Supplemental information

Synthesis and optimization of 1-substituted imidazo[4,5-c]quinoline TLR7 agonists

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Table of contents for supplemental data:

17a

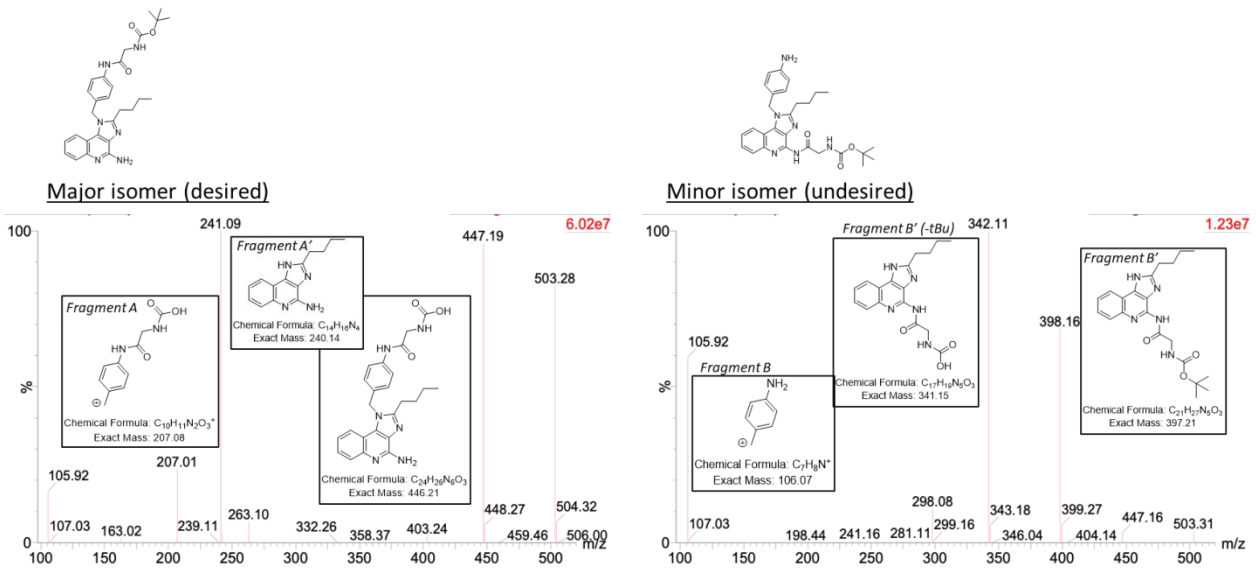


Figure S1: Daughter ion scan for isomers of **17a**

17c

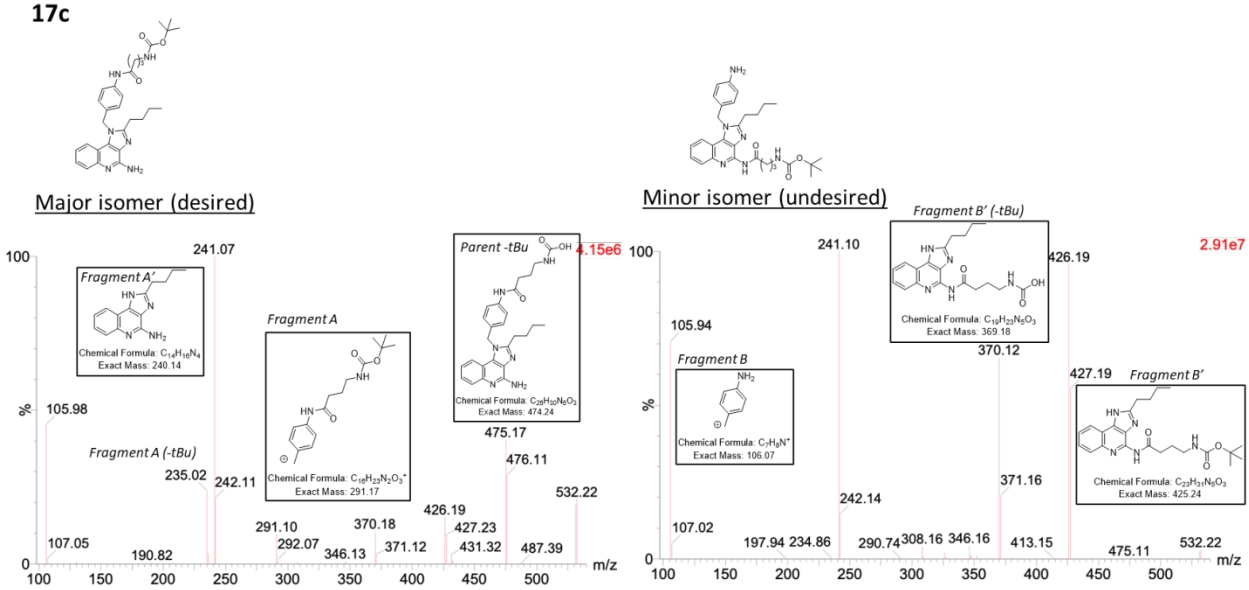
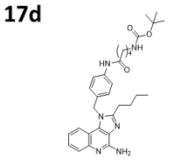
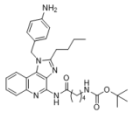


Figure S2: Daughter ion scan for isomers of **17c**

17d



Major isomer (desired)



Minor isomer (undesired)

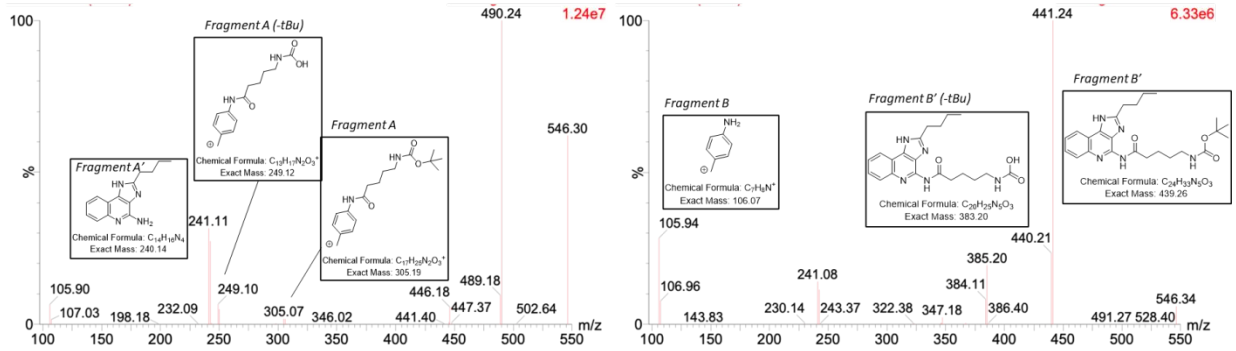
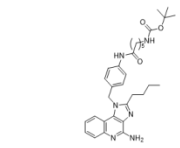


Figure S3: Daughter ion scan for isomers of **17d**

17e



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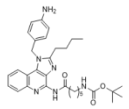

Minor isomer (undesired)

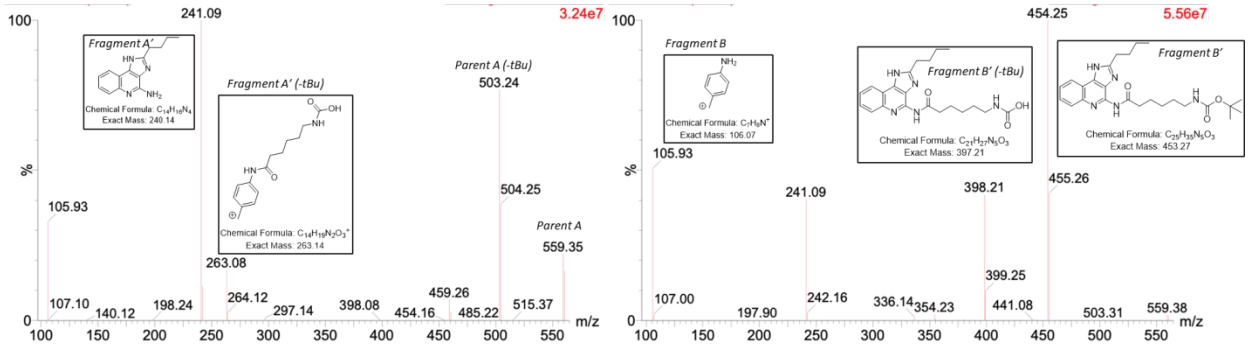

Figure S4: Daughter ion scan for isomers of **17e**

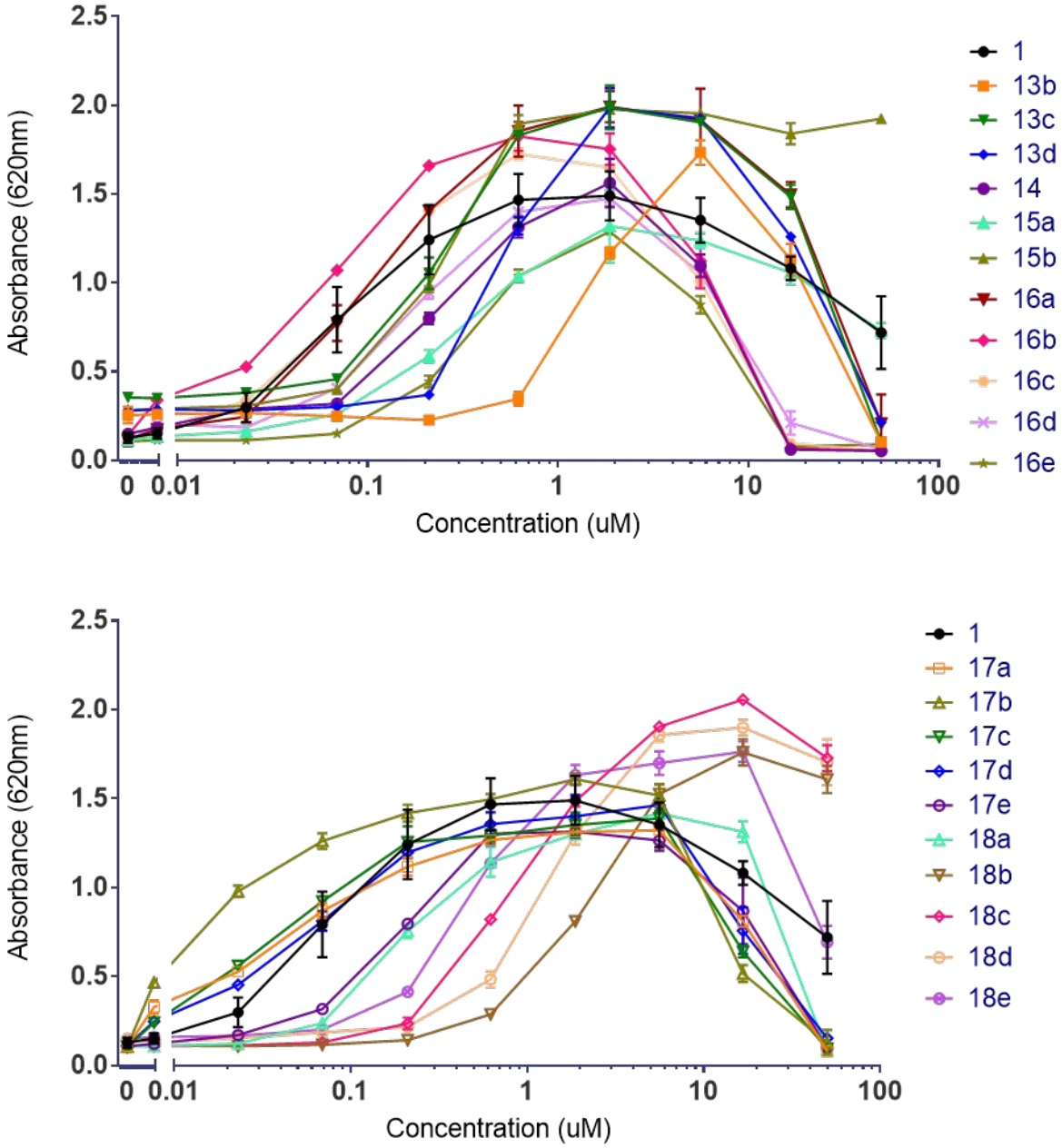

Figure S5. Full EC50 curves for Ramos Blue assays.

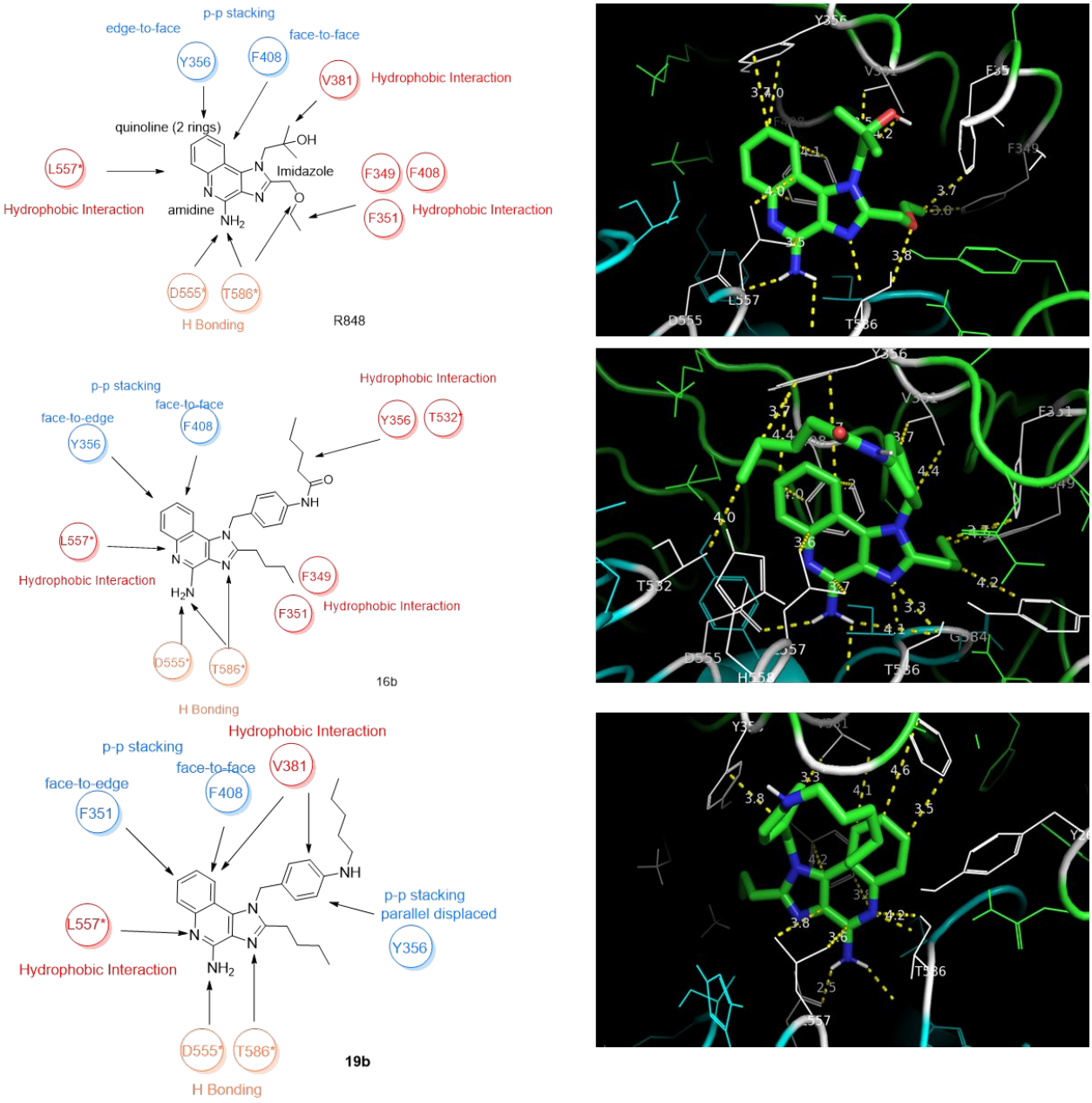

Figure S6. Docking results showing the key binding interactions for **R848**, **16b**, and **19b**.

Experimental details

General experimental conditions. All solvents and reagents were used as received from commercial vendors unless otherwise noted. Chemicals were purchased from TCI, MedChemExpress, or VWR. Compound **1** was prepared as previously described.[13] No unexpected or unusually high safety hazards were encountered. NMR was performed using a Bruker Avance III HD 400 MHz Proton NMR instrument, with a 9.1 Tesla shielded, superconducting magnet, 2 RF Channels, z-axis Pulsed Field Gradient, a 24-sample SampleCase Automation, and a 5mm Broadband/Fluorine Observe Probe. NMR was performed in D6-DMSO or in DMSO using WETDC_1 solvent suppression. Reaction monitoring and purity analysis was

conducted by UPLC-MS and HPLC-MS, as outlined below. Ramos blue cells (cat# rms-sp), RAW Dual cells (cat# rawd-ismip), HEK Blue hTLR7 cells (cat# hkb-htlr7), and HEK Blue hTLR8 cells (cat# hkb-htlr8) were purchased from InvivoGen and were cultured according to the manufacturer's instructions. The PAMPA assay kit was obtained from Corning (part# 353015).

Analytical HPLC-MS was performed using a Waters Autopurification system consisting of a 2545 binary gradient module, 2767 sample manager, 2998 UV/PDA detector, and SQD2 mass spectrometer. The separation was performed using an XBridge BEH C18 5µm (4.6 x 100 mm) column at 80 °C. Eluent was monitored by UV (210-600nm) and mass spectrometry (150-1800 Da, ES+/ES-). Solvents for the mobile phase were water containing 0.2% formic acid (solvent A) and acetonitrile (ACN) containing 0.2% formic acid (solvent B). The flow rate was 2.00 ml/min. The gradient was performed as follows: 5% B for 1.0 min (0-1.0 min) → 5% to 99% B over 2.8 min → 99% B for 0.1 min → 99% to 5% B over 0.1 min → 5% B for 1.0 min.

Analytical UPLC-MS was performed using a Waters Acquity H-Class UPLC ® with TUV detector and QDa mass spectrometer. The separation was performed using a BEH C18 1.7 µm column (2.1 x 50mm) at 80 °C. Eluent was monitored by UV (220 and 254nm) and mass spectrometry (150-1250 Da, ES+/ES-). Solvents for the mobile phase were water with 0.1% formic acid (solvent A) and acetonitrile (ACN) with 0.1% formic acid (solvent B). The flow rate was 0.8 ml/min. The gradient was performed as follows: 10%B for 0.8 min → 10% to 90% B over 3.6 min → 90% B for 0.1 min → 90% to 10% B over 0.05 min → 10% B for 0.45 min.

LCMS/MS Fragmentation was performed on a Waters Acquity H-Class UPLC ® with TUV detector and TQD mass spectrometer. The separation was conducted as outlined above. Samples were evaluated using a daughter ion scan to identify fragments of the parent ion.

Preparative HPLC was performed using a Waters Auto-purification system configured as described above. The separation was performed using an XBridge BEH C18 5 µm OBD (19 x 100 mm) prep column at room temperature. Eluent was monitored by UV (210-600nm), and mass spectrometry (150-2500 Da, ES+/ES-). Solvent for the mobile phase were water containing 0.05% TFA (solvent A) and CAN containing 0.05% TFA (solvent B). The flow rate was 20.0 ml/min. Gradient Used: 15% to 95% B over 7.0 min → 95% B for 0.5 min → 95% to 15% B over 0.5 min → 15% B for 1.0 min.

***tert*-Butyl (3-(((3-nitroquinolin-4-yl)amino)methyl)phenyl)carbamate (10b)**: To a solution of tert-butyl (3-(aminomethyl)phenyl)carbamate (1080 mg, 1 eq, 4.85mmol) in DCM (27.0 mL) was added 4-chloro-3-nitroquinoline (1000 mg, 1 eq, 4.82mmol) and triethylamine (975 mg, 1.34 mL, 2 eq, 9.64 mmol). The mixture was brown and became yellow once the triethylamine was added. The mixture was brought to reflux at 40 °C for 1 h. A yellow precipitate formed and LCMS indicated near-complete conversion by 60 min. The reaction was cooled to room temperature and concentrated to dryness. The yellow solid was suspended in water (140 mL), filtered under vacuum through a sintered funnel and dried in a desiccator to obtain the desired compound. (1550 mg, 82% yield) LCMS rt =3.50 min; m/z = 394.43 [M+H].

***tert*-Butyl (3-(((3-aminoquinolin-4-yl)amino)methyl)phenyl)carbamate (11b)**: A suspension of **10b** (650 mg, 1 eq, 1.65 mmol) in MeOH (20 mL)was treated with a pre-cooled suspension of

zinc (1100 mg, 10.2 eq, 16.8 mmol) and ammonium chloride (900 mg, 10.2 eq, 16.8 mmol) in MeOH (6 mL). The mixture was stirred at 0 °C for 20 min and monitored by HPLC. The mixture rapidly turned to a grey/green suspension. After 20 min the reaction mixture was filtered through celite and the solvent was evaporated *in vacuo*. The residue was dissolved in DCM, washed with water, and back-extracted into DCM. The organic layer was dried over $MgSO_4$.The solvent was removed under vacuum to obtain the desired compound (549 mg, 91% yield). LCMS rt = 2.66 min; m/z = 365.45 [M+H].

***tert*-Butyl (3-((2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)carbamate (12b)**:
Step 1: **11b** (549 mg, 1.0 eq, 1.51 mmol) in anhydrous EtOAc (16 mL), cooled to 0 °C, was added to triethylamine (198 mg, 273 µL, 1.3 eq, 1.96 mmol) also at 0 °C. This mixture was stirred for 10 min. Valeryl chloride (236 mg, 232 µL, 1.3 eq, 1.96 mmol) in EtOAc (5 mL) was added dropwise at 0 °C to the reaction mixture. The mixture was stirred for 40 min and monitored by HPLC. The solution was concentrated to dryness under reduced pressure and carried forward without purification. LCMS rt = 2.79 min; m/z = 449.57 [M+H].
Step 2: The crude product from the previous step (676 mg, 1 eq, 1.51mmol) was dissolved in EtOH (9 mL) and treated with sodium hydroxide (121 mg, 2 eq, 3.01 mmol) in H_2O (1.38 mL). The mixture was refluxed for 4 h and was monitored by HPLC. The reaction was diluted with water and the product was extracted with DCM. The organic layer was dried over $MgSO_4$ and evaporated to dryness. The amber-red product was purified using silica gel chromatography (MeOH/DCM) providing 647 mg (99.8% yield) of the title product (**12b**). LCMS rt = 3.15 min; m/z = 431.55 [M+H].

***tert*-Butyl (3-((4-amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)carbamate (13b)**: 3-Chlorobenzoperoxoic acid (382 mg, 2.90 eq, 1.55 mmol) was added to a solution of **12b** (230 mg, 1.0 eq, 0.535 mmol) in $CHCl_3$ (3 mL). The reaction mixture was stirred at 40 °C for 1 h. The material was re-dissolved in $CHCl_3$ (10 mL) and warmed to 50 °C. Ammonium hydroxide (20%) (2.81 g, 30 eq, 16.0 mmol) was added dropwise and the mixture was left to stir for 1 h at 50 °C. 4-Methylbenzenesulfonyl chloride (204 mg, 2 eq, 1.07 mmol) was added to this mixture and the resulting reaction was stirred rapidly at 50 °C for 4 h. The reaction was further diluted with $CHCl_3$ (40mL) and washed with aqueous bicarbonate and brine. The organic layer was dried with $MgSO_4$ and dried under reduced pressure. The crude product was purified by silica gel chromatography (20% DCM/MeOH). The pure fractions were dried under reduced pressure giving 165 mg of the title compound. (69% yield) LCMS rt = 2.94 min; m/z = 446.57 [M+H]. WETDC_1 NMR (400 MHz, DMSO) δH/ppm 8.94 (bs, 2H), 7.96 (d, *J* = 8.1 Hz, 1H), 7.82 (d, *J* = 8.1 Hz, 1H), 7.65 (t, *J* = 7.5 Hz, 1H), 7.47-7.35 (m, 3H), 7.25 (t, *J* = 7.7 Hz, 1H), 6.73 (d, *J* = 7.7 Hz, 1H), 5.93 (s, 2H), 2.98 (t, *J* = 7.5 Hz, 2H), 1.75 (quint, *J* = 7.5 Hz, 2H), 1.52-1.36 (m, 11H), 0.89 (t, *J* = 7.0 Hz, 3H).

1-(3-Aminobenzyl)-2-butyl-1H-imidazo[4,5-c]quinoline-4-amine (14): TFA (78 µL, 15 eq) was added to a solution of **13b** (30 mg, 67 µmol) in DCM. The reaction mixture was stirred at 23°C for 3 h. The resulting product mixture was dried under reduced pressure giving the desired material as a dark red-amber solid. (16.3 mg, 71% yield) LCMS rt = 2.61 min (97.9%); m/z = 346.45 [M+H]. ^{1}H NMR (400 MHz, DMSO) δH/ppm 9.00 (bs, 2H), 7.98 (d, *J* = 8.5 Hz, 1H), 7.81 (d, *J* = 8.5 Hz, 1H), 7.65 (t, *J* = 7.9 Hz, 1H), 7.39 (t, *J* = 7.5 Hz, 1H), 7.08 (t, *J* = 7.7 Hz, 1H), 6.59 (d, *J* = 8.1 Hz, 1H), 6.43 (d, *J* = 6.9 Hz, 1H), 6.29 (s, 1H), 5.84 (s, 2H), 1.75 (quint, *J* = 7.7 Hz, 2H), 1.41 (sextet, *J* = 7.7 Hz, 2H), 0.89 (t, *J* = 7.7 Hz, 3H)

N-(4-Methoxybenzyl)-3-nitroquinolin-4-amine (10c): 4-Chloro-3-nitroquinoline (1000 mg, 1 eq, 4.81 mol) was dissolved in DCM (15.0 mL) and treated with (4-methoxyphenyl)methanamine (725 mg, 690 µL, 1.10 eq, 5.28 mmol) followed by triethylamine (973 mg, 1.34 mL, 2 eq, 9.62 mmol). The reaction was stirred at 30℃ for 1 hour, concentrated to dryness, then triturated with water. The title compound was obtained by vacuum filtration (1250 mg, 4.04 mmol, 84.0% yield) as a bright yellow powder. LCMS rt = 3.23 min; m/z = 310.1 [M+H]. ^{1}H NMR (400 MHz, DMSO) δ_H/ppm 9.40 (t, *J* = 5.4 Hz, 1H), 9.02 (s, 1H), 8.54 (d, *J* = 8.4 Hz, 1H), 7.90 (d, *J* = 8.4 Hz, 1H), 7.83 (t, *J* = 7.5 Hz, 1H), 7.58 (t, *J* = 7.6 Hz, 1H), 7.27 (d, *J* = 8.6 Hz, 2H), 6.91 (d, *J* = 8.4 Hz, 2H), 4.84 (d, *J* = 5.7 Hz, 2H), 3.73 (s, 3H)

N4-(4-Methoxybenzyl)quinoline-3,4-diamine (11c): An approximately 500 mg portion of **10c** (501 mg, 1 eq, 1.62 mmol) was suspended in Methanol (30.0 mL). The solution was stirred and ammonium chloride (872 mg, 10.0 eq, 16.3 mmol) was added. This was then put into an ice bath and then Zinc (1063 mg, 10.0 eq, 16.3 mmol) was added. The reaction was kept for a total of 25 minutes and was monitored by UPLC, giving the desired product **11c** (534 mg, 118.0% yield). This was then washed with a 50:50 Methanol and DCM mixture through vacuum filtration over celite for removal of the excess Zinc. The solvent was then evaporated. This was now dissolved in 10% Methanol in DCM (40.0 mL) and partitioned against NaOH (40.0 mL). This was repeated 3 times and then the organic layer was dried using $MgSO_4$. This was then dried to recover the desired compound (308 mg, 57.6% recovery). LCMS rt = 1.64 min; m/z = 280.4 [M+H].

2-Butyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-c]quinoline (12c):
Step 1: 308 mg of **11c** was dissolved in ethyl acetate (5 mL). Triethylamine (145 mg, 200 µL, 1.3 eq, 1.43 mmol) was added and the reaction was chilled to 0°C and stirred. Valeryl chloride (152 mg, 150 µL, 1.15 eq, 1.26 mmol) was dissolved in 1.25 mL of ethyl acetate and cooled to 0°C then added dropwise to the reaction mixture. After stirring for 20 minutes at 0°C, the reaction was concentrated to dryness for a final weight of 576 mg of N-(4-((4-methoxybenzyl)amino)quinolin-3-yl)pentanamide that was used in the next step without further purification. LCMS rt = 2.59 min; m/z = 364.4 [M+H].
Step 2: The crude N-(4-((4-methoxybenzyl)amino)quinolin-3-yl)pentanamide from above (576 mg) was dissolved in ethanol (13 mL) and heated to 80°C. The reaction was treated with NaOH (127 mg, 2.89 eq, 3.19 mmol) dissolved in water (1.8 mL), also at 80°C. The reaction was heated at 80℃ for three and a half hours. After cooling, 25 mL of water was added and the product was extracted (3x) into DCM. The crude product was dried over magnesium sulfate, concentrated to dryness, and purified using silica gel chromatography (0% -> 10% MeOH in DCM) giving 154 mg of the title compound (44.9% yield). LCMS rt = 2.95 min; m/z = 346.3 [M+H]. ^{1}H NMR (400 MHz, DMSO) δ_H/ppm 9.20 (s, 1H), 8.17-8.10 (m, 2H), 7.62 (t, *J* = 7.7 Hz, 1H), 7.51 (t, *J* = 7.7 Hz, 1H), 6.97 (d, *J* = 8.5 Hz, 2H), 6.88 (d, *J* = 8.6 Hz, 2H), 5.90 (s, 2H), 3.68 (s, 3H), 2.97 (t, *J* = 7.8 Hz, 2H), 1.79 (quint, *J* = 7.4 Hz, 2H), 1.41 (sextet, *J* = 7.5 Hz, 2H), 0.90 (t, *J* = 7.3 Hz, 3H).

2-Butyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-c]quinolin-4-amine (13c): Compound **12c** (141 mg, 1 eq, 409 µmol) was dissolved in chloroform (2.5 mL) and treated with 3-chlorobenzoperoxoic acid (216 mg, 3.05 eq, 1.24 mmol). After vortexing, the reaction was

heated to 40℃ and stirred for 1 hour. The intermediate N-oxide was observed by LCMS (rt = 3.24 min; m/z = 362.3 [M+H]). The reaction was cooled to room temperature and then stirred vigorously and treated with ammonium hydroxide (405 mg, 460 µL, 28.2 eq, 11.5 mmol). After stirring vigorously for 1h, 4-methylbenzenesulfonyl chloride (161 mg, 2.01 eq, 846 µmol) was added and stirring was continued for 30 minutes. The reaction was concentrated to dryness, dissolved in 40 mL of chloroform, and washed with 40 mL of aqueous sodium bicarbonate and 40 mL of brine giving 229 mg of crude product. A portion of the product (70.6 mg) was further purified by prep HPLC providing a total of 31.9 mg of the title compound. LCMS rt = 2.77 min (90.9%); m/z = 361.5 [M+H]. ^{1}H NMR (400 MHz, DMSO) δH/ppm 8.01 (d, *J* = 8.2 Hz, 1H), 7.81 (d, *J* = 8.6 Hz, 1H), 7.64 (t, *J* = 7.5 Hz, 1H)**,** 7.40 (t, *J* = 7.5 Hz, 1H), 7.02 (d, *J* = 8.5 Hz, 2H), 6.90 (d, *J* = 8.6 Hz, 2H), 5.89 (s, 2H), 3.70 (s, 3H), 2.98 (t, *J* = 7.7 Hz, 2H), 1.73 (quint, *J* = 7.6 Hz, 2H), 1.39 (sextet, *J* = 7.4 Hz, 2H), 0.88 (t, *J* = 7.2 Hz, 3H)

4-((4-Amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenol (15a): Compound **13c** (23.5 mg) was dissolved in DCM (500 µL) under a nitrogen balloon. Upon cooling to 0°C, a solution of BBr_3 (20 µL) in 500 µL of DCM and was then added to the reaction dropwise. After warming to room temperature, the reaction was stirred overnight and treated with additional BBr_3 (3 eq.) and stirred for an addition 2h. The reaction was carefully quenched with 1 mL water and 1 mL of sodium bicarbonate. The product was extracted into DCM giving 5.2 mg of crude material (23% yield) which was further purified by prep HPLC giving 3.3 mg of the title compound. LCMS rt = 2.13 min (100%); m/z = 347.4 [M+H]. ^{1}H NMR (400 MHz, DMSO) δH/ppm 9.46 (s, 1H), 8.00 (d, *J* = 8.0 Hz, 1H), 7.78 (d, *J* = 8.0 Hz, 1H), 7.61 (t, *J* = 7.4 Hz, 1H), 7.37 (t, *J* = 7.0 Hz, 1H), 6.89 (d, *J* = 8.5 Hz, 2H), 6.72 (d, *J* = 8.4 Hz, 2H), 6.52 (s, 1H), 5.82 (s, 2H), 2.96 (t, *J* = 7.6 Hz, 2H), 1.72 (quint, *J* = 7.6 Hz, 2H), 1.39 (sextet, *J* = 7.3 Hz, 2H), 0.88 (t, *J* = 7.3 Hz, 3H)

N-(3-Methoxybenzyl)-3-nitroquinolin-4-amine (10d): A suspension of 4-chloro-3-nitroquinoline (1.50 g, 1.0 eq, 7.20 mmol) in DCM (22.0 mL) was treated with (3-methoxyphenyl)methanamine (986 mg, 0.92 mL, 1.0 eq, 7.20 mmol) and triethylamine (1.09 g, 1.50 mL, 1.50 eq, 10.8 mmol). The mixture was refluxed at 40 °C for 1h resulting in the formation of a bright yellow suspension. Upon cooling to rt, the solid was washed with water, filtered and dried under vacuum to give the title compound (2.18 g, 98%) as a bright yellow powder LC/MS rt = 3.25 min; m/z = 310.1 [M+H].

N4-(3-Methoxybenzyl)quinoline-3,4-diamine (11d): A suspension of **10d** (2.18 g, 1.0 eq, 7.04 mmol) in MeOH (25.0 mL) was treated with zinc (1.49 g, 4.0 eq, 28.0 mmol) and ammonium chloride (1.80 g, 4.0 eq, 28.0 mmol). The reaction mixture was stirred at room temperature for 10 min to give a grey suspension. The reaction mixture was filtered through celite and the solvent was evaporated *in vacuo*. The residue was dissolved in DCM and treated with 1M NaOH. The product was extracted (3x) into 10% MeOH in DCM, dried over $MgSO_4$, and concentrated to give 1.36 g (70%, yield) of the title compound as a black-brown oil that was used without further purification. LC/MS rt = 2.44; m/z = 280.1 [M+H].

2-Butyl-1-(3-methoxybenzyl)-1H-imidazo[4,5-c]quinoline (12d):
Step 1: Compound **11d** (1.36 g, 1.0 eq, 4.88 mmol) was dissolved in anhydrous EtOAc (45.0 mL), cooled to 0 °C, and treated with pre-chilled triethylamine (642 mg, 884 µL, 1.3 eq, 6.34

mmol). After stirring for 5 mins, valeryl chloride (883 mg, 868 µL, 1.5 eq, 7.32 mmol) in EtOAc (15.0 mL) was added dropwise at 0 °C and the reaction mixture was further stirred for 15 min. The reaction was quenched with ethanol and concentrated under reduced pressure forming a yellow-brown crystal that was used directly in the next step. LC/MS rt = 2.56 min; m/z = 364.4 [M+H].
Step 2: The crude product of step 1 (1.77 g, 1.0 eq, 4.88 mmol) was dissolved in EtOH (26.0 mL) and treated with sodium hydroxide (464 mg, 1.0 eq, 11.6 mmol) in H_2O (4.00 mL). The reaction mixture was refluxed at 80 °C for 24 h. Upon cooling, the reaction was treated with water (75 mL) and the product was extracted into EtOAc (75 mL x 3). The combined organic extracts were dried over $MgSO_4$ and evaporated to dryness and the title product was purified by silica gel chromatography (10% MeOH in DCM) providing the title compound as an amber resin (856mg, 51% yield). LC/MS rt = 2.97 min; m/z = 346.2 [M+H]. ^{1}H NMR (400 MHz, DMSO) δ_H/ppm 9.21 (s, 1H), 8.11 (d, *J* = 8.5 Hz, 2H), 7.61 (t, *J* = 7.7 Hz, 1H), 7.50 (t, *J* = 7.7 Hz, 1H), 7.21 (t, *J* = 8.0 Hz, 1H), 6.83 (d, *J* = 8.2 Hz, 1H), 6.66 (s, 1H), 6.49 (d, *J* = 7.7 Hz, 1H), 5.93 (s, 2H), 3.67 (s, 3H), 2.96 (t, *J* = 7.5 Hz, 2H), 1.78 (quint, *J* = 7.5 Hz, 2H), 1.40 (sextet, *J* = 7.4 Hz, 2H), 0.88 (t, *J* = 7.4 Hz, 3H).

2-Butyl-1-(3-methoxybenzyl)-1H-imidazo[4,5-c]quinoline-4-amine (13d): Compound **12d** (156 mg, 1.0 eq, 450 mmol) was dissolved in $CHCl_3$ (2.5 mL) and treated with 3-chlorobenzoperoxoic acid (173 mg, 1.6 eq, 700 mmol) and stirred at 40 °C for 1h. The temperature was increased to 50 °C, stirred vigorously, and treated with ammonium hydroxide (2.2 g, 2.5 mL, 28 eq, 13 mmol) dropwise followed by 4-methylbenzenesulfonyl chloride (174 mg, 2 eq, 920 µmol). The mixture was cooled to rt and stirred for 1 h. The product was extracted with chloroform, washed with aqueous bicarbonate, and dried over $MgSO_4$ giving 183 mg (112% yield) of crude product which was carried forward without further purification. A portion of the crude product was purified by preparative HPLC affording the title compound as a white residue. LC/MS rt = 2.78 min (98.5%); m/z = 361.2 [M+H]. ^{1}H NMR (400 MHz, DMSO) δ_H/ppm 7.97 (d, *J* = 7.9 Hz, 1H), 7.80 (d, *J* = 7.8 Hz, 1H), 7.63 (t, *J* = 7.8 Hz, 1H), 7.38 (t, *J* = 7.8 Hz, 1H), 7.24 (t, *J* = 8.0 Hz, 1H), 6.86 (d, *J* = 8.2, 1H), 6.71 (s, 1H), 6.54 (d, *J* = 8.0 Hz, 1H), 5.93 (s, 2H), 3.70 (s, 3H), 2.97 (t, *J* = 7.7 Hz, 2H), 1.72 (quint, *J* = 7.6 Hz, 2H), 1.38 (sextet, *J* = 7.4 Hz, 2H), 0.87 (t, *J* = 7.4 Hz, 3H).

3-((4-Amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenol (15b): A crude suspension of compound **13d** (106 mg, 1.0 eq, 293 µmol) in DCM (1.00 mL) was cooled to 0 °C under nitrogen and treated dropwise with BBr_3 (220 mg, 83 µL, 3.0 eq, 878 µmol) in DCM (0.70 mL). The mixture was stirred at 0 °C for 5 minutes then warmed to rt and stirred for an additional 1.5h. The reaction was quenched with ice water and aqueous bicarbonate and the product was extracted into DCM (10 mL x 2), dried over $MgSO_4$, and evaporated to dryness. The residue was purified by preparative HPLC affording the title compound as a fine white powder (35.0mg, 35% yield) LC/MS: Retention time = 2.57 min (100%): *m/z* = 347.2 [M+1]. ^{1}H NMR (400 MHz, DMSO) δ_H/ppm 7.97 (d, *J* = 7.8 Hz, 1H), 7.80 (d, *J* = 8.0 Hz, 1H), 7.64 (t, *J* = 7.8 Hz, 1H), 7.39 (t, *J* = 7.8 Hz, 1H), 7.14 (t, *J* = 7.8 Hz, 1H), 6.66 (d, *J* = 8.1 Hz, 1H), 6.52 (d, *J* = 7.9 Hz, 1H), 6.39 (s, 1H), 5.88 (s, 2H), 2.97 (t, *J* = 7.7 Hz, 2H), 1.72 (quint, *J* = 7.6 Hz, 2H), 1.39 (sextet, *J* = 7.4 Hz, 2H), 0.87 (t, *J* = 7.3, 3H).

N-(4-((4-Amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)acetamide (16a): Compound **1** (10.5 mg, 1 eq, 30.4 µmol) was dissolved in DCM (1.4 mL) and treated with acetic anhydride (9.31 mg, 10.2 µL, 3 eq, 91.2 µmol). After stirring at room temperature for 3 hours, the product was purified by preparative HPLC to obtain the desired product (2.8 mg, 24% yield). LCMS rt =2.71 min (90.2%); m/z = 388.4 [M+H]. WETDC_1 NMR (400 MHz, DMSO) δH/ppm 9.98 (s, 1H), 8.90 (bs, 2H), 7.98 (d, *J* = 8.3 Hz, 1H), 7.80 (d, *J* = 8.3 Hz, 1H), 7.65 (t, *J* = 7.6 Hz, 1H), 7.53 (d, *J* = 8.6 Hz, 2H), 7.40 (t, *J* = 7.6 Hz, 1H), 7.02 (d, *J* = 8.4 Hz, 2H), 5.90 (s, 2H), 2.98 (t, *J* = 7.8 Hz, 2H), 2.01 (s, 3H), 1.74 (quint, *J* = 7.5 Hz, 2H), 1.40 (sextet, *J* = 7.4 Hz, 2H), 0.88 (t, *J* = 7.3 Hz, 3H).

N-(4-((4-Amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)pentanamide (16b): Compound **1** (15.0 mg, 1 eq, 43.4 µmol) was dissolved in ethyl acetate (400 µL) and treated with triethylamine (5.7 mg, 7.9 µL, 1.3 eq, 57 µmol) and cooled to 0°C. Valeryl chloride (5.8 mg, 5.7 µL, 1.1 eq, 48 µmol) was dissolved in EtOAc (133 µL) and transferred to the reaction dropwise. The reaction was warmed to rt and stirred overnight. An additional 1.3 equivalents of triethylamine and 1.1 equivalents of valeryl chloride were added to the reaction in the same manner as above. After stirring for 2h, the reaction was purified by prep HPLC to obtain the title product (5.1 mg, 28% yield). LCMS rt = 2.82 min (100%); m/z = 430.6 [M+H]. ^{1}H NMR (400 MHz, DMSO) δ_H/ppm 9.72 (s, 1H), 8.75 (bs, 2H), 7.98 (d, J = 8.2 Hz, 1H), 7.79 (d, J = 8.2 Hz, 1H), 7.62 (t, J = 7.6 Hz, 1H), 7.54 (d, J = 8.2 Hz, 2H), 7.38 (t, J = 7.6 Hz, 1H), 7.01 (d, J = 8.0 Hz, 2H), 5.88 (s, 2H), 2.97 (t, J = 7.5 Hz, 2H), 2.27 (t, J = 7.3 Hz, 2H), 1.76 (quint, J = 7.3 Hz, 2H), 1.56 (quint J = 7.3 Hz, 2H), 1.41 (sextet, J = 7.3 Hz, 2H), 1.31 (sextet, J = 7.3 Hz, 2H), 0.92-0.84 (m, 6H). ^{13}C NMR (400 MHz, DMSO) δ_H/ppm 171.31, 158.80, 158.49, 156.86, 148.95, 138.81, 135.31, 134.01, 129.65, 129.36, 125.96, 124.66, 121.60, 119.49, 118.50, 112.44, 48.09, 36.03, 34.32, 29.25, 27.17, 26.22, 21.75**,** 21.72, 13.67, 13.62.

Ethyl (4-((4-amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)carbamate (16c): Compound **1** (14.1 mg, 1 eq, 40.8 µmol) was dissolved in DCM (2.0 mL) and treated with triethylamine (8.26 mg, 11.4 µL, 2 eq, 82 µmol) followed by ethyl chloroformate (8.9 mg, 7.9 µL, 2.0 eq, 82.0 µmol). After stirring for 12 hours at rt, an additional 2 equivalents of ethyl chloroformate were added and the reaction was stirred for an additional 1h. The crude mixture was then purified by preparative HPLC to obtain the title compound. (7.9 mg, 46% yield) LCMS rt = 2.77 min (95.6%); m/z = 418.5 [M+H]. WETDC_1 NMR (400 MHz, DMSO) δH/ppm 9.63 (s, 1H), 8.94 (bs, 2H), 7.99 (d, *J* = 8.6 Hz, 1H), 7.81 (d, *J* = 8.6 Hz, 1H), 7.65 (t, *J* = 7.8 Hz, 1H), 7.46-7.37 (m, 3H), 7.02 (d, *J* = 8.5 Hz, 2H), 6.53 (bs, 1H), 5.90 (s, 2H), 4.11 (q, *J* = 7.0 Hz, 2H), 2.98 (t, *J* = 7.6 Hz, 2H), 1.75 (quint, *J* = 7.3 Hz 2H), 1.42 (sextet, *J* = 7.4 Hz, 2H), 1.23 (t, *J* = 7.1 Hz, 3H) 0.89 (t, *J* = 7.4 Hz, 3H).

Isopropyl (4-((4-amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)carbamate (16d): The title compound was prepared according to the procedure described for compound **16c**. (4.9 mg, 41% yield) LCMS rt = 2.91 min (100%); m/z = 431.5 [M+H]. ^{1}H NMR (400 MHz, DMSO) δ_H/ppm 9.55 (s, 1H), 8.95 (bs, 2H), 7.96 (d, J = 8.0 Hz, 1H), 7.79 (d, J = 8.3 Hz, 1H), 7.63 (t, J = 7.7 Hz, 1H), 7.44-7.34 (m, 3H), 6.98 (d, J = 8.3 Hz, 2H), 5.87 (s, 2H), 4.84 (septet, J = 6.3 Hz, 1H), 2.97 (t, J = 7.4 Hz, 2H), 1.72 (quint, J = 7.5 Hz, 2H), 1.39 (sextet, J = 7.4 Hz, 2H), 1.21 (d, J =

6.5 Hz, 6H), 0.87 (t, J = 7.5 Hz, 3H). ^{13}C NMR (400 MHz, DMSO) δ_H/ppm 158.45, 156.87, 153.03, 148.78, 138.76, 135.29, 133.80, 129.34, 128.92, 126.03, 124.64, 124.59, 121.60, 118.55, 118.42, 112.39, 67.43, 48.00, 29.21, 26.17, 21.86, 21.68, 13.56.

Butyl 2-(4-((4-amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)acetate (16e): The title compound was prepared according to the procedure described for compound **16c**. (3.8 mg, 29% yield) LCMS rt = 3.00 min (100%); m/z = 446.6 [M+H]. WETDC_1 NMR (400 MHz, DMSO) δH/ppm 9.62 (s, 1H), 8.78 (bs, 2H), 7.97 (d, *J* = 8.3, 1H) 7.80 (d, *J* = 8.3 Hz, 1H), 7.63 (t, *J* = 7.7 Hz, 1H), 7.43 (d, *J* = 8.5 Hz, 2H), 7.38 (t, *J* = 7.7 Hz , 1H), 7.01 (d, *J* = 8.5 Hz, 2H), 6.54 (bs, 1H), 5.89 (s, 2H), 4.06 (t, *J* = 6.5 Hz, 2H), 2.99 (t, *J* = 7.6 Hz, 2H), 1.75 (quint, *J* = 7.5 Hz, 2H), 1.59 (quint, *J* = 6.7 Hz, 2H), 1.46-1.32 (m, 4H), 0.91 (t, *J* = 7.6 Hz, 3H), 0.89 (t, *J* = 7.3 Hz, 3H).

***tert*-Butyl(2-((4-((4-amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)amino)-2-oxoethyl)carbamate (17a)**: *Compound* **1** (15.2 mg, 1 eq, 44.0 µmol) was dissolved into DMA (2.0 mL) and treated with DIEA (5.6 mg, 7.6 µL, 0.99 eq, 44 µmol) followed by boc-glycine (7.7 mg, 1.0 eq, 44 µmol). EDC (16.9 mg, 2 eq, 88.0 µmol) was added and the reaction was stirred at room temperature for 12 hours then purified by preparative HPLC to obtain the title compound. (7.0 mg, 32% yield) LCMS rt = 2.74 min (86.2%); m/z = 503.6 [M+H] ^{1}H NMR (400 MHz, DMSO) δH/ppm 9.96 (s, 1H), 7.96 (d, *J* = 7.5 Hz, 1H), 7.80 (d, *J* = 7.7 Hz, 1H), 7.72 (t, *J* = 7.5 Hz, 1H), 7.54 (d, *J* = 8.3 Hz, 2H), 7.37 (t, *J* = 7.7 Hz, 1H), 7.03 (d, *J* = 8.1 Hz, 2H), 5.91 (s, 2H), 2.95 (t, *J* = 7.4 Hz, 4H), 1.72 (quint, 7.3 Hz, 2H), 1.48-1.22 (m, 11H), 0.87 (t, *J* = 7.5 Hz, 3H).

***tert*-Butyl (3-((4-((4-amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)amino)-3-oxopropyl)carbamate (17b)**: The title compound was prepared according to the general procedure described for compound **17a**. (4.8 mg, 22% yield) LCMS rt = 2.78 min (100%); m/z = 517.7 [M+H]. ^{1}H NMR (400 MHz, DMSO) δ_H/ppm 9.97 (s, 1H), 9.03 (bs, 2H), 7.95 (d, J = 8.4 Hz, 1H), 7.78 (d, J = 8.0 Hz, 1H), 7.62 (t, J = 7.8 Hz, 1H), 7.54 (d, J = 8.5 Hz, 2H), 7.37 (t, J = 7.6 Hz, 1H), 7.00 (d, J = 8.5 Hz, 2H), 5.89 (s, 2H), 3.17 (q, J = 6.5 Hz, 2H), 2.97 (t, J = 7.6 Hz, 2H), 2.42 (t, J = 7.0 Hz, 2H), 1.72 (quint, J = 7.6 Hz, 2H), 1.44-1.31 (m, 11H), 0.87 (t, J = 7.3 Hz, 3H). ^{13}C NMR (400 MHz, DMSO) δ_H/ppm 169.44, 158.89, 158.58, 156.90, 155.50, 148.97, 138.67, 135.35, 133.86, 129.77, 129.39, 125.96**,** 124.67, 124.63, 121.63, 119.56**,** 118.48, 118.38, 112.40, 77.59, 48.10, 36.71, 36.45, 35.76, 29.24, 28.20, 26.2, 21.72, 13.63.

***tert*-Butyl (4-((4-((4-amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)amino)-4-oxobutyl)carbamate (17c)**: The title compound was prepared according to the general procedure described for compound **17a**. (6.0 mg, 26% yield) LCMS rt = 2.80 min (89.8%); m/z = 531.6 [M+H]. WETDC_1 NMR (400 MHz, DMSO) δH/ppm 9.90 (s, 1H), 8.91 (bs, 2H), 7.98 (d, *J* = 8.4 Hz, 1H), 7.81 (d, *J* = 8.4 Hz, 1H), 7.65 (t, *J* = 7.7 Hz, 1H) 7.55 (d, *J* = 8.5 Hz, 2H), 7.40 (t, *J* = 7.7 Hz, 1H), 7.02 (d, *J* = 8.5 Hz, 2H), 5.91 (s, 2H), 3.01 (t, *J* = 7.7 Hz, 4H), 2.27 (t, *J* = 7.3 Hz, 2H), 1.75 (quint, *J* = 7.4 Hz, 2H), 1.66 (quint, *J* = 7.2 Hz, 2H), 1.46-1.33 (m, 11H), 0.89 (t, *J* = 7.3 Hz, 3H)

***tert*-Butyl(5-((4-((4-amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)amino)-5-oxopentyl)carbamate (17d)**: The title compound was prepared according to the general

procedure described for compound **17a**. (3.2 mg, 14% yield) LCMS rt =2.87 min (84.3%); m/z = 545.6 [M+H] WETDC_1 NMR (400 MHz, DMSO) δH/ppm 9.84 (s, 1H), 7.89 (d, J = 8.3Hz, 1H), 7.70 (d, J = 8.3Hz, 1H), 7.53 (t, J = 7.4Hz, 1H), 7.48 (d, J = 8.3Hz, 2H), 7.27 (t, J = 7.3Hz, 1H) , 6.94 (d, J = 8.3Hz, 2H), 5.82 (s, 2H), 2.94 (s, 6H), 1.67 (quint, J = 7.3Hz, 2H), 1.46 (t, J = 7.4Hz, 2H), 1.37-1.27 (m, 15H), 0.82 (t, J = 7.3Hz, 3H)

***tert*-Butyl(6-((4-((4-amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)amino)-6-oxohexyl)carbamate (17e)**: The title compound was prepared according to the general procedure described for compound **17a**. (5.6 mg, 23% yield) LCMS rt =2.90 min (96.4%); m/z =559.7 [M+H]. WETDC_1 NMR (400 MHz, DMSO) δH/ppm 9.82 (s, 1H), 8.81 (bs, 2H), 7.92 (d, *J* = 8.3 Hz, 1H) 7.75 (d, *J* = 8.3 Hz, 1H), 7.58 (t, *J* = 7.6 Hz, 1H), 7.49 (d, *J* = 8.5 Hz, 2H), 7.33 (t, *J* = 7.6 Hz, 1H) 6.95 (d, *J* = 8.5 Hz, 2H), 5.84 (s, 2H), 2.95 (t, *J* = 7.7 Hz 6H), 1.68 (quint, *J* = 7.5 Hz, 2H), 1.49 (quint, *J* = 7.5 Hz, 2H), 1.38-1.28 (m, 15H), 0.83 (t, *J* = 7.2 Hz, 3H)

2-Amino-N-(4-((4-amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)acetamide (18a): Compound **17a** (4.7 mg, 1 *eq*, 9.4 μmol) was dissolved in DCM (400 μL) and treated with TFA (59 mg, 40 μL, 56 eq, 0.52 mmol). The reaction mixture was stirred for one hour then concentrated to dryness to obtain the desired product. (2.4 mg, 64% yield). LCMS rt = 2.30 min (100%); m/z = 403.5 [M+H] ^{1}H NMR (400 MHz, DMSO) δH/ppm 10.50 (s, 1H), 7.94 (d, *J* = 7.5 Hz, 1H), 7.76 (d, *J* = 7.5 Hz, 1H), 7.63-7.57 (m, 1H), 7.55 (d, *J* = 8.6 Hz, 2H), 7.37-7.26 (m, 1H), 7.07 (d, *J* = 8.5 Hz, 2H) 5.91 (s, 2H), 2.95 (t, *J* = 7.2 Hz, 4H), 1.72 (quint, *J* = 7.5 Hz, 2H), 1.39 (sextet, *J* = 7.5 Hz, 2H), 0.87 (t, *J* = 7.0 Hz, 3H)

3-Amino-N-(4-((4-amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)propenamide (18b): The title compound was prepared according to the general procedure described for compound **18a**. (1.9 mg, 81% yield) LCMS rt = 2.78 min (100%); m/z = 417.5 [M+H]. WETDC_1 NMR (400 MHz, DMSO) δH/ppm 10.13 (s, 1H), 9.16 (bs, 2H), 7.86 (d, *J* = 8.2 Hz, 1H), 7.69 (d, *J* = 8.2 Hz, 1H), 7.55-7.47 (m, 3H), 7.24 (t, *J* = 7.4 Hz, 1H), 6.97 (d, *J* = 8.5 Hz, 2H), 5.84 (s, 2H), 4.05 (d, *J* = 4.8 Hz, 2H), 3.13 (d, *J* = 4.3 Hz, 2H), 2.91 (t, *J* = 7.7 Hz, 2H), 1.67 (quint, *J* = 7.6 Hz, 2H), 1.34 (sextet, J = 7.4 Hz, 2H), 0.82 (t, J = 7.4 Hz, 3H)

4-Amino-N-(4-((4-amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)butanamide (18c): The title compound was prepared according to the general procedure described for compound **18a**. (2.8 mg, 91% yield) LCMS rt = 2.81 min (100%); m/z =431.3 [M+H]. WETDC_1 NMR (400 MHz, DMSO) δH/ppm 10.01 (s, 1H), 9.03 (bs, 2H), 7.91 (d, *J* = 8.3 Hz, 1H), 7.81 (d, *J* = 8.3 Hz, 1H), 7.65 (t, *J* = 7.8 Hz, 1H), 7.56 (d, *J* = 8.5 Hz, 2H), 7.39 (t, *J* = 7.8 Hz, 1H), 7.04 (d, *J* = 8.5 Hz, 2H), 5.92 (s, 2H), 3.01 (t, *J* = 7.7 Hz, 4H), 2.40 (t, *J* = 7.2 Hz, 2H), 1.83 (quint, *J* = 7.6 Hz, 2H), 1.75 (quint, *J* = 7.6 Hz, 2H), 1.41 (sextet, *J* = 7.4 Hz, 2H), 0.89 (t, *J* = 7.4 Hz, 3H)

5-Amino-N-(4-((4-amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)pentanamide (18d): The title compound was prepared according to the general procedure described for compound **18a**. (1.7 mg, 100% yield) LCMS rt =3.42 min (100%); m/z =445.5 [M+H]. WETDC_1 NMR (400 MHz, DMSO) δH/ppm 9.96 (s, 1H), 8.94 (bs, 2H), 7.97 (d, *J* = 8.4 Hz, 1H) 7.81 (d, *J* = 8.4 Hz, 1H), 7.65 (t, *J* = 7.5 Hz, 1H), 7.56 (d, *J* = 8.4 Hz, 2H), 7.39 (t, *J* = 7.5 Hz, 1H), 7.03 (d, *J* = 8.4 Hz, 2H), 5.91 (s, 2H), 3.01 (t, *J* = 7.7 Hz,

6H), 1.75 (quint, *J* = 7.4 Hz, 2H), 1.65-1.52 (m, 2H), 1.41 (sextet, *J* = 7.4 Hz, 2H), 1.27 (quint, *J* = 6.0 Hz, 2H), 0.89 (t, *J* = 7.2 Hz, 3H).

6-Amino-N-(4-((4-amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)hexanamide (18e): The title compound was prepared according to the general procedure described for compound **18a**. (2.5 mg, 100% yield) LCMS rt =2.33 min (100%); m/z =459.5 [M+H]. WETDC_1 NMR (400 MHz, DMSO) δH/ppm 9.92 (s, 1H), 9.01 (bs, 2H), 7.98 (d, *J* = 8.2 Hz, 1H), 7.81 (d, *J* = 8.3 Hz, 1H), 7.65 (t, *J* = 7.6 Hz, 1H), 7.56 (d, *J* = 8.3 Hz, 2H), 7.39 (t, *J* = 7.7 Hz, 1H), 7.03 (d, *J* = 8.3 Hz, 2H), 5.91 (s, 2H), 3.01 (t, *J* = 7.7 Hz, 4H), 2.29 (t, *J* = 7.3 Hz, 2H), 1.75 (quint, *J* = 7.6 Hz, 2H), 1.64-1.48 (m, 4H), 1.41 (q, *J* = 7.4 Hz, 2H) 1.33 (q, *J* = 8.1 Hz, 2H), 0.90 (t, *J* = 7.3 Hz, 3H)

2-butyl-1-(4-(propylamino)benzyl)-1H-imidazo[4,5-c]quinolin-4-amine (19a): Compound **1** (15.1 mg, 1 eq, 43.7 μmol) and propionaldehyde (2.6 mg, 3.3 μL, 1.0 eq, 44 μmol) were dissolved in anhydrous MeOH (500 μL) and treated with acetic acid (1.4 mg, 1.3 μL, 0.52 eq, 23 μmol). After 20 minutes, sodium cyanoborohydride (8.3 mg, 3.0 eq, 0.13 mmol) was added and the reaction was stirred overnight at rt. The reaction was partitioned between DCM and aqueous sodium bicarbonate. The organic layer was dried with magnesium sulfate, filtered, and then concentrated to dryness. The crude product was purified by preparative HPLC (using a formic acid additive in place of TFA) to yield the title compound. (8.5 mg, 50% yield). LCMS rt = 2.99 (100%); m/z = 388.3 [M+H]. WETDC_1 NMR (400 MHz, DMSO) δH/ppm 8.19 (s, 2H), 7.91 (d, *J* = 7.5 Hz, 1H), 7.63 (d, *J* = 7.1 Hz, 1H), 7.41 (t, *J* = 7.4 Hz, 1H), 7.15 (t, *J* = 7.5 Hz, 1H), 6.77 (d, *J* = 8.4 Hz, 2H), 6.49 (d, *J* = 8.0 Hz, 2H), 5.68 (s, 2H), 2.95-2.86 (m, 4H), 1.73 (quint, *J* = 7.7 Hz, 2H), 1.50 (sextet, *J* = 7.5 Hz, 2H), 1.39 (sextet, *J* = 8.0 Hz, 2H), 0.89 (t, *J* = 7.5 Hz, 6H).

2-butyl-1-(4-(pentylamino)benzyl)-1H-imidazo[4,5-c]quinolin-4-amine (19b): The title compound was prepared according to the general procedure described for compound **19a** providing 8.0 mg of the title compounds (44%). LCMS rt = 3.19 (100%); m/z = 416.4 [M+H]. WETDC_1 NMR (400 MHz, DMSO) δH/ppm 8.20 (s, 2H), 7.90 (d, *J* = 7.5 Hz, 1H), 7.62 (d, *J* = 7.9 Hz, 1H), 7.40 (t, *J* = 7.5 Hz, 1H), 7.13 (t, *J* = 7.5 Hz, 1H), 6.77 (d, *J* = 8.4 Hz, 2H), 6.49 (d, *J* = 8.6 Hz, 2H), 5.67 (s, 2H), 2.91 (t, *J* = 7.3 Hz, 4H), 1.72 (quint, *J* = 7.4 Hz, 2H), 1.49 (quint, *J* = 7.0 Hz, 2H), 1.39 (sextet, *J* = 7.6 Hz, 2H), 1.33-1.25 (m, 4H), 0.89 (t, *J* = 7.4 Hz, 3H), 0.86 (t, *J* = 7.3 Hz, 3H).

2-butyl-1-(4-(hexylamino)benzyl)-1H-imidazo[4,5-c]quinolin-4-amine (19c): The title compound was prepared according to the general procedure described for compound **19a**, except using 3 equivalents of aldehyde, providing 8.4 mg of the title compounds (23% yield). LCMS rt = 2.91 (100%); m/z = 430.4 [M+H]. WETDC_1 NMR (400 MHz, DMSO) δH/ppm 8.21 (s, 1H), 7.89 (d, *J* = 7.4 Hz, 1H), 7.61 (d, *J* = 7.4 Hz, 1H), 7.39 (t, *J* = 7.4 Hz, 1H), 7.11 (t, *J* = 7.4 Hz, 1H), 6.77 (d, *J* = 8.2 Hz, 2H), 6.49 (d, *J* = 8.0 Hz, 2H), 5.67 (s, 2H), 1.72 (quint, *J* = 7.2 Hz, 2H), 1.48 (quint, *J* = 7.0 Hz, 2H), 1.39 (sextet, *J* = 7.2 Hz, 2H), 1.34-1.20 (m, 6H), 0.89 (t, *J* = 7.4 Hz, 3H) 0.85 (t, *J* = 7.2 Hz, 3H).

2-butyl-1-(4-(heptylamino)benzyl)-1H-imidazo[4,5-c]quinolin-4-amine (19d): The title compound was prepared according to the general procedure described for compound **19a** except

using 3 equivalents of aldehyde, providing 2.3 mg of the title compounds (12% yield). LCMS rt = 3.15 (90%); m/z = 444.4 [M+H]. WETDC_1 NMR (400 MHz, DMSO) δH/ppm 7.91 (d, *J* = 8.3 Hz, 1H), 7.62 (d, *J* = 8.2 Hz, 1H), 7.41 (t, *J* = 7.5 Hz, 1H), 7.14 (t, *J* = 7.4 Hz, 1H), 6.77 (d, *J* = 8.3 Hz, 2H), 6.48 (d, *J* = 8.4 Hz, 2H), 5.68 (s, 2H), 2.92 (t, *J* = 7.4 Hz, 4H), 1.73 (quint, *J* = 7.5 Hz, 2H), 1.48 (quint, *J* = 6.9 Hz, 2H), 1.39 (sextet, *J* = 7.4 Hz, 2H), 1.34-1.18 (m, 8H), 0.89 (t, *J* = 7.5 Hz, 3H) 0.85 (t, *J* = 7.2 Hz, 3H).

Ramos Blue Cell Assay: Ramos Blue cells were seeded in triplicate at a density of $1x10^6$ cells/mL. Compounds were prepared at 10x the test concentration using a 3x serial dilution in PBS containing 10% v/v DMSO. Cells were suspended in IMDM + 10% FBS media at a concentration of $1.11x10^6$ cells/mL. The cells (135 uL) were added to each test well followed by 15 uL of test compound. Plates were incubated for 72 hours at 37°C then centrifuged at 2000rpm for 10min. A 40ul aliquot of supernatant was added to 160uL* Quanti-blue solution (prepared via manufacturer's instructions) and allowed to incubate at 37°C for 4 hours and 24 hours. Absorbance readings (620 nm) were taken at each time point.

PAMPA Permeability Assay: Prior to performing the PAMPA assay, we performed a serial dilution of several test compounds in 10% MeOH in PBS to ensure linearity of absorbance at 320 and 280 nm. The assay was performed using slight modifications of the manufacturer's instructions. (Corning #353015) Compounds were stored as 5 or 10 mM stock solution in DMSO. PBS containing 10% methanol was used to dilute each compound to 300uL of a 200 μM working solution. (total organic for each stock solution was 12-14%, including the DMSO) This 300 uL working solution (the donor solution) was added to the receiver plate. 200μL/well of 10% Methanol in PBS was added to the pre-coated filter plate (acceptor solution). The plates were then coupled and incubated at 37°C for 5 hours. The plates were then separated and both the acceptor solution and donor solutions were transferred to 96 well plates for storage. Analysis was performed by measuring the 280nm and 320nm absorbance. The absorbance of each well was measured in comparison to a background blank well containing 10% MeOH in PBS. The compound concentration was established in comparison to a fixed 200 μM standard solution for each compound.

TLR7 docking studies: In silico molecular docking of TLR7 agonists was performed using AutoDockTools 1.5.7 and Autodockvina. Protein preparation: The X-ray crystal structure of monkey TLR7 in complex with R848 was downloaded from the protein data bank (PDB code: R848). All the co-crystallized ligands/chains (i.e C, D, and E) were removed from the structure and Chains A and B (the dimeric units of TLR7) were further optimized by deleting all the water molecules, the addition of hydrogen, and commutation of charges by Autodock Tools 1.5.7. The dimeric protein was then finally converted to a "pdbqt" format. Ligand preparation: The 2D structure of the ligands were converted to an optimized 3D structure using Avagadro and finally saved as pdb files. Using Autodock Tools 1.5.7, the ligands were further optimized by the addition of polar hydrogens and kolhmann charges. The ligands were finally converted to the "pdbqt" format. Grid generation.The docking was performed with a grid box of 60×40×40 points with a spacing of 0.508, centered on the R848 binding site. Visualization: For 3D visualization, the generated output files were opened in PyMOL and interactions between ligand and protein were visualized in their respective binding pockets.

PBMC / Cytokine Release Assay: Cytokine release in human PBMCs was measured by ELISA. PBMCs (20 million) were thawed and re-suspended into 10 mL of RPMI1640 media supplemented with 10% FBS. 100 uL of the cell suspension was added to each well of a 96-well plate ($2x10^5$ cells/well). A 20 μM stock solution of each compound was prepared in 5% DMSO in DPBS. The appropriate serial dilutions were performed at 10x the final test concentration. Compound (11 uL) was added to PBMCs and cells were incubated for 24h at 37°C under 5% CO_2. TNFα and IFNα (all subtypes) was quantitated by ELISA (R&D systems / DY21005 and PBL / PBL41135-1). For the IFNα alpha ELISA (PBL), the supernatant sample was diluted 10X. For the TNF alpha ELISA (Duoset), the supernatant was diluted 5X. Readings were taken on the plate reader for absorbance at 450nm and 570nm.

TLR7/8 Activation: Specificity of **1** and **17b** for the TLR7 versus TLR8 pathway were measured in HEK-Blue hTLR7 and hTLR8 cells as previously described.[13]

Activation of PBMC-derived macrophages: 25 million PBMCs were thawed and resuspended in Monocyte Attachment Medium (Promocell) at a density of 1.5 million cells/cm^2. Cells were plated in a 6-well tissue-culture treated plated. After incubating for 90 minutes, the media was removed and adherent cells (largely monocytes) were washed gently with PBS. (suspension cells were discarded) Cells were incubated for 6 days in RPMI 1640 with 10%FBS with 1x pen/strep and 50 ng/ml GM-CSF. The media was replaced after 3-4 days. After 6 day total incubation, the cells were washed with PBS and then removed using DetachKit (Promocell) and cell lifters. Cells were resuspended at $2.5x10^5$ cells/mL in RPMI 1640 + 10%FBS. 100ul of the cell suspension was added to each well of a tissue-culture treated 96 well plate. After 24 hours the cells were washed with PBS.
Stock solutions of small molecule were prepared at 2mM in DMSO. The solutions were further diluted in 5%DMSO in DPBS to obtain a working concentration of 20μM. Compound (11 uL) was added to each well at 10x the final test concentration. After 6 hours the plate centrifuged (300 rcf) and the supernatant was removed and frozen at -80°C until analysis.
Cytokine analysis was performed at a 3x dilution using a simple Plex Cartridge (Protein-Simple, ELLA).

Activation of RAW-dual macrophages: The NFκB reporter murine macrophage line RAW-dual (Invivogen) was maintained in high glucose DMEM media supplemented with 10% FBS. RAW-dual cells were rinsed with DPBS and harvested with a cell scraper. After centrifugation, the cells were resuspended to 0.1 million per mL in fresh growth media and seeded to a 96-well plate with a volume of 100 uL. Compounds **1**, **16b**, **16d**, and **17b** were diluted in DPBS with 5% DMSO, serial diluted to the appropriate concentration (10x the final test concentration), dosed to corresponding wells (11 μL), and mixed gently. The cells were incubated at 37°C/5%CO_2 for 24 hours. After the incubation, 40 uL of supernatant samples was collected and mixed with 160 uL of QuantiBlue solution (Invivogen). Absorbance at 630 nM was recorded after 2 hours of incubation to evaluate NFκB activation. Data was analyzed and plotted via GraphPad Prism software.

HPLC and NMR Characterization

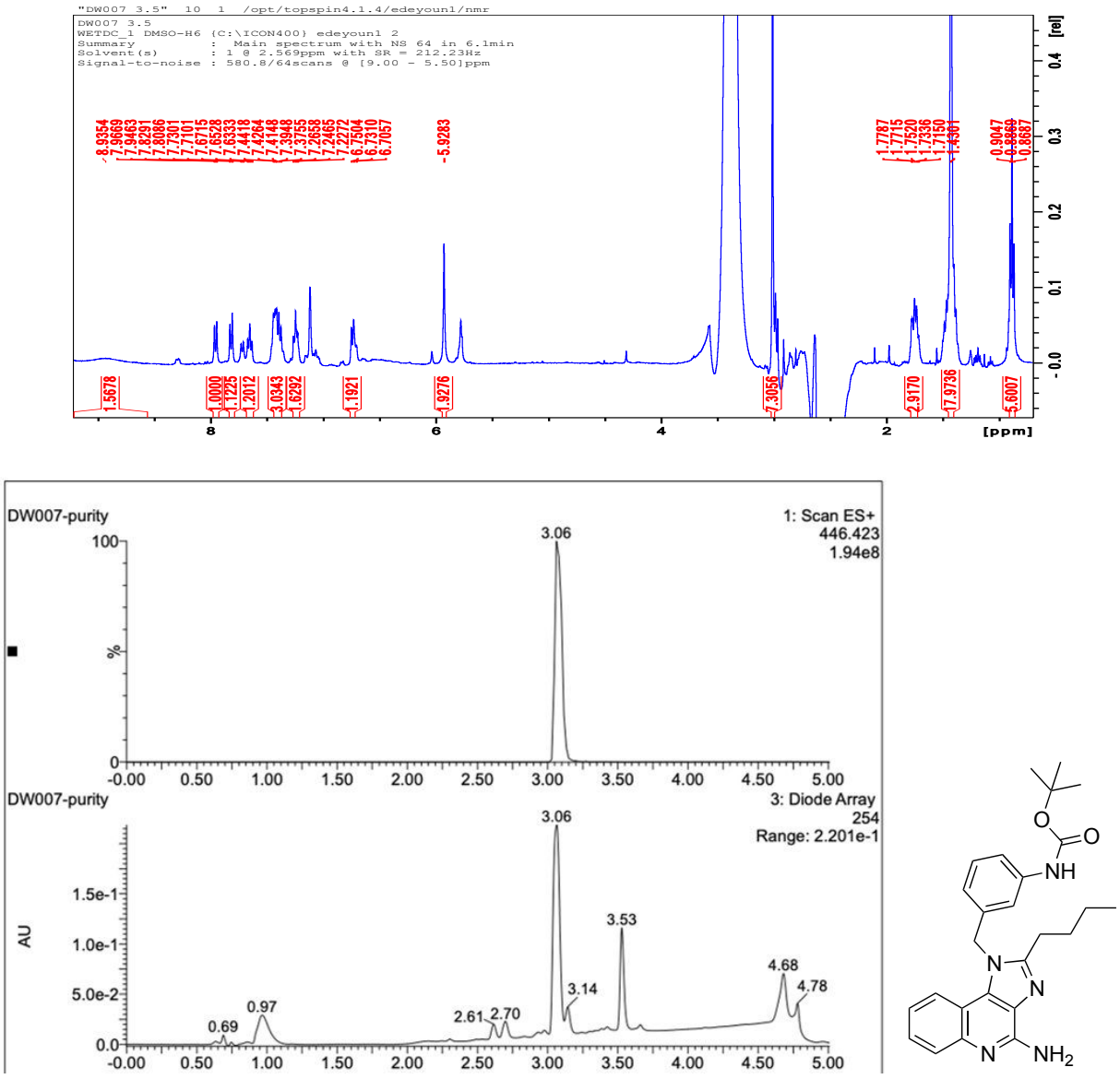

Figure S7: HPLC and NMR Characterization of Compound **13b,** MW = 446.4 [M+H]

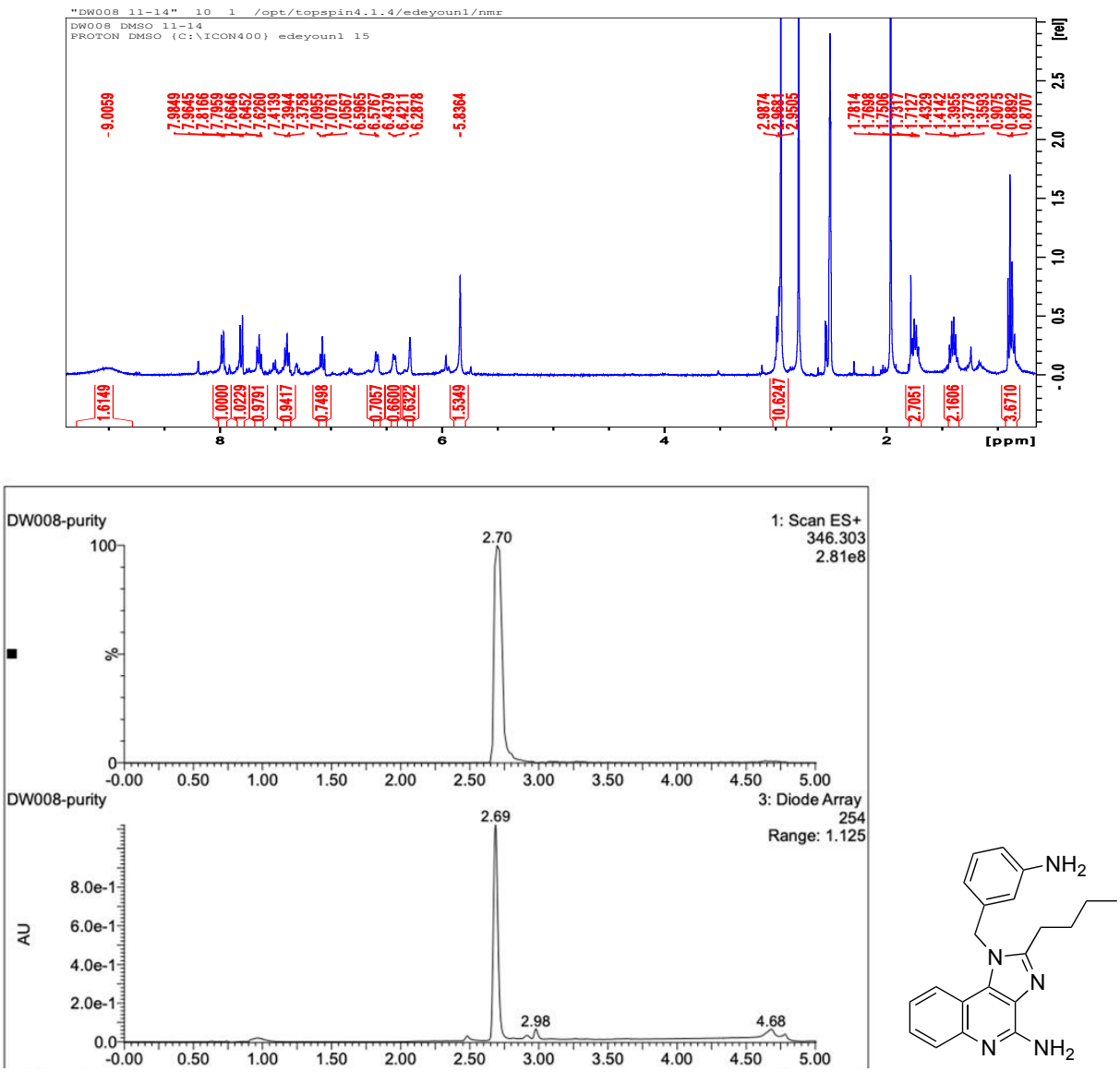

Figure S8: HPLC and NMR Characterization of Compound **14,** MW = 346.3 [M+H]

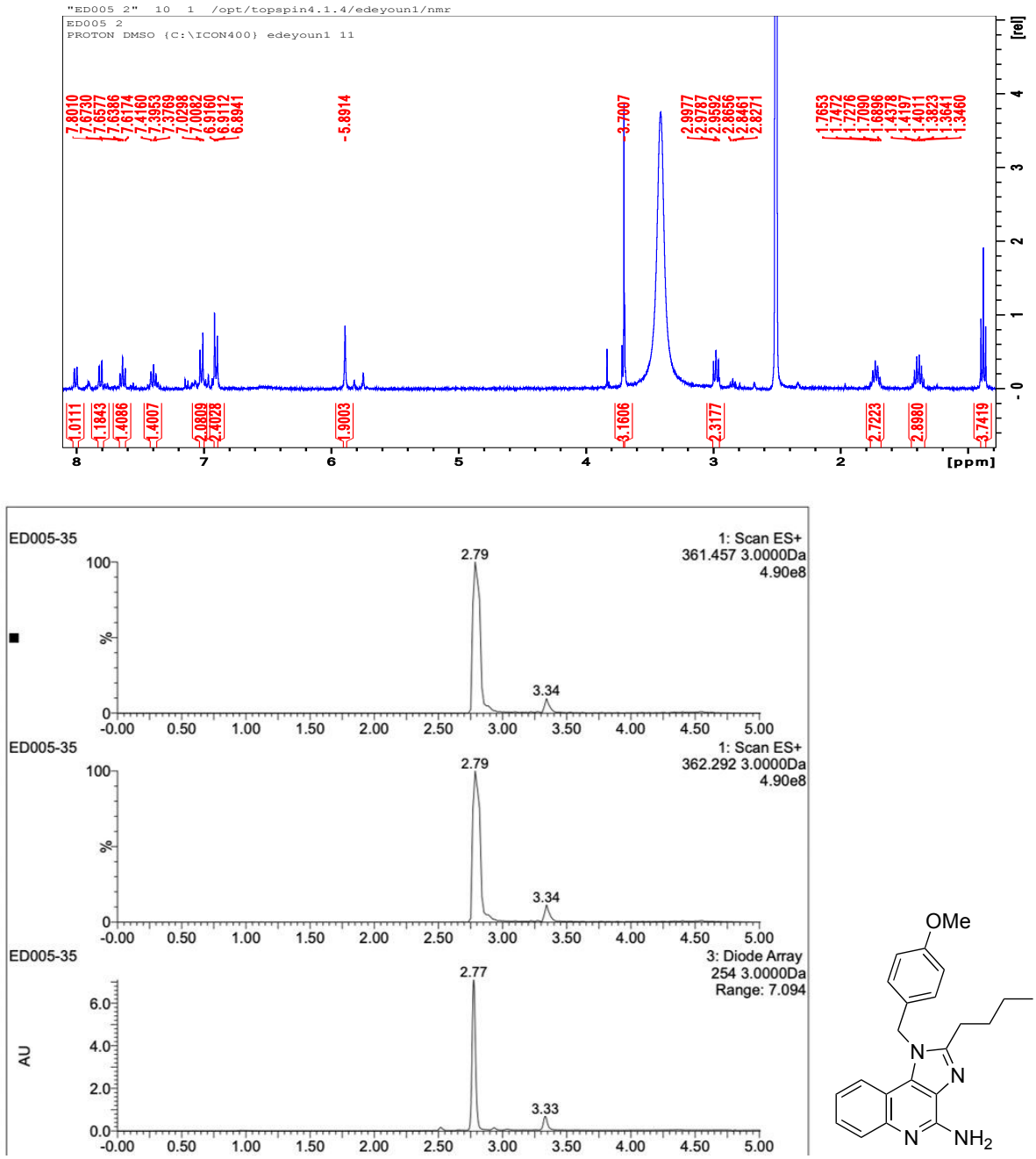

Figure S9: HPLC and NMR Characterization of Compound **13c**, MW = 361.5 [M+H]

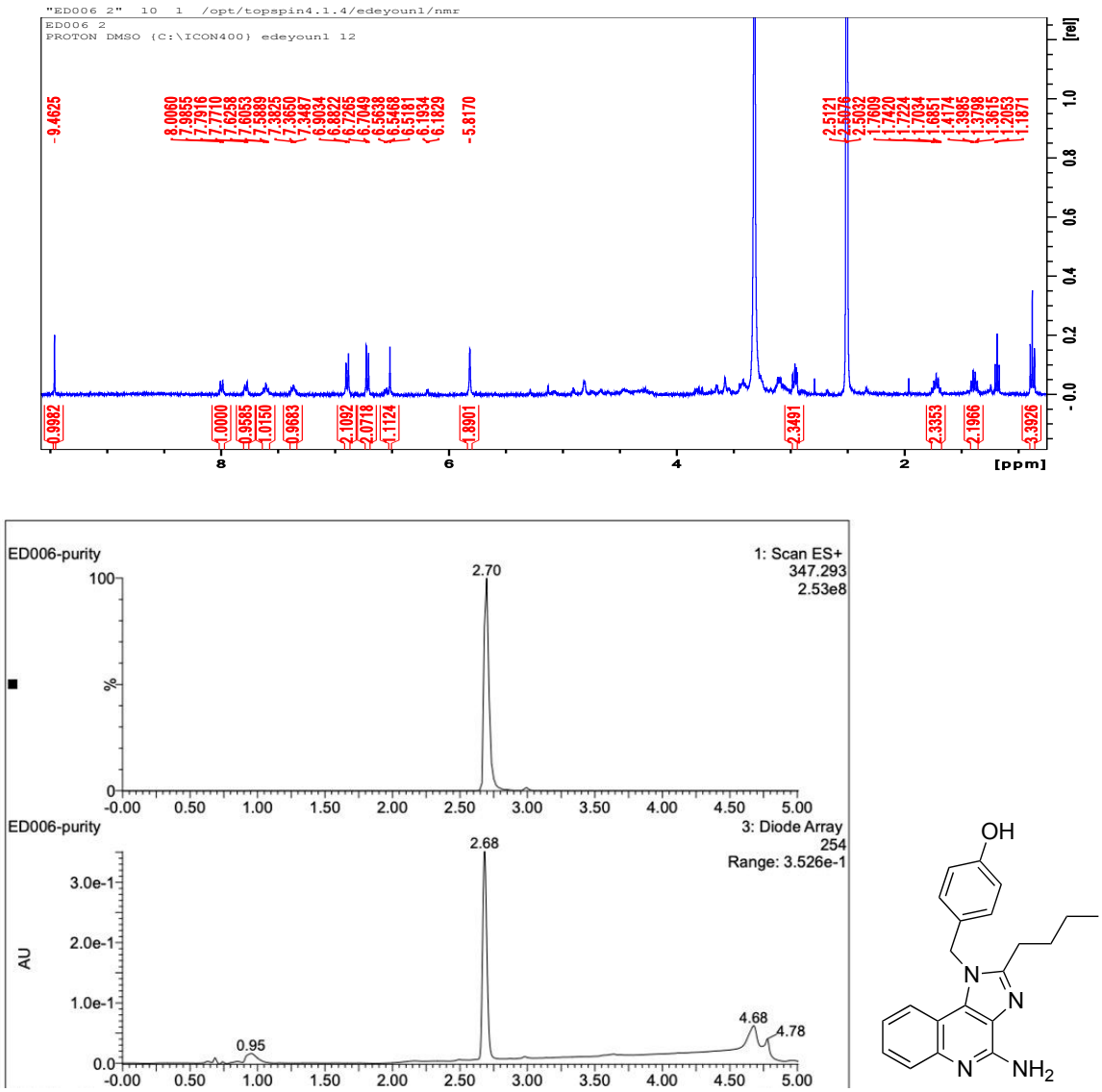

Figure S10: HPLC and NMR Characterization of Compound **15a,** MW = 347.3 [M+H]

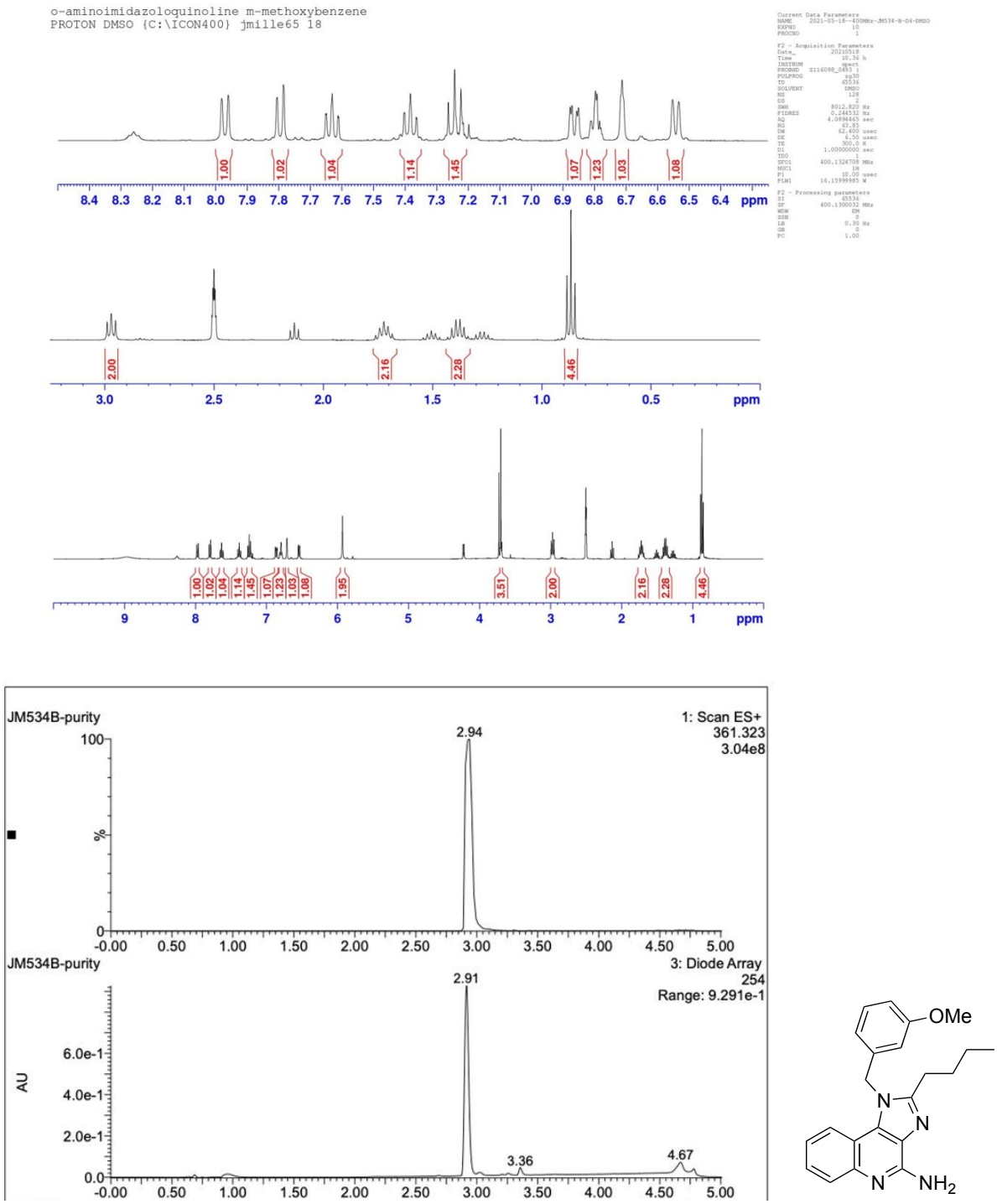

Figure S11: HPLC and NMR Characterization of Compound **13d,** MW = 361.3 [M+H]

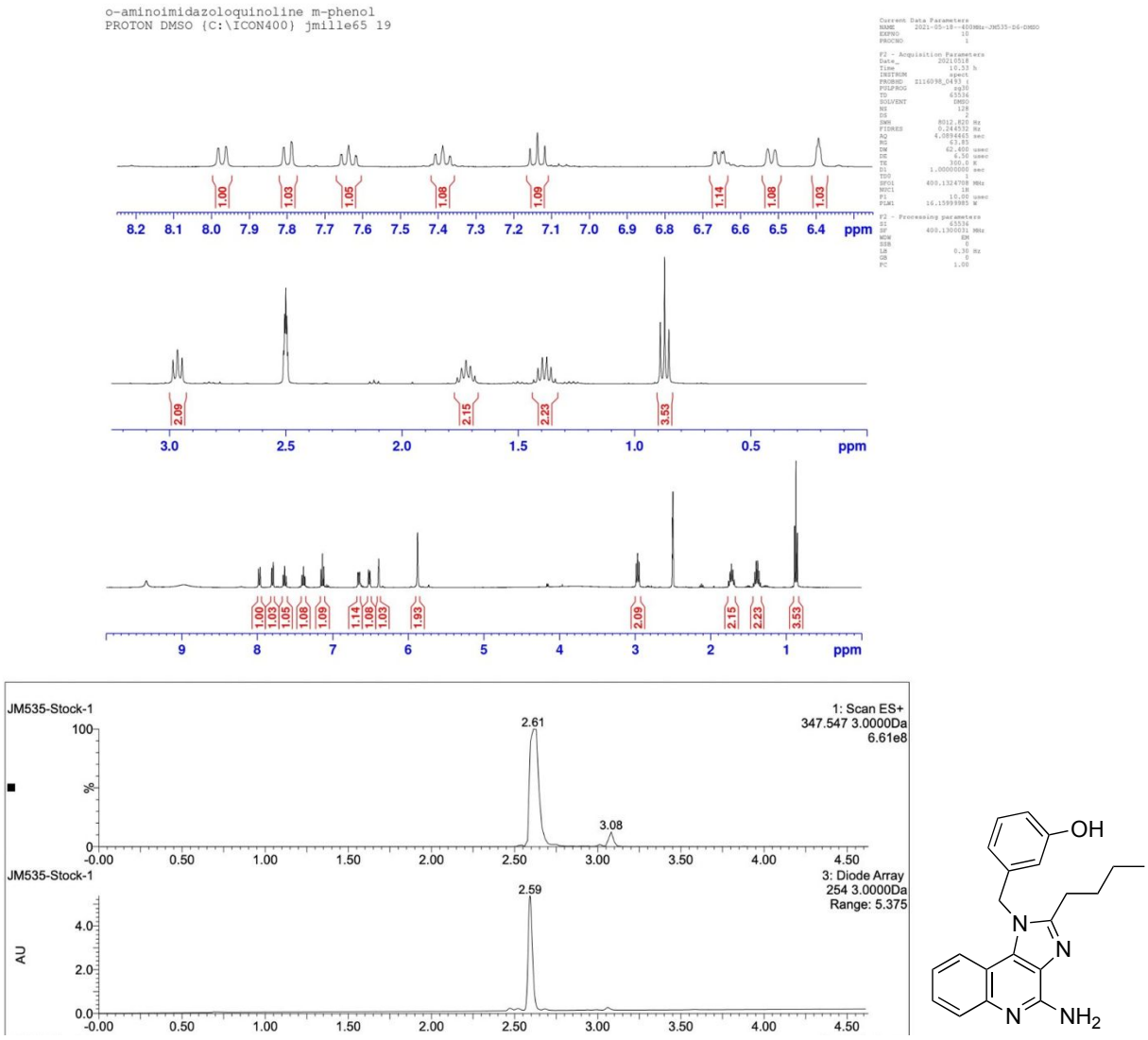

Figure S12: HPLC and NMR Characterization of Compound **15b,** MW = 347.5 [M+H]

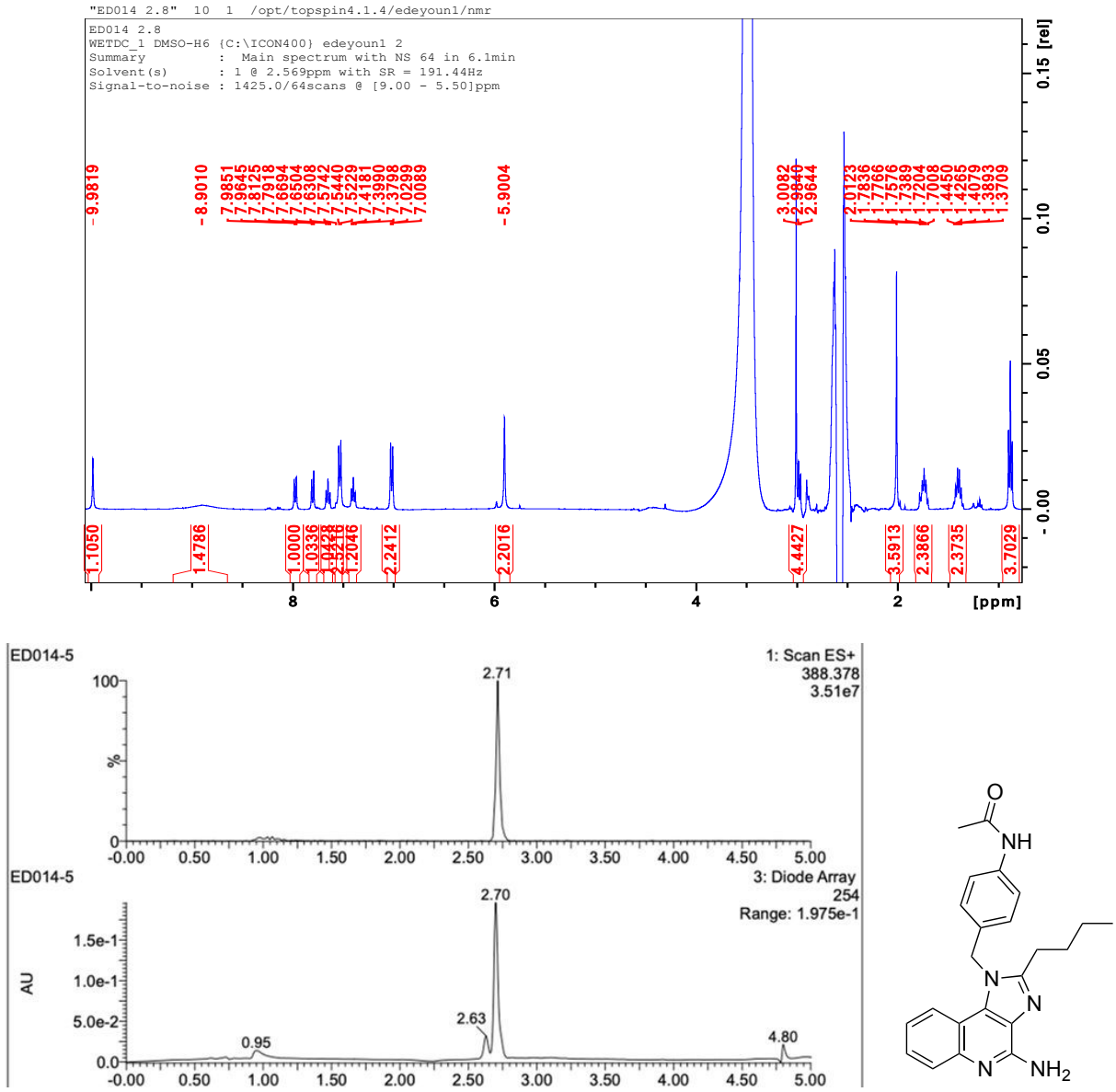

Figure S13: HPLC and NMR Characterization of Compound **16a,** MW = 388.4 [M+H]

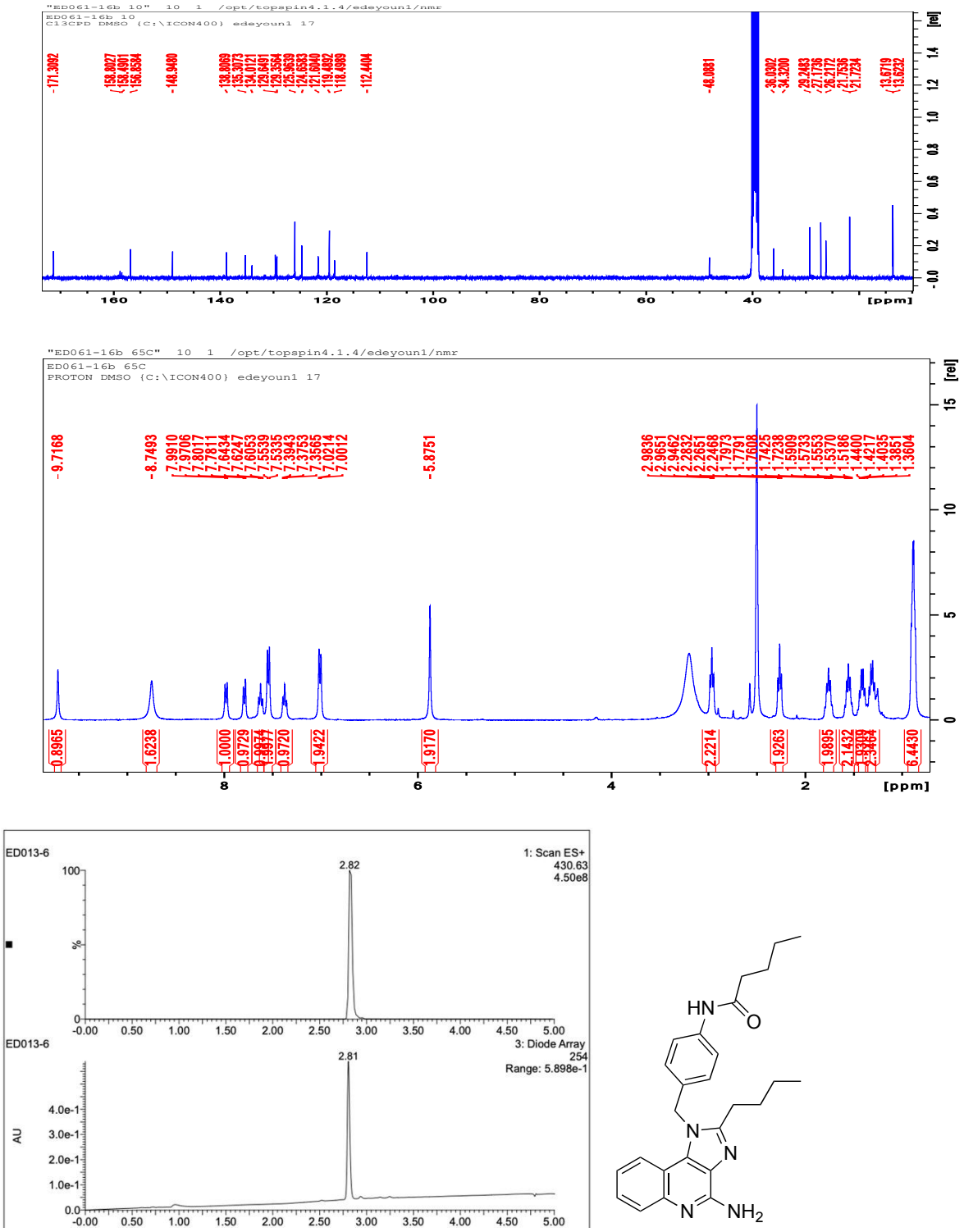

Figure S14: HPLC and NMR Characterization of Compound **16b,** MW = 430.6 [M+H]

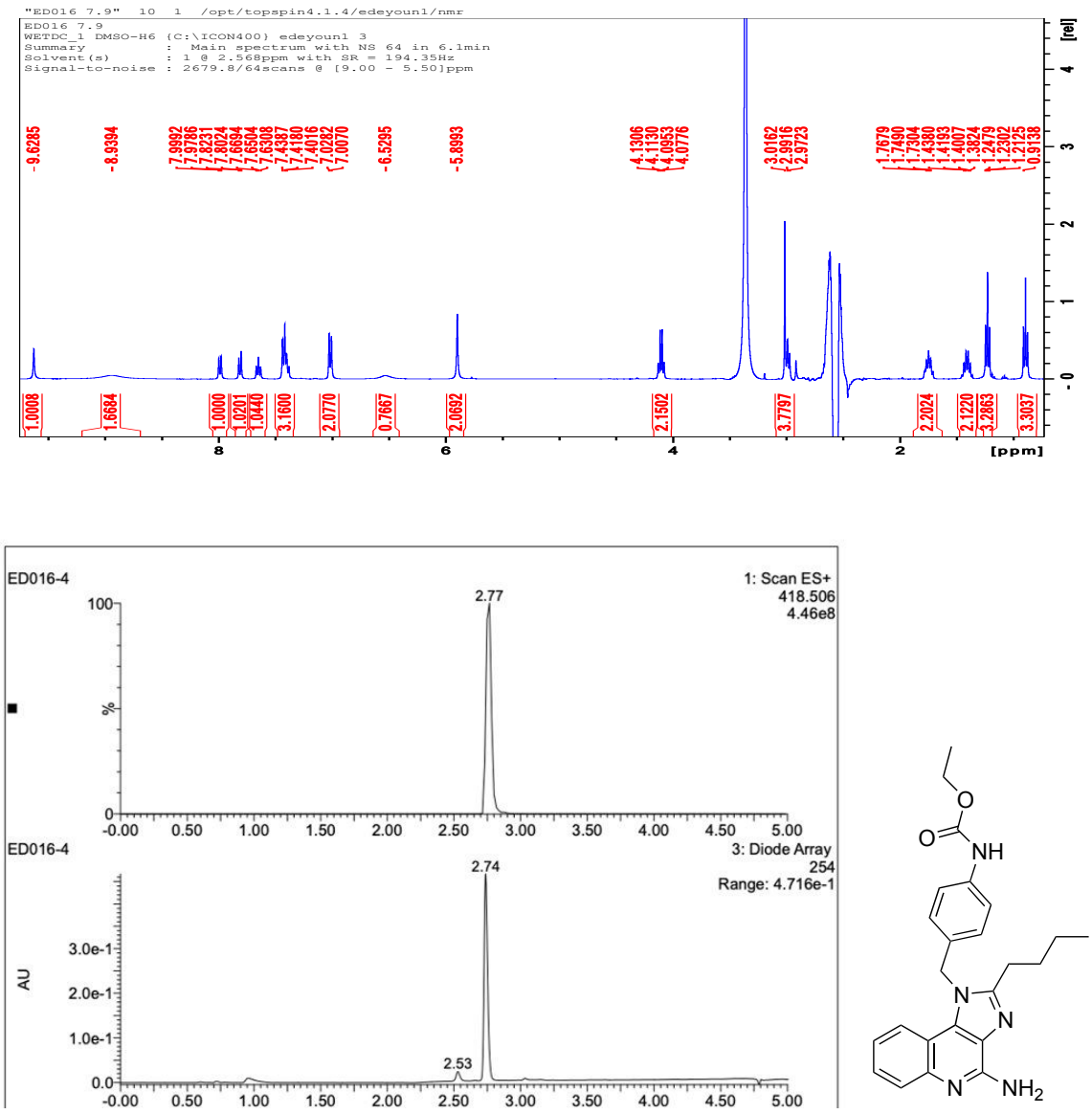

Figure S15: HPLC and NMR Characterization of Compound **16c,** MW = 418.5 [M+H]

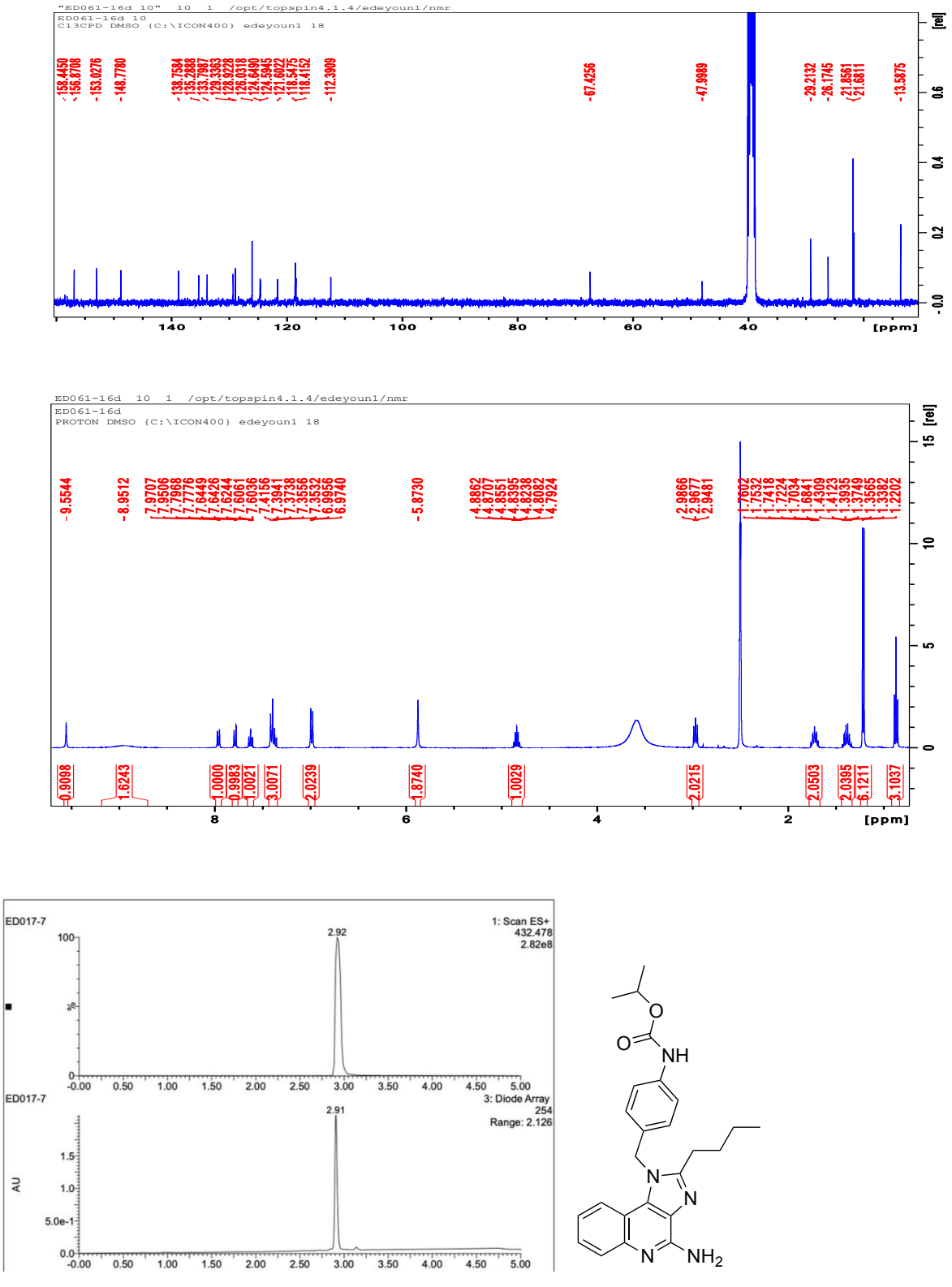

Figure S16: HPLC and NMR Characterization of Compound **16d,** MW = 432.5 [M+H]

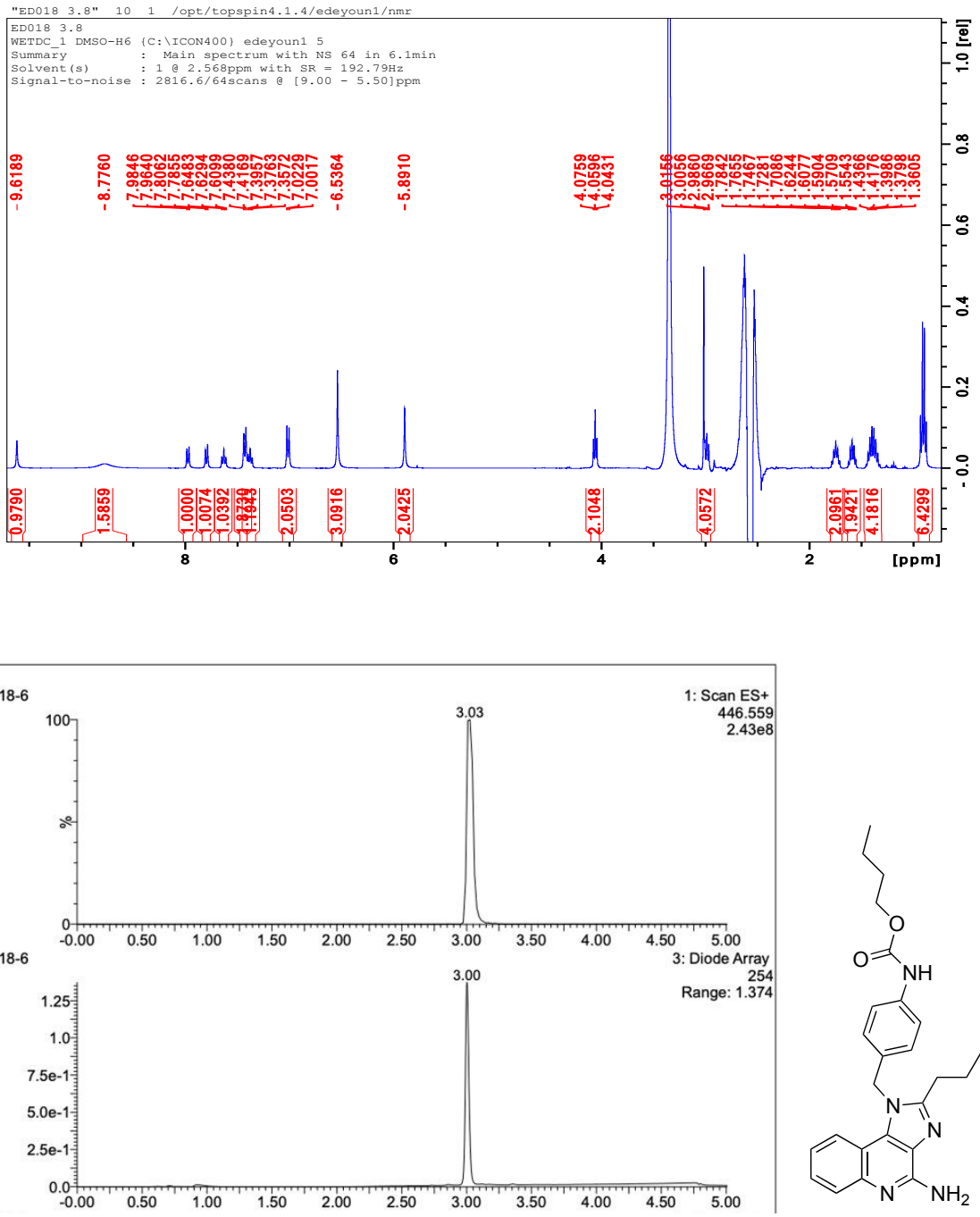

Figure S17: HPLC and NMR Characterization of Compound **16e,** MW = 446.6 [M+H]

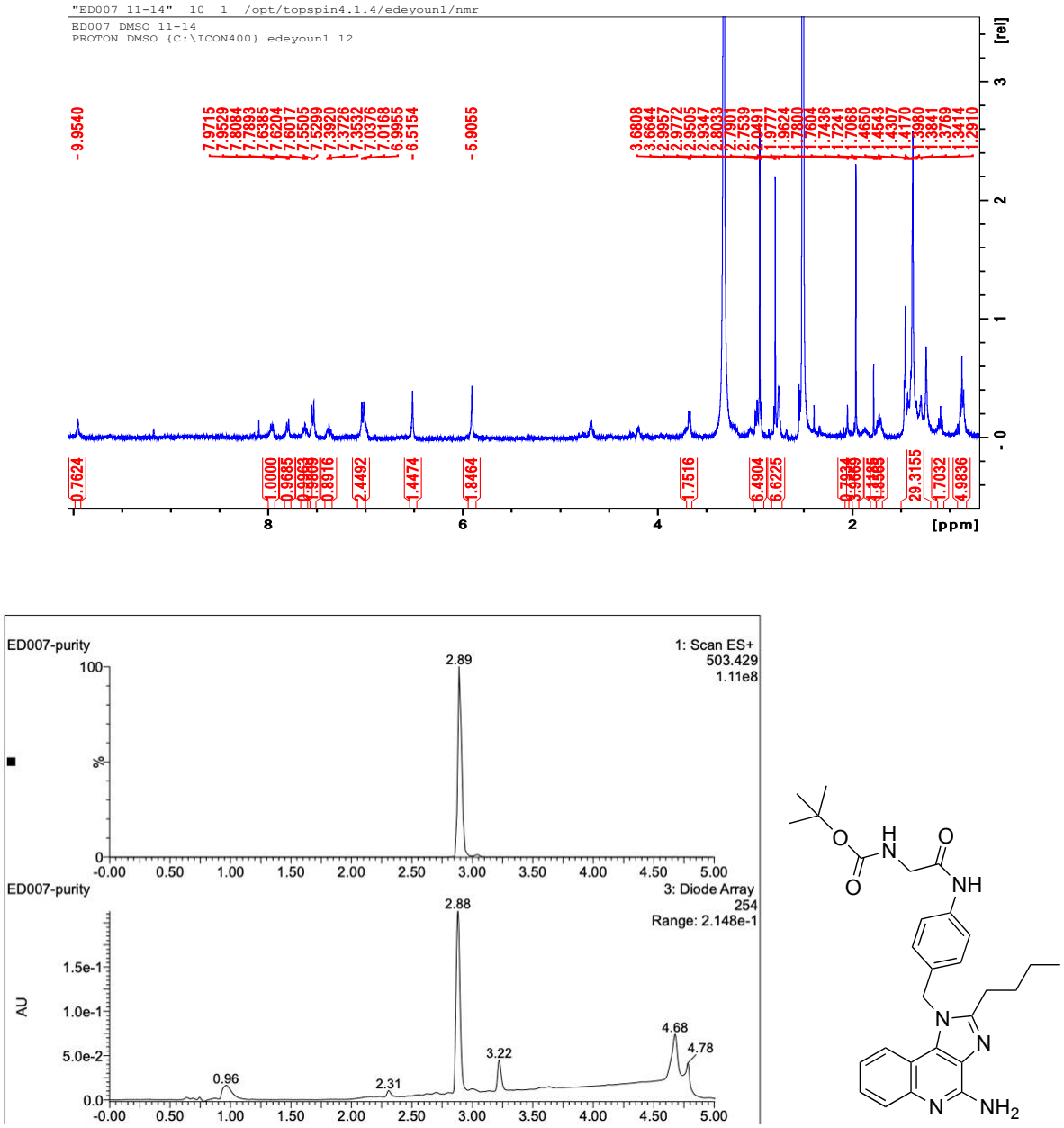

Figure S18: HPLC and NMR Characterization of Compound **17a,** MW = 503.4 [M+H]

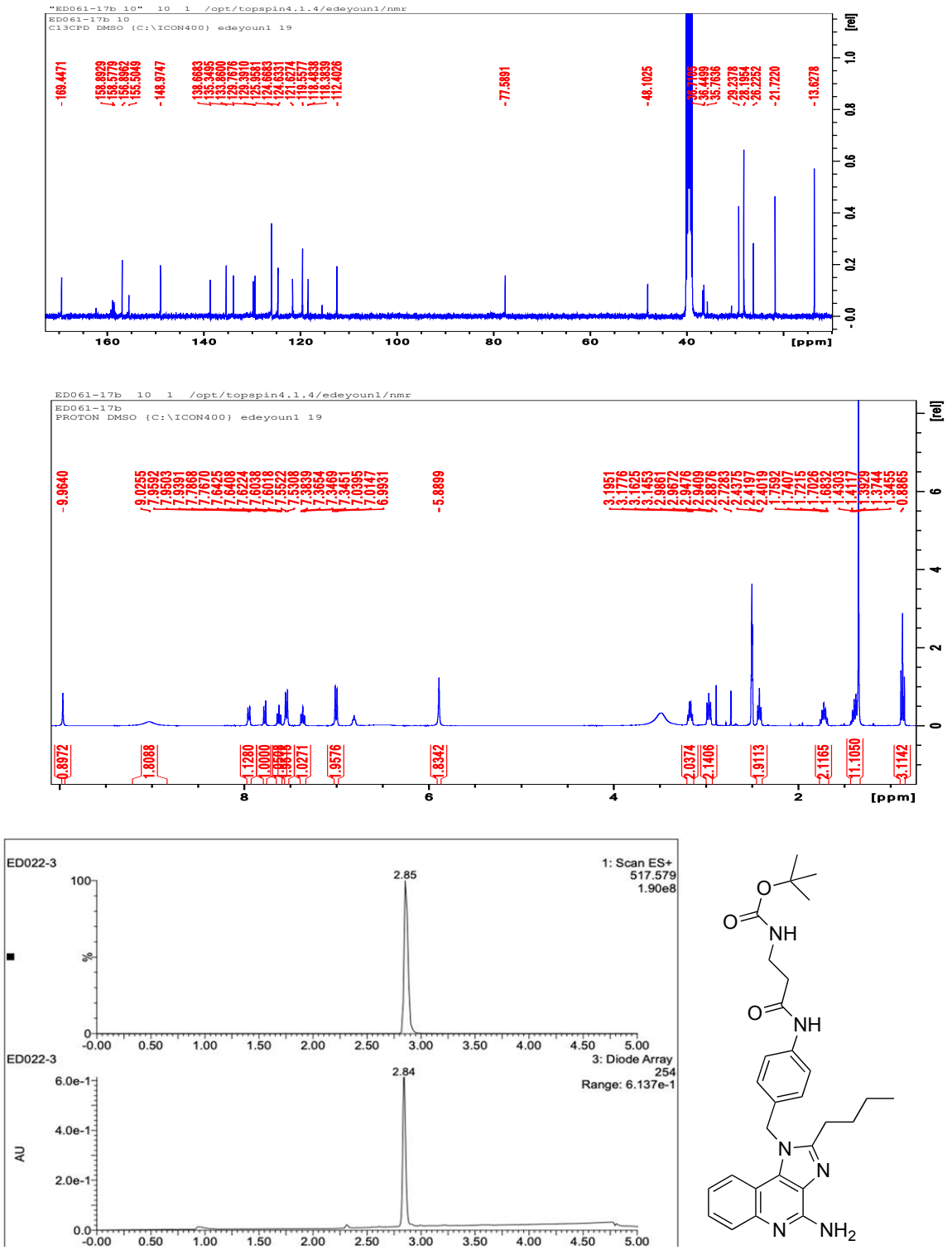

Figure S19: HPLC and NMR Characterization of Compound **17b,** MW = 517.6 [M+H]

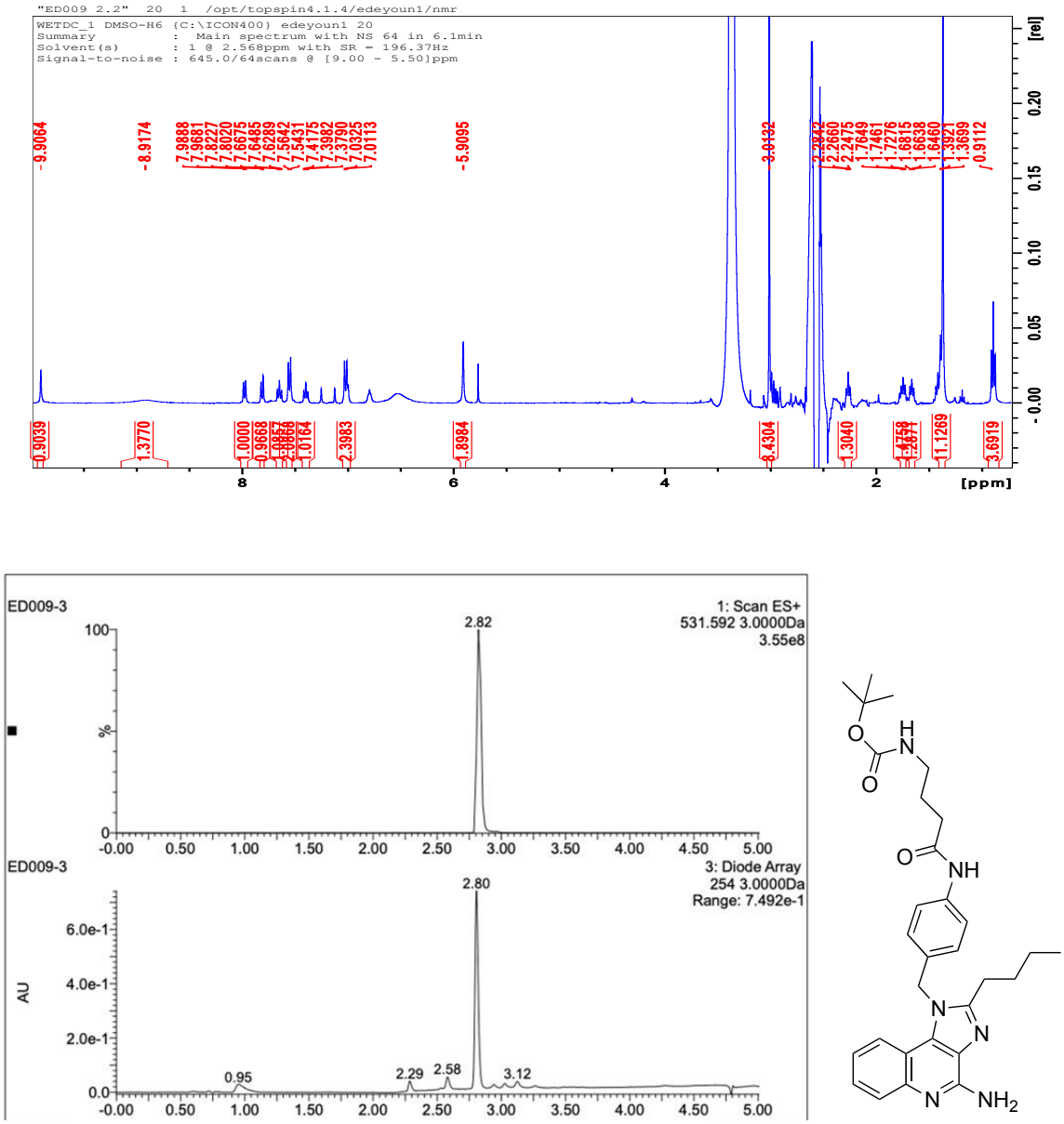

Figure S20: HPLC and NMR Characterization of Compound **17c,** MW = 531.6 [M+H]

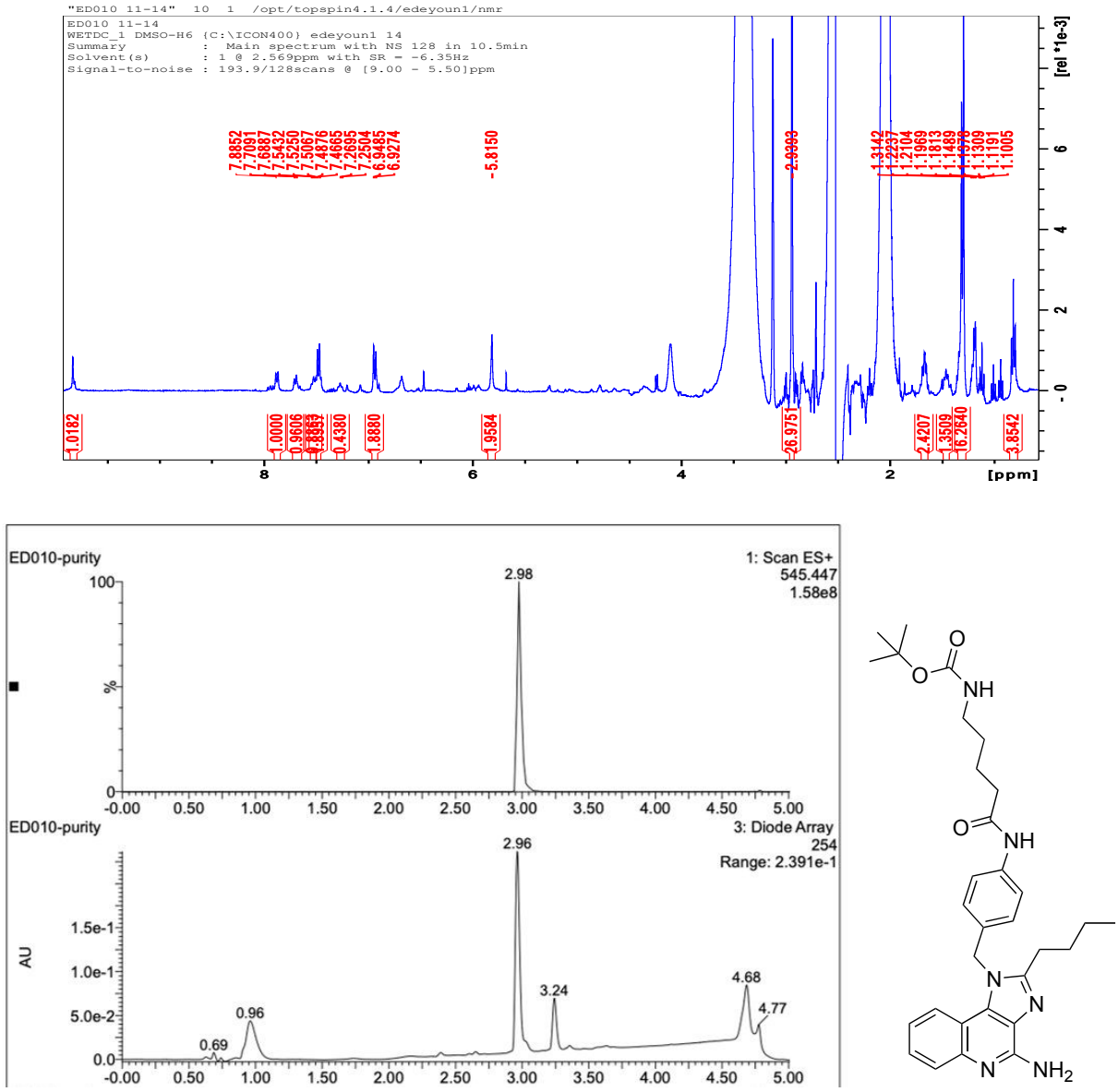

Figure S21: HPLC and NMR Characterization of Compound **17d,** MW = 545.4 [M+H]

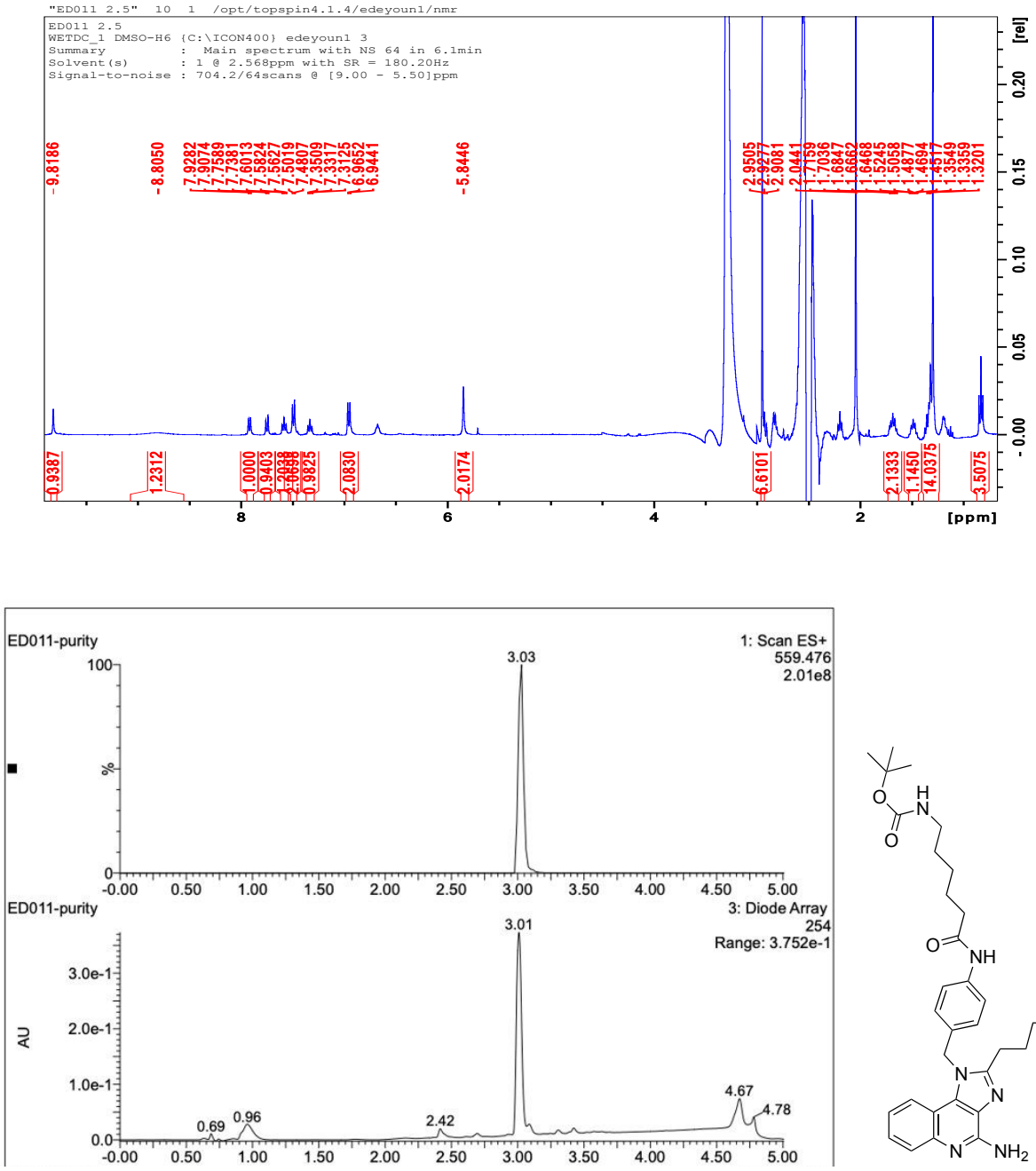

Figure S22: HPLC and NMR Characterization of Compound **17e,** MW = 559.5 [M+H]

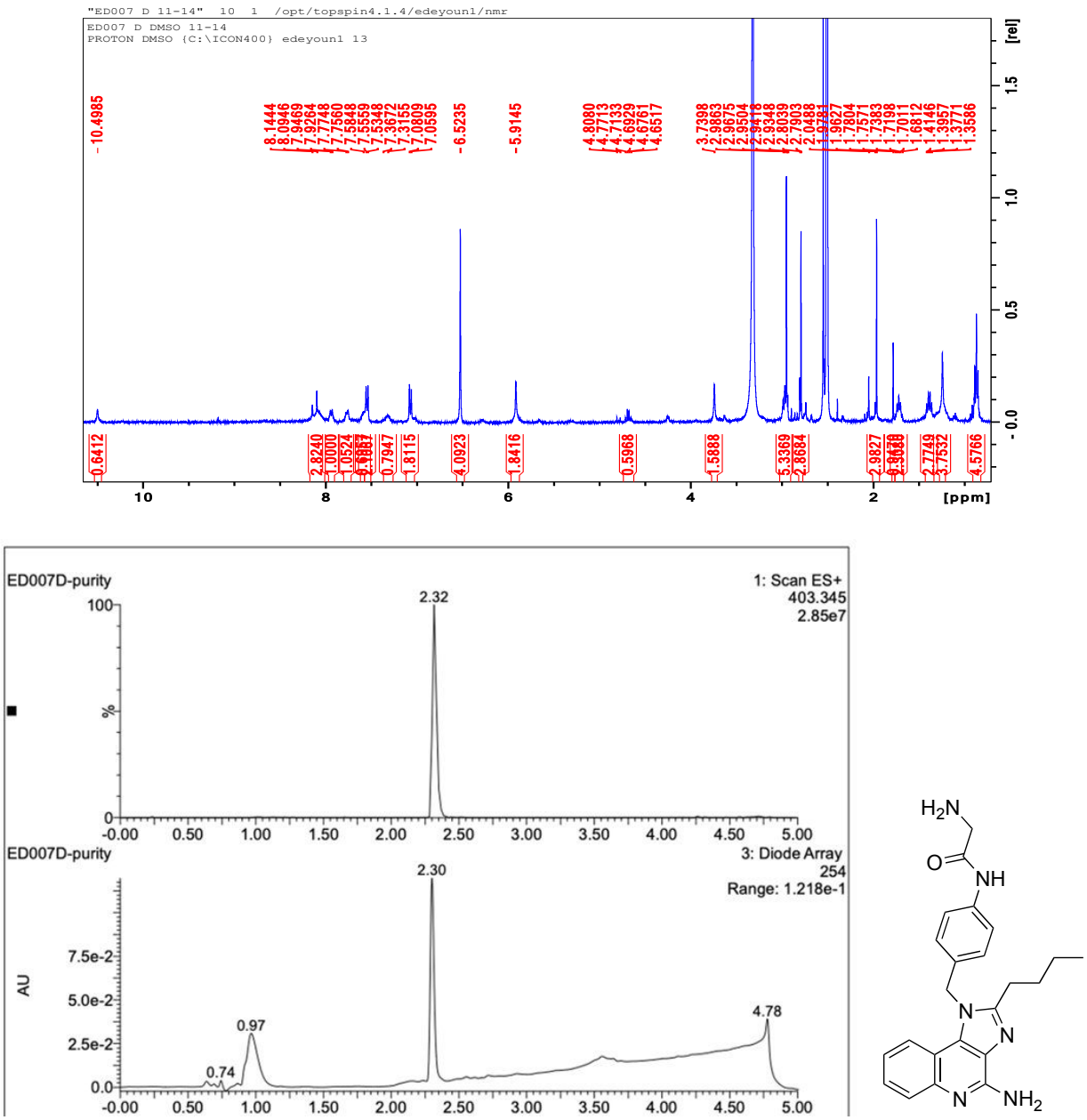

Figure S23: HPLC and NMR Characterization of Compound **18a,** MW = 403.3 [M+H]

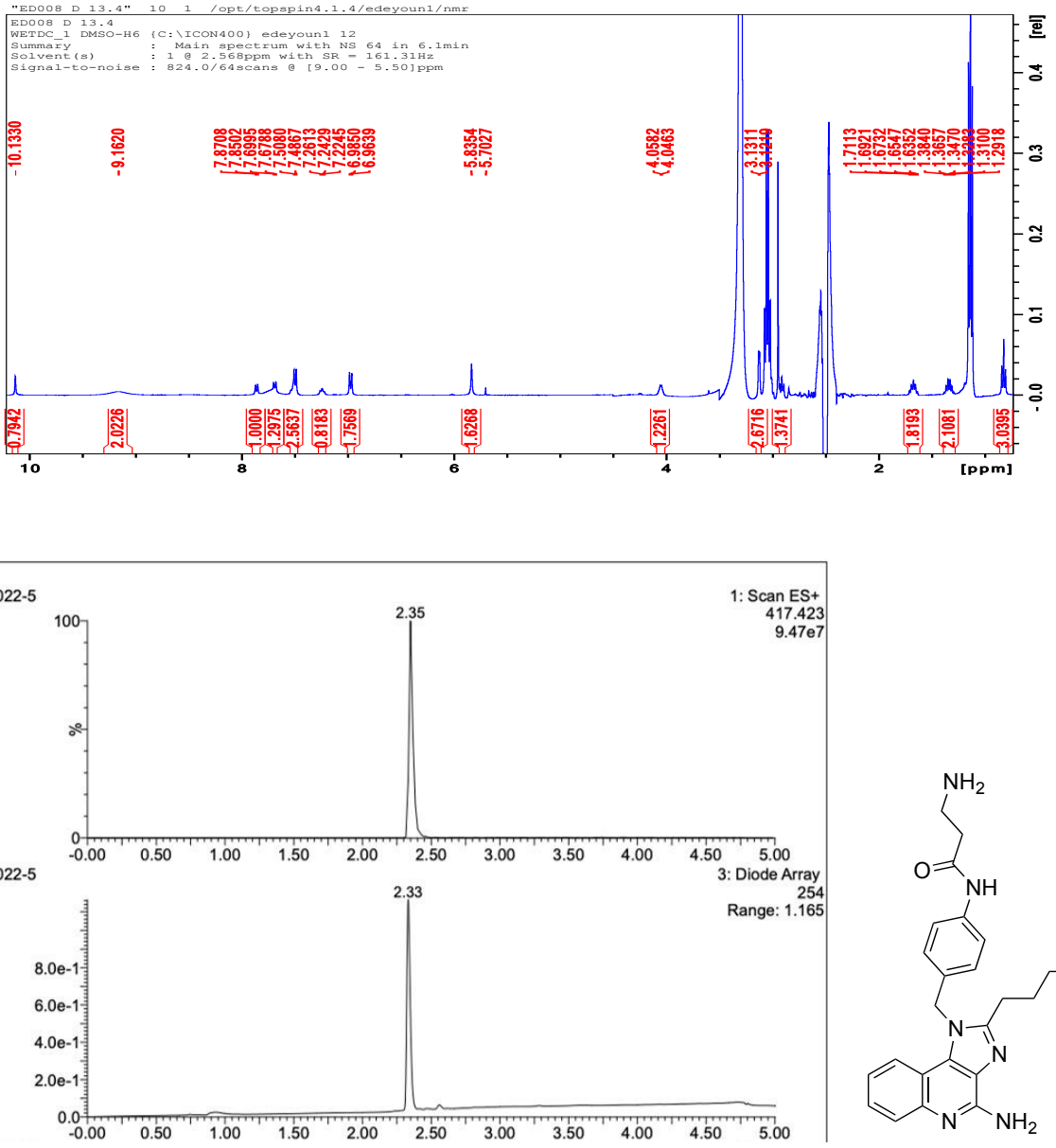

Figure S24: HPLC and NMR Characterization of Compound **18b,** MW = 417.4 [M+H]

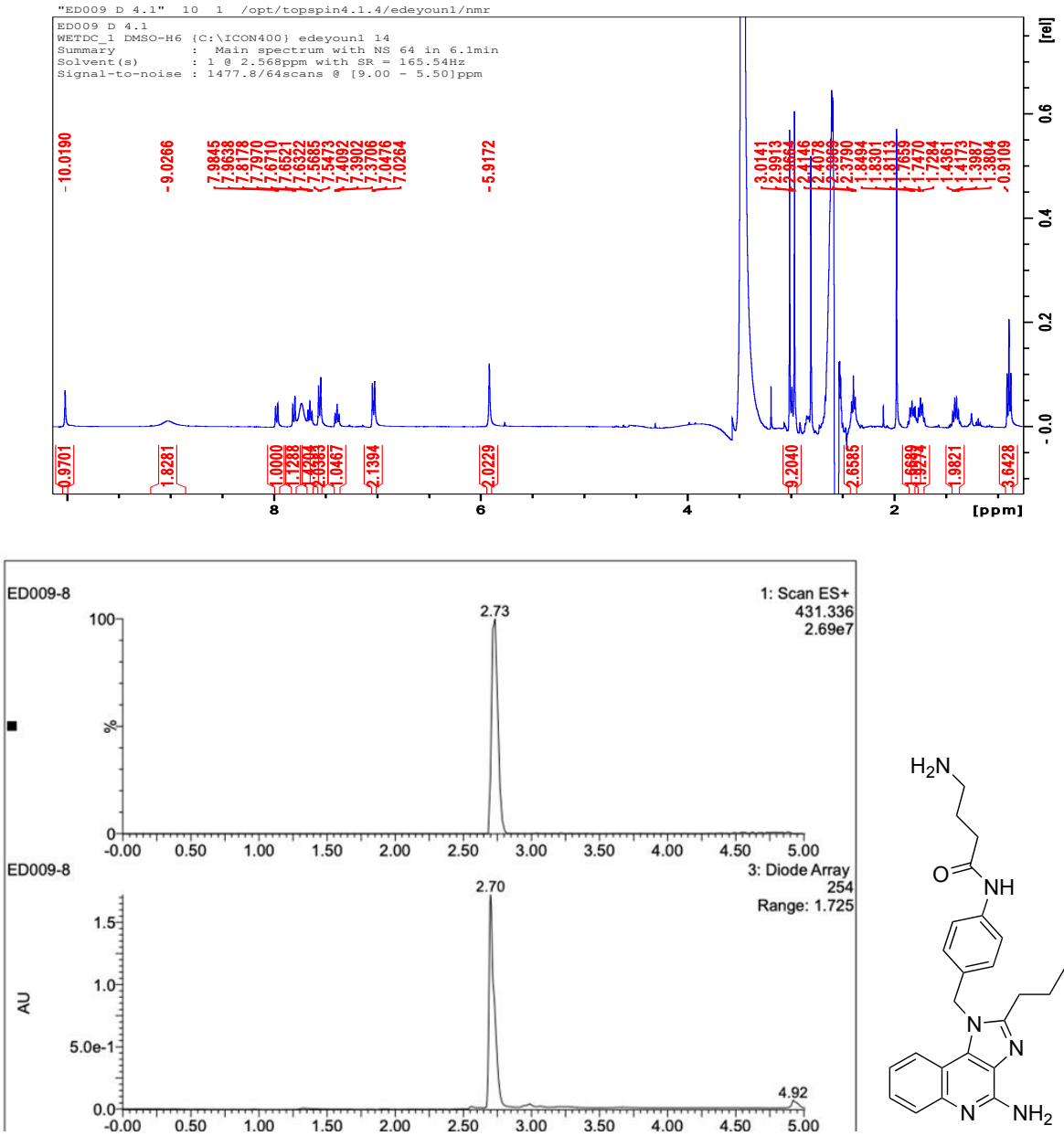

Figure S25: HPLC and NMR Characterization of Compound **18c,** MW = 431.3 [M+H]

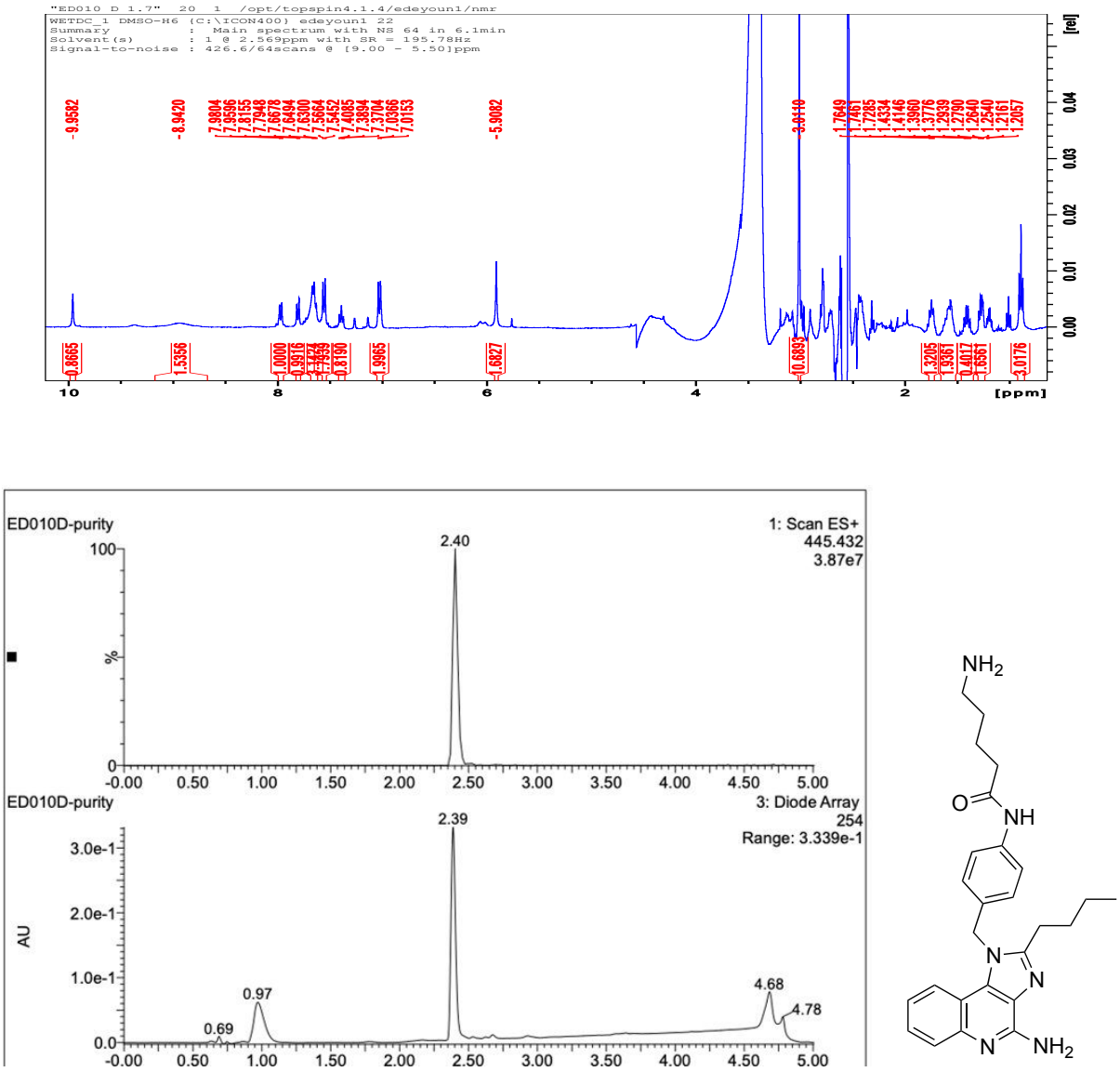

Figure S26: HPLC and NMR Characterization of Compound **18d,** MW = 445.4 [M+H]

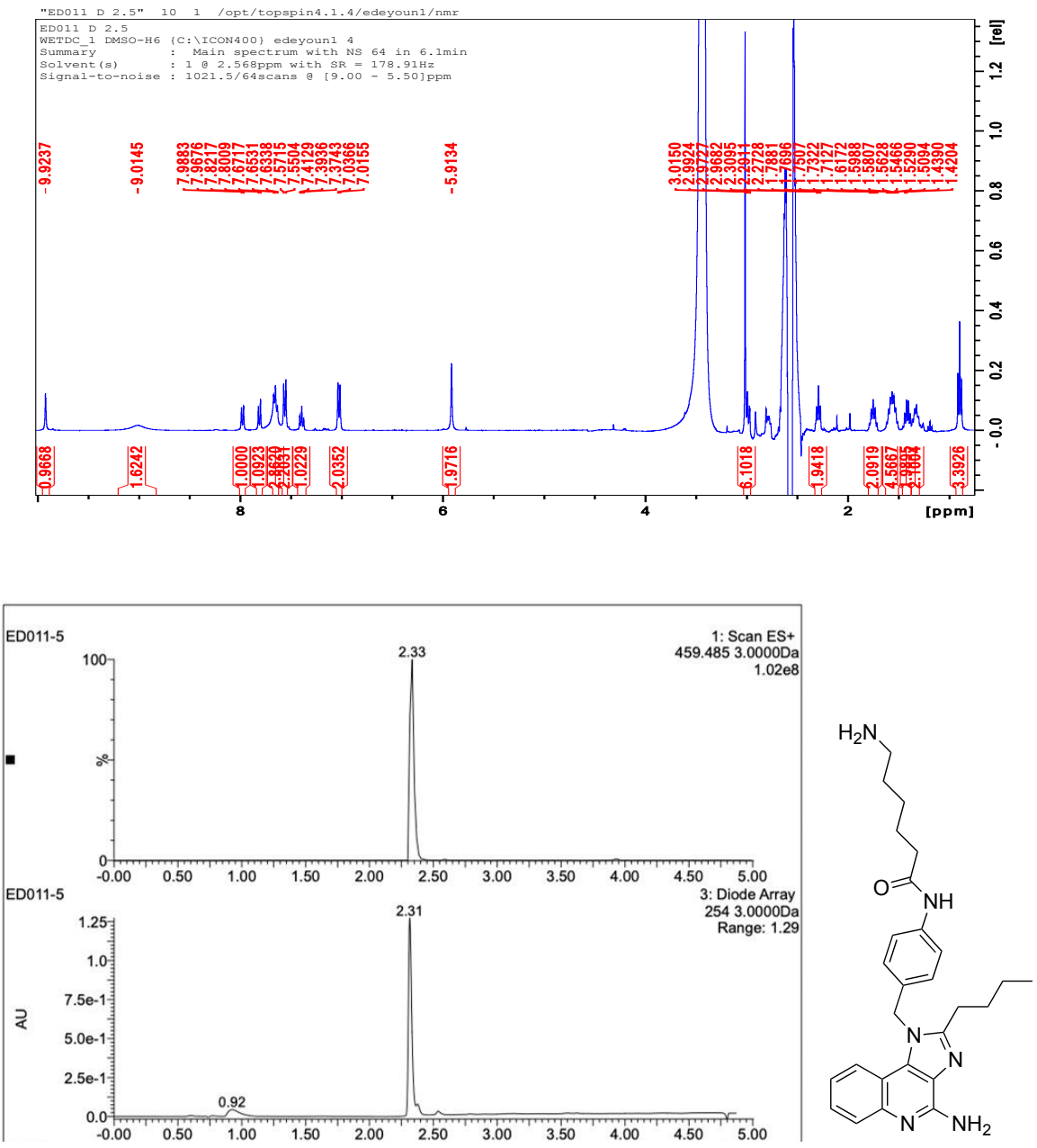

Figure S27: HPLC and NMR Characterization of Compound **18e,** MW = 459.5 [M+H]

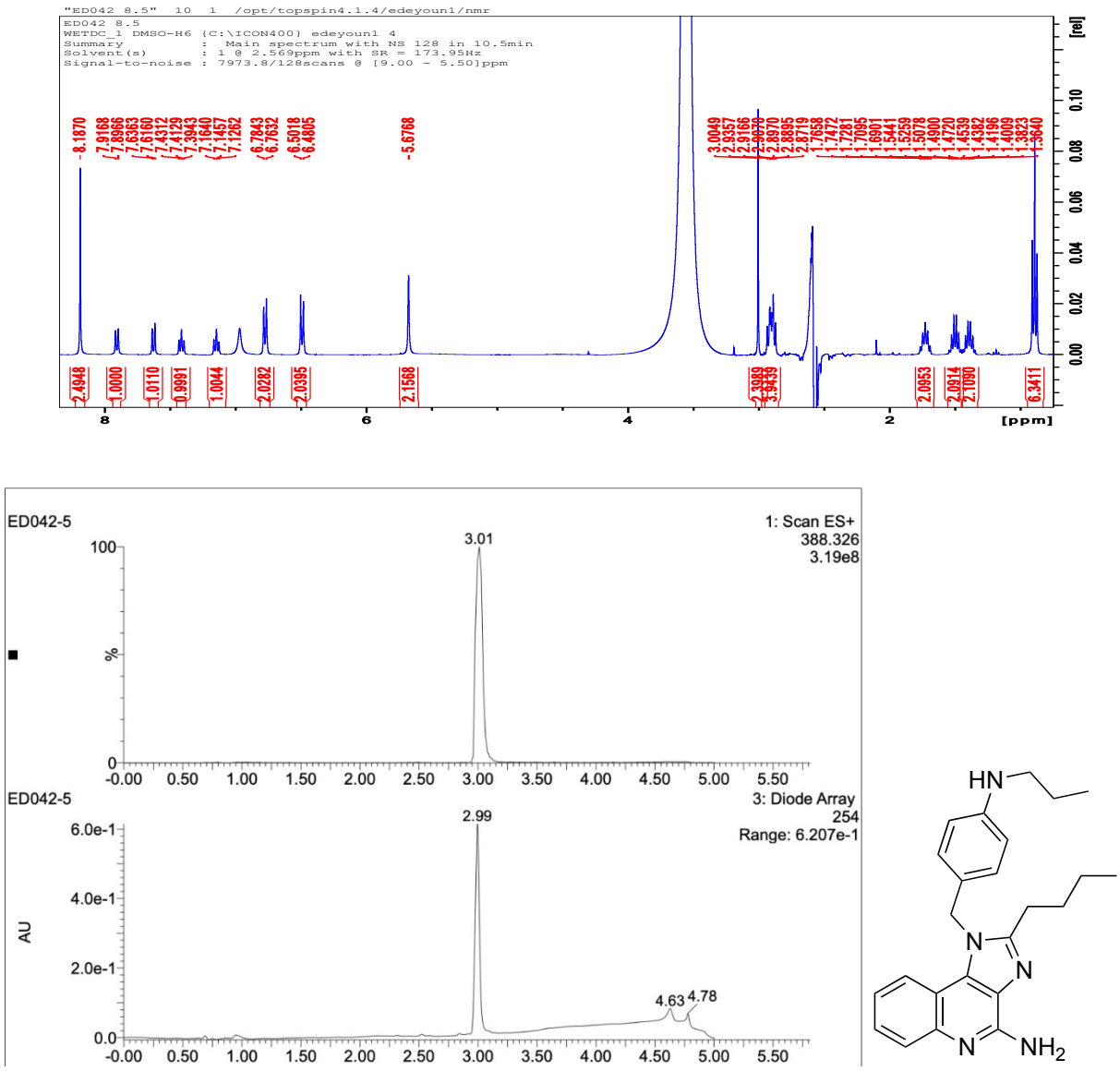

Figure S28: HPLC and NMR Characterization of Compound **19a,** MW = 388.3 [M+H]

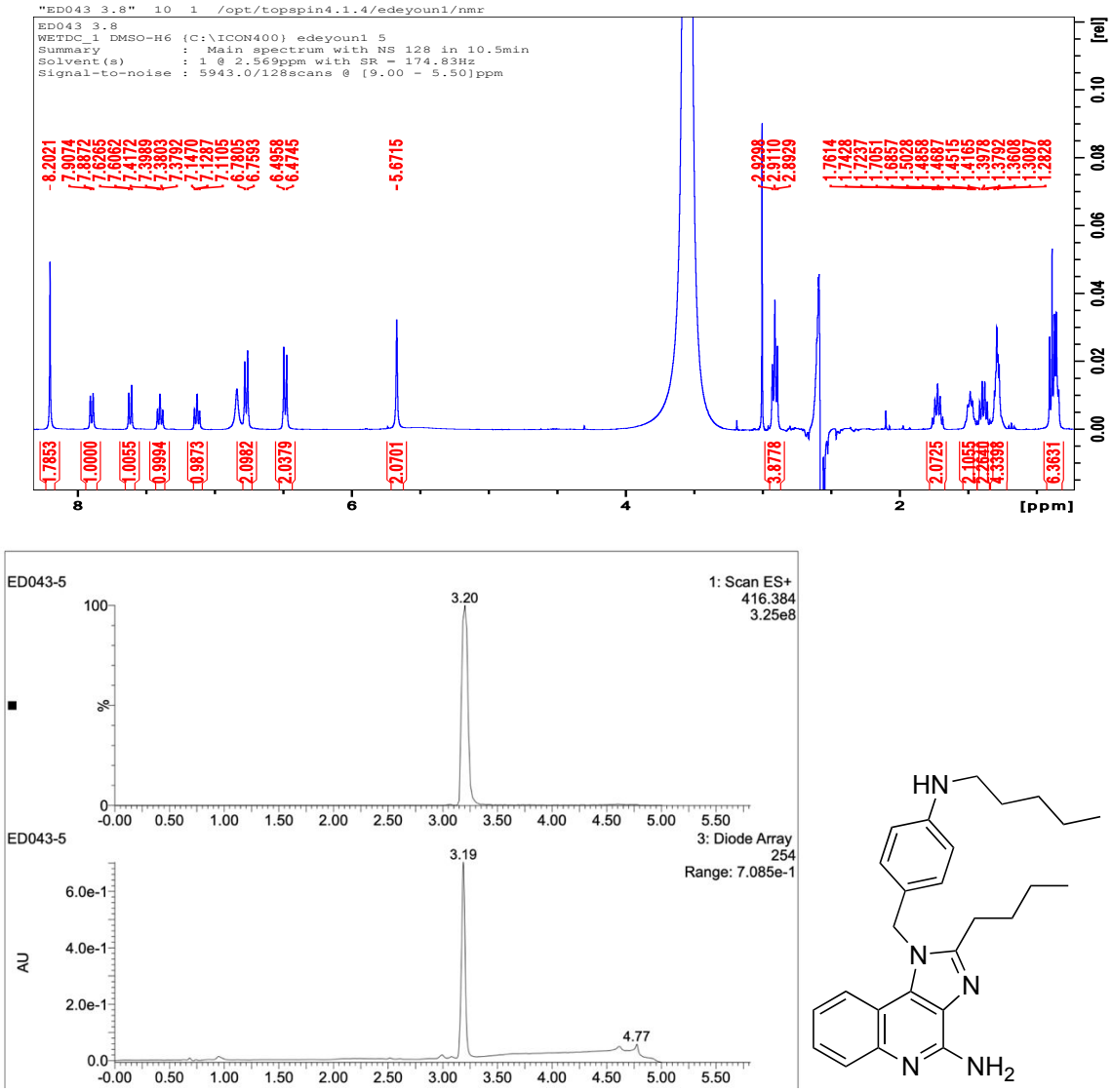

Figure S29: HPLC and NMR Characterization of Compound **19b,** MW = 416.4 [M+H]

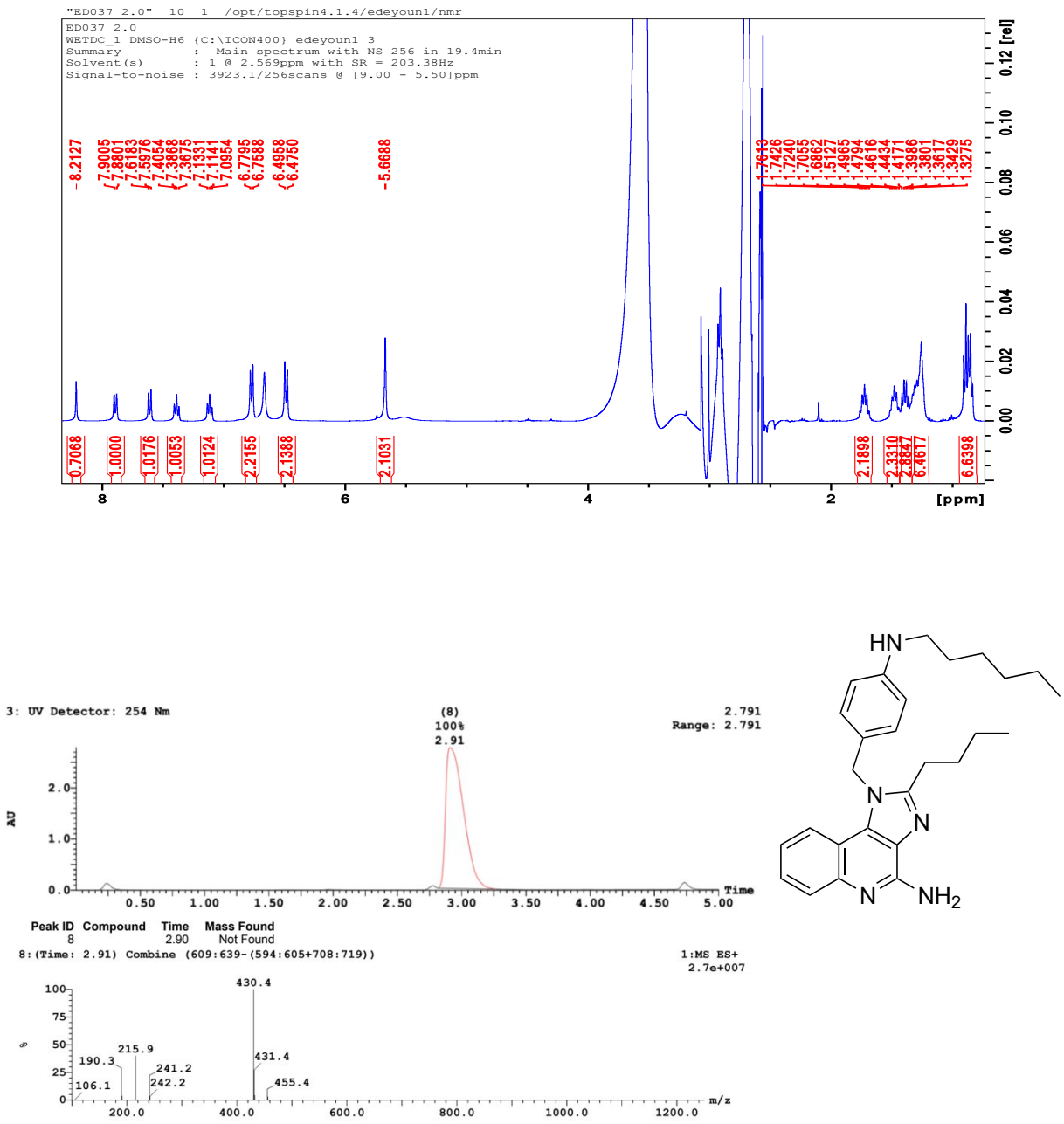

Figure S30: HPLC and NMR Characterization of Compound **19c,** MW = 429.6 [M+H]

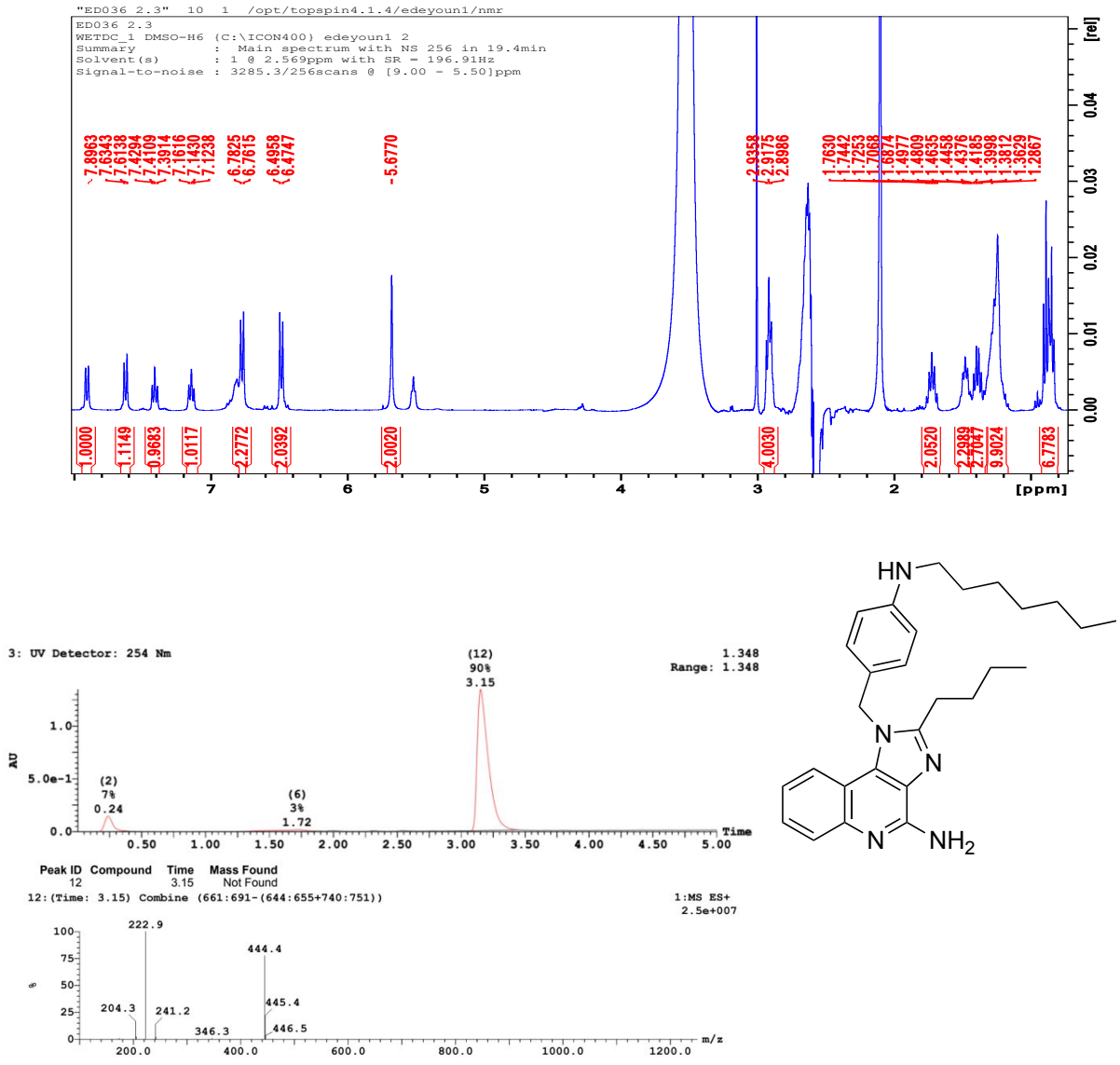

Figure S31: HPLC and NMR Characterization of Compound **19d,** MW = 443.6 [M+H]