

Design and Synthesis of N1-Modified Imidazoquinoline Agonists for Selective Activation of Toll-like Receptor 7 and 8

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Supporting Information

Chemical Synthesis

General Experimental Conditions and Materials: Bulk solvents were purchased from Fisher Chemical, Macron, and VWR; general chemicals were purchased from Fisher Chemical, Sigma-Aldrich, Acros Organics, and Malinkrodt Chemicals and were used as received. SiliaFlash® P60 silica gel was purchased from Silicycle. Aminomalononitrile *p*-toluenesulfonate and 1,1,1-trimethoxypentane was purchased from TCI America. 4-Bromobenzoic acid, iron powder (325-mesh), and diiodomethane were purchased from Alfa Aesar. Bis(pinacolato) diboron was purchased from Oakwood Products Inc. 2-Dicyclohexylphosphino-2',6'-dimethoxybiphenyl (Sphos) was purchased from Sigma-Aldrich. Palladium catalysts were purchased from Chem-Impex, Sigma-Aldrich, and Strem Chemicals. 3-amino-1-propanol was purchased from Sigma-Aldrich. 1-(*N*-Boc-aminomethyl)-4-(aminomethyl)benzene, 4-amino-1-butanol, 2-(ethylthio)ethylamine, and 3-(methylthio)propylamine were purchased from Acros Organics. 2-(Methylthio)ethylamine was purchased from Maybridge Chemical Company. Imiquimod (**1**) was synthesized as described in the Hays, et al. patent.¹ Imidazoquinolines **12a**, **12b**, and **12e** were synthesized as reported in our previous work.^{2,3} Amino acid methyl esters **7k-o**,⁴ **u**,⁵ **v**⁶ can be prepared according to the respective literature procedures and mono-protected diamines **7p-s** were prepared according to Lee et al.⁷ or purchased from Acros Organics and Sigma-Aldrich. An anhydrous solvent dispensing system (MBraun MB-SPS-800) was used for drying THF and toluene and these solvents were dispensed under nitrogen. Thin layer chromatography (TLC) was performed on 0.25 mm hard-layer silica GF plates. Developed plates were visualized with a hand-held UV lamp. Column chromatography conditions are based on TLC results. ¹H NMR spectra were recorded on a Varian 400 MHz spectrometer. Proton chemical shifts are reported in ppm from internal standards of residual chloroform (7.26 ppm), dimethylsulfoxide (2.50 ppm), or methanol (3.31 ppm). Proton chemical data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, hex = hextet, m = multiplet, br = broad), coupling constant, and integration. High resolution mass spectra were obtained on either on an Agilent TOF II TOF/MS instrument or a Bruker BioTOF II TOF/MS instrument, both equipped with an ESI or APCI interface.

Purity was determined using HPLC/MS analysis for the lead compound of the series (**12b**) and all key compounds evaluated in the cytokine assays (**12h**, **12p**, **12t**, and imiquimod). Compounds **12h** and **12p** are also representative of the sulfide and amino substituted derivatives. Reverse-phase HPLC was performed using a two phase gradient technique. Mobile phase A was 0.1% aqueous formic acid and mobile phase B was 0.1% formic acid in acetonitrile. Initial conditions were 100% A with a continuous gradient over 16 min to 90% B. The column was allowed to re-equilibrate to 100% A for at least 8 min following each run. Final

compounds were run on a Hamilton Robotics PRP-1 250 x 4.1 mm column with a 7 μ m particle size with a PRP-1 12-20 μ m guard column and detected by 250 nm UV and confirmed by ESI positive mode MS detection. Purity and retention times for **12b**, **12h**, **12p**, **12t**, and imiquimod were 99.0%/9.9 min, 96.8%/11.5 min, 95.6%/7.1 min, 95.8%/8.1 min, and 94.0%/9.2 min, respectively. All other compounds were evaluated for purity by qualitative analysis of NMR spectra which are included in the Supporting Information.

General Procedure for the Synthesis of 8. To a suspension of aminomalononitrile *p*-toluenesulfonate (2.0 g, 7.9 mmol, 1.0 equiv) in THF (70 mL) at 25 °C was added NEt₃ (1.4 mL, 10.0 mmol, 1.27 equiv) in one portion. The mixture was stirred briefly until it became a homogeneous solution. To this solution was added 1,1,1-trimethoxypentane (2.18 g, 13.4 mmol, 1.7 equiv), and the solution was heated at reflux for 3.5 h under argon before being cooled to 25 °C. NEt₃ (1.4 mL, 10.0 mmol, 1.27 equiv) and **7** were added sequentially, and the mixture was stirred for 18 h. Some primary amine compounds required additional heating or other additives for reasonable yields. Solvent was removed *in vacuo* and the crude residue was redissolved in CH₂Cl₂ (50 mL) and washed with saturated aqueous Na₂CO₃ (20 mL). The organics were separated and the aqueous layer was extracted with CH₂Cl₂ (3 x 20 mL). Combined organic fractions were concentrated *in vacuo*. The crude residue was purified by flash column chromatography on silica gel. The product was dried for at least 24 h *in vacuo* (3–5 mbar).

General Procedure for the Synthesis of 9. The dried imidazole (**8**) (3.0 mmol, 1.0 equiv) was dissolved/suspended in CHCl₃ (15 mL). To this mixture, CH₂I₂ (10.0 g, 37.3 mmol, 12.4 equiv) was added followed by a solution of isoamyl nitrite (2.11 g, 18.0 mmol, 6.0 equiv) in CHCl₃ (15 mL). The mixture was heated to 80 °C for 1 h under argon and then allowed to cool to 25 °C. The mixture was concentrated *in vacuo* and the crude residue was purified by flash column chromatography on silica gel.

General Procedure for the Synthesis of 12. To a solution of methyl 3-amino-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate (1.8 mmol, 3.0 equiv) in THF (25 mL) was added K₂CO₃ (1.8 mmol, 3.0 equiv), imidazole (**9**) (0.6 mmol, 1.0 equiv), Pd(PPh₃)₂Cl₂ (0.09 mmol, 0.15 equiv), and H₂O (10 mL) sequentially. The mixture was heated to 90 °C under argon for 3 h or until when TLC analysis indicated complete conversion. The mixture was cooled to 25 °C and diluted with EtOAc (30 mL) and H₂O (20 mL). The organics were separated and the aqueous layer was extracted with EtOAc (3 x 20 mL). Combined organic fractions were concentrated *in vacuo*. The crude residue was chromatographed on a short silica plug to remove polar impurities. The resulting oil was dried for at least 24 h *in vacuo* (3–5 mbar) and used without further purification.

To a round-bottom flask containing the dried oil (~0.33 mmol, 1.0 equiv) was added 4 N HCl in dioxane (2.5 mL, 10.0 mmol, 30 equiv). The mixture was heated at reflux for 4 h under argon and then cooled to 25 °C. NaHCO₃ powder was added to quench the acid. The reaction mixture was partitioned between 5% MeOH in EtOAc (25 mL) and H₂O (20 mL). The aqueous layer was extracted with 5% MeOH in EtOAc (3 x 20 mL). Combined organic fractions were concentrated *in vacuo*. The crude residue was purified by flash column chromatography on silica gel.

General Procedure for the Synthesis of 12p-t. To a solution of methyl 3-amino-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate (1.8 mmol, 3.0 equiv) in THF (25 mL) was added K₂CO₃ (1.8 mmol, 3.0 equiv), imidazole (**9p-t**) (0.6 mmol, 1.0 equiv), Pd(PPh₃)₂Cl₂ (0.09 mmol, 0.15 equiv), and H₂O (10 mL) sequentially. The mixture was heated to 90 °C under argon for 3 h or until when TLC analysis indicated complete conversion. The mixture was cooled to 25

°C and diluted with EtOAc (30 mL) and H₂O (20 mL). The organics were separated and the aqueous layer was extracted with EtOAc (3 x 20 mL). Combined organic fractions were concentrated *in vacuo*. The crude residue was chromatographed on a short silica plug to remove polar impurities. The resulting oil was dried for at least 24 h *in vacuo* (3–5 mbar) and used without further purification.

To a round-bottom flask containing the dried oil (~0.33 mmol, 1.0 equiv) was added anhydrous MeOH (0.5 mL) which was lightly heated until the oil was completely dissolved. Then 4 N HCl in dioxane (2.5 mL, 10 mmol, 30 equiv) was added dropwise under argon. The Boc-protecting group was removed and a precipitate sometimes formed. With light heating, anhydrous MeOH was added dropwise until most of the solid was dissolved. The mixture was heated at reflux for 4 h under argon and then cooled to 25 °C. NaHCO₃ powder was added to quench the acid. The reaction mixture was partitioned between 5% MeOH in EtOAc (25 mL) and H₂O (20 mL). The aqueous layer was extracted with 5% MeOH in EtOAc (3 x 20 mL). Combined organic fractions were concentrated *in vacuo*. The crude residue was purified by flash column chromatography on silica gel.

Modification to imidazole synthesis (used in 2 cases)

Modified Procedure for the Synthesis of 8k,v. To a suspension of aminomalononitrile *p*-toluenesulfonate (2.0 g, 7.9 mmol, 1.0 equiv) in 70 mL of THF at 25 °C was added NEt₃ (1.4 mL, 10.0 mmol, 1.27 equiv) in one portion. The mixture was stirred briefly until it became a homogeneous solution. To this solution was added 1,1,1-trimethoxypentane (2.18 g, 13.4 mmol, 1.7 equiv), and the solution was heated at reflux for 3.5 h under argon before being cooled to 25 °C. NEt₃ (25 mL) and primary amine were added sequentially, and the mixture was heated at reflux for 18 h under argon then allowed to cool to 25 °C. Solvent was removed *in vacuo* and the crude residue was redissolved in CH₂Cl₂ (50 mL) and washed with saturated aqueous Na₂CO₃ (20 mL). The organics were separated and the aqueous layer was extracted with CH₂Cl₂ (3 x 20 mL). Combined organic fractions were concentrated *in vacuo*. The crude residue was purified by flash column chromatography on silica gel. The product was dried for at least 24 h *in vacuo* (3–5 mbar).

Intermediates

Methyl 4-bromo-5-nitrobenzoate: To a round bottom flask containing 4-bromobenzoic acid (10 g, 50 mmol, 1 equiv) was added H₂SO₄ (70 mL) and cooled to 0°C in an ice bath. Nitric acid (6.3 mL, 100 mmol, 2 equiv) was added drop wise and the reaction mixture was allowed to warm to 25°C over 16 h. After which the reaction was poured onto ice, filtered and washed with H₂O to afford the title compound as a white solid that was carried on directly to the next step.

To a round bottom flask containing the crude 4-bromo-3-nitrobenzoic acid in MeOH (200 mL) was added H₂SO₄ (cat.) and refluxed for 8 h. After which the reaction was diluted with H₂O and filtered to afford the title compound as a faint yellow solid in 81% yield: ¹H (DMSO-*d*₆, 400 MHz) δ 8.48 – 8.44 (m, 1H), 8.09 – 8.04 (m, 2H), 3.90 (s, 3H).

Methyl 3-amino-4-bromobenzoate: To a solution of **methyl 4-bromo-5-nitrobenzoate** (9.1 mmol, 1 equiv) in MeOH (23 mL, 0.4 M) was added iron powder (27.3 mmol, 3 equiv), 0.2M HCl (9 mL, 1M), and was heated at 80 °C for 1 h. The reaction was allowed to cool to 25 °C, filtered through Celite, diluted with H₂O (20 mL) and extracted with EtOAc (3 x 100 mL). The combined organic fractions were concentrated *in vacuo* and the crude residue was purified by silica gel column chromatography to give the product as a white solid in 86% yield: ¹H (CDCl₃, 400 MHz) δ 7.49 – 7.39 (m, 2H), 7.01 (dd, *J* = 2.3, 8.2 Hz, 1H), 5.66 (s, 2H), 3.80 (s, 3H).

Methyl 3-amino-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate: To a vial containing **methyl 4-bromo-5-nitrobenzoate** (600 mg, 2.7 mmol, 1 equiv), Bis(pinacolato)diboron (750 mg, 0.6 mmol, 1.1 equiv), Pd(dppf)Cl₂ (110 mg, 0.2 mmol, 0.05 equiv), and KOAc (800 mg, 8 mmol, 3 equiv) was added toluene (50 mL, 0.1 M) and heated at 80 °C for 16 h. After which the reaction was filtered through Celite and the residue was washed with EtOAc (100 mL). The organic layer was concentrated and the crude residue was flashed through a silica plug with 40:60 EtOAc/Hex and carried on directly to the next step.

5-amino-2-butyl-1-(3-hydroxypropyl)-1H-imidazole-4-carbonitrile (8f). The title compound was prepared according to the general procedure using 3-amino-1-propanol as an off white solid in 63.2% yield: ¹H NMR (DMSO-*d*₆, 400 MHz) δ 0.83 (t, *J* = 7.4 Hz, 3H), 1.28 (hex, *J* = 7.4 Hz, 2H), 1.51 (quin, *J* = 7.4 Hz, 2H), 1.65 (quin, *J* = 6.2 Hz, 2H), 2.45 (t, *J* = 7.4 Hz, 2H), 3.34 (dd, *J* = 5.1, 5.9 Hz, 2H), 3.74 (t, *J* = 7.4 Hz, 2H), 4.83 (t, *J* = 5.1 Hz, 1H), 5.95 (s, 2H).

5-amino-2-butyl-1-(4-hydroxybutyl)-1H-imidazole-4-carbonitrile (8g). The title compound was prepared according to the general procedure using 4-amino-1-butanol as an off white solid in 55.9% yield: ¹H NMR (CDCl₃, 400 MHz) δ 0.94 (t, *J* = 7.4 Hz, 3H), 1.40 (hex, *J* = 7.4 Hz, 2H), 1.61 (quin, *J* = 6.3 Hz, 2H), 1.70 (quin, *J* = 7.4 Hz, 2H), 1.82 (quin, *J* = 7.4 Hz, 2H), 2.50 (br s, 1H), 2.54 (t, *J* = 7.4 Hz, 2H), 3.77 (t, *J* = 5.5 Hz, 2H), 3.83 (t, *J* = 7.4 Hz, 1H), 4.40 (s, 2H).

5-amino-2-butyl-1-(2-(methylthio)ethyl)-1H-imidazole-4-carbonitrile (8h). The title compound was prepared according to the general procedure using 2-(methylthio)ethylamine as a tan solid in 78.5% yield: ¹H NMR (DMSO-*d*₆, 400 MHz) δ 0.86 (t, *J* = 7.4 Hz, 3H), 1.31 (hex, *J* = 7.4 Hz, 2H), 1.56 (quin, *J* = 7.4 Hz, 2H), 2.02 (s, 3H), 2.50 (t, *J* = 7.4 Hz, 2H), 2.65 (t, *J* = 7.4 Hz, 2H), 3.91 (t, *J* = 7.4 Hz, 2H), 6.01 (s, 2H).

5-amino-2-butyl-1-(3-(methylthio)propyl)-1H-imidazole-4-carbonitrile (8i). The title compound was prepared according to the general procedure using 3-(methylthio)propylamine as a tan solid in 56.0% yield: ¹H NMR (DMSO-*d*₆, 400 MHz) δ 0.87 (t, *J* = 7.4 Hz, 3H), 1.32 (hex, *J* = 7.4 Hz, 2H), 1.56 (quin, *J* = 7.4 Hz, 2H), 1.77 (quin, *J* = 7.4 Hz, 2H), 2.04 (s, 3H), 2.38–2.48 (m, 4H), 3.78 (t, *J* = 7.4 Hz, 2H), 6.03 (s, 2H).

5-amino-2-butyl-1-(2-(ethylthio)ethyl)-1H-imidazole-4-carbonitrile (8j). The title compound was prepared according to the general procedure using 2-(ethylthio)ethylamine as a tan solid in 66.2% yield: ¹H NMR (CDCl₃, 400 MHz) δ 0.94 (t, *J* = 7.4 Hz, 3H), 1.22 (t, *J* = 7.4 Hz, 3H), 1.41 (hex, *J* = 7.4 Hz, 2H), 1.75 (quin, *J* = 7.8 Hz, 2H), 2.46 (q, *J* = 7.4 Hz, 2H), 2.55 (t, *J* = 7.4 Hz, 2H), 2.81 (t, *J* = 6.3 Hz, 2H), 3.97 (t, *J* = 6.3 Hz, 2H), 4.18 (s, 2H).

5-amino-2-butyl-1-(2-methoxy-2-oxoethyl)-1H-imidazole-4-carbonitrile (8k). The title compound was prepared according to the modified procedure using glycine methyl ester HCl as an amber solid in 47.5% yield: ¹H NMR (CDCl₃, 400 MHz) δ 0.91 (t, *J* = 7.4 Hz, 3H), 1.36 (hex, *J* = 7.4 Hz, 2H), 1.64 (quin, *J* = 7.8 Hz, 2H), 2.52 (t, *J* = 7.4 Hz, 2H), 3.82 (s, 3H), 3.84 (br s, 2H), 4.50 (s, 2H).

5-amino-2-butyl-1-(3-methoxy-3-oxopropyl)-1H-imidazole-4-carbonitrile (8l). To a suspension of aminomalononitrile *p*-toluenesulfonate (0.52 g, 2.0 mmol, 1.0 equiv) in 10 mL of THF at 25 °C was added NEt₃ (0.5 mL, 3.6 mmol, 1.8 equiv) in one portion. The mixture was stirred briefly until it became a homogeneous solution. To this solution was added 1,1,1-trimethoxypropane (0.59 g, 3.6 mmol, 1.8 equiv), and the solution was heated at reflux for 3.5 h

under argon before being cooled to 25 °C. A solution of NEt₃ (0.5 mL, 3.6 mmol, 1.8 equiv) in MeCN (10 mL) was then added to the reaction followed immediately by the addition of a suspension of β-alanine methyl ester (prepared from HCl salt) (0.82 g, 8.0 mmol, 4.0 equiv) in H₂O (10 mL). The mixture was stirred for 18 h under argon at 25 °C. Most of the organic solvents were removed *in vacuo* and the crude residue was redissolved in CH₂Cl₂ (25 mL) and washed with saturated aqueous Na₂CO₃ (15 mL). The organics were separated and the aqueous layer was extracted with CH₂Cl₂ (3 x 15 mL). Combined organic fractions were concentrated *in vacuo*. The crude residue was purified by flash column chromatography on silica gel and dried for 24 h *in vacuo* (3–5 mbar) to give a tan solid in 28.2% yield: ¹H NMR (CDCl₃, 400 MHz) δ 0.94 (t, *J* = 7.4 Hz, 3H), 1.41 (hex, *J* = 7.4 Hz, 2H), 1.73 (quin, *J* = 7.4 Hz, 2H), 2.52 (t, *J* = 7.8 Hz, 2H), 2.76 (t, *J* = 5.8 Hz, 2H), 3.72 (s, 3H), 4.03 (t, *J* = 5.8 Hz, 2H), 4.54 (s, 2H).

5-amino-2-butyl-1-(4-methoxy-4-oxobutyl)-1*H*-imidazole-4-carbonitrile (8m). To a suspension of aminomalononitrile *p*-toluenesulfonate (2.0 g, 7.9 mmol, 1.0 equiv) in THF (70 mL) at 25 °C was added NEt₃ (1.8 mL, 12.9 mmol, 1.63 equiv) in one portion. The mixture was stirred briefly until it became a homogeneous solution. To this solution was added 1,1,1-trimethoxypentane (2.35 g, 14.5 mmol, 1.84 equiv), and the solution was heated at reflux for 3.5 h under argon before cooling to 65 °C. Next, more NEt₃ (1.8 mL, 12.9 mmol, 1.63 equiv) was added, immediately followed by methyl 4-aminobutanoate (prepared from HCl salt) (3.0 g, 25.6 mmol, 3.24 equiv) suspended in H₂O (15 mL). The reaction was allowed to cool to 25 °C and stirred for 18 h. Most of the THF and NEt₃ was removed *in vacuo* and the crude residue was redissolved in CH₂Cl₂ (50 mL) and washed with saturated aqueous Na₂CO₃ (20 mL). The organics were separated and the aqueous layer was extracted with CH₂Cl₂ (3 x 20 mL). Combined organic fractions were concentrated *in vacuo*. The crude residue was purified by flash column chromatography on silica gel and dried for 48 h *in vacuo* (3–5 mbar) to give a tan solid in 22.3% yield: ¹H NMR (CDCl₃, 400 MHz) δ 0.94 (t, *J* = 7.4 Hz, 3H), 1.40 (hex, *J* = 7.4 Hz, 2H), 1.70 (quin, *J* = 7.4 Hz, 2H), 1.95 (quin, *J* = 7.8 Hz, 2H), 2.42 (t, *J* = 6.2 Hz, 2H), 2.53 (t, *J* = 7.8 Hz, 2H), 3.73 (s, 3H), 3.76 (t, *J* = 7.8 Hz, 2H), 4.45 (s, 2H).

5-amino-2-butyl-1-(5-methoxy-5-oxopentyl)-1*H*-imidazole-4-carbonitrile (8n). To a suspension of aminomalononitrile *p*-toluenesulfonate (3.0 g, 11.8 mmol, 1.0 equiv) in THF (70 mL) at 25 °C was added NEt₃ (2.6 mL, 18.6 mmol, 1.58 equiv) in one portion. The mixture was stirred briefly until it became a homogeneous solution. To this solution was added 1,1,1-trimethoxypentane (3.6 g, 22.2 mmol, 1.88 equiv), and the solution was heated at reflux for 3.0 h under argon before cooling to 65 °C. Next, more NEt₃ (2.6 mL, 18.6 mmol, 1.58 equiv) was added, immediately followed by methyl 5-aminopentanoate HCl (2.0 g, 11.9 mmol, 1.01 equiv) suspended in H₂O (20 mL). The reaction heated at 60 °C and stirred for 18 h. Most of the THF and NEt₃ was removed *in vacuo* and the crude residue was redissolved in CH₂Cl₂ (60 mL) and washed with saturated aqueous Na₂CO₃ (20 mL). The organics were separated and the aqueous layer was extracted with CH₂Cl₂ (3 x 20 mL). Combined organic fractions were concentrated *in vacuo*. The crude residue was purified by flash column chromatography on silica gel and dried for 48 h *in vacuo* (3–5 mbar) to give a tan solid in 26.5% yield: ¹H NMR (CDCl₃, 400 MHz) δ 0.89 (t, *J* = 7.4 Hz, 3H), 1.35 (hex, *J* = 7.4 Hz, 2H), 1.60–1.75 (m, 6H), 2.34 (t, *J* = 7.0 Hz, 2H), 2.49 (t, *J* = 7.4 Hz, 2H), 3.64 (s, 3H), 3.72 (t, *J* = 7.0 Hz, 2H), 4.31 (s, 2H).

5-amino-2-butyl-1-(6-methoxy-6-oxohexyl)-1*H*-imidazole-4-carbonitrile (8o). To a suspension of aminomalononitrile *p*-toluenesulfonate (2.0 g, 7.9 mmol, 1.0 equiv) in THF (40 mL) at 25 °C was added NEt₃ (1.8 mL, 12.9 mmol, 1.63 equiv) in one portion. The mixture was stirred briefly until it became a homogeneous solution. To this solution was added 1,1,1-trimethoxypentane (2.29 g, 14.1 mmol, 1.79 equiv), and the solution was heated at reflux for 3.5

h under argon before cooling to 65 °C. Next, more THF (50 mL) and NEt₃ (1.8 mL, 12.9 mmol, 1.63 equiv) was added, immediately followed by methyl 6-aminohexanoate (prepared from HCl salt) (1.75 g, 12.1 mmol, 1.53 equiv). The reaction was heated back to reflux and stirred for 18 h. Solvent was removed *in vacuo* and the crude residue was redissolved in CH₂Cl₂ (60 mL) and washed with saturated aqueous Na₂CO₃ (20 mL). The organics were separated and the aqueous layer was extracted with CH₂Cl₂ (3 x 20 mL). Combined organic fractions were concentrated *in vacuo*. The crude residue was purified by flash column chromatography on silica gel and dried for 72 h *in vacuo* (3–5 mbar) to give a brown oil in 18.9% yield: ¹H NMR (CDCl₃, 400 MHz) δ 0.94 (t, *J* = 7.4 Hz, 3H), 1.30–1.45 (m, 4H), 1.60–1.75 (m, 6H), 2.34 (t, *J* = 7.0 Hz, 2H), 2.53 (t, *J* = 7.4 Hz, 2H), 3.67 (s, 3H), 3.72 (t, *J* = 7.0 Hz, 2H), 4.31 (s, 2H).

5-amino-1-((*N*-Boc)2-aminoethyl)-2-butyl-1H-imidazole-4-carbonitrile (8p). The title compound was prepared according to the general procedure using *N*-Boc-1,2-diaminoethane as an off-white solid in 50.2% yield: ¹H NMR (CDCl₃, 400 MHz) δ 0.92 (t, *J* = 7.4 Hz, 3H), 1.38 (hex, *J* = 7.8 Hz, 2H), 1.43 (s, 9H), 1.67 (quin, *J* = 7.8 Hz, 2H), 2.50 (t, *J* = 7.8 Hz, 2H), 3.29 (q, *J* = 6.6 Hz, 2H), 3.86 (t, *J* = 6.6 Hz, 2H), 4.43 (br s, 2H), 4.89 (br s, 1H).

5-amino-1-((*N*-Boc)3-aminopropyl)-2-butyl-1H-imidazole-4-carbonitrile (8q). The title compound was prepared according to the general procedure using *N*-Boc-1,3-diaminopropane as a tan waxy solid in 48.7% yield: ¹H NMR (CDCl₃, 400 MHz) δ 0.93 (t, *J* = 7.4 Hz, 3H), 1.39 (hex, *J* = 7.4 Hz, 2H), 1.44 (s, 9H), 1.70 (quin, *J* = 7.4 Hz, 2H), 1.86 (quin, *J* = 6.3 Hz, 2H), 2.51 (t, *J* = 7.4 Hz, 2H), 3.16 (q, *J* = 5.9 Hz, 2H), 3.79 (t, *J* = 7.4 Hz, 2H), 4.19 (br s, 2H), 4.72 (br s, 1H).

5-amino-1-((*N*-Boc)4-aminobutyl)-2-butyl-1H-imidazole-4-carbonitrile (8r). The title compound was prepared according to the general procedure using *N*-Boc-1,4-diaminobutane as a tan solid in 30.0% yield: ¹H NMR (CDCl₃, 400 MHz) δ 0.93 (t, *J* = 7.4 Hz, 3H), 1.39 (hex, *J* = 7.4 Hz, 2H), 1.43 (s, 9H), 1.56 (quin, *J* = 5.8 Hz, 2H), 1.60–1.75 (m, 4H), 2.52 (t, *J* = 7.4 Hz, 2H), 3.22 (q, *J* = 5.8 Hz, 2H), 3.79 (t, *J* = 7.8 Hz, 2H), 4.55 (br s, 2H), 4.77 (br s, 1H).

5-amino-1-((*N*-Boc)5-aminopentyl)-2-butyl-1H-imidazole-4-carbonitrile (8s). The title compound was prepared according to the general procedure using *N*-Boc-1,5-diaminopropane as a tan solid in 26.7% yield: ¹H NMR (CDCl₃, 400 MHz) δ 0.93 (t, *J* = 7.4 Hz, 3H), 1.32–1.41 (m, 4H), 1.43 (s, 9H), 1.51 (quin, *J* = 6.7 Hz, 2H), 1.63–1.78 (m, 4H), 2.52 (t, *J* = 7.8 Hz, 2H), 3.14 (q, *J* = 6.3 Hz, 2H), 3.67 (t, *J* = 8.2 Hz, 2H), 4.29 (br s, 2H), 4.66 (br s, 1H).

5-amino-1-(4-(*N*-Boc-aminomethyl)benzyl)-2-butyl-1H-imidazole-4-carbonitrile (8t). The title compound was prepared according to the general procedure using 1-(*N*-Boc-aminomethyl)-4-(aminomethyl)benzene as an off white solid in 43.9% yield: ¹H NMR (DMSO-*d*₆, 400 MHz) δ 0.75 (t, *J* = 7.4 Hz, 3H), 1.19 (hex, *J* = 7.4 Hz, 2H), 1.35 (s, 9H), 1.39 (quin, *J* = 7.4 Hz, 2H), 2.32 (t, *J* = 7.4 Hz, 2H), 4.05 (d, *J* = 5.9 Hz, 2H), 5.01 (s, 2H), 6.11 (s, 2H), 6.95 (d, *J* = 7.8 Hz, 2H), 7.17 (d, *J* = 7.8 Hz, 2H), 7.32 (s, 1H).

5-amino-2-butyl-1-(4-(methoxycarbonyl)benzyl)-1H-imidazole-4-carbonitrile (8u). The title compound was prepared according to the general procedure using methyl 4-(aminomethyl)benzoate (prepared from HCl salt) as a tan solid in 35.3% yield: ¹H NMR (CDCl₃, 400 MHz) δ 0.86 (t, *J* = 7.4 Hz, 3H), 1.33 (hex, *J* = 7.4 Hz, 2H), 1.63 (quin, *J* = 7.4 Hz, 2H), 2.54 (t, *J* = 7.8 Hz, 2H), 3.91 (s, 3H), 3.93 (br s, 2H), 5.07 (s, 2H), 7.10 (d, *J* = 8.2 Hz, 2H), 8.03 (d, *J* = 8.2 Hz, 2H).

5-amino-2-butyl-1-(4-(methoxycarbonyl)2-phenylethyl)-1H-imidazole-4-carbonitrile

(8v). The title compound was prepared according to the modified procedure using methyl 4-(2-aminoethyl)benzoate HCl as a waxy amber solid in 24.8% yield: ^1H NMR (CDCl_3 , 400 MHz) δ 0.88 (t, J = 7.4 Hz, 3H), 1.32 (hex, J = 7.4 Hz, 2H), 1.61 (quin, J = 7.4 Hz, 2H), 2.33 (t, J = 7.8 Hz, 2H), 3.02 (t, J = 6.6 Hz, 2H), 3.56 (br s, 2H), 3.90 (s, 3H), 4.00 (t, J = 6.7 Hz, 2H), 7.10 (d, J = 7.4 Hz, 2H), 7.97 (d, J = 7.0 Hz, 2H).

2-butyl-1-(3-hydroxypropyl)-5-iodo-1*H*-imidazole-4-carbonitrile (9f). The title compound was prepared according to the general procedure using **8f** as a waxy orange solid in 11.5% yield: ^1H NMR (CDCl_3 , 400 MHz) δ 0.94 (t, J = 7.4 Hz, 3H), 1.40 (hex, J = 7.4 Hz, 2H), 1.73 (quin, J = 7.4 Hz, 2H), 1.93 (quin, J = 6.7 Hz, 2H), 2.77 (t, J = 7.8 Hz, 2H), 3.70 (t, J = 5.5 Hz, 2H), 4.08 (t, J = 7.4 Hz, 1H).

2-butyl-1-(4-hydroxybutyl)-5-iodo-1*H*-imidazole-4-carbonitrile (9g). The title compound was prepared according to the general procedure using **8g** as a waxy orange solid in 8.1% yield: ^1H NMR (CDCl_3 , 400 MHz) δ 0.94 (t, J = 7.4 Hz, 3H), 1.40 (hex, J = 7.4 Hz, 2H), 1.63 (quin, J = 6.7 Hz, 2H), 1.73 (quin, J = 7.4 Hz, 2H), 1.80 (quin, J = 8.2 Hz, 2H), 2.73 (t, J = 7.8 Hz, 2H), 3.71 (t, J = 6.2 Hz, 2H), 3.97 (t, J = 7.8 Hz, 1H).

2-butyl-5-iodo-1-(2-(methylthio)ethyl)-1*H*-imidazole-4-carbonitrile (9h). The title compound was prepared according to the general procedure using **8h** as a brown waxy solid in 20.6% yield: ^1H NMR (CDCl_3 , 400 MHz) δ 0.94 (t, J = 7.4 Hz, 3H), 1.41 (hex, J = 7.4 Hz, 2H), 1.74 (quin, J = 7.4 Hz, 2H), 2.14 (s, 3H), 2.70–2.80 (m, 4H), 4.11 (t, J = 7.4 Hz, 2H).

2-butyl-5-iodo-1-(3-(methylthio)propyl)-1*H*-imidazole-4-carbonitrile (9i). The title compound was prepared according to the general procedure using **8i** as a brown waxy solid in 19.1% yield: ^1H NMR (CDCl_3 , 400 MHz) δ 0.96 (t, J = 7.4 Hz, 3H), 1.41 (hex, J = 7.4 Hz, 2H), 1.75 (quin, J = 7.8 Hz, 2H), 1.98 (quin, J = 7.0 Hz, 2H), 2.14 (s, 3H), 2.55 (t, J = 6.7 Hz, 2H), 2.75 (t, J = 7.4 Hz, 2H), 4.05 (t, J = 7.8 Hz, 2H).

2-butyl-5-iodo-1-(2-(ethylthio)ethyl)-1*H*-imidazole-4-carbonitrile (9j). The title compound was prepared according to the general procedure using **8j** as a brown waxy solid in 16.8% yield: ^1H NMR (CDCl_3 , 400 MHz) δ 0.94 (t, J = 7.4 Hz, 3H), 1.26 (t, J = 7.4 Hz, 3H), 1.40 (hex, J = 7.4 Hz, 2H), 1.73 (quin, J = 7.4 Hz, 2H), 2.54 (q, J = 7.4 Hz, 2H), 2.72–2.82 (m, 4H), 3.97 (t, J = 7.4 Hz, 2H).

2-butyl-5-iodo-1-(2-methoxy-2-oxoethyl)-1*H*-imidazole-4-carbonitrile (9k). The title compound was prepared according to the general procedure using **8k** as a copper waxy solid in 33.9% yield: ^1H NMR (CDCl_3 , 400 MHz) δ 0.92 (t, J = 7.4 Hz, 3H), 1.38 (hex, J = 7.4 Hz, 2H), 1.70 (quin, J = 7.4 Hz, 2H), 2.66 (t, J = 7.4 Hz, 2H), 3.81 (s, 3H), 4.68 (s, 2H).

2-butyl-5-iodo-1-(3-methoxy-3-oxopropyl)-1*H*-imidazole-4-carbonitrile (9l). The title compound was prepared according to the general procedure using **8l** as a copper waxy solid in 26.3% yield: ^1H NMR (CDCl_3 , 400 MHz) δ 0.96 (t, J = 7.4 Hz, 3H), 1.42 (hex, J = 7.4 Hz, 2H), 1.74 (quin, J = 7.4 Hz, 2H), 2.74 (t, J = 7.4 Hz, 2H), 2.78 (t, J = 7.8 Hz, 2H), 3.73 (s, 3H), 4.25 (t, J = 7.4 Hz, 2H).

2-butyl-5-iodo-1-(4-methoxy-4-oxobutyl)-1*H*-imidazole-4-carbonitrile (9m). The title compound was prepared according to the general procedure using **8m** as a copper oil in 35.8% yield: ^1H NMR (CDCl_3 , 400 MHz) δ 0.95 (t, J = 7.4 Hz, 3H), 1.42 (hex, J = 7.4 Hz, 2H), 1.75 (quin, J = 7.4 Hz, 2H), 2.00 (quin, J = 7.8 Hz, 2H), 2.43 (t, J = 7.4 Hz, 2H), 2.77 (t, J = 7.8 Hz, 2H), 3.72 (s, 3H), 4.00 (t, J = 7.8 Hz, 2H).

2-butyl-5-iodo-1-(5-methoxy-5-oxopentyl)-1H-imidazole-4-carbonitrile (9n). The title compound was prepared according to the general procedure using **8n** as a copper oil in 36.4% yield: ^1H NMR (CDCl_3 , 400 MHz) δ 0.86 (t, J = 7.4 Hz, 3H), 1.32 (hex, J = 7.4 Hz, 2H), 1.58–1.72 (m, 6H), 2.31 (t, J = 6.2 Hz, 2H), 2.64 (t, J = 7.0 Hz, 2H), 3.59 (s, 3H), 3.87 (t, J = 7.0 Hz, 2H).

2-butyl-5-iodo-1-(6-methoxy-6-oxohexyl)-1H-imidazole-4-carbonitrile (9o). The title compound was prepared according to the general procedure using **8o** as a copper oil in 24.8% yield: ^1H NMR (CDCl_3 , 400 MHz) δ 0.95 (t, J = 7.4 Hz, 3H), 1.30–1.50 (m, 4H), 1.60–1.80 (m, 6H), 2.34 (t, J = 7.4 Hz, 2H), 2.71 (t, J = 7.4 Hz, 2H), 3.67 (s, 3H), 3.90 (t, J = 7.8 Hz, 2H).

1-((N-Boc)2-aminoethyl)-2-butyl-5-iodo-1H-imidazole-4-carbonitrile (9p). The title compound was prepared according to the general procedure using **8p** as a tan waxy solid in 28.8% yield: ^1H NMR (CDCl_3 , 400 MHz) δ 0.94 (t, J = 7.4 Hz, 3H), 1.40 (hex, J = 7.8 Hz, 2H), 1.43 (s, 9H), 1.73 (quin, J = 7.4 Hz, 2H), 2.74 (t, J = 7.8 Hz, 2H), 3.40 (q, J = 5.9 Hz, 2H), 4.09 (t, J = 5.9 Hz, 2H), 4.77 (br s, 1H).

1-((N-Boc)3-aminopropyl)-2-butyl-5-iodo-1H-imidazole-4-carbonitrile (9q). The title compound was prepared according to the general procedure using **8q** as a tan waxy solid in 18.2% yield: ^1H NMR (CDCl_3 , 400 MHz) δ 0.95 (t, J = 7.4 Hz, 3H), 1.43 (hex, J = 7.4 Hz, 2H), 1.46 (s, 9H), 1.74 (quin, J = 7.4 Hz, 2H), 1.87 (quin, J = 6.3 Hz, 2H), 2.72 (t, J = 7.8 Hz, 2H), 3.23 (q, J = 6.3 Hz, 2H), 3.76 (t, J = 7.4 Hz, 2H), 4.65 (br s, 1H).

1-((N-Boc)4-aminobutyl)-2-butyl-5-iodo-1H-imidazole-4-carbonitrile (9r). The title compound was prepared according to the general procedure using **8r** as a copper solid in 17.0% yield: ^1H NMR (CDCl_3 , 400 MHz) δ 0.93 (t, J = 7.4 Hz, 3H), 1.38 (hex, J = 7.4 Hz, 2H), 1.42 (s, 9H), 1.54 (quin, J = 7.4 Hz, 2H), 1.60–1.78 (m, 4H), 2.70 (t, J = 7.4 Hz, 2H), 3.16 (q, J = 6.6 Hz, 2H), 3.92 (t, J = 7.8 Hz, 2H), 4.67 (br s, 1H).

1-((N-Boc)5-aminopentyl)-2-butyl-5-iodo-1H-imidazole-4-carbonitrile (9s). The title compound was prepared according to the general procedure using **8s** as a copper solid in 15.5% yield: ^1H NMR (CDCl_3 , 400 MHz) δ 0.93 (t, J = 7.4 Hz, 3H), 1.33–1.42 (m, 4H), 1.42 (s, 9H), 1.53 (quin, J = 7.0 Hz, 2H), 1.63–1.78 (m, 4H), 2.70 (t, J = 7.4 Hz, 2H), 3.12 (q, J = 6.7 Hz, 2H), 3.88 (t, J = 7.8 Hz, 2H), 4.60 (br s, 1H).

1-(4-(N-Boc-aminomethyl)benzyl)-2-butyl-5-iodo-1H-imidazole-4-carbonitrile (9t). The title compound was prepared according to the general procedure using **8t** as an amber solid in 47.6% yield: ^1H NMR (CDCl_3 , 400 MHz) δ 0.86 (t, J = 7.4 Hz, 3H), 1.31 (hex, J = 7.4 Hz, 2H), 1.45 (s, 9H), 1.63 (quin, J = 7.4 Hz, 2H), 2.63 (t, J = 7.4 Hz, 2H), 4.31 (d, J = 5.5 Hz, 2H), 4.89 (br s, 1H), 5.16 (s, 2H), 6.92 (d, J = 8.2 Hz, 2H), 7.27 (d, J = 7.8 Hz, 2H).

2-butyl-5-iodo-1-(4-(methoxycarbonyl)benzyl)-1H-imidazole-4-carbonitrile (9u*). The title compound was prepared according to the general procedure using **8u** as a waxy amber solid in 37.9% yield: ^1H NMR (CDCl_3 , 400 MHz) δ 0.87 (t, J = 7.4 Hz, 3H), 1.33 (hex, J = 7.4 Hz, 2H), 1.64 (quin, J = 7.4 Hz, 2H), 2.64 (t, J = 7.8 Hz, 2H), 3.93 (s, 3H), 5.24 (s, 2H), 7.04 (d, J = 8.2 Hz, 2H), 8.05 (d, J = 8.2 Hz, 2H).

2-butyl-1-(4-(hydroxycarbonyl)benzyl)-5-iodo-1H-imidazole-4-carbonitrile (9u). To a solution of **9u*** (0.65 g, 1.54 mmol, 1.0 equiv) in THF (30 mL) was added 0.5 N aqueous KOH

(10 mL). This mixture was stirred for 40 h at 25 °C. The solution was acidified to a pH of 6 by adding concentrated aqueous HCl dropwise. A fine white precipitate began to form and the THF was removed *in vacuo*. The solid was filtered and washed with cold H₂O (10 mL) and EtOAc (10 mL) and dried *in vacuo* to give a light yellow solid in 73.2% yield: ¹H NMR (DMSO-*d*₆, 400 MHz) δ 0.75 (t, *J* = 7.4 Hz, 3H), 1.21 (hex, *J* = 7.4 Hz, 2H), 1.46 (quin, *J* = 7.4 Hz, 2H), 2.62 (t, *J* = 7.8 Hz, 2H), 5.36 (s, 2H), 7.08 (d, *J* = 7.8 Hz, 2H), 7.90 (d, *J* = 7.0 Hz, 2H), 12.97 (s, 2H).

2-butyl-5-iodo-1-(4-(methoxycarbonyl)2-phenylethyl)-1H-imidazole-4-carbonitrile (9v*). The title compound was prepared according to the general procedure using **8v** as a waxy amber solid in 25.9% yield: ¹H NMR (CDCl₃, 400 MHz) δ 0.87 (t, *J* = 7.4 Hz, 3H), 1.29 (hex, *J* = 7.4 Hz, 2H), 1.60 (quin, *J* = 7.4 Hz, 2H), 2.38 (t, *J* = 7.4 Hz, 2H), 3.03 (t, *J* = 7.0 Hz, 2H), 3.91 (s, 3H), 4.13 (t, *J* = 7.1 Hz, 2H), 7.11 (d, *J* = 7.8 Hz, 2H), 7.97 (d, *J* = 7.8 Hz, 2H).

2-butyl-1-(4-(hydroxycarbonyl)2-phenylethyl)-5-iodo-1H-imidazole-4-carbonitrile (9v). To a solution of **9v*** (0.11 g, 0.25 mmol, 1.0 equiv) in THF (20 mL) was added 0.3 N aqueous KOH (10 mL). This mixture was stirred for 72 h at 25 °C. The solution was acidified to a pH of 6 by adding concentrated aqueous HCl dropwise. A fine white precipitate began to form and the THF was removed *in vacuo*. The solid was filtered and washed with cold H₂O (10 mL) and dried *in vacuo* to give a light yellow solid in 89.6% yield: ¹H NMR (DMSO-*d*₆, 400 MHz) δ 0.79 (t, *J* = 7.4 Hz, 3H), 1.19 (hex, *J* = 7.4 Hz, 2H), 1.43 (quin, *J* = 7.4 Hz, 2H), 2.38 (t, *J* = 7.8 Hz, 2H), 3.00 (t, *J* = 7.0 Hz, 2H), 4.19 (t, *J* = 7.0 Hz, 2H), 7.18 (d, *J* = 7.8 Hz, 2H), 7.83 (d, *J* = 8.2 Hz, 2H), 12.87 (s, 2H).

2-butyl-5-iodo-1-(5-hydroxy-5-oxopentyl)-1H-imidazole-4-carbonitrile (9w). To a solution of **9n** (190 mg, 0.49 mmol, 1.0 equiv) in THF (20 mL) was added a solution of potassium hydroxide (110 mg, 1.95 mmol, 4.0 equiv) in H₂O (20 mL). The mixture was left to stir for 72 h at 25 °C. The solution was acidified to a pH of 6 by adding 1.5 N aqueous HCl dropwise. The color of the mixture turned from light orange to light yellow. A yellow solid slowly precipitated out of the solution. The mixture was filtered and the product was washed with cold water and dried *in vacuo* to give a light yellow solid in 95.5% yield: ¹H NMR (CDCl₃, 400 MHz) δ 0.95 (t, *J* = 7.4 Hz, 3H), 1.41 (hex, *J* = 7.4 Hz, 2H), 1.67–1.82 (m, 6H), 2.44 (t, *J* = 7.0 Hz, 2H), 2.73 (t, *J* = 7.4 Hz, 2H), 3.94 (t, *J* = 7.2 Hz, 2H).

Imidazoquinolines

4-amino-2-butyl-1-(2-(ethanoyloxy)propyl)-7-methoxycarbonyl-1H-imidazo[4,5-c]quinoline (12c). **12b** (56.0 mg, 0.16 mmol, 1.0 equiv), dicyclohexylcarbodiimide (100 mg, 0.48 mmol, 3.0 equiv), and 4-dimethylaminopyridine (71 mg, 0.58 mmol, 3.6 equiv) were added to a small vial with a stir bar. While stirring, a solution of acetic acid (1.5 mL) in DCM (11 mL) was added in one portion. This mixture was stirred for 1 h at 25 °C. Solvent was removed *in vacuo* and the crude residue was purified by flash column chromatography on silica gel to give a white solid in 31.8% yield: *R*_f = 0.40 (EtOAc); ¹H NMR (CD₃OD, 400 MHz) δ 1.05 (t, *J* = 7.4 Hz, 3H), 1.47 (d, *J* = 6.6 Hz, 3H), 1.55 (hex, *J* = 7.4 Hz, 2H), 1.61 (s, 3H), 1.94 (quin, *J* = 7.4 Hz, 2H), 3.03 (t, *J* = 7.4 Hz, 2H), 3.96 (s, 3H), 4.77 (d, *J* = 5.8 Hz, 2H), 5.41 (tq, *J* = 6.6, 5.8 Hz, 1H), 7.92 (d, *J* = 8.6 Hz, 1H), 8.25 (d, *J* = 8.6 Hz, 1H), 8.36 (s, 1H). HRMS (ESI⁺): calcd C₂₁H₂₇N₄O₄ [M + H]⁺ 399.2027, found 399.2023 (error 1.0 ppm).

4-amino-2-butyl-1-(2-(octanoyloxy)propyl)-7-methoxycarbonyl-1H-imidazo[4,5-c]quinoline (12d): To a vial containing **12b** (44.9 mg, 0.13 mmol, 1 equiv) and octanoic acid (0.63 mL, 0.4 mmol, 3.1 equiv) in CH₂Cl₂ (10 mL, 0.01M) was added dicyclohexylcarbodiimide (82 mg, 0.4 mmol, 3.1 equiv) and 4-dimethylaminopyridine (48 mg, 0.4 mmol, 3.1 equiv) and the

reaction was stirred for 1 h. After which the reaction was filtered, concentrated *in vacuo* and purified by column chromatography to give a white solid in 52% yield: $R_f = 0.67$ (EtOAc); ^1H (CDCl₃, 400 MHz) δ 8.52 (d, $J = 1.8$ Hz, 1H), 8.07 (d, $J = 8.8$ Hz, 1H), 7.95 (dd, $J = 1.8, 8.6$ Hz, 1H), 5.51 (s, 2H), 5.44 – 5.34 (m, 1H), 4.68 (dd, $J = 9.4, 15.5$ Hz, 1H), 4.51 (dd, $J = 4.1, 15.5$ Hz, 1H), 3.97 (s, 3H), 3.04 – 2.89 (m, 2H), 2.11 – 1.89 (m, 4H), 1.54 (hex, $J = 7.6$ Hz, 2H), 1.49 (d, $J = 6.3$ Hz, 3H), 1.38 – 0.99 (m, 13H), 0.89 (t, $J = 7.2$, 3H); ^{13}C (CDCl₃, 100 MHz) δ 172.6, 167.2, 154.8, 151.6, 144.3, 133.0, 129.5, 128.2, 128.0, 122.1, 119.7, 118.5, 68.8, 52.2, 49.5, 34.1, 31.5, 29.8, 28.8, 28.7, 27.3, 24.6, 22.7, 22.5, 17.9, 14.0, 13.9; HRMS (APCI⁺): calcd C₂₇H₃₉N₄O₄ [M + H]⁺ 483.2966, found 483.2989 (error 4.81 ppm).

4-Amino-2-butyl-1-(3-hydroxypropyl)-7-methoxycarbonyl-1H-imidazo[4,5-c]quinoline (12f). The title compound was prepared according to the general procedure using **9f** as a white solid in 17.9% yield over two steps: $R_f = 0.18$ (5:95 MeOH/EtOAc); ^1H NMR (DMSO- d_6 , 400 MHz) δ 0.92 (t, $J = 7.4$ Hz, 3H), 1.41 (hex, $J = 7.4$ Hz, 2H), 1.78 (quin, $J = 7.4$ Hz, 2H), 1.93 (quin, $J = 5.8$ Hz, 2H), 2.93 (t, $J = 7.4$ Hz, 2H), 3.49 (dd, $J = 5.8, 4.7$ Hz, 2H), 3.86 (s, 3H), 4.56 (t, $J = 7$ Hz, 2H), 4.81 (t, $J = 4.7$ Hz, 1H), 6.64 (br s, 2H), 7.71 (d, $J = 8.7$ Hz, 1H), 8.15 (s, 1H), 8.21 (d, $J = 8.7$ Hz, 1H). HRMS (ESI⁺): calcd C₁₉H₂₅N₄O₃ [M + H]⁺ 357.1921, found 357.1925 (error 0.9 ppm).

4-Amino-2-butyl-1-(4-hydroxybutyl)-7-methoxycarbonyl-1H-imidazo[4,5-c]quinoline (12g). The title compound was prepared according to the general procedure using **9g** as a white solid in 37.5% yield over two steps: $R_f = 0.15$ (5:95 MeOH/EtOAc); ^1H NMR (CD₃OD, 400 MHz) δ 1.04 (t, $J = 7.4$ Hz, 3H), 1.53 (hex, $J = 7.4$, 2H), 1.72 (quin, $J = 7.4$ Hz, 2H), 1.90 (quin, $J = 7.8$ Hz, 2H), 2.00 (quin, $J = 7.8$ Hz, 2H), 3.01 (t, $J = 7.4$ Hz, 2H), 3.63 (t, $J = 7.8$ Hz, 2H), 3.95 (s, 3H), 4.61 (t, $J = 7.8$ Hz, 2H), 7.91 (dd, $J = 8.6, 1.6$ Hz, 1H), 8.17 (d, $J = 8.6$ Hz, 1H), 8.34 (d, $J = 1.6$ Hz, 1H). HRMS (ESI⁺): calcd C₂₀H₂₇N₄O₃ [M + H]⁺ 371.2078, found 371.2077 (error 0.1 ppm).

4-amino-2-butyl-1-(2-(methylthio)ethyl)-7-methoxycarbonyl-1H-imidazo[4,5-c]quinoline (12h). The title compound was prepared according to the general procedure using **9h** as an off white solid in 29.3% yield: $R_f = 0.30$ (EtOAc); ^1H NMR (CDCl₃, 400 MHz) δ 1.02 (t, $J = 7.4$ Hz, 3H), 1.52 (hex, $J = 7.4$ Hz, 2H), 1.89 (quin, $J = 7.4$ Hz, 2H), 2.13 (s, 3H), 2.99 (t, $J = 7.4$ Hz, 2H), 3.01 (t, $J = 7.4$ Hz, 2H), 3.97 (s, 3H), 4.68 (t, $J = 7.4$ Hz, 2H), 6.03 (br s, 2H), 7.96 (s, 2H), 8.53 (s, 1H). HRMS (ESI⁺): calcd C₁₉H₂₅N₄O₂S [M + H]⁺ 373.1693, found 373.1708 (error 4.0 ppm).

4-amino-2-butyl-1-(3-(methylthio)propyl)-7-methoxycarbonyl-1H-imidazo[4,5-c]quinoline (12i). The title compound was prepared according to the general procedure using **9i** as an off white solid in 18.3% yield: $R_f = 0.35$ (EtOAc); ^1H NMR (CDCl₃, 400 MHz) δ 1.02 (t, $J = 7.4$ Hz, 3H), 1.52 (hex, $J = 7.4$ Hz, 2H), 1.89 (quin, $J = 7.4$ Hz, 2H), 2.18 (s, 3H), 2.21 (tt, $J = 6.3, 7.8$ Hz, 2H), 2.65 (t, $J = 6.3$ Hz, 2H), 2.96 (t, $J = 7.4$ Hz, 2H), 3.97 (s, 3H), 4.62 (t, $J = 7.8$ Hz, 2H), 5.45 (br s, 2H), 7.94 (dd, $J = 8.6, 1.6$ Hz, 1H), 8.11 (d, $J = 8.6$ Hz, 1H), 8.52 (d, $J = 1.6$ Hz, 1H). HRMS (ESI⁺): calcd C₂₀H₂₇N₄O₂S [M + H]⁺ 387.1849, found 387.1851 (error 0.5 ppm).

4-amino-2-butyl-1-(2-(ethylthio)ethyl)-7-methoxycarbonyl-1H-imidazo[4,5-c]quinoline (12j). The title compound was prepared according to the general procedure using **9j** as an off white solid in 37.6% yield: $R_f = 0.35$ (EtOAc); ^1H NMR (CDCl₃, 400 MHz) δ 1.01 (t, $J = 7.4$ Hz, 3H), 1.24 (t, $J = 7.4$ Hz, 3H), 1.52 (hex, $J = 7.4$ Hz, 2H), 1.88 (quin, $J = 7.4$ Hz, 2H), 2.54 (q, $J = 7.4$ Hz, 2H), 2.97 (t, $J = 7.4$ Hz, 2H), 3.02 (t, $J = 7.4$ Hz, 2H), 3.96 (s, 3H), 4.65 (t, $J = 7.4$ Hz, 2H), 5.53 (br s, 2H), 7.94 (s, 2H), 8.52 (s, 2H). HRMS (ESI⁺): calcd C₂₀H₂₇N₄O₂S [M + H]⁺ 387.1849, found 387.1862 (error 3.4 ppm).

4-amino-2-butyl-1-(2-methoxy-2-oxoethyl)-7-methoxycarbonyl-1H-imidazo[4,5-c]quinoline (12k). Pd₂(dba)₃ (0.06 g, 0.10 mmol, 0.14 equiv) and 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl (Sphos) (0.07 g, 0.17 mmol, 0.24 equiv) were added to a flask with THF (10 mL) and stirred at 25 °C for 1 h. To this mixture a solution of **9k** (0.25 g, 0.72 mmol, 1.0 equiv) and methyl 3-amino-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate (2.2 mmol, 3.0 equiv) in THF (10 mL) was added, followed immediately by Cs₂CO₃ (1.07 mmol, 1.5 equiv) and H₂O (9 mL). Refluxed for 2 h and allowed to cool to 25 °C. Diluted with H₂O (20 mL) and EtOAc (20 mL) and separated. Extracted the aqueous layer with EtOAc (3 x 10 mL). Combined organic fractions were concentrated *in vacuo*. The crude residue was chromatographed on a short silica plug to remove polar impurities. The resulting oil was dried for 96 h *in vacuo* (3–5 mbar) and used without further purification.

To a round-bottom flask containing the dried oil (~0.40 mmol, 1.0 equiv) was added 4 N HCl in dioxane (4 mL, 16 mmol, 40 equiv). The mixture was heated at reflux for 4 h under argon and then cooled to 25 °C. NaHCO₃ powder was added to quench the acid. The reaction mixture was partitioned between 5% MeOH in EtOAc (25 mL) and H₂O (20 mL). The aqueous layer was extracted with 5% MeOH in EtOAc (3 x 20 mL). Combined organic fractions were concentrated *in vacuo*. The crude residue was purified by flash column chromatography on silica gel giving a white solid in 10.7% yield: *R*_f = 0.35 (EtOAc); ¹H NMR (CDCl₃, 400 MHz) δ 0.98 (t, *J* = 7.4 Hz, 3H), 1.48 (hex, *J* = 7.4, 2H), 1.84 (quin, *J* = 7.4 Hz, 2H), 2.87 (t, *J* = 7.4 Hz, 2H), 3.81 (s, 3H), 3.94 (s, 3H), 5.27 (s, 2H), 6.48 (br s, 2H), 7.78 (d, *J* = 8.2 Hz, 1H), 7.95 (dd, *J* = 8.2, 1.6 Hz, 1H), 8.42 (d, *J* = 1.6 Hz, 1H). HRMS (ESI⁺): calcd C₁₉H₂₃N₄O₄ [*M* + *H*]⁺ 371.1714, found 371.1735 (error 5.8 ppm).

4-amino-2-butyl-1-(3-methoxy-3-oxopropyl)-7-methoxycarbonyl-1H-imidazo[4,5-c]quinoline (12l). Pd₂(dba)₃ (0.07 g, 0.10 mmol, 0.14 equiv) and 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl (Sphos) (0.11 g, 0.17 mmol, 0.24 equiv) were added to a flask with THF (10 mL) and stirred at 25 °C for 1 h. To this mixture a solution of **9l** (0.21 g, 0.58 mmol, 1.0 equiv) and methyl 3-amino-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate (1.75 mmol, 3.0 equiv) in THF (12 mL) was added, followed immediately by Cs₂CO₃ (1.07 mmol, 1.5 equiv) and H₂O (9 mL). Refluxed for 3 h and allowed to cool to 25 °C. Diluted with H₂O (20 mL) and EtOAc (20 mL) and separated. Extracted the aqueous layer with EtOAc (3 x 10 mL). Combined organic fractions were concentrated *in vacuo*. The crude residue was chromatographed on a short silica plug to remove polar impurities. The resulting oil was dried for 40 h *in vacuo* (3–5 mbar) and used without further purification.

To a round-bottom flask containing the dried oil (~0.40 mmol, 1.0 equiv) was added 4 N HCl in dioxane (4 mL, 16 mmol, 40 equiv). The mixture was heated at reflux for 4 h under argon and then cooled to 25 °C. NaHCO₃ powder was added to quench the acid. The reaction mixture was partitioned between 5% MeOH in EtOAc (25 mL) and H₂O (20 mL). The aqueous layer was extracted with 5% MeOH in EtOAc (3 x 20 mL). Combined organic fractions were concentrated *in vacuo*. The crude residue was purified by flash column chromatography on silica gel giving a white solid in 5.5% yield: *R*_f = 0.35 (EtOAc); ¹H NMR (CDCl₃, 400 MHz) δ 1.01 (t, *J* = 7.4 Hz, 3H), 1.52 (hex, *J* = 7.4 Hz, 2H), 1.85 (quin, *J* = 7.4 Hz, 2H), 2.95 (t, *J* = 7.4 Hz, 2H), 2.97 (t, *J* = 7.4 Hz, 2H), 3.74 (s, 3H), 3.96 (s, 3H), 4.81 (t, *J* = 7.4 Hz, 2H), 5.50 (br s, 2H), 7.96 (s, 2H), 8.53 (s, 1H). HRMS (ESI⁺): calcd C₂₀H₂₅N₄O₄ [*M* + *H*]⁺ 385.1870, found 385.1869 (error 0.3 ppm).

4-amino-2-butyl-1-(4-methoxy-4-oxobutyl)-7-methoxycarbonyl-1H-imidazo[4,5-c]quinoline (12m). The title compound was prepared according to the general procedure using **9m** as a white solid in 23.2% yield: *R*_f = 0.32 (EtOAc); ¹H NMR (CDCl₃, 400 MHz) δ 1.01 (t, *J* = 7.4 Hz, 3H), 1.52 (hex, *J* = 7.4, 2H), 1.88 (quin, *J* = 7.4 Hz, 2H), 2.23 (quin, *J* = 6.8 Hz, 2H), 2.52 (t, *J* = 6.8 Hz, 2H), 2.93 (t, *J* = 7.4 Hz, 2H), 3.74 (s, 3H), 3.96 (s, 3H), 4.55 (t, *J* = 6.8 Hz,

2H), 5.43 (br s, 2H), 7.96 (d, $J = 8.6$ Hz, 1H), 8.07 (d, $J = 8.6$ Hz, 1H), 8.52 (s, 1H). HRMS (ESI⁺): calcd C₂₁H₂₇N₄O₄ [M + H]⁺ 399.2027, found 399.2049 (error 5.5 ppm).

4-amino-2-butyl-1-(5-methoxy-5-oxopentyl)-7-methoxycarbonyl-1H-imidazo[4,5-c]quinoline (12n). The title compound was prepared according to the general procedure using **9n** as a white solid in 7.0% yield: $R_f = 0.30$ (EtOAc); ¹H NMR (CDCl₃, 400 MHz) δ 1.01 (t, $J = 7.4$ Hz, 3H), 1.52 (hex, $J = 7.4$, 2H), 1.76–1.91 (m, 4H), 1.97 (quin, $J = 7.4$ Hz, 2H), 2.40 (t, $J = 7.4$ Hz, 2H), 2.92 (t, $J = 7.4$ Hz, 2H), 3.66 (s, 3H), 3.97 (s, 3H), 4.47 (t, $J = 7.4$ Hz, 2H), 5.46 (br s, 2H), 7.95 (s, 2H), 8.52 (s, 1H). HRMS (ESI⁺): calcd C₂₂H₂₉N₄O₄ [M + H]⁺ 413.2183, found 413.2202 (error 4.6 ppm).

4-amino-2-butyl-1-(6-methoxy-6-oxohexyl)-7-methoxycarbonyl-1H-imidazo[4,5-c]quinoline (12o). The title compound was prepared according to the general procedure using **9o** as a white solid in 21.3% yield: $R_f = 0.30$ (EtOAc); ¹H NMR (CDCl₃, 400 MHz) δ 0.97 (t, $J = 7.4$ Hz, 3H), 1.40–1.55 (m, 4H), 1.68 (quin, $J = 7.4$ Hz, 2H), 1.76–1.96 (m, 4H), 2.31 (t, $J = 7.0$ Hz, 2H), 2.85 (t, $J = 7.4$ Hz, 2H), 3.64 (s, 3H), 3.93 (s, 3H), 4.36 (t, $J = 7.4$ Hz, 2H), 5.90 (br s, 2H), 7.84 (s, 2H), 8.44 (s, 1H). HRMS (ESI⁺): calcd C₂₃H₃₁N₄O₄ [M + H]⁺ 427.2340, found 427.2342 (error 0.5 ppm).

4-amino-2-butyl-1-(2-aminoethyl)-7-methoxycarbonyl-1H-imidazo[4,5-c]quinoline (12p). The title compound was prepared according to the general procedure using **9p** as a yellow-white solid in 14.1% yield: $R_f = 0.60$ (5:5:10:80 H₂O/NEt₃/MeOH/EtOAc); ¹H NMR (CD₃OD, 400 MHz) δ 1.02 (t, $J = 7.4$ Hz, 3H), 1.53 (hex, $J = 7.4$ Hz, 2H), 1.89 (quin, $J = 7.4$ Hz, 2H), 3.02 (t, $J = 7.4$ Hz, 2H), 3.11 (t, $J = 7.4$ Hz, 2H), 3.93 (s, 3H), 4.61 (t, $J = 7.4$ Hz, 2H), 7.89 (dd, $J = 8.6$, 1.6 Hz, 1H), 8.17 (d, $J = 8.6$ Hz, 1H), 8.32 (d, $J = 1.6$ Hz, 1H). HRMS (ESI⁺): calcd C₁₈H₂₄N₅O₂ [M + H]⁺ 342.1925, found 342.1913 (error 3.3 ppm).

4-amino-2-butyl-1-(3-aminopropyl)-7-methoxycarbonyl-1H-imidazo[4,5-c]quinoline (12q). The title compound was prepared according to the general procedure using **9q** as a white solid in 2.5% yield: $R_f = 0.50$ (5:5:10:80 H₂O/NEt₃/MeOH/EtOAc); ¹H NMR (CD₃OD, 400 MHz) δ 1.03 (t, $J = 7.4$ Hz, 3H), 1.53 (hex, $J = 7.4$ Hz, 2H), 1.89 (quin, $J = 7.4$ Hz, 2H), 2.19 (quin, $J = 7.4$ Hz, 2H), 2.99 (t, $J = 7.4$ Hz, 2H), 3.05 (t, $J = 7.4$ Hz, 2H), 3.94 (s, 3H), 4.67 (t, $J = 7.4$ Hz, 2H), 7.90 (d, $J = 8.6$ Hz, 1H), 8.13 (d, $J = 8.6$ Hz, 1H), 8.31 (s, 1H). HRMS (ESI⁺): calcd C₁₉H₂₆N₅O₂ [M + H]⁺ 356.2081, found 356.2068 (error 3.8 ppm).

4-amino-2-butyl-1-(4-aminobutyl)-7-methoxycarbonyl-1H-imidazo[4,5-c]quinoline (12r). The title compound was prepared according to the general procedure using **9r** as a white solid in 10.3% yield: $R_f = 0.40$ (5:5:10:80 H₂O/NEt₃/MeOH/EtOAc); ¹H NMR (CD₃OD, 400 MHz) δ 1.04 (t, $J = 7.4$ Hz, 3H), 1.54 (hex, $J = 7.4$ Hz, 2H), 1.65 (quin, $J = 7.4$ Hz, 2H), 1.85–2.00 (m, 4H), 2.70 (t, $J = 6.6$ Hz, 2H), 3.02 (t, $J = 7.4$ Hz, 2H), 3.96 (s, 3H), 4.61 (t, $J = 7.4$ Hz, 2H), 7.93 (d, $J = 8.6$ Hz, 1H), 8.15 (d, $J = 8.6$ Hz, 1H), 8.36 (s, 1H). HRMS (ESI⁺): calcd C₂₀H₂₈N₅O₂ [M + H]⁺ 370.2238, found 370.2235 (error 0.8 ppm).

4-amino-2-butyl-1-(5-aminopentyl)-7-methoxycarbonyl-1H-imidazo[4,5-c]quinoline (12s). The title compound was prepared according to the general procedure using **9s** as a white solid in 6.2% yield: $R_f = 0.35$ (5:5:10:80 H₂O/NEt₃/MeOH/EtOAc); ¹H NMR (CD₃OD, 400 MHz) δ 1.03 (t, $J = 7.4$ Hz, 3H), 1.46–1.62 (m, 6H), 1.82–1.98 (m, 4H), 2.69 (t, $J = 6.6$ Hz, 2H), 2.98 (t, $J = 7.4$ Hz, 2H), 3.95 (s, 3H), 4.55 (t, $J = 7.4$ Hz, 2H), 7.89 (d, $J = 8.6$ Hz, 1H), 8.08 (d, $J = 8.6$ Hz, 1H), 8.33 (s, 1H). HRMS (ESI⁺): calcd C₂₁H₃₀N₅O₂ [M + H]⁺ 384.2394, found 384.2387 (error 1.8

ppm).

4-Amino-1-(4-(aminomethyl)benzyl)-2-butyl-7-methoxycarbonyl-1H-imidazo[4,5-c]quinoline (12t). The title compound was prepared according to the general procedure using **9t** as a yellow solid in 23.6% yield: $R_f = 0.55$ (5:5:10:80 H₂O/NEt₃/MeOH/EtOAc); ¹H NMR (CD₃OD, 400 MHz) δ 0.93 (t, $J = 7.4$ Hz, 3H), 1.44 (hex, $J = 7.4$ Hz, 2H), 1.79 (quin, $J = 7.4$ Hz, 2H), 2.98 (t, $J = 7.4$ Hz, 2H), 3.79 (s, 2H), 3.90 (s, 3H), 5.88 (s, 2H), 7.03 (d, $J = 7.8$ Hz, 2H), 7.32 (d, $J = 7.8$ Hz, 2H), 7.61 (d, $J = 9.0$ Hz, 1H), 7.83 (d, $J = 9.0$ Hz, 1H), 8.29 (s, 1H). HRMS (ESI+): calcd C₂₄H₂₈N₅O₂ [M + H]⁺ 418.2238, found 418.2234 (error 0.9 ppm).

4-Amino-2-butyl-1-(4-(hydroxycarbonyl)benzyl)-7-methoxycarbonyl-1H-imidazo[4,5-c]quinoline (12u). The title compound was prepared according to the general procedure using **9u** as a white solid in 42.3% yield: $R_f = 0.53$ (3:7:90 AcOH/MeOH/EtOAc); ¹H NMR (DMSO- *d*₆, 400 MHz) δ 0.80 (t, $J = 7.5$ Hz, 3H), 1.33 (hex, $J = 7.5$ Hz, 2H), 1.66 (quin, $J = 7.5$ Hz, 2H), 2.93 (t, $J = 7.5$ Hz, 2H), 3.11 (s, 2H), 3.83 (s, 3H), 6.02 (s, 2H), 7.15 (d, $J = 7.8$, 2H), 7.69 (d, $J = 8.2$ Hz, 1H), 7.84 (d, $J = 7.8$ Hz, 2H), 7.95 (d, $J = 8.2$, 1H), 8.28 (s, 1H). HRMS (ESI+): calcd C₂₄H₂₅N₄O₄ [M + H]⁺ 433.1876, found 433.1865 (error 1.2 ppm).

4-Amino-2-butyl-1-(4-(hydroxycarbonyl)2-phenylethyl)-7-methoxycarbonyl-1H-imidazo[4,5-c]quinoline (12v). The title compound was prepared according to the general procedure using **9v** as a white solid in 13.6% yield: $R_f = 0.56$ (3:7:90 AcOH/MeOH/EtOAc); ¹H NMR (CD₃OD, 400 MHz) δ 0.87 (t, $J = 7.4$ Hz, 3H), 1.29 (hex, $J = 7.4$ Hz, 2H), 1.63 (quin, $J = 7.4$ Hz, 2H), 2.38 (t, $J = 7.5$ Hz, 2H), 3.33 (t, $J = 6.3$ Hz, 2H), 3.99 (s, 3H), 4.93 (t, $J = 6.3$ Hz, 2H), 7.06 (d, $J = 8.2$, 2H), 7.85 (d, $J = 8.2$ Hz, 2H), 8.19 (d, $J = 9$ Hz, 1H), 8.42 (s, 1H), 8.43 (d, $J = 9$ Hz, 1H). HRMS (ESI+): calcd C₂₅H₂₇N₄O₄ [M + H]⁺ 447.2027, found 447.2031 (error 0.9 ppm).

4-amino-2-butyl-1-(5-hydroxy-5-oxopentyl)-7-methoxycarbonyl-1H-imidazo[4,5-c]quinoline (12w). The title compound was prepared according to the general procedure using **9w** as a white solid in 10.9% yield: $R_f = 0.40$ (3:7:90 AcOH/MeOH/EtOAc); ¹H NMR (CD₃OD, 400 MHz) δ 1.02 (t, $J = 7.4$ Hz, 3H), 1.52 (hex, $J = 7.4$ Hz, 2H), 1.77 (quin, $J = 7.4$ Hz, 2H), 1.83–2.00 (m, 4H), 2.22 (t, $J = 7.4$ Hz, 2H), 3.01 (t, $J = 7.4$ Hz, 2H), 3.94 (s, 3H), 4.57 (t, $J = 7.4$ Hz, 2H), 7.93 (d, $J = 8.6$ Hz, 1H), 8.14 (d, $J = 8.6$ Hz, 1H), 8.33 (s, 1H). HRMS (ESI+): calcd C₂₁H₂₇N₄O₄ [M + H]⁺ 399.2027, found 399.2046 (error 4.8 ppm).

Computational Chemistry

Calculated Octanol-Water Partition Coefficient

The imidazoquinoline heterocyclic scaffold containing the C2-butyl and C7-methylester groups was model built and relaxed with default parameters using the xLEaP program in AMBER 16.⁸ From this template, N1 functionalization was appended in an extended conformation to minimize steric contacts and relaxed with default parameters within xLEaP. The structures were subsequently optimized with *ab-initio* molecular orbital calculations using the HF/6-31+G** basis set in Gaussian 03 and imported using Maestro for evaluation using QuikProp within the Schrodinger suite of programs.^{9,10} Log *P* values (octanol-water) were calculated via QikProp 4.6.¹⁰

Table S1. Calculated logP values using Schrodinger QikProp software.											
Cmpd	logP	Cmpd	logP	Cmpd	logP	Cmpd	logP	Cmpd	logP	Cmpd	logP
12a	3.10	12e	2.03	12h	3.65	12k	2.36	12p	1.54	12u	3.57
12b	2.41	12f	2.28	12i	4.08	12l	2.81	12q	1.86	12v	3.92
12c	3.24	12g	2.65	12j	4.01	12m	2.95	12r	2.22	12w	2.83
12d	5.41					12n	3.49	12s	2.59		
						12o	3.86	12t	3.07		

Biological Assays

TLR7/8-NF- κ B reporter assay

Human embryonic kidney (HEK) cells stably transfected with human TLR7 or TLR8 and an NF- κ B responsive *secreted embryonic alkaline phosphatase* (SEAP) gene (HEK-Blue-hTLR7/8) were purchased from InvivoGen (San Diego, CA). The procedure used to measure TLR7 or TLR8 agonist activity was conducted as described by Hood et al.¹¹ HEK-TLR7/8 cells were stimulated in triplicate with compound concentrations ranging from 0.0137-180 μ M in a 96-well plate in DMEM containing 10% HI- FBS and 0.01% Normocin (InvivoGen) for 8-12 h. Following an initial 2-fold dilution of the highest concentration, three-fold serial dilutions were applied thereafter (from 0.0137 to 90 μ M). Additional concentrations were added to complete the dose response curve if any activity was detected at the higher concentrations. Imiquimod and resiquimod were used as positive controls. Following treatment, 20 μ L of the supernatant from each well was incubated with Quanti-blue substrate solution (InvivoGen) at 37 °C for 1 h and absorbance was read at 650 nm using a Synergy plate reader (Biotek, Winooski, VT). Data analysis was performed using Prism 6.0 (GraphPad Software, La Jolla California USA, www.graphpad.com). Reporter cell viability was also monitored for the duration of the assay and compounds **12g**, **12j-l**, and **12p-s** displayed cell toxicity and associated reductions in optical densities at the higher dose concentrations.

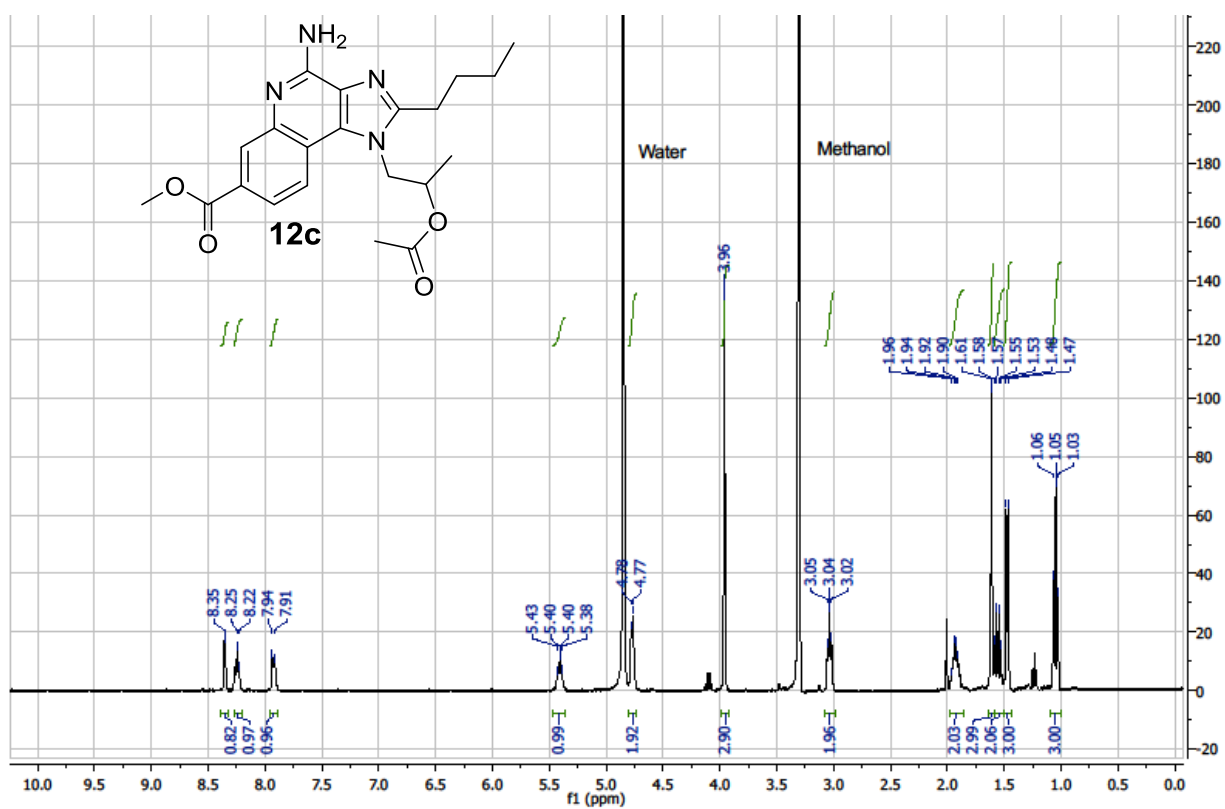
Preparation and stimulation of PBMC

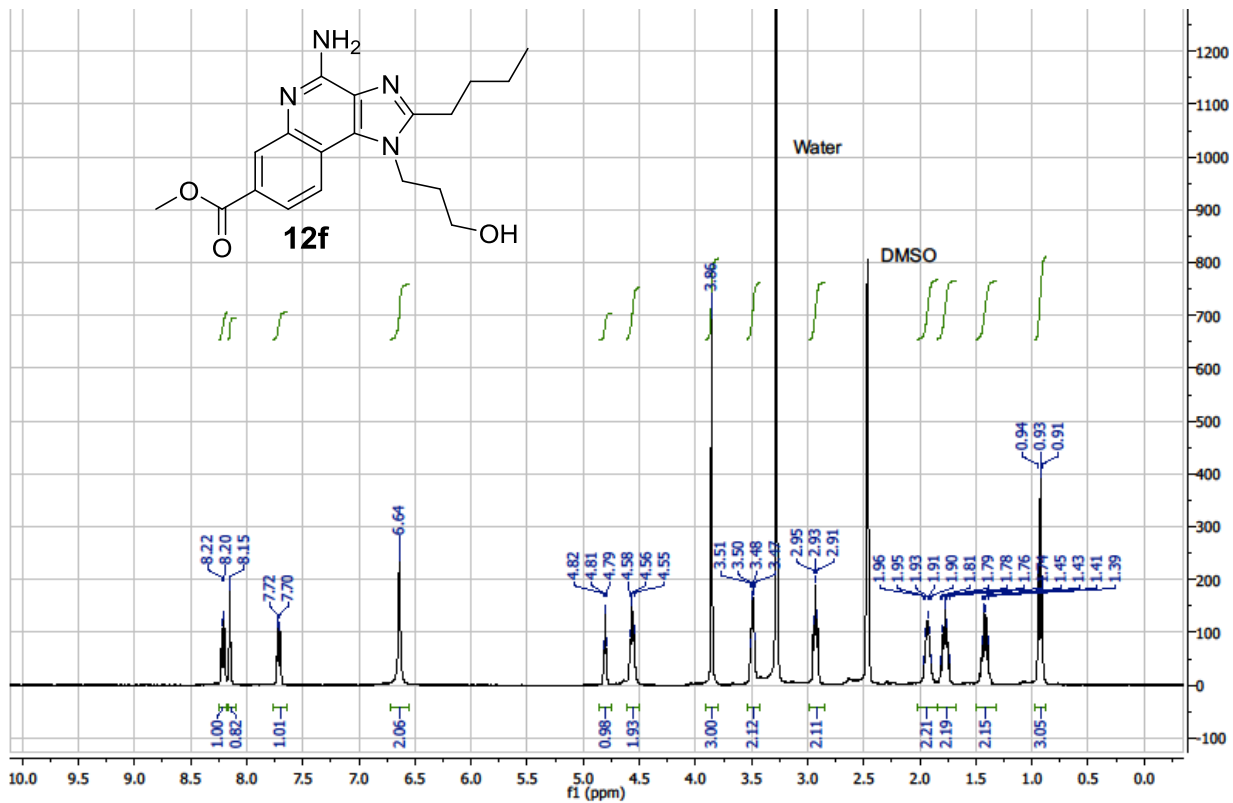
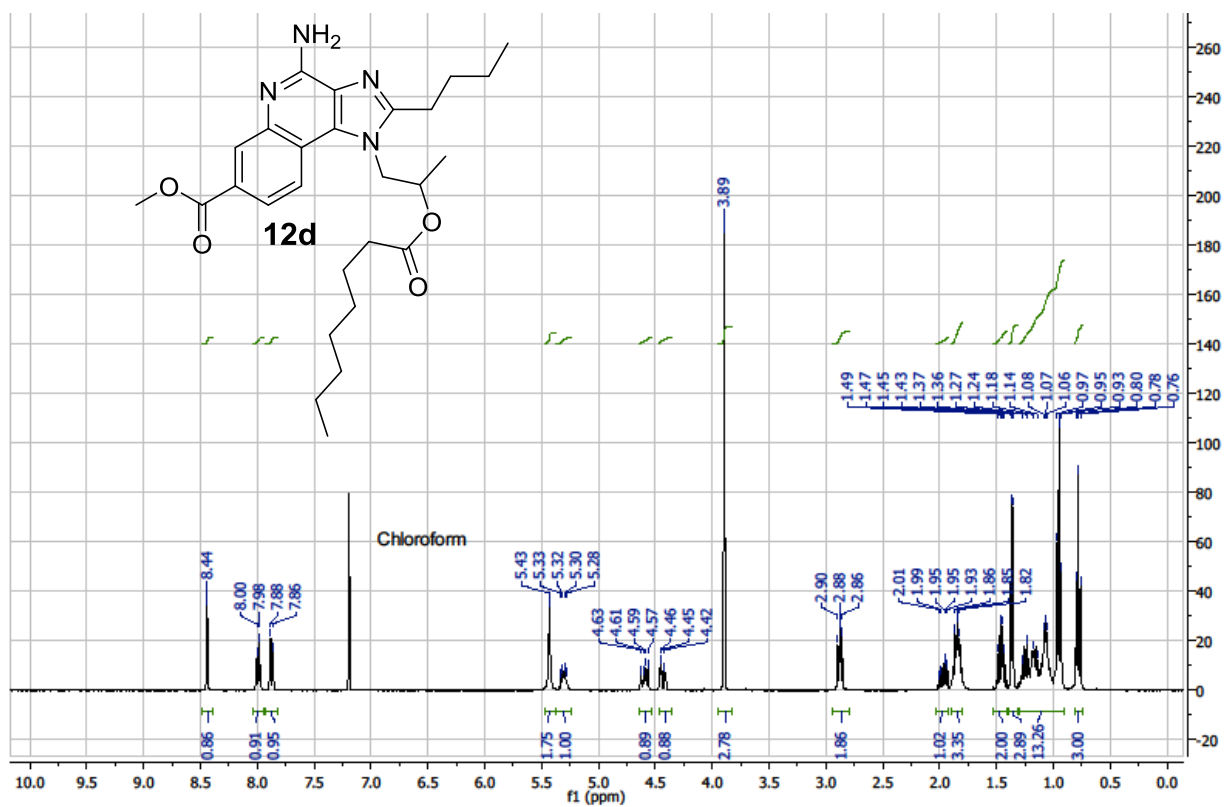
Human PBMC were isolated from heparinized blood by standard density-gradient centrifugation over Ficoll-Paque Plus (Pharmacia, Uppsala, Sweden). Briefly, peripheral blood was drawn by venipuncture into green top heparin tubes. The blood was diluted 1:1 with PBS, and then layered upon Ficoll-Paque Plus in 50 ml centrifuge tubes. The tubes were spun at 2000 rpm for 20 min, after which the buffy coats were collected and washed twice in PBS. The isolated PBMC were counted and added to the wells of a 24-well plate (5×10^5 cells/1 ml/well) in RPMI 1640 supplemented with 10% FBS, penicillin, streptomycin, sodium pyruvate, nonessential amino acids, and HEPES. TLR agonists were prepared in DMSO and added to the wells (in triplicate) so that the final concentration was between 50 μ M and 0.39 μ M. Some wells only had DMSO added. After 24 h, culture supernatants were collected, aliquoted, and frozen at -80°C until analysis. Cell toxicity was not noted for the compounds tested.

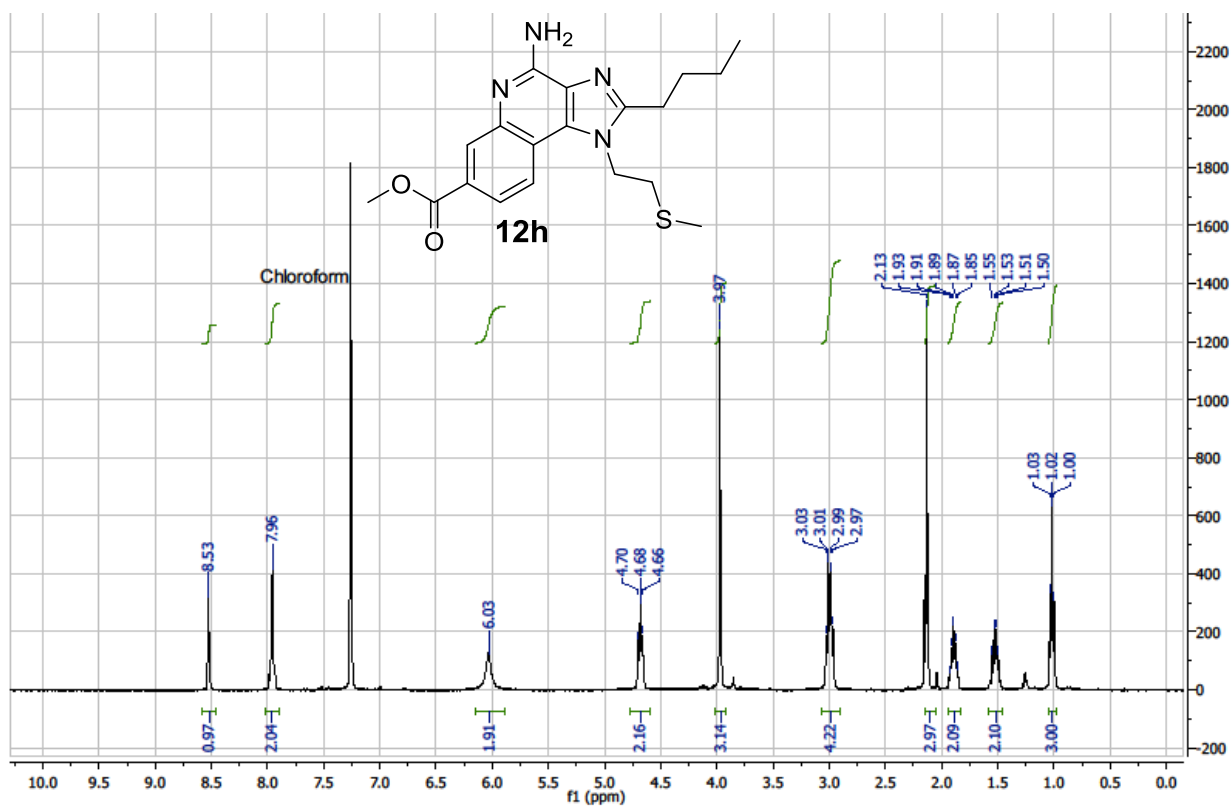
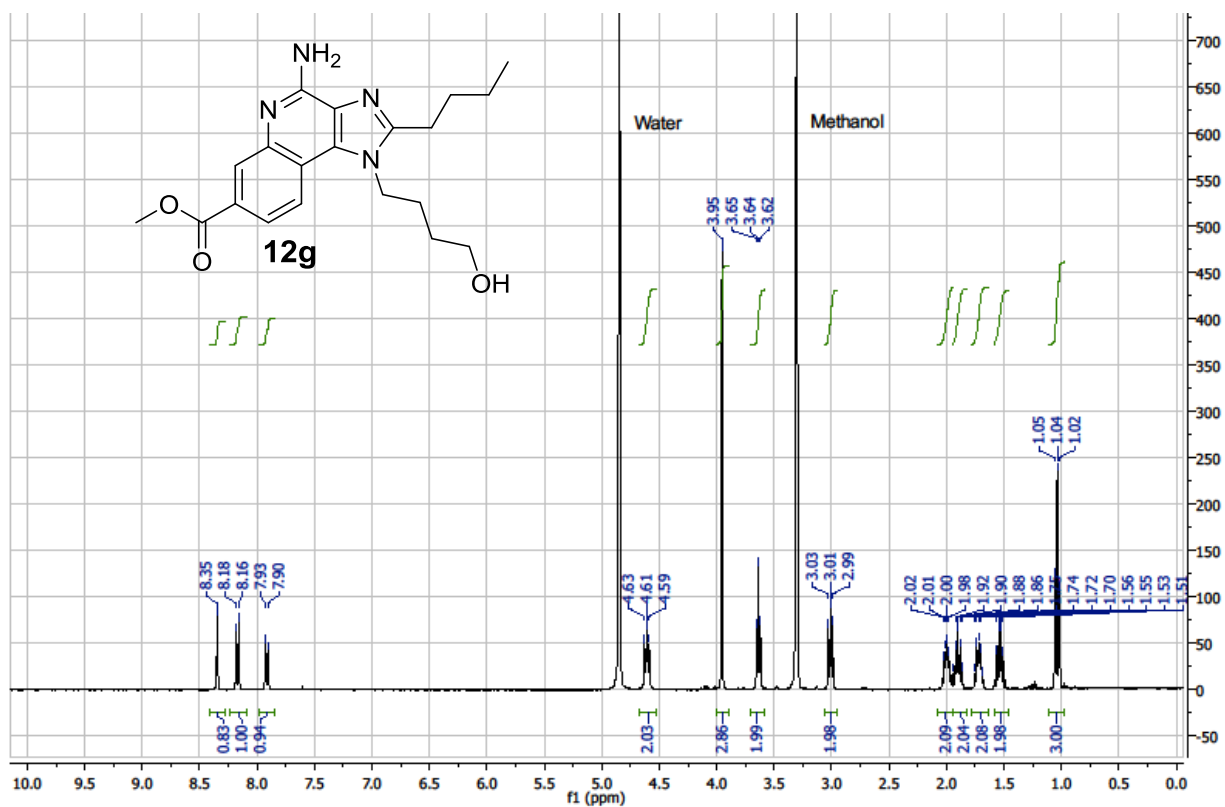
Quantitation of IL-1 β , IL-12p70, IFN γ , and TNF α

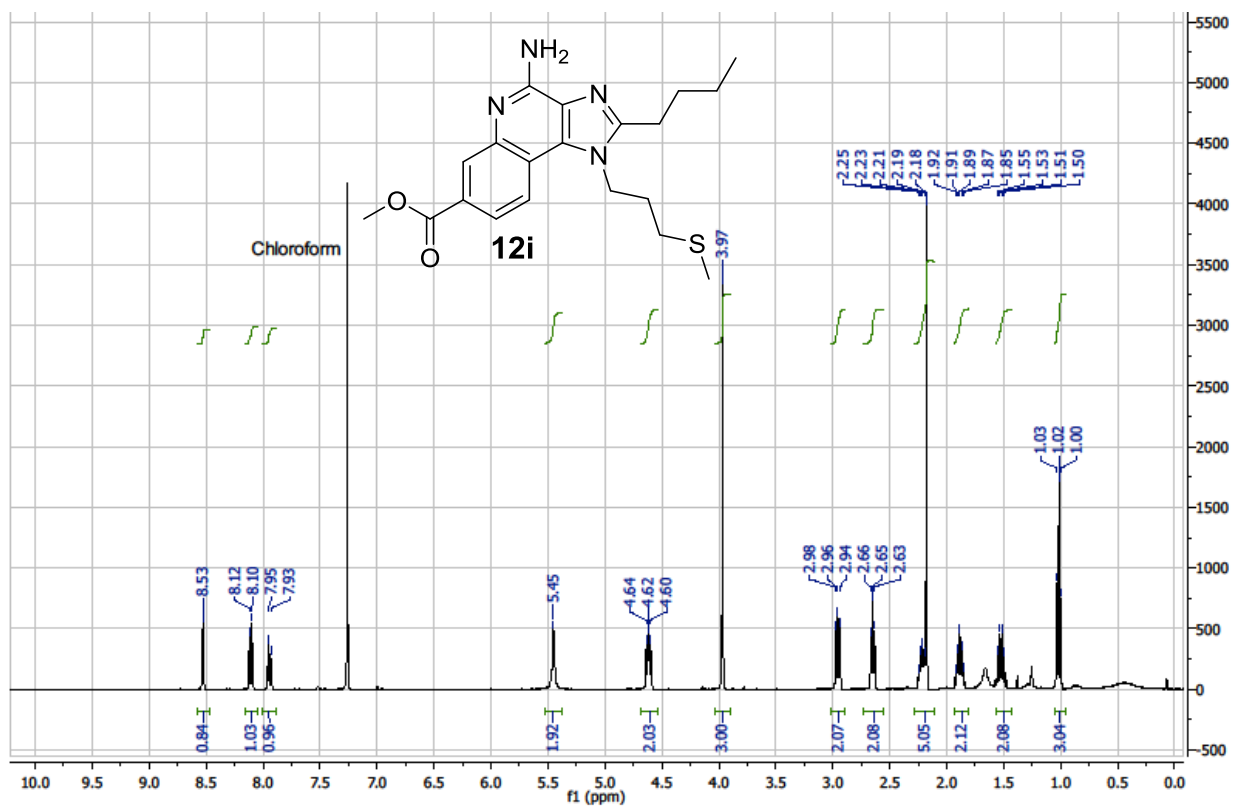
The amount of cytokine present in the supernatants after stimulation was determined by ELISA (BioLegend).

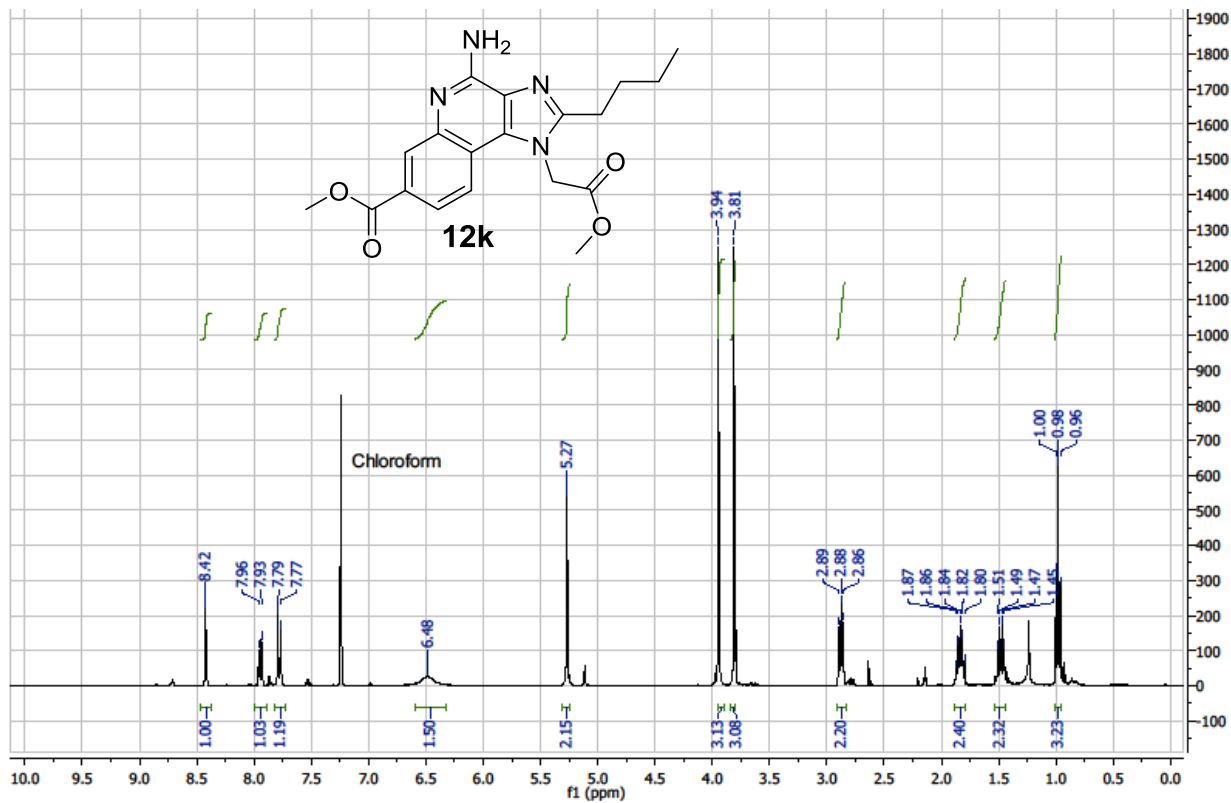
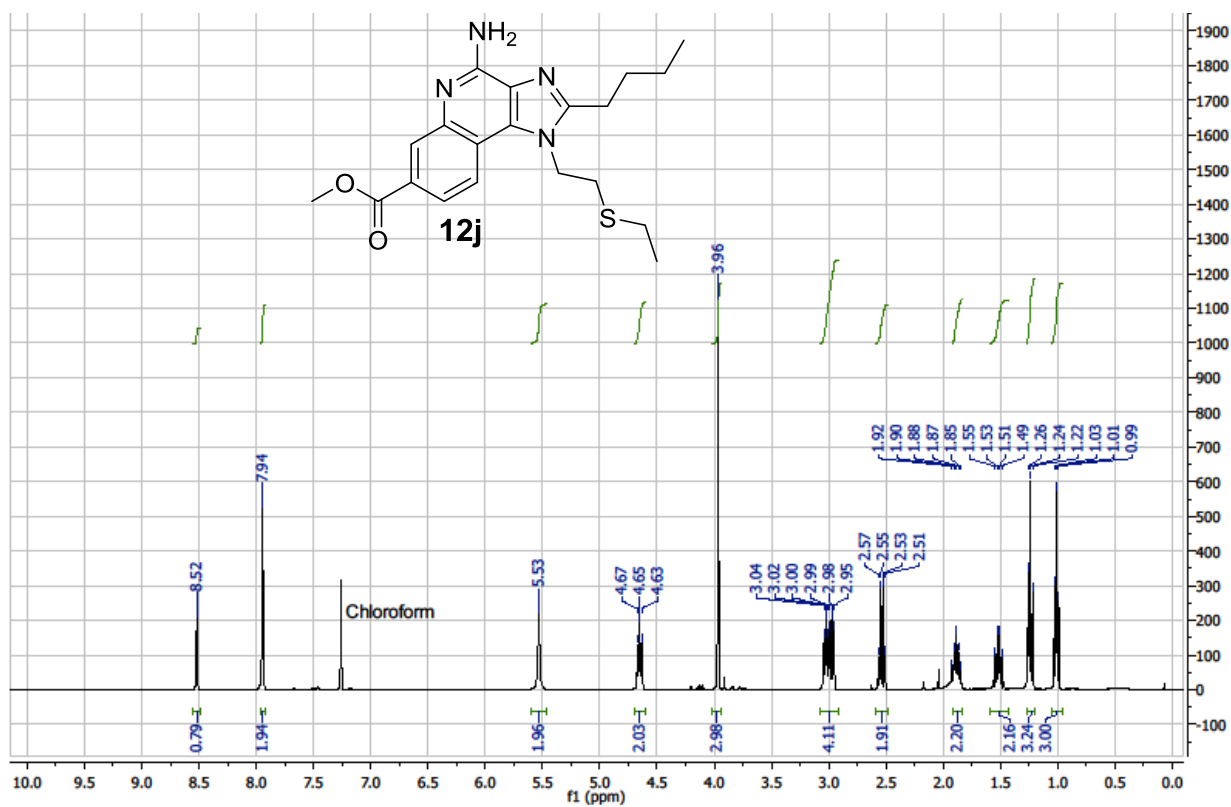
¹H NMR Spectra (Varian 400 MHz spectrometer.)

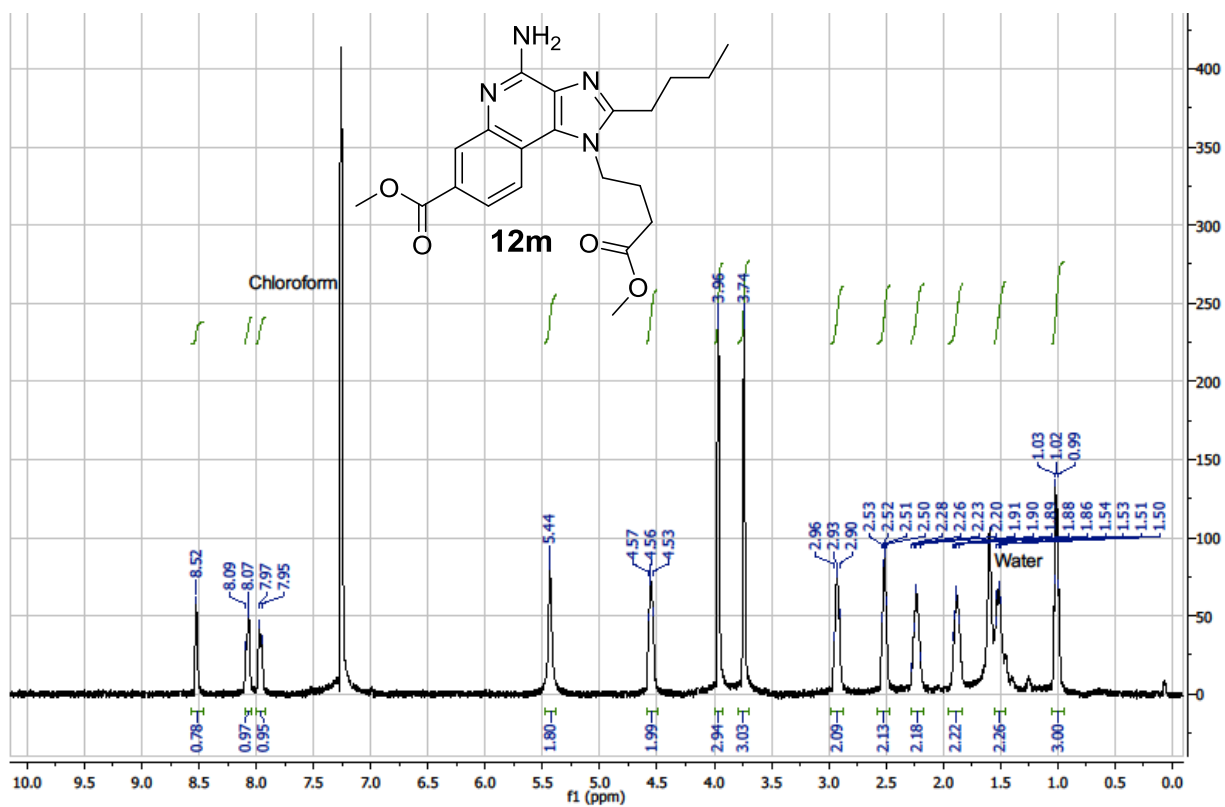
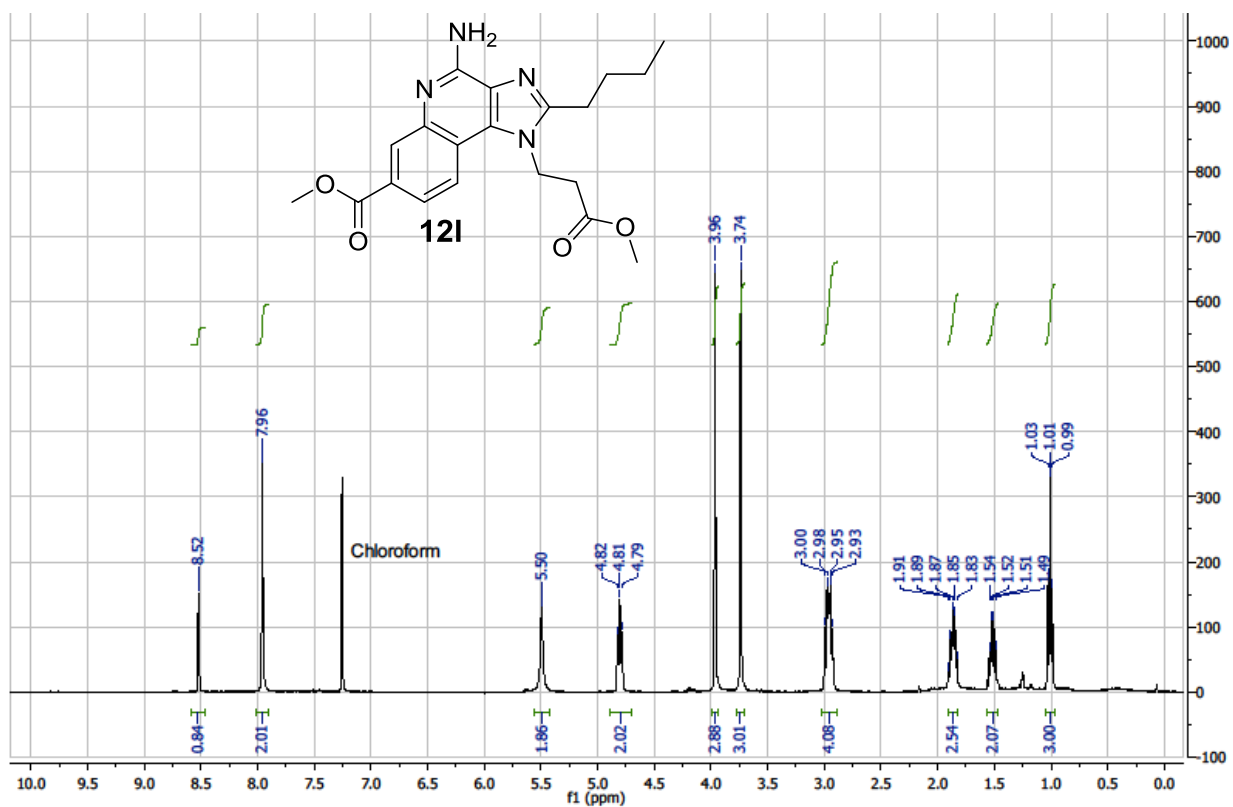


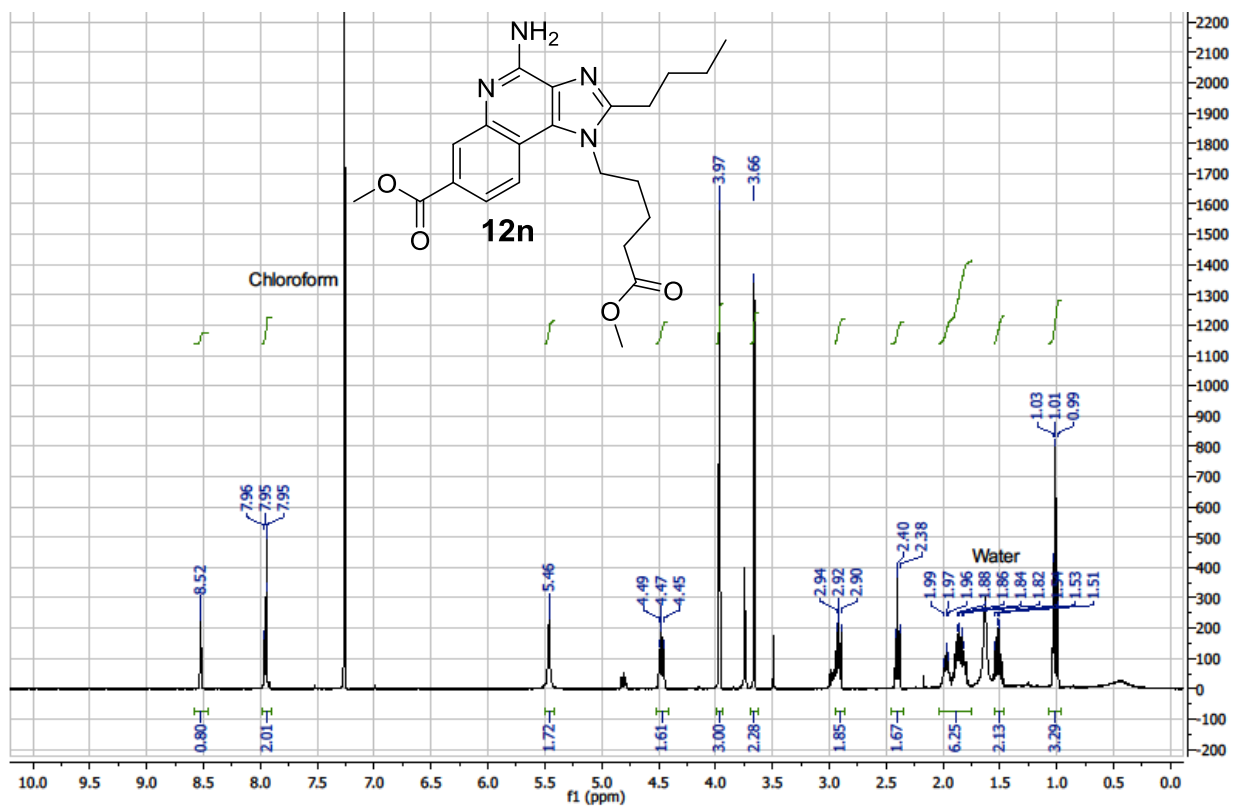


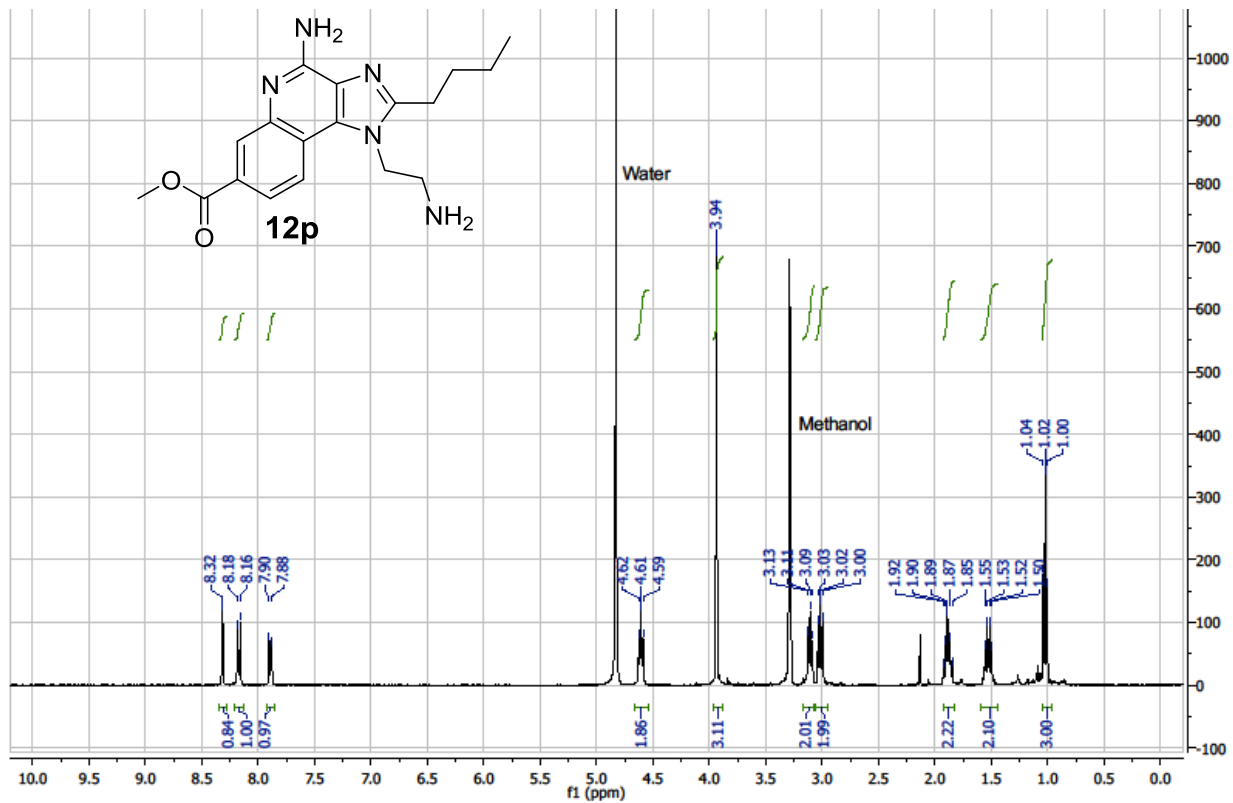
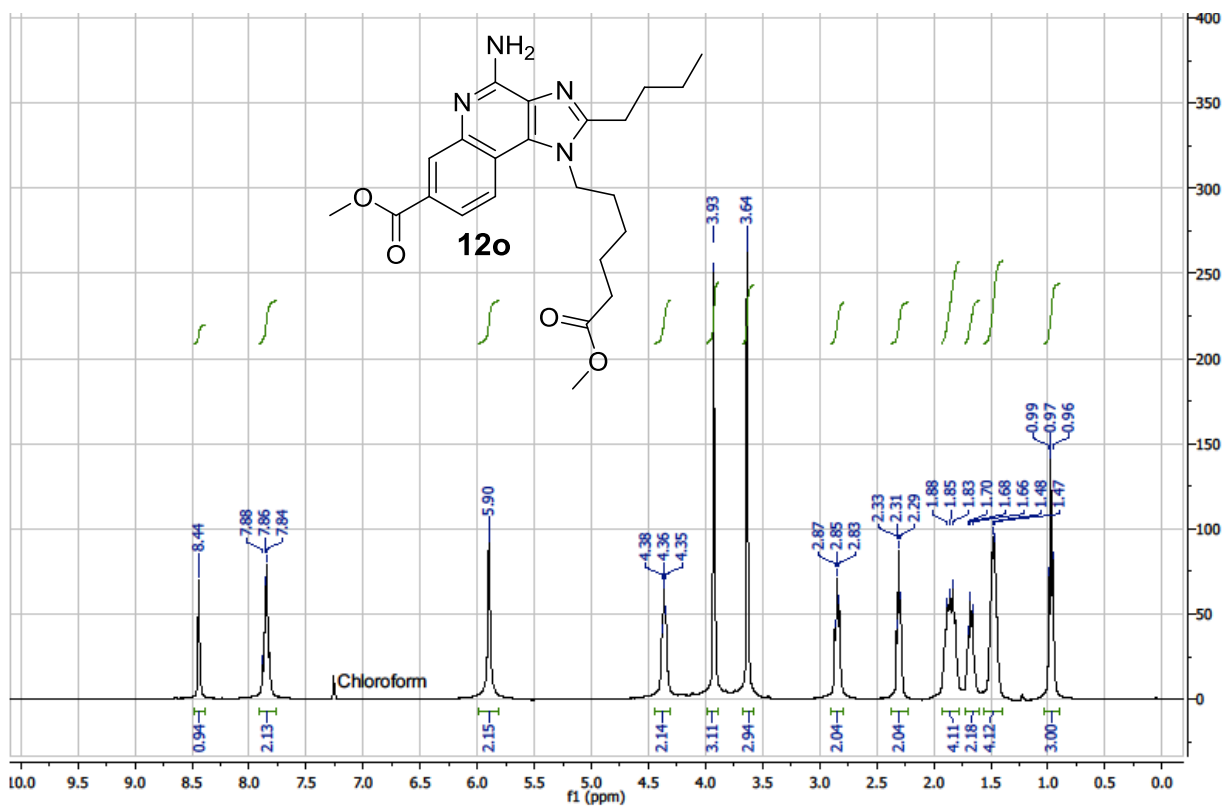


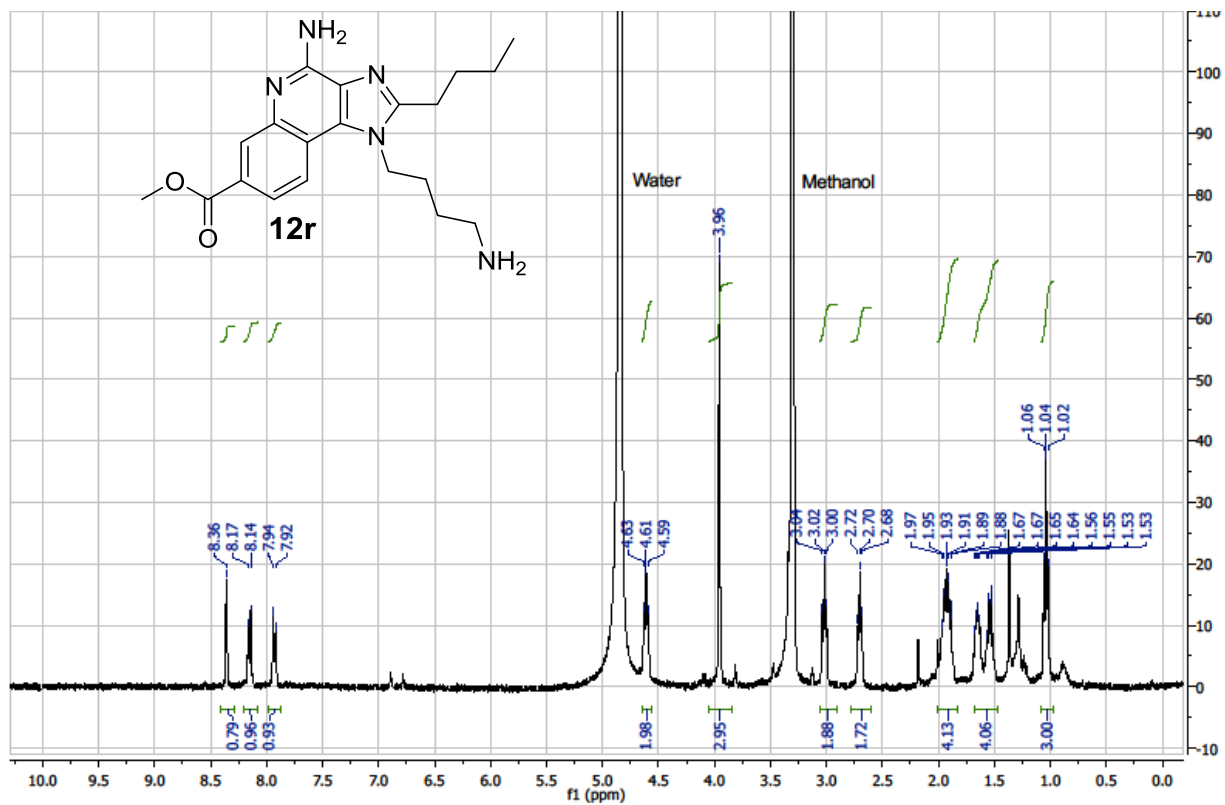
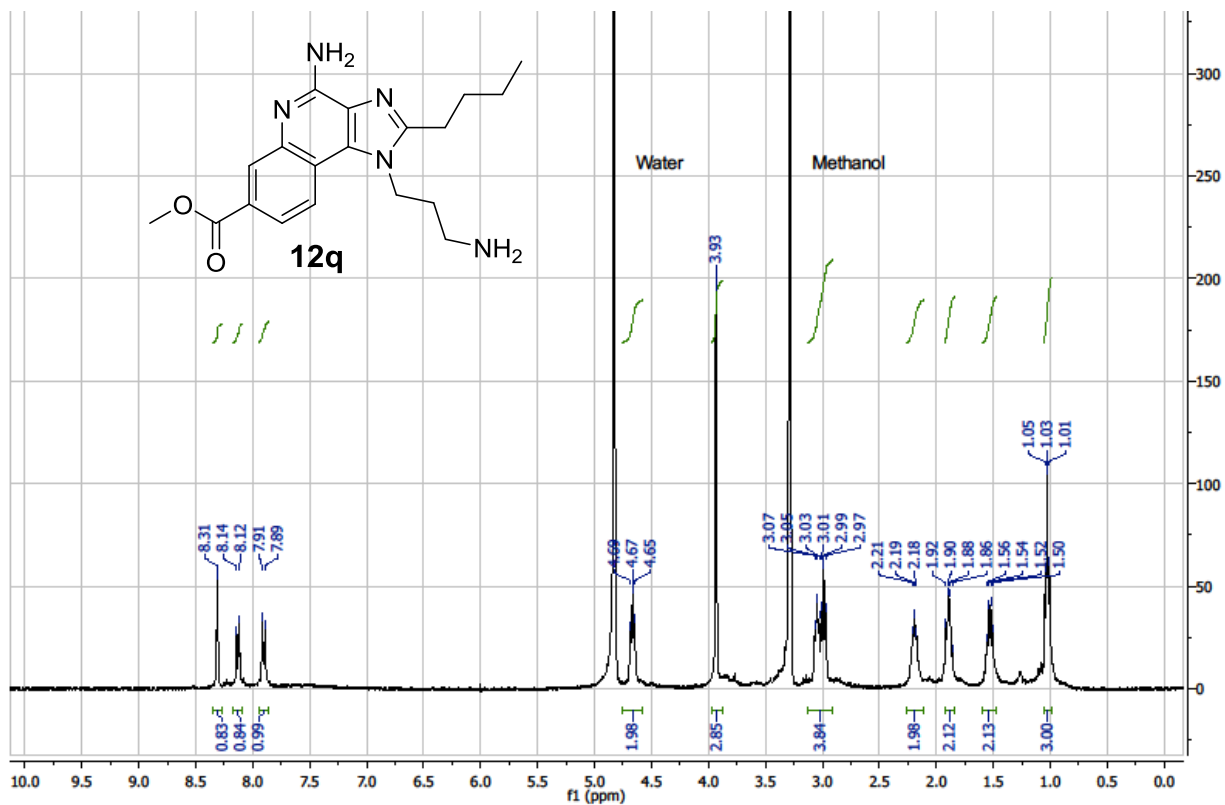


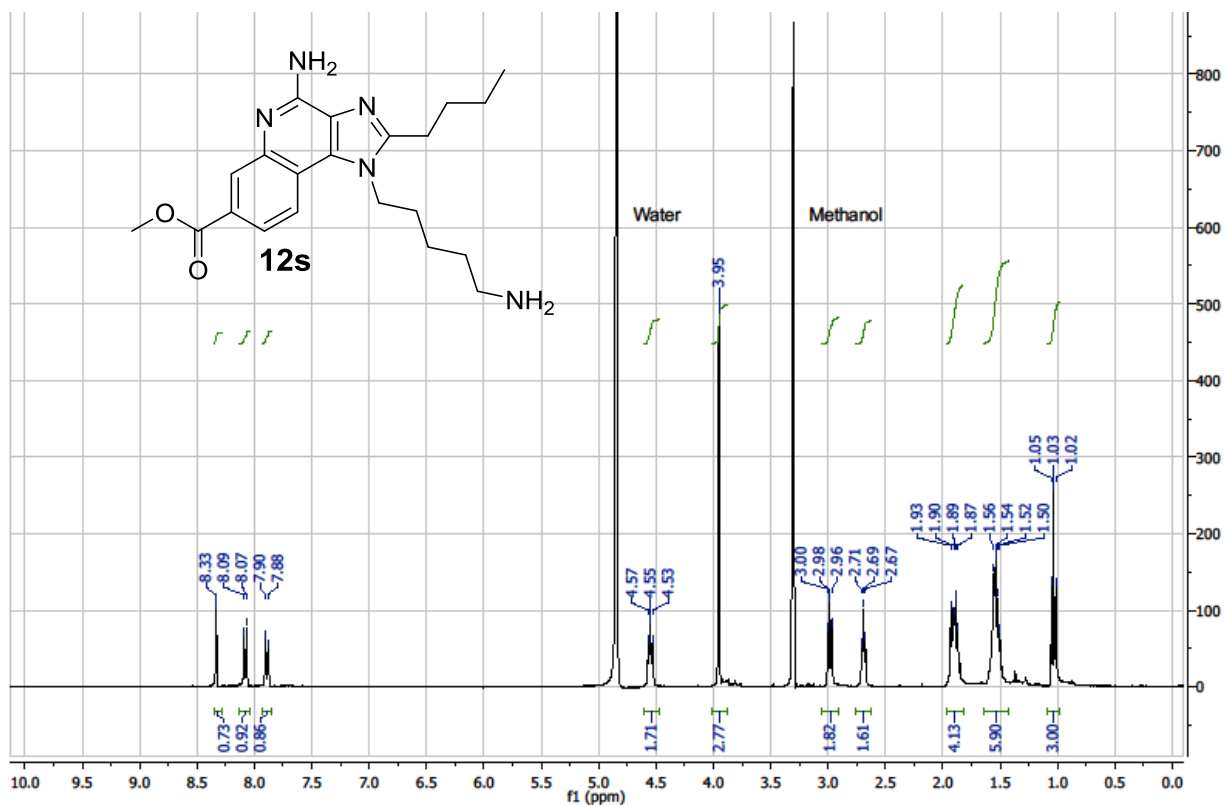


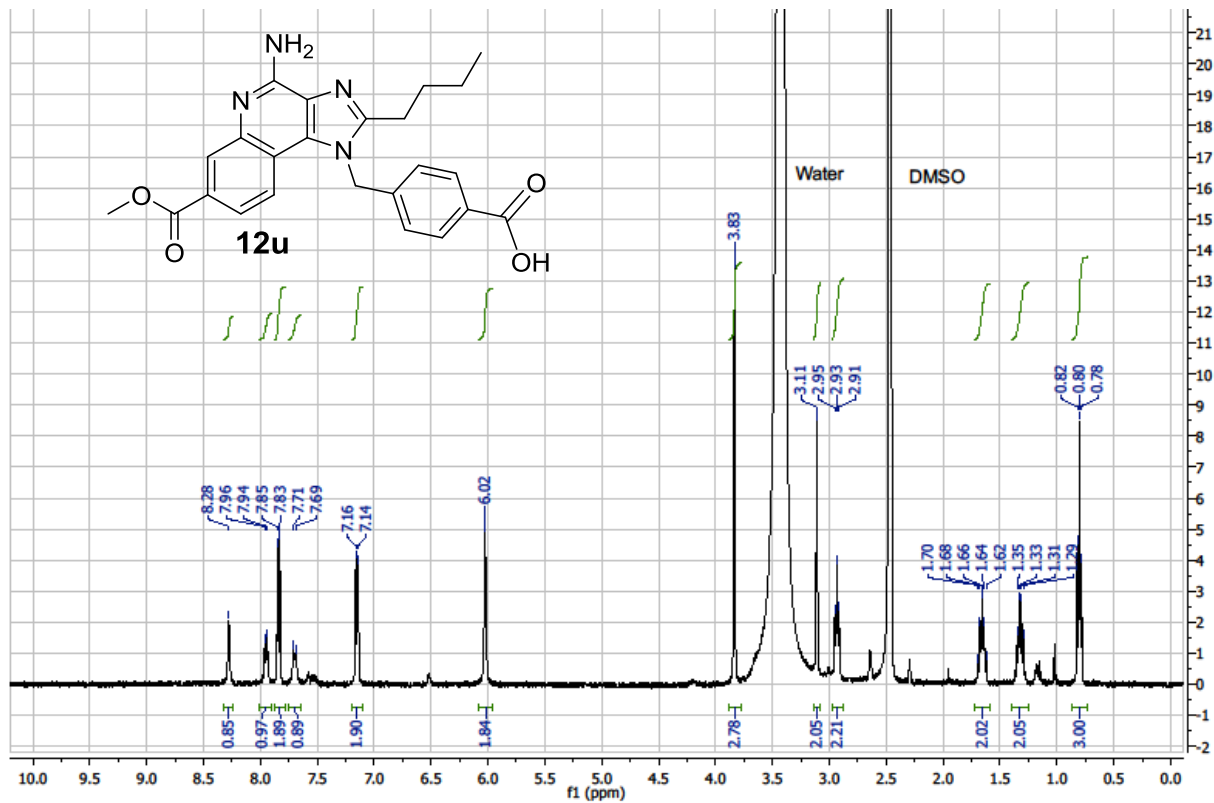
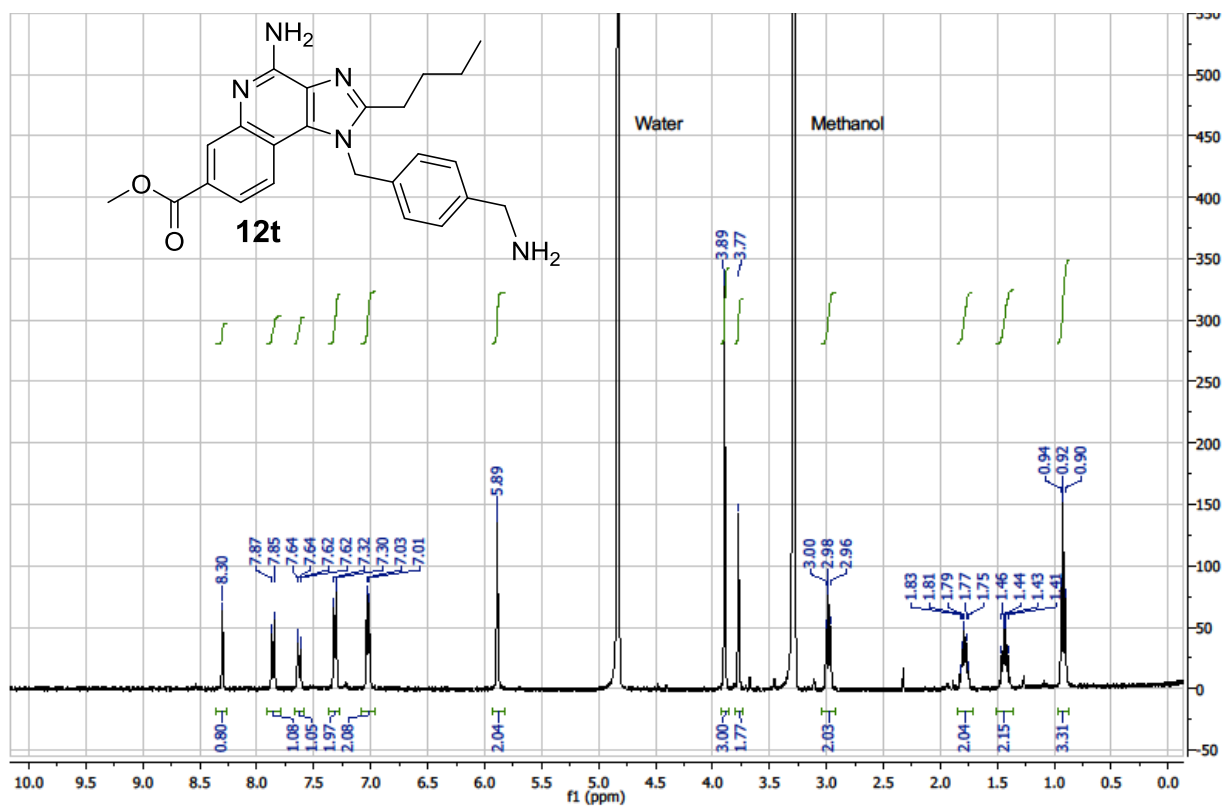


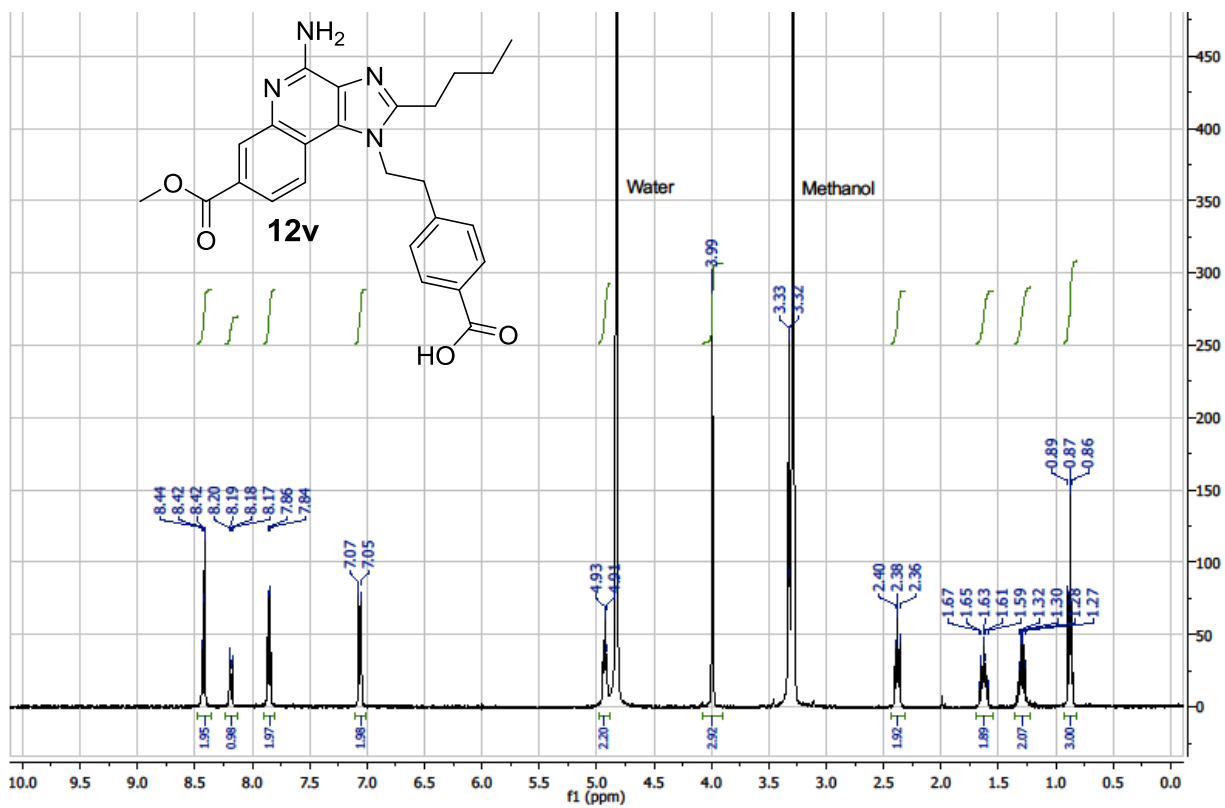


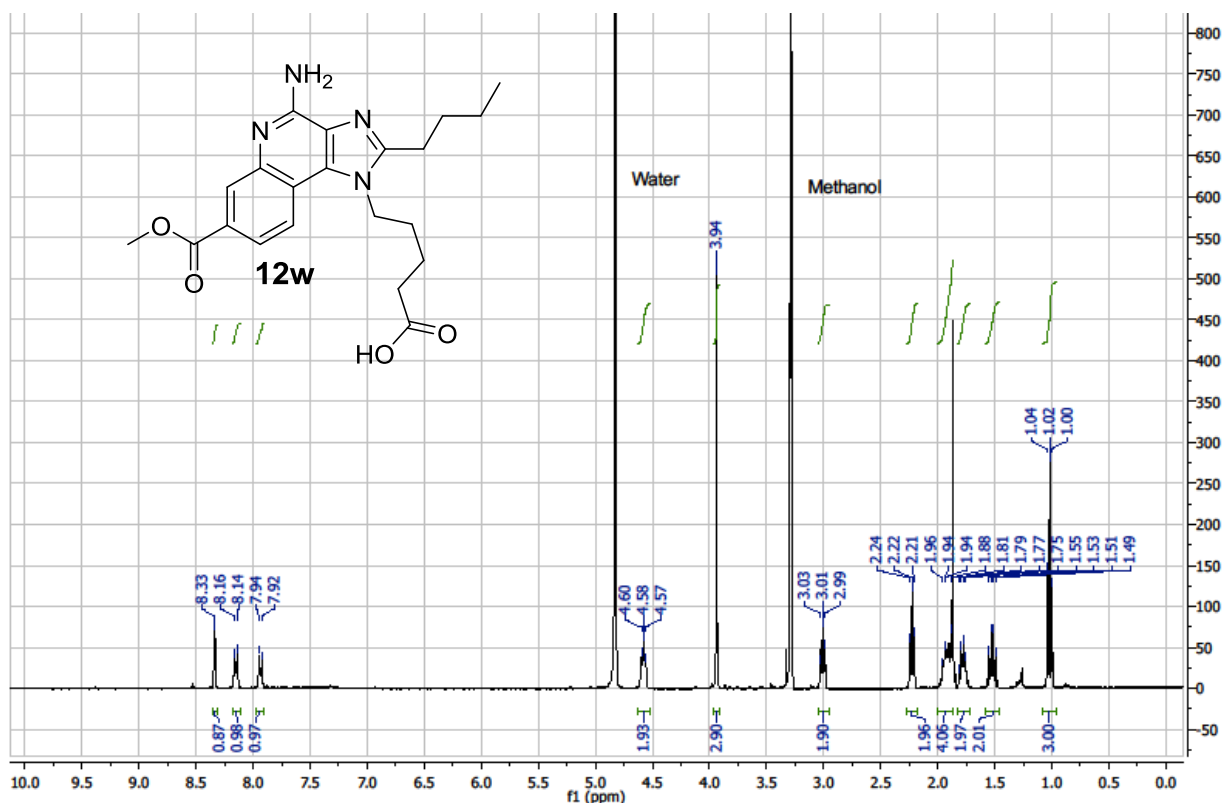












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