
-0,002
0,00
5,00
10,00
15,00
20,00
25,00
30,00
35,00
40,00
45,00
50,00
Minutes

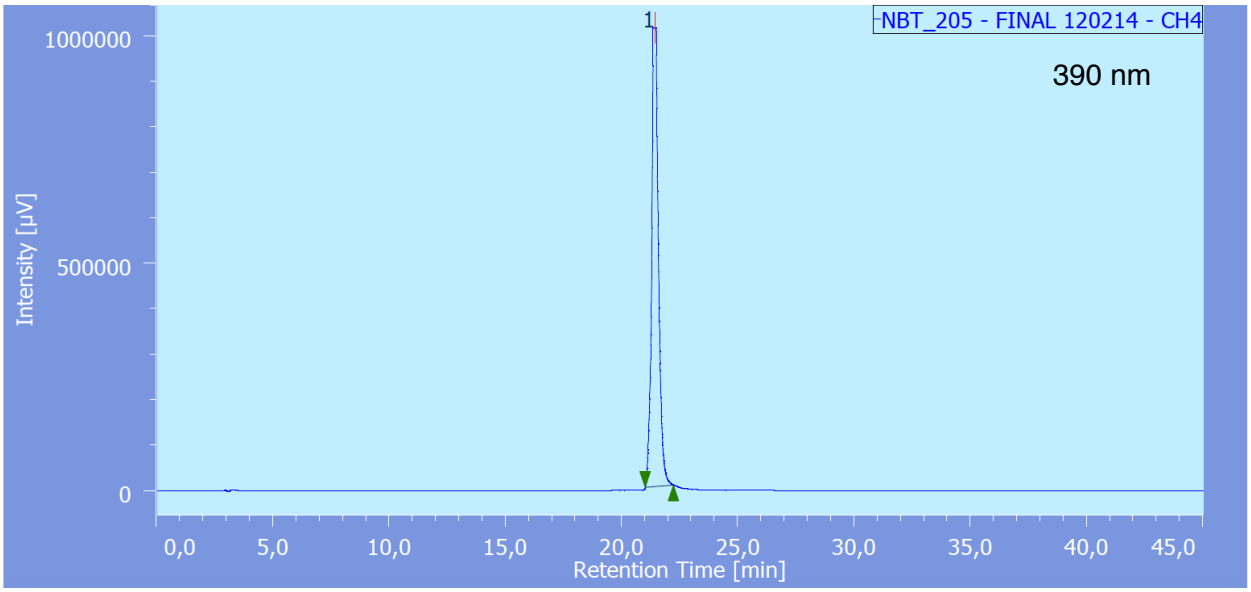

390 nm
30,237
AU
0,045
0,040
0,035
0,030
0,025
0,020
0,015
0,010
0,005
0,000
0,00
5,00
10,00
15,00
20,00
25,00
30,00
35,00
40,00
45,00
50,00
Minutes

FCU

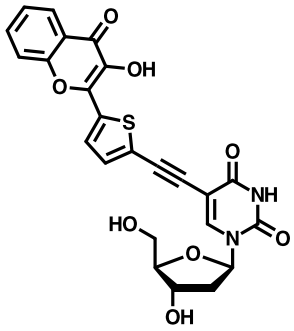

O
OH
O
O
O
NH
HO
N
O
O
OH

FCU

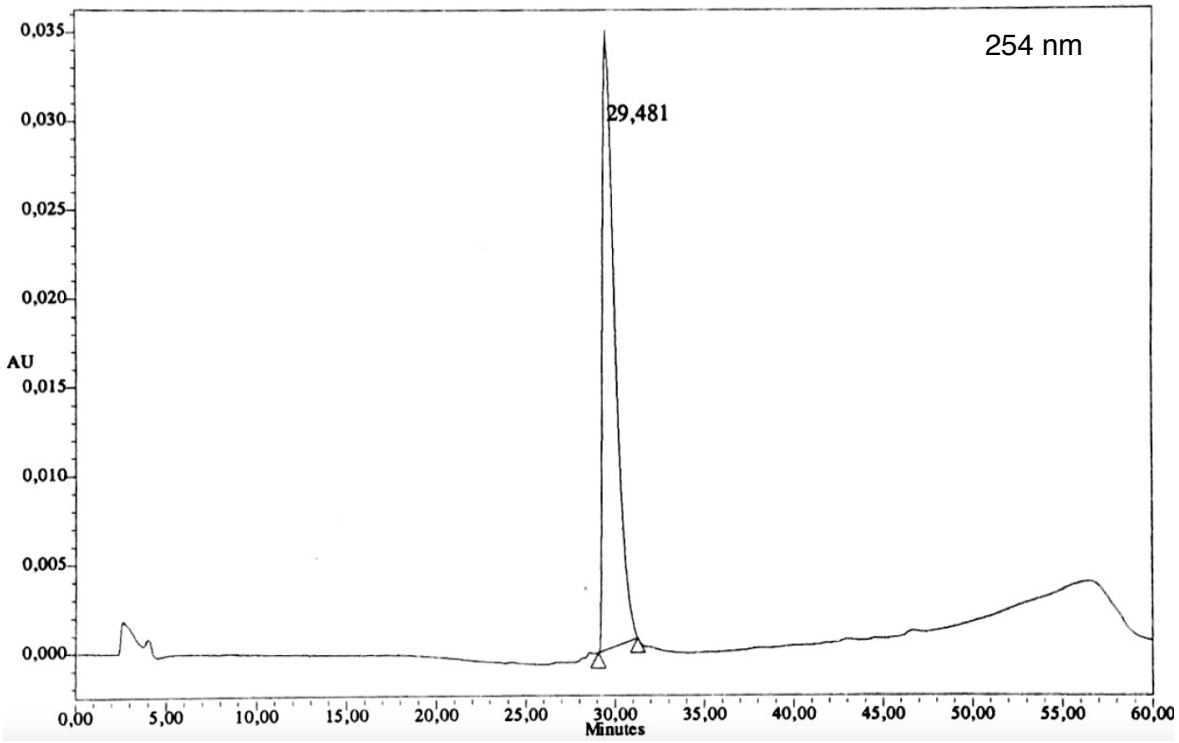

NBT_205 - FINAL 120214 - CH1
254 nm
1
600000
400000
200000
0
Intensity [µV]
0,0
5,0
10,0
15,0
20,0
25,0
30,0
35,0
40,0
45,0
Retention Time [min]

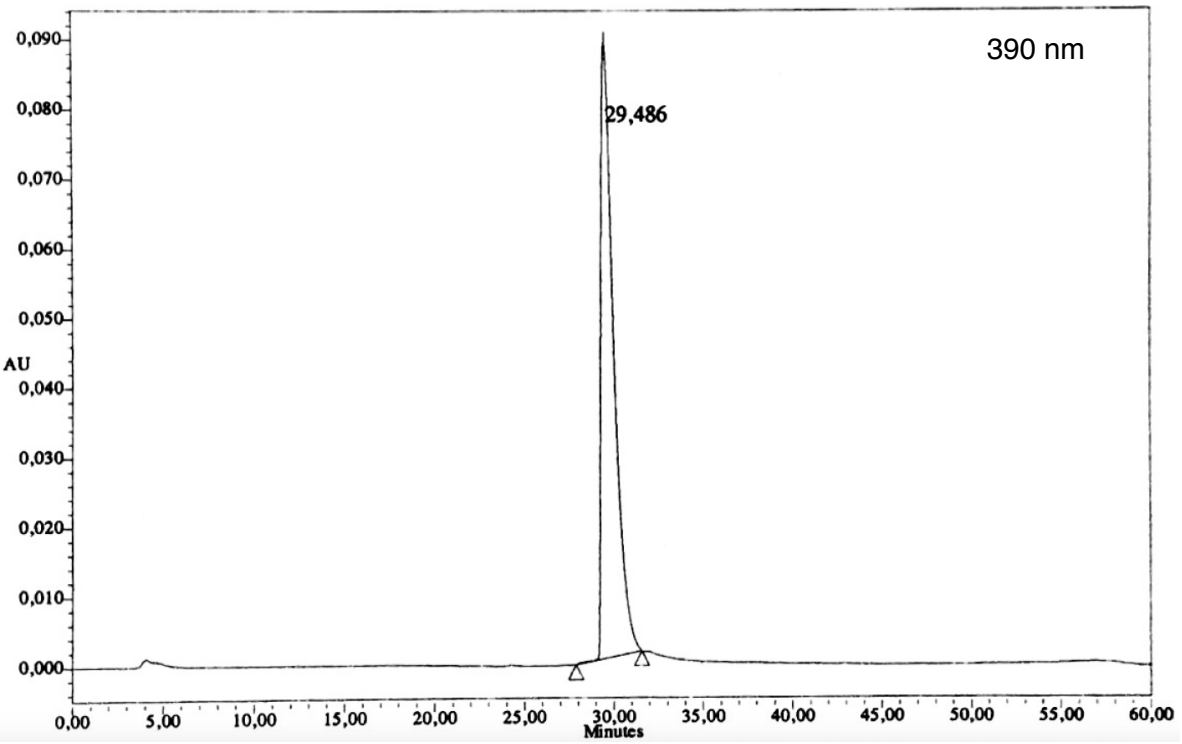

NBT_205 - FINAL 120214 - CH4
390 nm
1
1000000
500000
0
Intensity [µV]
0,0
5,0
10,0
15,0
20,0
25,0
30,0
35,0
40,0
45,0
Retention Time [min]

TCU


O
OH
O
S
O
NH
HO
N
O
O
OH

TCU


254 nm
29,481
AU
0,035
0,030
0,025
0,020
0,015
0,010
0,005
0,000
0,00
5,00
10,00
15,00
20,00
25,00
30,00
35,00
40,00
45,00
50,00
55,00
60,00
Minutes


390 nm
29,486
AU
0,090
0,080
0,070
0,060
0,050
0,040
0,030
0,020
0,010
0,000
0,00
5,00
10,00
15,00
20,00
25,00
30,00
35,00
40,00
45,00
50,00
55,00
60,00
Minutes