Supporting Information for:

Phenoxide-bridged Zinc(II)-Bis(dipicolylamine) Probes for Molecular Imaging of Cell Death

Kasey J. Clear,[†] Kara M. Harmatys,[†] Douglas R. Rice,[†] William R. Wolter,[‡] Mark A. Suckow,[‡] Yuzhen Wang[‖], Mary Rusckowski[‖], and Bradley D. Smith*[,†]

[†]*Department of Chemistry and Biochemistry, 236 Nieuwland Science Hall, University of Notre Dame, Notre Dame, Indiana 46556, United States*
[‡]*Freimann Life Science Center, 400 Galvin Life Science, University of Notre Dame, Notre Dame, Indiana 46556, United States*
[‖]*Division of Nuclear Medicine, Department of Radiology, University of Massachusetts Medical School, Worcester, MA, 01655, USA*

Contents

A. FRET titration curves

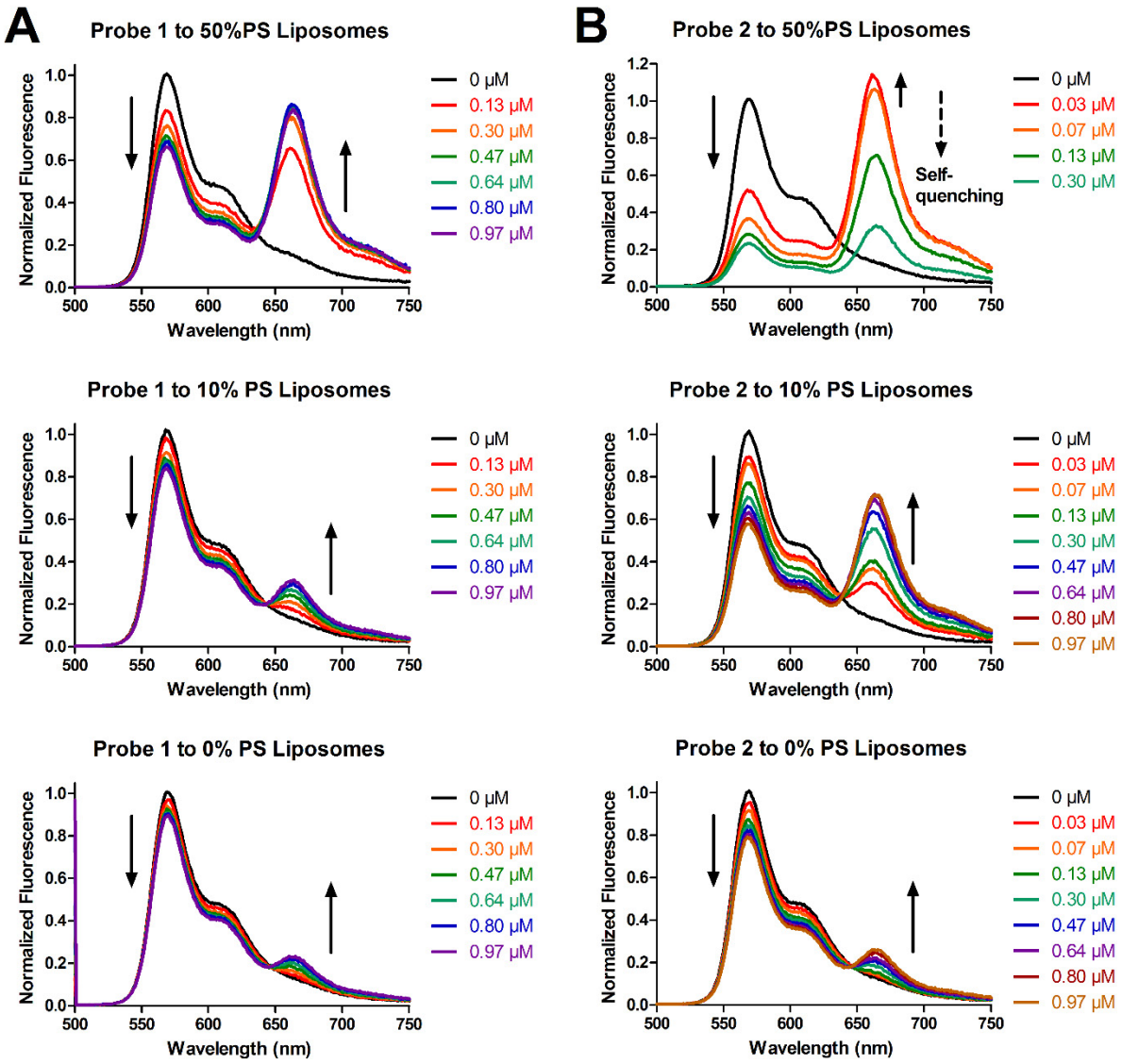


Figure S1: Representative fluorescence spectra for FRET titrations in response to addition of 0-1 μM of Probe **1** (panel A) or **2** (panel B) to liposomes composed of varying amounts of POPS. Liposomes were composed of 50% PS (50:2:47:1 POPS:PEG_{2000}DPPE:POPC:$DiIC_{18}$), 10% PS (10:2:87:1 POPS:PEG_{2000}DPPE:POPC:$DiIC_{18}$), 0% PS (2:97:1 PEG_{2000}DPPE:POPC:$DiIC_{18}$). All experiments were performed in HEPES buffer (10 mM, 137 mM NaCl, 3.2 mM KCl, pH 7.4) at 25°C. Excitation was at 480 nm. Note: A loss of FRET signal at 660 nm due to self-quenching of probe **2** was observed with 50% PS liposomes (panel B, top frame).

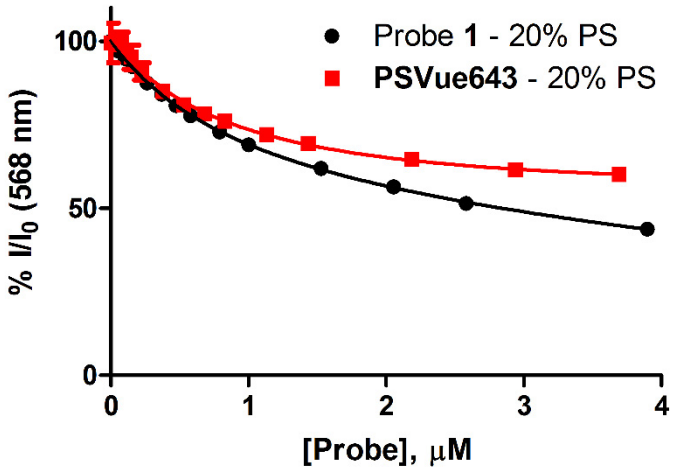


Figure S2: Comparison of Probe **1** and **PSVue643** association with liposomes containing 20% POPS by FRET assay. See Figure 1 legend for experimental summary. Liposomes were composed of 20% PS (20:2:77:1 POPS:PEG_{2000}DPPE:POPC:$DiIC_{18}$).

B. Aggregation and quenching of probe 2 in presence of PS-rich liposomes

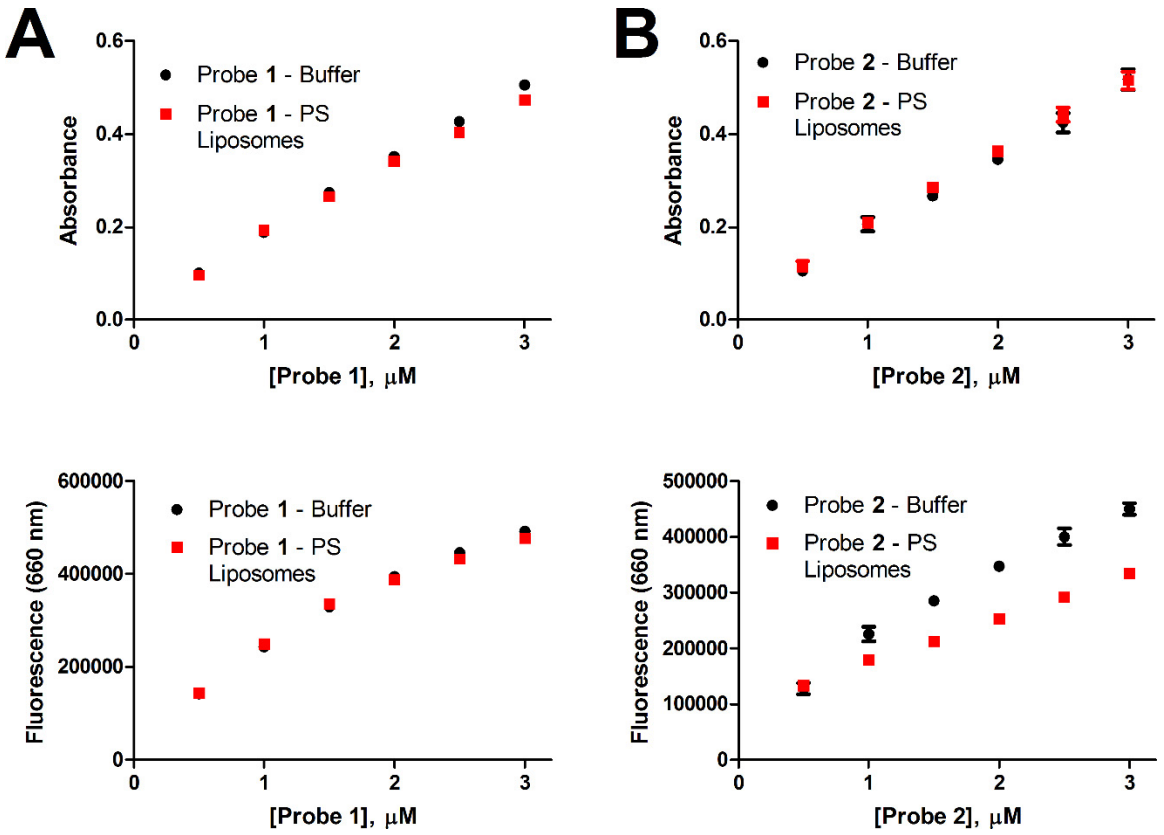
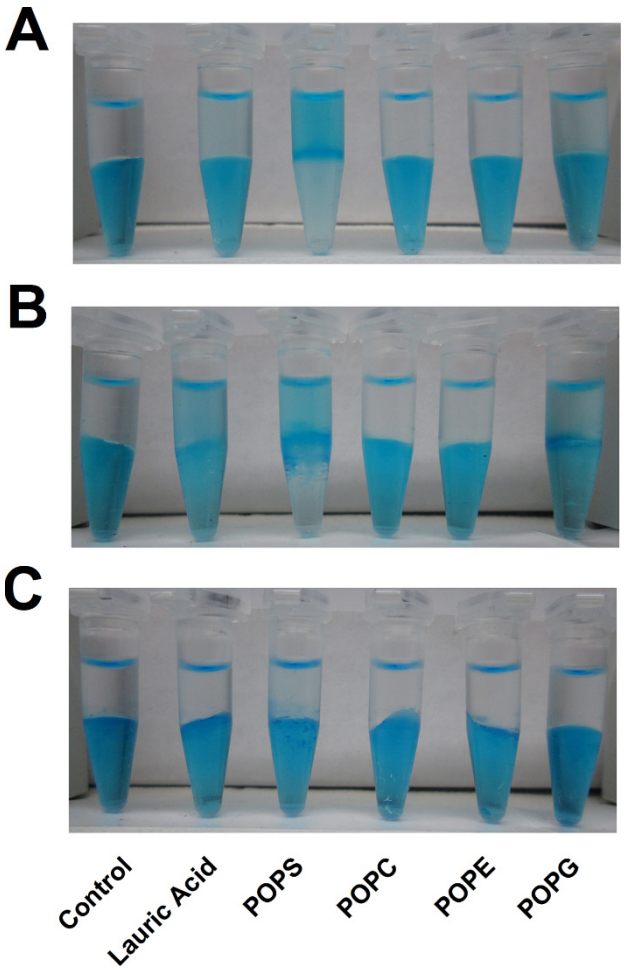


Figure S3: Absorbance and fluorescence changes upon addition of increasing concentrations of probe **1** (A) and **2** (B) to liposomes containing 20% POPS or buffer alone. Absorbance (644 nm, *upper*) and fluorescence (635/660 nm, *lower*) is plotted as a function of probe concentration under the two conditions. Liposomes were composed of 20% PS (20:2:78 POPS:PEG_{2000}DPPE:POPC). All experiments were performed in HEPES buffer (10 mM, 137 mM NaCl, 3.2 mM KCl, pH 7.4) at 25°C. Probe **2** measurements are reported as the average and standard deviation of three measurements and for most points on the graphs the error bars are smaller than the symbols.

C. Octanol partitioning and log *P* calculation



Probe	log *P* (25 ºC)					
	Control	Lauric Acid	POPS	POPC	POPE	POPG
1	-2.2	-1.0	0.5	-2.0	-2.0	-1.0
2	-1.9	-0.4	1.0	-2.3	-1.6	-0.3
PSVue643	-3.0	-2.2	-1.2	-2.9	-2.8	-2.1

Figure S4: (*top*) Color photographs of aqueous:organic partitioning of 10 µM probe **1** (A), **2** (B), or **PSVue643** (C) in the presence of 50 µM lipids in 1:1 octanol:buffer. The control experiments contained no polar lipid. The aqueous layer was composed of 5 mM TES Buffer, 140 mM NaCl at pH 7.3. (*lower*) log *P* values for each probe-lipid combination calculated as indicated in Figure 3.

D. Cell viability data

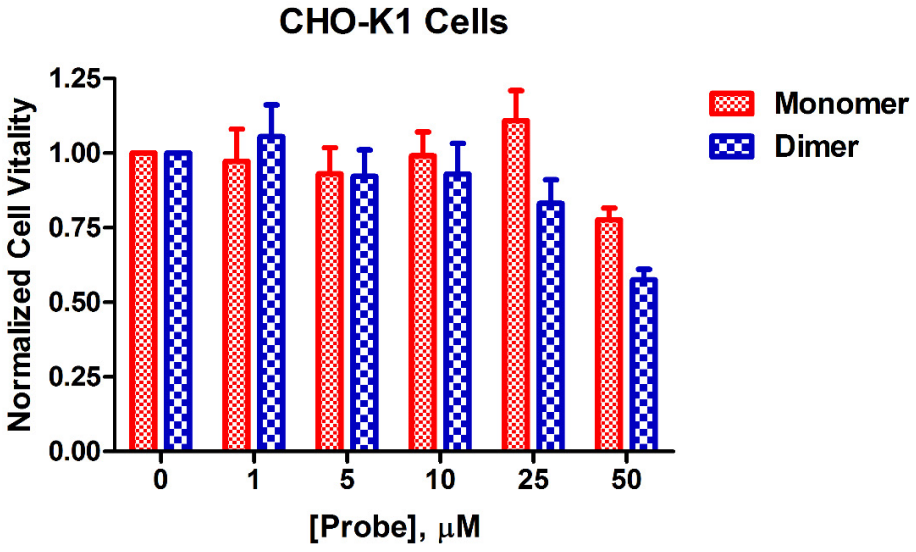


Figure S5. Cell viability of CHO-K1 cells treated with either **1** (red) or **2** (blue) for 18.5 h at 37 °C.

E. Additional cell microscopy and cytometry

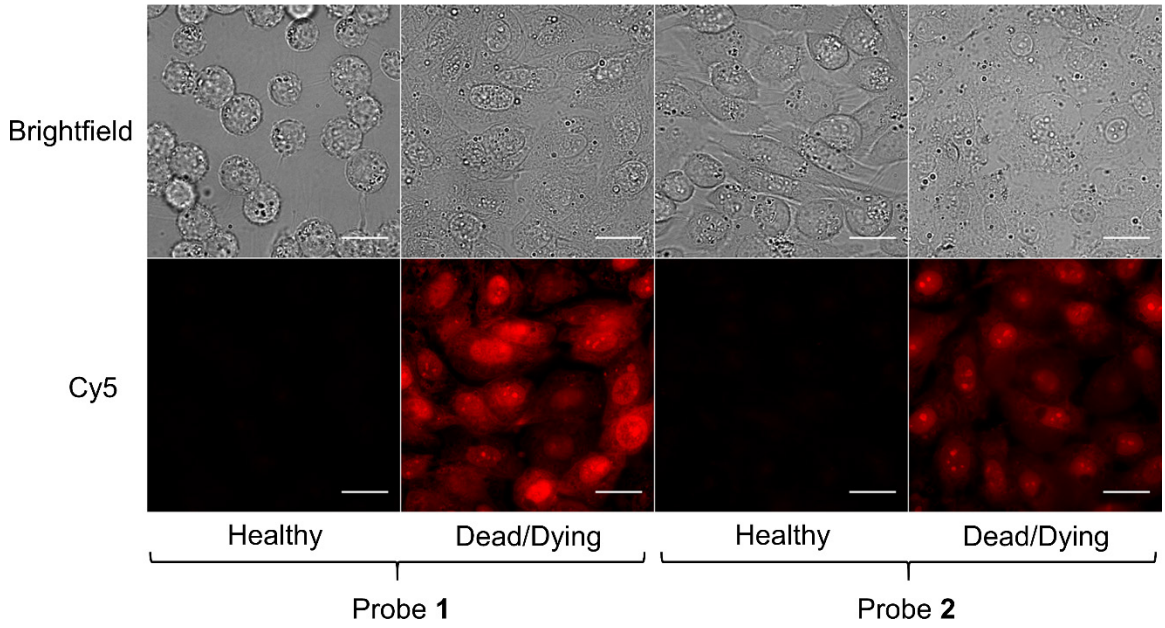


Figure S6: Fluorescence micrographs (Bright field = top; Cy5 = bottom) of healthy (A/E, C/G) or dead and dying CHO-K1 cells stained with 5 μM of either **1** (left two panels) or **2** (right two panels). The dead and dying cells were treated with camptothecin (15 μM) for 18 h, then incubated with 5 μM of either probe for 15 min at 37 °C and washed with HEPES buffer. Scale bar = 25 μm.

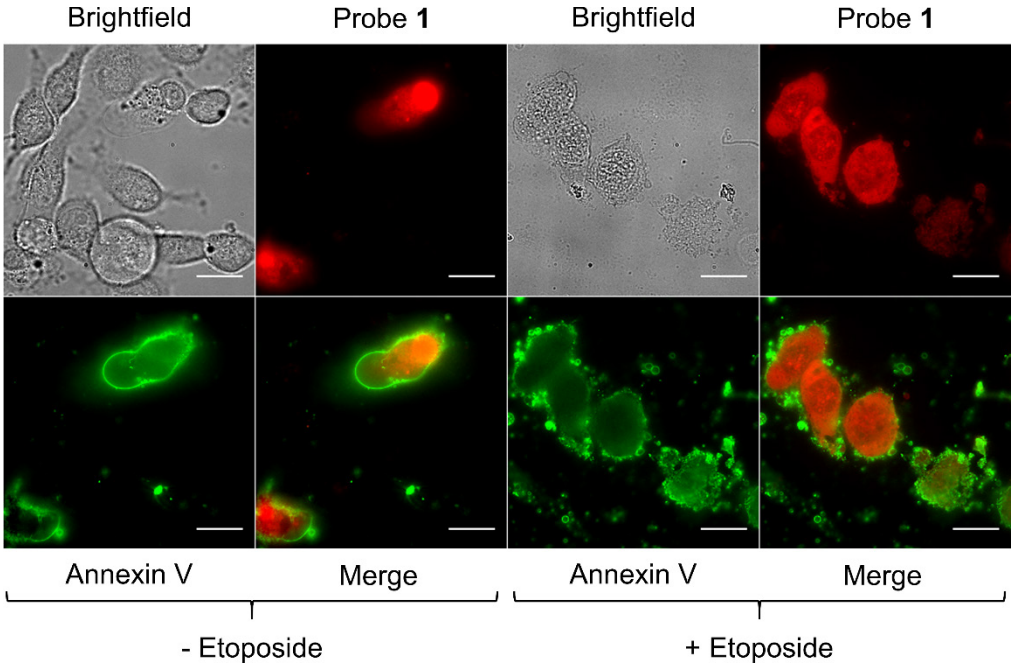


Figure S7: Fluorescence costaining micrographs of healthy (left) or dead and dying (right) MDA-MB-231 cells stained with 5 μM of **1** (red fluorescence) and Annexin V-AlexaFluor480 (green fluorescence). The dead and dying cells were treated with etoposide (15 μM) for 6 h, then incubated with 5 μM of **1** and Annexin V probes for 15 min and washed with HEPES buffer.

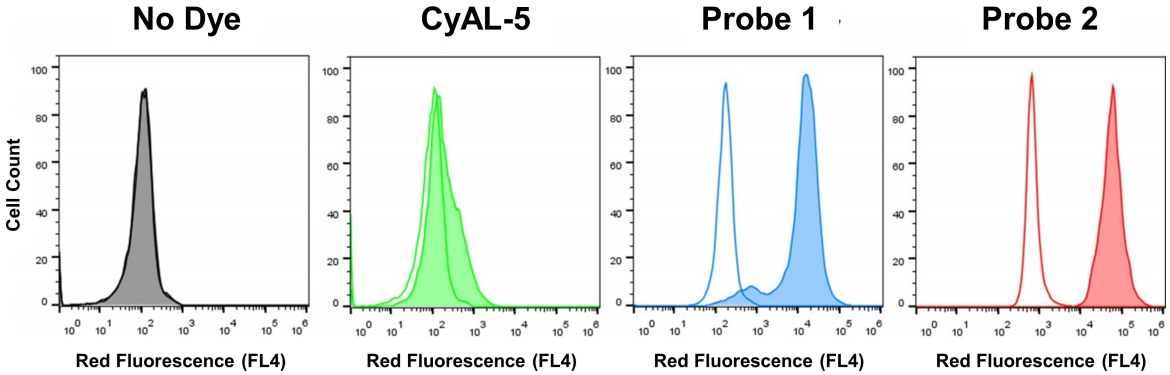


Figure S8. Combined histogram plot depicting flow cytometry results for different populations of CHO-K1 cells treated with 5 μM of dye; cells left untreated (gray), cells stained with **CyAL-5** control dye (green), cells stained with monomeric probe **1** (blue), and cells stained with dimeric probe **2** (red) for 15 min in PBS (1 % DMSO). Solid-filled histograms indicate dead/dying cell populations treated prior with etoposide (15 μM, 13 h). Unfilled histogram plots indicate healthy cell populations.

F. Animal optical imaging data and histology

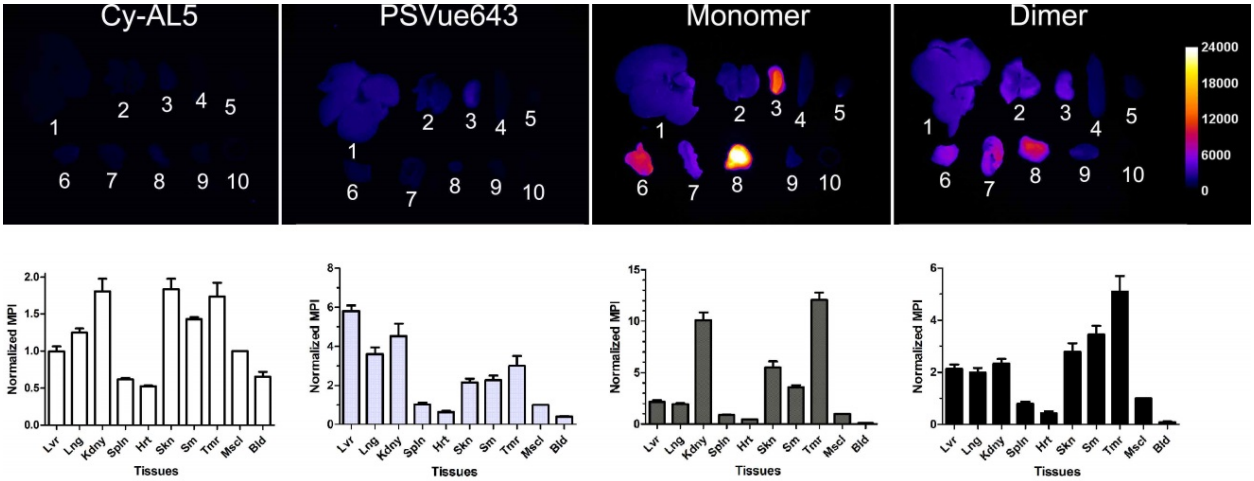


Figure S9. Ex vivo fluorescence images of excised organs taken from rats bearing a subcutaneous prostate tumor 24 h after intravenous dosing (150 nmol) with untargeted **CyAL-5** control dye, **PSVue643**, Monomer (**1**), or Dimer (**2**) (upper). Organs are listed as the following: 1, Liver (Lvr); 2, Lung (Lng); 3, Kidney (Kdny); 4, Spleen (Spln); 5, Heart (Hrt); 6, Skin (Skn); 7, Small Intestine (Sm); 8, Tumor (Tmr); 9, Muscle (Mscl); 10, Blood (Bld). Bar graphs showing ex vivo tissue distribution of **CyAL-5**, **PSVue643**, Monomer (**1**), and Dimer (**2**) (lower). Error bars are standard error of the mean (N = 4, 4, 10, 6, respectively of each probe) of the mean pixel intensities (MPI) normalized to muscle.

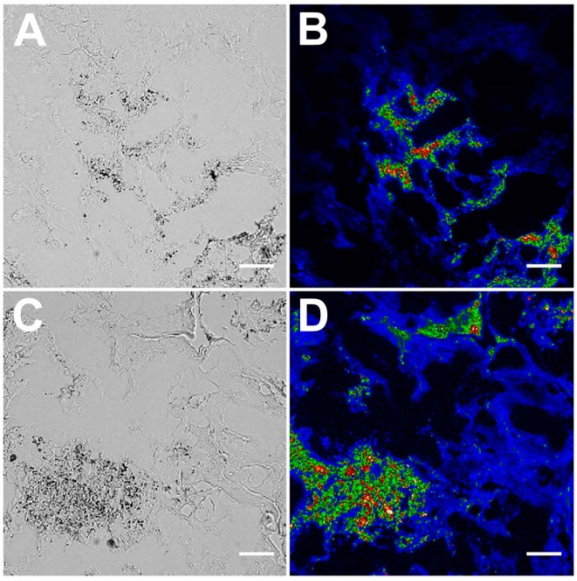


Figure S10. Coregistered micrographs of histological slices (5 µm) of tumor core from rat prostate tumor model. The brightfield images (A, C) show necrotic cells as darker regions that colocalize with deep-red fluorescence emission of **1** (B) and **2** (D). Scale bar = 130 µm

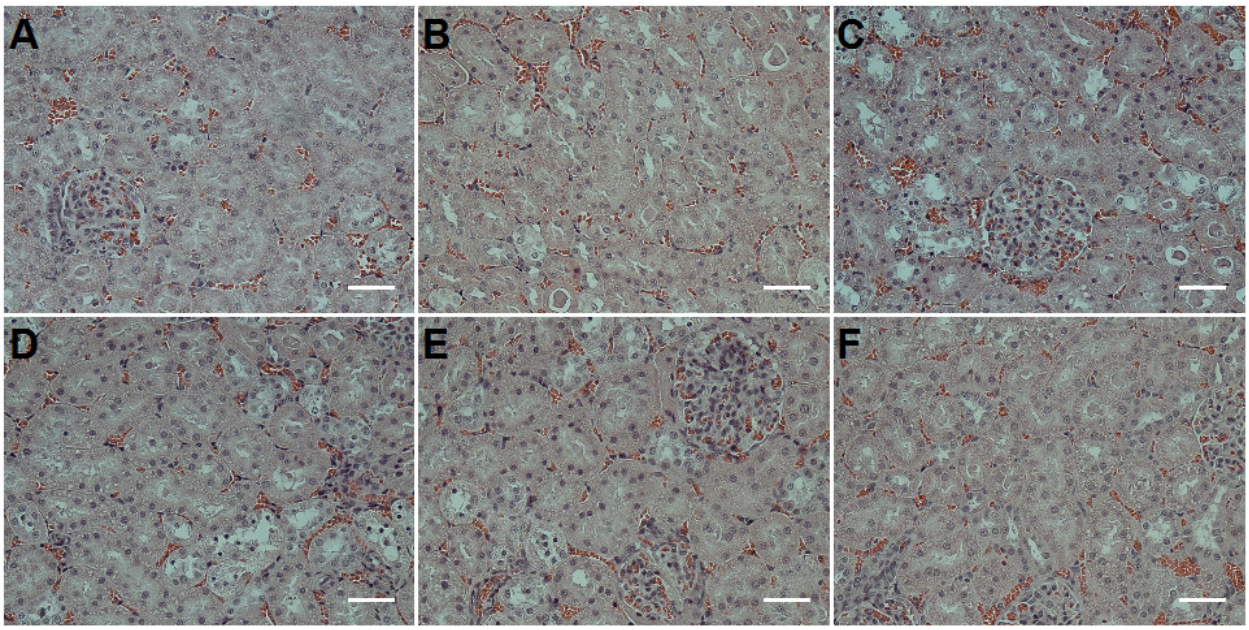


Figure S11. Haematoxylin/eosin staining of excised rat kidneys showing that probes do not induce cell death in the kidney. Representative histological slices of kidney from Lobund-Wistar rats that were euthanized at 24 h after intravenous injections of (A) saline, (B) **CyAL-5** control, (C**) PSVue643**, (D) **2**, and (E) **1**. The slice shown in (F) is from a rat that was euthanized 7 days after treatment with probe **1**. In each treatment, rats were injected with 150 nmol of probe. Scale bars = 50 µm. N = 3.

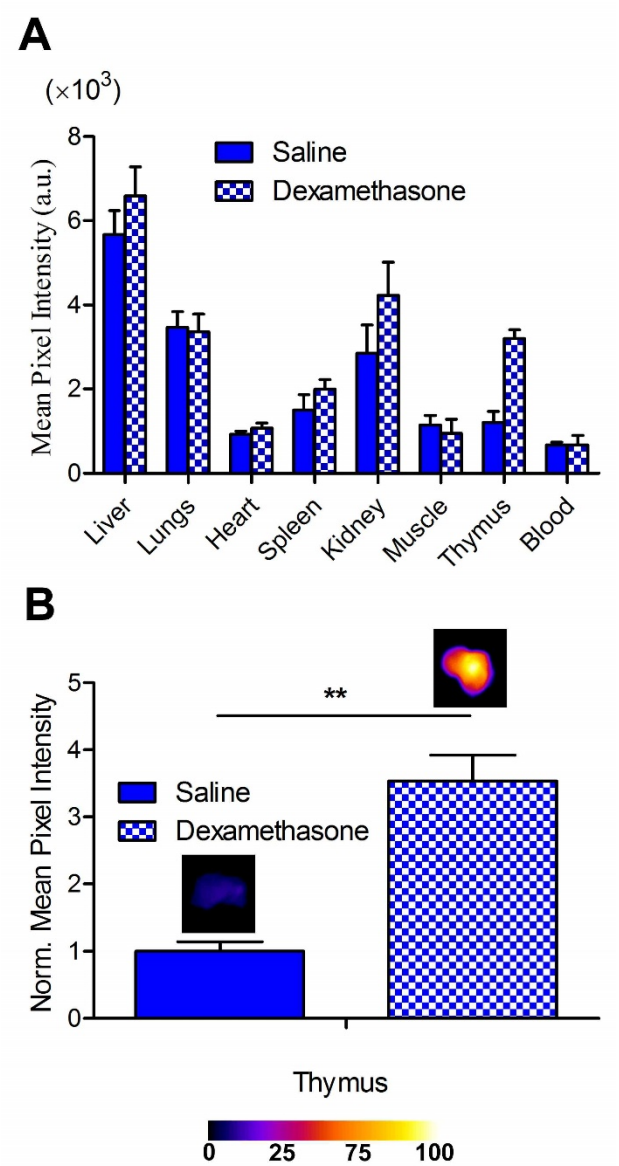


Figure S12: Comparison of probe **1** localization between dexamethasone and saline treatment in a thymus atrophy model of cell death in immunocompetent mice. (A) Biodistribution of **1** in excised organs taken from the saline-treated (solid blue) or dexamethasone-treated (checked blue) cohorts 3 h after intravenous dosing. (B) Mean pixel intensities for probe fluorescence in the excised thymi. Error bars are the standard error of the mean. N = 3 for both cohorts. P values ≤ 0.01 (*),≤ 0.001 (**) or ≤ 0.0001 (***) are considered statistically significant. SKH1 mice were given intraperitoneal injections of dexamethasone at a dose of 50 mg/kg or saline. After 24 h, the imaging probe (10 nmol) was injected via the tail vain, and after 3 h mice were sacrificed and organs were imaged using a planar fluorescence imaging station.

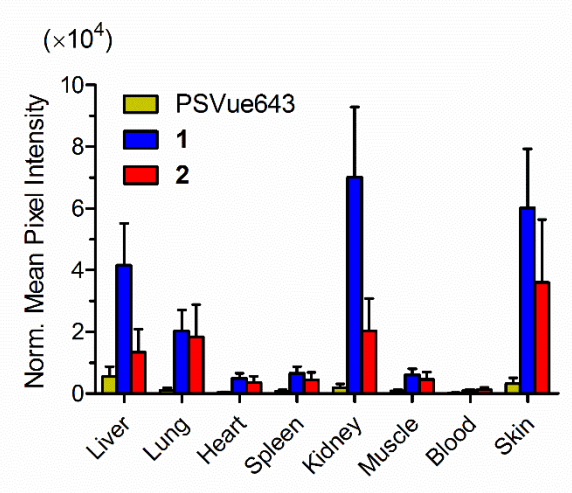


Figure S13: Probe localization in healthy SKH1 hairless mice 24 h after probe injection. Error bars are standard error of the mean. N = 3. Each cohort was given a retroorbital intravenous injection of fluorescent probe (20 nmol) in water (1% DMSO). The animals were euthanized 24 h later and the organ biodistributions were determined by imaging the excised tissues using a planar fluorescence imaging station with deep-red filter set.

G. Additional radiolabeling and stability data

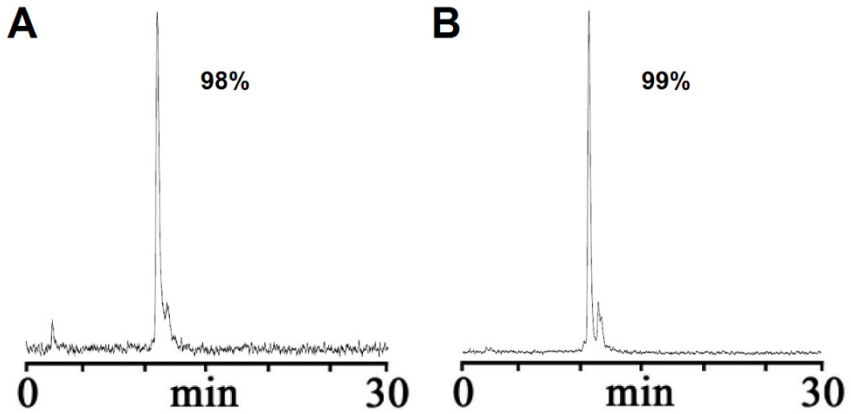


Figure S14: Radiopurity of **9** and **10**. HPLC radiochromatograms and radiopurities of **9** (A) and **10** (B). The retention times are 11.5 and 11.8 min, with radiopurities of 98% and 99%, respectively. The uncomplexed ^{111}In eluted at ~3 min, and the tailing observed with **9** and **10** at ~12 min is an artifact of the HPLC instrument. The samples were judged pure enough for immediate animal dosing without further purification.

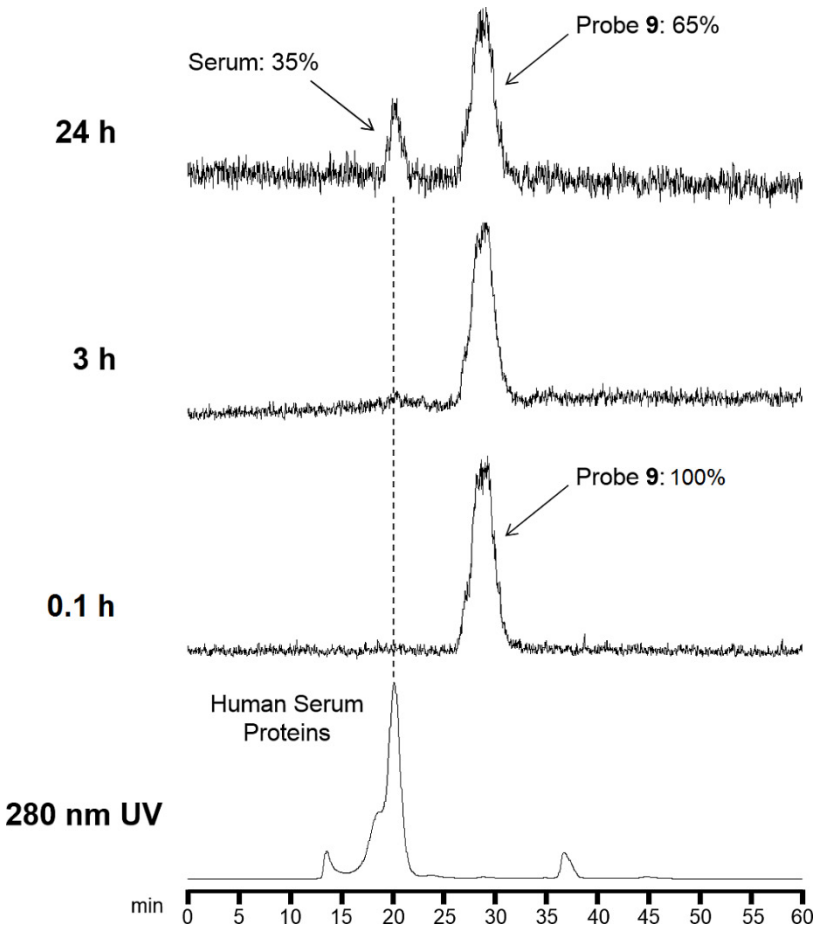
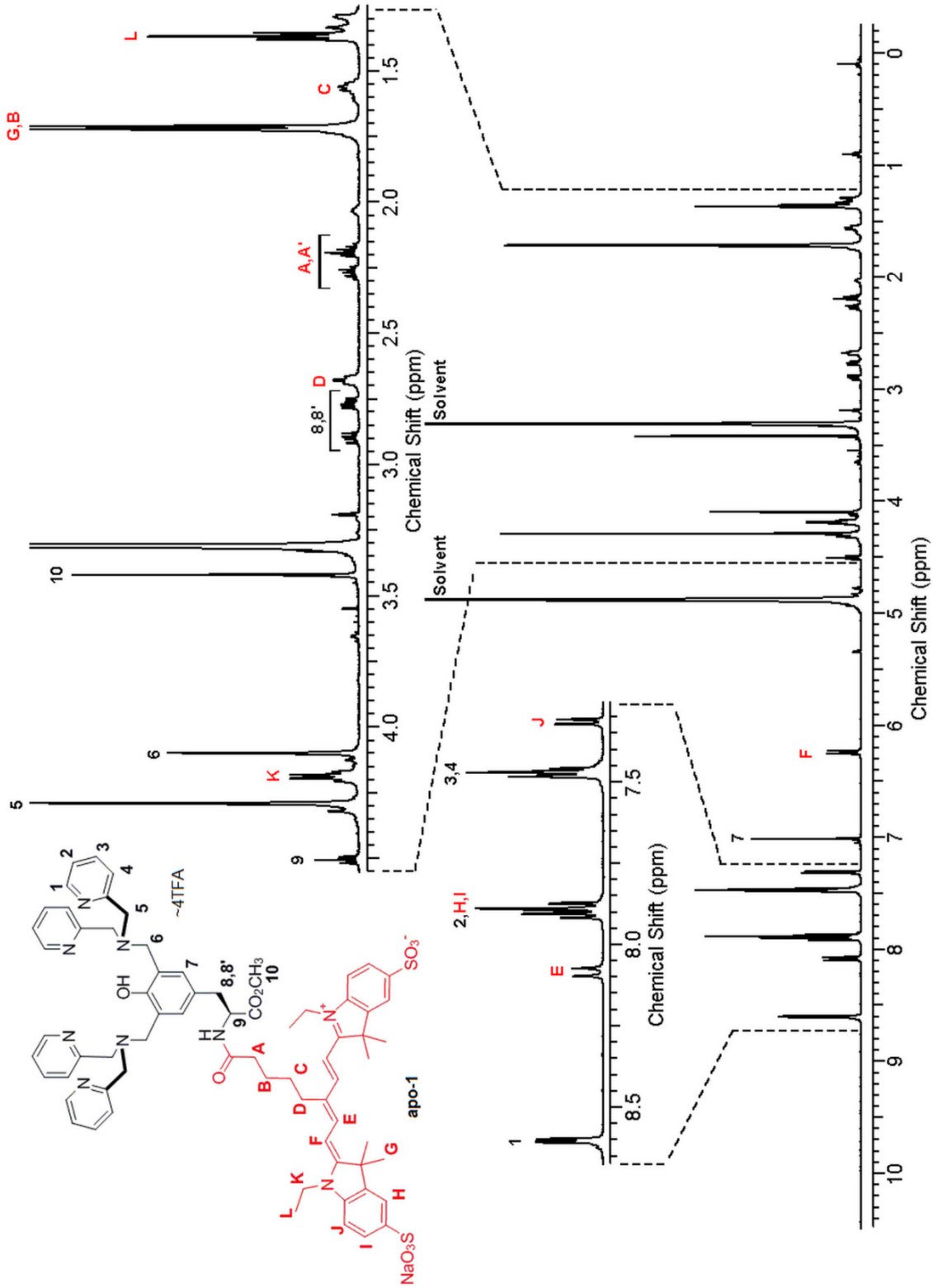
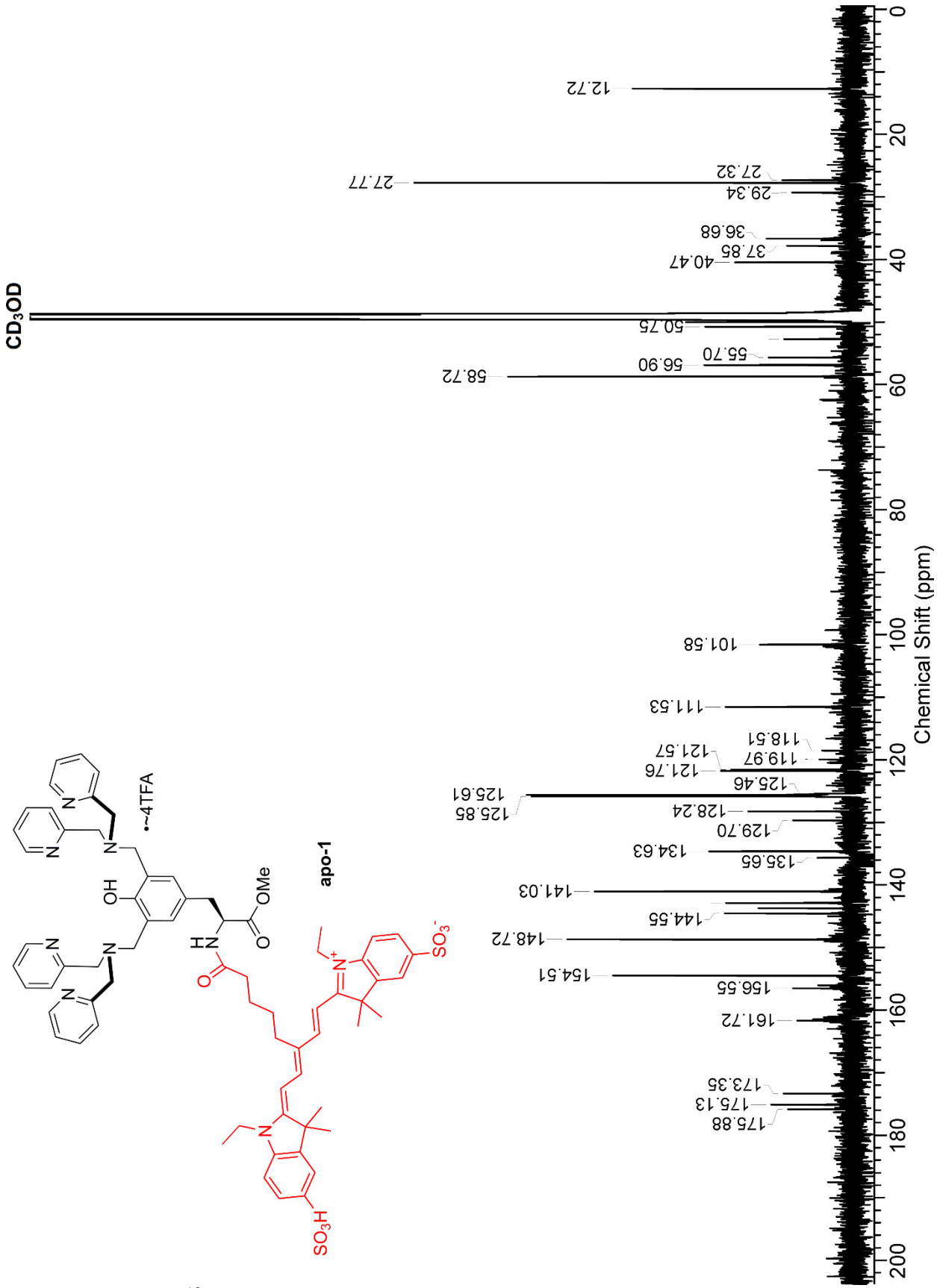


Figure S15: Serum stability and transfer for ^{111}In complex **9**. Size exclusion HPLC ultraviolet- and radio- chromatograms of **9** mixed with human serum after incubation at 37 °C for 0 to 24 hours.

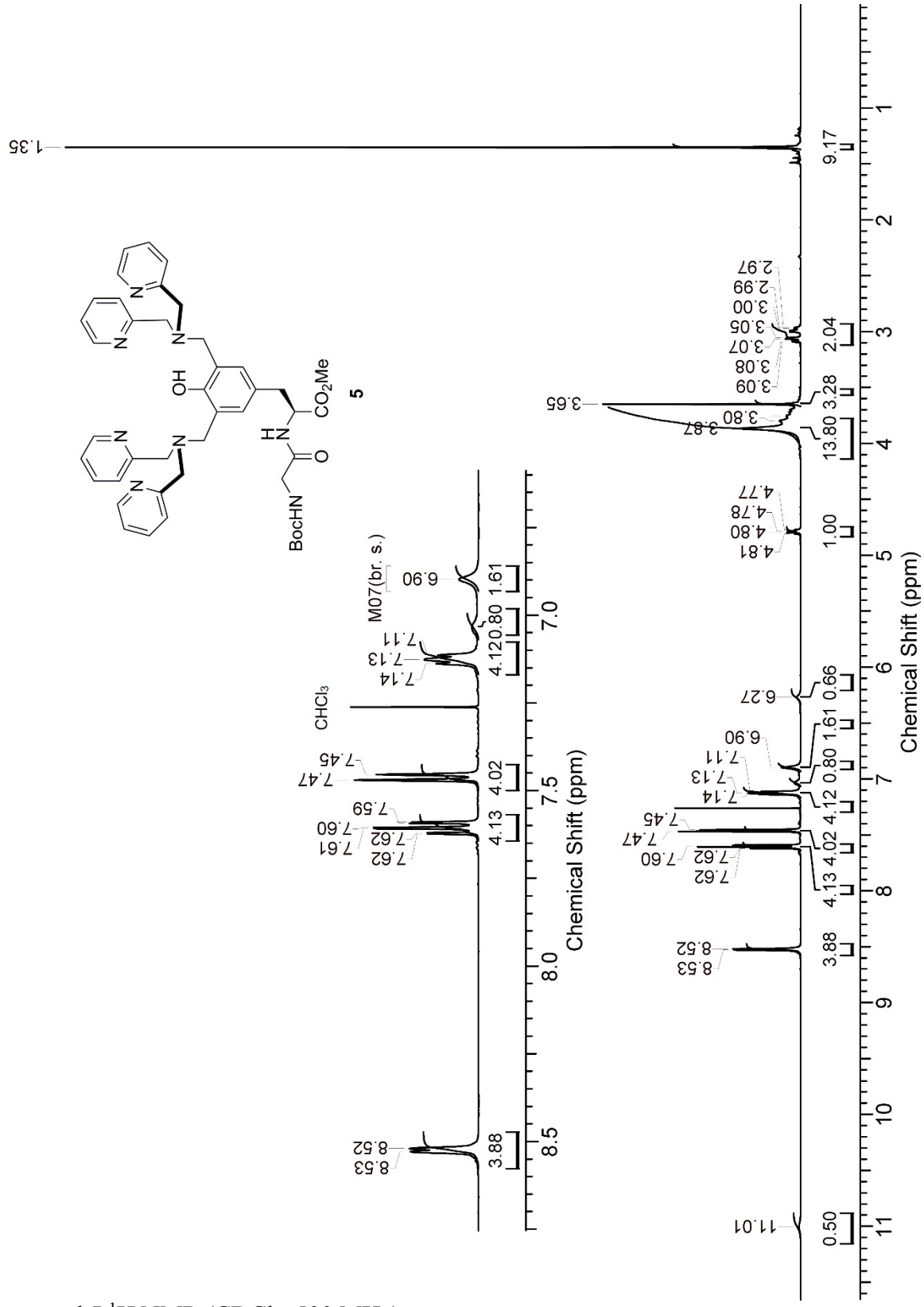
H. $^{1}H/^{13}C$ NMR Spectra



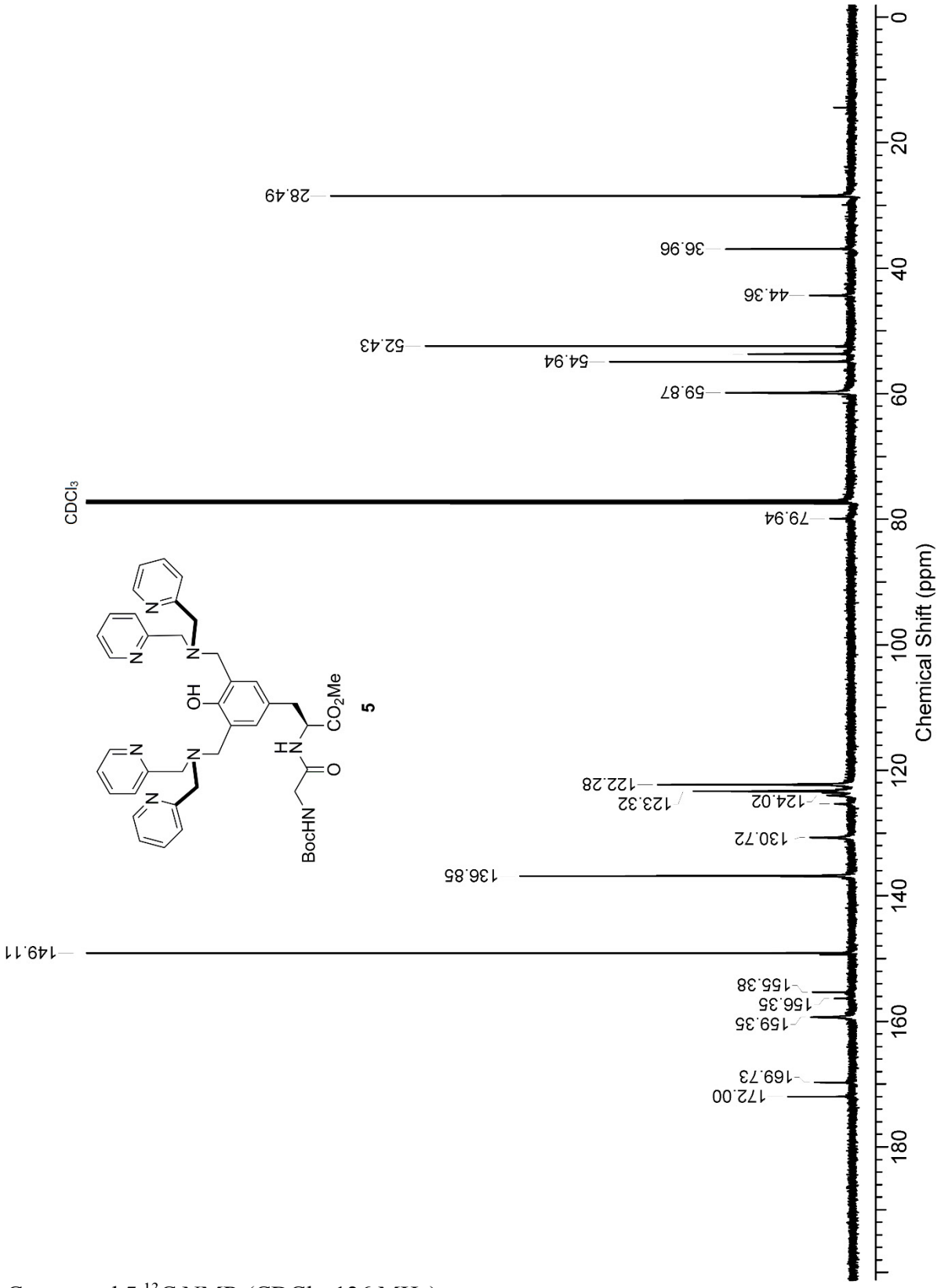
Compound **apo-1** ^{1}H NMR (CD_3OD, 600 MHz)



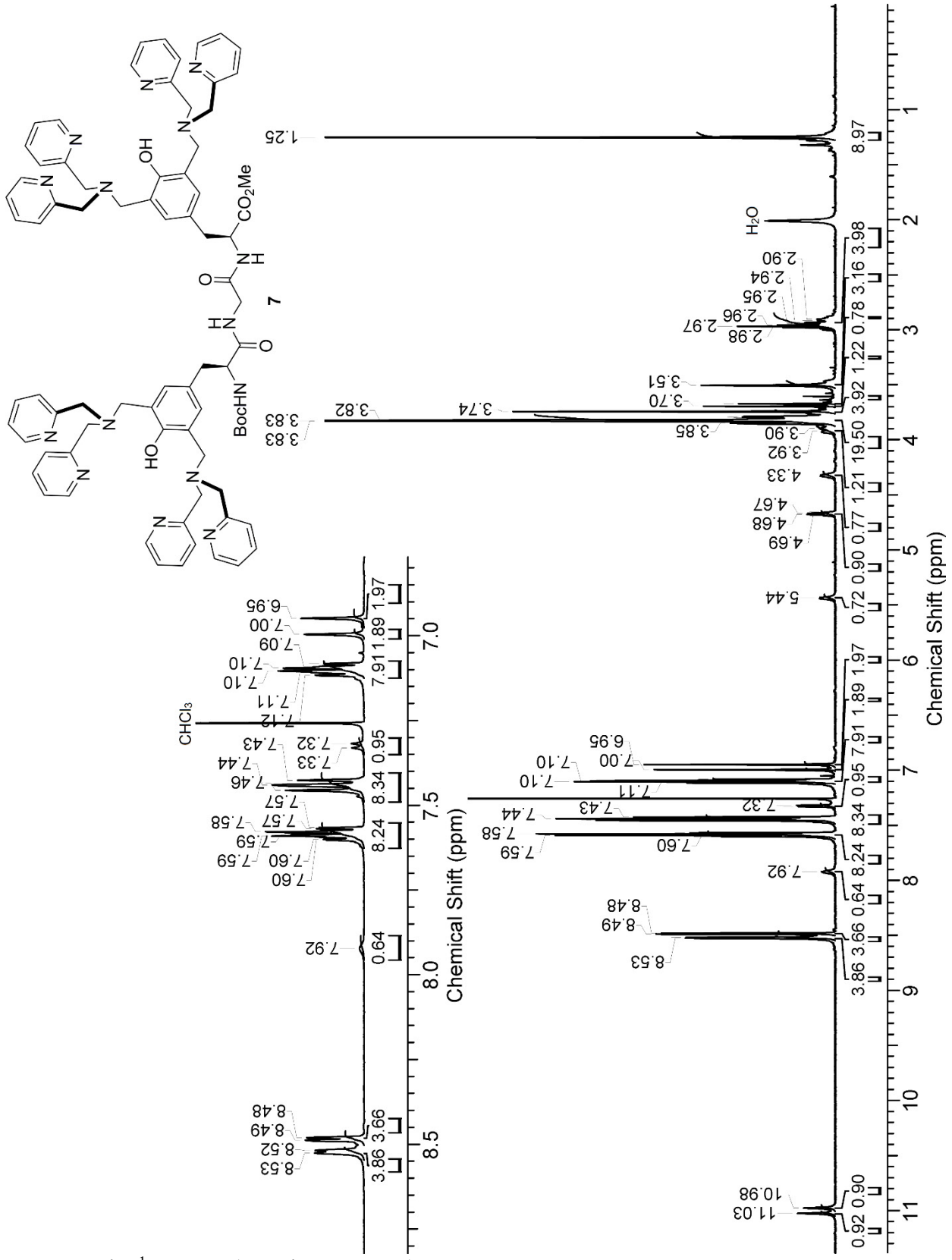
Compound a**po -1** ^{13}C NMR (CD_3OD, 151 MHz)



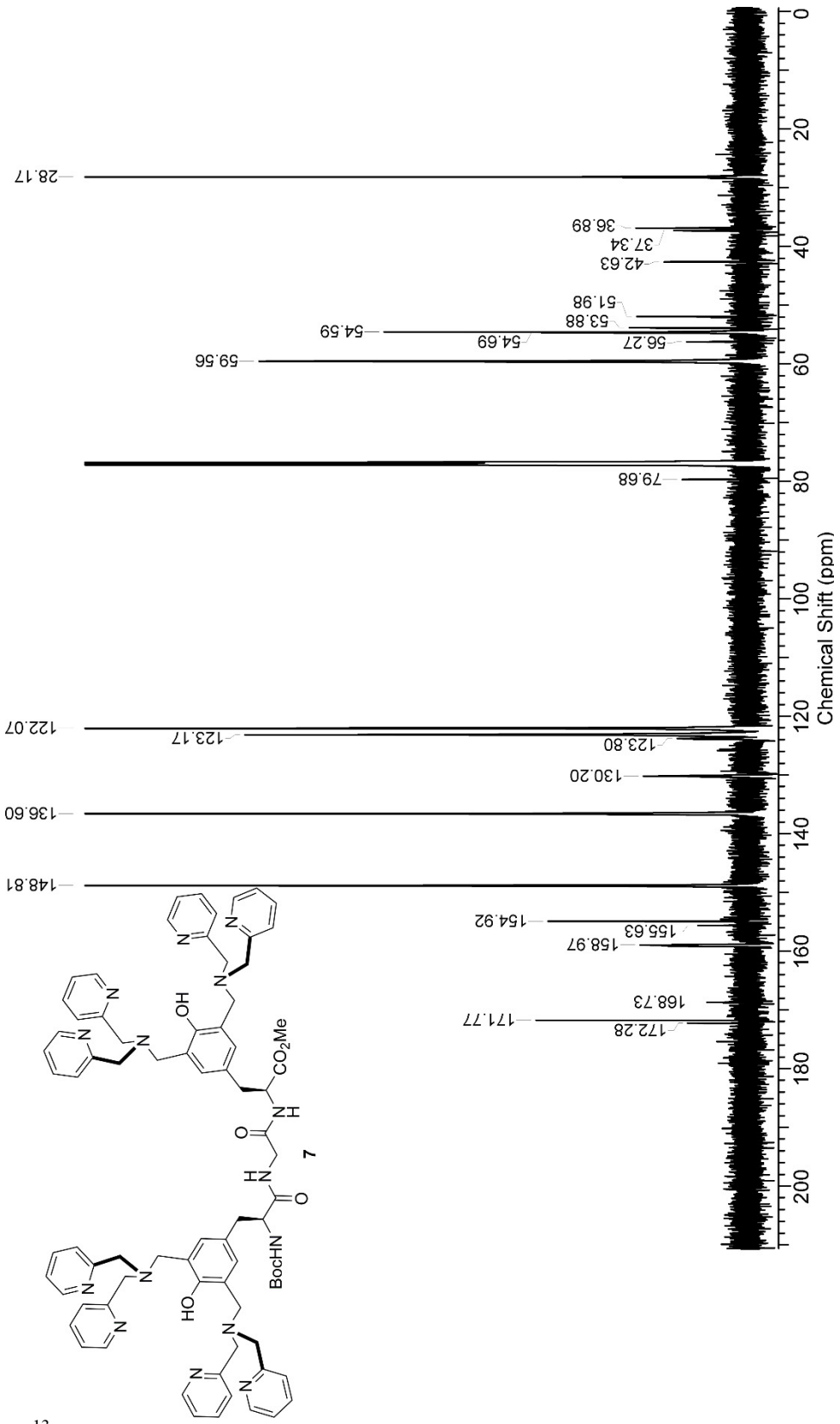
Compound **5** ^{1}H NMR ($CDCl_3$, 500 MHz)



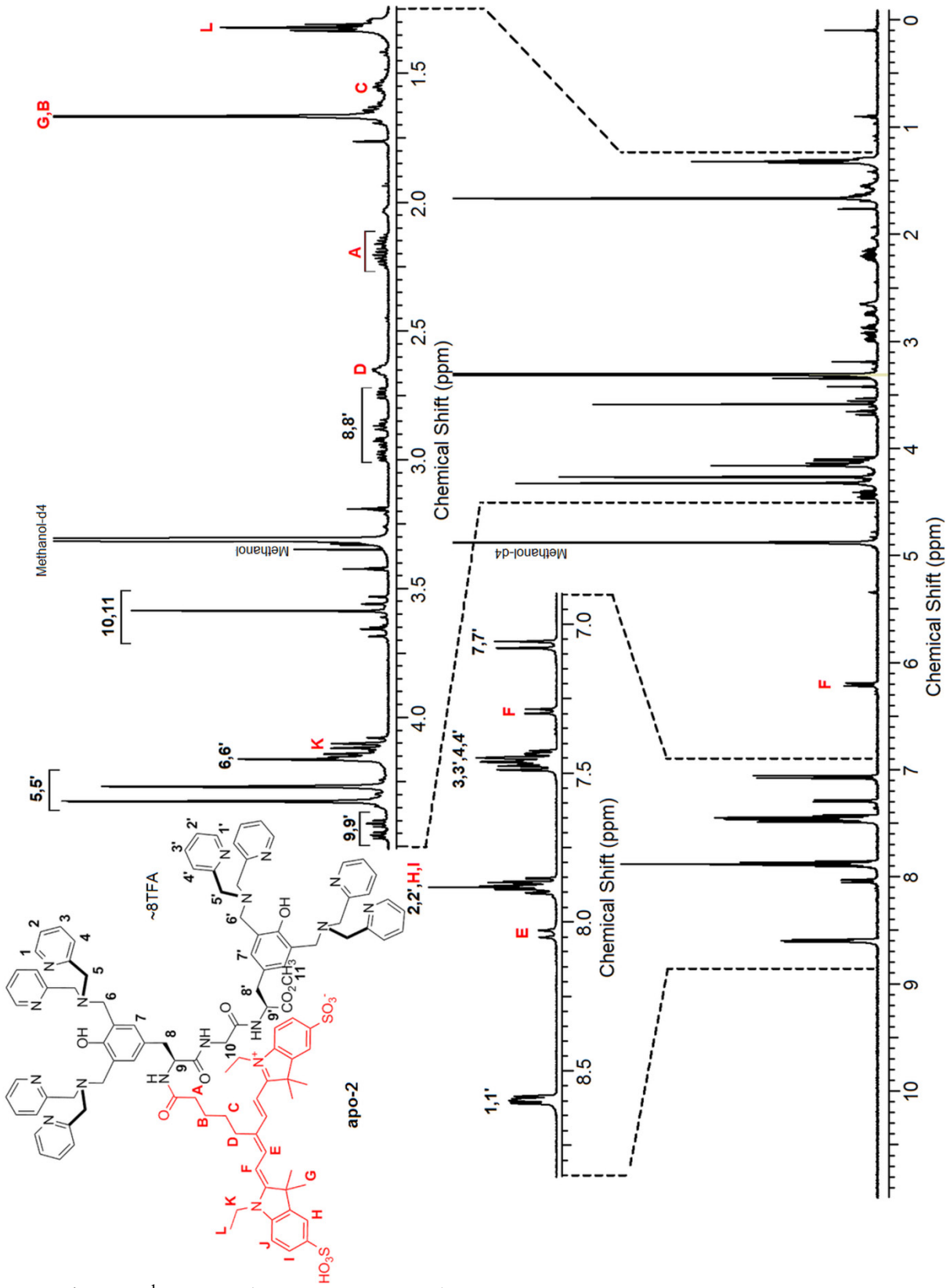
Compound **5** ^{13}C NMR ($CDCl_3$, 126 MHz)



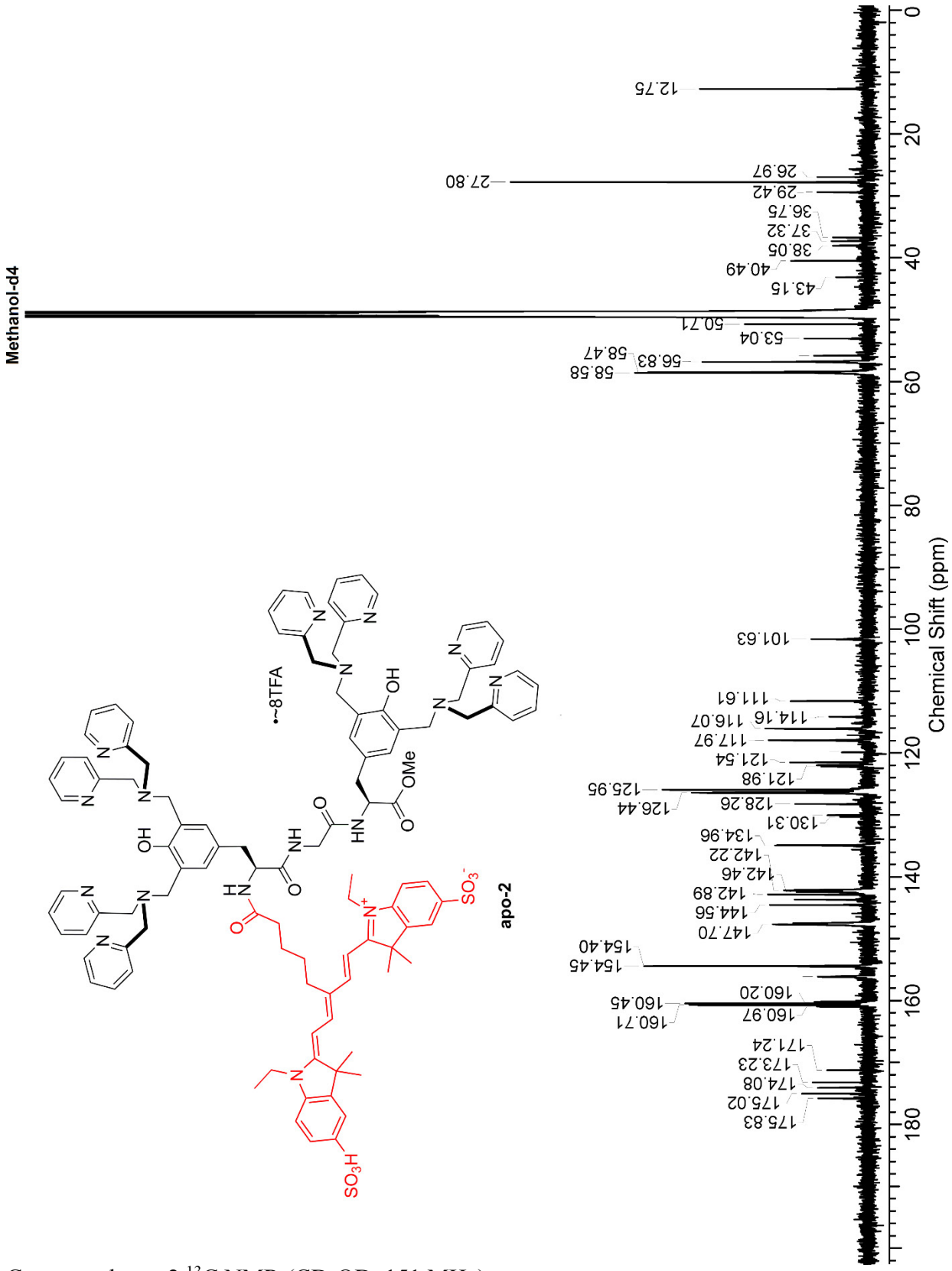
Compound **7** ^{1}H NMR ($CDCl_3$, 500 MHz)



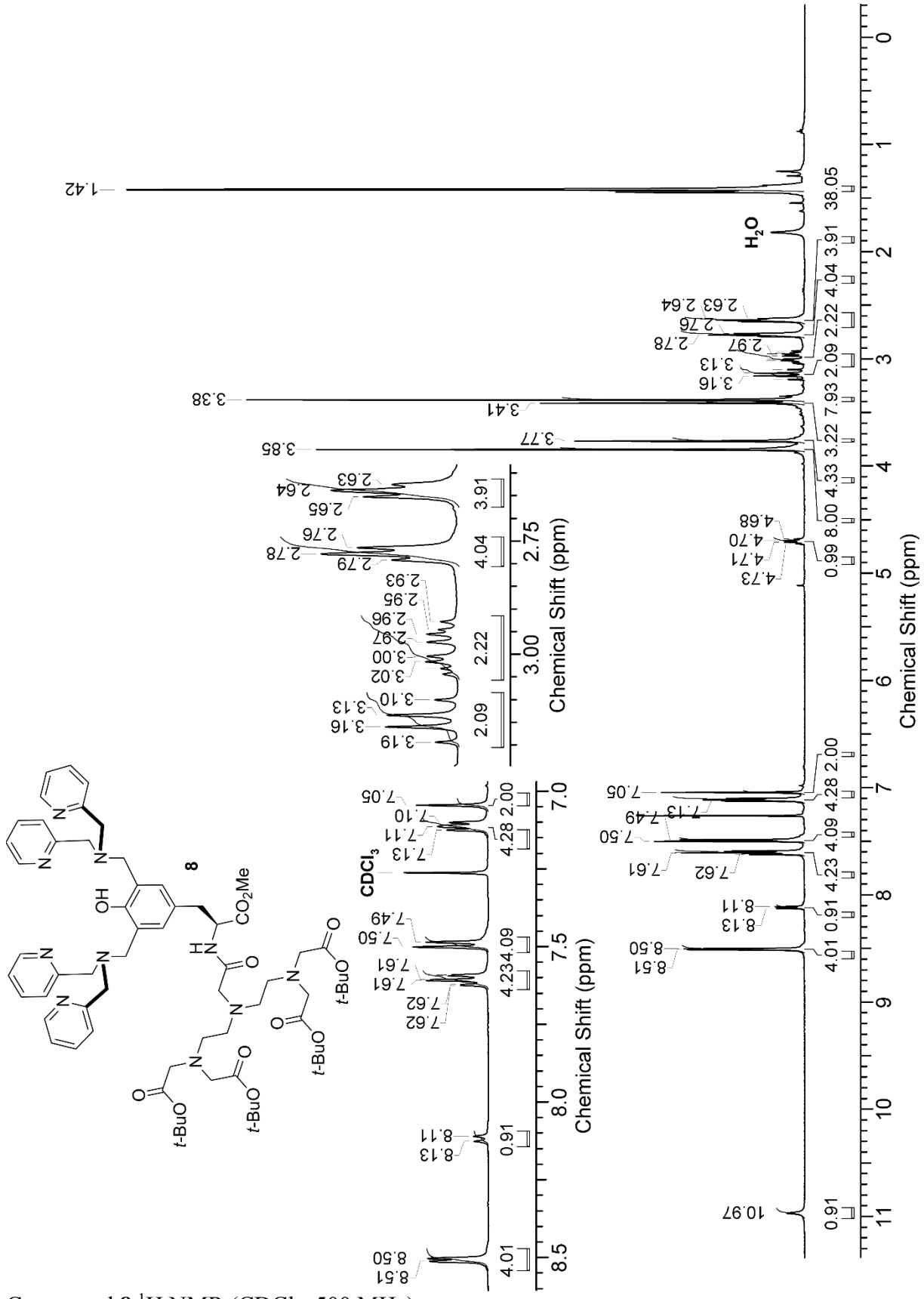
Compound **7** ^{13}C NMR ($CDCl_3$, 151 MHz)



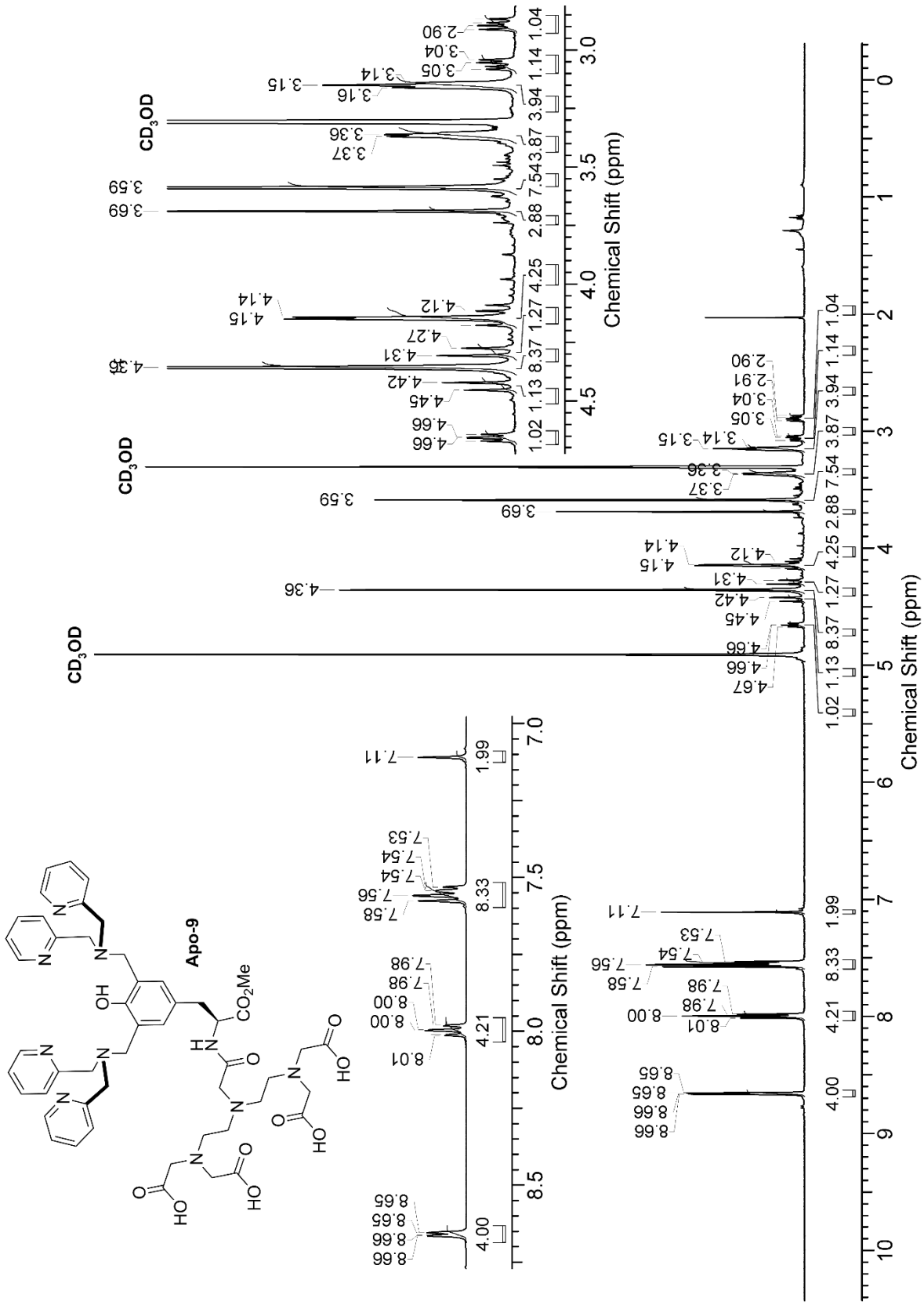
Compound **apo-2** ^{1}H NMR (CD_3OD, 600 MHz)



Compound **apo-2** ^{13}C NMR (CD_3OD, 151 MHz)



Compound **8** ^{1}H NMR ($CDCl_3$, 500 MHz)



Compound **Apo-9** ^{1}H NMR (CD_3OD, 500 MHz)