SUPPORTING INFORMATION

Biocompatible Infinite Coordination Polymer Nanoparticle –Nucleic Acid Conjugates For Antisense Gene Regulation

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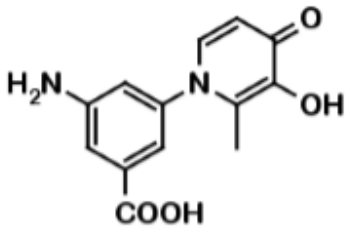
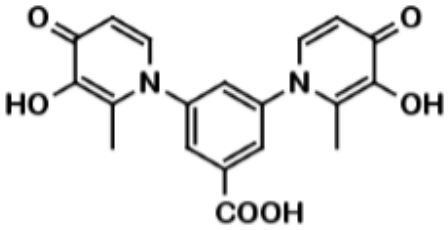
GENERAL MATERIALS AND METHODS

3,5-Diaminobenzoic acid was purchased from TCI America (Portland, OR). 4-Azido-butan-1-amine was purchased from Synthonix, Inc. (Wake Forest, NC). All reagents for oligonucleotide synthesis were purchased from Glen Research (Sterling, VA) and used according to manufacturer instructions. Buffer solutions were purchased from Invitrogen (Carlsbad, CA). Deuterated solvents were purchased from Cambridge Isotope Laboratories Inc. (Andover, MA). Gold nanoparticles were purchased from Ted Pella (Redding, CA). Amicon® Ultra centrifugal filter units were purchased from EMD Millipore (Billerica, MA). All other reagents were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. ^{1}H NMR spectra were recorded on a Bruker Avance 400 MHz NMR spectrometer. ^{1}H NMR spectra were referenced internally to residual proton signals in the deuterated solvents. ^{13}C NMR spectra for compounds **3** and **4** were collected on an Agilent DD2 500 MHz NMR spectrometer operating at an internal temperature of 100° C. Electrospray ionization (ESI) mass spectra were recorded on an Agilent 6120 LC-TOF instrument in positive ionization mode. UV-Vis spectra and thermal denaturation curves were collected on an Agilent Cary 5000 UV-Vis spectrometer in quartz cuvettes having a path length of 1 cm. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-ToF) data was collected on a Bruker AutoFlex III MALDI-ToF mass spectrometer employing 2,5-dihydroxyacetophenone (DHAP) as the matrix material. FTIR spectra were collected on a Perkin-Elmer Spectrum 100 FTIR spectrometer. AFM images were collected on a Bruker Dimension Icon atomic force microscope in non-contact mode equipped with a POINTPROBE-PLUS® Silicon-SPM-Sensor. TEM images were collected on a Hitachi H8100 transmission electron microscope operating at an accelerating voltage of 200kV. TEM and EDX data were collected on a Hitachi HD2300 STEM equipped with two Thermo Scientific X-ray EDX detectors. Dynamic light scattering (DLS) and zeta potential measurements were collected on a Zetasizer Nano ZS (Malvern Instruments Ltd). ICP-MS data were collected on a Thermo X-series II ICP-MS. Elemental analysis was conducted off-site by Intertek Pharmaceutical Services (Whitehouse, NJ).

SYNTHESIS

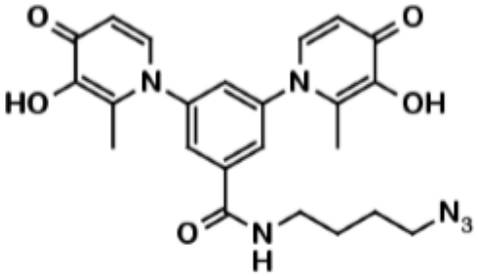


Diaminobenzoic acid *mono*-hydroxypyridinone (2) To a 100 mL round-bottomed flask with a magnetic stirrer was added 3,5-diaminobenzoic acid (5.00 g, 32.86 mmol), maltol (8.70 g, 69.00 mmol) and 30 mL of acidic n-propanol (49:1 propanol/12M HCl). The reaction vessel was fitted with a water-cooled condenser and the mixture heated to reflux for 16 h. The resulting suspension was vacuum-filtered while hot and the solids washed with acetone (200 mL) to yield 4.84 g of **(1)** as a tan powder (18.60 mmol, 57%). Propanol may be substituted with 5:1 EtOH/H_2O affording similar yields. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.52 (d, J = 7.4 Hz, 1H), 7.28 – 7.26 (m, 1H), 6.92 (t, J = 1.7 Hz, 1H), 6.67 (t, J = 2.1 Hz, 1H), 6.16 (d, J = 7.3 Hz, 1H), 5.77 (s, 2H), 1.96 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.96, 167.24, 150.62, 145.45, 142.79, 138.07, 133.21, 129.02, 115.64, 115.35, 114.34, 111.30, 13.61. HRMS-ESI (*m/z*): $[M+H]^+$ calculated for $C_{13}H_{13}N_2O_4$ 261.0870, found 261.0875.



Diaminobenzoic acid *bis*-hydroxypyridinone (3) To a 100 mL round-bottomed flask with a magnetic stirrer was added **(2)** (5.90 g, 22.67 mmol), maltol (3.57 g, 28.34 mmol), and 30 mL of acidic 2-ethoxyethanol (49:1 ethoxyethanol/12M HCl). The reaction vessel was fitted with a water-cooled condenser and the mixture heated to reflux for 64 h. The resulting suspension was vacuum-filtered while hot and the solids washed with water (50 mL), followed by acetone (50 mL), to afford the crude product as a fine brown solid. The *bis* product was selectively isolated by precipitation from boiling pyridine (100 mL), filtration, and further precipitation from hot dimethylformamide (100 mL) and drying *in vacuo* to afford 0.98 g of **(3)** (2.66 mmol, 12%) as a grey powder sparingly soluble in methanol, soluble in hot DMSO and DMF. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.03 (d, J = 2.0 Hz, 2H), 8.00 (t, J = 2.0 Hz, 1H), 7.64 (d, J = 7.4 Hz, 2H), 6.22 (d, J = 7.4 Hz, 2H), 2.02 (s, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.43, 165.59, 145.52, 142.92, 138.79, 138.26, 134.82, 130.74, 130.47, 128.55, 111.45, 111.28, 13.79, 13.60.
HRMS-ESI (*m/z*): $[M+H]^+$ calculated for $C_{19}H_{17}N_2O_6$ 369.1081, found 369.1084.

Note: the presence of additional signals in the ^{13}C NMR spectrum greater than the predicted number (11) is likely due to hindered rotation of the ortho-substituted HOPO rings giving rise to conformational isomers.



Diaminobenzoic acid *bis*-HP azide (4) To a 50 mL round-bottomed flask with a magnetic stirrer was added **(3)** (0.400 g, 1.09 mmol) fully dissolved in anhydrous DMSO (30 mL). HATU (0.414 g, 1.09 mmol) and diisopropylethylamine (0.48 mL, 2.73 mmol) were subsequently added and the reaction vessel was capped with a rubber septum. After 5 minutes, 4-azidobutan-1-amine (0.187 g, 1.64 mmol) was injected via syringe and the mixture allowed to stir for 4 h under N_2. The organic phase was diluted with 1 volume of water and allowed to stand for 1 h. The resulting grey precipitate was collected by vacuum filtration and washed extensively with water (150 mL), followed by acetonitrile (100 mL), and allowed to dry on the filter. The obtained azide monomer **(4)** was used without further purification. (0.283 g, 0.61 mmol, 56%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.72 (t, J = 5.7 Hz, 1H), 8.00 (d, J = 2.0 Hz, 2H), 7.91 (t, J = 1.9 Hz, 1H), 7.63 (d, J = 7.3 Hz, 2H), 6.23 (d, J = 7.4 Hz, 2H), 3.41 – 3.19 (m, 4H), 2.03 (s, 6H), 1.60 – 1.53 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.41, 164.21, 145.55, 142.74, 138.81, 138.27, 137.87, 128.68, 128.54, 126.92, 126.78, 111.39, 111.23, 51.14, 39.56, 26.72, 26.37, 13.81, 13.62. HRMS-ESI (*m/z*): $[M+H]^+$ calculated for $C_{23}H_{25}N_6O_5$ 465.1881, found 465.1881. FTIR (KBr): ν_{max} 2093 cm^{-1} (N=N=N stretch)

Synthesis of DABA-bis-HP-N_3 ICP particles (ICP-N_3 NPs) In a typical experiment, an aqueous stock solution of DABA-bis-HP-N_3 was prepared consisting of 2.28 mM ligand and 24.5 mM NaOH. The ligand is freely soluble in water as its disodium salt. A stock solution of $Fe(NO_3)_3{\cdot}9H_2O$ was prepared consisting of 10.80 mM Fe^{3+} and 4 mM HCl (as stabilizer). To a glass vial was added 877 µL ligand stock solution, followed by 1 mL Milli-Q H_2O, followed by 123 µL Fe(III) stock and the resulting orange-red mixture (2 mL) shaken for 10 minutes. The as-synthesized particles have a mean diameter ranging from 10-20 nm (DLS). Particles were purified by filtration through an Amicon Ultra 15 mL centrifugal filter with a nominal molecular weight cutoff (MWCO) of 100 kDa, washing with 3 x 3 mL portions of Milli-Q H_2O, spinning at 5000 rcf for 10 minutes each. The particles were resuspended in 2 mL of H_2O to give an approximate azide concentration of 1 mM. The particle solution was lyophilized and the resulting dark red powder characterized by FTIR (KBr), showing the characteristic azide stretch at 2093 cm^{-1} is retained after the nanoparticle synthesis.

CHARACTERIZATION OF ICP-N_3 NANOPARTICLES

To determine the stoichiometry of metal-ligand binding, we conducted a titration wherein samples each containing a fixed concentration of 200 µM DABA-bis-HP-N_3 (**4**) in 1 mL H_2O were prepared with increasing amounts of $Fe(NO_3)_3{\cdot}9H_2O$ ranging from 0 to 220 µM. The absorbance at 460 nm was measured for each sample. The LMCT band at 460 nm is characteristic of the *tris*-HOPO-Fe^{3+} coordination complex. The equivalence point was reached at 133 µM (0.66 equiv.), consistent with Fe_2L_3 stoichiometry (*Figure S1*). Further increase in

absorbance is due to the presence of uncoordinated iron precursor salt. Additionally, we conducted elemental analysis on a lyophilized sample of the particles to assess their composition. Calc'd for $C_{69}H_{66}Fe_2N_{18}O_{15}$: C 55.28%, H 4.44%, N 16.82%. Found C 49.10%, H 4.18%; N 14.18%. The lower observed organic content may be explained by the porous nature of the ICP particles and their ability to entrap polar solvent molecules, e.g. H_2O. Lastly, we studied the bare ICPs by energy dispersive X-ray spectroscopy (Hitachi H2300-A STEM) (*Figure S2*).

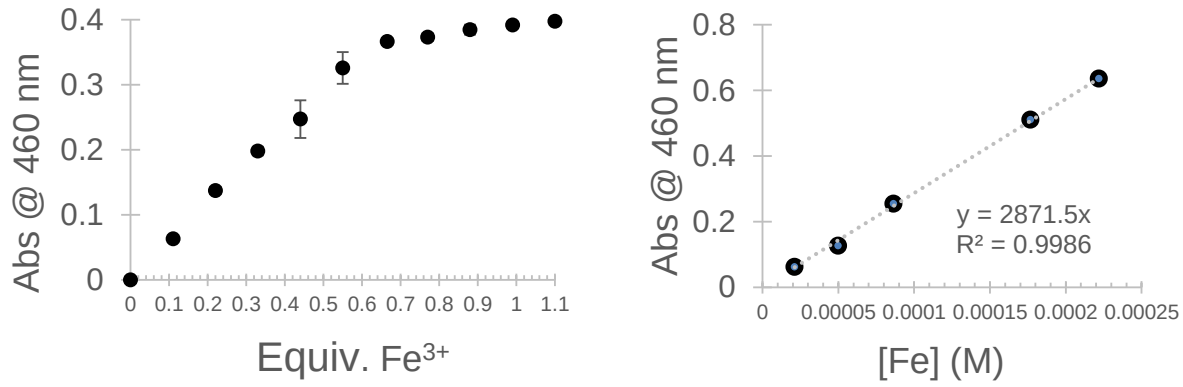


Figure S1. Left: titration of ligand **4** with iron(III). Right: determination of ε_{460} of ICP-N_3 particles

ICP-MS and UV-Vis were used in tandem to determine the extinction coefficient ε_{460} of the particles in Milli-Q H_2O. Briefly, five samples of ICP-N_3 particles in H_2O were prepared at varying dilutions and the absorbance at 460 nm was measured by UV-Vis. Subsequently, the iron concentration of each sample was determined by ICP-MS. Each sample was prepared in a matrix consisting of 3% HNO_3, 5 ppb indium (internal standard), and deionized water. The iron concentration was plotted vs. A_{460}, and the data was fit by a simple linear regression model. The slope of the line corresponds to $\varepsilon_{460} \approx 2870$ L·mol^{-1} cm^{-1} arising from the LMCT of the ICP-N_3 particles, allowing for spectroscopic determination of iron concentration.

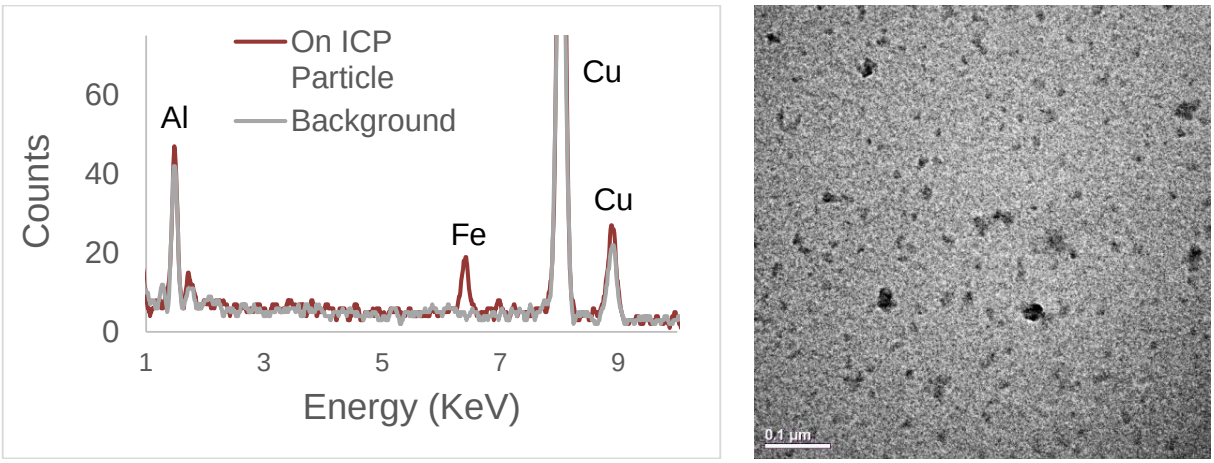


Figure S2. Left: EDX spectrum of background (copper TEM grid and aluminum holder) and ICP-N_3 particle. Right: TEM image of the same ICP-N_3 particle sample. Scale bar = 100 nm.

The weight of the particles produced by the above procedure is expected to be in the range of 10-1000 kDa, since a small portion of the as-synthesized particles pass through a 100 kDa cutoff filter. Supporting this observation, the predicted degree of polymerization for ditopic 3,4-HOPOs is approximately 1000 repeat units under the reaction conditions given above, when estimated from literature stability constants of the 3,4-HOPO-Fe(III) complex.[1,2]

OLIGONUCLEOTIDE SYNTHESIS AND CHARACTERIZATION

All DNA synthesis was carried out on a BioAutomation MM48 DNA synthesizer, according to the standard manufacturer trityl-on protocol with an additional 5 minute coupling time for non-nucleosidic phosphoramidites. Ac-dC and dmf-dG phosphoramidites were used to enable room-temperature deprotection of the nucleobases. Oligonucleotides were synthesized on 1 μmol scale and deprotected in concentrated NH_4OH (30%) for 17 hours at room temperature, except for poly(CCT)-Cy5-containing oligonucleotides, which were deprotected for 2 hours at room temperature. The resulting crude oligonucleotides were purified on a Varian Prostar HPLC fitted with a DynaMax Microsorb C18 Column, employing a gradient of 0-75% acetonitrile in triethylammonium acetate buffer (pH 7.0) over 45 minutes. The optical absorbance of the eluent was monitored at 254/310 nm for DBCO-containing oligonucleotides, 254/649 nm for Cy5-containing oligonucleotides, and 254/280 nm for all other oligonucleotides. DBCO-terminated oligonucleotides were lyophilized, resuspended in H_2O, and conjugated immediately to ICP-N_3 nanoparticles. Disulfide-terminated oligonucleotides were lyophilized, reduced to the free thiol and conjugated to AuNPs as described in previous reports.[3]

Oligo Name	Sequence	Calc. FW	Found FW
A-DBCO	5'-DBCO-TEG-A_4-AATCCTTATCAATATTT	6942	6951
B-DBCO	5'-DBCO-TEG-A_4-AAATATTGATAAGGATT	7080	7082
A-SH	5'-HS-$(CH_2)_6$-A_4-AATCCTTATCAATATTT	6699	6689
HER2-DBCO	5'-DBCO-TEG-CTC-CAT-GGT-GCT-CAC	5075	5070
NONT-DBCO	5'-DBCO-TEG-GAG-CTG-CAC-GCT-GCC-GTC-A	6360	6369
Cy5-DBCO	5'-DBCO-TEG-CCTCCTCCT-Cy5-CCTCCTCCT	6337	6341
CCT-Cy5-SH	5'-HS-$(CH_2)_6$-CCTCCTCCT-Cy5-CCTCCTCCT	6094	6098
Cy5-T_{20}-SH*	5'-Cy5-TTTTTTTTTTTTTTTTTTTT-$(CH_2)_3$-SH	7084	7084

Table S1. All oligonucleotides synthesized for conjugation to ICP-NPs and AuNPs with mass determined by MALDI-ToF.
*Purchased from TriLink BioTechnologies (San Diego, CA)

SYNTHESIS AND CHARACTERIZATION OF AUNP-DNA CONJUGATES

AuNP-SNAs synthesized in this study were prepared according to established protocols.[3] For AuNP-SNAs employed in cell uptake experiments, the number of oligonucleotides/AuNP were determined by fluorescence measurements. Oligonucleotide loading on AuNP-SNAs was quantified using a 5nM solution of Cy5 labeled AuNP-SNAs. The Au core was dissolved using 100mM KCN diluted in deionized water. The mixture was then incubated at room temperature for 20 minutes, and the resultant fluorescence measured against a standard curve. The standard curve consisted of the equivalent oligonucleotide sequence at a range of concentrations, dissolved in water, treated with KCN and incubated in the same manner as the SNAs. All fluorescence measurements were made using a Synergy H4 fluorescent plate reader (BioTek).

Loading on the CCT-Cy5-AuNPs used as a positive control for cell uptake experiments was 113 strands/particle (**CCT-Cy5-AuNP**). Loading on the Cy5-T_{20}-AuNPs was 157 strands/particle (**Cy5-T_{20}-AuNP**). Similar values were assumed for the non-fluorescent AuNP-SNA (**A-AuNP**).

SYNTHESIS OF DNA-ICP CONJUGATES

In a typical procedure, a solution was prepared containing 100 µM of the desired cyclooctyne-DNA, 0.5M NaCl, and ICP-N_3 particles (500 µM in azide) in 2 mL Milli-Q H_2O. The resulting clear, orange solution was shaken for 16 h at 25°C. The reaction mixture was purified by ultrafiltration through an Amicon® Ultra 15 mL centrifugal filter (100 kDa MWCO), washing with 4 x 3 mL portions of 0.1M Tris buffer (pH 8.0), spinning at 5000 rcf for 10 minutes. The particles were resuspended in 1 mL of 0.1M Tris (pH 8.0). DNA-ICP particles remain colloidally stable at high salt concentrations (up to 1M NaCl), in contrast to the bare ICP-N_3 particles, which sediment within minutes in 1M NaCl. This observation indicates a stabilizing DNA surface layer has been successfully conjugated to the particle.

CHARACTERIZATION OF DNA-ICP CONJUGATES

The size, charge, and DNA-loading of DNA-ICP particles were analyzed by DLS, zeta potential, and UV-Vis. The DNA concentration of a particle solution was determined by UV-Vis using the ratio (A_{260}/A_{460}). Bare particles in Milli-Q H_2O possess $A_{260}/A_{460} \approx 5.4$. DNA-decorated particles were synthesized having a ratio A_{260}/A_{460} varying from 10.9 to 15.5, indicating the presence of DNA attached to the particles. Extensive washing was conducted to ensure no free DNA remained in solution. Loading of Cy5-containing DNA was significantly lower, potentially due to the steric bulk of the dye label. Finally, the zeta potential of the bare and DNA-loaded ICPs was compared, with all samples prepared at identical dilution in 10 mM Tris buffer (pH 8.0) and 0.1M NaCl. The following data were collected for the ICP particles synthesized for this study:

ICP Particle Type	ζ_{avg} (mV)	d_H (nm)	%A_{260} DNA
Bare	-18.9	14 ± 2	-
A-ICP	-35.2	31 ± 10	53%
B-ICP	-33.7	32 ± 8	60%
Her2-ICP	-31.1	31 ± 13	61%
NonTarget-ICP	-33.4	31 ± 11	65%
Cy5-ICP	-23.7	n/a*	14%

Table S2. Characterization of DNA-ICP conjugates. *DLS was not suitable for analysis of Cy5-containing particles. AFM imaging revealed particles with a similar size distribution. (37 ± 11 nm, NanoScope Analysis software)

THERMAL DENATURATION STUDIES

DNA-ICP particles bearing complementary sequences with a 17 base-pair overlap (**A-ICP** and **B-ICP**) were mixed at varying salt concentrations in 0.1M Tris buffer (pH 8.0) and heated from 20°C to 80°C at a rate of 0.25°C per minute. At room temperature, insoluble aggregates formed within 30-60 minutes of mixing the complementary DNA-ICPs. Upon heating, a sharp melting transition was observed, consistent with high DNA surface loading of the ICP particles. The same behavior was not observed for a pair of DNA-ICPs with mismatched sequences (**B-ICP** and **NonTarget-ICP**). The free DNA duplex has a melting temperature of 54°C in 0.3M NaCl, compared to >60°C for the DNA-ICPs, depending on the NaCl concentration. The same experiment was repeated using a gold nanoparticle/DNA-ICP pair (**A-AuNP** and **B-ICP**). The results are shown below at increasing NaCl concentrations (*Figure S3*).

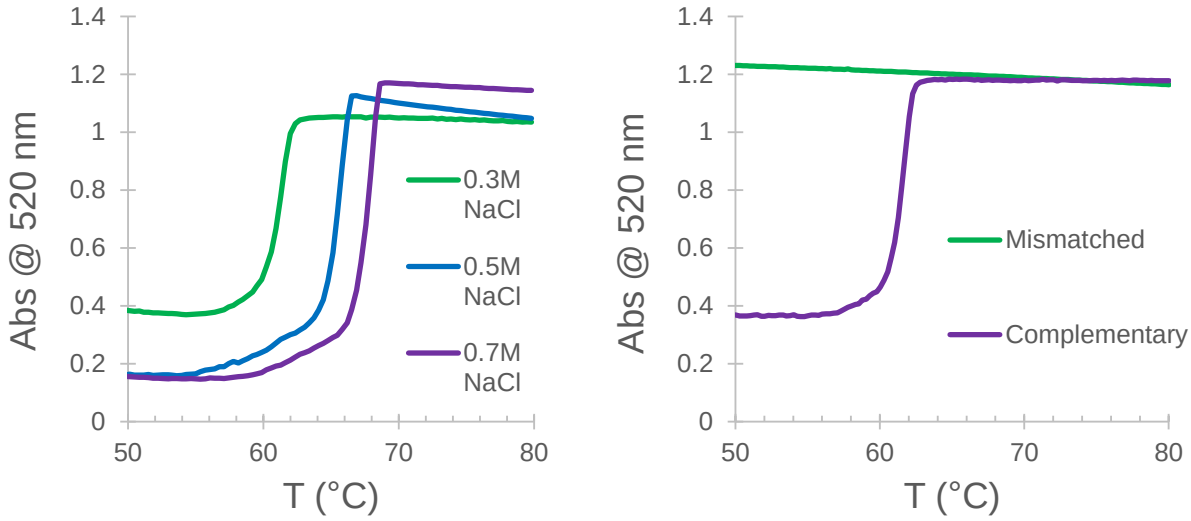


Figure S3. Thermal denaturation of complementary (left) and non-complementary (right) ICP/AuNP-DNA conjugates

IMAGES OF ICP-N_3 AND DNA-ICP PARTICLES

AFM imaging was carried out by drop-casting a solution of particles in H_2O and air-drying the droplet on freshly cleaved muscovite mica (Ted Pella). TEM imaging was carried out by drop-casting a solution of particles in H_2O and air-drying the droplet on a carbon-coated TEM grid (Ted Pella). The bare particles exhibited a strong tendency to aggregate into network-like structures upon drying, whereas the DNA-functionalized particles appeared more dispersed on the substrate under the same conditions.

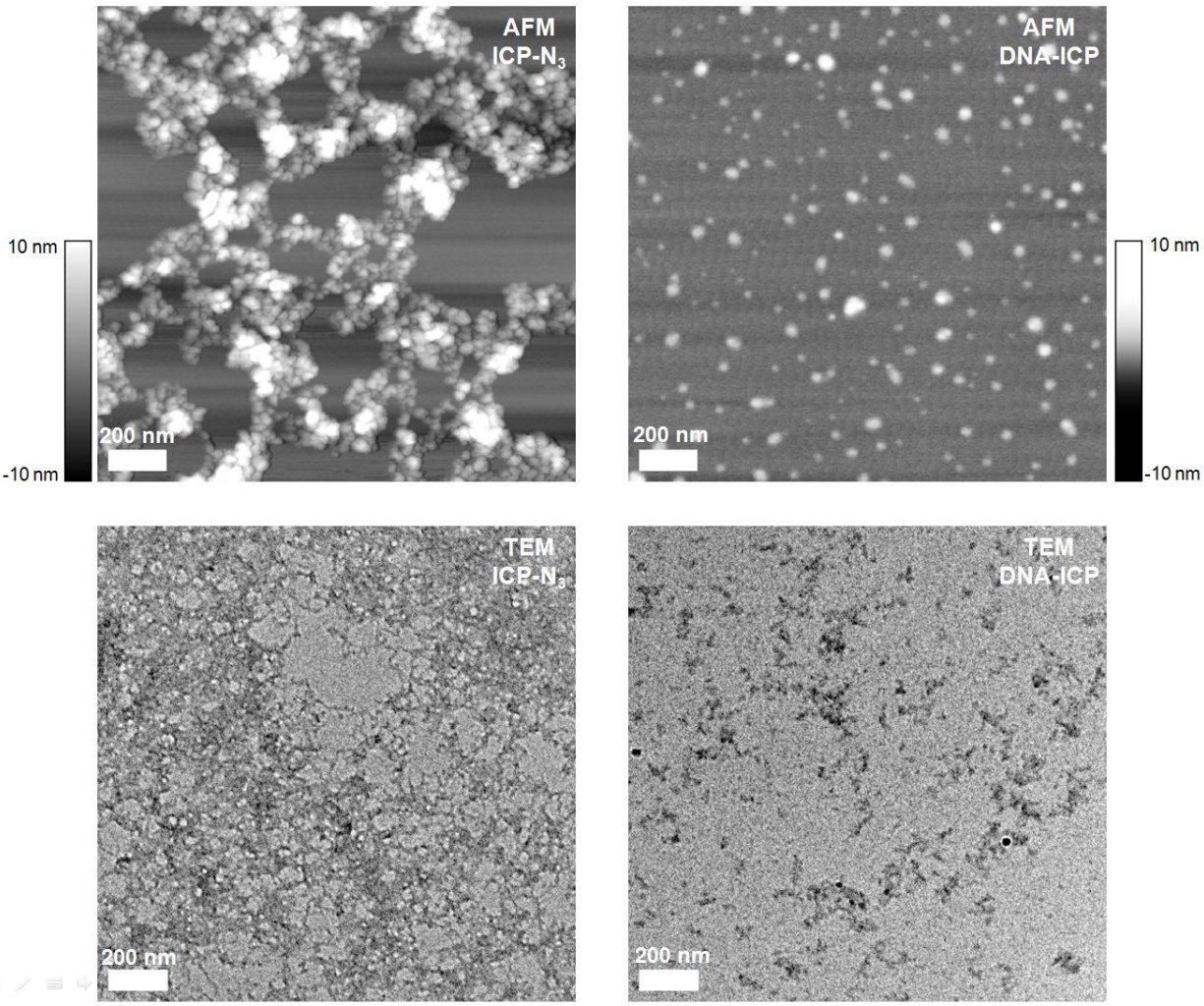


Figure S4. AFM images. Top left: Bare N_3-ICPs. Top right: DNA-ICPs. Bottom left: Bare N_3-ICPs. Bottom right: DNA-ICPs. Note that the oligonucleotides attached to the particle surface are largely transparent to the electron beam and therefore do not contribute to the apparent size of the particles.

MTT TOXICITY ASSAY OF ICP LIGANDS

To ensure the parent ligand comprising the particle core did not exhibit cellular toxicity, an MTT assay was performed. SKOV-3 cells were seeded in a 96-well plate at a population of $5x10^3$ cells per well. After 24 h, the cells were treated with 0.1 mL of a solution of compound **3** or **4** (diluted from DMSO stock solution into Opti-MEM) and incubated at 37°C for 24 h. After incubation, the compound was removed from the cells and replaced with 0.1 mL of complete McCoy's 5A medium (supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin). Cell viability was measured by MTT assay at 48 h and 72 h following the addition of compound **3** or **4** to the cells.

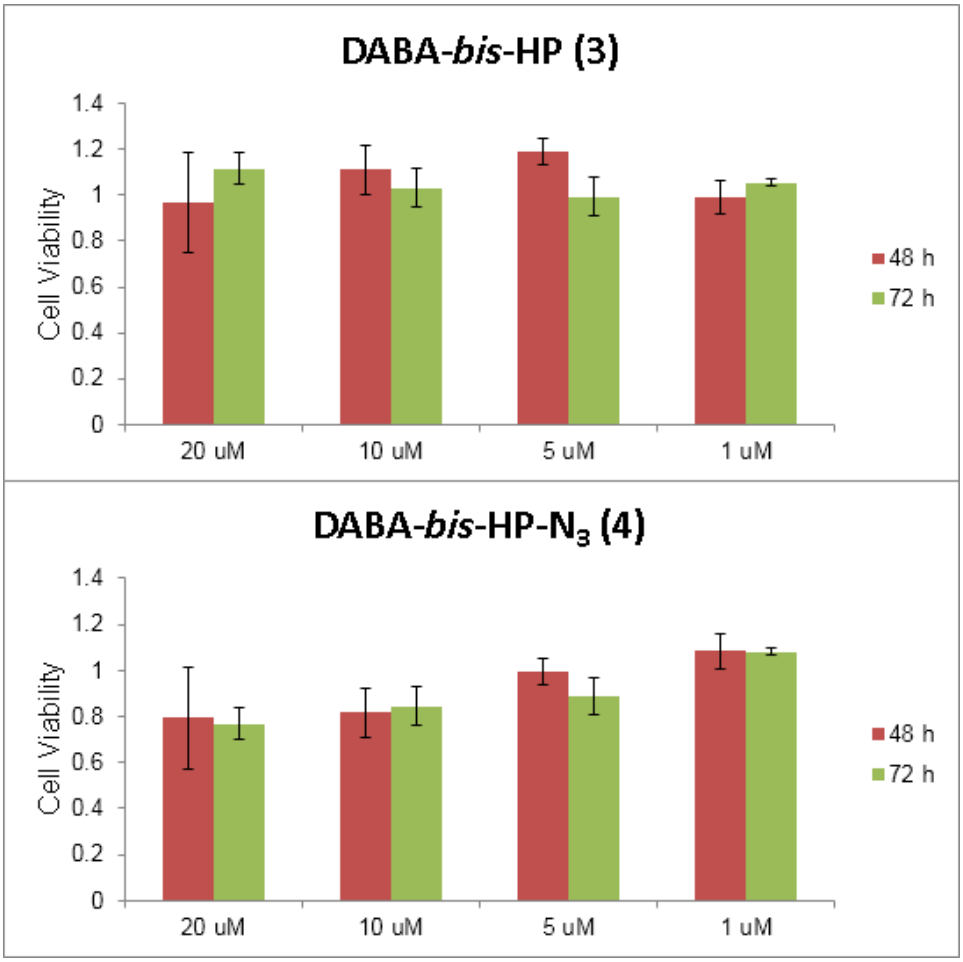


Figure S5. Viability of cells treated with ICP ligands **3** and **4** at various time points and concentrations (normalized to DMSO)

Briefly, cells were incubated with 0.1 mL of complete McCoy's 5A medium. 10 µL of MTT solution (5 mg/mL MTT in 1x PBS; Molecular Probes) was added into each well of cells and cells were incubated at 37°C for 2 h. After incubation, 0.1 mL of SDS-HCl solution (0.1 g/mL SDS in 0.01 M HCl) was added to each well to solubilize the formazan product, and cells were further incubated at 37°C overnight. After overnight incubation, the absorbance of the cell lysate was measured at 570 nm using a Synergy H4 Multimode Microplate Reader (Biotek). The relative cell viability was calculated compared to vehicle-treated cells. Reported values represent the mean ± SD of three replicates.

CELL CULTURE AND UPTAKE STUDIES

For visualizing cell uptake by confocal microscopy, ovarian cancer (SKOV-3), cervical cancer (HeLa) and C166 mouse endothelial cells were cultured in DMEM supplemented with 10% Fetal Bovine Serum (Atlanta Biologicals) and 1% Penicillin/streptomycin (Life Technologies).

All microscopy was performed using an SP5 laser scanning confocal microscope. Cellular images were obtained by culturing HeLa cells in supplemented Dulbecco's Modified Eagle Medium (DMEM, Life Technologies) at approximately 30% confluency in Nunc Lab-Tek II borosilicate-bottom chamber slides (Thermo Scientific). Cells were allowed to attach for 24 hours, after which they were washed once with PBS and resuspended in OptiMEM. Cells were then treated with either linear DNA, AuNP-SNAs, or DNA-ICPs at a concentration of 100 nM (DNA basis). After 24 hours the cells were washed once with OptiMEM, and resuspended in DMEM containing Hoechst 33258 (Life Technologies). All images are of live cells. Equivalent methods were used for flow cytometry, however cells were not treated with Hoechst and instead were trypsinized for 3 minutes in 05% trypsin-EDTA (GIBCO), resuspended in Opti-MEM, and analyzed using a Guava Easycyte 8HT (Millipore) equipped with a 633nm laser.

For naked-eye visualization of cell uptake, MCF-7 and SKOV-3 cells were plated in 6 well plates (~100,000 cells/well). After incubating the cells for 24 hours in DMEM + 10% FBS containing medium, the cell media was changed to Opti-MEM and the following concentrations of DNA-ICPs were added to individual wells (DNA basis): 0.0, 0.1, 0.5, 1.0, 2, and 5 µM. The particles were incubated in cells for 24 hours, after which the cells were washed thrice in PBS, cells were replenished with fresh media, and the cells were incubated for an additional 48 hours. Thereafter, cells were rigorously washed to remove any extracellular ICP particles, trypsinized, and immediately transferred to 1.5 ml Eppendorf tubes containing PBS. The cells were then centrifuged at 1100 RPM for 5 minutes to form cellular pellets. Pictures of the cell pellet were taken against a white background to show a concentration dependent increase in ICP uptake.

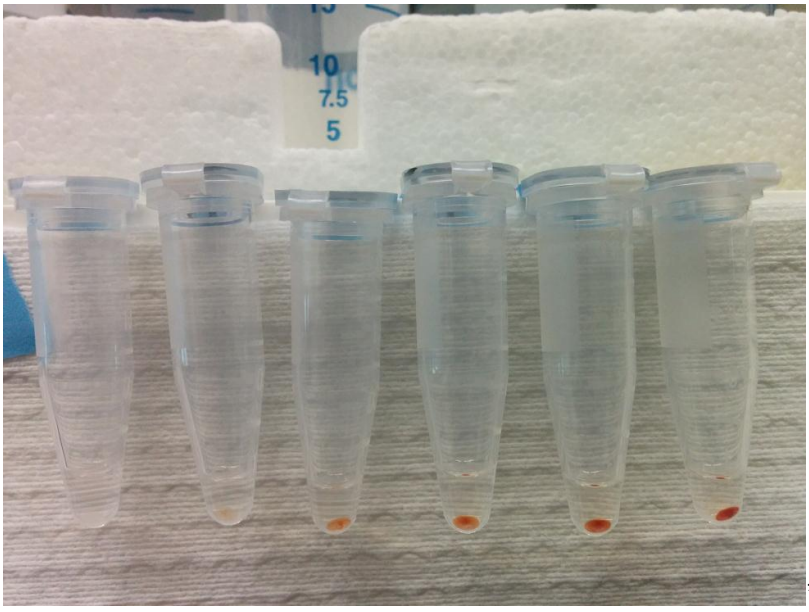


Figure S6. Pelleted MCF-7 cells incubated with DNA-ICPs (left to right): 0 µM, 0.1 µM, 0.5 µM, 1.0 µM, 2.0 µM, 5.0 µM

ANALYSIS OF CELL UPTAKE IN SKOV-3 CELLS

Prior to gene regulation experiments, we repeated the uptake studies in SKOV-3 cells utilizing confocal microscopy and flow cytometry. The results were similar to those observed for other cell lines.

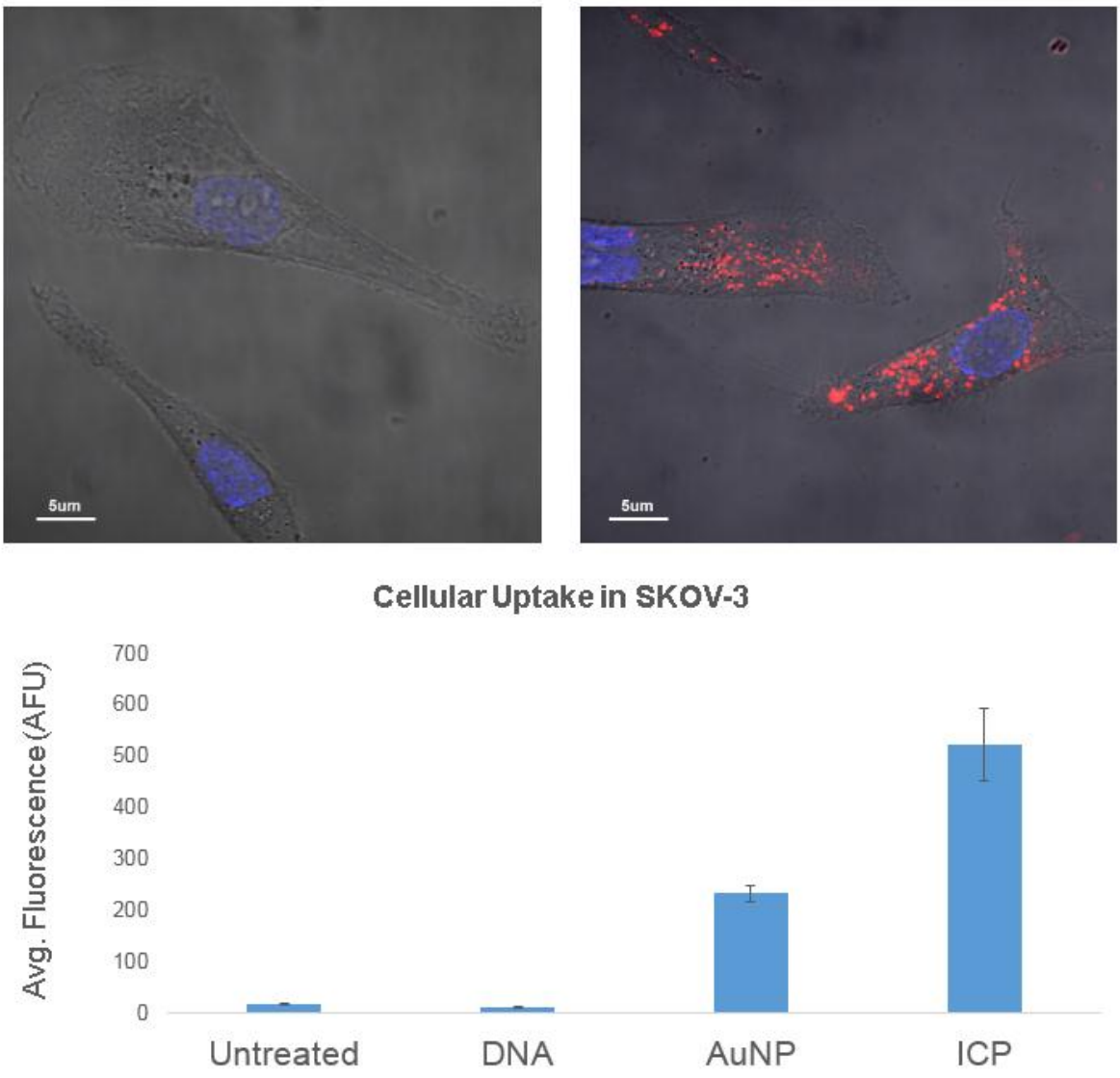


Figure S7. Top left: cells treated with fluorescent ssDNA. Top right: cells treated with DNA-ICPs bearing the same sequence. Bottom: flow cytometry analysis comparing untreated cells with Cy5-labeled ssDNA, AuNP-SNAs, and DNA-ICPs. All treatments 100 nM total (DNA basis).

ADDITIONAL IMAGES OF CELL UPTAKE

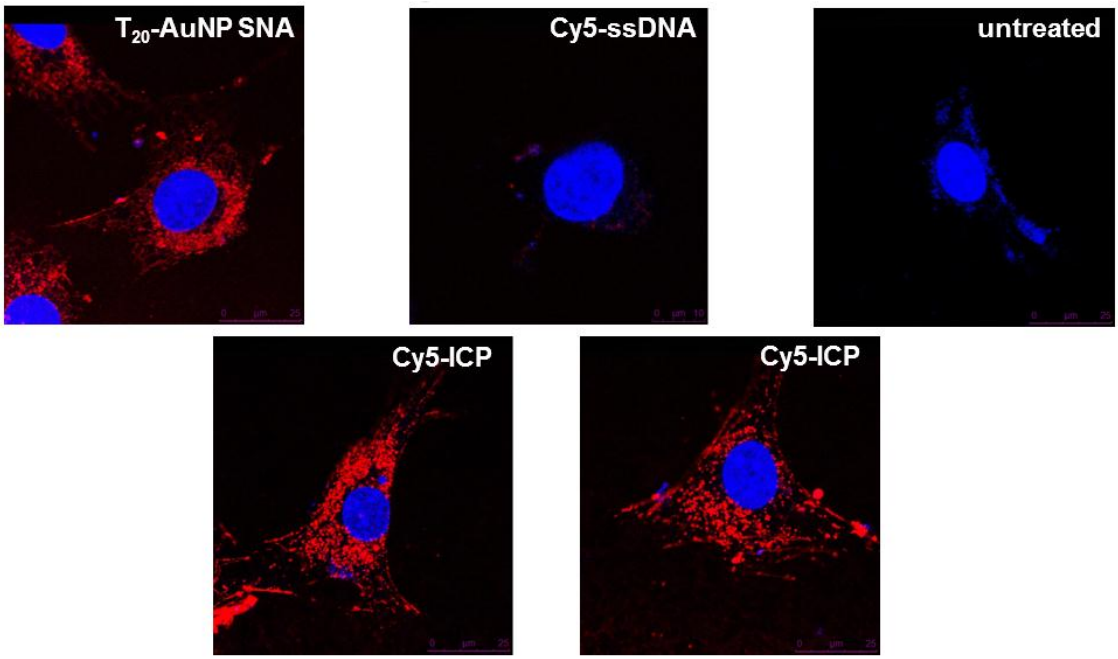


Figure S8. Cell uptake (C166 mouse endothelial) of Cy5-labeled AuNP-SNAs (top left) compared to free DNA (top middle), unreated cells (top right), and CCT-Cy5-DNA ICPs (bottom). All treatments 100 nM total (DNA basis).

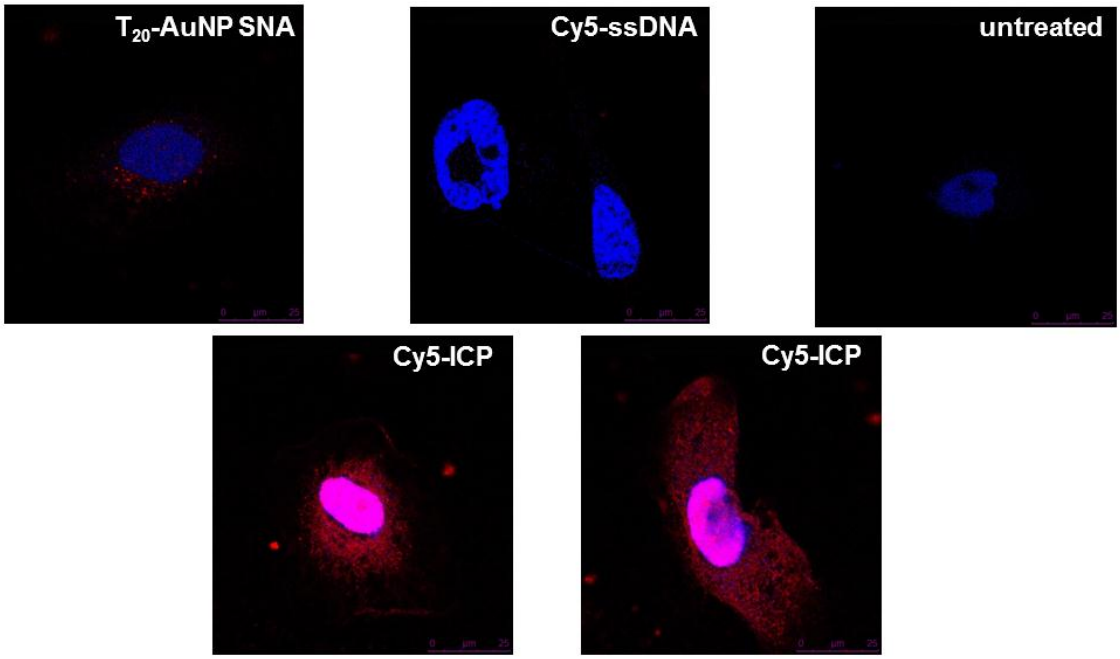


Figure S9. Cell uptake (HeLa cells) of Cy5-labeled AuNP-SNAs (top left) compared to free DNA (top middle), unreated cells (top right), and CCT-Cy5-DNA ICPs (bottom). All treatments 100 nM total (DNA basis).

Note: The above images were taken from z-stack data sets where the focus (z dimension) was collected at various intervals encompassing the full height of the cell(s). Each image is meant to be representative of the total DNA content inside the cell.

WESTERN BLOT AND GENE KNOCKDOWN ANALYSIS

SKOV3 cells were obtained from American Tissue Culture Collection (ATCC). The cells were incubated in 5% CO_2 at 37˚C in McCoy's 5A medium supplemented with 10% heat-inactivated FBS. Cells were cultured in 6 well cell culture plates (BD Biosciences) with 100,000 cells per well seeded 24 hours before treatment with ICPs. Medium was replaced with Opti-MEM (Life technologies) immediately prior to treatment with ICPs or Lipofectamine RNAimax (Life technologies) DNA. Lipofectamine transfection was performed according to manufacturer's instructions to deliver 25 pmole of DNA. After 12 hours, the medium was replaced with fresh media (McCoy's 5A with 10% FBS) and the cells incubated for another 48 hours. The cells were then washed three times with PBS, trypsinized and the pellet was resuspended in 100 µL of mammalian cell lysis buffer (Cell Signalling) containing protease and phosphatase inhibitor (Thermo Scientific). The whole cell lysates were then purified and collected by centrifugation and frozen at -80 ˚C. Protein concentrations were determined using the BCA Protein Assay Kit (Pierce). Equal amounts of protein samples (25 µg) were fractionated by 4-20% precast gradient gel (Bio-Rad) and transferred to nitrocellulose membranes (Thermo Scientific). Membranes were dried overnight, rehydrated in PBS, then blocked for 1 hour at room temperature in blocking buffer (LI-COR Biosciences). Proteins were detected with rabbit primary antibodies against HER2 (1000:1) (Cell Signaling), mouse antibody against beta-tubulin (1000:1) (Thermo Scientific) and anti-rabbit or anti-mouse IgG-dye conjugated secondary antibodies (10,000:1) (LI-COR Biosciences). The fluorescence signal was recorded and quantified using the Odyssey Infrared Imaging System (LI-COR Biosciences) and quantified using Image Studio software (LI-COR Biosciences).

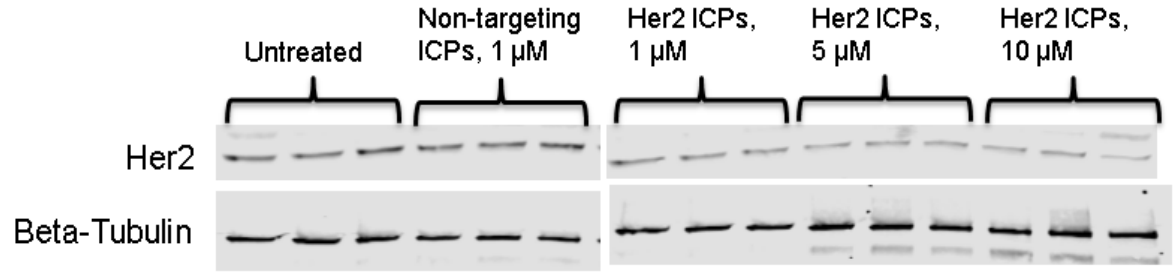


Figure S10. Western Blot analysis of SKOV-3 cells treated with varying types and concentrations of DNA-ICP particles.

AGAROSE GEL ANALYSIS OF DNA-ICP DEGRADATION

To visualize the amount of nucleic acids released from DNA-ICP particles under acidic conditions, an agarose gel experiment was carried out. Briefly, five aliquots of DNA-ICPs (**B-ICP**) containing 12.2 µM total DNA were diluted 1:1 with the following buffer solutions (100 mM each): KHP buffer, pH 4.0; HEPES buffer, pH 5.5; MES buffer, pH 6.0, PBS buffer, pH 7.4, or Tris buffer, pH 8.0. As a control, a sample was prepared with the single-stranded oligonucleotide (**B-DBCO**) containing the same total amount of DNA in deionized H_2O. The solutions were allowed to stand at RT for 48 h, then were subsequently diluted again 1:1 with 0.5x TAE buffer. The samples were then analyzed in 1% agarose gel run at 90V for 1 hour in 0.5x TAE buffer. Agarose gels were imaged on a Fujifilm FLA-5100 gel imager and 1X SYBR® Gold stain was used to visualize oligonucleotides.

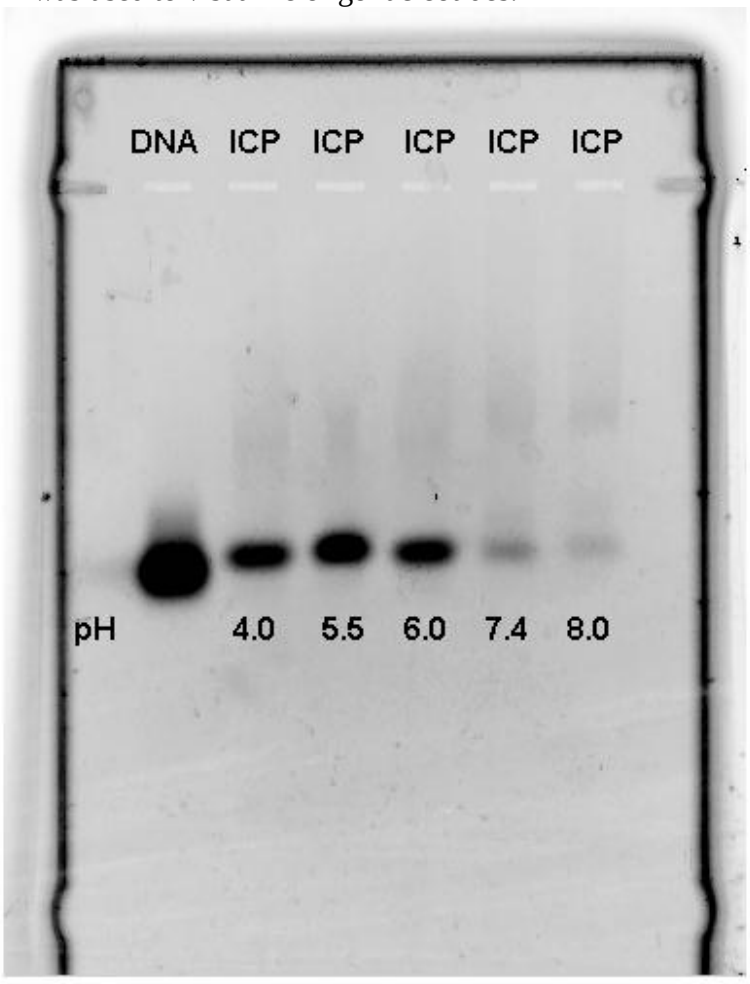


Figure S11. Agarose gel analysis of ICP degradation after 48 hours. Note that the DNA released from the ICP is slightly retarded in the gel due to its higher molecular weight after reaction with the ligand (**4**). Identical results were obtained after 72 h.

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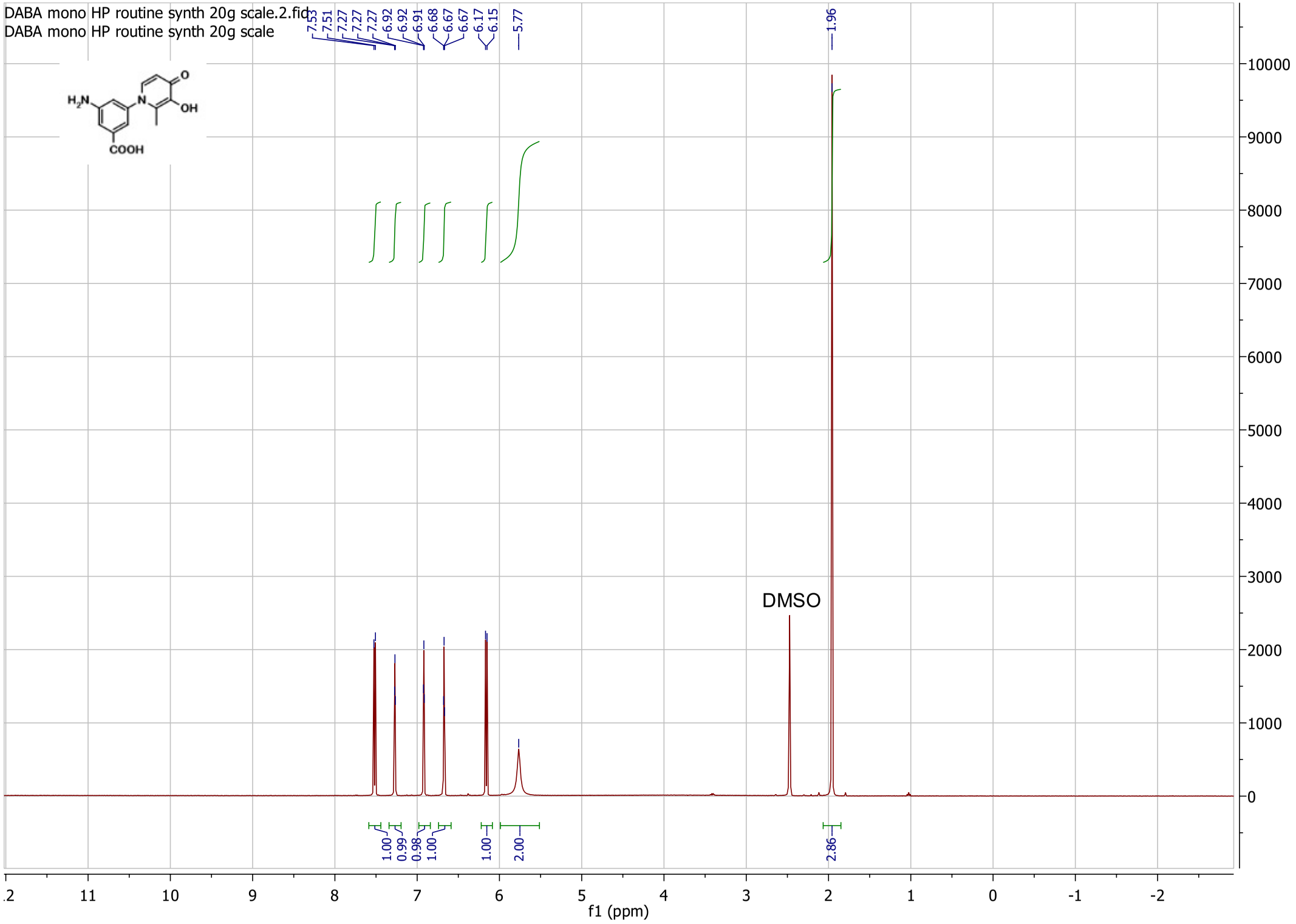
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DABA mono HP routine synth 20g scale
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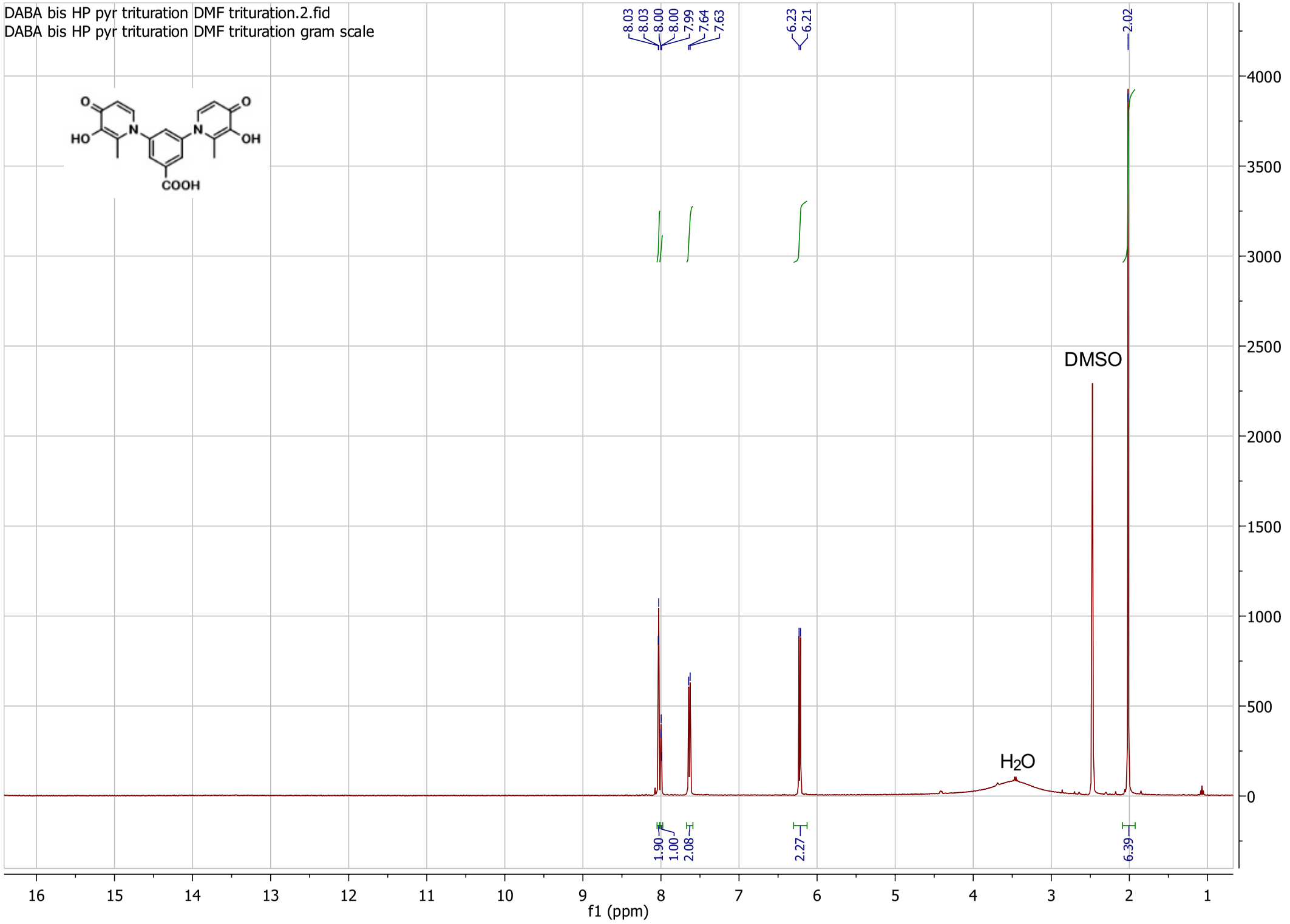
7.51
7.27
7.27
7.27
6.92
6.92
6.91
6.68
6.67
6.67
6.17
6.15
5.77
1.96
DMSO
1.00
0.99
0.98
1.00
1.00
2.00
2.86
f1 (ppm)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DABA bis HP pyr trituration DMF trituration gram scale
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8.03
8.00
8.00
7.99
7.64
7.63
6.23
6.21
2.02
HO
OH
COOH
DMSO
H2O
1.90
1.00
2.08
2.27
6.39
f1 (ppm)

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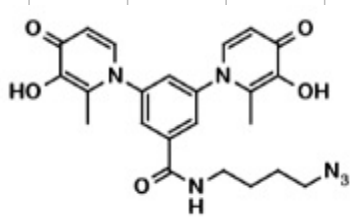
DABA BisHP azidobutanamide DMSO+HATUv 12-5-13



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DMSO

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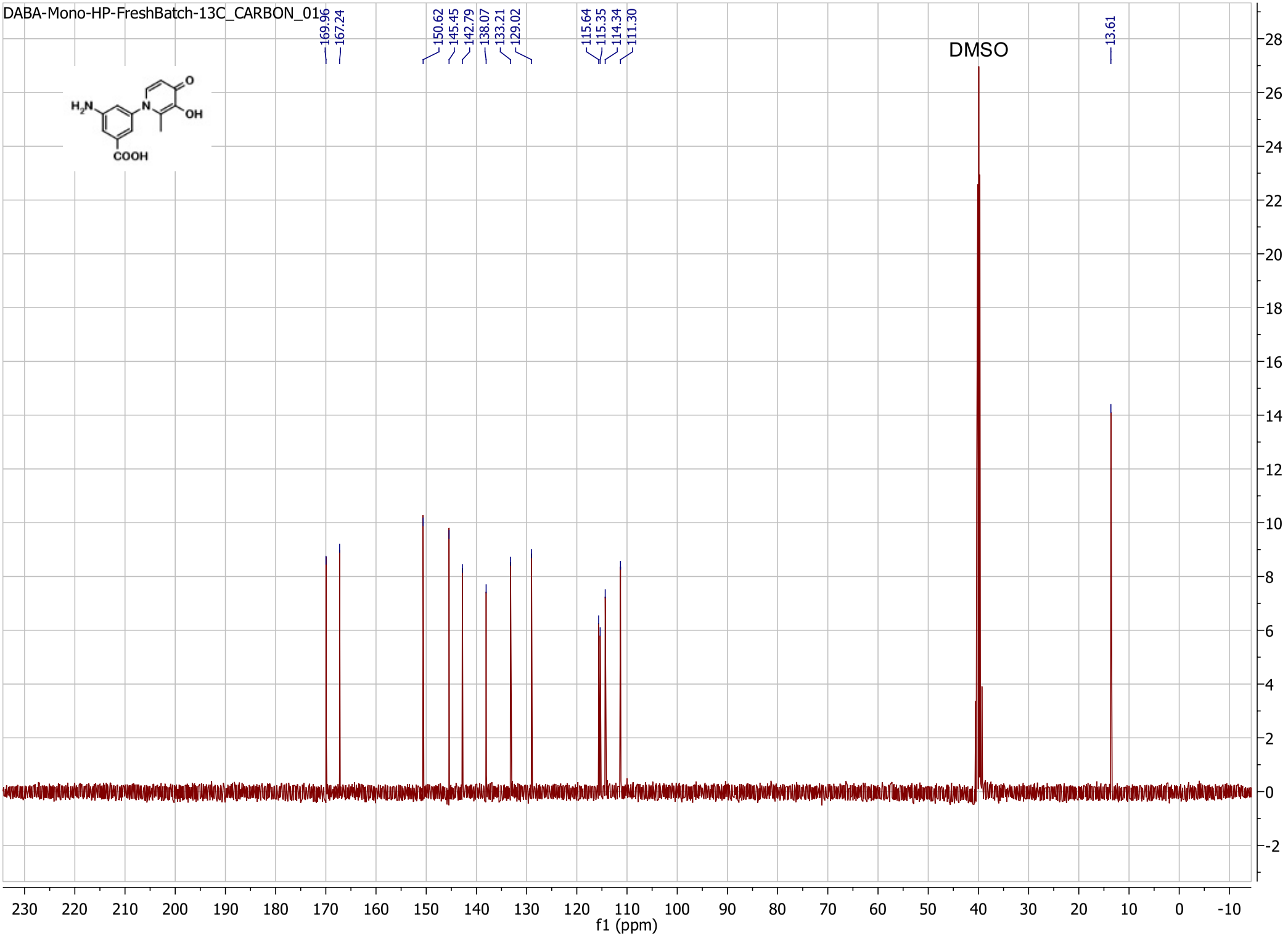
f1 (ppm)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115.35
114.34
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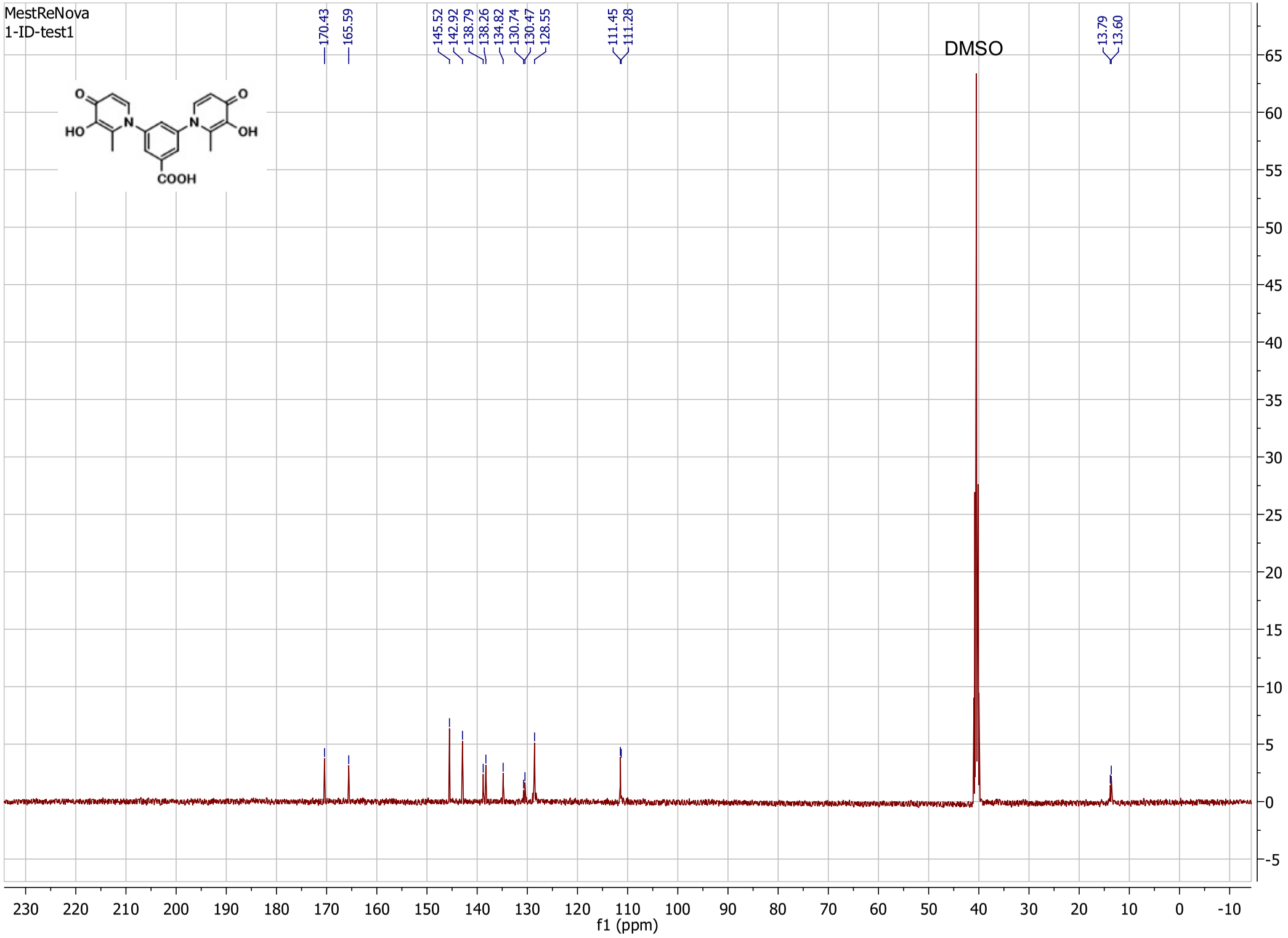
DMSO
13.61
H2N
N
O
OH
COOH
f1 (ppm)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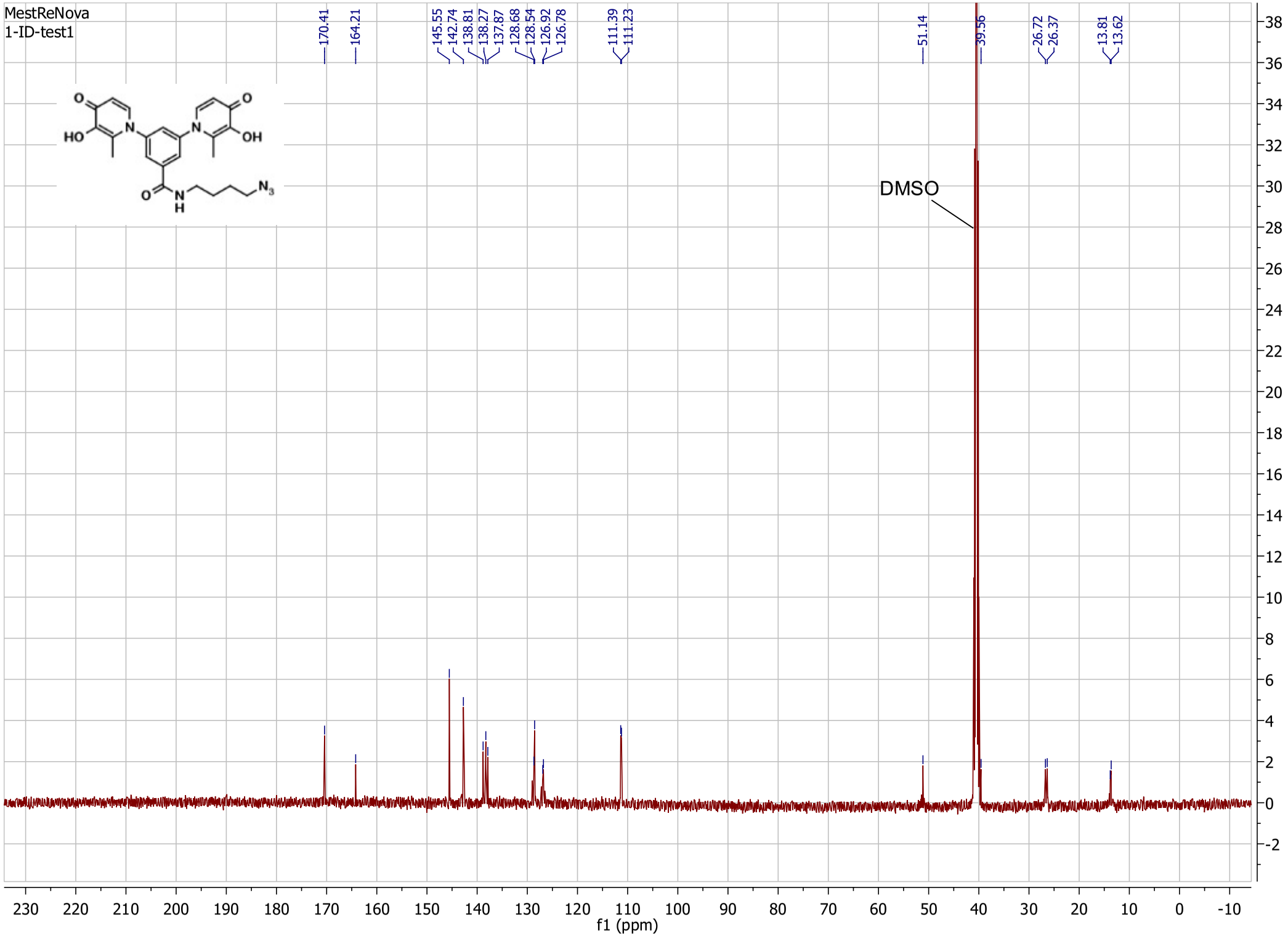
13.60
HO
OH
COOH
f1 (ppm)


MestReNova
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126.92
126.78
111.39
111.23
51.14
39.56
26.72
26.37
13.81
13.62
DMSO
HO
OH
O
N
H
N_3
f1 (ppm)