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Supplementary Information

Synthesis and spectral characterization of environmentally responsive fluorescent deoxycytidine analogs

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Table of Contents

General Remarks S3

^{1}H and ^{13}C NMR spectra S5

Fluorescence spectra.....S20

Fluorescence spectra used for polarity sensitivity measurements.....S21

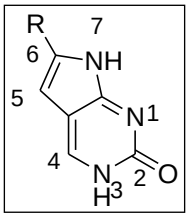
Determination of fluorescence quantum yields and molar extinction coefficients.....S22

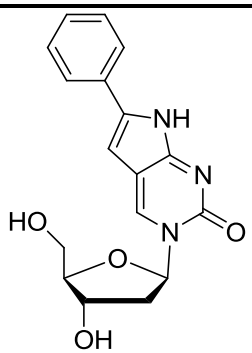
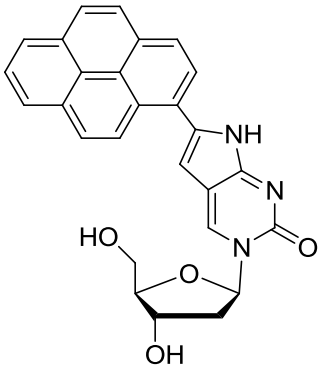
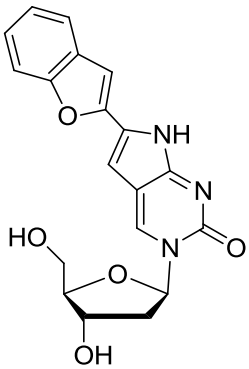
Determination of polarity sensitivities S23

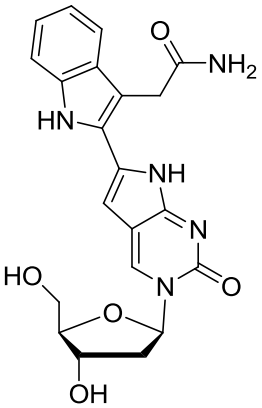
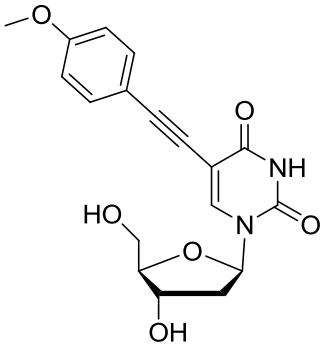
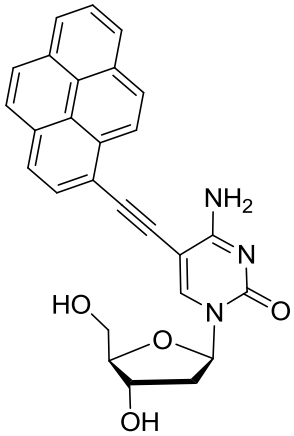
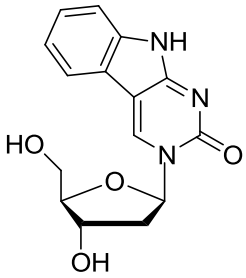
Determination of response to viscosity changes S27

General Remark

The compound 3*H*-pyrrolo[2,3-d]pyrimidin-2(7H)-one is abbreviated as pyrrolocytosine and the corresponding deoxynucleoside as pyrrolodeoxycytidine.

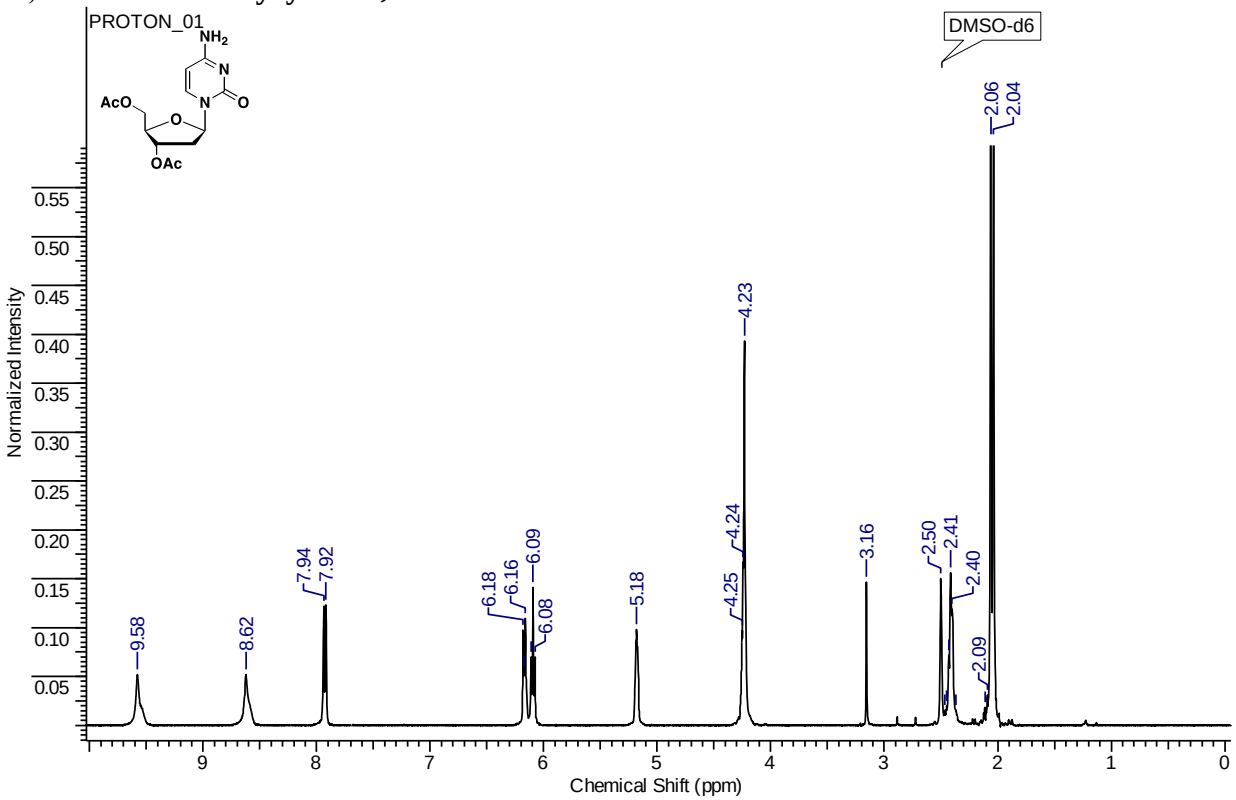


Compound Number	Structure	Trivial Abbreviation
1b		**PhpC**
1c		**PypC**
1d		**BFpC**

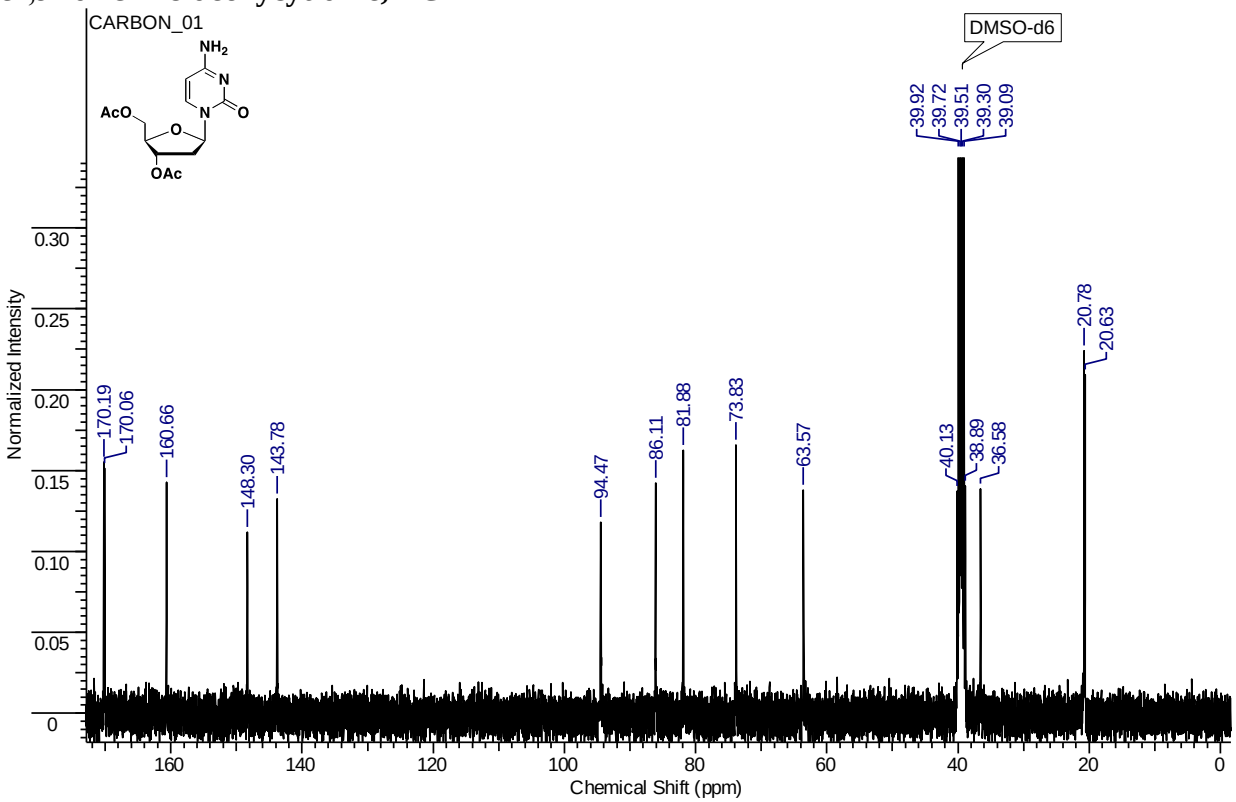
1e		**IndolylpC**
2a		***p*-MeOPhEtdU**
2b		**PyEtdC**
3a		**benzopC**

^{1}H AND ^{13}C NMR Spectra

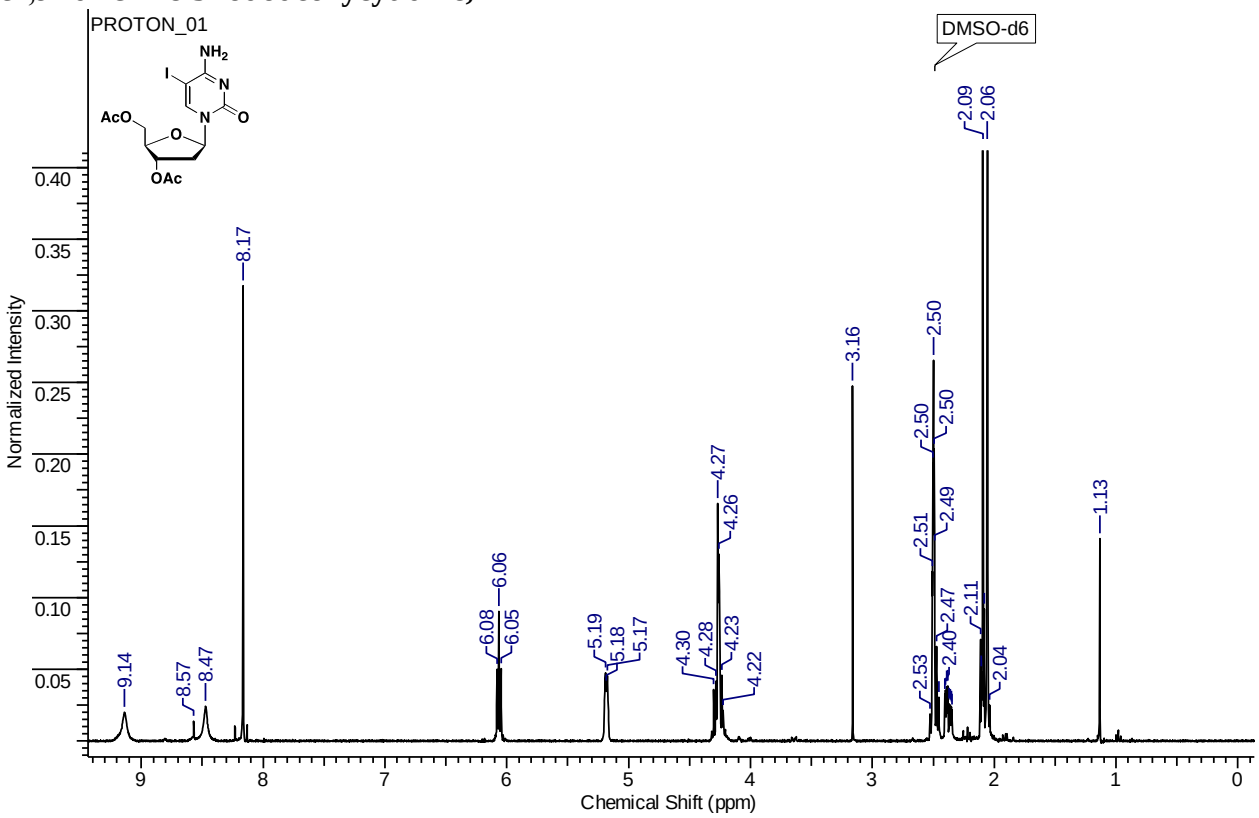
5',3'-di-*O*-Ac-deoxycytidine, ^{1}H NMR



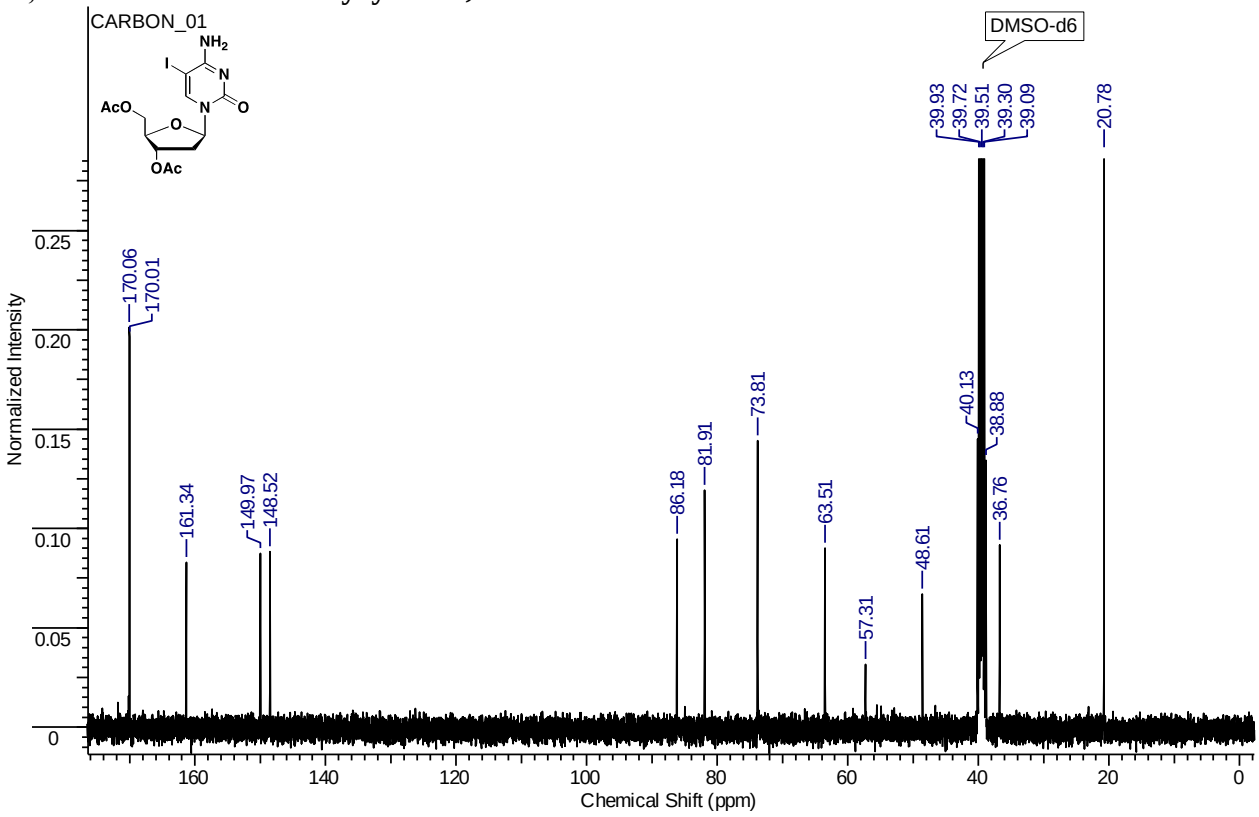
5',3'-di-*O*-Ac-deoxycytidine, ^{13}C NMR



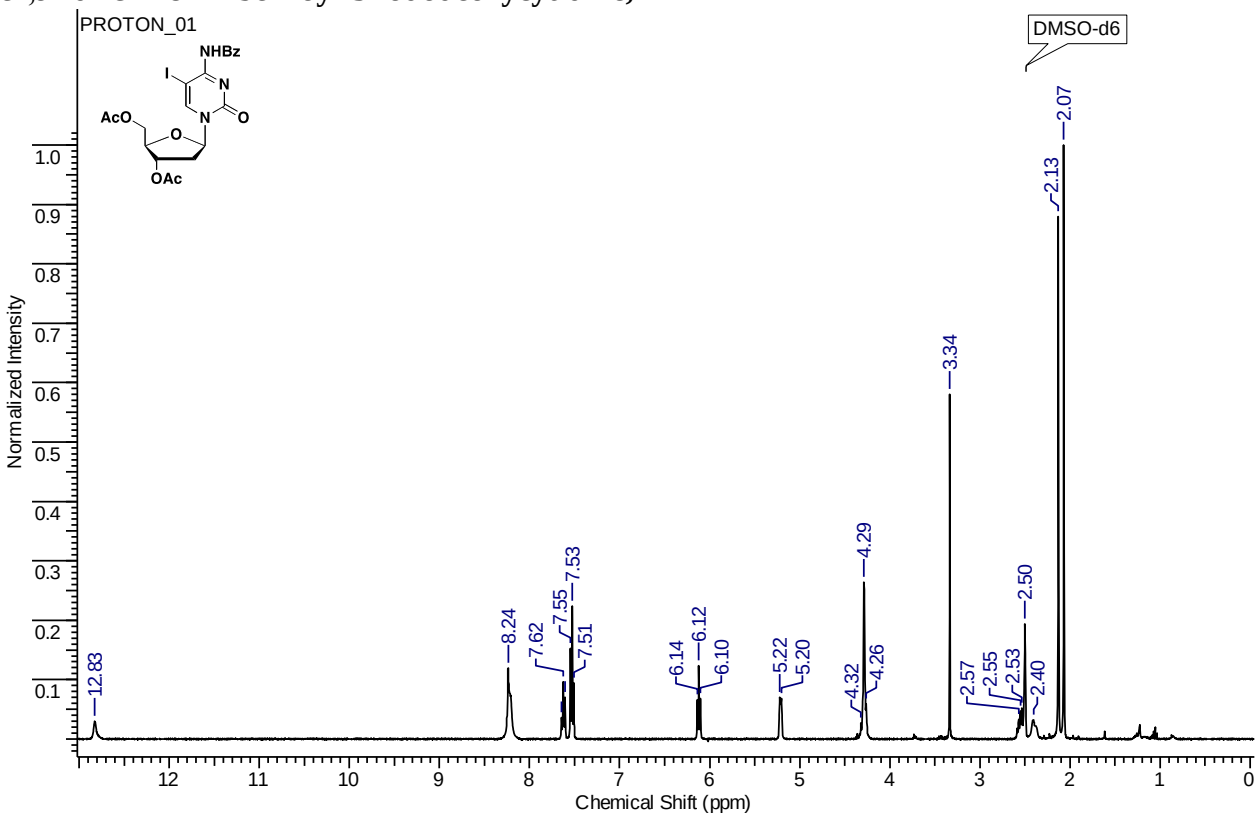
5',3'-di-*O*-Ac-5-iododeoxycytidine, ^{1}H NMR



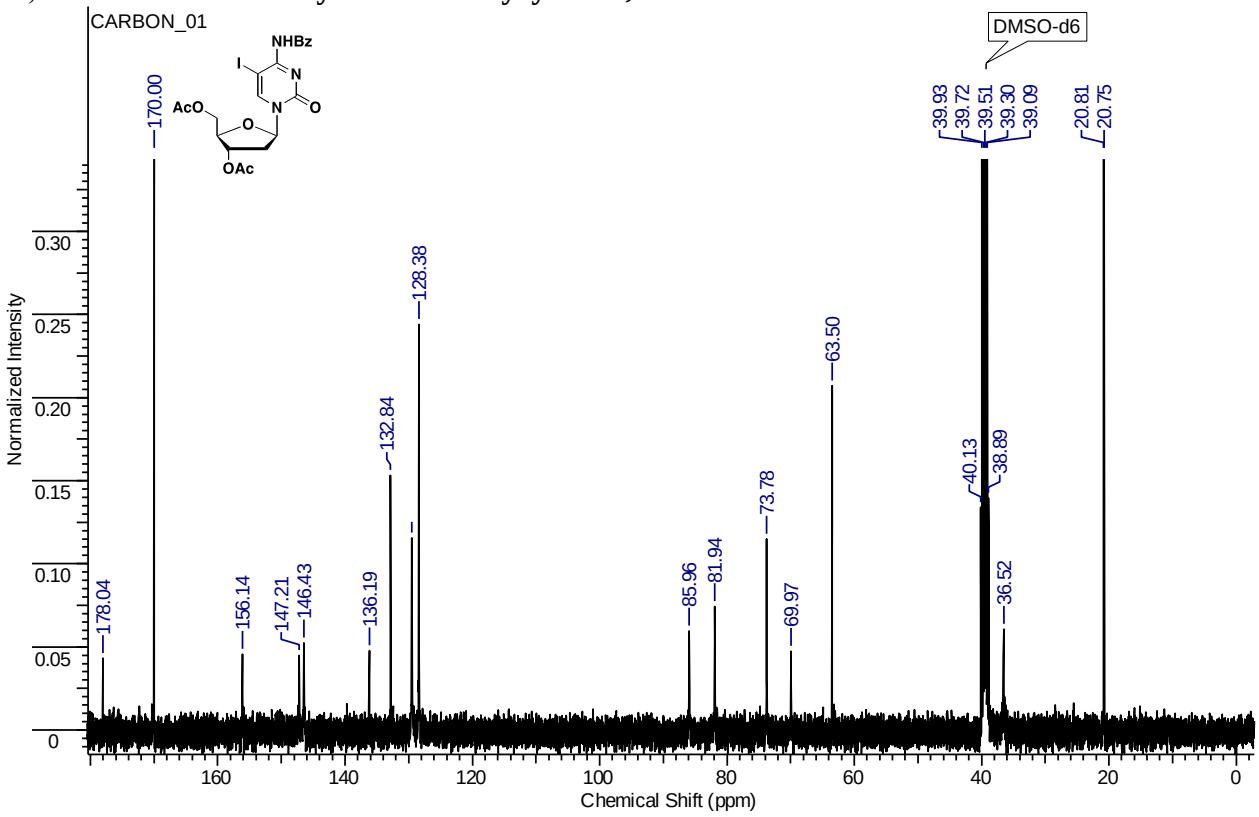
5',3'-di-*O*-Ac-5-iododeoxycytidine, ^{13}C NMR



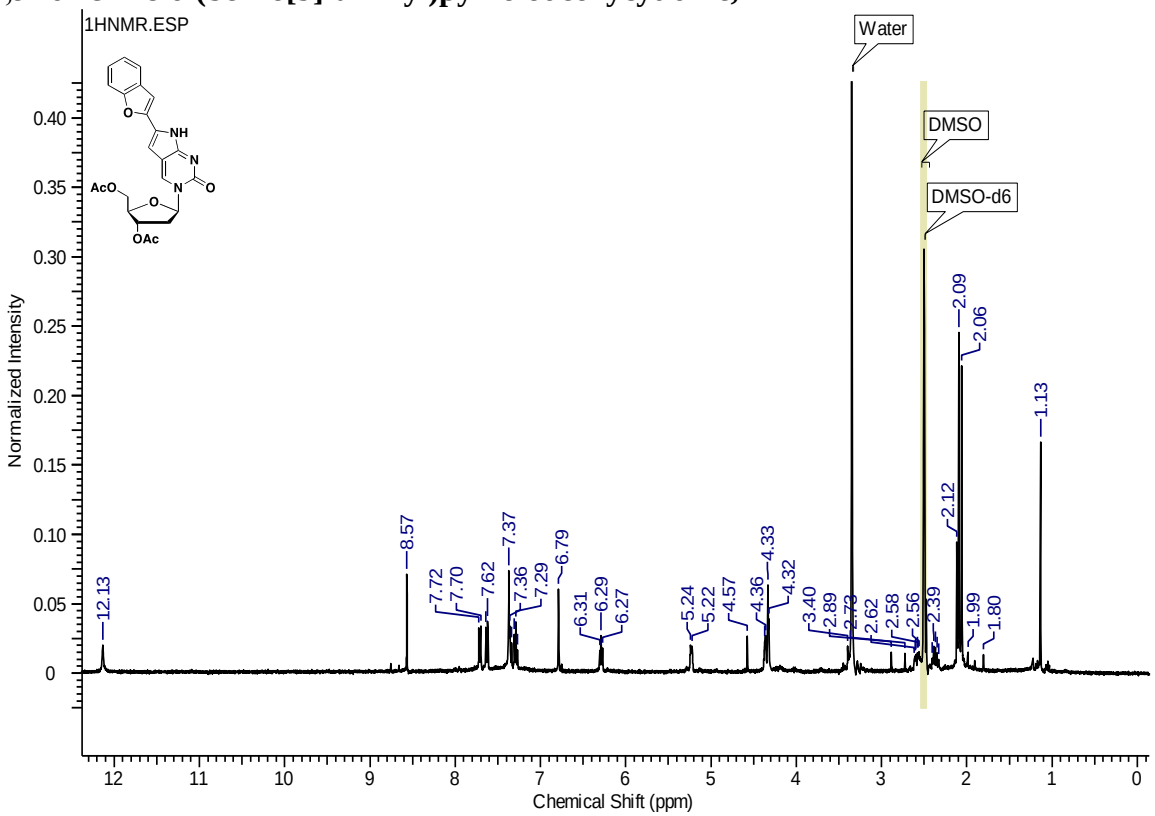
5',3'-di-*O*-Ac-*N*^{4}-benzoyl-5-iododeoxycytidine, ^{1}H NMR



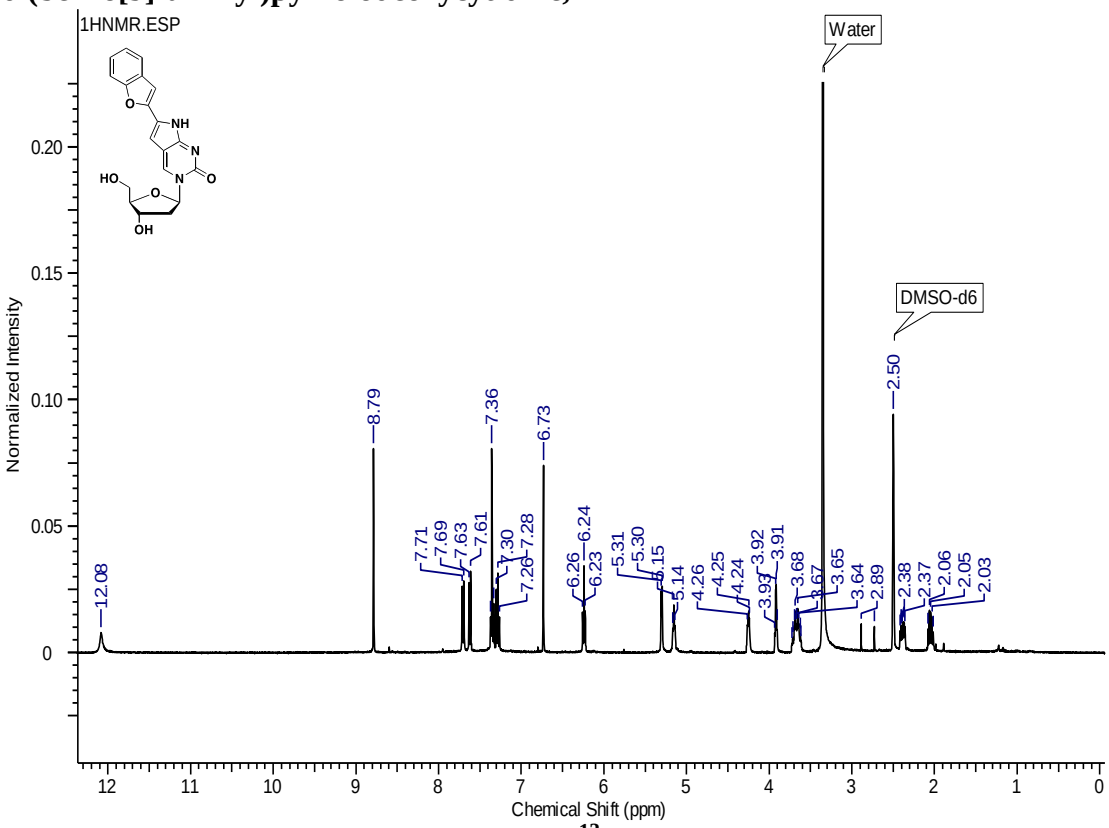
5',3'-di-*O*-Ac-*N*^{4}-benzoyl-5-iododeoxycytidine, ^{13}C NMR



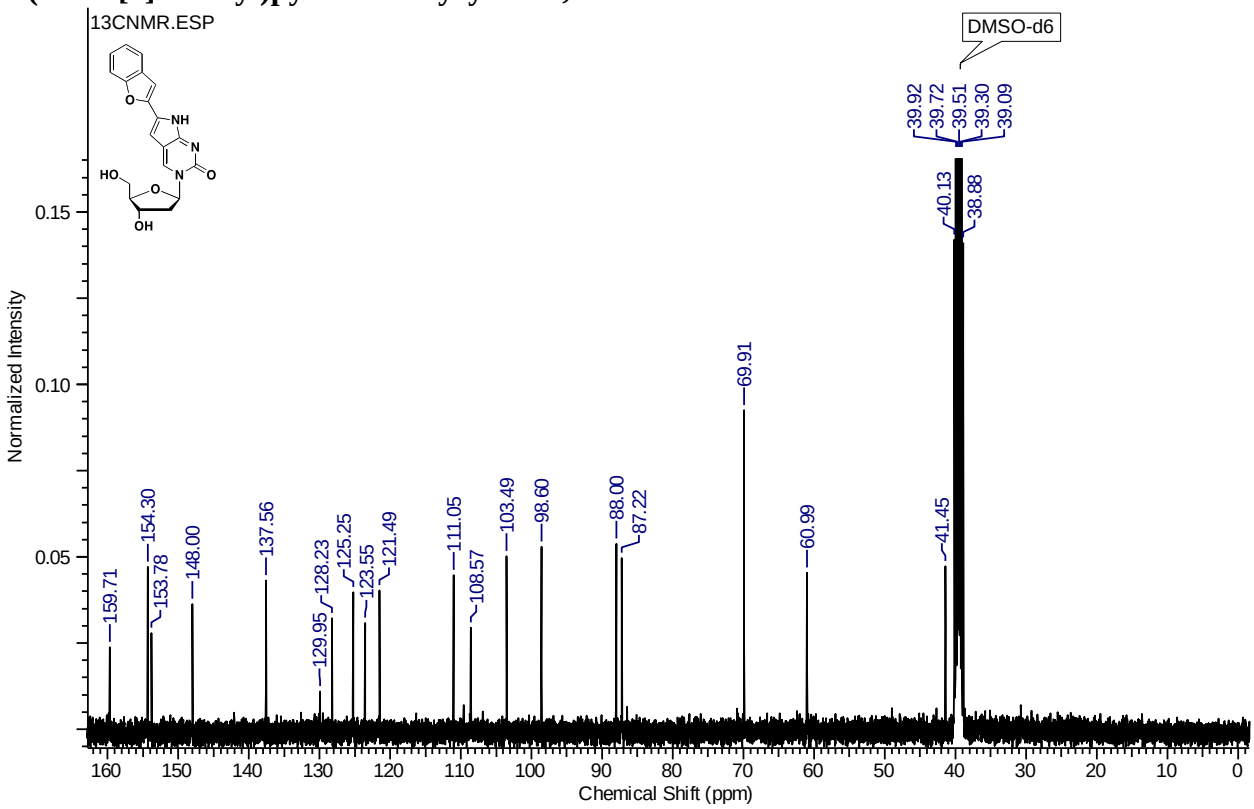
5',3'-di-*O*-Ac-6-(benzo[*b*]fur-2-yl)pyrrolodeoxycytidine, ^{1}H NMR



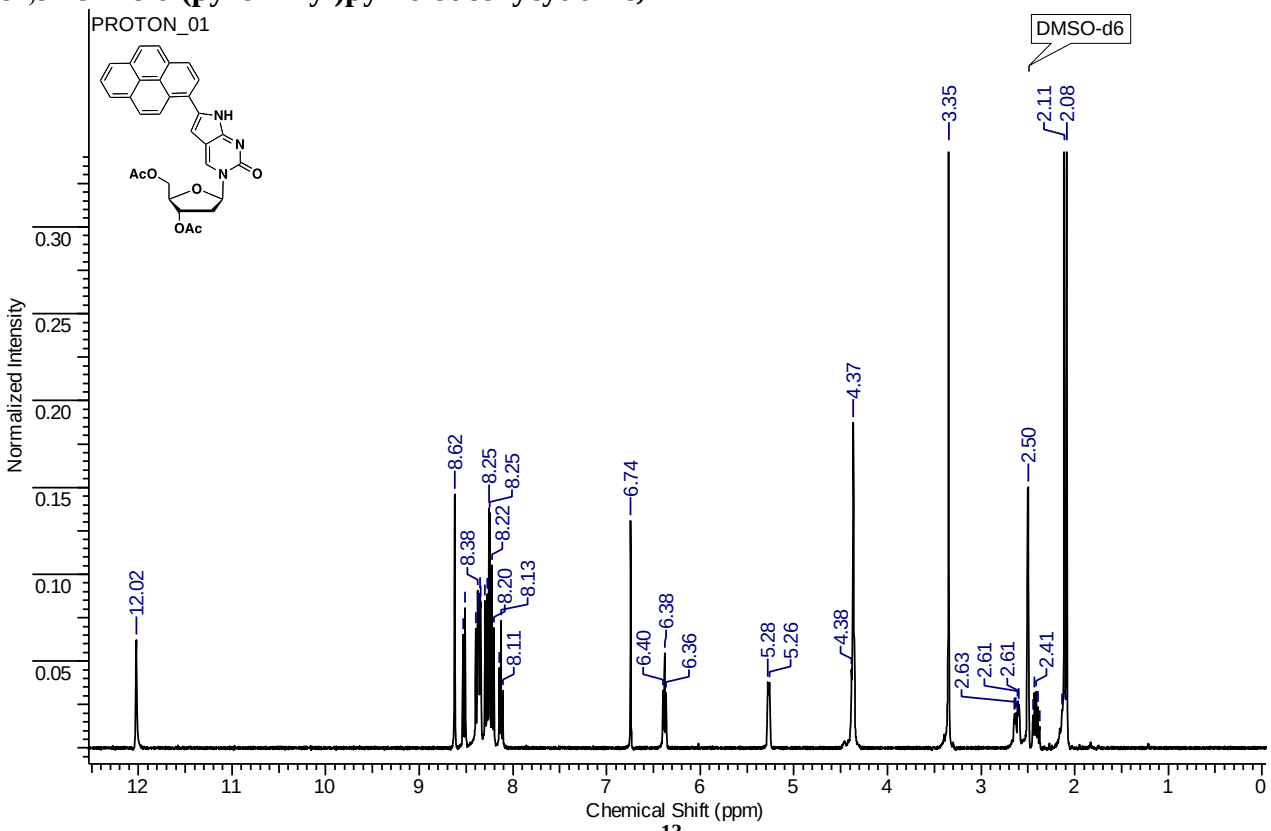
6-(benzo[*b*]fur-2-yl)pyrrolodeoxycytidine, ^{1}H NMR



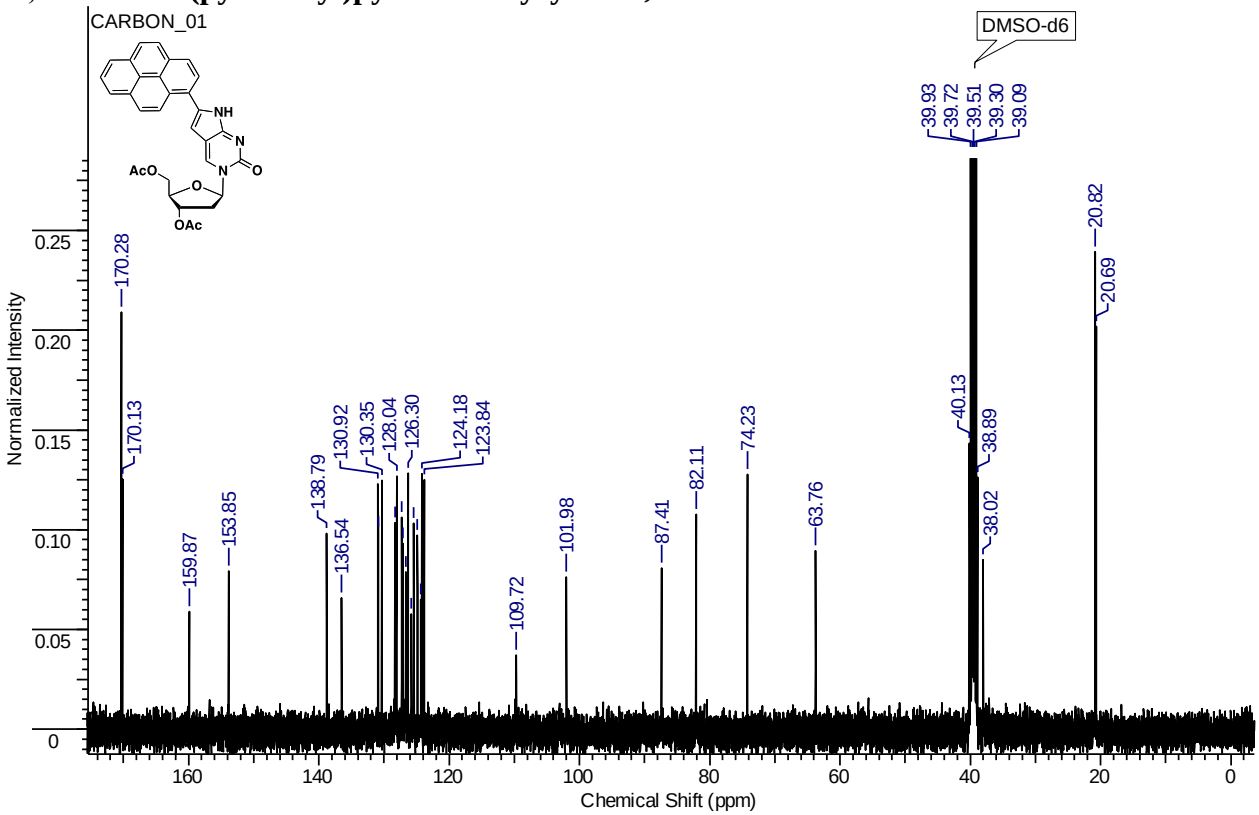
6-(benzo[*b*]fur-2-yl)pyrrolodeoxycytidine, ^{13}C NMR



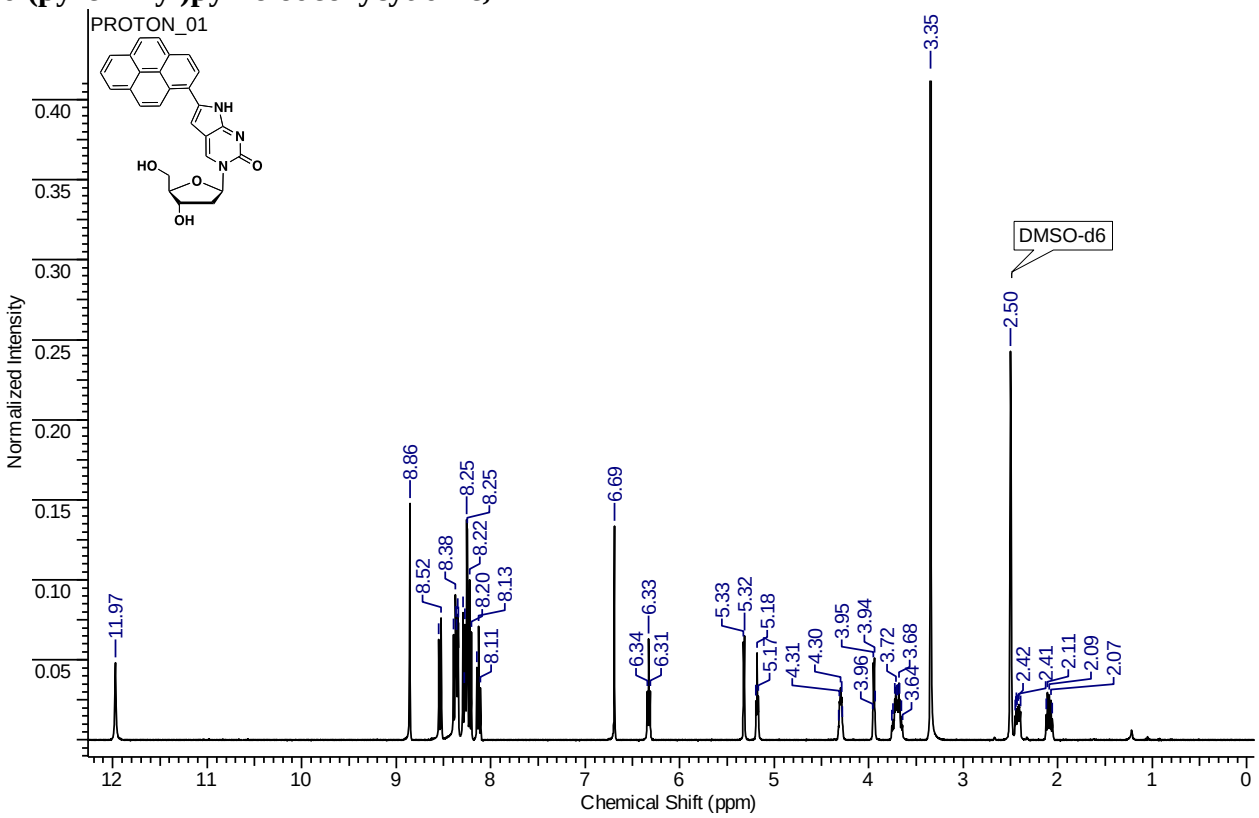
3',5'-*O*-Ac-6-(pyren-1-yl)pyrrolodeoxycytidine, ^{1}H NMR



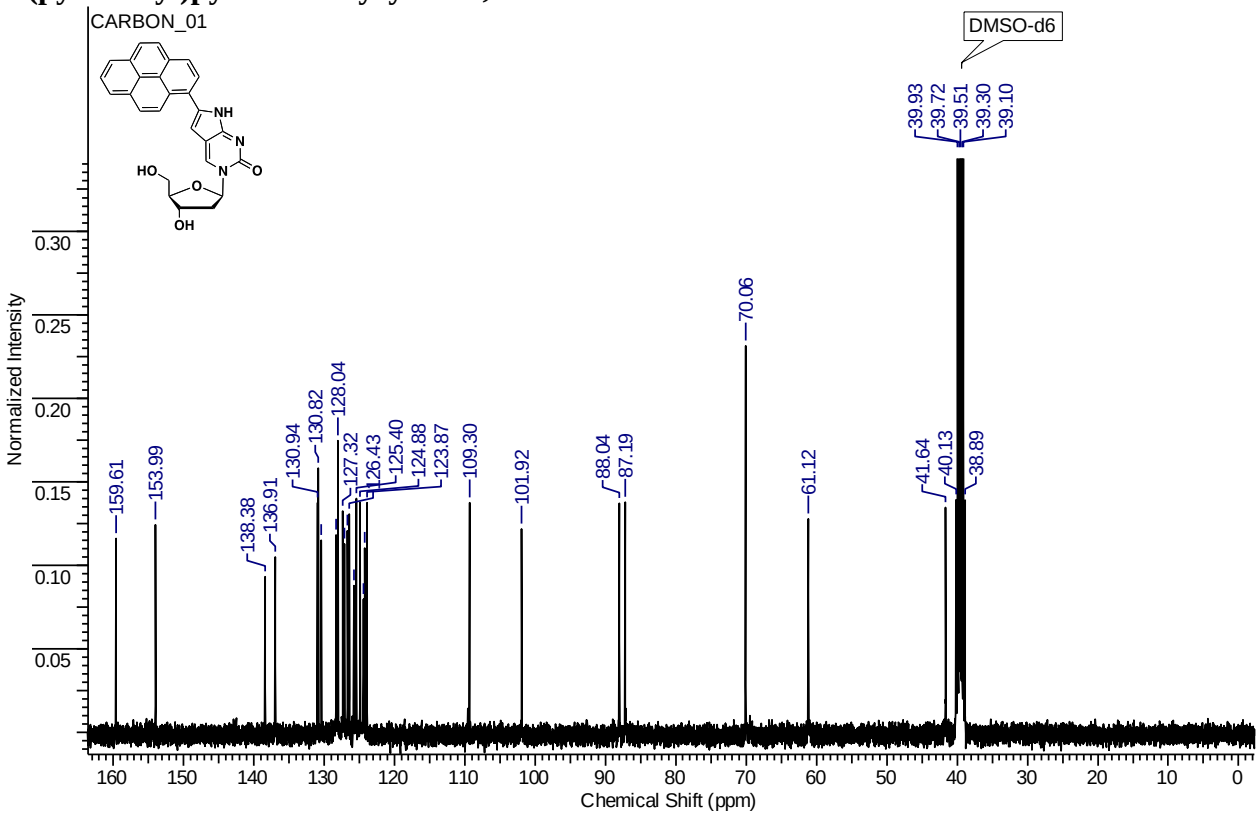
3',5'-*O*-Ac -6-(pyren-1-yl)pyrrolodeoxycytidine, ^{13}C NMR



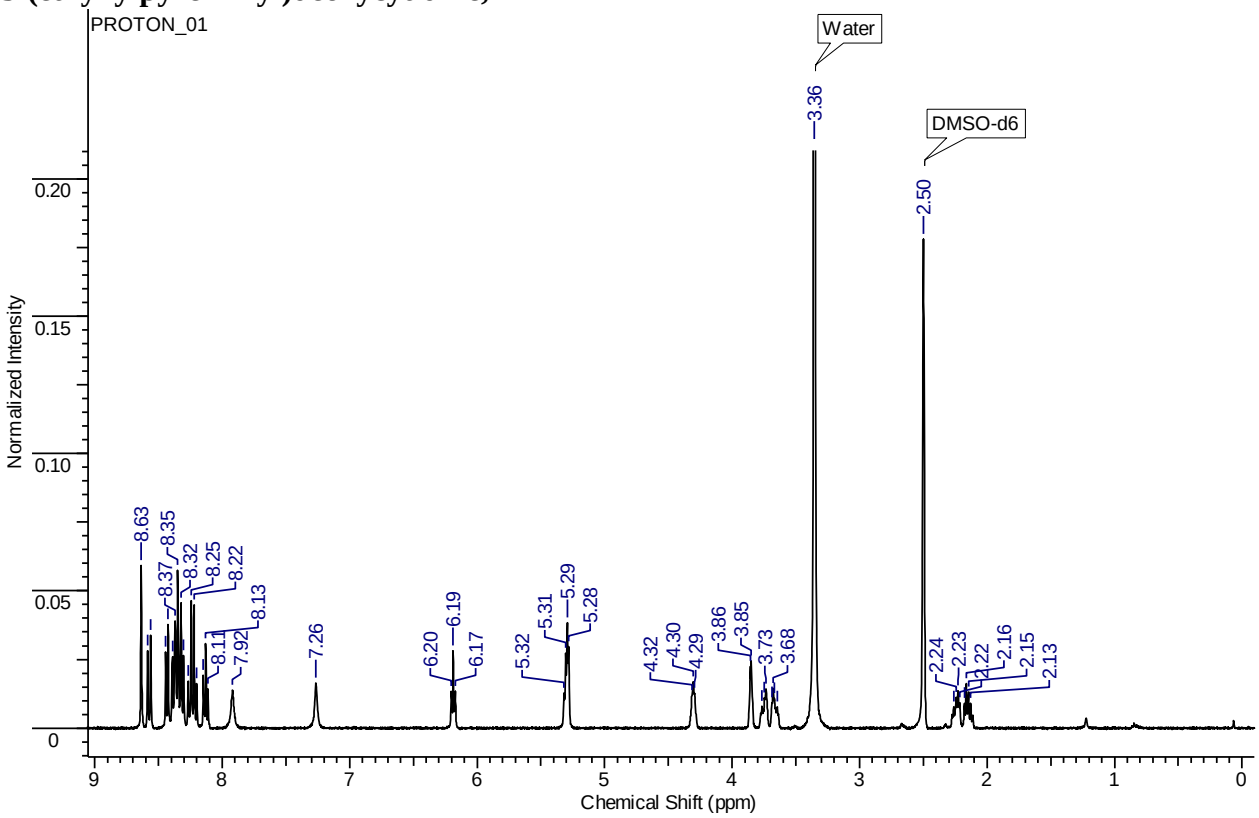
6-(pyren-1-yl)pyrrolodeoxycytidine, ^{1}H NMR



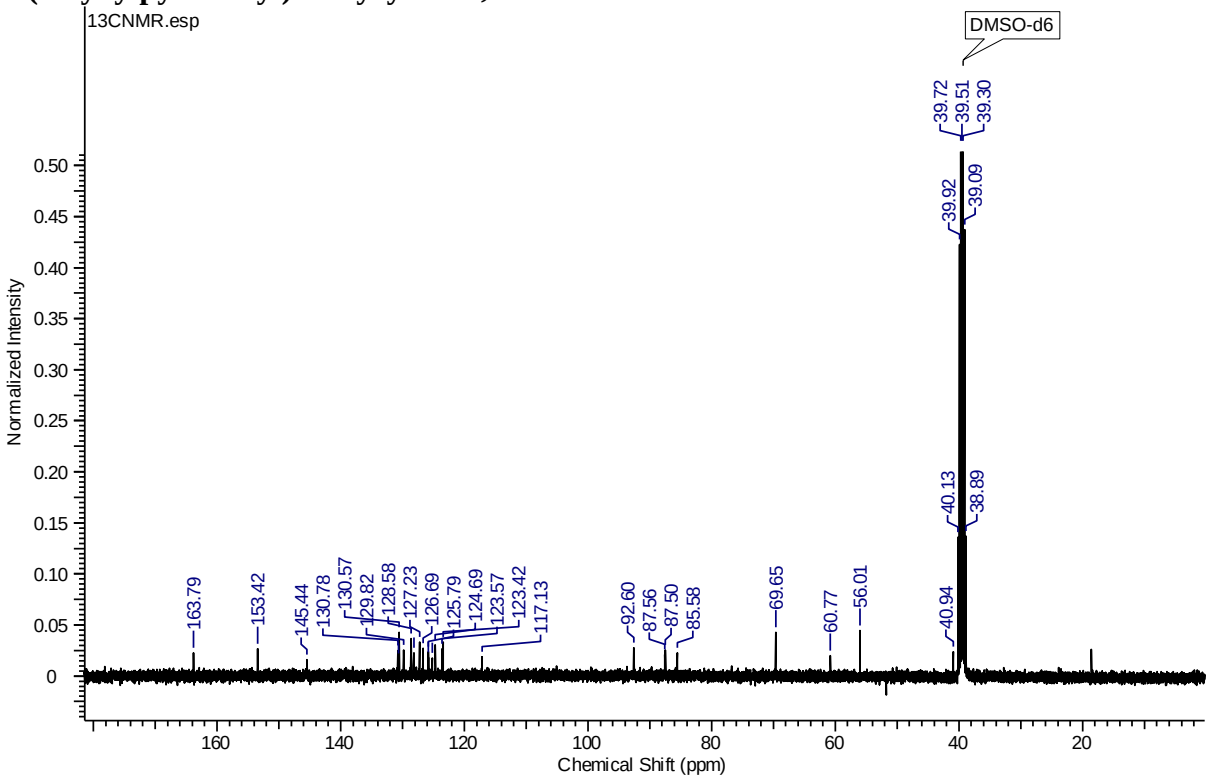
6-(pyren-1-yl)pyrrolodeoxycytidine, ^{13}C NMR



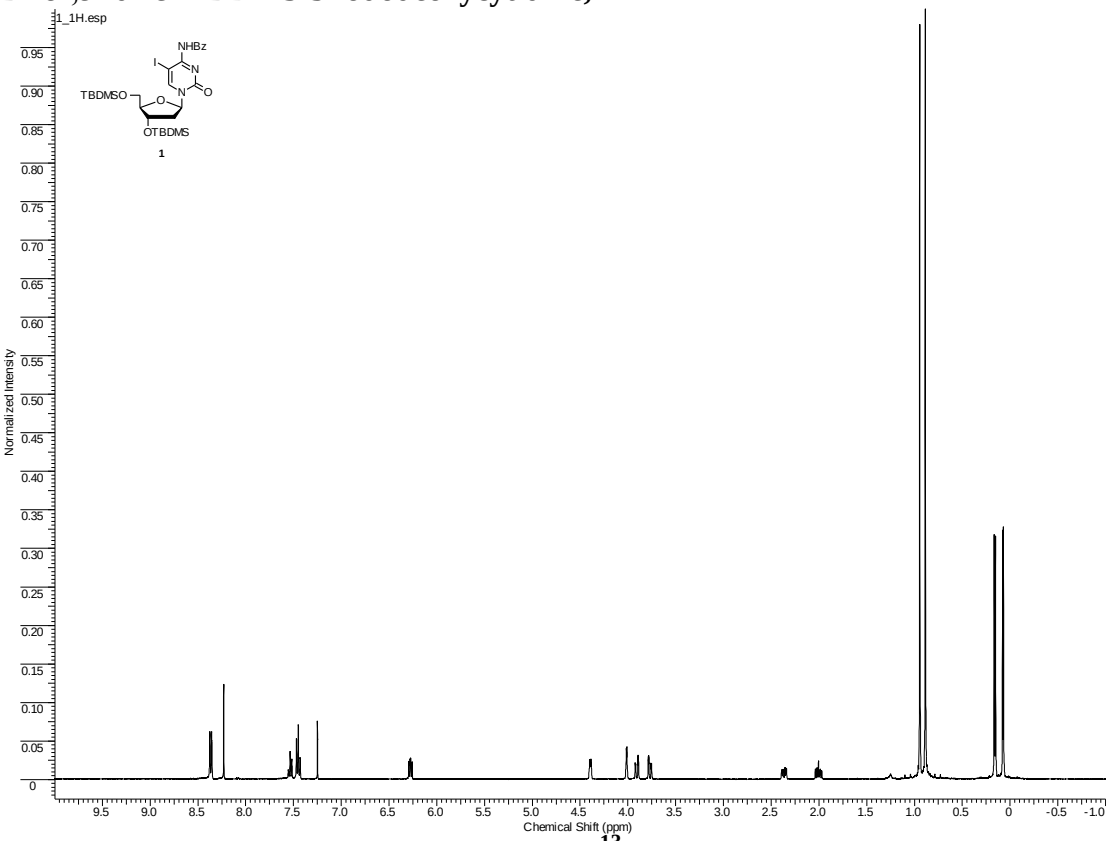
5-(ethynylpyren-1-yl)deoxycytidine, ^{1}H NMR



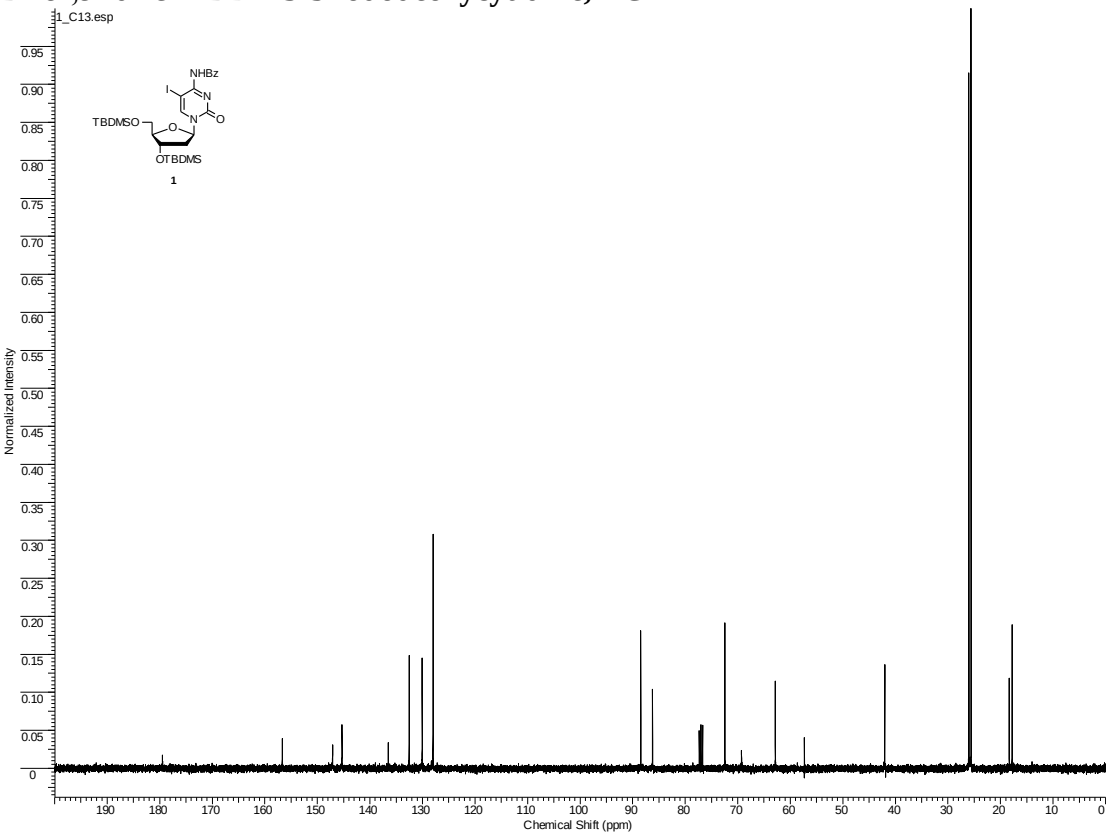
5-(ethynylpyren-1-yl)deoxycytidine, ^{13}C NMR



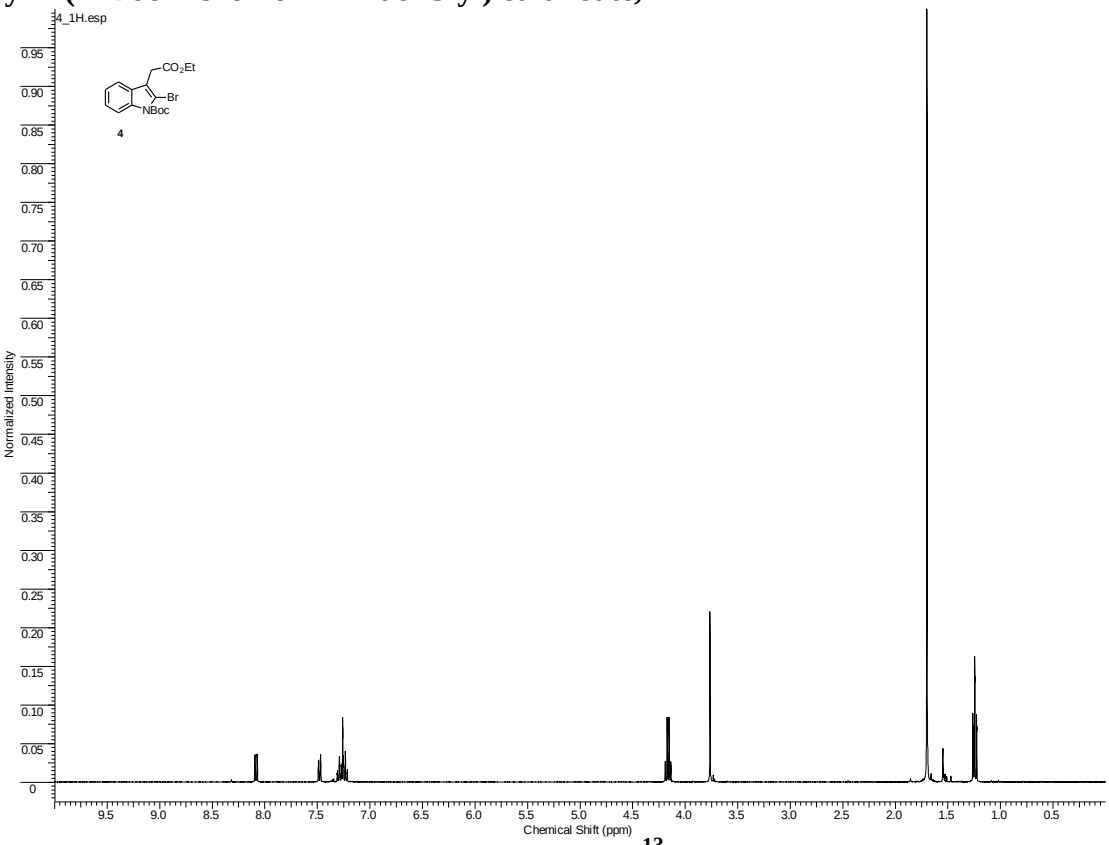
N^4-Bz-3′,5′-di-*O*-TBDMS-5-iododeoxycytidine, ^{1}H NMR



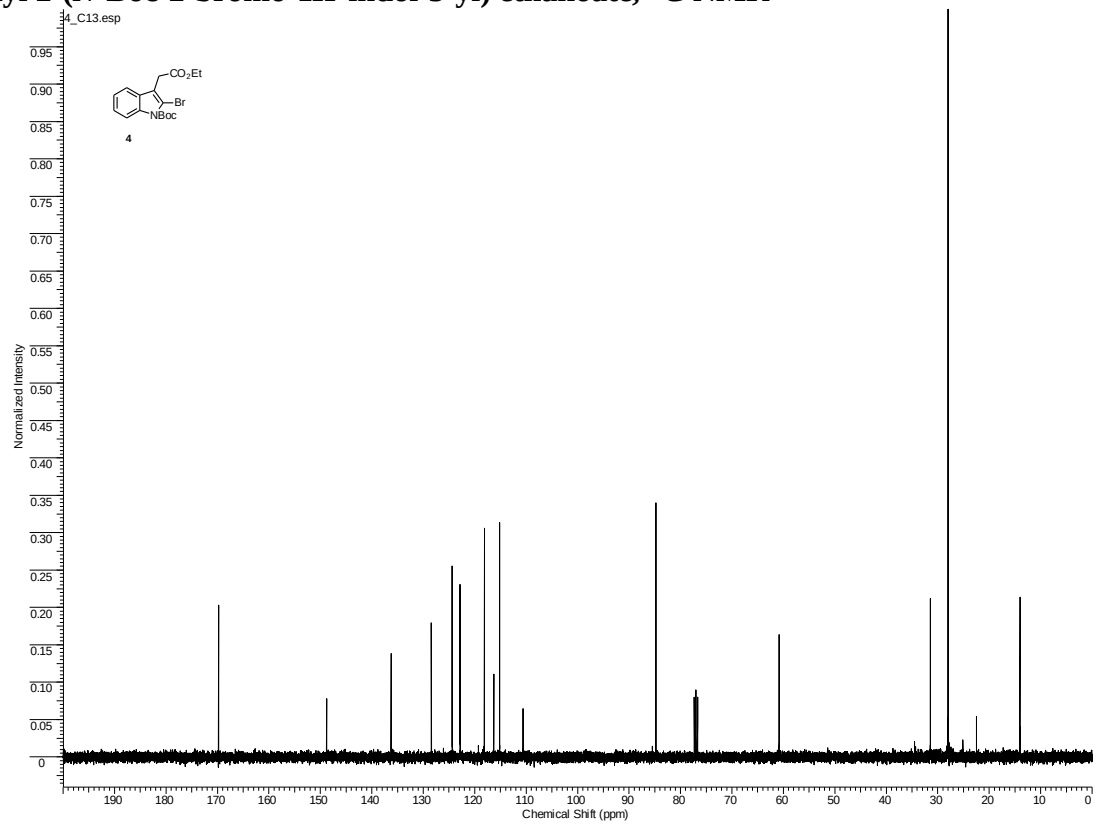
N^4-Bz-3′,5′-di-*O*-TBDMS-5-iododeoxycytidine, ^{13}C NMR



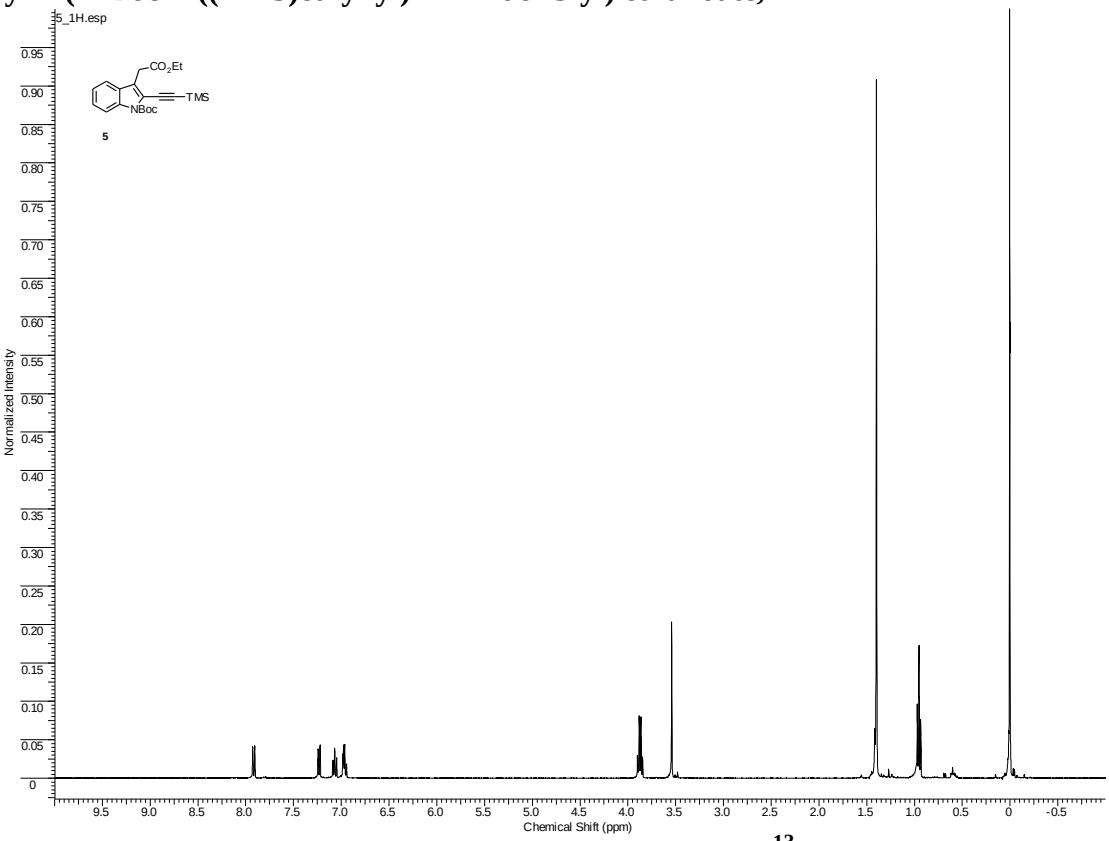
Ethyl 2-(*N*-Boc-2-bromo-1*H*-indol-3-yl) ethanoate, ^{1}H NMR



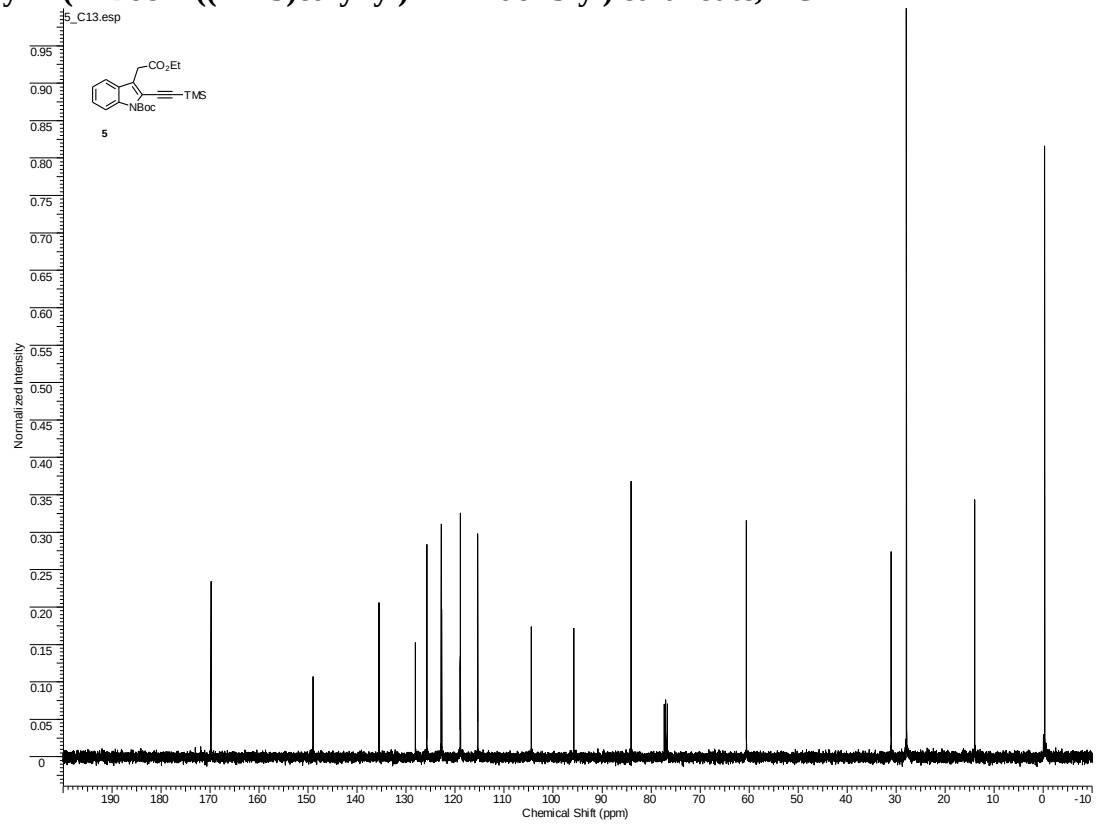
Ethyl 2-(*N*-Boc-2-bromo-1*H*-indol-3-yl) ethanoate, ^{13}C NMR



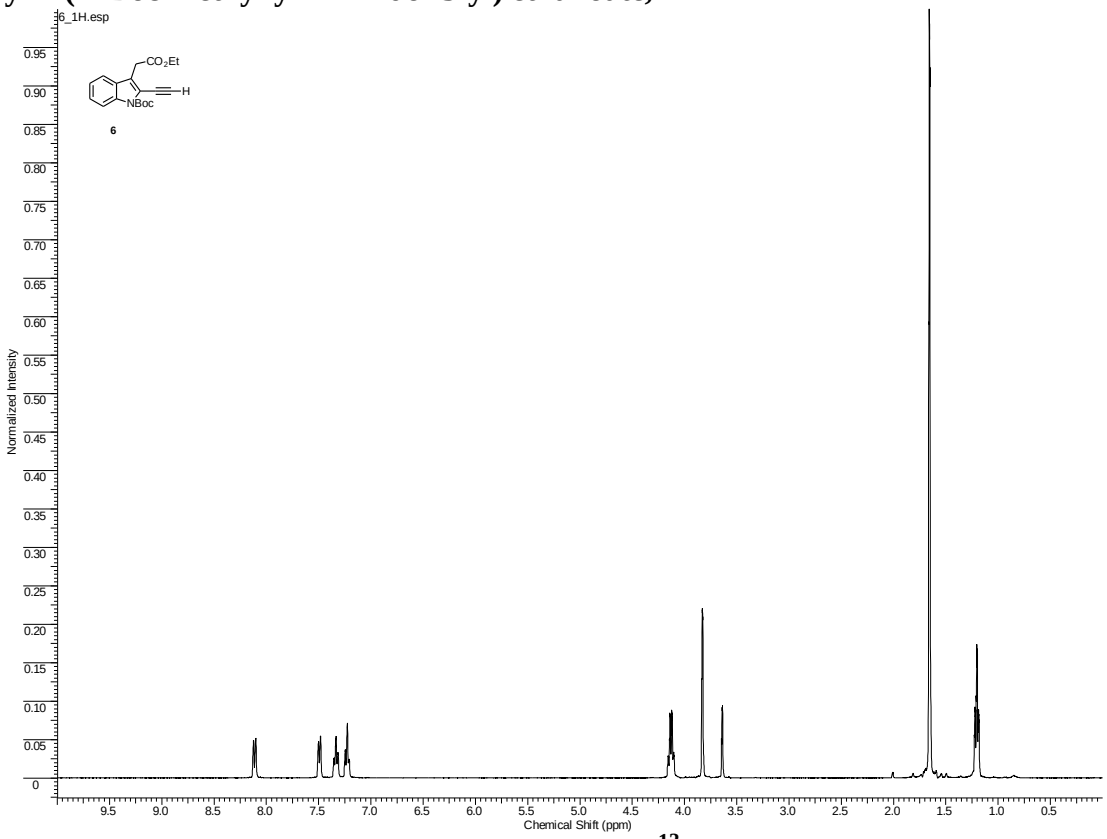
Ethyl 2-(*N*-Boc-2-((TMS)ethynyl)-1*H*-indol-3-yl) ethanoate, ^{1}H NMR



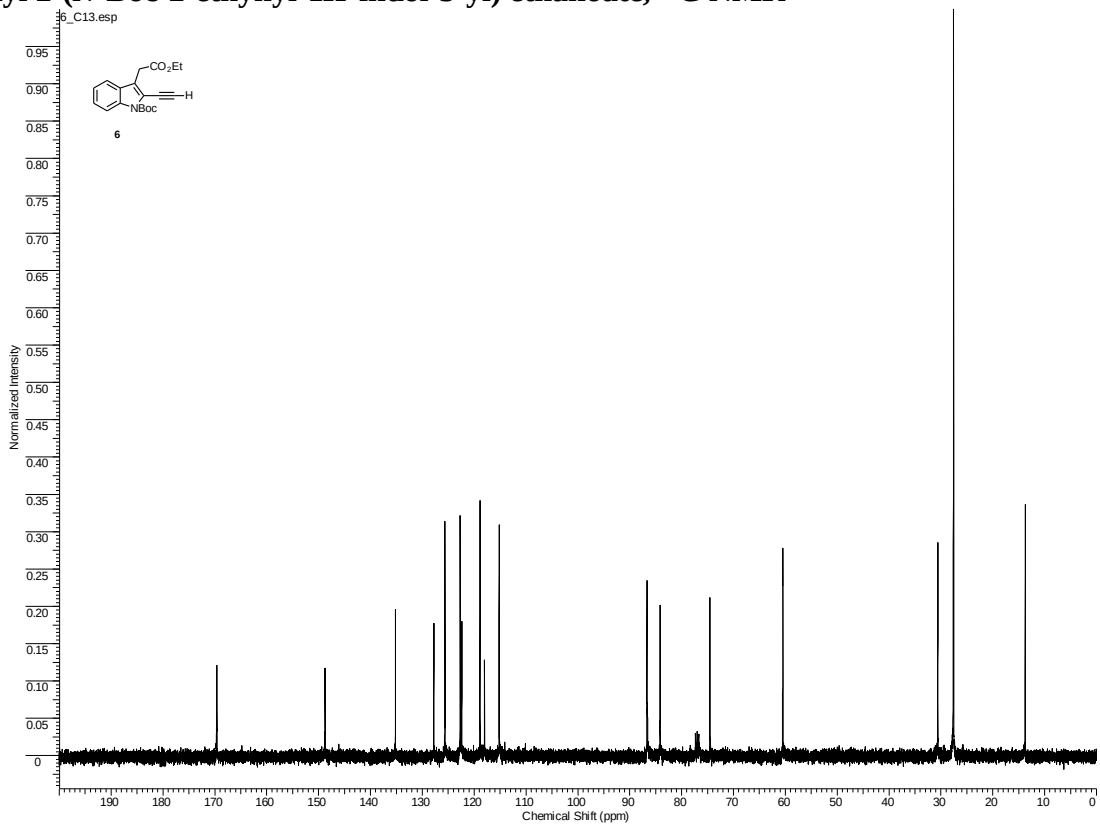
Ethyl 2-(*N*-Boc-2-((TMS)ethynyl)-1*H*-indol-3-yl) ethanoate, ^{13}C NMR



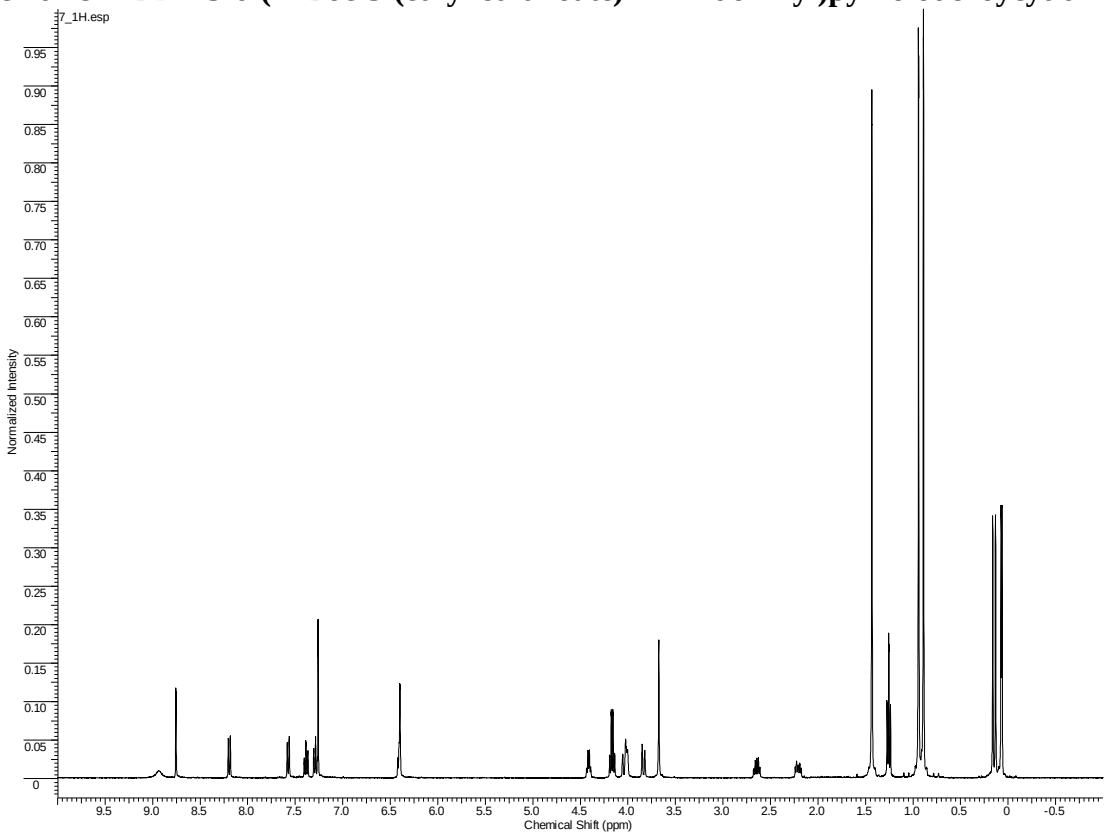
Ethyl 2-(*N*-Boc-2-ethynyl-1*H*-indol-3-yl) ethanoate, ^{1}H NMR



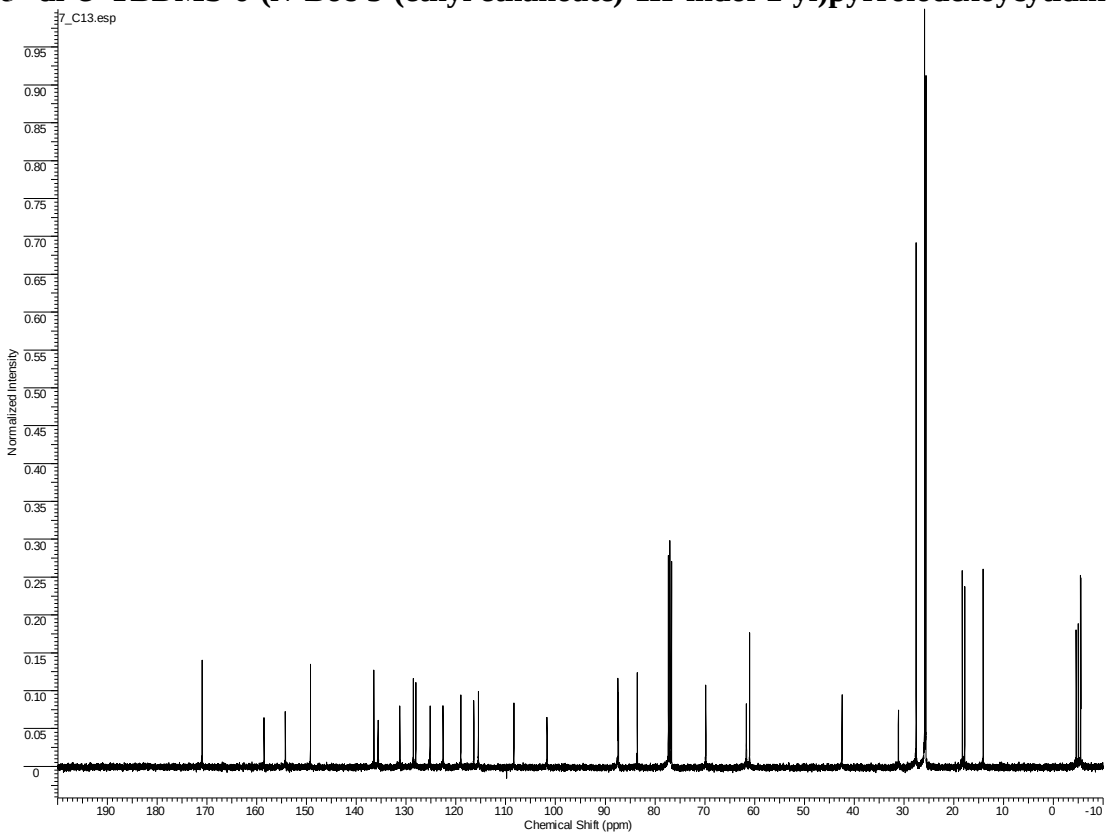
Ethyl 2-(*N*-Boc-2-ethynyl-1*H*-indol-3-yl) ethanoate, ^{13}C NMR



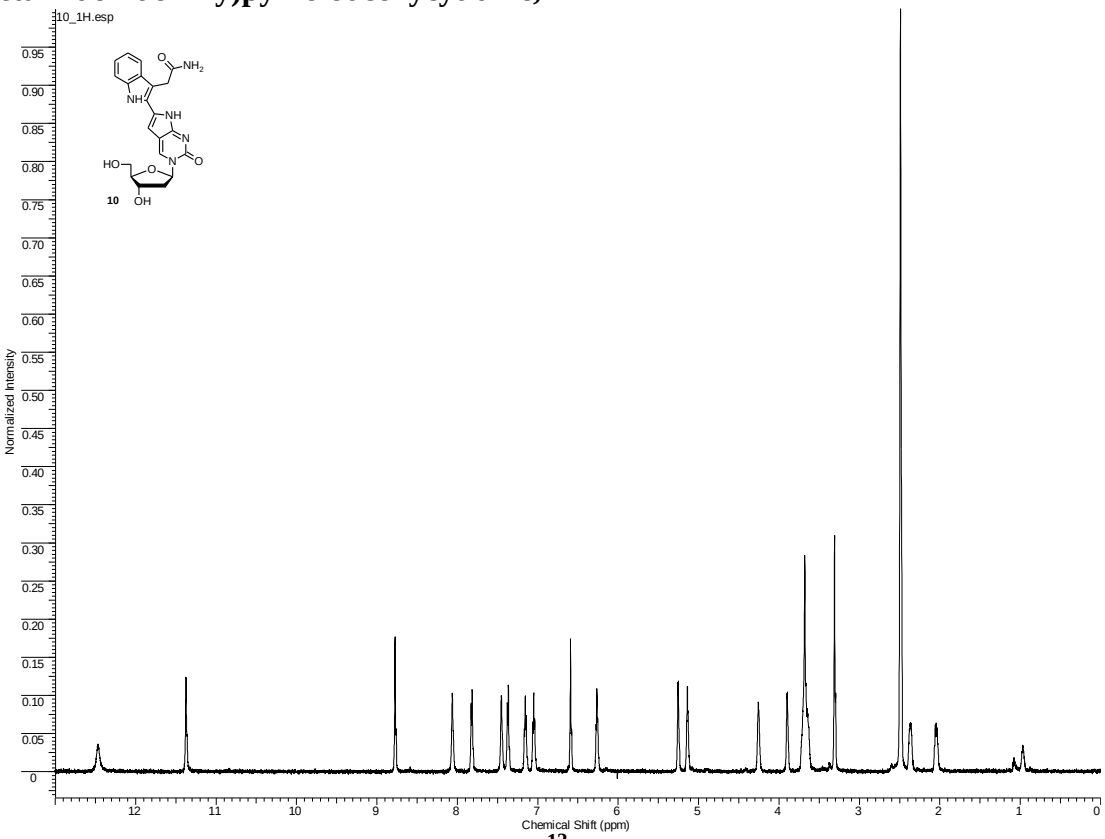
3',5'-di-*O*-TBDMS-6-(*N*-Boc-3-(ethyl ethanoate)-1*H*-indol-2-yl)pyrrolodexoycytidine, ^{1}H NMR



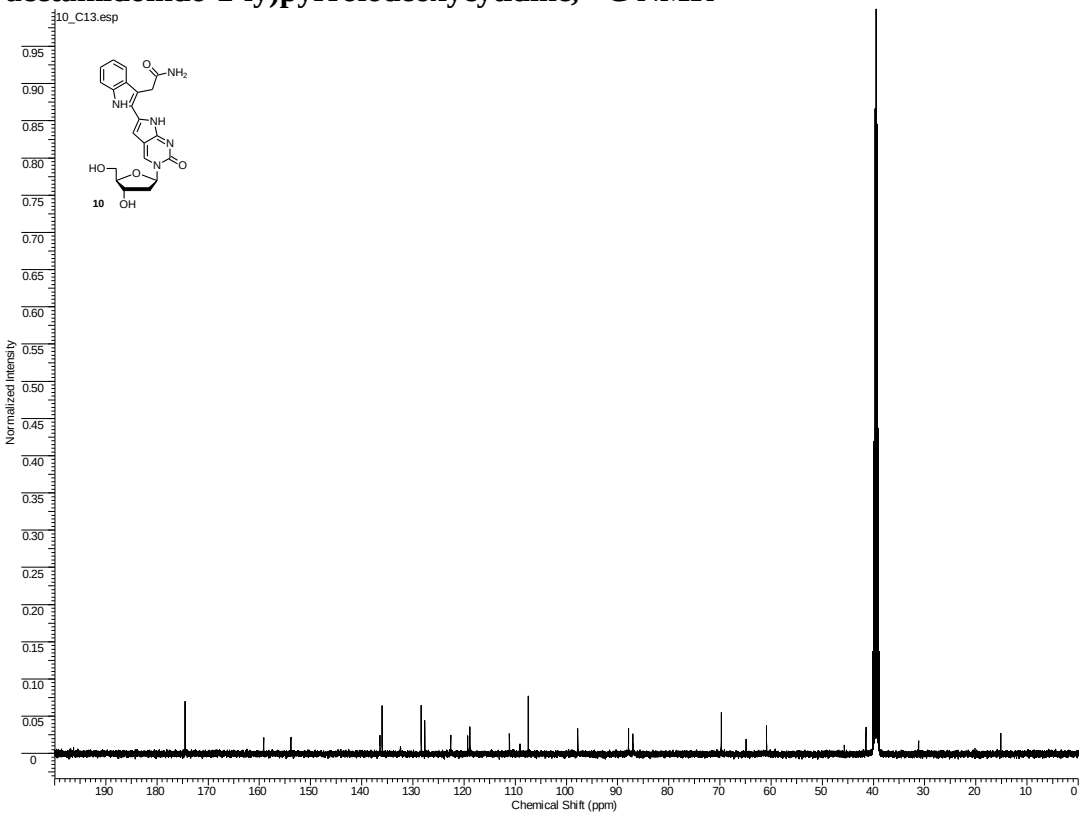
3',5'-di-*O*-TBDMS-6-(*N*-Boc-3-(ethyl ethanoate)-1*H*-indol-2-yl)pyrrolodexoycytidine, ^{13}C NMR



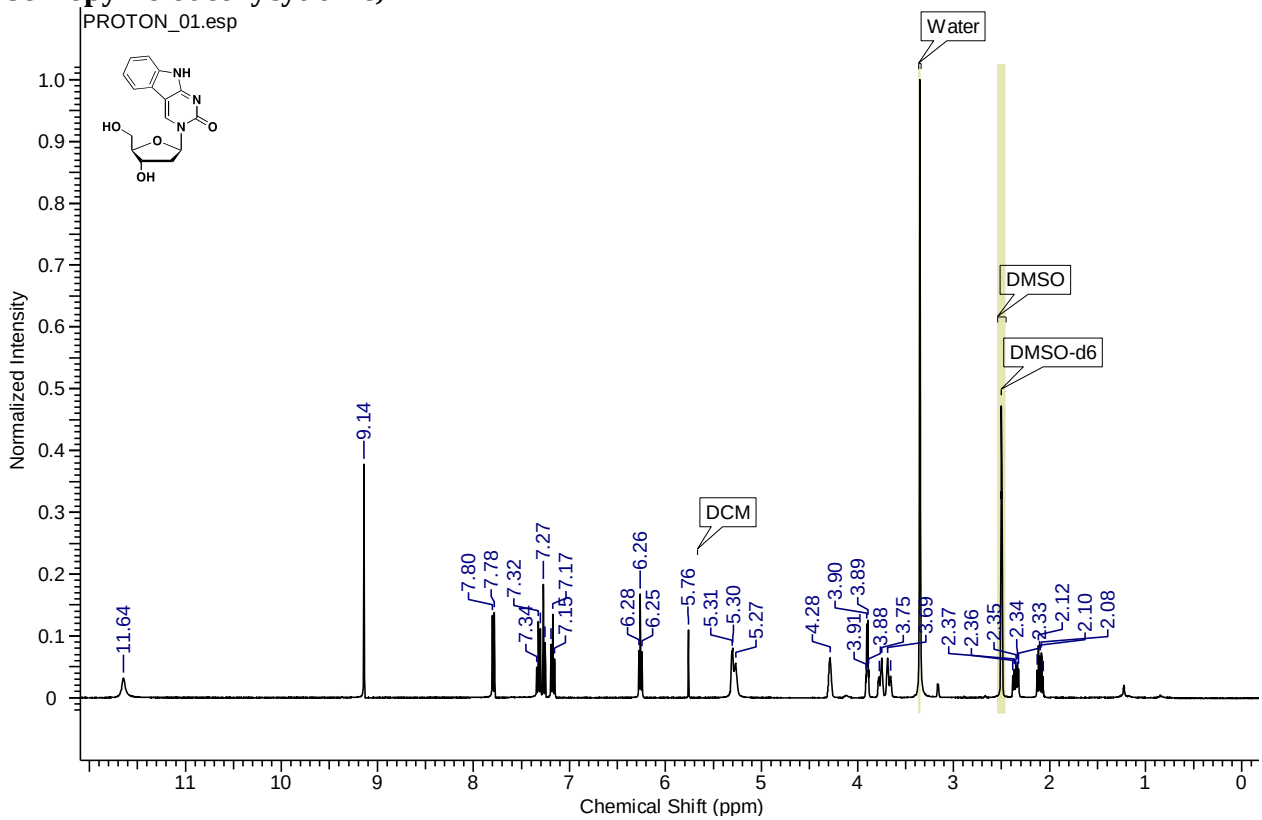
6-(3-acetamidoindo-2-ly)pyrrolodeoxycytidine, ^{1}H NMR



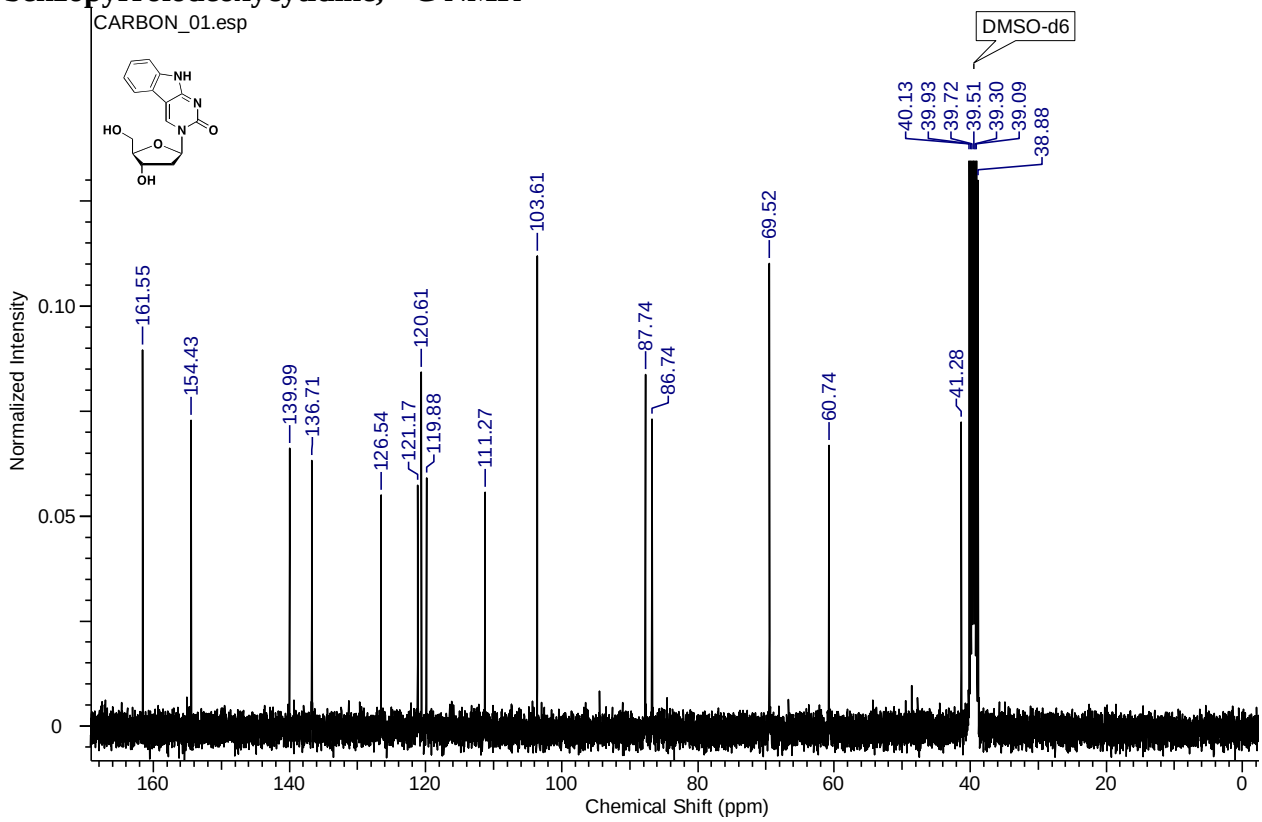
6-(3-acetamidoindo-2-ly)pyrrolodeoxycytidine, ^{13}C NMR



benzopyrrolodeoxycytidine, ^{1}H NMR



benzopyrrolodeoxycytidine, ^{13}C NMR



Fluorescence Spectra

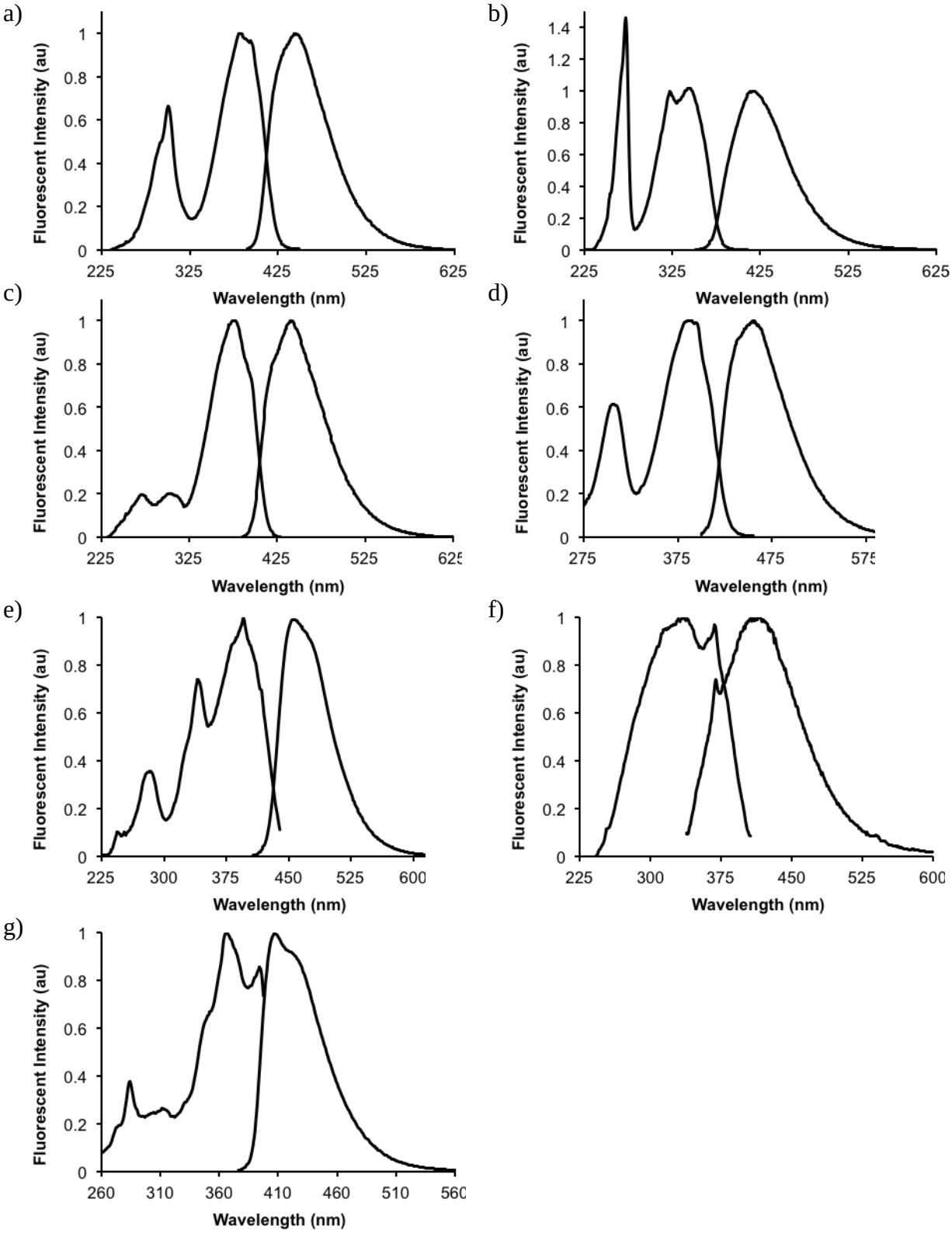


Figure S1: Normalized steady state excitation and emission spectra of a) **BFpC**, b) **BenzopC**, c) **PhpC**, d) **IndolylpC**, e) **PypC**, f) ***p*-MeOPhEtdU**, g) **PyEtdC** in dioxane

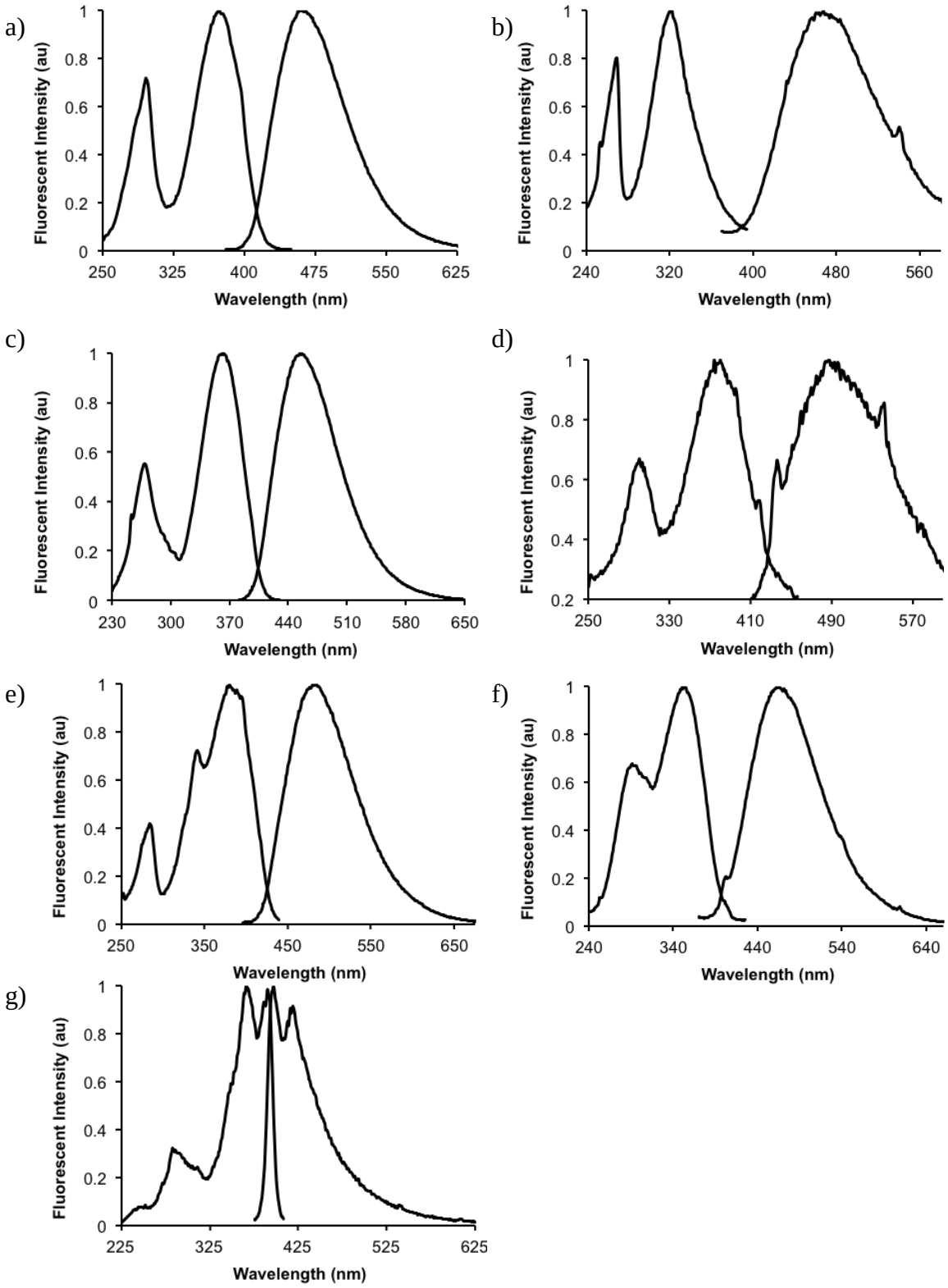


Figure S2: Normalized steady state excitation and emission spectra of a) **BFpC**, b) **BenzopC**, c) **PhpC**, d) **IndolylpC**, e) **PypC** (9:1 Water:Dioxane), f) ***p*-MeOPhEtdU**, g) **PyEtdC** (9:1 Water:Dioxane) in water.

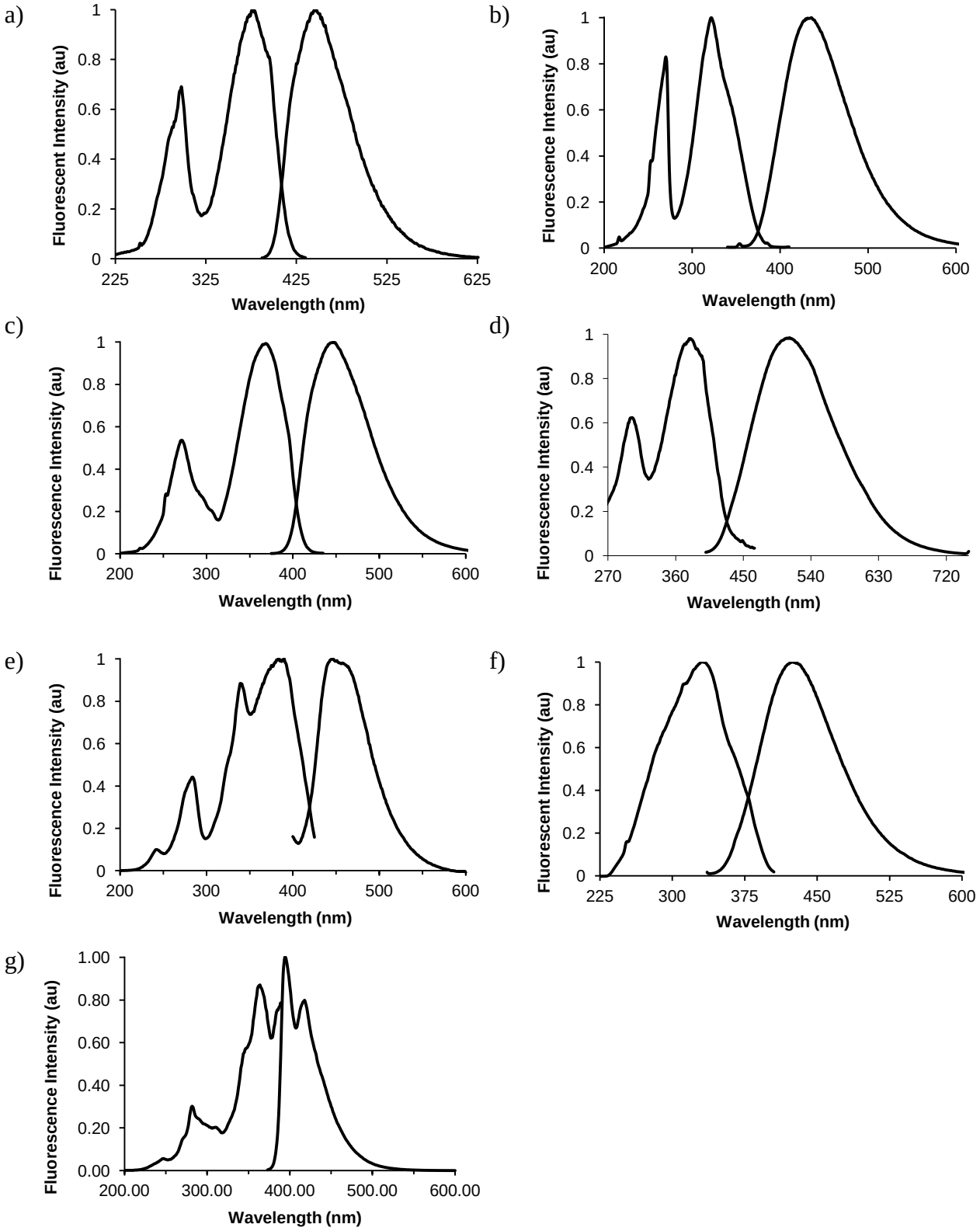


Figure S3: Normalized steady state excitation and emission spectra of a) **BFpC**, b) **BenzopC**, c) **PhpC**, d) **IndolylpC**, e) **PypC**, f) ***p*-MeOPhEtdU**, g) **PyEtdC** in ethanol.

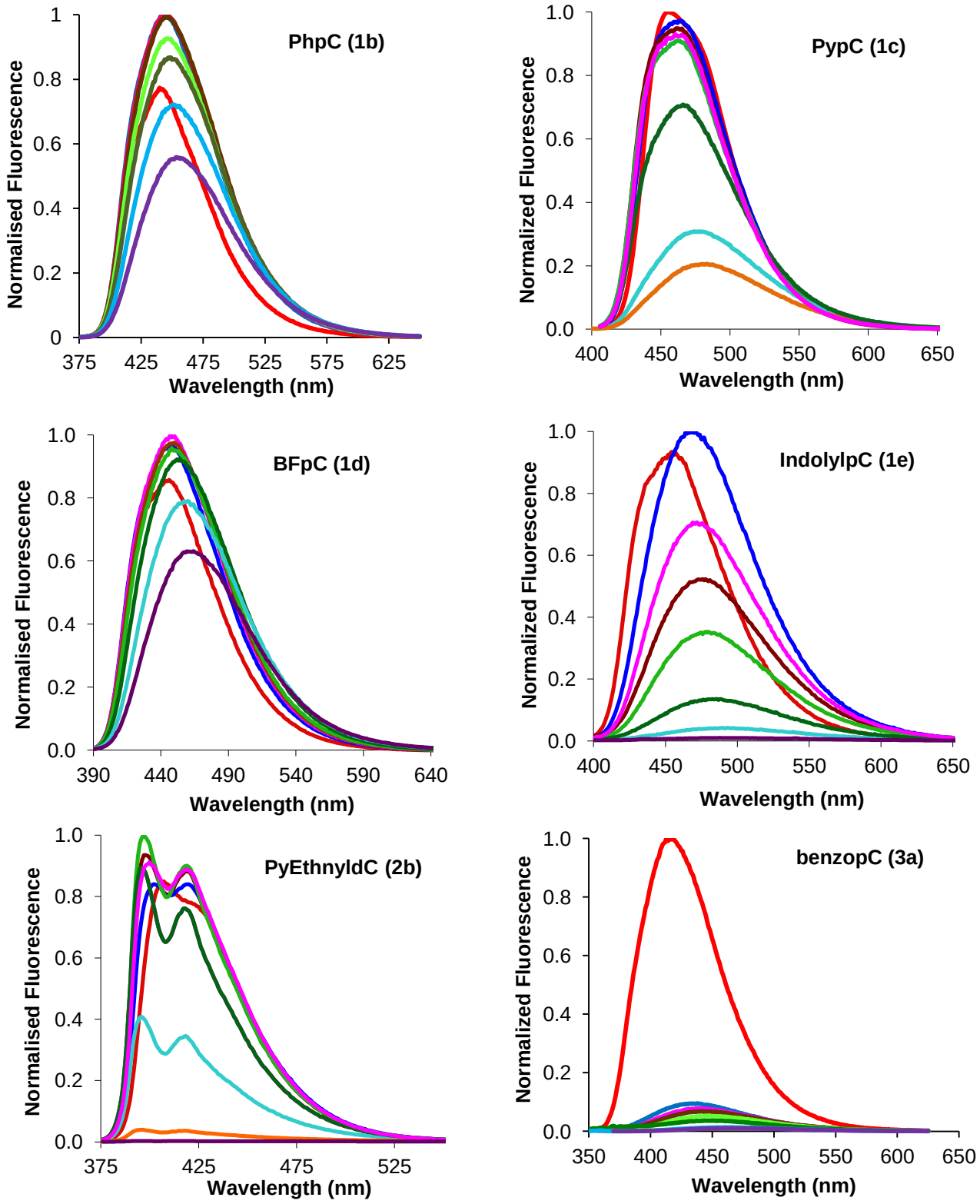


Figure S4: Fluorescence emission spectra in dioxane/water mixtures. 100% dioxane, red; 90% dioxane/10% water, blue; 80% dioxane/20% water, pink; 70% dioxane/30% water, brown; 60% dioxane/40% water, light green; 40% dioxane/60% water, dark green; 20% dioxane/80% water, turquoise; 10% dioxane/90% water, orange; 100% water, purple.

Determination of Fluorescence Quantum Yields and Molar Extinction Coefficients

Relative fluorescence quantum efficiencies were determined for each nucleoside analogue in ethanol, water and dioxane. All quantum efficiencies measured in water were determined using quinine bisulfate in 0.5 M H_2SO_4 ($\Phi = 0.546$[1]) as a reference. Quantum efficiencies in organic solvents were measured as follows: **IndolylpC**, **PypC** and **PyEtdC** were determined using dichloroanthacene ($\Phi = 0.58$[2]) as a standard, ***p*-MeOPhEtdU** and **BenzopC** pyrene ($\Phi = 0.53$[3]) was used as a standard, and **BFpC** and **PhpC** diphenylanthracene ($\Phi = 0.95$[4]) was used as a standard. In each case the equation (1) was used to calculate the relative fluorescence quantum efficiency for each nucleoside analogue using seven samples of varying concentration:

(1) $$\Phi_s = \frac{I_s}{I_{ref}} \cdot \frac{Abs_{ref}}{Abs_s} \cdot \Phi_{ref}$$

where Φ, I and Abs stand for fluorescence quantum efficiency, integrated fluorescence intensity and UV-Vis absorbance at λ_{ex}. *Reference* and *sample* are denoted by *ref* and *s* respectively.

Molar extinction coefficients were estimated by constructing Beer's Law plots of concentration versus absorbance for each nucleoside analogue. Generally, a carefully weighed sample (several milligrams in mass) was dissolved in a volumetric flask to give a solution with an absorbance > 2. Successive dilutions (using a minimum of six concentrations with absorbance between 0.02 - 2.0) were used to estimate each extinction coefficient.

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[1] Eaton, D. F. *Pure & Appl. Chem.*, **1988**, 60, 1107-1114.
[2] Ware, W. R.; Baldwin, B. A. *J. Chem. Phys*. **1965**, 43, 1194-1197
[3] Dawseon, W. R.; Windsor, M. W**.** *J. Phys. Chem.***1968**, 72, 3251-3260.
[4] Morris, J. V.; Mahaney, M. A.; Huber, J. R. *J. Phys. Chem*. **1976**, 80, 969-974

Determination of Polarity Sensitivities

Samples were prepared by dissolving a known mass of each nucleoside analogue in DMSO to acquire concentrations of 1.09×10^{-3} M, 4.15×10^{-3} M, 7.64×10^{-3} M, 2.60×10^{-3} M, 2.21×10^{-3} M, 2.79×10^{-3} M and 2.21×10^{-3} M for **BFpC**, **BenzopC**, **PhpC**, **IndolylpC**, **PypC**, ***p*-MeOPhEtdU**, **PyEtdC**, respectively. Varying amounts of the stock DMSO solution (400 µl, 375 µl, 175 µl, 140 µl, 600 µl, 400 µl and 300 µl respectively depending on fluorescence quantum yields and molar extinction coefficients of the individual compounds) was then diluted in 5 mL of dioxane using a volumetric flask. 200 µl of this solution was then dispensed in into additional 5 mL volumetric flasks. Appropriate volumes of water and dioxane were then added to give solutions of 100%, 90% 80%, 70%, 60%, 40%, 20% and 10% dioxane in water. A 100% water sample was also prepared using an equivalent amount of the DMSO solution. The $E_T(30)$ values were obtained from the literature.[5] Excitation and emission spectra for each sample were then obtained with the measurement of the maximum of measured over a narrow window (aprrox. 50 nm) and averaged ten times. Plots of solvent polarity, in $E_T(30)$ versus Stokes shift, in cm^{-1} were then constructed (**Figure S5**) and the slope calculated to provide a numerical representation of solvent polarity sensitivity in $cm^{-1}/(kcal\ mol^{-1})$. This procedure is similar to the method reported by Tor and colleagues.[6]

Select points were excluded if the fluorescence emission was too low to reliably find a maximum in either the excitation or emission spectrum. The 100% water and 100% dioxane points were removed from the ***p*-MeOPhEtdU** plot due to excessive changes in the electronic structure between the neat and binary solutions preventing the selection of a constant electronic transition to monitor. In the cases of **PypC** and **PyEtdC** a significant shift in the trend can be observed. As such the largest continuously liner segment was used to represent the polarity sensitivity of the compound.

[5] Casassas, E.; Fonrondona, G.; de Juan, A. *J. Solution Chem*. **1992**, 21, 147-162.

[6] Noé, M. S.; Rios, A. C.; Tor, Y. *Org. Lett.* **2012**, 14, 3150-3153.

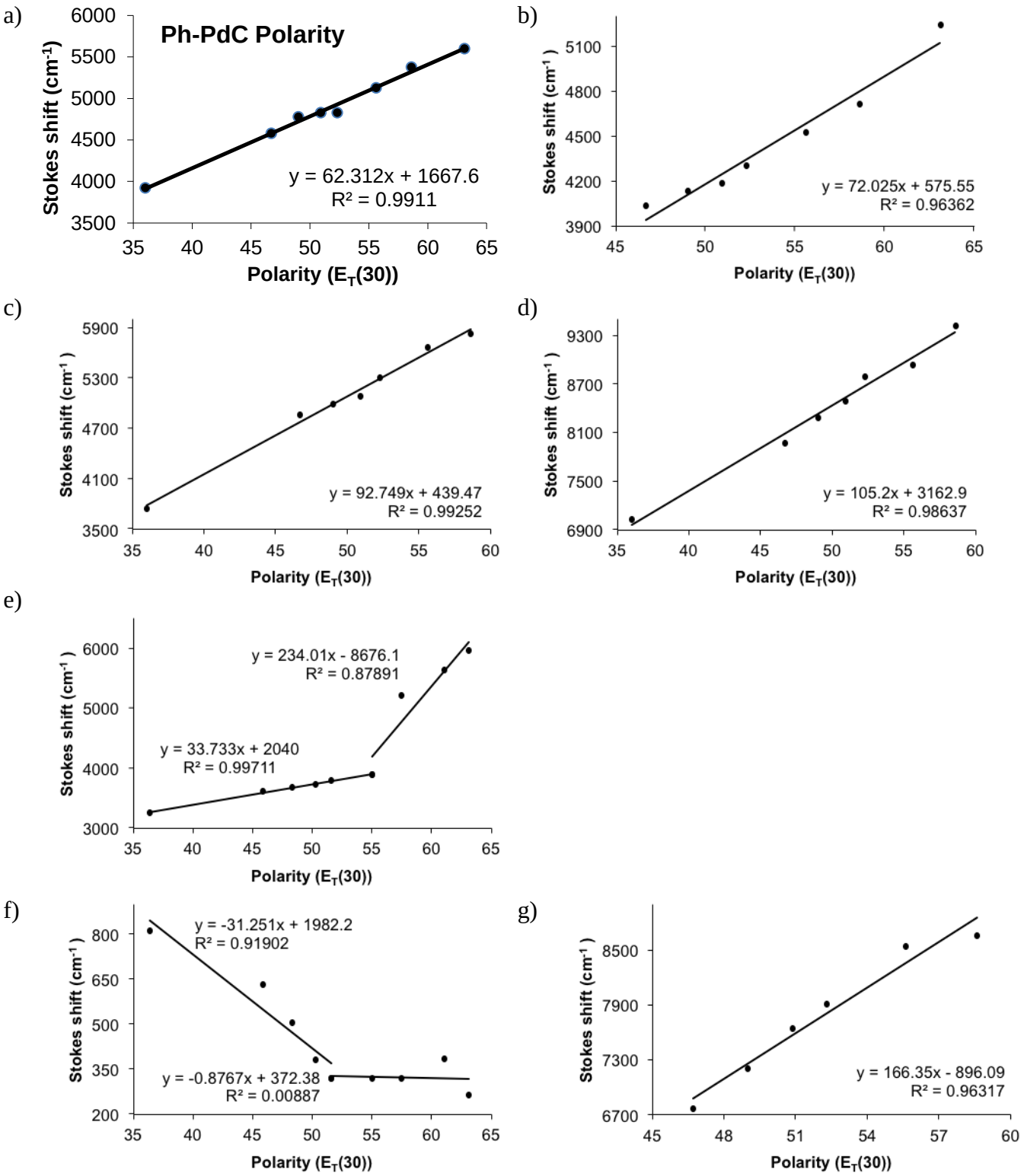

Figure S5: Plots of solvent polarity ($E_T(30)$) versus Stokes shift (cm^{-1}) for a) **PhpC**, b) **BFpC**, c) **IndolylpC**, d) **BenzopC**, e) **PypC**, f) **PyEtdC** and g) ***p*-MeOPhEtdU**. The magnitude of the slope represents the sensitivity to polarity change.

Determination of Response to Viscosity

Stock solutions of 70%, 50%, 40%, 20%, 15% and 10% methanol in glycerol were prepared in sealed vials and stirred overnight to ensure solutions were uniform. A known amount of ***p*-MeOPhEtdU** was then dissolved in DMSO to obtain a stock solution of concentration of 2.79×10^{-3} M. 20 µl of the stock solution was then used to stain 5 mL solutions of 100%, 70%, 50%, 40%, 20%, 15% and 10% methanol in glycerol. UV-Vis absorbance, excitation and emission spectra were then obtained with the emission maximum being averaged over a small window (50 nm). Integrated emission intensities were then normalized using the equation (2). The log of the normalized emission intensities were then plotted (**Figure S6**) versus the log of the solvent viscosity, obtained using equation (3).

(2) $$I_{norm} = \frac{0.1}{Abs_{\lambda_{ex}}} \cdot I$$

(3) $$ln\eta_{mix} = \sum_{i=1}^{2} w_i \cdot ln\eta_i$$

I, *Abs*, *η*, and *w* stand for integrated fluorescence intensity, absorbance at λ_{ex}, viscosity and weight fraction respectively. *Normalized* and *mixture* are denoted by *norm* and *mix* respectively. Values of 1317 cp and 0.583 cp[7] have been used for the viscosity of glycerol and methanol respectively. Values of 1.25 kg/L and 0.79 kg/L have been used for the density of glycerol and methanol respectively.

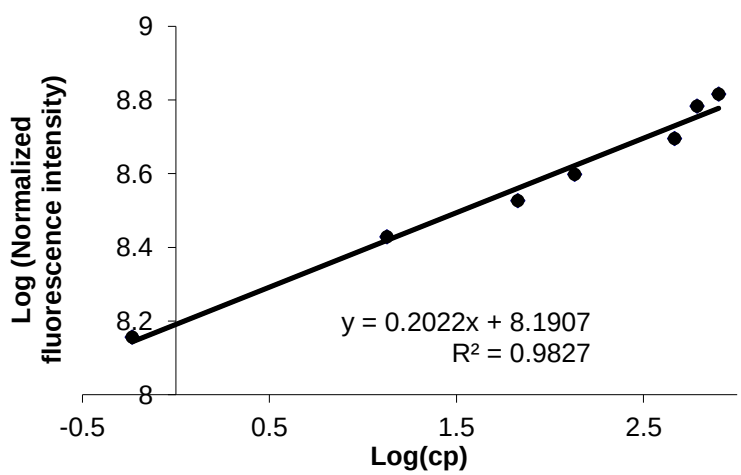

Figure S6: Double log plot of solvent viscosity versus normalized integrated fluorescence intensity of compound **2a** 5-(*p*-methoxyphenylethynyl)dU. Linearity indicates behavior representative of fluorescent molecular rotors.

[7] Viscosity values taken from in order to facilitate comparison: Sinkeldam, R.W.; Wheat, A.J.; Boyaci, H.; Tor, Y. *ChemPhysChem* , **2011**, *12*(3), 567-570.