Supporting Information

Fluorescent, chirality-responsive, and water-soluble cage as a multifunctional molecular container for drug delivery

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General Experimental Details.

Starting materials were purchased from commercial suppliers were used without further purification. NMR spectra were recorded on a spectrometer operating at 400 MHz spectra on a Bruker ascend spectrometer. Isothermal titration calorimetry (ITC) was carried out using a VP-ITC (Malvern) at 25 ˚C, and computer fitting of the data were performed using the VP-ITC analyze software. UV/vis spectra were done on Agilent Cary-100 spectrometer. Fluorescence spectra were performed by using a Horiba Fluorolog-3 spectrometer. The Circular dichroism (CD) spectra were performed by using JASCO J-1500. Fluorescence microscopy images were taken by Fluorescence microscopy (DM505, Nikon Co., Ltd., Otawara, Tochigi, Japan).

Chemicals and reagents

Acadesine (**ACA**) and Sofosbuvir (**SOF**) were purchased from Aladdin. Gemcitabine (**GEM**), Lamivudine (**LAM**), Zidovudine (**AZT**), Tenofovir (**TFV**), and Lenalidomide (**LEN**) were purchased from Innochem. Clofarabine (**CLF**), Abacavir (**ABC**), Camptothecin (**CPT**) and Irinotecan (**CPT-11**) were purchased from Bidepharm. **1**•8Cl^- was synthesized by previously reported procedures.[1]

McCOY's 5A, DMEM, PBS, MTT, and trypsin, were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fetal bovine serum (FBS) was purchased from Excell Bio (Shanghai, China). The cell membrane fluorescent probe DiIC18(3) and nuclear dye DAPI were purchased from Yeasen (Shanghai, China).

Test Method for Phase Solubility.

Solubility enhancement was mainly studied by using the method of phase solubility method. Firstly, we prepare 20.0 mM and 15.0 mM of the aqueous solution of **1**, and then diluting the solution obtained the cage the gradient of concentration, respectively is 20.0 mM, 15.0 mM, 10.0 mM, 7.50 mM, 5.00 mM, 3.75 mM, and 2.50 mM. Next, we stirred solutions containing known concentrations of **1** with an excess of drug until equilibrium was achieved. Excess insoluble drug was removed by filtering, and the

concentration of soluble drug was determined by ^{1}H NMR integration of drug peak versus 1,3,5-trimethoxybenzene, which was selected as an internal standard. These data are used to plot phase solubility diagrams for each drug. The actual concentration of the cage was determined by internal standard method.

Cell lines and cell culture

The human colon cancer cell line HCT-116 was obtained from the Shanghai Institute of Cell Biology at the Chinese Academy of Sciences and was cultured in McCOY's 5A medium with 10% (v/v) FBS. The human breast cancer cell line MCF-7 was obtained from Shanghai Genechem Co., Ltd., which was cultured in DMEM medium with 10% (v/v) FBS. The cells were maintained at 37 °C in a humidified incubator with 5% CO_2.

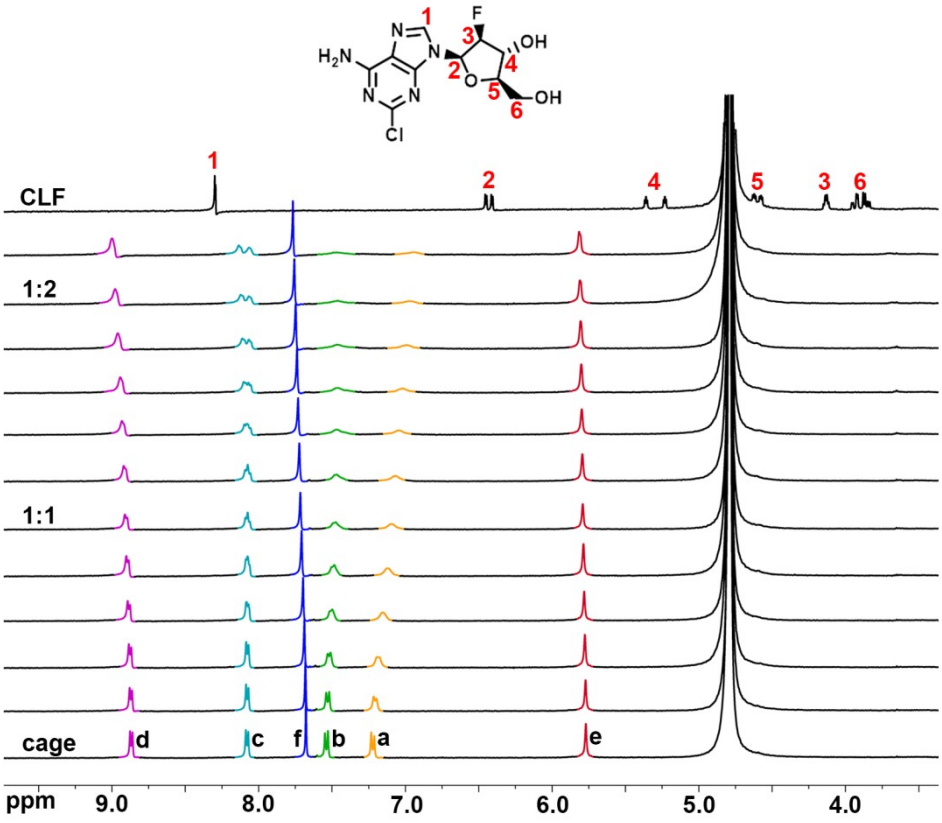


Figure S1. ^{1}H NMR spectra (400 MHz, 298 K, D_2O) recorded for **1**•8Cl^- (0.4 mM) titrated with **CLF** (0-2.5 equiv).

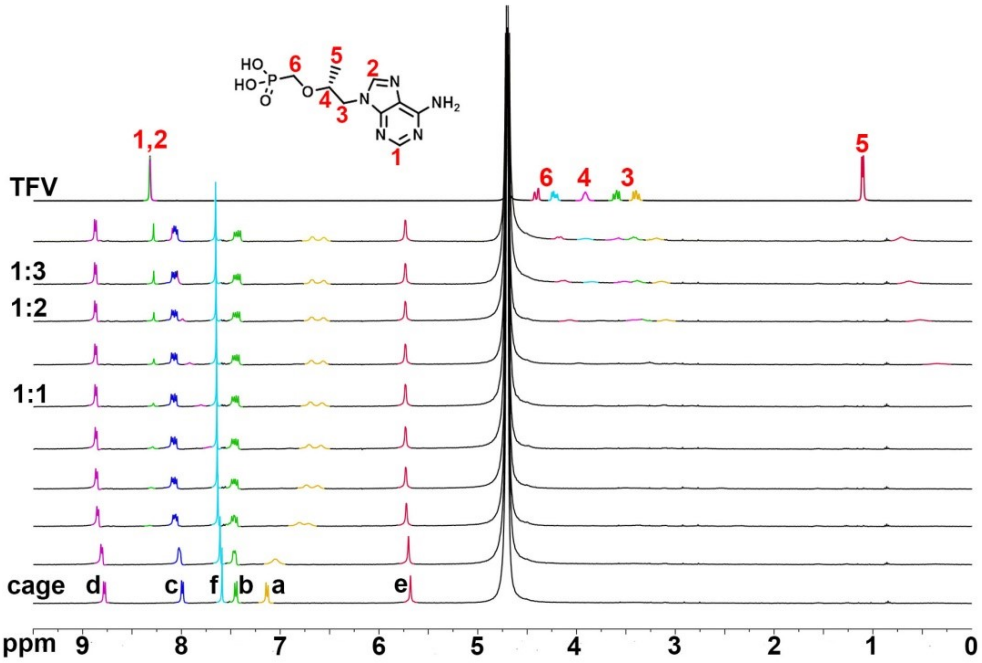


Figure S2. ^{1}H NMR spectra (400 MHz, 298 K, D_2O) recorded for **1**•8Cl^- (0.4 mM) titrated with **TFV** (0-3.5 equiv).

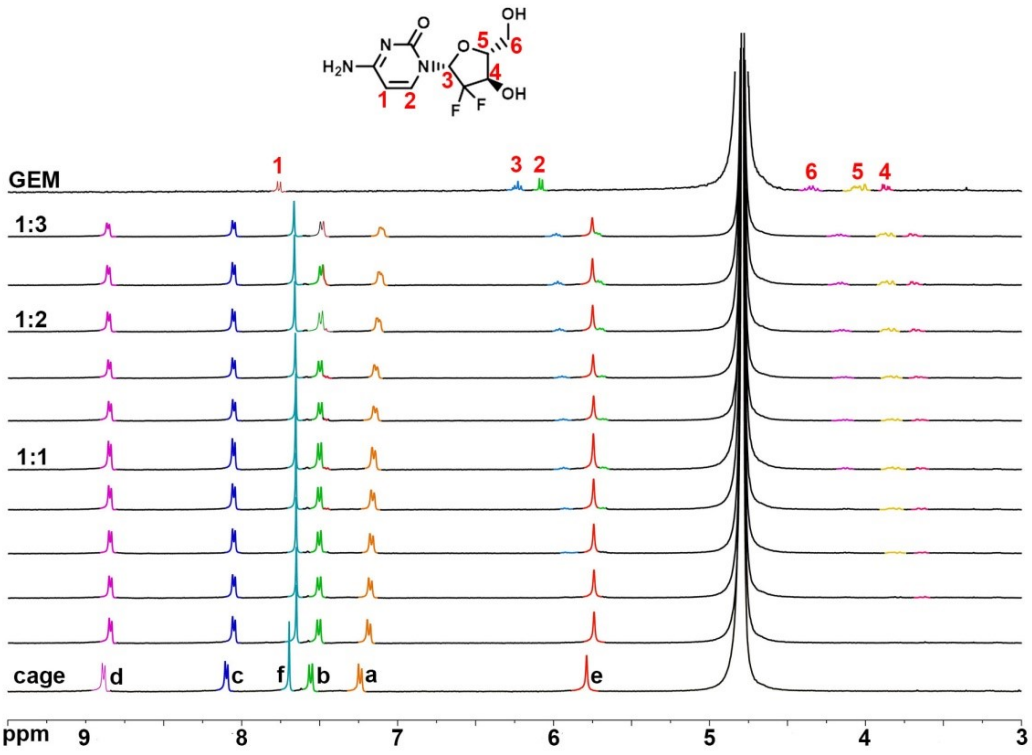


Figure S3. ^{1}H NMR spectra (400 MHz, 298 K, D_2O) recorded for **1**•8Cl^{-} (0.4 mM) titrated with **GEM** (0-3.0 equiv).

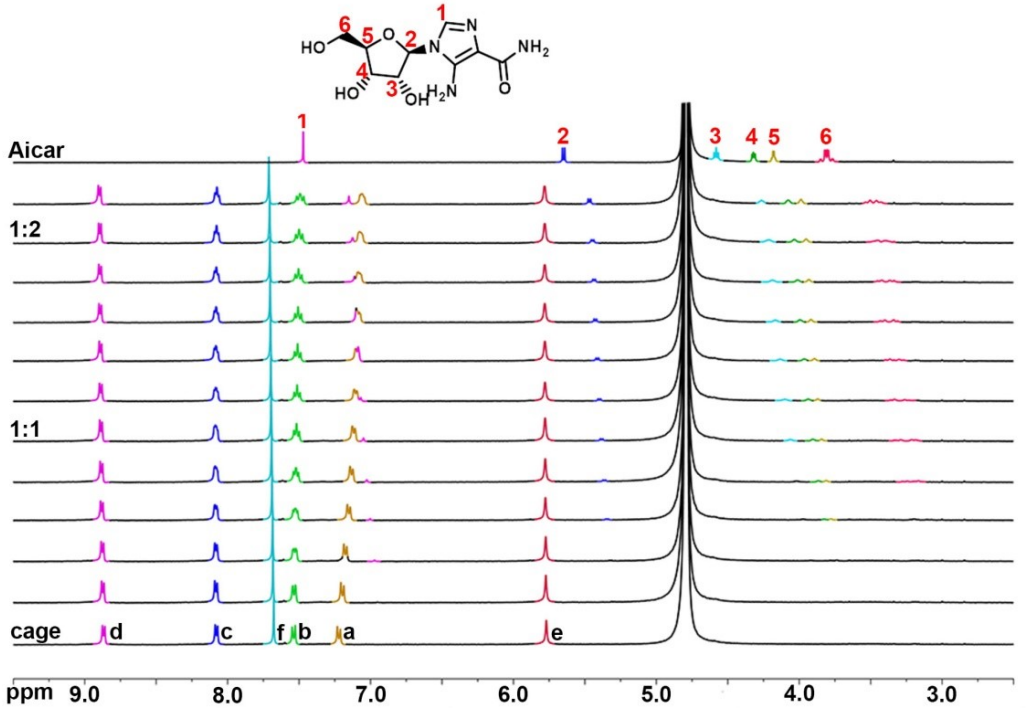


Figure S4. ^{1}H NMR spectra (400 MHz, 298 K, D_2O) recorded for **1**•8Cl^{-} (0.4 mM) titrated with **ACA** (0-2.5 equiv).

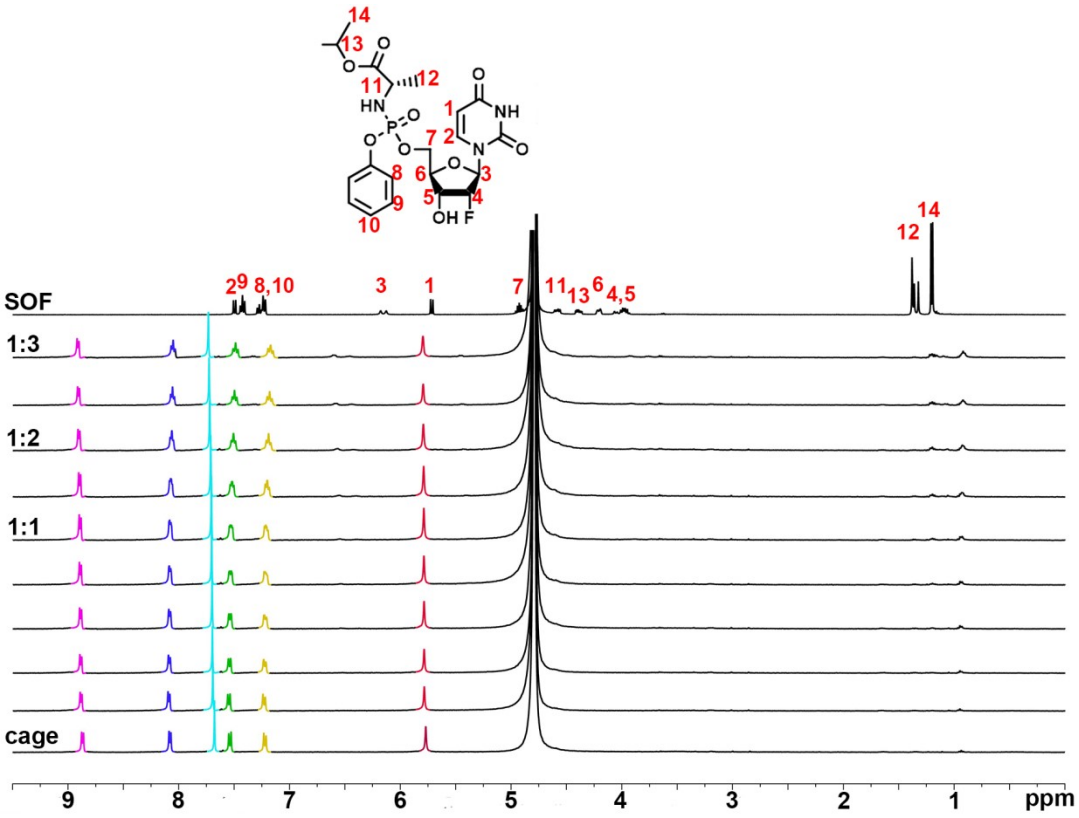


Figure S5. ^{1}H NMR spectra (400 MHz, 298 K, D_2O) recorded for **1**•8Cl$^-$ (0.4 mM) titrated with **SOF** (0-3.0 equiv).

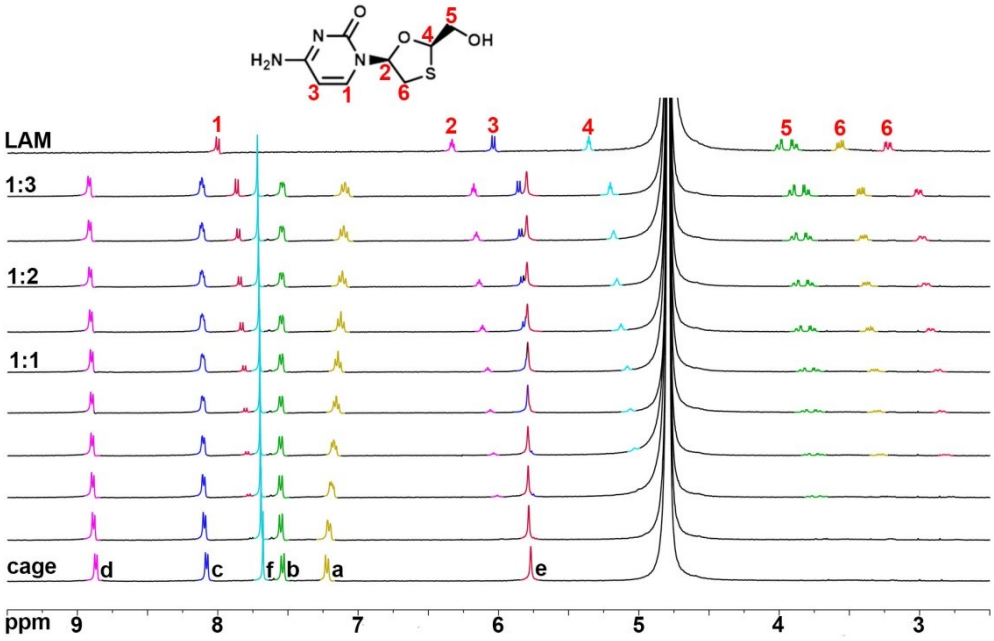


Figure S6. ^{1}H NMR spectra (400 MHz, 298 K, D_2O) recorded for **1**•8Cl$^-$ (0.4 mM) titrated with **LAM** (0-3.0 equiv).

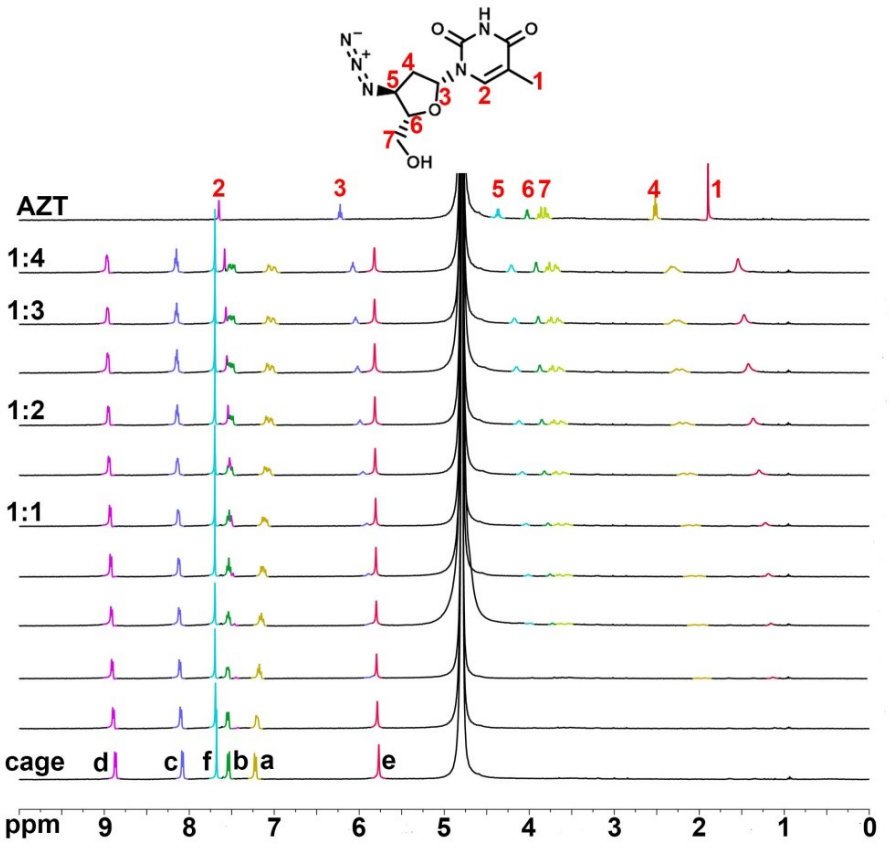
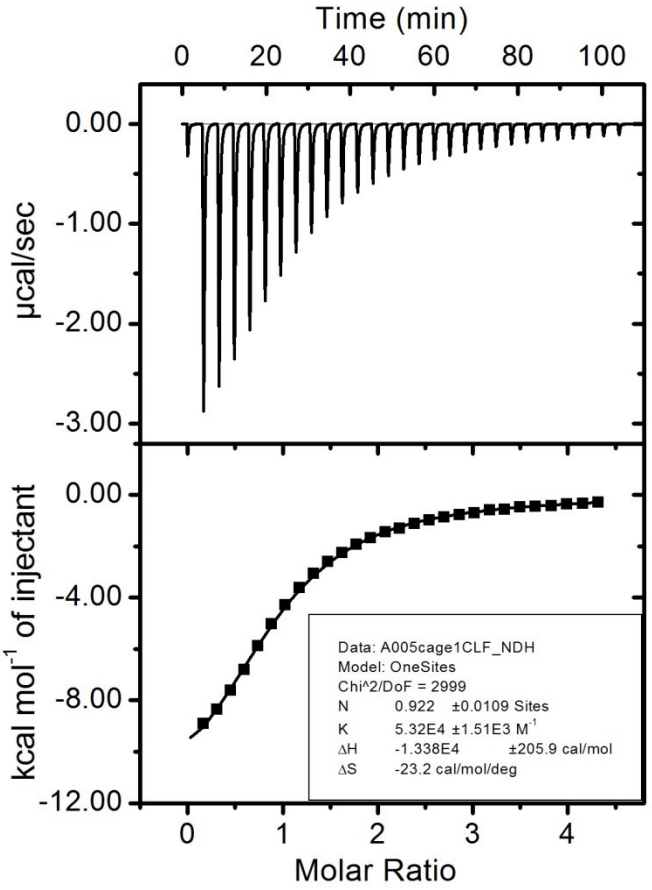
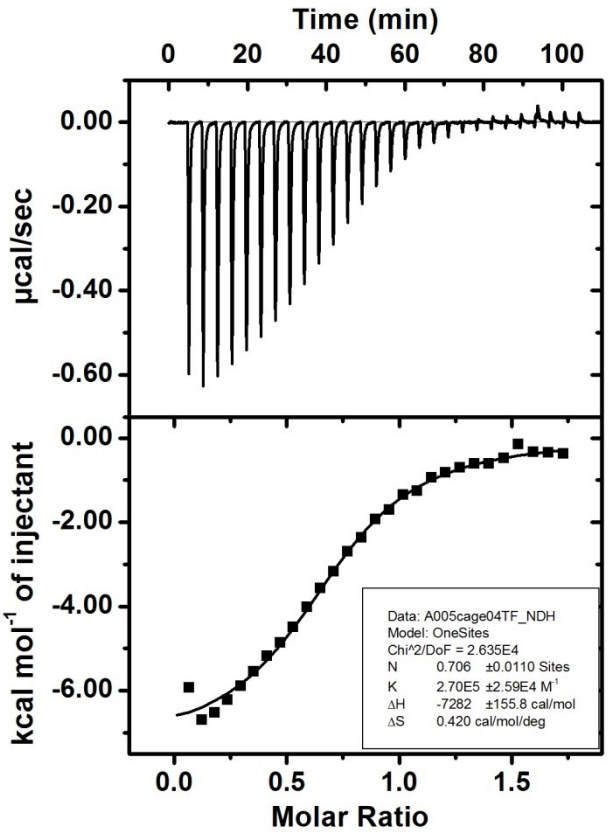


Figure S7. ^{1}H NMR spectra (400 MHz, 298 K, D_2O) recorded for **1**•8Cl^- (0.4 mM) titrated with **AZT** (0-4.0 equiv).



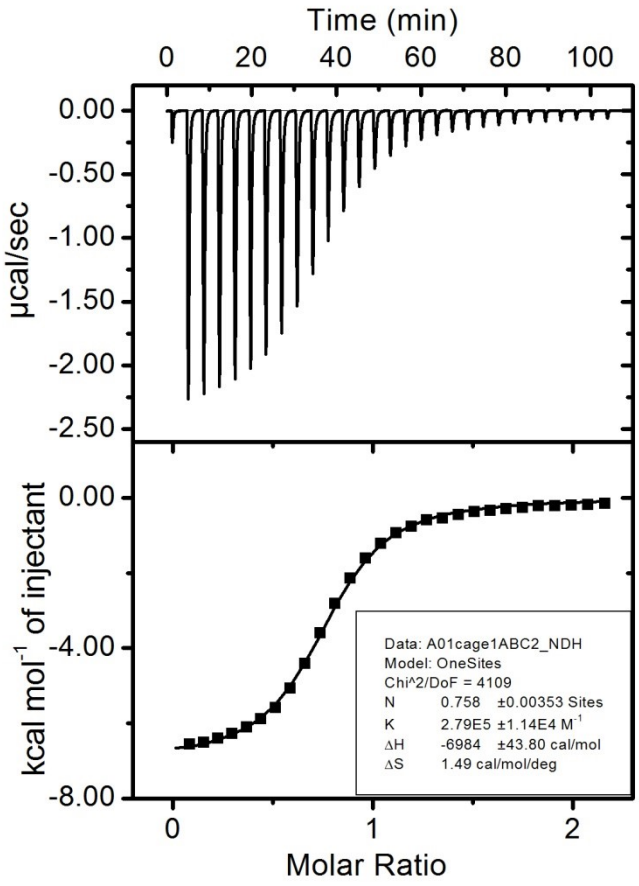
Thermodynamic parameters	1	2	3
K_a (10^4 M^{-1})	5.32 ± 0.15	6.36 ± 0.43	5.83 ± 0.40
ΔH (cal mol^{-1})	-133880 ± 205.9	-13690 ± 684.8	-11400 ± 201.9
ΔS (cal mol^{-1} deg^{-1})	-23.2	-23.9	-16.4

Figure S8. ITC data for the titration of **1**•8Cl^- (0.05 mM) in the cell with a solution of **CLF** (1.0 mM) in the syringe in water at 298K. The thermodynamic parameters for ITC experiments were repeated three times.



Thermodynamic parameters	1	2	3
K_a (10^5 M^{-1})	2.70 ± 0.26	4.00 ± 0.37	4.44 ± 0.31
ΔH (cal mol^{-1})	-7282 ± 155.8	-7950 ± 151.8	-7093 ± 87.80
ΔS (cal mol^{-1} deg^{-1})	0.420	1.03	2.05

Figure S9. ITC data for the titration of **1**•8Cl^- (0.05 mM) in the cell with a solution of **TFV** (0.4 mM) in the syringe in water at 298K. The thermodynamic parameters for ITC experiments were repeated three times.



Thermodynamic parameters	1	2	3
K_a (10^5 M^{-1})	2.79 ± 0.11	3.28 ± 0.12	3.78 ± 0.18
ΔH (cal mol^{-1})	-6984 ± 43.80	-6914 ± 36.15	-7315 ± 68.52
ΔS (cal mol^{-1} deg^{-1})	1.49	2.05	0.985

Figure S10. ITC data for the titration of **1** (0.1 mM) in the cell with a solution of **ABC** (1.0 mM) in the syringe in water at 298K. The thermodynamic parameters for ITC experiments were repeated three times.

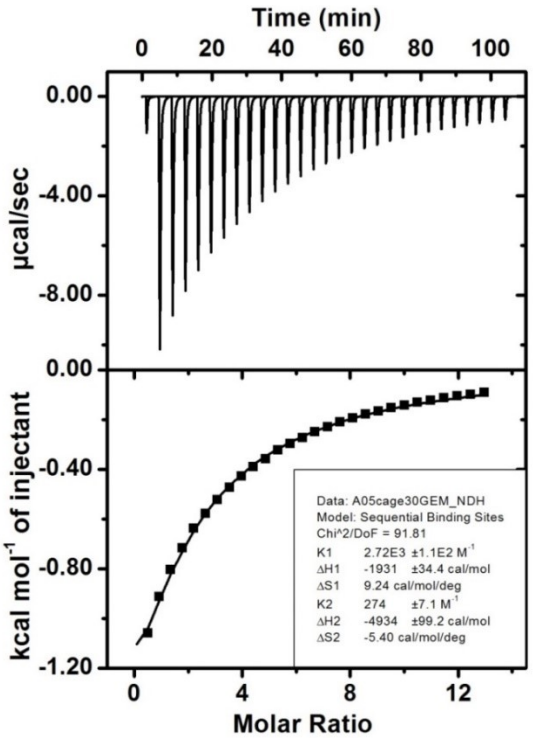


Figure S11. ITC data for the titration of **1**•8Cl⁻ (0.5 mM) in the cell with a solution of **GEM** (1.0 mM) in the syringe in water at 298K. Continuous mode simulation is used.

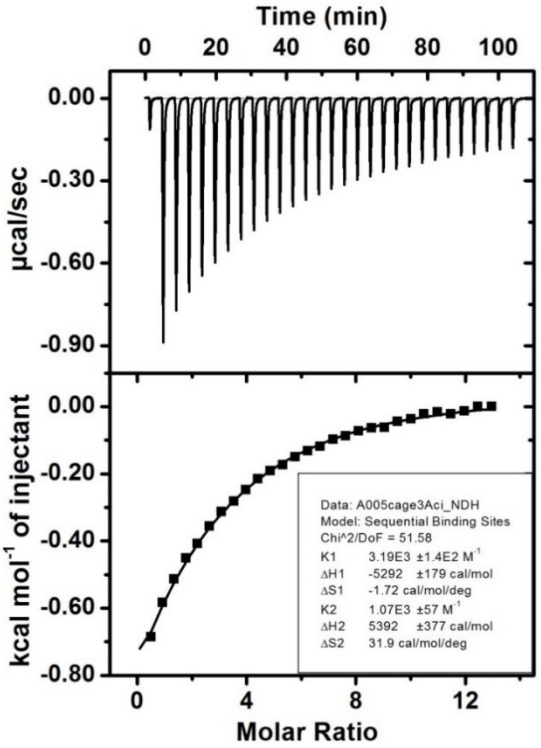


Figure S12. ITC data for the titration of **1**•8Cl⁻ (0.05 mM) in the cell with a solution of **ACA** (3.0 mM) in the syringe in water at 298K. Continuous mode simulation is used.

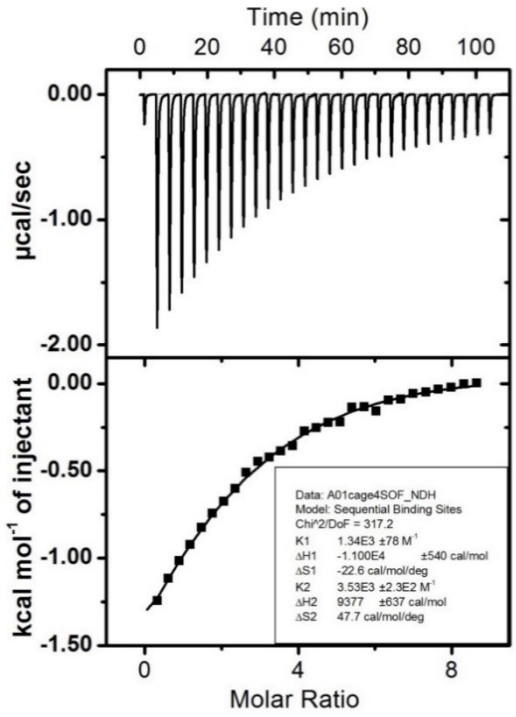


Figure S13. ITC data for the titration of **1**•8Cl⁻ (0.1 mM) in the cell with a solution of **SOF** (4.0 mM) in the syringe in water at 298K. Continuous mode simulation is used.

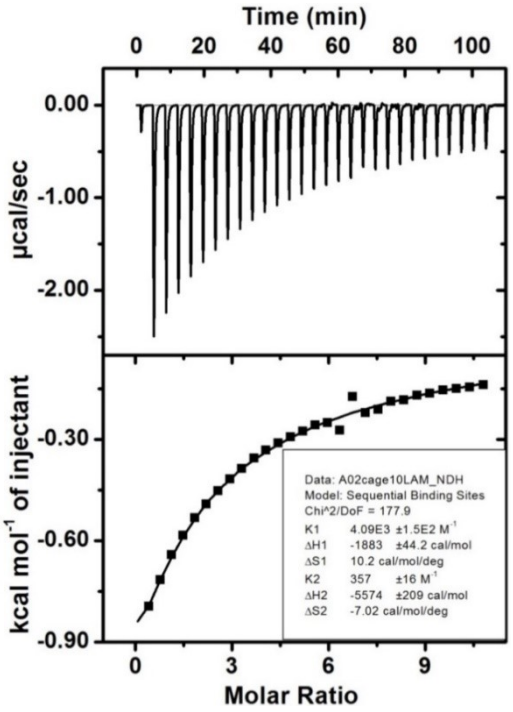


Figure S14. ITC data for the titration of **1**•8Cl⁻ (0.2 mM) in the cell with a solution of **LAM** (10.0 mM) in the syringe in water at 298K. Continuous mode simulation is used.

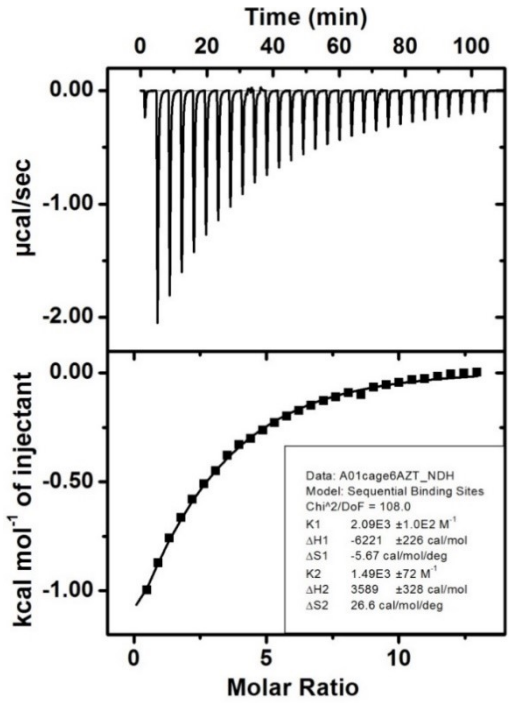


Figure S15. (a) ITC data for the titration of **1**•8Cl^- (0.1 mM) in the cell with a solution of **AZT** (6.0 mM) in the syringe in water at 298K. Continuous mode simulation is used.

Table S1. The binding constants (K_a) and thermodynamic parameters for the 1:1 host-guest complexes of **1** and drugs in H_2O at 298 K.

	K **(×10^5 M^{-1})**	**ΔH (kcal mol^{-1})**	**ΔS (cal mol^{-1} K^{-1})**	**ΔG (kcal mol^{-1})**
1⊃CLF	0.58 ± 0.04	-12.8 ± 1.0	-21.2 ± 3.4	-6.5 ± 0.04
1⊃TFV	3.71 ± 0.74	-7.44 ± 0.37	1.17 ± 0.67	-7.79 ± 0.35
1⊃ABC	3.28 ± 0.40	-7.07 ± 0.17	1.51 ± 0.44	-7.5 ± 0.07

Table S2. The binding constants (K_a) and thermodynamic parameters for the 1:2 host-guest complexes of **1** and drugs in H_2O at 298 K.

	K **(×10³ M⁻¹)**	**ΔH (kcal mol⁻¹)**	**ΔS (cal mol⁻¹ K⁻¹)**	**ΔG (kcal mol⁻¹)**
1⊃GEM$_2$	K_1 = 2.72 ± 0.11	-1.93±0.03	9.24	-4.69
	K_2 = 0.27 ± 0.07	-4.93±0.10	-5.40	-3.32
1⊃ACA$_2$	K_1 = 3.19 ± 0.14	-5.29 ± 0.18	-1.72	-4.78
	K_2 = 1.07 ± 0.06	5.39 ± 0.38	31.9	-4.13
1⊃SOF$_2$	K_1 = 1.34 ± 0.08	-11.0 ± 0.54	-22.6	-4.27
	K_2 = 3.53 ± 0.23	9.38 ± 0.64	47.7	-4.84
1⊃LAM$_2$	K_1 = 4.09 ± 0.15	-1.88 ± 0.04	10.2	-4.93
	K_2 = 0.36 ± 0.02	-5.57 ± 0.21	-7.02	-3.49
1⊃AZT$_2$	K_1 = 2.09 ± 0.10	-6.22 ± 0.23	-5.67	-4.53
	K_2 = 1.49 ± 0.07	3.59 ± 0.33	26.6	-4.33

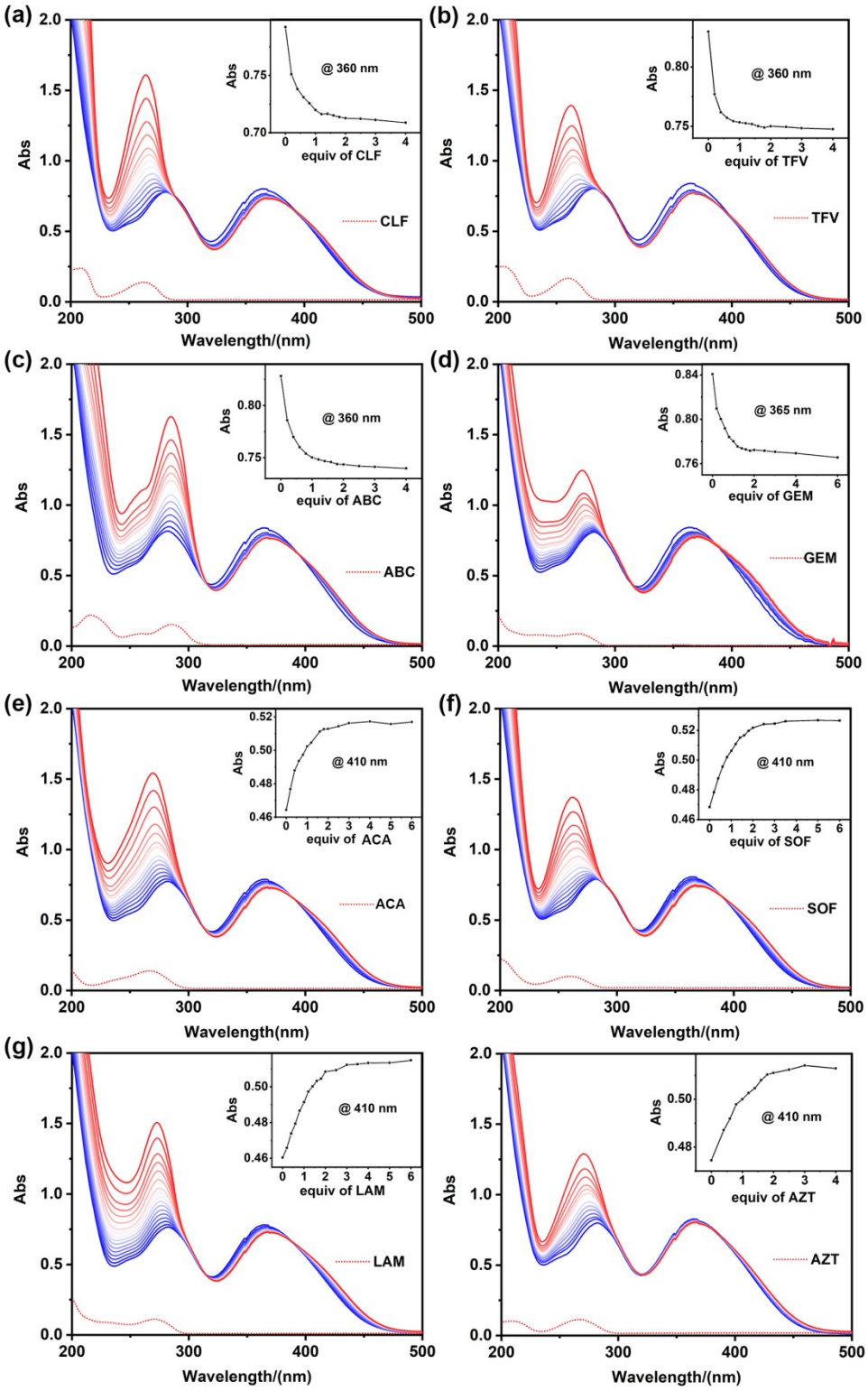


Figure S16. UV-vis spectra of **1**•8Cl^- (10 μM) with different guests: (a) **CLF**, (b) **TFV**, (c) **ABC**, (d) **GEM**, (e) **ACA**, (f) **SOF**, (g) **LAM**, and (h) **AZT**. Insert: Plots of Abs vs the equiv of guests, respectively.

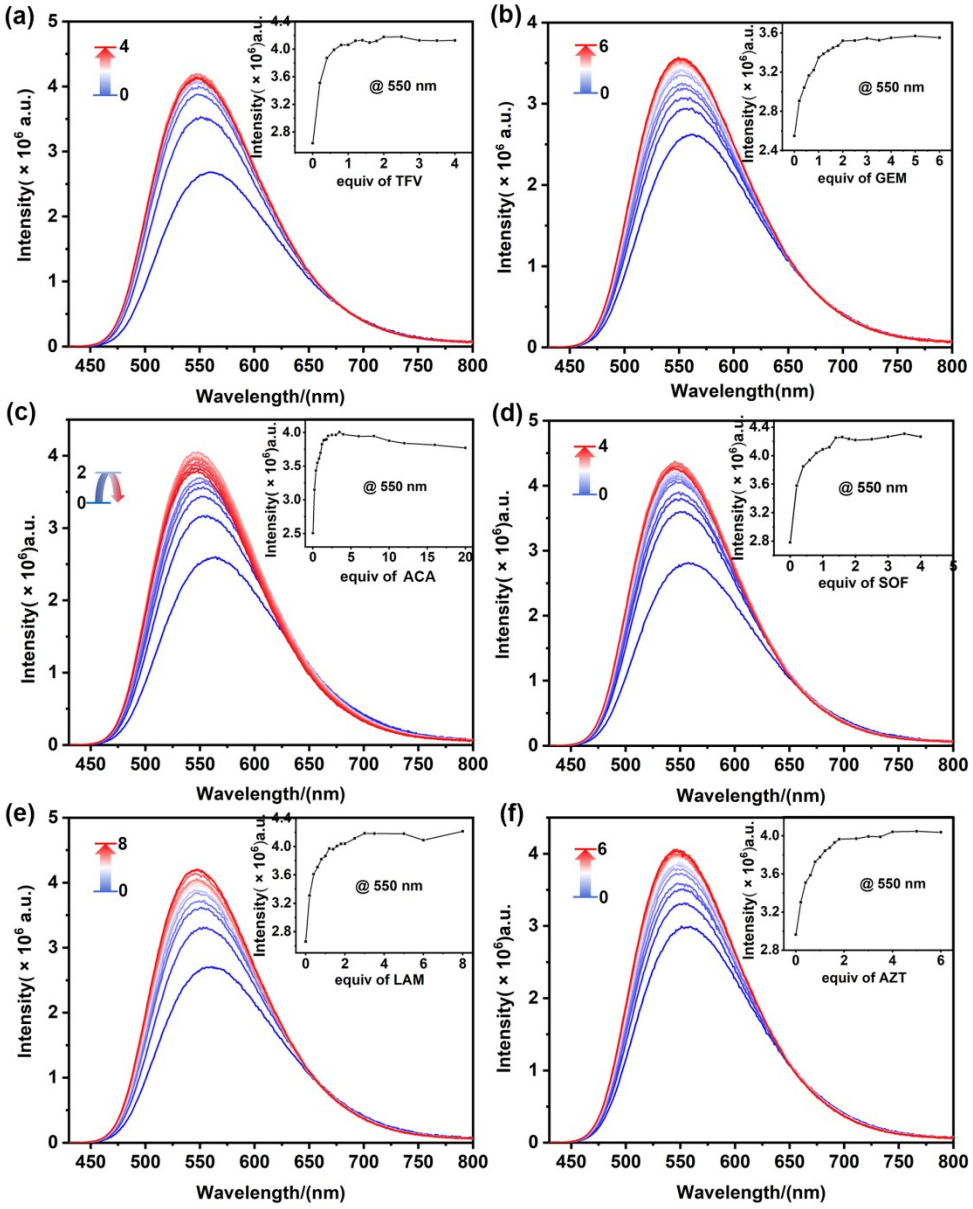


Figure S17. Fluorescence spectra of 1 **1**•8Cl⁻ (10 μM) with different guests: (a) **TFV**, (b) **GEM**, (c) **ACA**, (d) **SOF**, (e) **LAM**, and (f) **AZT**. Inset: Plots of fluorescence intensity vs the equiv of guests, respectively.

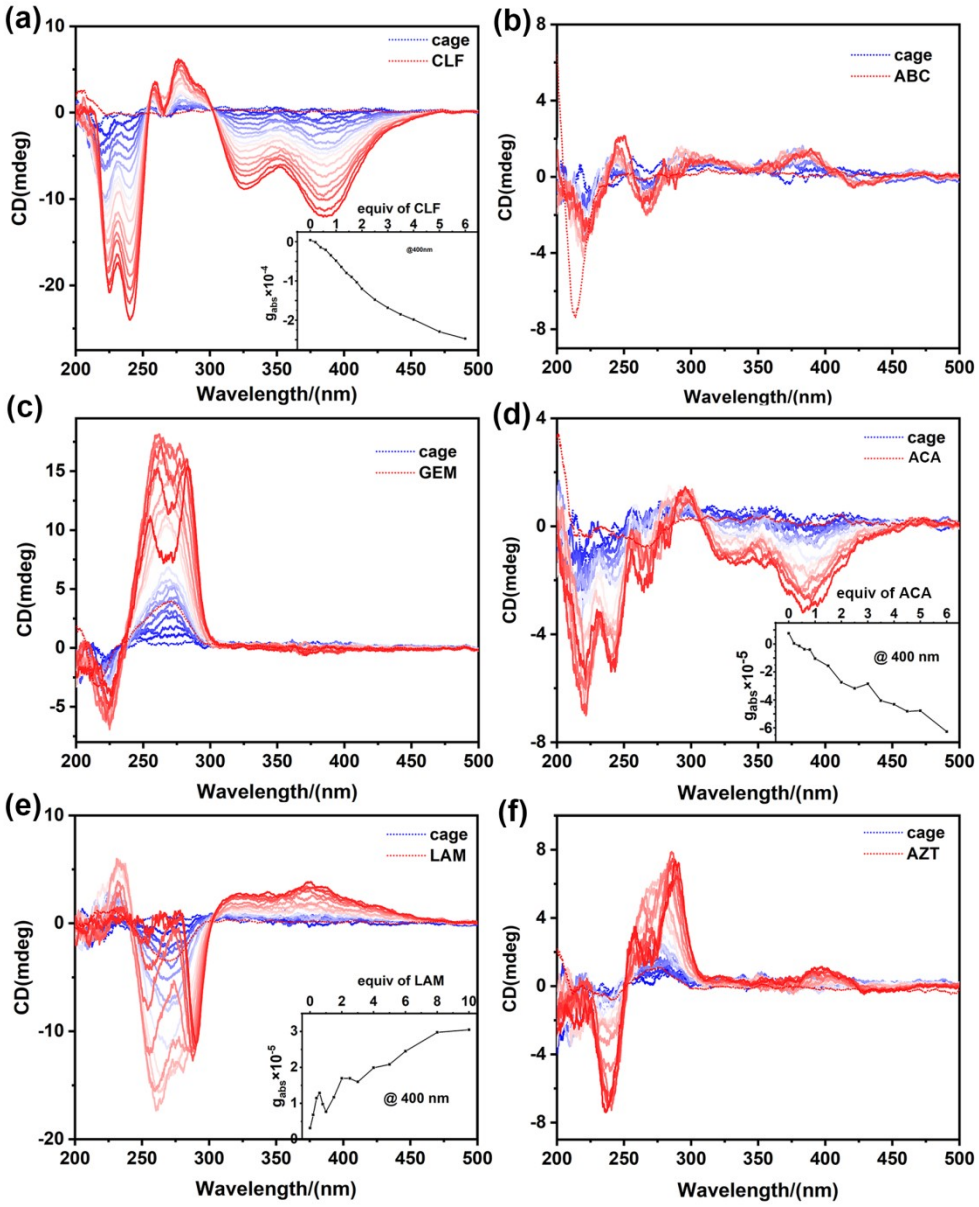


Figure S18. CD titration of **1**•8Cl^- (20 μM) with different guests: (a) **CLF**, (b) **ABC**, (c) **GEM**, (d) **ACA**, (e) **LAM**, and (f) **AZT**. Inset: Plots of g_{abs} versus the equiv of guests.

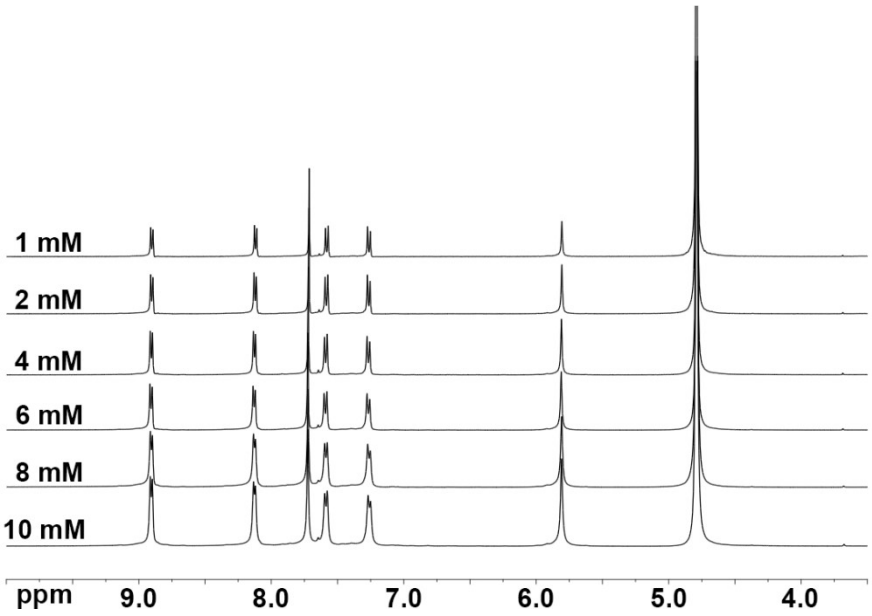


Figure S19. [1] NMR recorded at different concentrations of **1** (400 MHz, 298K).

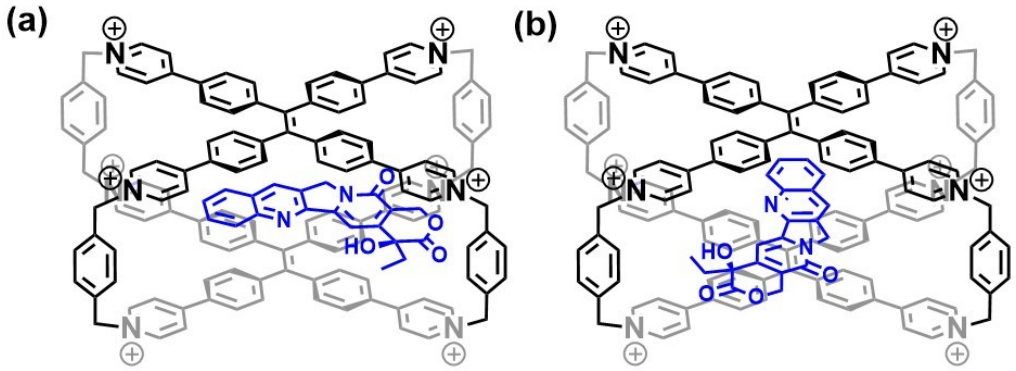


Figure S20. Two possible structures of **1⊃CPT**: (a) parallel and (b) vertical positions.

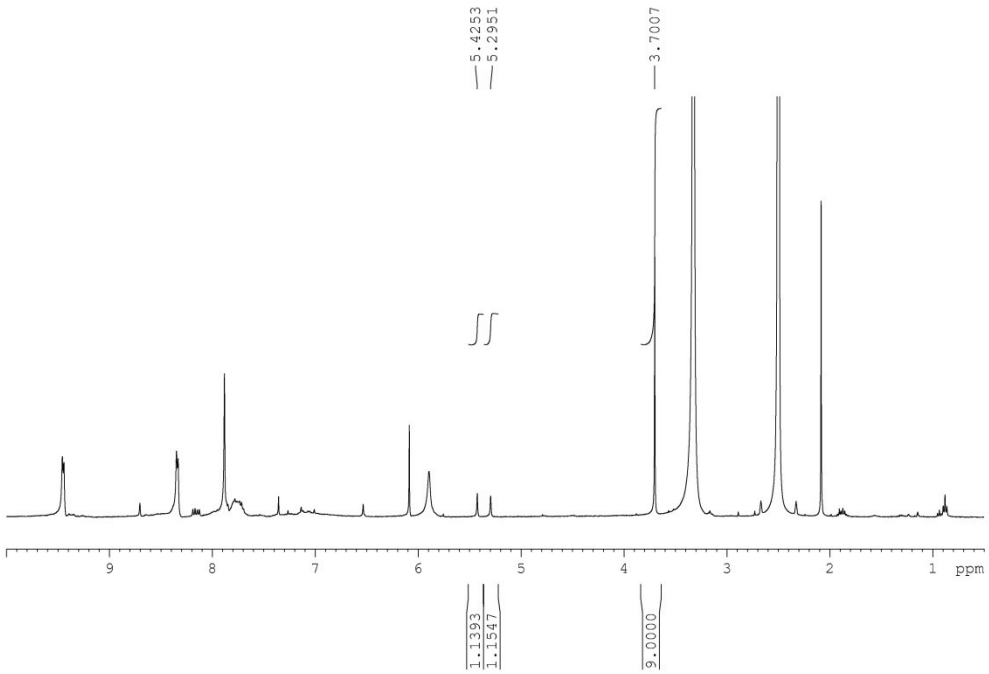


Figure S21. [1]H NMR recorded for Camptothecin with **1** (16.12 mM) (400 MHz, 20 mM 1,3,5-trimethoxy-benzen as reference).

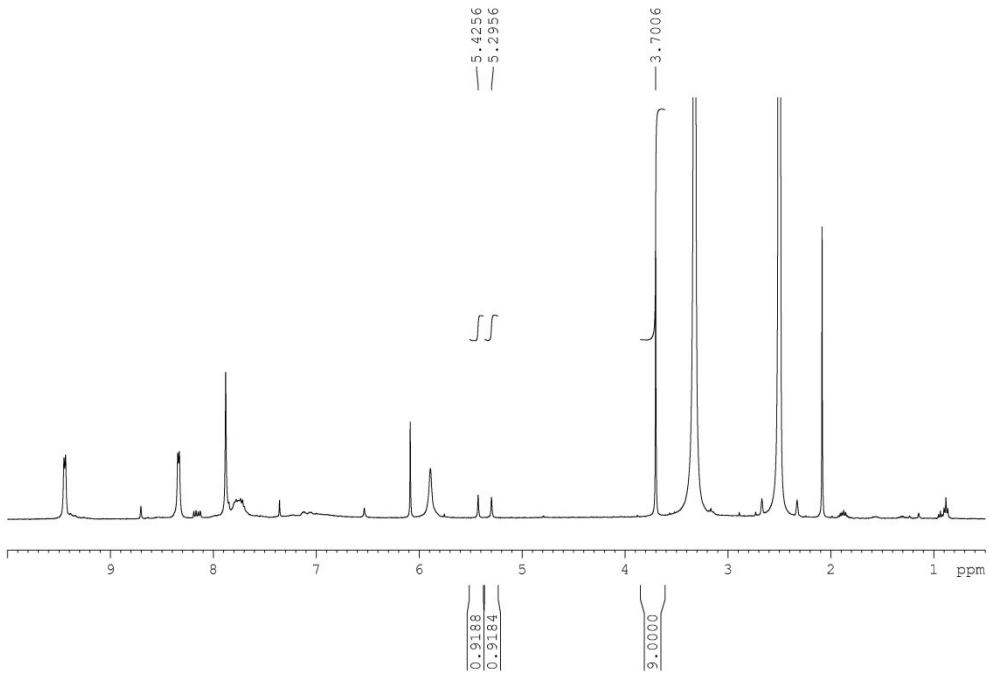


Figure S22. [1]H NMR recorded for Camptothecin with **1** (13.26 mM) (400 MHz, 20 mM 1,3,5-trimethoxy-benzen as reference).

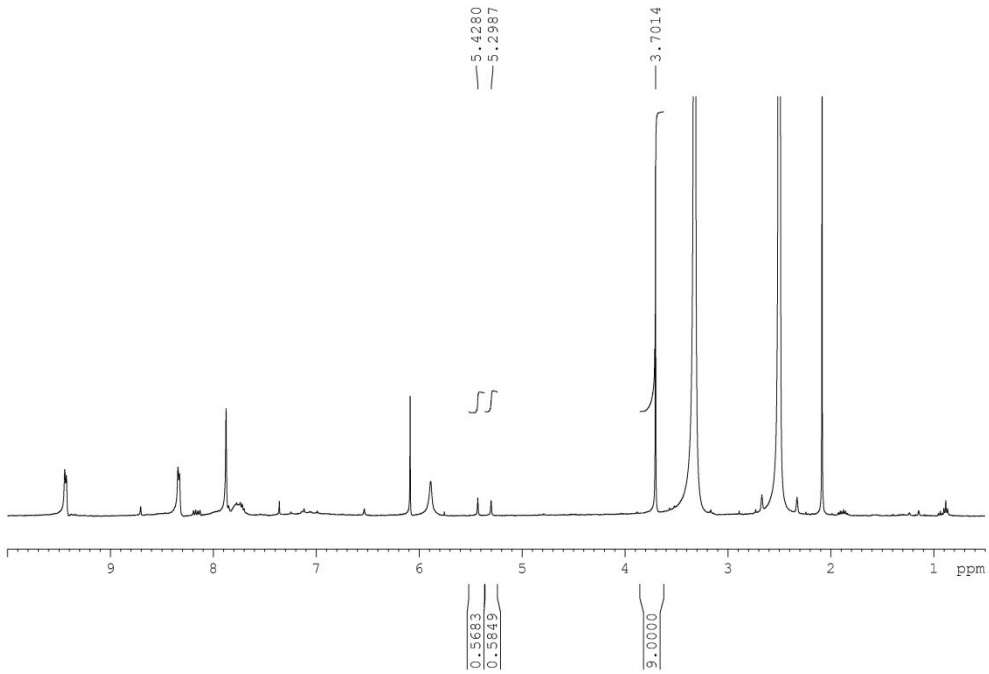


Figure S23. [1]H NMR recorded for Camptothecin with **1** (8.06 mM) (400 MHz, 20 mM 1,3,5-trimethoxy-benzen as reference).

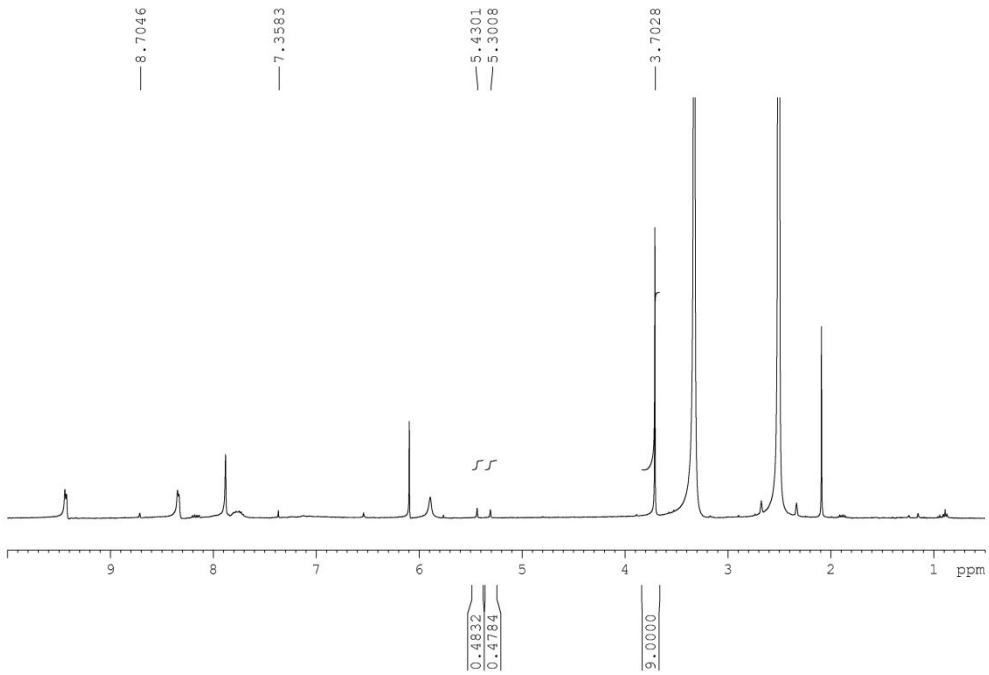


Figure S24. [1]H NMR recorded for Camptothecin with **1** (6.63 mM) (400 MHz, 20 mM 1,3,5-trimethoxy-benzen as reference).

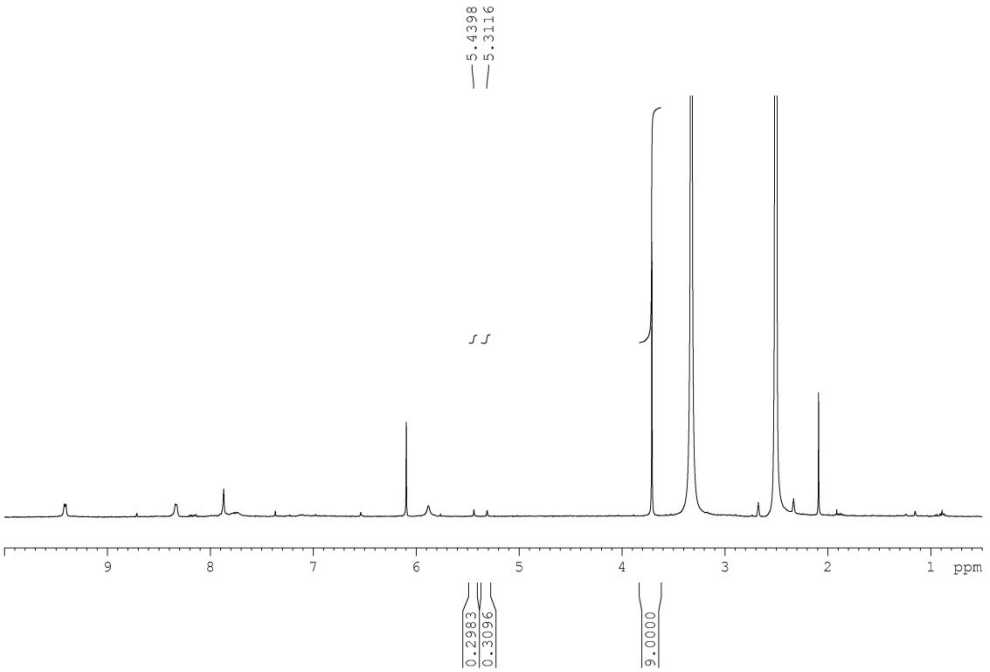


Figure S25. [1]H NMR recorded for Camptothecin with **1** (4.03 mM) (400 MHz, 20 mM 1,3,5-trimethoxy-benzen as reference).

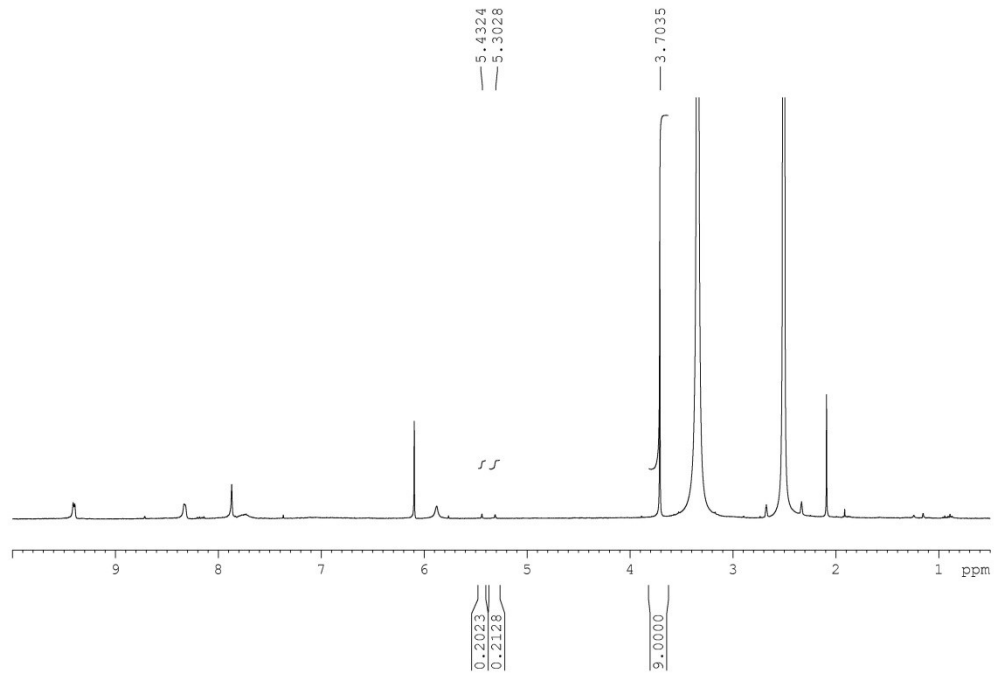


Figure S26. [1]H NMR recorded for Camptothecin with **1** (3.315 mM) (400 MHz, 20 mM 1,3,5-trimethoxy-benzen as reference).

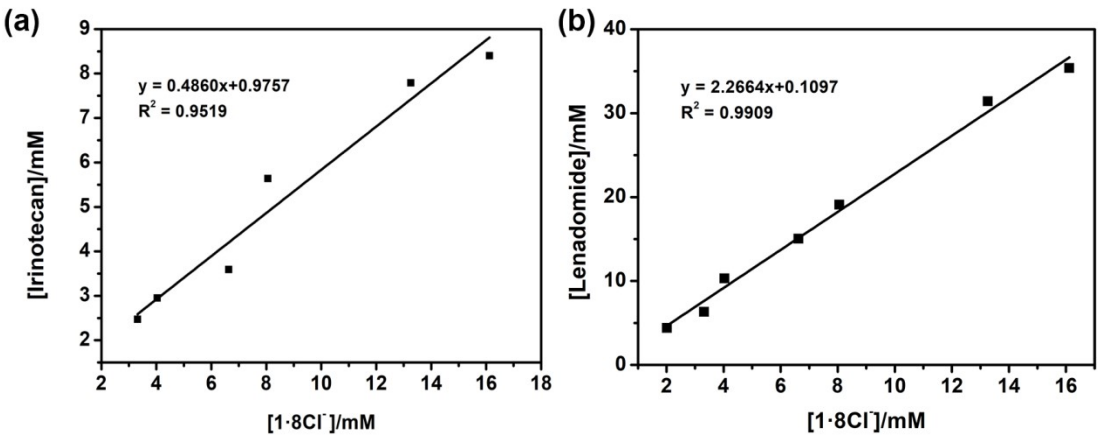


Figure S27. PSD of (a) **CPT-11** and (b) **LEN** with **1**•8Cl^- (400 MHz, DMSO, 1,3,5-trimethoxy-benzen as reference).

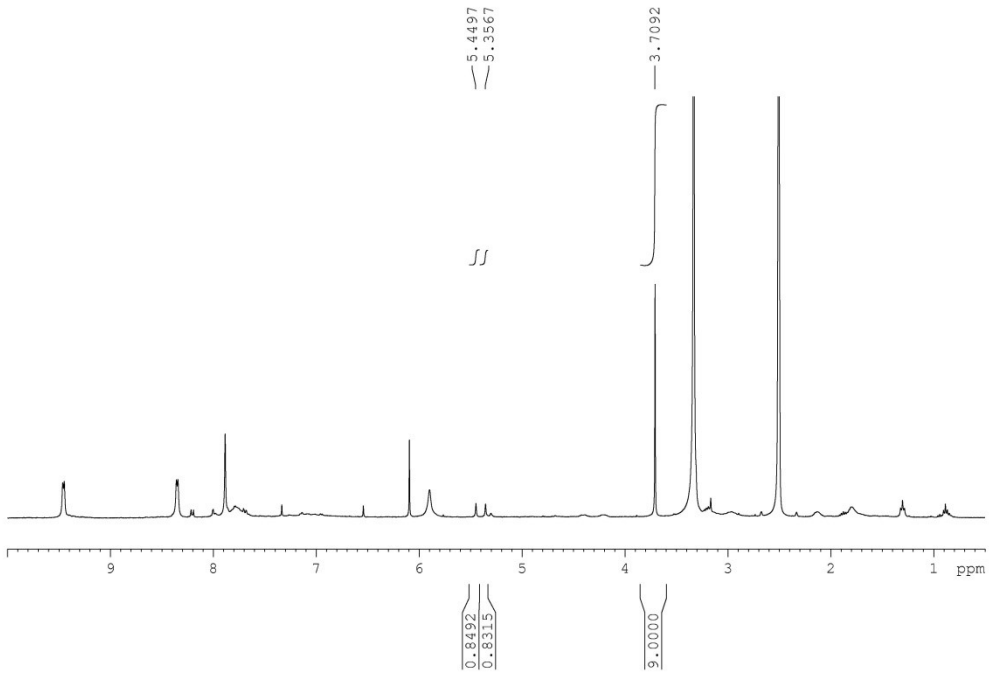


Figure S28. ^{1}H NMR recorded for Irinotecan with **1** (16.12 mM) (400 MHz, 20 mM, 1,3,5-trimethoxy-benzen as reference).

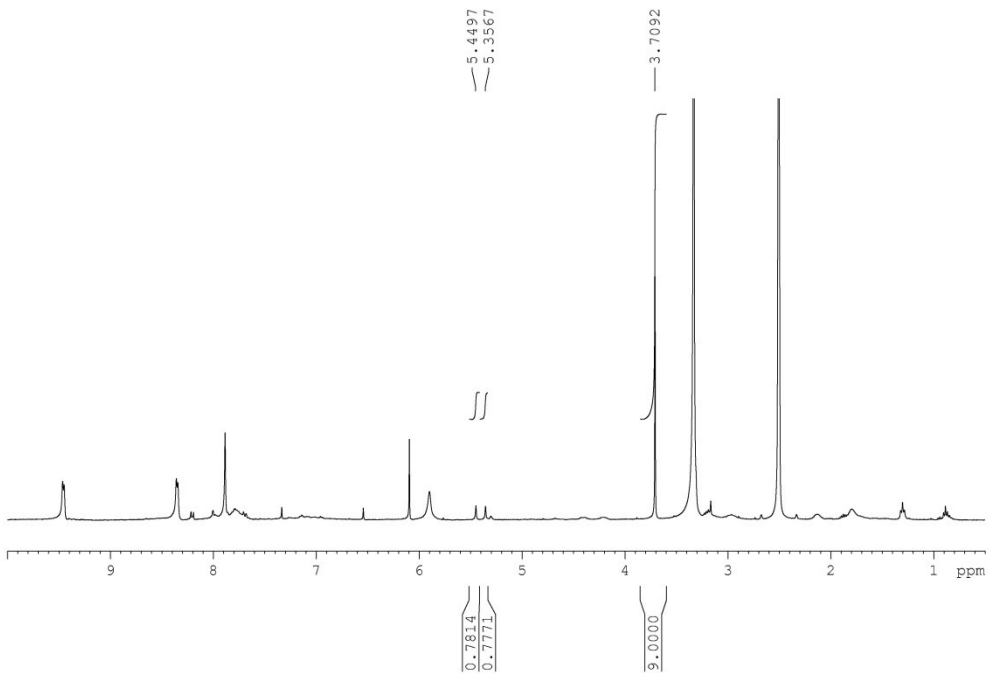


Figure S29. [1]H NMR recorded for Irinotecan with **1** (13.26 mM) (400 MHz, 20 mM, 1,3,5-trimethoxy-benzen as reference).

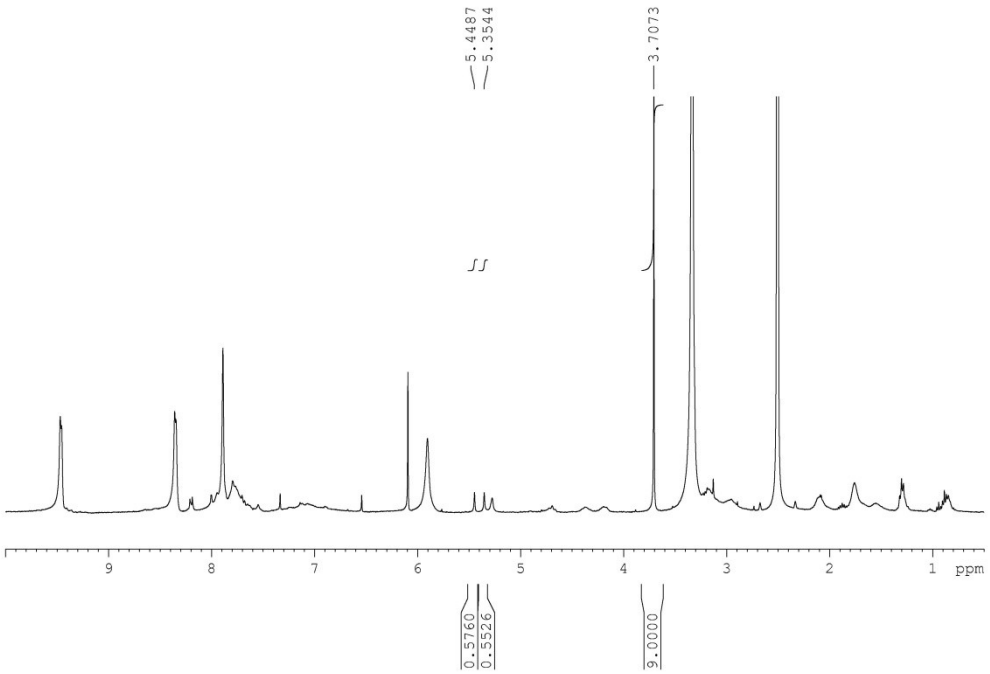


Figure S30. [1]H NMR recorded for Irinotecan with **1** (8.06 mM) (400 MHz, 20 mM, 1,3,5-trimethoxy-benzen as reference).

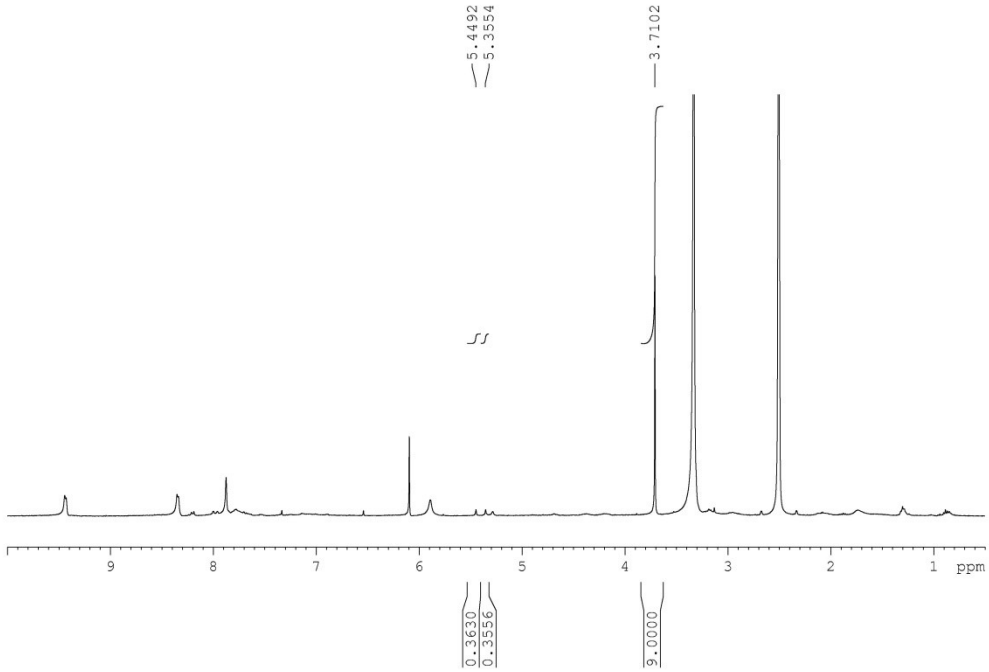


Figure S31. [1]H NMR recorded for Irinotecan with **1** (6.63 mM) (400 MHz, 20 mM, 1,3,5-trimethoxy-benzen as reference).

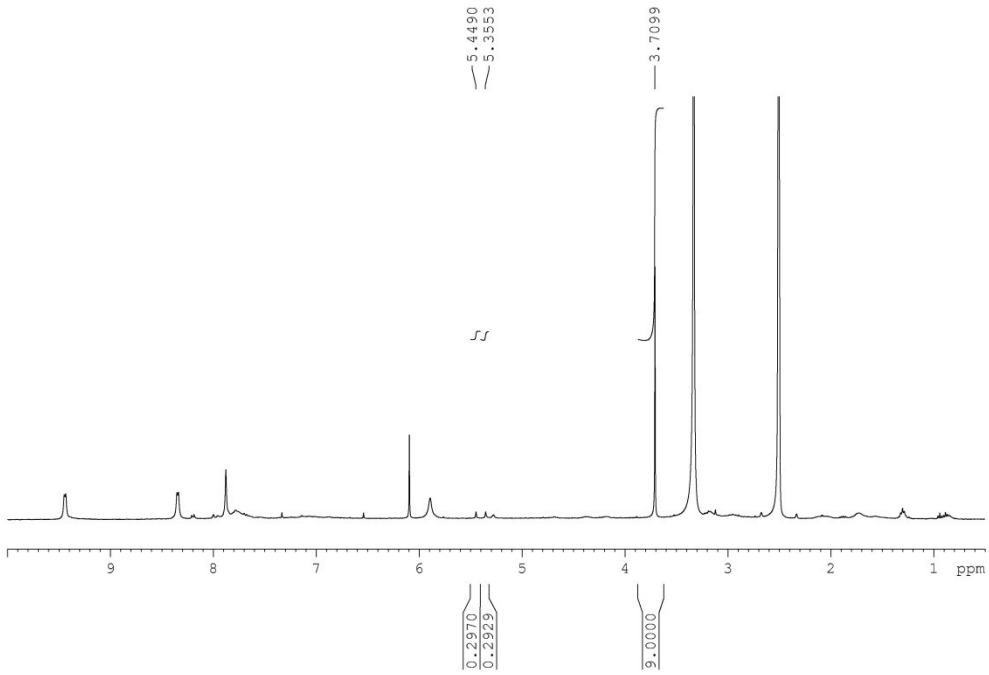


Figure S32. [1]H NMR recorded for Irinotecan with **1** (4.03 mM) (400 MHz, 20 mM, 1,3,5-trimethoxy-benzen as reference).

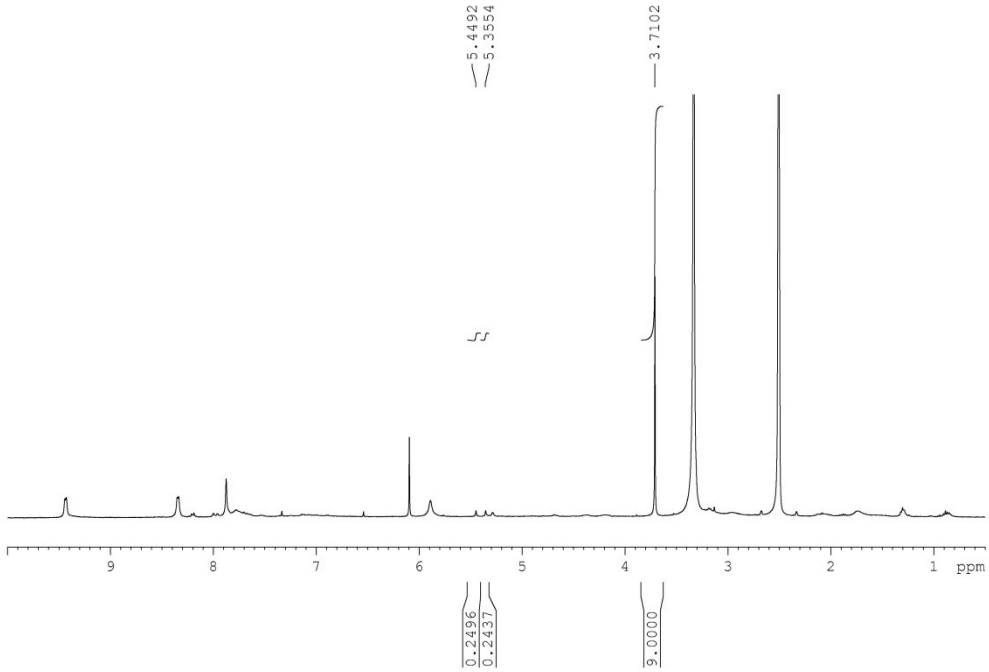


Figure S33. [1]H NMR recorded for Irinotecan with **1** (3.315 mM) (400 MHz, 20 mM, 1,3,5-trimethoxy-benzen as reference).

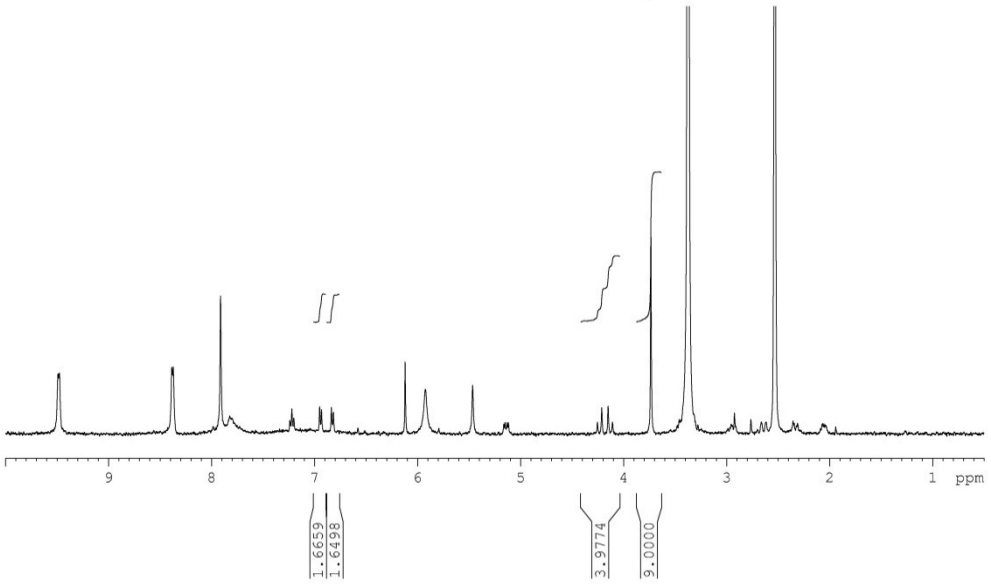


Figure S34. [1]H NMR recorded for Lenalidomide with **1** (16.12 mM) (400 MHz, 20 mM, 1,3,5-trimethoxy-benzen as reference).

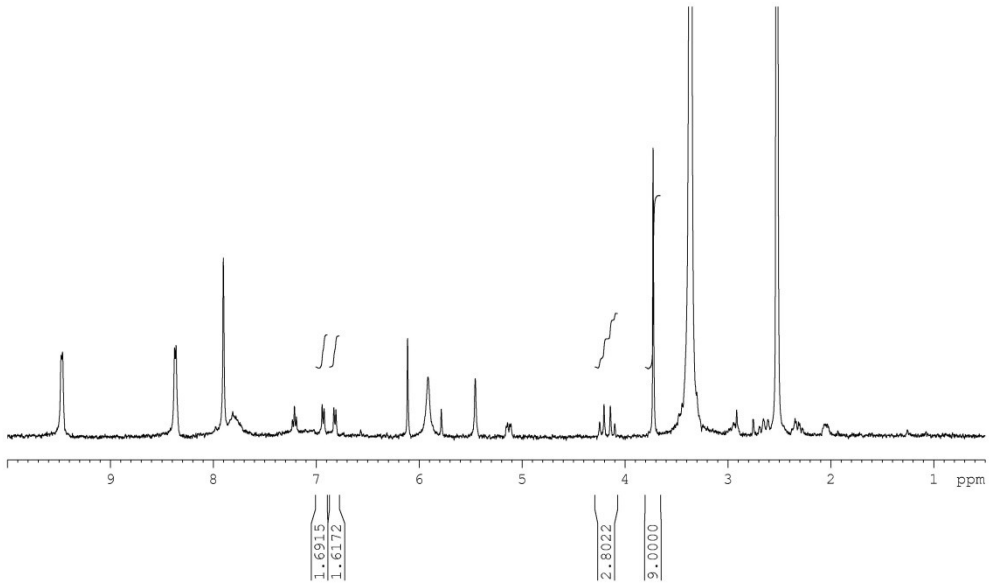


Figure S35. ^{1}H NMR recorded for Lenalidomide with **1** (13.26 mM) (400 MHz, 20 mM, 1,3,5-trimethoxy-benzen as reference).

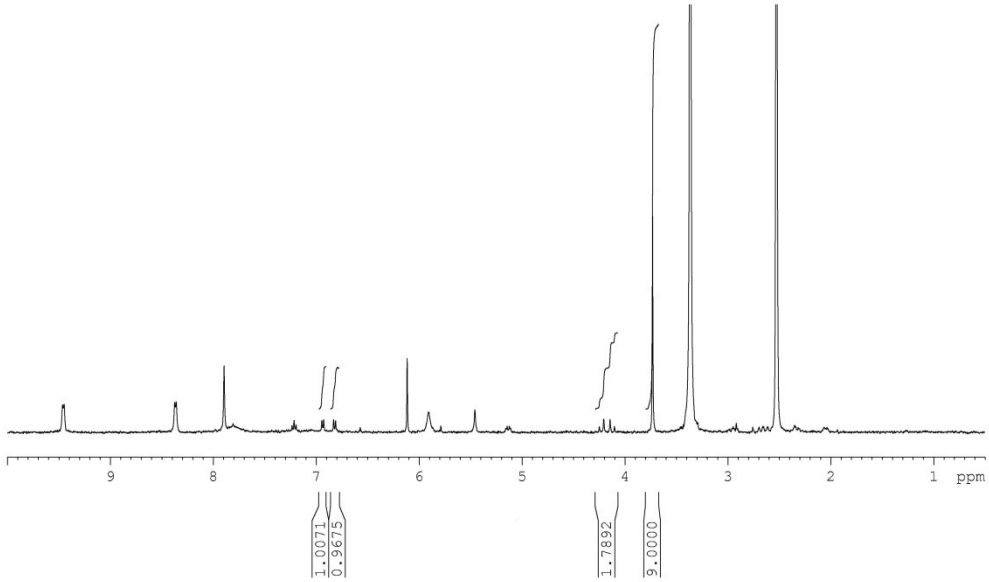


Figure S36. [1]H NMR recorded for Lenalidomide with **1** (8.06 mM) (400 MHz, 20 mM, 1,3,5-trimethoxy-benzen as reference).

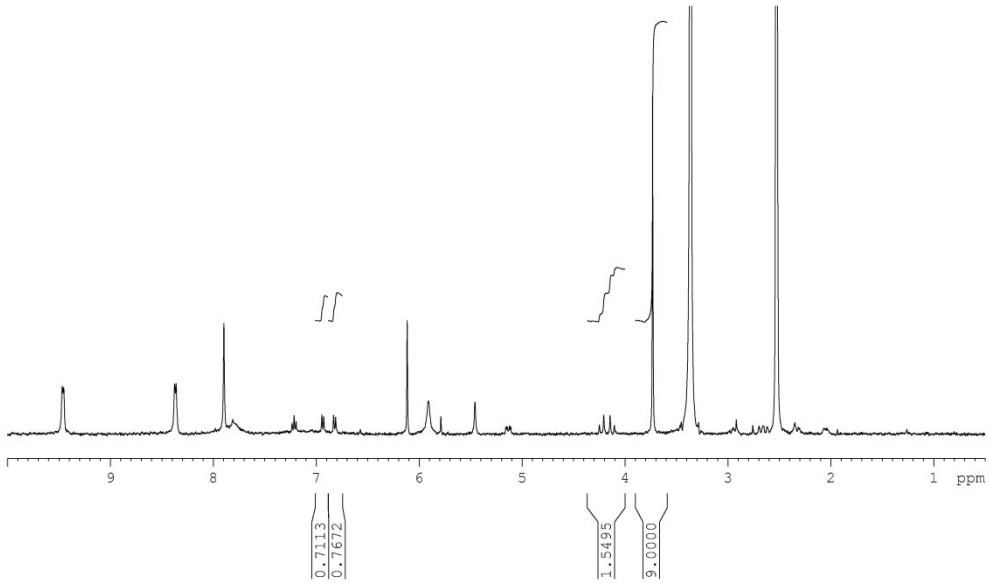


Figure S37. [1]H NMR recorded for Lenalidomide with **1** (6.63 mM) (400 MHz, 20 mM, 1,3,5-trimethoxy-benzen as reference).

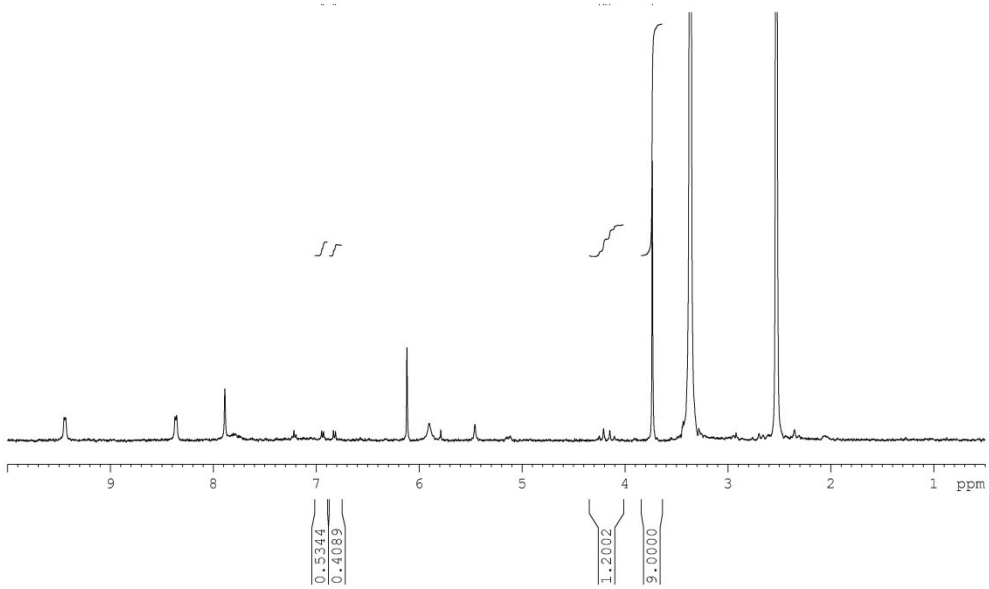


Figure S38. [1]H NMR recorded for Lenalidomide with **1** (4.03 mM) (400 MHz, 20 mM, 1,3,5-trimethoxy-benzen as reference).

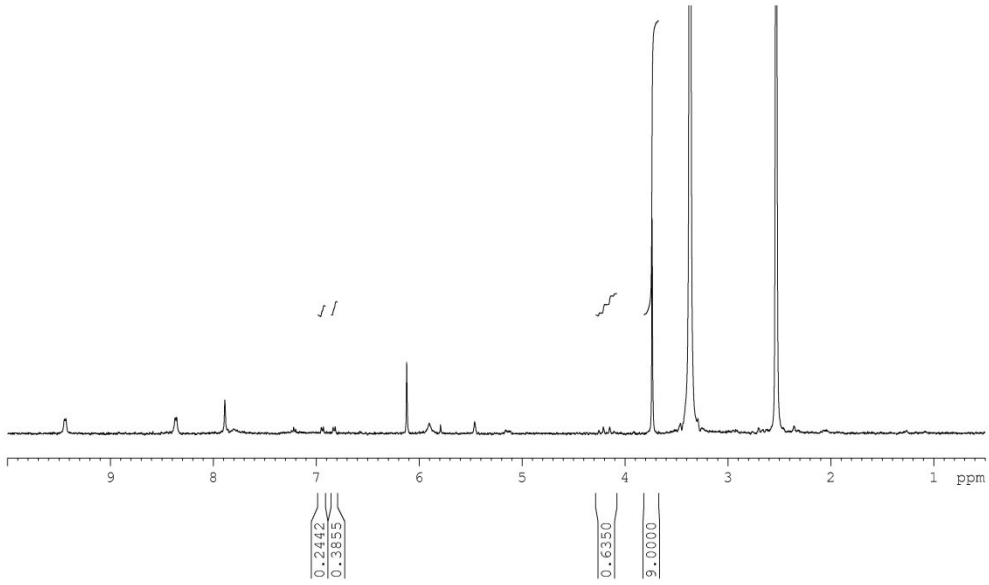


Figure S39. [1]H NMR recorded for Lenalidomide with **1** (3.315 mM) (400 MHz, 20 mM, 1,3,5-trimethoxy-benzen as reference).

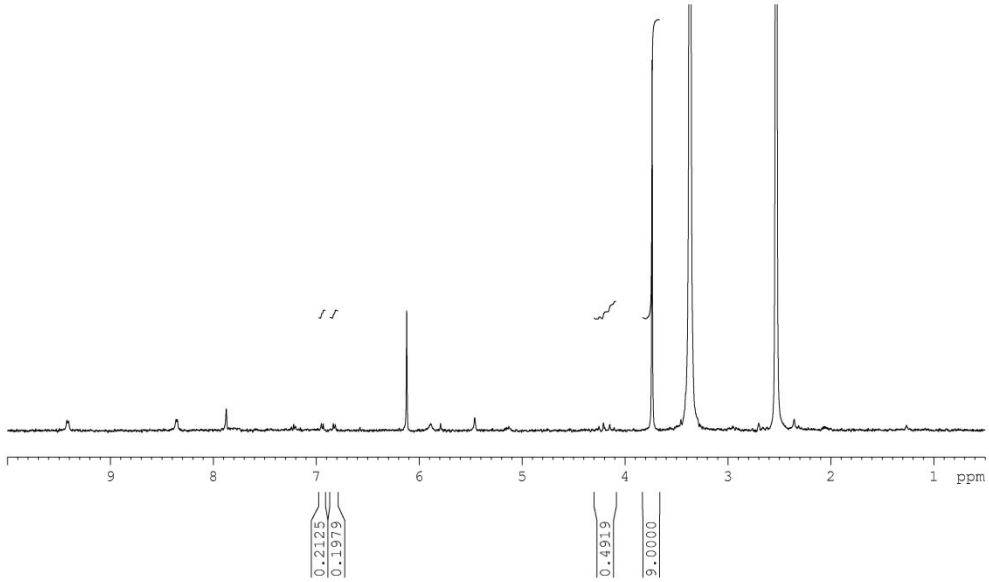


Figure S40. ^{1}H NMR recorded for Lenalidomide with **1** (2.015 mM) (400 MHz, 20 mM, 1,3,5-trimethoxy-benzen as reference).