Electronic Supplementary Material (ESI) for RSC Advances

Supporting Information for

A Selective Turn-On Fluorescence Strategy for the Detection of 5-Hydroxymethyl-2'-deoxycytidine

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Materials, methods and instruments

N, *N*-dimethylformamide (DMF), ammonium acetate, glacial acetic acid, paraformaldeyde, isopropanol were bought from SCRC (Shanghai, China). All the other compounds and reagents were purchased from Sigma-Aldrich.

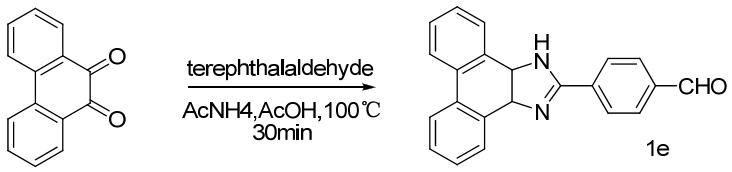
^{1}H and ^{13}C NMR spectra were recorded on Varian Mercury 300 and 400 spectrometers, respectively. HRMS were recorded on a Bruker Daltonics, Inc. APEXIII 7.0 TESLA FTMS and Varian ProMALDI. Fluorescence emission spectra were collected on PerkinElmer LS 55. UV-Vis absorption spectra were collected on SHIMADZU UV-2550. Quartz cuvettes with 400μL volume were used for emission measurements. Unless otherwise specified, all spectra were taken at room temperature. Fluorescence quantum yields were determined by standard methods, using quinine sulfate (Φ = 0.56) in 100mM H_2SO_4 as standard.

Visualization Assay

The concentrations of compounds **1e**, **1f**, **2e** and **2f** were 10μM in wate. The picture was taken under UV irradiation by a canon camera.

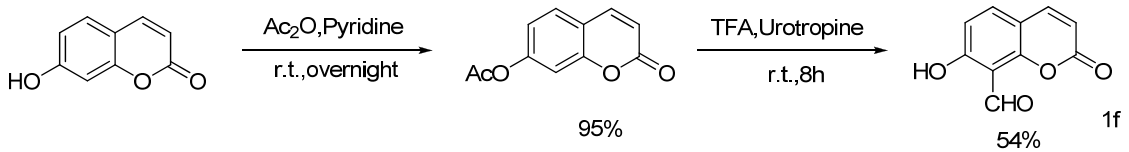
General procedure for the synthesis of hmC and Compounds 2a-2f

Compound **1e** was prepared by the literature method[1].



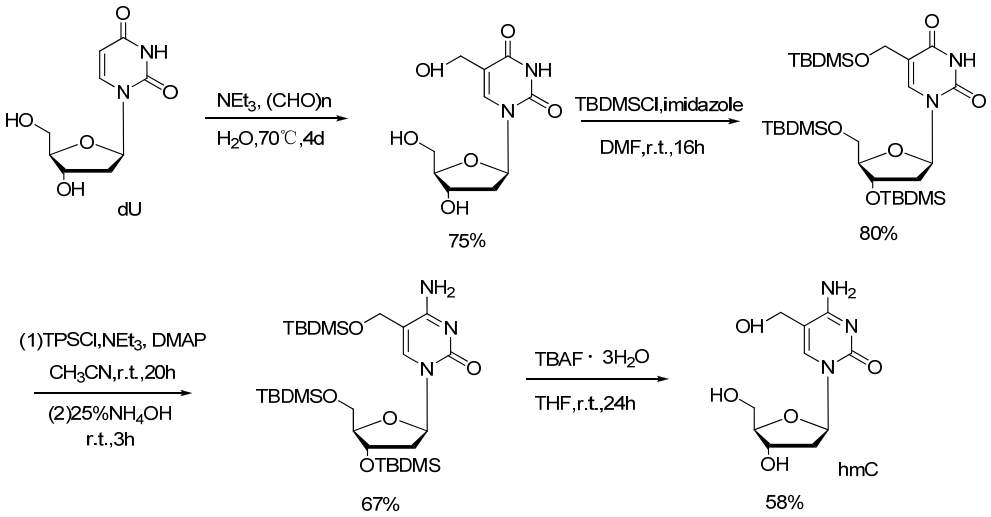
72%

Compound **1f** was prepared by the literature method[2].

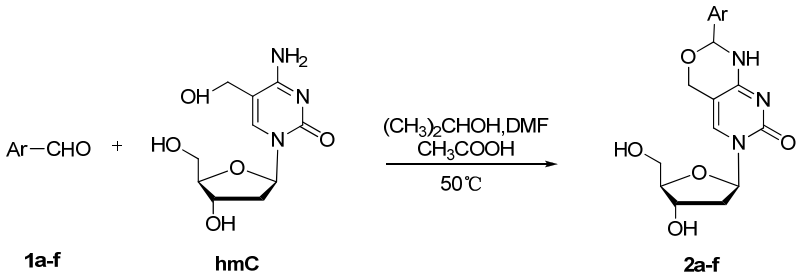


54%

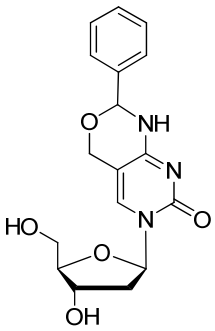
Compound **hmC** was prepared by the literature method[3].



Synthesis of other compounds is described below.



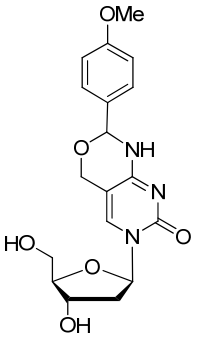
Compound 2a:



Benzaldehyde (compound **1a**, 212 mg, 2 mmol, 2 eq.) was added to a solution of **hmC** (257 mg, 1 mmol, 1eq.) in the mixture of isopropanol (2mL) and DMF (1mL). Then, a drop of acetic acid was added and the solution was stirred at 50ºC for 24 hours. Next, the mixture was concentrated *in vacuo* and purified by silica gel column chromatography (DCM/MeOH, 5:1, v/v) to afford the product as a white solid (120 mg, 0.35 mmol). Yield 35%.

^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) 7.73 (s, 1 H), 7.42(s, 5 H), 6.19 (br, 1 H), 5.89 (s, 1 H), 5.22 (s,1 H), 4.97 (br, 1 H), 4.62-4.50 (m, 2 H), 4.20 (s, 1 H), 3.77 (s, 1 H), 3.56 (s, 2 H), 2.11 (br, 1 H), 1.99 (br, 1 H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 160.4, 155.1, 138.7, 135.9, 129.7, 129.0, 127.7, 100.0, 87.8, 85.4, 85.3, 70.9, 61.9; HRMS (ESI) calcd for $C_{17}H_{19}N_3O_5$ $[M+H]^+$: 346.1406; found: 346.1398.

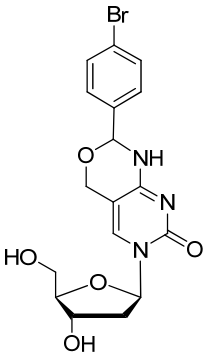
Compound 2b:



The preparative procedure was the same as for compound **2a** except that 4-anisaldehyde (compound **1b**) was the benzaldehyde derivative used. Yield 30%.

^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) 7.72 (s, 1 H), 7.34 (d, 2 H, *J*=8.1 Hz), 6.96 (d, 2 H, *J*=8.1 Hz), 6.19 (br, 1 H), 5.83 (s, 1 H), 5.22 (d,1 H, *J*=3 Hz), 4.97 (br, 1 H), 4.60-4.53 (m, 2 H), 4.20 (s, 1 H), 3.77 (s, 4 H), 3.55 (s, 2 H), 2.10 (br, 1 H), 1.98 (br, 1 H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 160.7, 160.6, 160.4, 155.2, 130.8, 129.1, 114.3, 100.0, 87.8, 70.9, 61.9, 55.7; HRMS (ESI) calcd for $C_{18}H_{21}N_3O_6$ $[M+H]^+$: 376.1515; found: 376.1503.

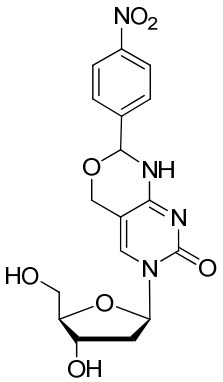
Compound 2c:



The preparative procedure was the same as for compound **2a** except that 4-bromobenzaldehyde (compound **1c**) was the benzaldehyde derivative used and reaction time was 12h. Yield 58%.

^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) 7.73 (s, 1 H), 7.62 (d, 2 H, *J*=6.6 Hz), 7.38 (d, 2 H, *J*=7.2 Hz), 6.18 (br, 1 H), 5.89 (s, 1 H), 5.23 (s,1 H), 4.99 (br, 1 H), 4.61-4.55 (m, 2 H), 4.20 (s, 1 H), 3.77 (s, 1 H), 3.55 (s, 2 H), 2.11 (br, 1 H), 1.98 (br, 1 H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 160.2, 155.0, 138.1, 135.9, 131.8, 129.9, 122.9, 100.0, 87.8, 85.4, 83.5, 79.7, 70.9, 63.2, 61.9; HRMS (ESI) calcd for $C_{17}H_{18}BrN_3O_5$ $[M+Na]^+$: 446.0320; found: 446.0322.

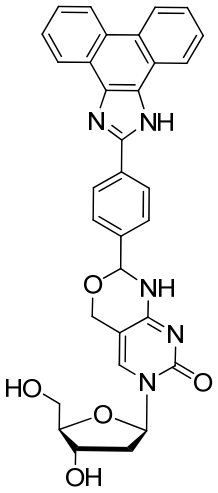
Compound 2d:



The preparative procedure was the same as for compound **2a** except that 4-nitrobenzaldehyde (compound **1d**) was the benzaldehyde derivative used and reaction time was 12h. Yield 62%.

^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) 8.28 (d, 2 H, *J*=7.2 Hz), 7.73-7.71 (m, 3 H), 6.18 (br, 1 H), 6.06 (s, 1 H), 5.23 (s,1 H), 4.98 (br, 1 H), 4.63-4.57 (m, 2 H), 4.21 (s, 1 H), 3.84-3.77 (m, 1 H), 3.56 (br, 2 H), 1.99 (br, 1 H), 1.97 (br, 1 H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 160.2, 154.7, 148.4, 145.8, 130.0, 128.4, 125.0, 123.3, 100.0, 88.0, 87.2, 84.5, 70.9, 63.2, 61.9; HRMS (ESI) calcd for $C_{17}H_{18}N_4O_7$ $[M+H]^+$: 391.1253; found: 391.1248.

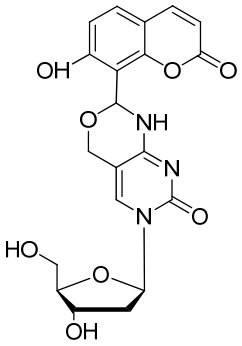
Compound 2e:



The preparative procedure was the same as for compound **2a** except that compound **1e** was the benzaldehyde derivative used. Yield 65%.

^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) 8.87 (t, 2 H, *J*=9 Hz), 8.58 (t, 2 H, *J*=7.5 Hz), 8.37 (d, 2 H, *J*=8.1 Hz), 7.77-7.66 (m, 7 H), 6.22 (br, 1 H), 6.01 (s, 1 H), 5.24 (d, 1 H, *J*=3.9 Hz), 5.01 (br, 1 H), 4.69-4.64 (m, 2 H), 4.22 (s, 1 H), 3.78 (s, 1 H), 3.57 (br, 2 H), 2.12 (br, 1 H), 2.02 (br, 1 H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 160.2, 153.0, 149.0, 131.8, 128.5, 127.8, 126.8, 122.7, 88.0, 85.6, 71.1, 63.5, 62.1; HRMS (ESI) calcd for $C_{32}H_{27}N_5O_5$ $[M-H]^-$: 560.1934; found: 560.1942.

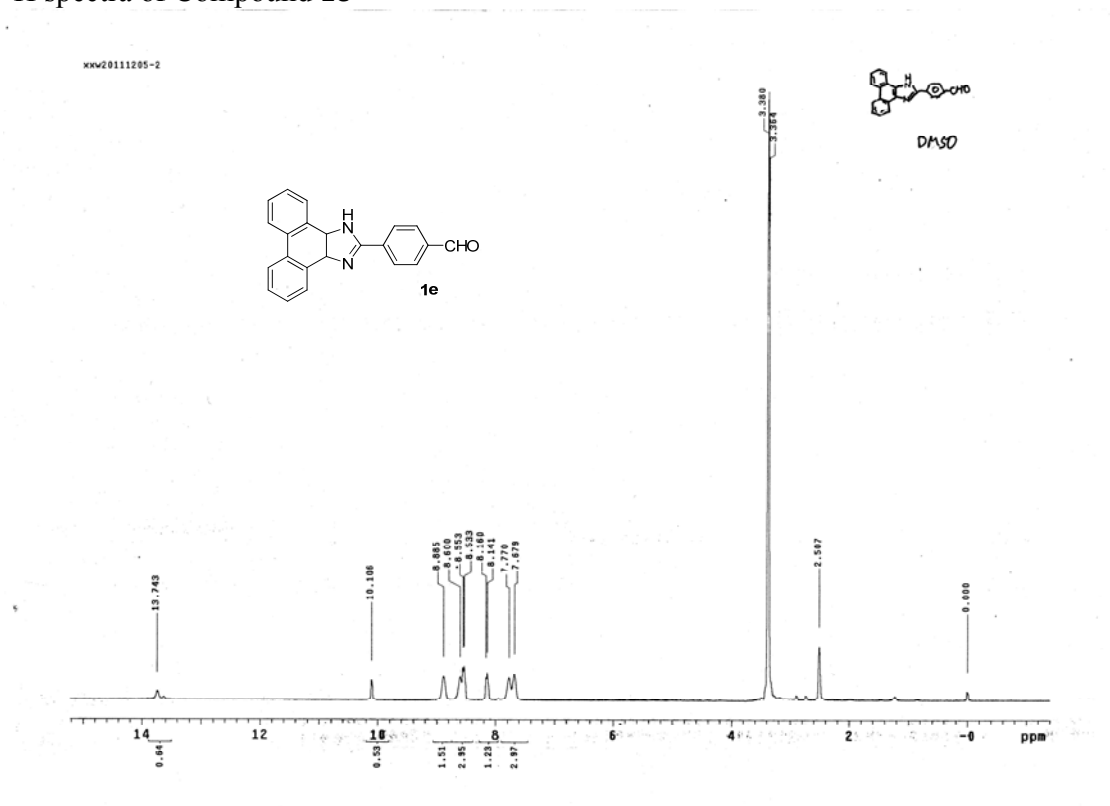
Compound 2f:



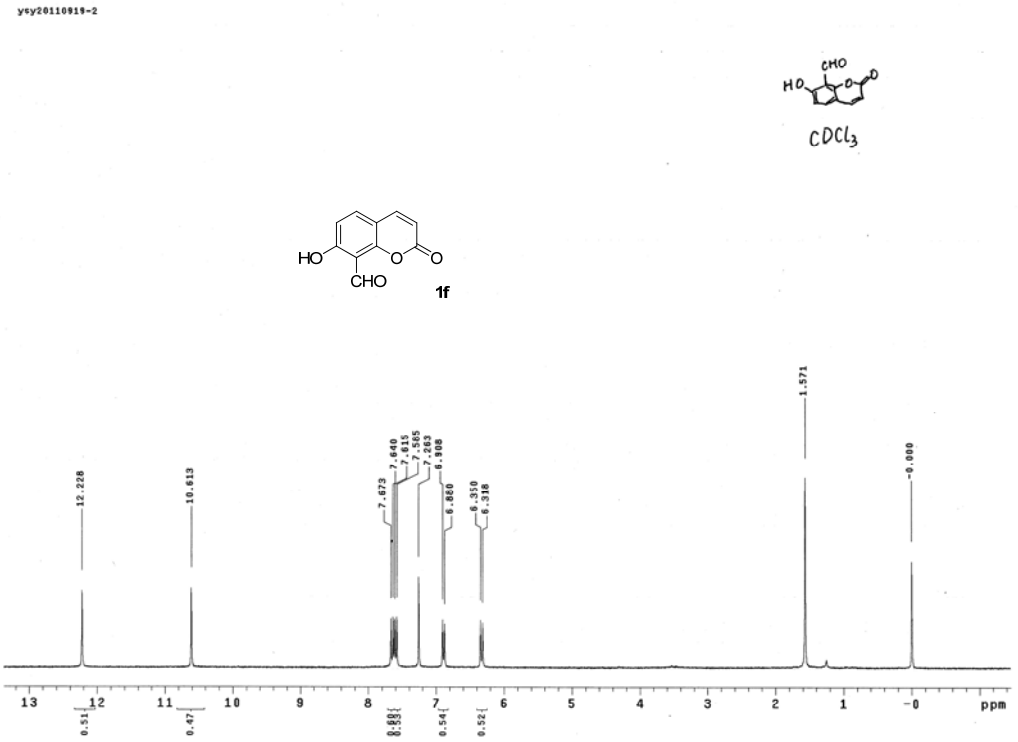
The preparative procedure was the same as for compound **2a** except that compound **1f** was the benzaldehyde derivative used. Yield 75%.

^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) 7.95 (br, 1 H), 7.70 (s, 1 H), 7.56 (br, 1 H), 6.86 (br, 1 H), 6.35 (d, 1 H, *J*=4.2 Hz), 6.23 (br, 2 H), 5.24 (s, 1 H), 5.01 (br, 1 H), 4.67 (s, 2 H), 4.21 (s, 1 H), 3.76 (s, 1 H), 3.56 (br, 2 H), 2.09 (br, 1 H), 2.00 (br, 1 H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 160.5, 154.8, 145.4, 131.0, 113.9, 112.1, 111.9, 111.1, 87.9, 85.4, 79.9, 71.2, 64.9, 62.1; HRMS (ESI) calcd for $C_{20}H_{19}N_3O_8$ $[M+Na]^+$: 452.1070; found: 452.1059.

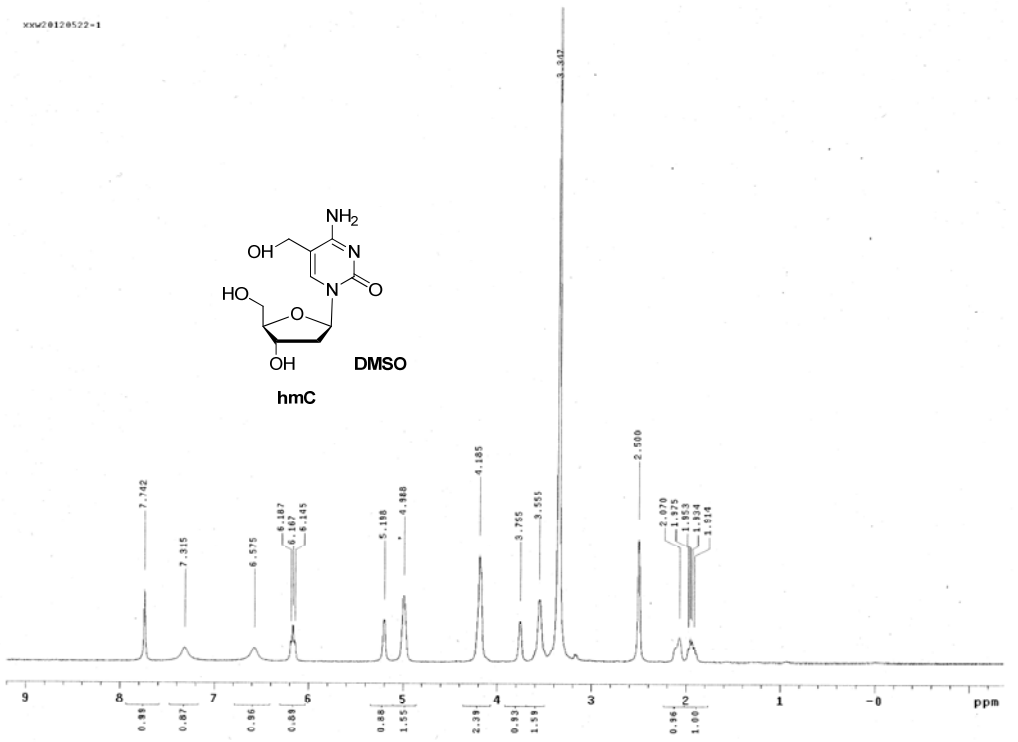
^{1}H spectra of Compound **1e**



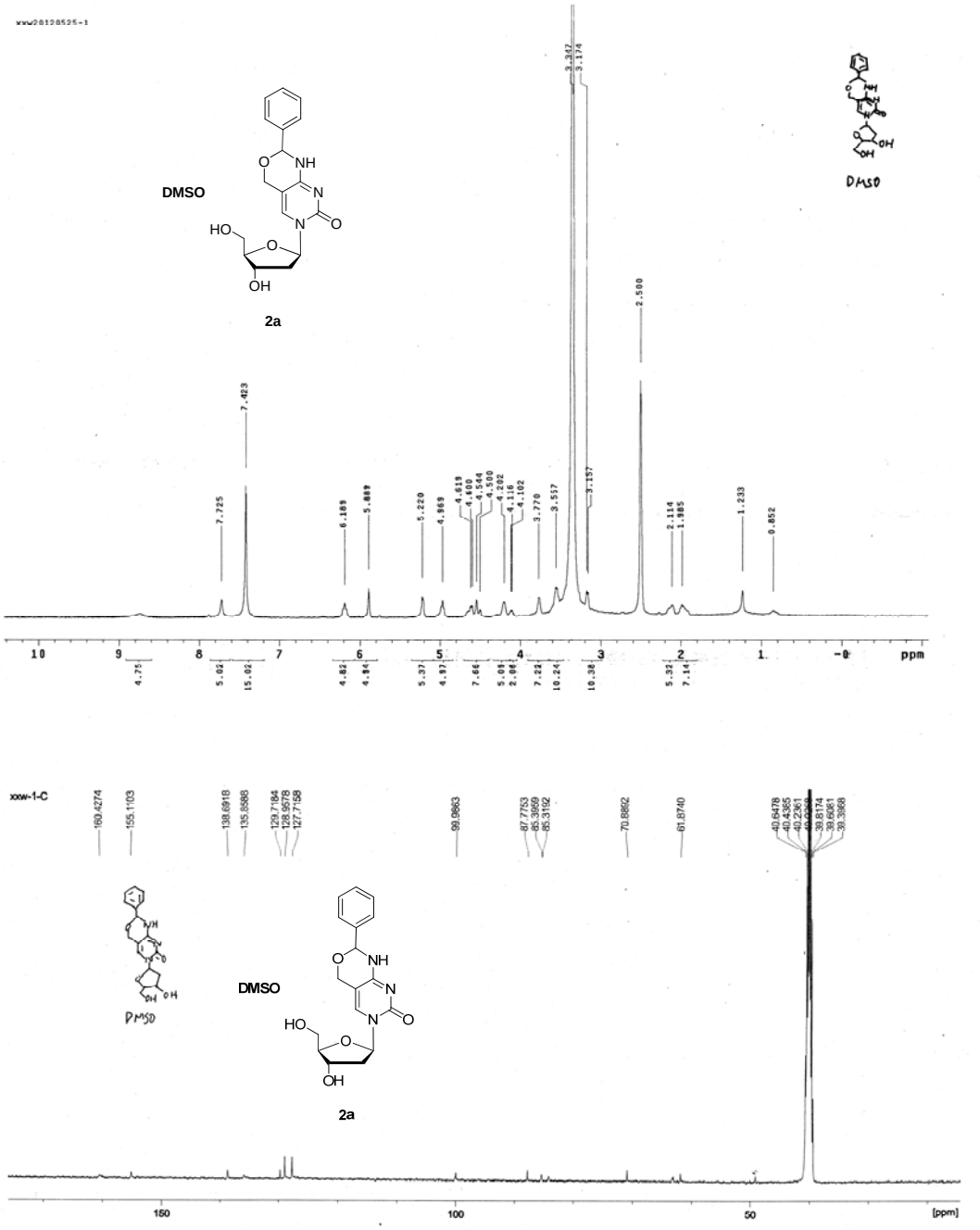
^{1}H spectra of Compound **1f**



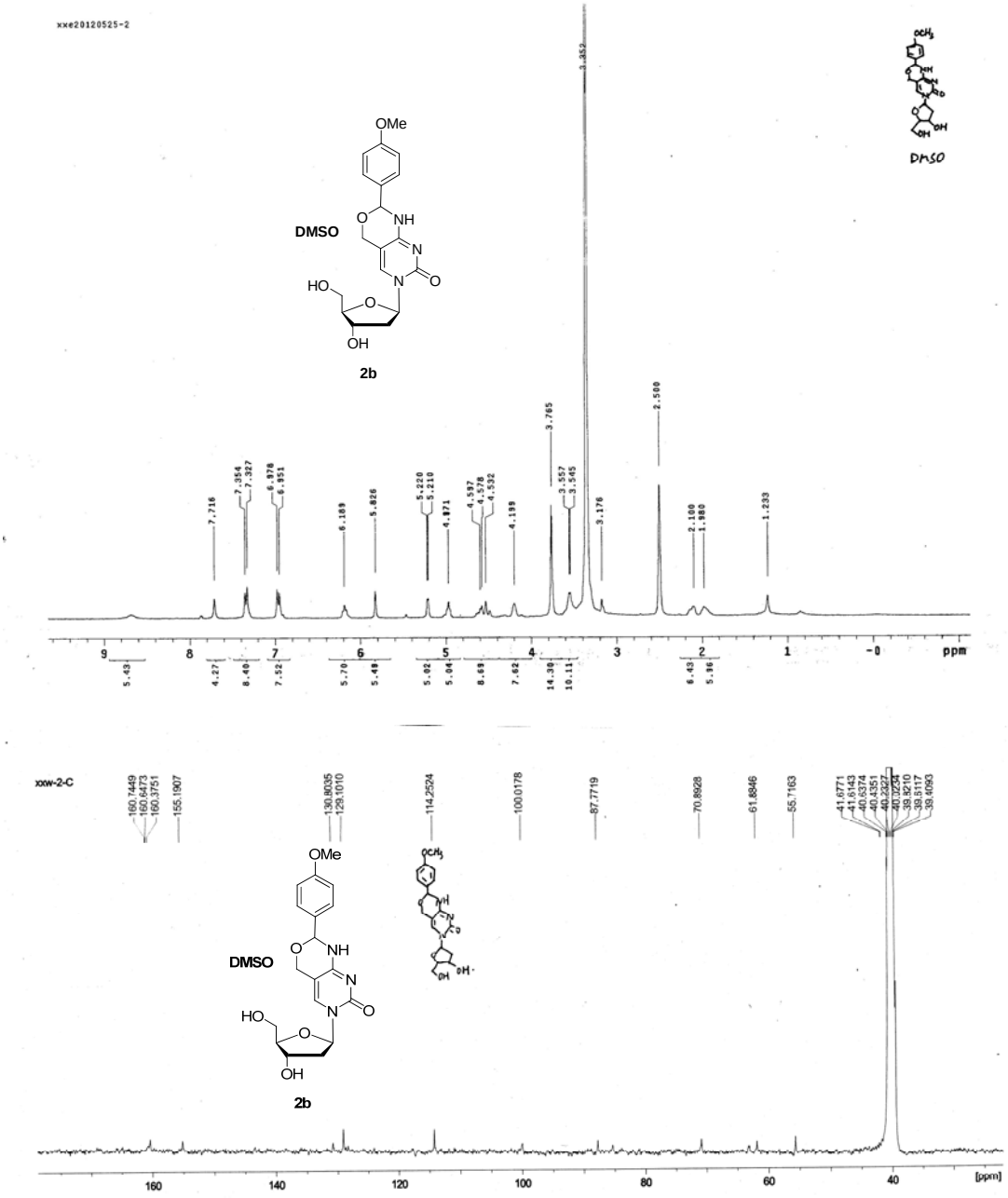
^{1}H spectra of Compound **hmC**



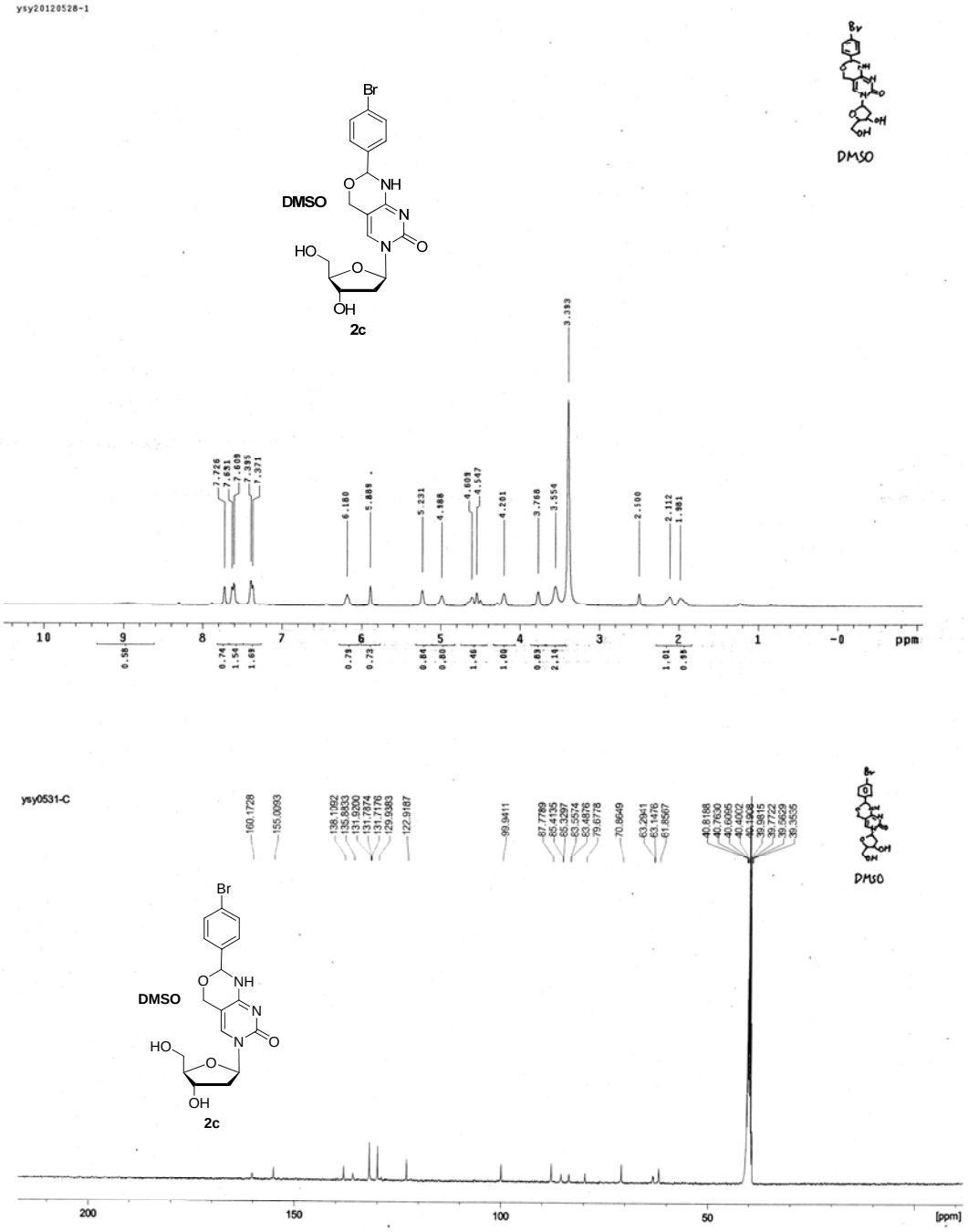
^{1}H and ^{13}C NMR spectra of Compound **2a**



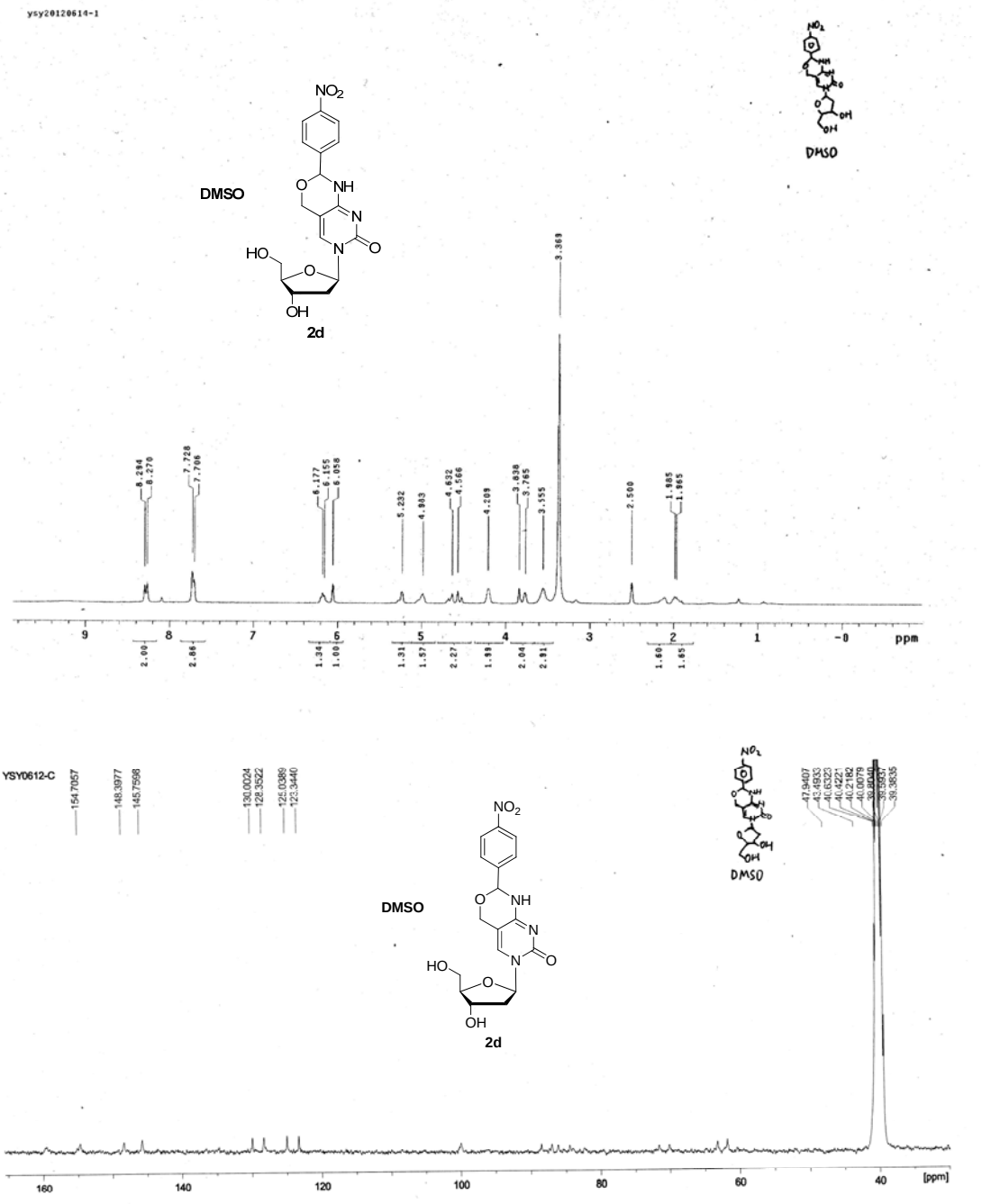
^{1}H and ^{13}C NMR spectra of Compound **2b**



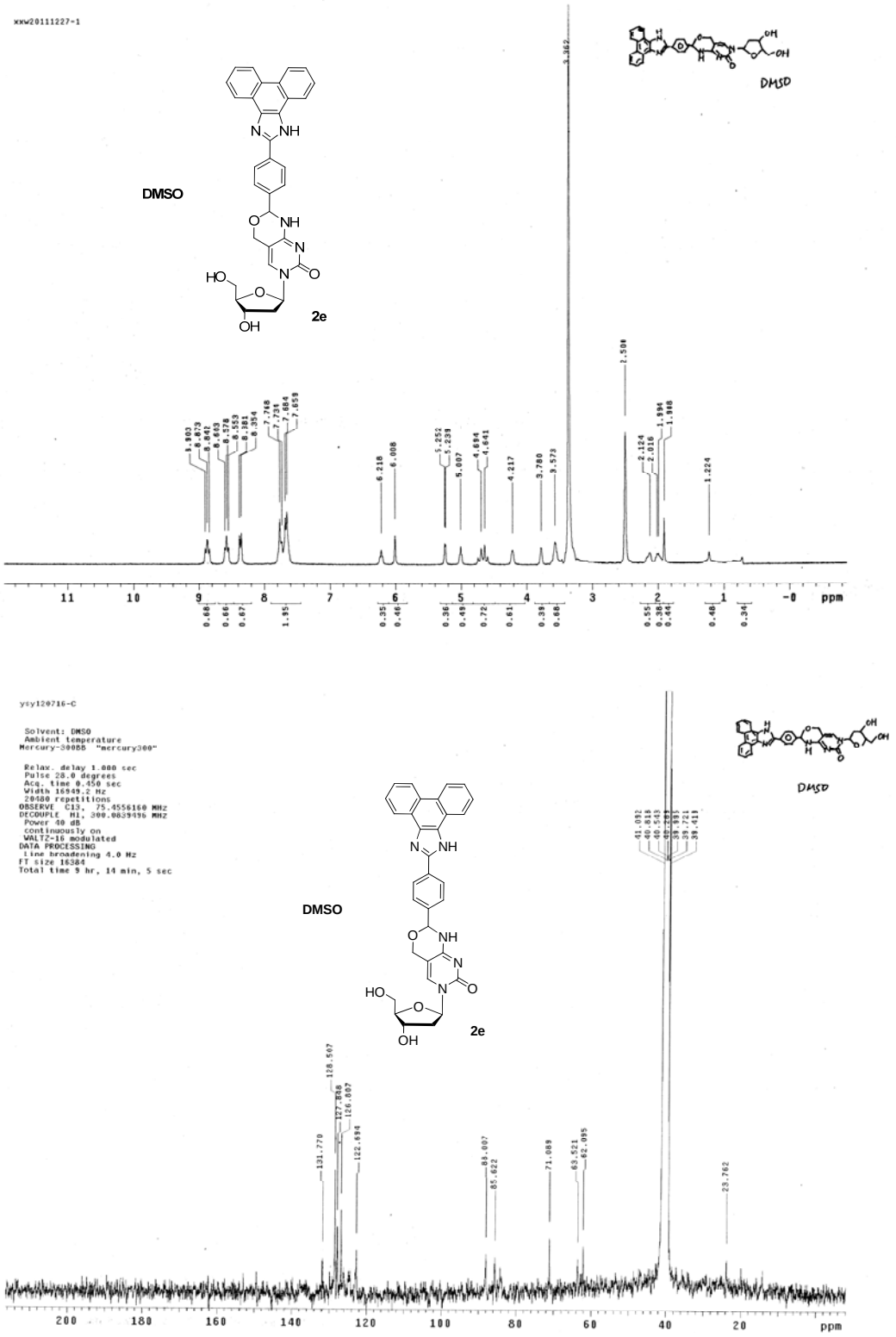
^{1}H and ^{13}C NMR spectra of Compound **2c**



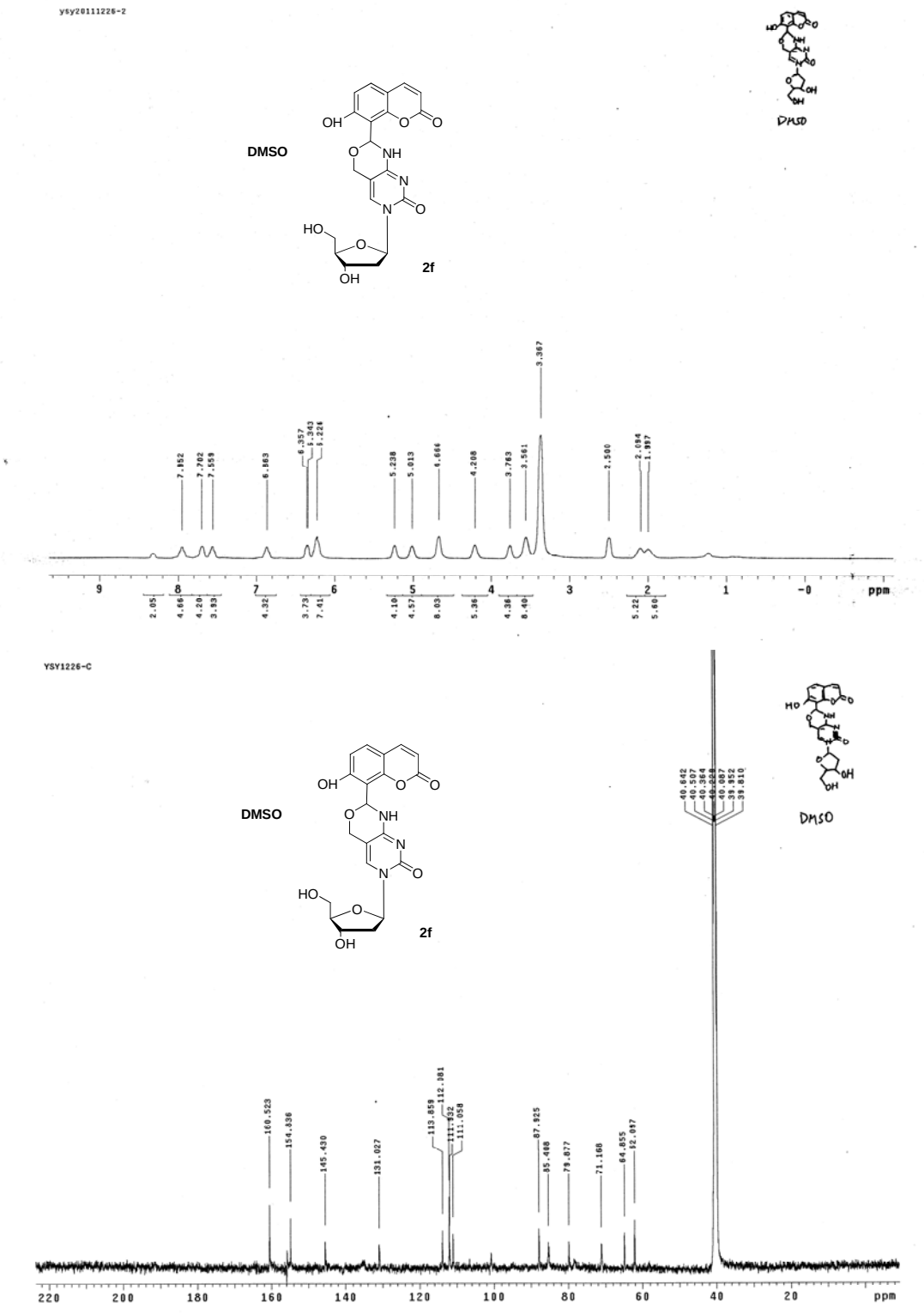
^{1}H and ^{13}C NMR spectra of Compound **2d**



^{1}H and ^{13}C NMR spectra of Compound **2e**



^{1}H and ^{13}C NMR spectra of Compound **2f**



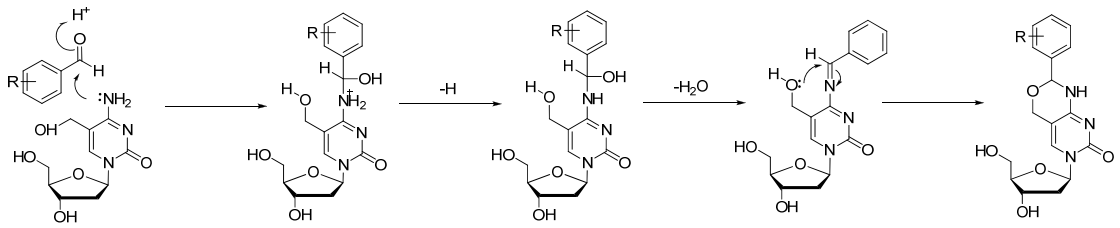


Fig. S1 The putative mechanism for the formation of heterocyclic nucleosides.

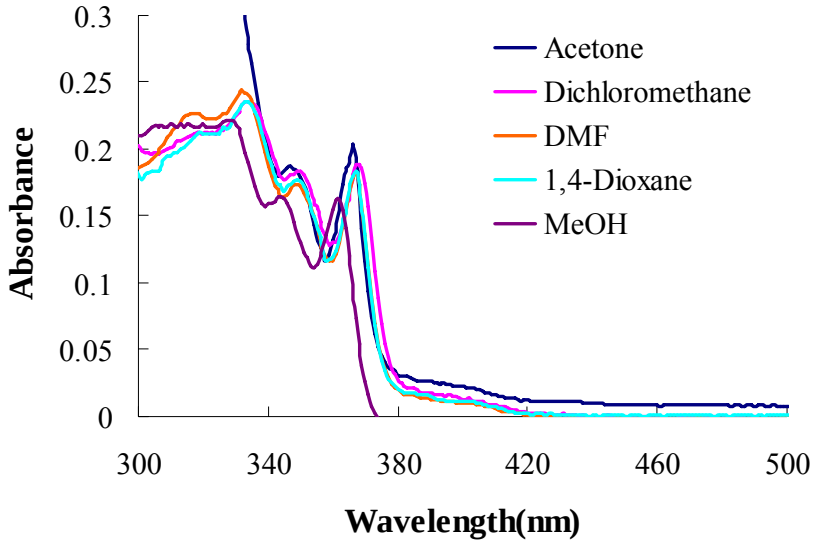


Fig. S2 UV-Vis absorption spectra of compound **2e** in organic solvents.

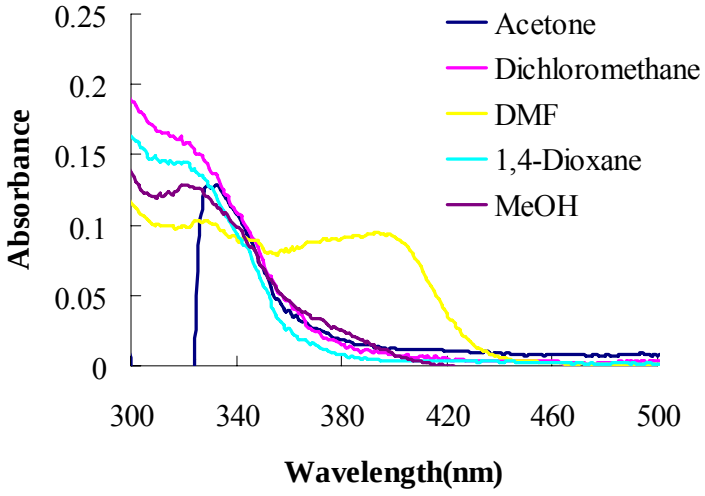


Fig. S3 UV-Vis absorption spectra of compound **2f** in organic solvents.

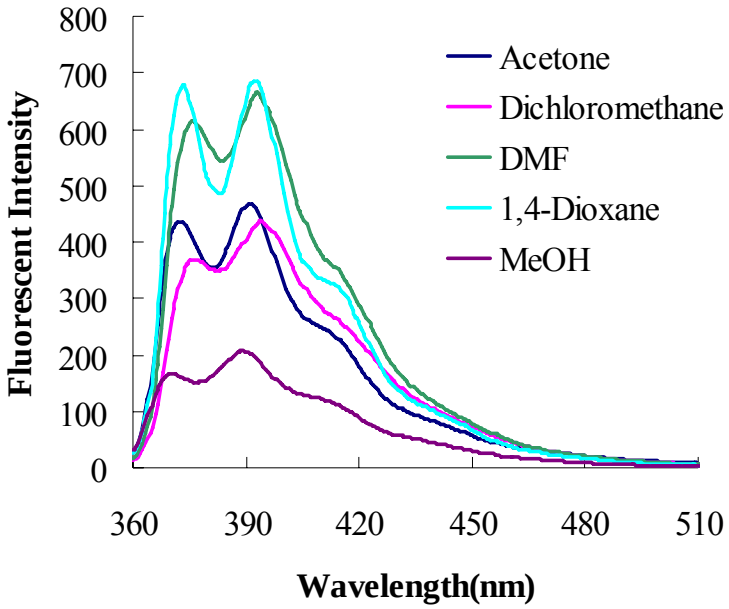


Fig. S4 Fluorescence emission spectra of compound **2e** in organic solvents (λ_{ex}=325nm).

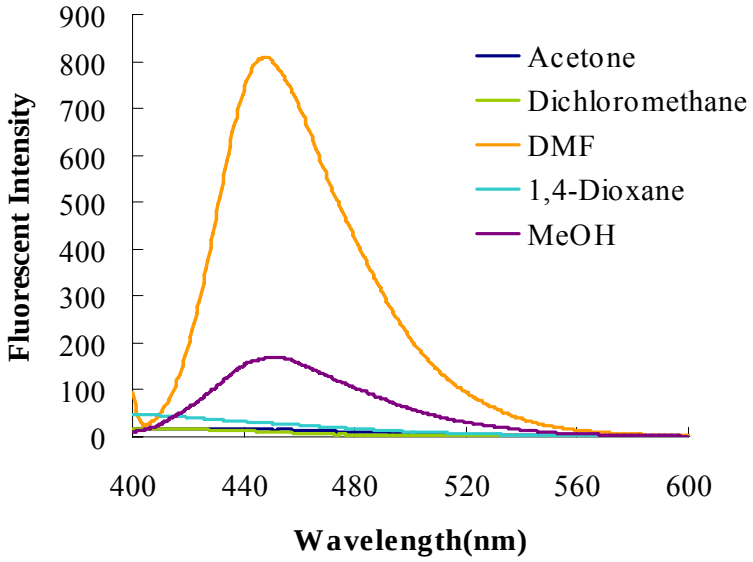


Fig. S5 Fluorescence emission spectra of compound **2f** in organic solvents (λ_{ex}=325nm).

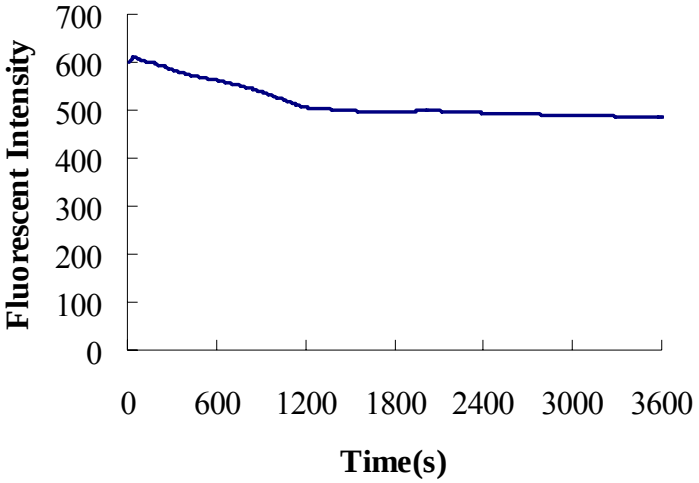


Fig. S6 Time course of the fluorescence response at 404nm of compound **2e** (λ_{ex}=325nm).

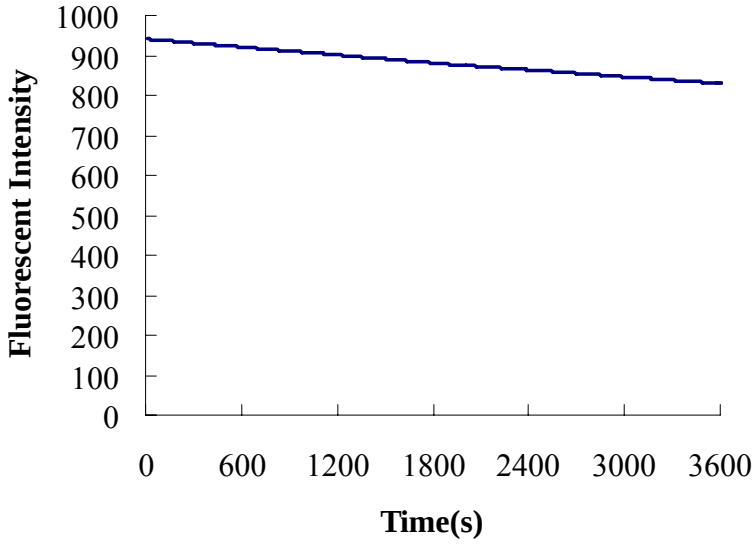


Fig. S7 Time course of the fluorescence response at 448nm of compound **2f** (λ_{ex}=325nm).

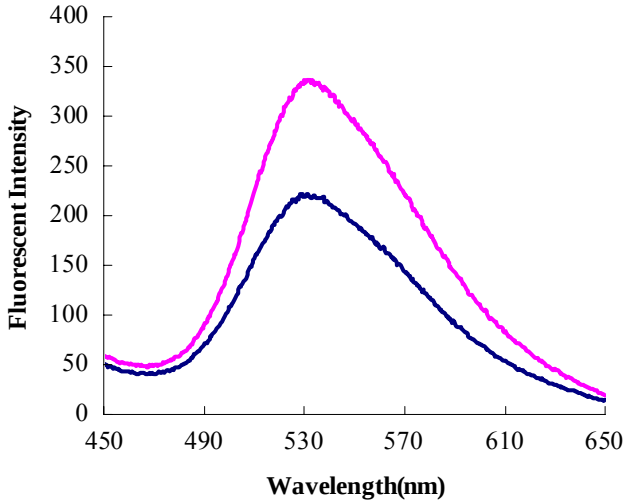


Fig. S8 Fluorescence emission spectra of compound **1e** (100μM) in the absence (red curve) and presence (blue curve) of the **hmC** (100μM) in the aqueous buffer (10mM CH_3COONH_4, pH=4.5) (λ_{ex}=325nm).

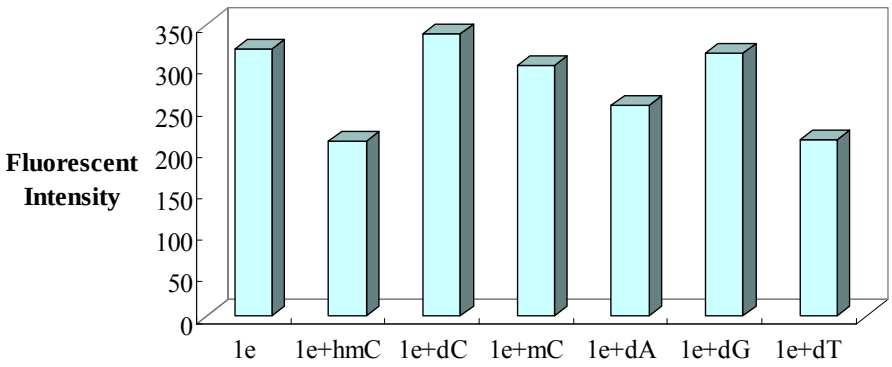
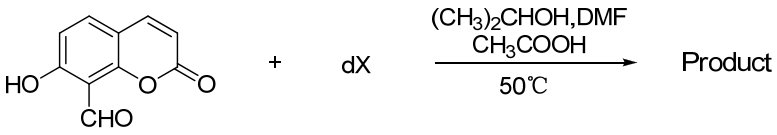


Fig. S9 Selectivity studies: compound **1e** alone, **1e**+**hmC**, **1e**+**dC**, **1e**+**mC**, **1e**+**dA**, **1e**+**dG**, **1e**+**dT**. All concentrations are 100μM (λ_{ex}=325nm).

Table S1 The reactions between compound **1f** and nucleotides



Entry	dX	Product[a]	Yield(%)[b]
1	hmC	Compound 2f	75
2	dC	NR	–
3	mC	NR	–
4	dA	NR	–
5	dG	NR	–
6	dT	NR	–
7	mixture[c]	Compound 2f	68

[a] The reactions were monitored by TLC. [b] Yields of isolated products.
[c] The mixture of **hmC**, **dC**, **mC**, **dA**, **dG** and **dT**.

References:

1 W. Lin, L. Long, L. Yuan, Z. Cao, B. Chen and W. Tan, *Org. Lett.*, 2008, **10**, 5577.

2 K. S. Lee, T. K. Kim, J. H. Lee, H. J. Kim and J. I. Hong, *Chem. Commun.*, 2008, **14**, 6173.

3 Y. E. Safadi, J. C. Paillart, G. Laumond, A. M. Aubertin, A. Burger, R. Marquet and V. Vivet-Boudou, *J. Med. Chem.*, 2010, **53**, 1534.