Cooperativity and Site-Selectivity of Intramolecular Hydrogen Bonds on the Fluorescence Quenching of Modified GFP Chromophores

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(Supporting Information 18 pages)

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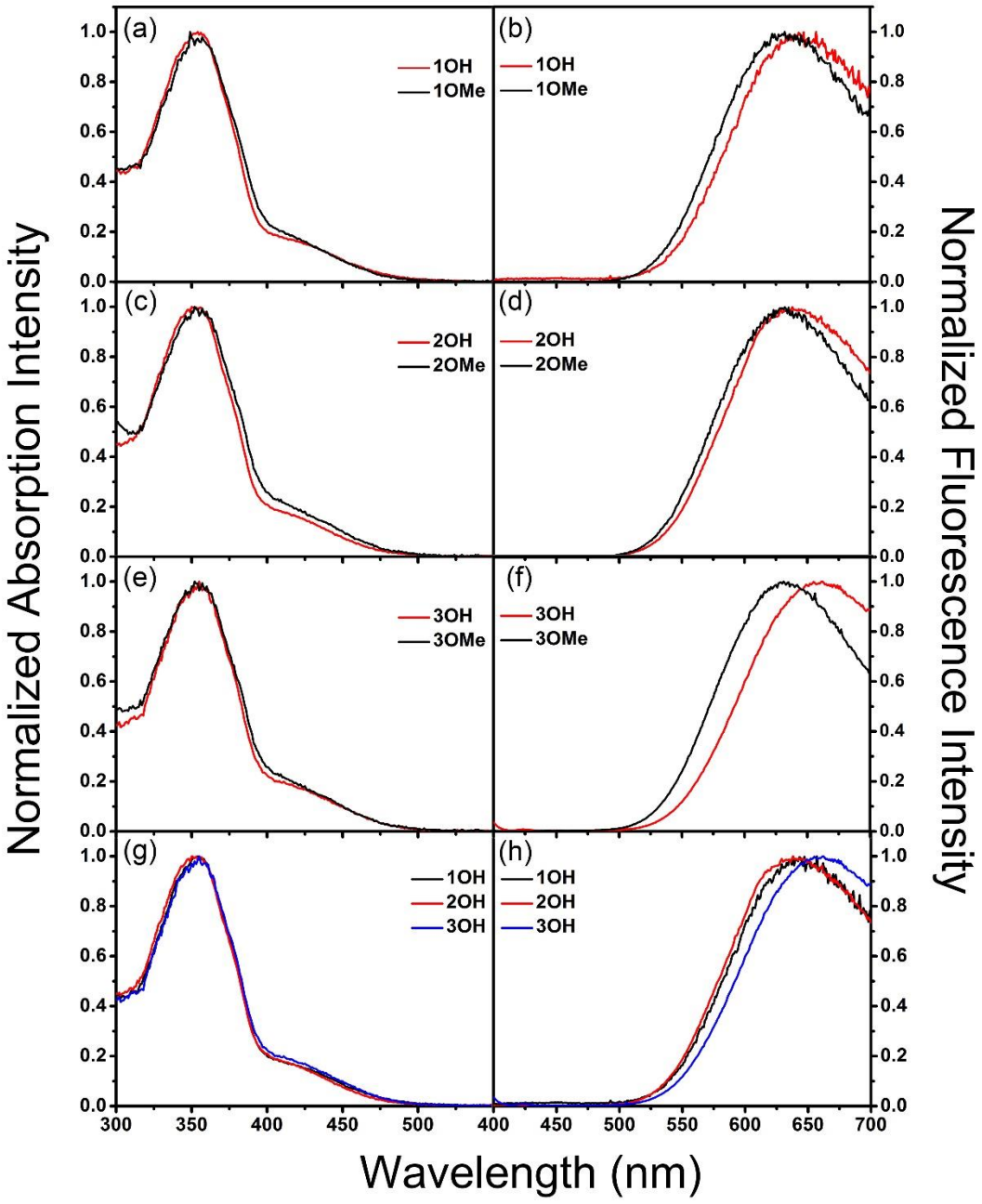

Figure S1. Normalized absorption and emission spectra of **1OH**–**3OH** and **1OMe**–**3Ome** in CH_3CN**.**

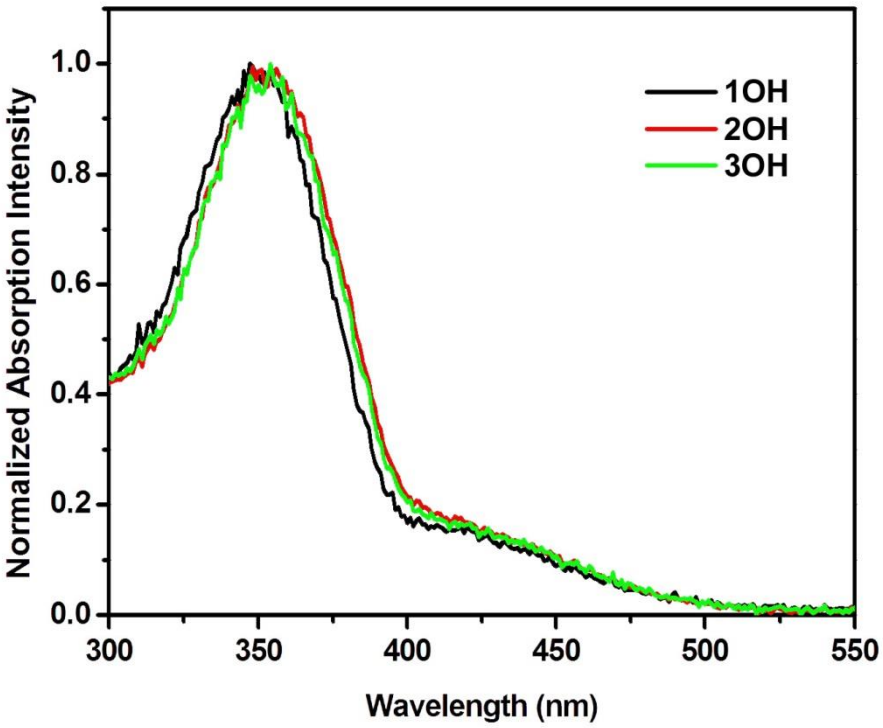

Figure S2. Normalized absorption spectra of **1OH**–**3OH** in MeOH.

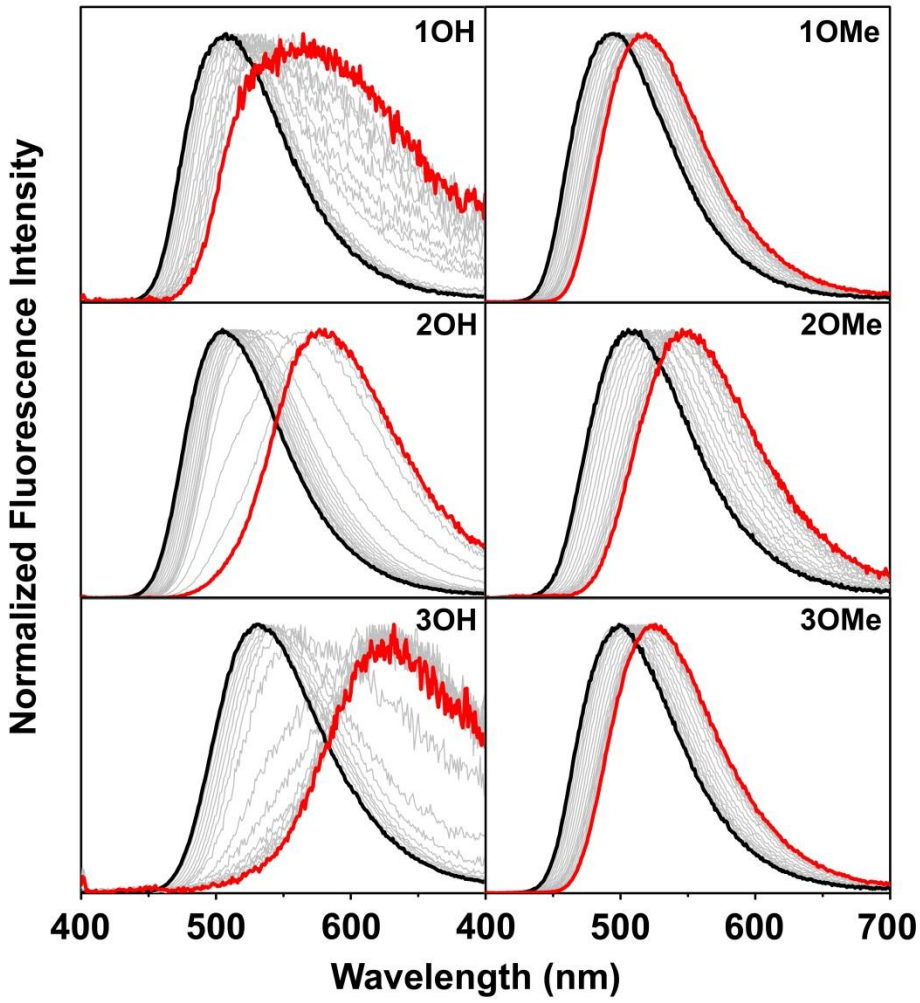

Figure S3. Normalized fluorescence spectra of **1OH–3OH** and **1OMe–3OMe** in methylcyclohexane. The spectra were recorded from 298 K (black) to 128 K (red). excitation wavelength is 350 nm.

Table S1. Crystallographic data of **1OH**−**3OH**.

	1OH	**2OH**	**3OH**
Empirical formula	$C_{15}H_{19}N_3O_2$	$C_{16}H_{21}N_3O_2$	$C_{17}H_{23}N_3O_3$
Formula weight	273.33	287.36	317.38
Temperature	295(2) K	295(2) K	200(2) K
Wavelength	1.54178 Å	1.54178 Å	1.54178 Å
Crystal system	Monoclinic	Triclinic	Monoclinic
Space group	P2(1)	P-1	$P2_1/n$
Unit cell dimensions	a = 9.4598(2) Å α = 90°	a = 7.4623(8) Å α = 100.622(9)°	a = 9.3568(4) Å α = 90°
	b = 7.61760(10) Å β = 95.575(2)°	b = 7.9403(9) Å β = 92.419(9)°	b = 15.0678(6) Å β = 100.597(4)°
	c = 10.0179(2) Å γ = 90°	c = 14.3735(15) Å γ = 112.362(10)°	c = 11.9128(5) Å γ = 90°
Volume, Z	718.48(2) $Å^3$, 2	768.19(14) $Å^3$, 2	1650.90(12) $Å^3$, 4
Density (calculated)	1.263 Mg/m^3	1.242 Mg/m^3	1.277 Mg/m^3
Absorption coefficient	0.693 mm^{-1}	0.671 mm^{-1}	0.721 mm^{-1}
F(0 0 0)	292	308	680
Crystal size (mm^3)	0.25 x 0.20 x 0.15	0.25 x 0.20 x 0.15	0.20 x 0.15 x 0.10
Θ range for data collection	4.43 to 67.97°	3.15 to 68.00°	4.78 to 67.99°
Limiting indices	$-10 \le h \le 11$, $-9 \le k \le 9$, $-11 \le l \le 12$	$-8 \le h \le 8$, $-8 \le k \le 9$, $-17 \le l \le 15$	$-11 \le h \le 10$, $-18 \le k \le 17$, $-14 \le l \le 11$
Reflections collection	4668	5153	6078
Independent reflections	2566 [R(int) = 0.0143]	2781 [R(int) = 0.0352]	3005 [R(int) = 0.0225]
Completeness to Θ = 67.99°	100.0 %	99.5 %	99.8 %
Absorption correction	Semi-empirical from equivalents	Semi-empirical from equivalents	Semi-empirical from equivalents

Max. and min. transmission	1.00000 and 0.75593	1.00000 and 0.58358	1.00000 and 0.90285
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data / restraints / parameters	2566 / 1 / 185	2781 / 2 / 193	3005 / 1 / 217
Goodness-of-fit on F^2	0.786	1.168	1.014
Final R indices [I>2σ(I)]	R1 = 0.0365, wR2 = 0.1129	R1 = 0.0881, wR2 = 0.2219	R1 = 0.0595, wR2 = 0.1673
R indices (all data)	R1 = 0.0373, wR2 = 0.1146	R1 = 0.1336, wR2 = 0.2529	R1 = 0.0723, wR2 = 0.1793
Largest diff. peak and hole	0.183 and -0.161 e.Å $^{-3}$	0.542 and -0.313 e.Å $^{-3}$	0.772 and -0.262 e.Å $^{-3}$

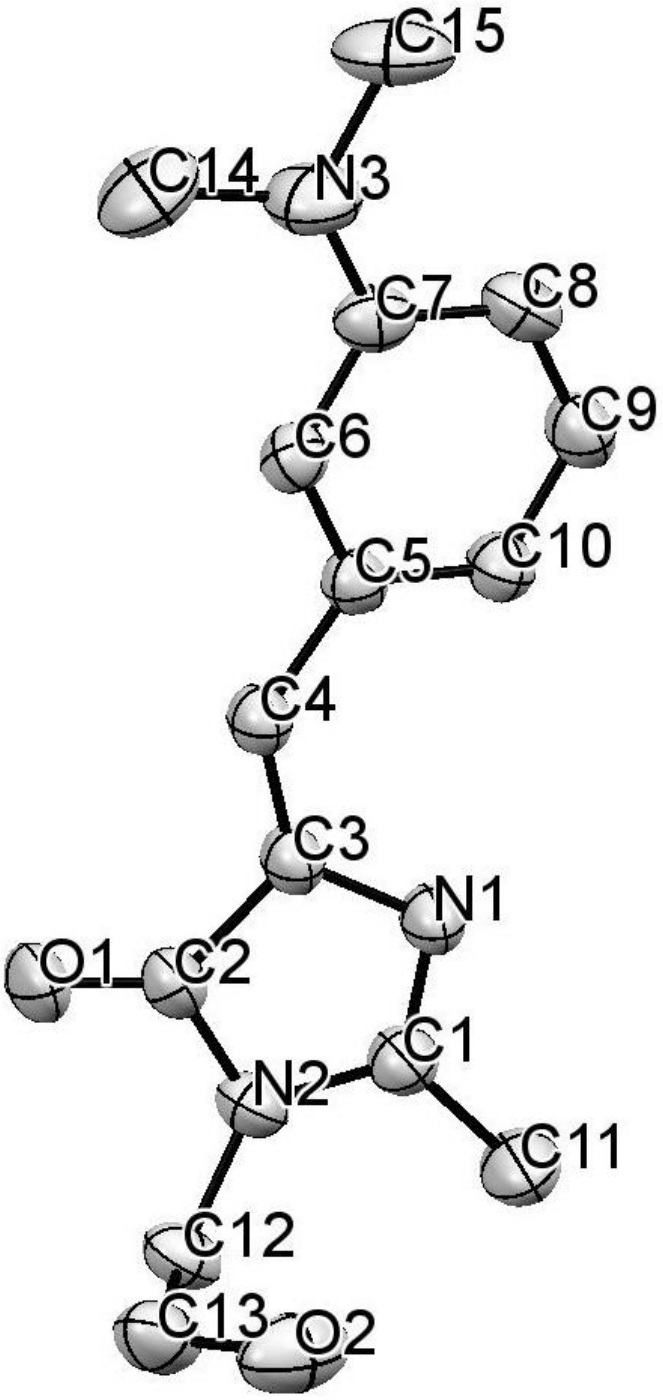

Figure S4. Thermal ellipsoid plot (30%) of the crystal of **1OH**. Hydrogen atoms are omitted for clarity.

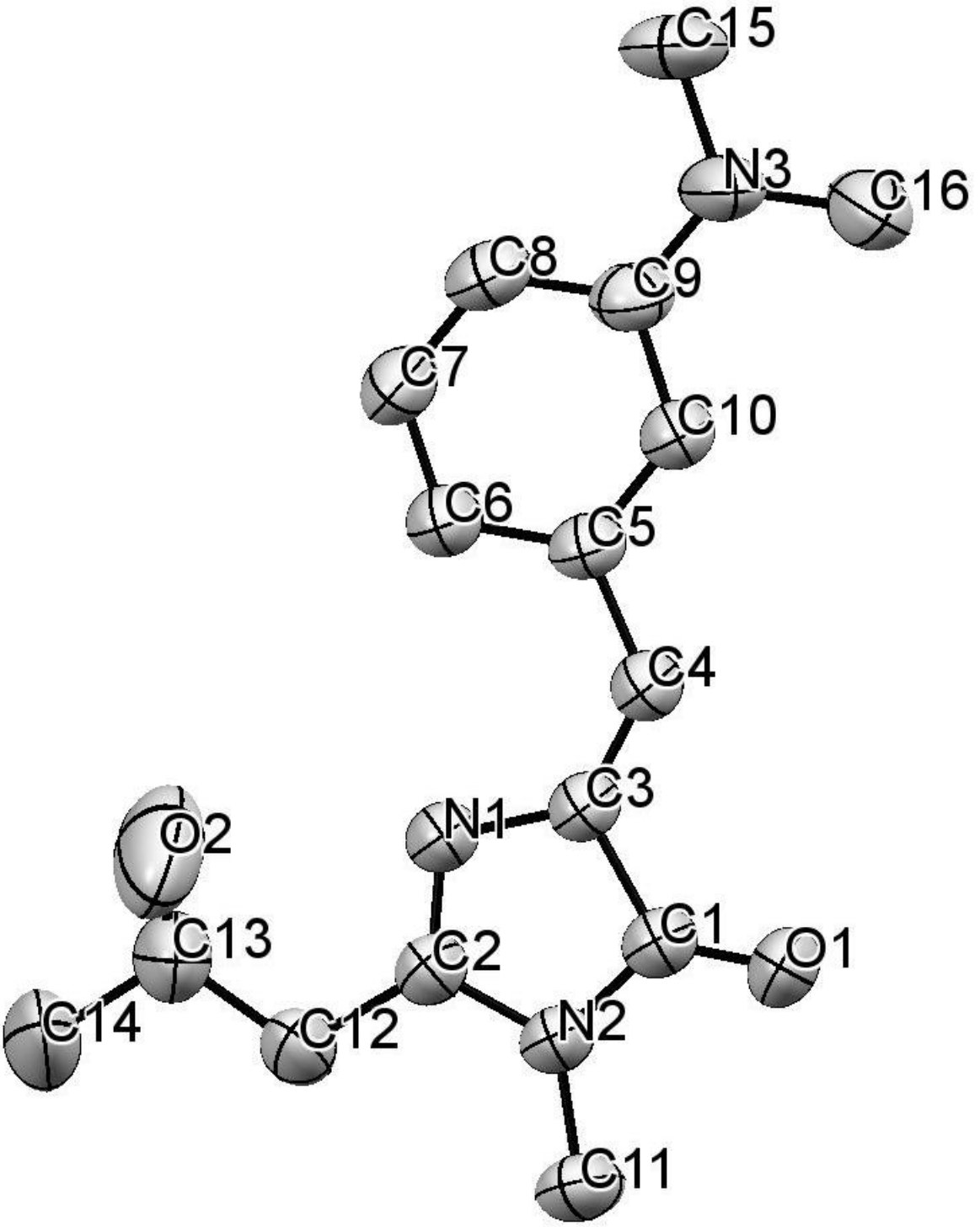

Figure S5. Thermal ellipsoid plot (30%) of the crystal of **2OH**. Hydrogen atoms are omitted for clarity.

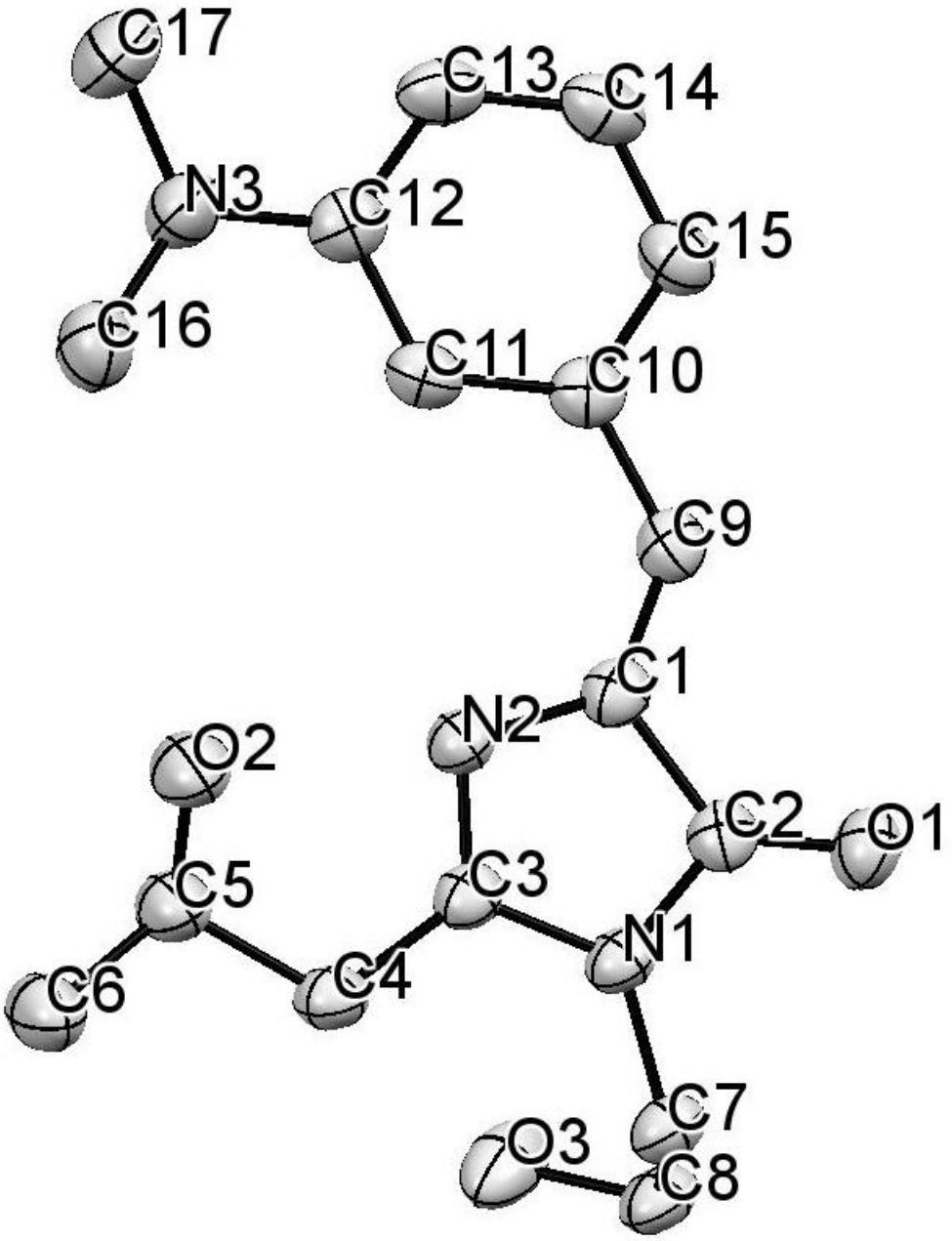

Figure S6. Thermal ellipsoid plot (30%) of the crystal of **3OH**. Hydrogen atoms are omitted for clarity.

Figure S7. Unit cell packing of **1OH**.

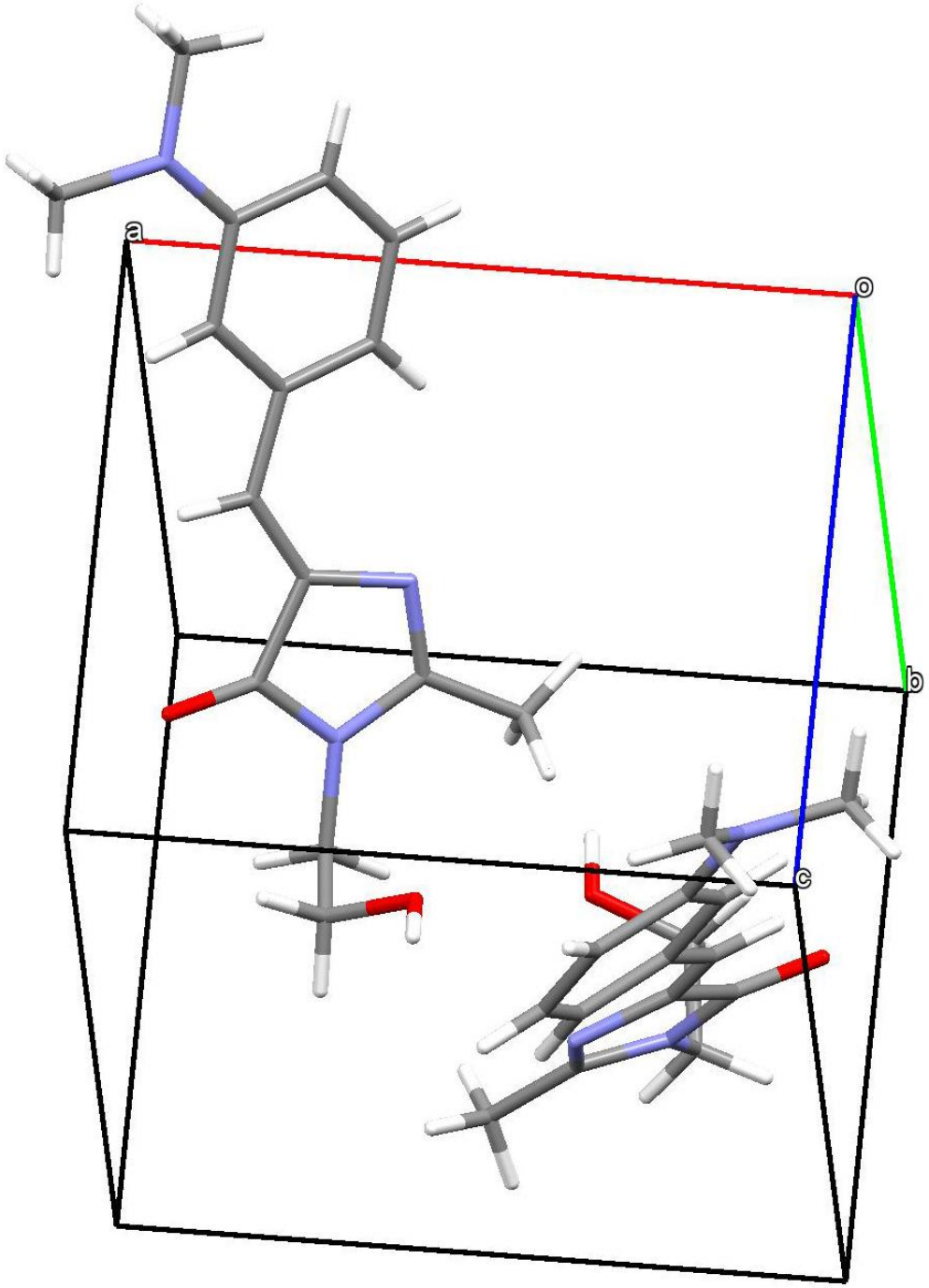

Figure S8. Unit cell packing of **2OH**.

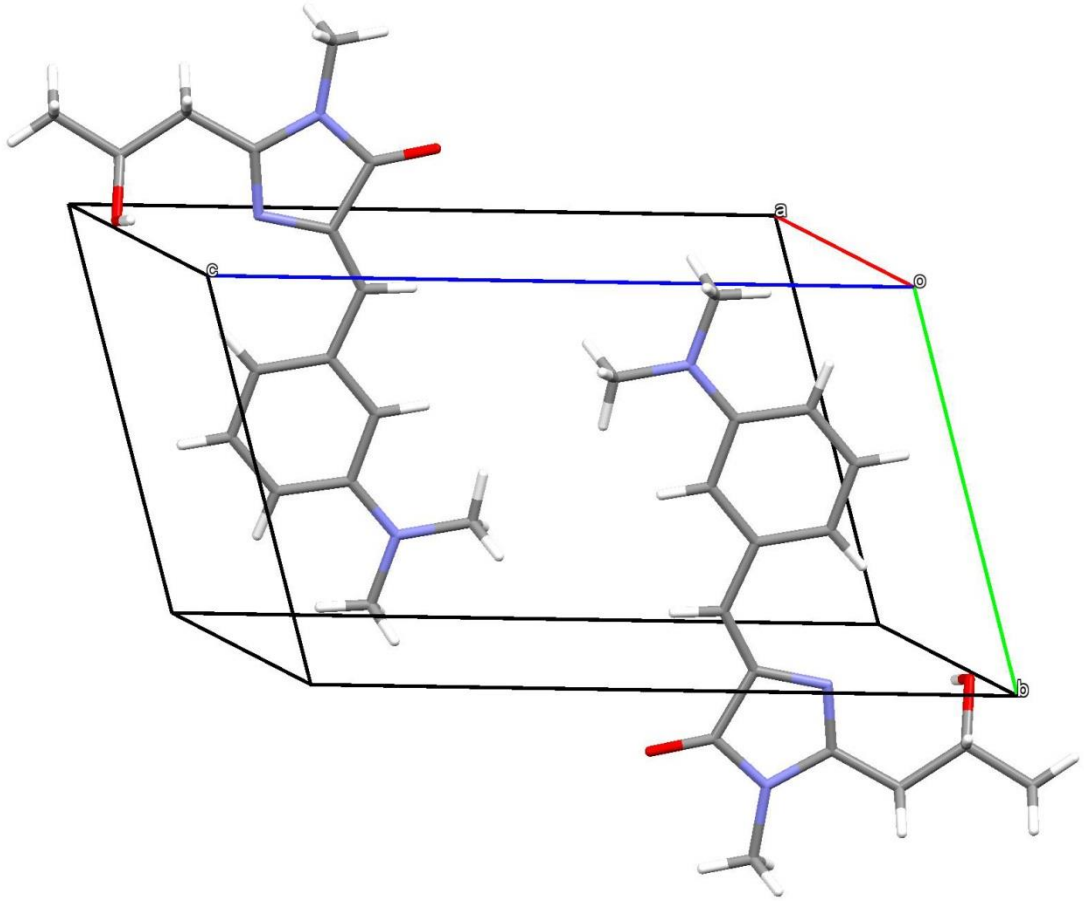

Figure S9. Unit cell packing of **3OH**.

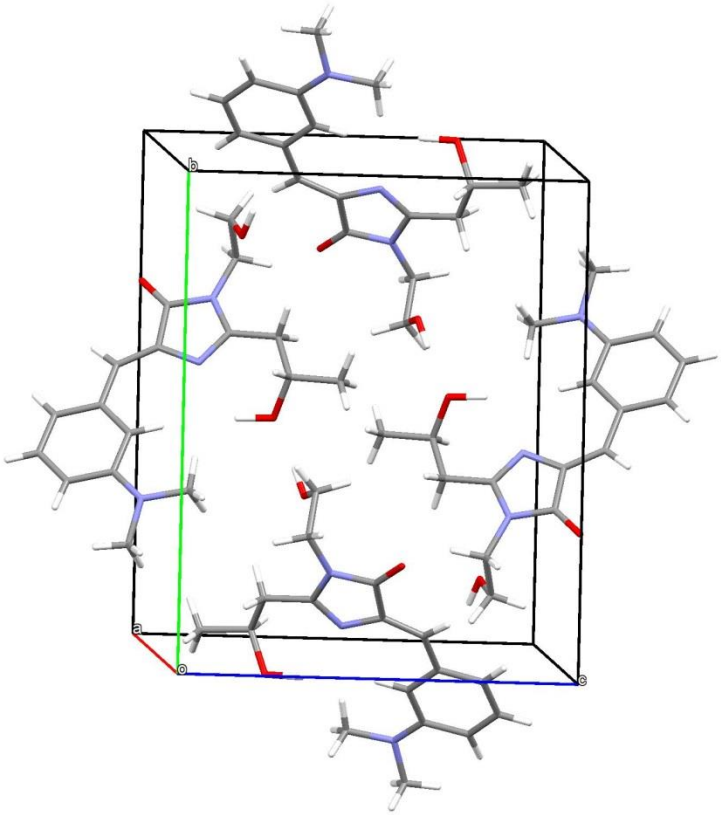

Figure S10. ^{1}H NMR spectra of **4** in $CDCl_3$.

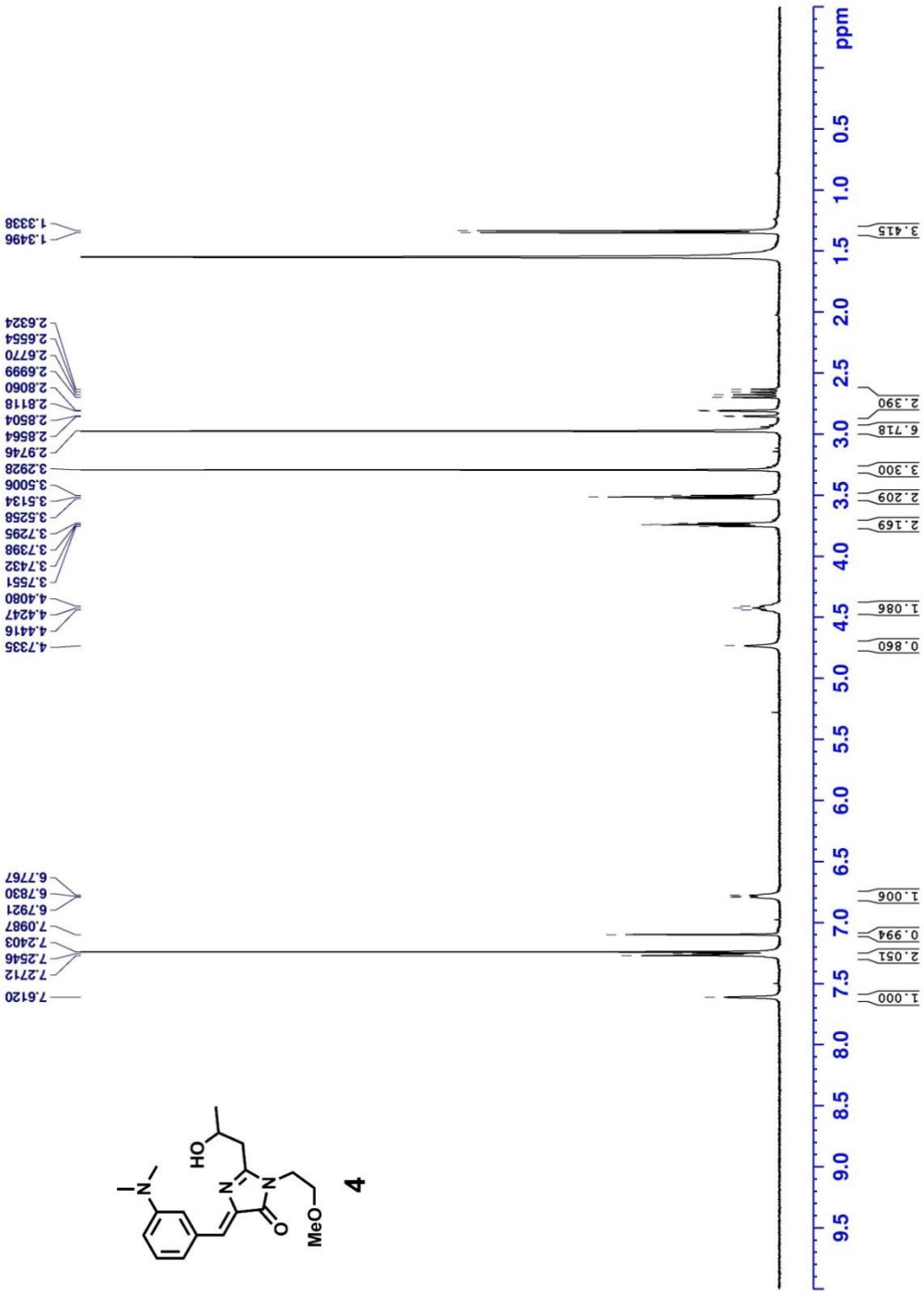

Figure S11. ^{13}C NMR spectra of **4** in $CDCl_3$.

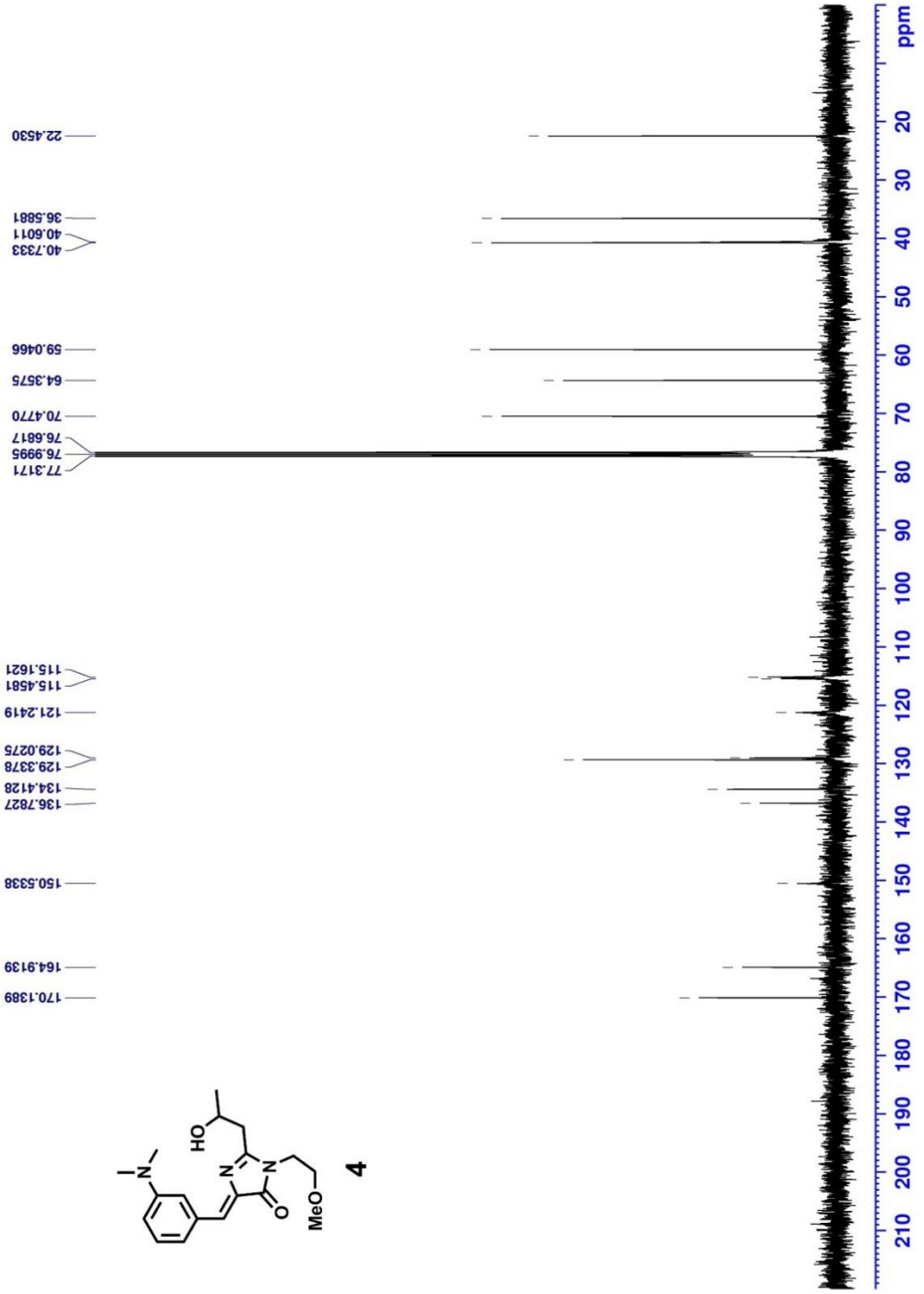

Figure S12. ^{1}H NMR spectra of **3OMe** in $CDCl_3$.

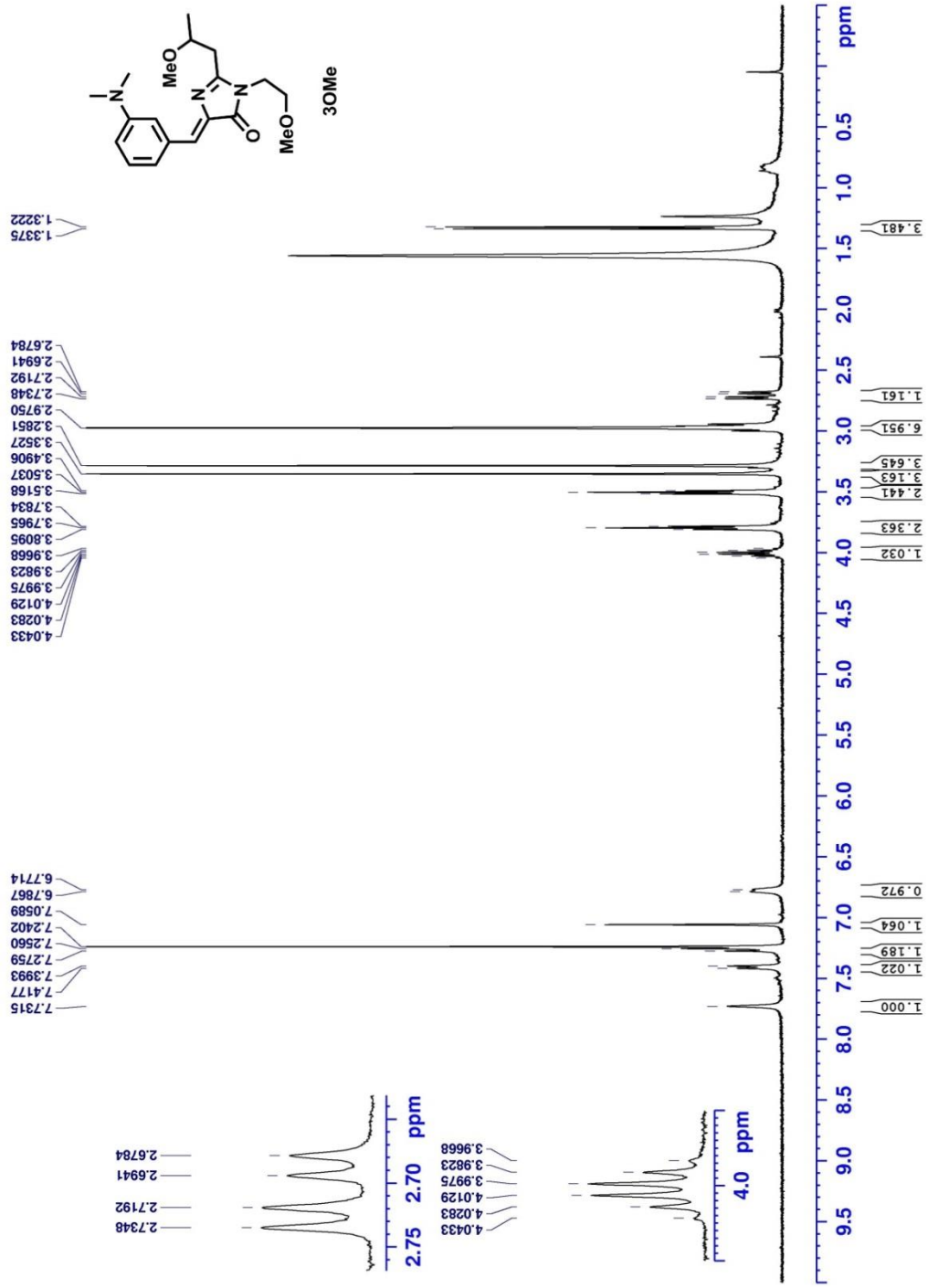

Figure S13. ^{13}C NMR spectra of **3OMe** in $CDCl_3$.

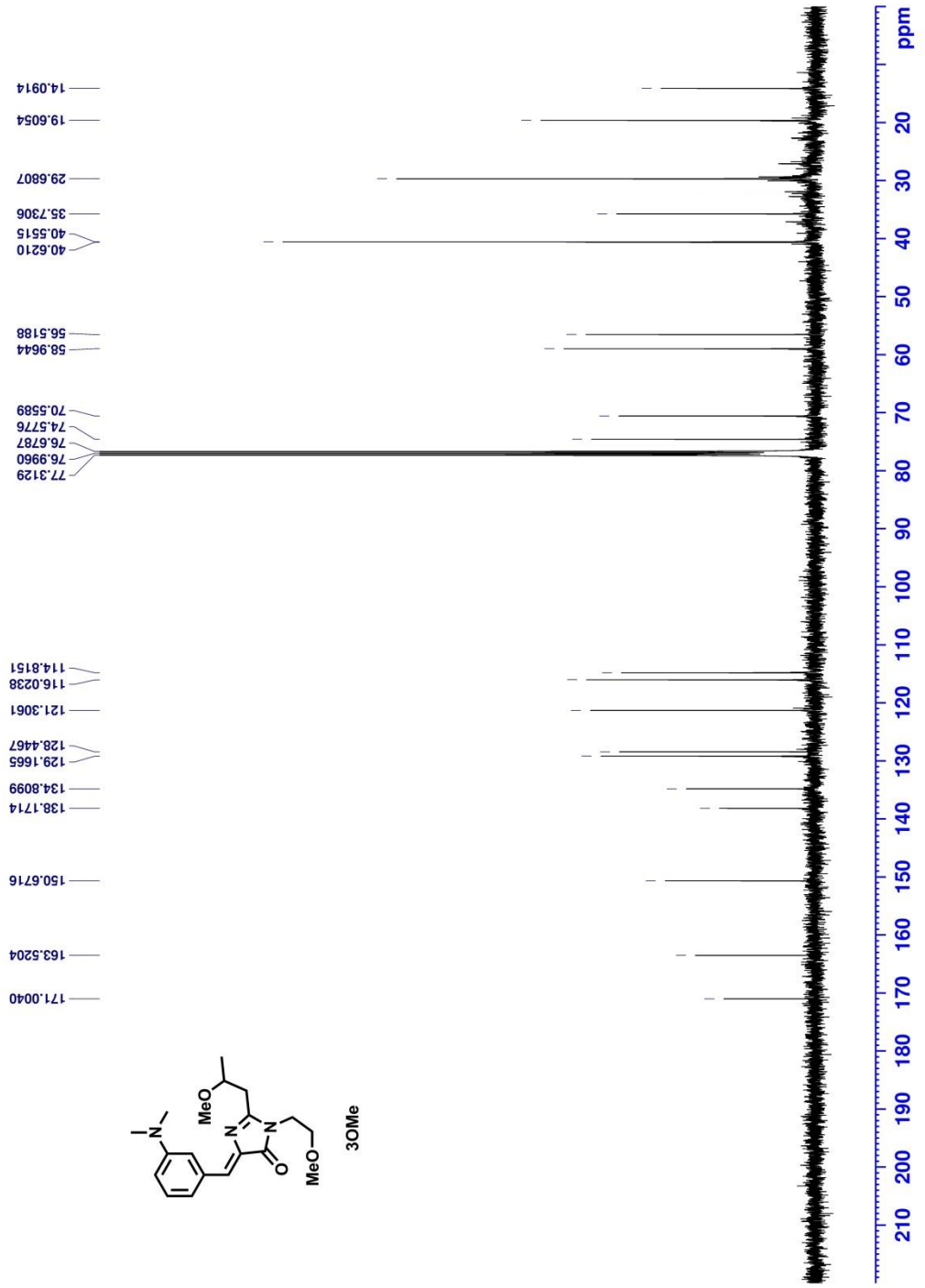

Figure S7. ^{1}H NMR spectra of **3OH** in DMSO-d_6.

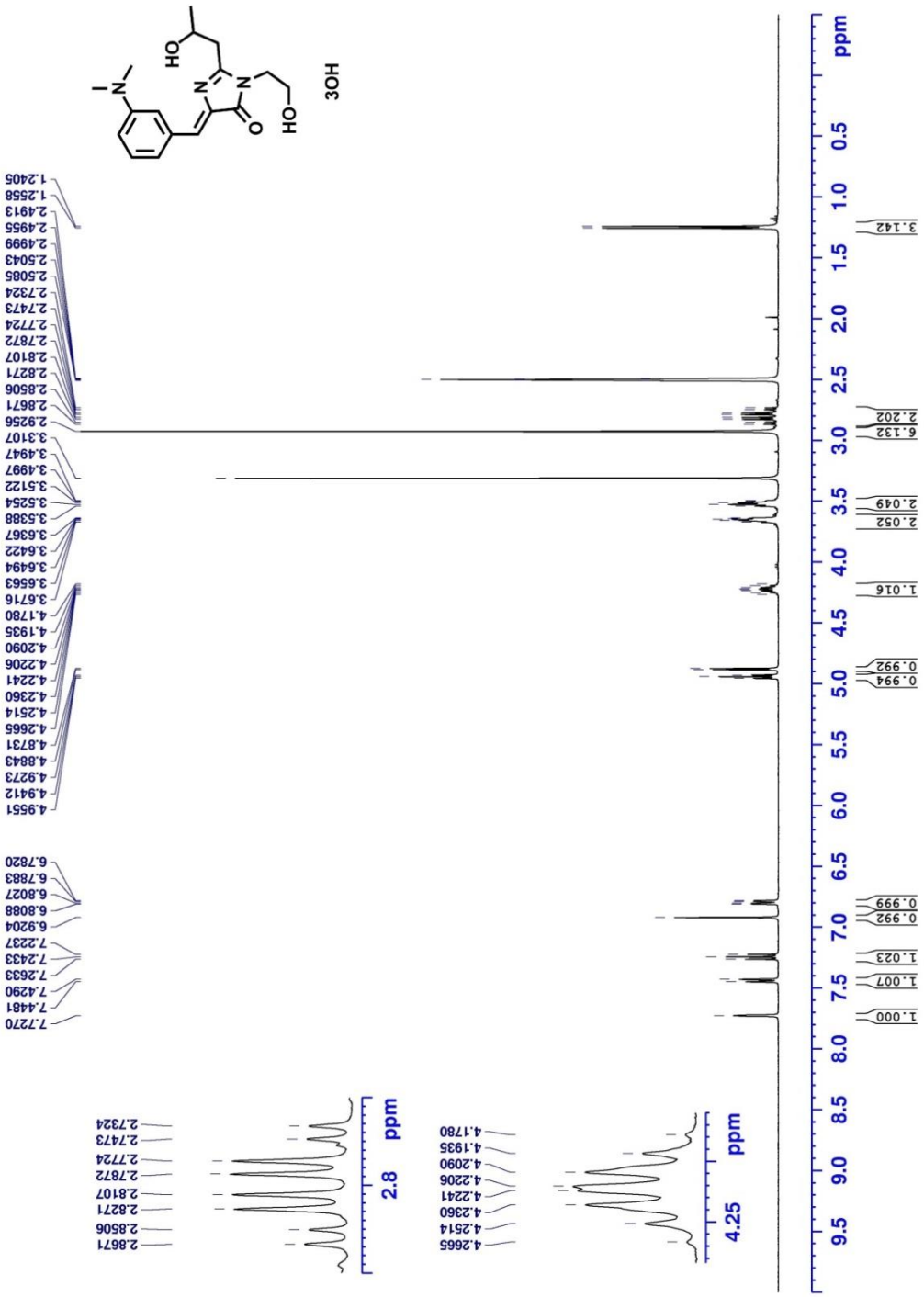

Figure S8. ^{13}C NMR spectra of **3OH** in DMSO-d_6.