Design, Synthesis and Biological Characterization of a Caspase 3/7 Selective Isatin Labeled with 2-[^{18}F]fluoroethylazide

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TITLE RUNNING HEAD: 2-[^{18}F]fluoroethyazide-labeled isatins.

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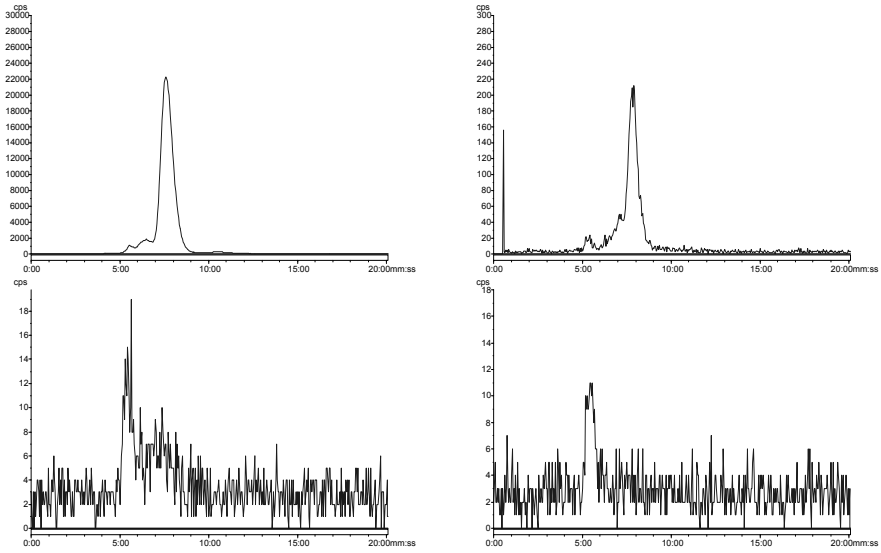


Figure S1. In vivo metabolism analysis of [^{125}I]**4** by radio-HPLC. Top row, left: [^{125}I]**4** control; right, plasma 2 mins sample; bottom row, left, plasma 10 mins sample; right, plasma 30 mins sample.

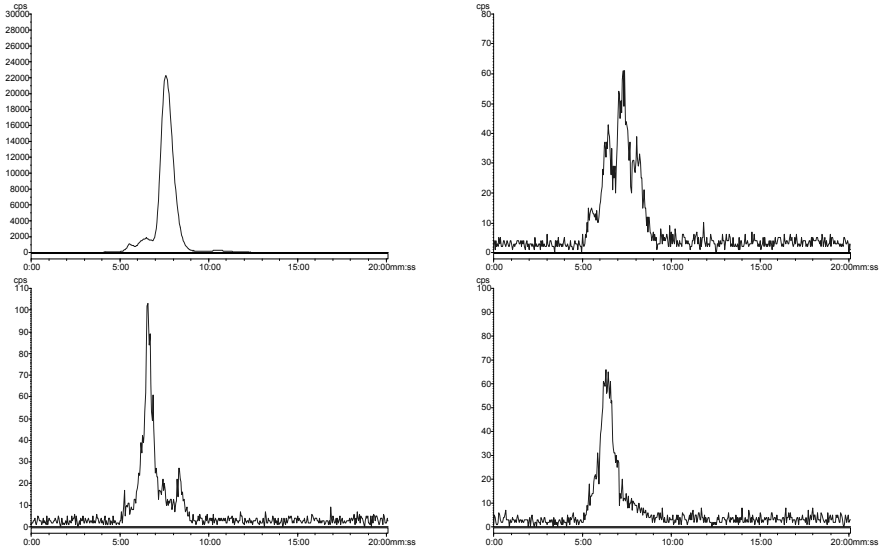


Figure S2. In vivo metabolism analysis of [^{125}I]**4** by radio-HPLC. Top row, left: [^{125}I]**4** control; right, liver 2 mins sample; bottom row, left, liver 10 mins sample; right, liver 30 mins sample.

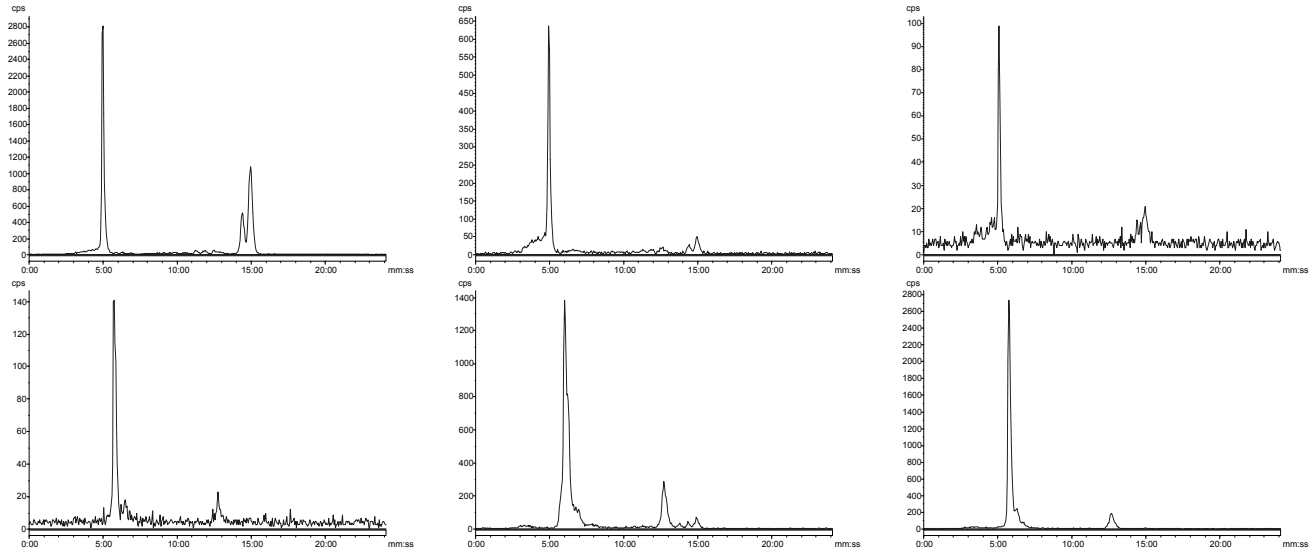


Figure S3. *In vivo* metabolism of [^{18}F]**15** assessed in liver and urine by radio-HPLC. Top row liver extracts, bottom row urine extracts, left to right 2, 15 and 60 minutes respectively.

Experimental Section

Chemistry. Reagents and solvents were purchased from Sigma-Aldrich (Gillingham, United Kingdom) and used without further purification. Potassium hydroxide and potassium carbonate were stored in a vacuum dessicator over phosphorus pentoxide. All reactions were carried out under argon unless otherwise stated. Petroleum ether refers to the fraction that is distilled between 40°C and 60°C. Automated flash chromatography was performed on a CombiFlash Companion machine (Companion Presearch Ltd.), using RediSep 4 g or 12 g normal phase silica cartridges (flow rate 12 mL/min or 26 mL/min). Manual flash chromatography was carried out using Davisil neutral silica (60 Å, 60-200 micron, Fisher Scientific, Loughborough, UK), solvent mixtures are quoted as volume/volume. ^{1}H NMR Spectra were obtained on a Bruker Avance 600 MHz NMR machine and spectra are referenced to residual solvent. Coupling constants (*J*) are given in Hertz (Hz). GC-MS data was acquired under electron ionization using an Agilent 6890N system. Mass

spectra were obtained in positive electrospray ionization mode on a WatersMicromass LCT Premier or a Finnigan MAT 900 XLT machine. Melting points were determined in capillary tubes on a Stuart Scientific SMP1 melting point apparatus and are uncorrected. Solvent mixtures for thin layer chromatography (TLC) are quoted as volume/volume and samples were developed on aluminium backed neutral silica plates (0.2 mm thickness) (Fluka, Seelze, Germany). Purity analysis for compounds **2, 4, 5, 6, 10b-10h, 11b-11h, 12, 13, 14, 15** and **16** was evaluated by analytical HPLC; compounds **9b-9h** were analyzed by GC-MS with only a single peak observed in the GC trace. Tabulated values of purity can be found below and the purity of all compounds was greater than 95%.

(*S*)-*tert*-Butyl 2-(3-fluorophenoxymethyl)pyrrolidine-1-carboxylate (9c). The carboxylate **9c** was prepared according to the procedure for **9b** except using 3-fluorophenol. Chromatography (hexanes/ethyl acetate) afforded the title compound as a colorless oil (0.32 g, 54%). HRMS (ESI) = 296.1657 $(M + H)^+$. Calcd. for $C_{16}H_{23}FNO_3$ 296.1656 ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.20-7.15 (m, 1 H), 6.73-6.69 (m, 1 H), 6.65-6.59 (m, 2 H), 4.18-4.03 (m, 2 H), 3.97-3.74 (m, 1 H), 3.48-3.29 (m, 2 H), 2.05-1.79 (m, 4 H), 1.48 (s, 9 H). TLC (UV_{254}) R_f = 0.40 (3:1 hexanes/ethyl acetate).

(*S*)-*tert*-Butyl 2-(2,4-difluorophenoxymethyl)pyrrolidine-1-carboxylate (9d). The carboxylate **9d** was prepared according to the procedure for **9b** except using 2,4-difluorophenol. Chromatography (hexanes/ethyl acetate) afforded the title compound as a colorless oil (1.92 g, 58%). HRMS (ESI) = 314.1560 $(M + H)^+$. Calcd. for $C_{16}H_{22}F_2NO_3$ 314.1562. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.08-6.76 (m, 3 H), 4.21-3.87

(m, 3 H), 3.47-3.32 (m, 2 H), 2.16-1.85 (m, 4 H), 1.48 (s, 9 H). TLC (UV_{254}) R_f = 0.51 (2:1 hexanes/ethyl acetate).

(*S*)-*tert*-Butyl 2-(3,5-difluorophenoxymethyl)pyrrolidine-1-carboxylate (9e). The carboxylate **9e** was prepared according to the procedure for **9b** except using 3,5-difluorophenol. Chromatography (hexanes/ethyl acetate) afforded the title compound as a colorless oil (0.31 g, 53%). HRMS (ESI) = 314.1563 $(M + H)^+$. Calcd. for $C_{16}H_{22}F_2NO_3$ 314.1562. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.44-6.29 (m, 3 H), 4.11-3.98 (m, 2 H), 3.91-3.72 (m, 1 H), 3.42-3.24 (m, 2H), 1.99-1.79 (m, 4 H), 1.42 (s, 9 H). TLC (UV_{254}) R_f = 0.56 (2:1 hexanes/ethyl acetate).

(*S*)-*tert*-Butyl 2-(4-tetrahydropyranyloxymethyl)pyrrolidine-1-carboxylate (9f). The carboxylate **9f** was prepared according to the procedure for **9b** except using 4-hydroxytetrahydropyran. Chromatography (ethyl acetate) afforded the title compound as a colorless oil (0.14 g, 25%). HRMS (ESI) = 286.2012 $(M + H)^+$. Calcd. for $C_{15}H_{28}NO_4$ 286.2013. ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.97-3.83 (m, 2 H), 3.64-3.23 (m, 9 H), 1.97-1.72 (m, 5 H), 1.60-1.52 (m, 2 H), 1.46 (s, 9 H). TLC (I_2) R_f = 0.62 (ethyl acetate).

(*S*)-5-(2-(3-Fluorophenoxymethyl)-pyrrolidine-1-sulfonyl)isatin (10c). The isatin **10c** was prepared according to the procedure for **10b** except using **9c** to give an orange solid (93 mg, 46%). Mp: 201-203°C. HRMS (ESI) = 405.0933 $(M + H)^+$. Calcd. for $C_{19}H_{18}FN_2O_5S$ 405.0920. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.14 (br, 1 H), 8.09-8.06 (m, 2 H), 7.21 (q, J = 7.2 Hz, 1 H), 7.03 (d, J = 7.8 Hz, 1 H), 6.67-6.62 (m, 2 H), 6.55 (dt, J = 10.8 Hz, J = 2.4 Hz, 1 H), 4.17 (dd, J = 9 Hz, J = 3 Hz, 1 H), 4.01-3.97 (m, 1 H), 3.94 (dd, J = 9 Hz, J = 7.2 Hz, 1 H), 3.54-3.50 (m, 1 H), 3.27-3.25 (m,

1 H), 2.08-1.96 (m, 2 H), 1.88-1.77 (m, 2 H). TLC (UV_{254}) R_f = 0.36 (2:1 ethyl acetate/hexanes). HPLC t_R = 8.27 min.

(*S*)-5-(2-(2,4-Difluorophenoxymethyl)-pyrrolidine-1-sulfonyl)isatin (10d). The isatin **10d** was prepared according to the procedure for **10b** except using **9d** to give an orange solid (0.86 g, 34%). Mp: 185-187°C. HRMS (ESI) = 423.0834 $(M + H)^+$. Calcd. for $C_{19}H_{17}F_2N_2O_5S$ 423.0826. ^1H NMR (600 MHz, $CDCl_3$) δ 8.08-8.06 (m, 2 H), 7.97 (br, 1 H), 7.03 (d, J = 9 Hz, 1 H), 6.97-6.92 (m, 1 H), 6.87-6.77 (m, 2 H), 4.21 (dd, J = 8.4 Hz, J = 2.4 Hz, 1 H), 4.03-3.97 (m, 2 H), 3.56-3.52 (m, 1 H), 3.23-3.17 (m, 1 H), 2.11-2.01 (m, 2 H), 1.88-1.75 (m, 2 H). TLC (UV_{254}) R_f = 0.46 (2:1 ethyl acetate/hexanes). HPLC t_R = 8.12 min.

(*S*)-5-(2-(3,5-Difluorophenoxymethyl)-pyrrolidine-1-sulfonyl)isatin (10e). The isatin **10e** was prepared according to the procedure for **10b** except using **9e** to give an orange solid (112 mg, 53%). Mp: 196-198°C. HRMS (ESI) = 423.0834 $(M + H)^+$. Calcd. for $C_{19}H_{17}F_2N_2O_5S$ 423.0826. ^1H NMR (600 MHz, $CDCl_3$) δ 8.10-8.06 (m, 2 H), 8.04 (br, 1 H), 7.05 (d, J = 8.4 Hz), 6.45-6.38 (m, 3 H), 4.18 (dd, J = 8.4 Hz, J = 2.4 Hz, 1 H), 3.99-3.92 (m, 2 H), 3.56-3.49 (m, 1 H), 3.24-3.17 (m, 1 H), 2.04-1.92 (m, 2 H), 1.86-1.77 (m, 2 H). TLC (UV_{254}) R_f = 0.36 (2:1 ethyl acetate/hexanes). HPLC t_R = 9.18 min.

(*S*)-5-(2-(Tetrahydro-2*H*-pyran-4-yloxymethyl)-pyrrolidine-1-sulfonyl)isatin (10f). The isatin **10f** was prepared according to the procedure for **10b** except using **9f** to give an orange gum (63 mg, 32%). HRMS (ESI) = 395.1282 $(M + H)^+$. Calcd. for $C_{18}H_{23}N_2O_6S$ 395.1277. ^1H NMR (600 MHz, $CDCl_3$) δ 8.17 (br, 1 H), 8.10-8.08 (m, 2

H), 7.07 (d, *J* = 8.4 Hz, 1 H), 3.94-3.90 (m, 2 H), 3.77-3.73 (m, 1 H), 3.70 (dd, *J* = 9.6 Hz, *J* = 3 Hz, 1 H), 3.56-3.51 (septet, *J* = 4.2 Hz, 1 H), 3.48-3.44 (m, 3 H), 3.13-3.11 (m, 1 H), 2.05-1.87 (m, 4 H), 1.72-1.64 (m, 2 H), 1.60-1.52 (m, 2 H). TLC (UV_{254}) R_f = 0.26 (4:1 ethyl acetate/hexanes). HPLC t_R = 2.65 min.

(*S*)-5-(2-(Pyrimidin-4-yloxymethyl)-pyrrolidine-1-sulfonyl)isatin (10g). The isatin **10g** was prepared according to the procedure for **10b** except using **9g.** Chromatography (ethyl acetate) gave an orange gum (51 mg, 27%). HRMS (ESI) = 389.025 $(M + H)^+$. Calcd. for $C_{17}H_{17}N_4O_5S$ 389.020. ^1H NMR (600 MHz, $CDCl_3$) δ 8.79 (s, 1 H), 8.45 (d, *J* = 5.4 Hz, 1 H), 8.12 (d, *J* = 1.8 Hz, 1 H), 8.09 (dd, *J* = 8.4 Hz, *J* = 1.8 Hz, 1 H), 7.95 (br, 1 H), 7.03 (d, *J* = 8.4 Hz, 1 H), 6.72 (d, *J* = 5.4 Hz, 1 H), 4.57 (dd, *J* = 10.8 Hz, *J* = 4.8 Hz, 1 H), 4.39 (dd, *J* = 10.8 Hz, *J* =7.2 Hz, 1 H), 4.08-4.04 (m, 1 H), 3.53-3.46 (m, 1 H), 3.27-3.22 (m, 1 H), 2.01-1.93 (m, 2 H), 1.84-1.74 (m, 2 H). TLC (UV_{254}) R_f = 0.49 (9:1 ethyl acetate/methanol). HPLC t_R = 1.90 min.

(*S*)-5-(2-(2-Propynyloxymethyl)-pyrrolidine-1-sulfonyl)isatin (10h). The isatin **10h** was prepared according to the procedure for **10b** except using **9h** to give an orange gum (92 mg, 53%). HRMS (ESI) = 349.0867 $(M + H)^+$. Calcd. for $C_{16}H_{17}N_2O_5S$ 349.067. ^1H NMR (600 MHz, $CDCl_3$) δ 8.10-8.07 (m, 2 H), 7.84 (br, 1 H), 7.04 (d, *J* = 8.4 Hz, 1 H), 4.16 (s, 2 H), 3.83-3.79 (m, 1 H), 3.72 (dd, *J* = 9.6 Hz, *J* = 3.6 Hz, 1 H), 3.54 (dd, *J* = 9.6 Hz, *J* = 3.6 Hz, 1 H), 3.45-3.43 (m, 1 H), 3.19-3.15 (m, 1 H), 2.46 (t, *J* = 2.4 Hz, 1 H), 1.96-1.89 (m, 2 H), 1.74-1.67 (m, 2 H). TLC (UV_{254}) R_f = 0.28 (2:1 ethyl acetate/hexanes). HPLC t_R =2.93 min.

(*S*)-1-(4-Fluorobenzyl)-5-(2-(3-fluorophenoxymethyl)-pyrrolidine-1-sulfonyl)isatin (11c). The isatin **11c** was prepared according to the procedure for **11b**

except using **10c** to give an orange gum (31 mg, 61%). HRMS (ESI) = 513.1298 (M + H)$^+$. Calcd. for $C_{26}H_{23}F_2N_2O_5S$ 513.1296. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.02 (d, *J* = 1.8 Hz, 1 H), 7.98 (dd, *J* = 8.4 Hz, *J* = 1.8 Hz, 1 H), 7.33-7.29 (m, 2 H), 7.18 (m, 1 H), 7.09-7.04 (m, 2 H), 6.86 (d, *J* = 8.4 Hz, 1 H), 6.66-6.62 (m, 2 H), 6.53 (dt, *J* = 10.8, *J* = 2.4 Hz, 1 H), 4.88 (s, 2 H), 4.15 (dd, *J* = 9.6 Hz, *J* = 3.6 Hz, 1 H), 3.98-3.88 (m, 2 H), 3.51-3.47 (m, 1 H), 3.23-3.18 (m, 1 H), 2.08-1.97 (m, 2 H), 1.84-1.72 (m, 2 H). TLC (UV_{254}) R_f = 0.64 (2:1 ethyl acetate/hexanes). HPLC t_R = 12.57 min.

(*S*)-1-(4-Fluorobenzyl)-5-(2-(2,4-difluorophenoxymethyl)-pyrrolidine-1-sulfonyl)isatin (11d). The isatin **11d** was prepared according to the procedure for **11b** except using **10d** to give an orange gum (32 mg, 60%). HRMS (ESI) = 531.1204 (M + H)$^+$. Calcd. for $C_{26}H_{22}F_3N_2O_5S$ 531.1202. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.03 (d, *J* = 1.8 Hz, 1 H), 7.98 (dd, *J* = 8.4 Hz, *J* = 1.8 Hz, 1 H), 7.33-7.30 (m, 2 H), 7.09-7.06 (m, 2 H), 6.95-6.91 (m, 1 H), 6.88 (d, *J* = 8.4 Hz, 1 H), 6.81-6.76 (m, 2 H), 4.93 (d, *J* = 16.2 Hz, 1 H), 4.92 (d, *J* = 16.2 Hz, 1 H), 4.18 (dd, *J* = 9 Hz, *J* = 3 Hz, 1 H), 4.00-3.95 (m, 2 H), 3.51-3.49 (m, 1 H), 3.21-3.17 (m, 1 H), 2.09-1.98 (m, 2 H), 1.85-1.74 (m, 2 H). TLC (UV_{254}) R_f = 0.67 (2:1 ethyl acetate/hexanes). HPLC t_R = 12.50 min.

(*S*)-1-(4-Fluorobenzyl)-5-(2-(3,5-difluorophenoxymethyl)-pyrrolidine-1-sulfonyl)isatin (11e). The isatin **11e** was prepared according to the procedure for **11b** except using **10e** to give an orange gum (31 mg, 58%). HRMS (ESI) = 531.1213 (M + H)$^+$. Calcd. for $C_{26}H_{22}F_3N_2O_5S$ 531.1202. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.04 (d, *J* = 1.8 Hz, 1 H), 7.99 (dd, *J* = 8.4 Hz, *J* = 1.8 Hz, 1 H), 7.34-7.29 (m, 2 H), 7.09-7.06 (m, 2 H), 6.90 (d, *J* = 8.4 Hz, 1 H), 6.44-6.38 (m, 3 H), 4.93 (s, 2 H), 4.18-4.15 (m, 1 H), 3.94-3.89 (m, 2 H), 3.51-3.48 (m, 1 H), 3.19-3.15 (m, 1 H), 2.03-1.93 (m, 2 H), 1.83-

1.73 (m, 2 H). TLC (UV_{254}) R_f = 0.64 (2:1 ethyl acetate/hexanes). HPLC t_R = 13.00 min.

(*S*)-1-(4-Fluorobenzyl)-5-(2-(tetrahydro-2*H*-pyran-4-yloxymethyl)-pyrrolidine-1-sulfonyl)isatin (11f). The isatin **11f** was prepared according to the procedure for **11b** except using **10f** to give an orange gum (26 mg, 52%). HRMS (ESI) = 503.1646 $(M + H)^+$. Calcd. for $C_{25}H_{28}FN_2O_6S$ 503.1652. ^1H NMR (600 MHz, $CDCl_3$) δ 8.06 (d, *J* = 1.8 Hz, 1 H), 8.01 (dd, *J* = 8.4 Hz, *J* = 1.8 Hz, 1 H), 7.34-7.31 (m, 2 H), 7.10-7.06 (m, 2 H), 6.91 (d, *J* = 8.4 Hz, 1 H), 4.87 (s, 2 H), 3.93-3.88 (m, 2 H), 3.73-3.70 (m, 1 H), 3.67 (dd, *J* = 9 Hz, *J* = 3 Hz, 1 H), 3.55-3.49 (m, 1 H), 3.47-3.41 (m, 4 H), 3.10-3.05 (m, 1 H), 1.97-1.85 (m, 4 H), 1.70-1.63 (m, 2 H), 1.59-1.54 (m, 2 H). TLC (UV_{254}) R_f = 0.55 (4:1 ethyl acetate/hexanes). HPLC t_R = 8.58 min.

(*S*)-1-(4-Fluorobenzyl)-5-(2-(pyrimidin-4-yloxymethyl)-pyrrolidine-1-sulfonyl)isatin (11g). The isatin **11g** was prepared according to the procedure for **11b** except using **10g** to give an orange gum (12 mg, 24%). HRMS (ESI) = 497.1287 $(M + H)^+$. Calcd. for $C_{24}H_{22}FN_4O_5S$ 497.1295. ^1H NMR (600 MHz, $CDCl_3$) δ 8.77 (s, 1 H), 8.43 (d, *J* = 5.4 Hz, 1 H), 8.08 (d, *J* = 1.8 Hz, 1 H), 8.01 (dd, *J* = 8.4 Hz, *J* = 1.8 Hz, 1 H), 7.34-7.32 (m, 2 H), 7.09-7.06 (m, 2 H), 6.89 (d, *J* = 8.4 Hz, 1 H), 6.70 (d, *J* = 5.4 Hz, 1 H), 4.90 (s, 2 H), 4.55 (dd, *J* = 10.8 Hz, *J* = 4.2 Hz, 1 H), 4.37 (dd, *J* = 10.8 Hz, *J* = 7.8 Hz, 1 H), 4.05-4.01 (m, 1 H), 3.50-3.46 (m, 1 H), 3.22-3.19 (m, 1 H), 1.98-1.87 (m, 2 H), 1.81-1.72 (m, 2 H). TLC (UV_{254}) R_f = 0.32 (2:1 ethyl acetate). HPLC t_R = 7.45 min.

(*S*)-1-(4-Fluorobenzyl)-5-(2-(2-propynyloxymethyl)-pyrrolidine-1-sulfonyl)isatin (11h). The isatin **11h** was prepared according to the procedure for **11b** except using **10h** to give an orange gum. Recrystallization from ethyl acetate/hexanes furnished the desired product as orange needles (93 mg, 58%). Mp: 115-117°C HRMS (ESI) = 457.1236 $(M + H)^+$. Calcd. for $C_{23}H_{22}FN_2O_5S$ 457.1233. ^1H NMR (600 MHz, $CDCl_3$) δ 8.07 (d, *J* = 1.8 Hz, 1 H), 8.01 (dd, *J* = 8.4 Hz, *J* = 1.8 Hz, 1 H), 7.43-7.31 (m, 2 H), 7.09-7.04 (m, 2 H), 6.90 (d, *J* = 8.4 Hz, 1 H), 4.91 (s, 2 H), 4.13 (d, *J* = 2.4 Hz, 2 H), 3.81-3.77 (m, 1 H), 3.69 (dd, *J* = 9.6 Hz, *J* = 4.2 Hz, 1 H), 3.51 (dd, *J* = 9 Hz, *J* = 7.2 Hz, 1 H), 3.43-3.40 (m, 1 H), 3.16-3.12 (m, 1 H), 2.43 (t, *J* = 2.4 Hz, 1 H), 1.94-1.87 (m, 2 H), 1.75-1.66 (m, 2 H). TLC (UV_{254}) R_f = 0.62 (2:1 ethyl acetate/hexanes). HPLC t_R = 8.80 min.

(*S*)-1-(2-Propynyl)-5-(2-(2,4-difluorophenoxymethyl)-pyrrolidine-1-sulfonyl)isatin (13). The isatin **13** was prepared according to the procedure for **12** except using **10d** to give an orange solid (0.48 g, 70%). Mp: 101-103°C. HRMS (ESI) = 461.0976 $(M + H)^+$. Calcd. for $C_{22}H_{19}F_2N_2O_5S$ 461.0983. ^1H NMR (600 MHz, $CDCl_3$) δ 8.13 (dd, *J* = 8.4 Hz, *J* = 1.8 Hz, 1 H), 8.07 (d, *J* = 1.8 Hz, 1 H), 7.24 (d, *J* = 8.4 Hz, 1 H), 6.96-6.92 (m, 1 H), 6.85-6.78 (m, 2 H), 4.59 (dd, *J* = 18 Hz, *J* = 2.4 Hz, 1 H), 4.57 (dd, *J* = 18 Hz, *J* = 2.4 Hz, 1 H), 4.24-4.20 (m, 1 H), 4.03-3.98 (m, 2 H), 3.55-3.52 (m, 1 H), 3.26-3.21 (m, 1 H), 2.37 (t, *J* = 2.4 Hz, 1 H), 2.12-2.01 (m, 2 H), 1.88-1.76 (m, 2 H). TLC (UV_{254}) R_f = 0.63 (2:1 ethyl acetate/hexanes). HPLC t_R = 9.60 min.

(*S*)-1-((1-(2-Fluoroethyl)-1*H*-[1,2,3]-triazol-4-yl)methyl)-5-(2(2,4-difluorophenoxymethyl)-pyrrolidine-1-sulfonyl)isatin (15). The isatin **15** was prepared according to the procedure for **14** except using **13** to give an orange gum. Recrystallization from ethyl acetate/hexanes gave an orange solid (94 mg, 57%). Mp:

130-131°C. HRMS (ESI) = 550.1381 $(M + H)^+$. Calcd. for $C_{24}H_{23}F_3N_5O_5S$ 550.1372. ^1H NMR (600 MHz, $CDCl_3$) δ 8.08 (dd, *J* = 8.4 Hz, *J* = 1.8 Hz, 1 H), 8.02 (d, *J* = 1.8 Hz, 1 H), 7.77 (s, 1 H), 7.52 (d, *J* = 8.4 Hz, 1 H), 6.95-6.91 (m, 1 H), 6.82-6.75 (m, 2 H), 5.05 (s, 2 H), 4.79 (dt, *J* = 46.2 Hz, *J* = 4.8 Hz, 2 H), 4.67 (dt, *J* = 26.4 Hz, *J* = 4.8 Hz, 2 H), 4.20 (dd, *J* = 9.6 Hz, *J* = 3 Hz, 1 H), 4.01-3.94 (m, 2 H), 3.53-3.50 (m, 1 H), 3.22-3.17 (m, 1 H), 2.10-1.98 (m, 2 H), 1.85-1.74 (m, 2 H). TLC (UV_{254}) R_f = 0.47 (ethyl acetate). HPLC t_R = 8.45 min.

(*S*)-1-(4-Fluorobenzyl)-5-(2-((1-(2-fluoroethyl)-1*H*-[1,2,3]-triazol-4-yl)methoxymethyl)-pyrrolidine-1-sulfonyl)isatin (16). The isatin **16** was prepared according to the procedure for **14** except using **11h** to give an orange gum (26 mg, 48%). HRMS (ESI) = 546.1632 $(M + H)^+$. Calcd. for $C_{25}H_{26}F_2N_5O_5S$ 546.1623. ^1H NMR (600 MHz, $CDCl_3$) δ 8.03 (d, *J* =1.8 Hz, 1 H), 8.00 (dd, *J* = 8.4 Hz, *J* = 1.8 Hz, 1 H), 7.67 (s, 1 H), 7.35-7.33 (m, 2 H), 7.08-7.05 (m, 2 H), 6.90 (d, *J* = 8.4 Hz, 1 H), 4.95 (s, 2 H), 4.81 (dt, *J* = 46.8 Hz, *J* = 4.8 Hz, 2 H), 4.67 (dt. *J* = 26.4 Hz, *J* = 4.8 Hz, 2 H), 4.62 (d, *J* = 12 Hz, 1 H), 4.60 (d, *J* = 12 Hz, 1 H), 3.81-3.78 (m, 1 H), 3.65 (dd, *J* = 9.6 Hz, *J* = 4.2 Hz, 1 H), 3.51 (dd, *J* = 9.6 Hz, *J* = 7.2 Hz, 1 H), 3.40-3.37 (m, 1 H), 3.19-3.15 (m, 1 H), 1.92-1.87 (m, 2 H), 1.72-1.66 (m, 2 H). TLC (UV_{254}) R_f = 0.35 (ethyl acetate). HPLC t_R = 6.58 min.

Table S1. HPLC purity analysis of intermediate isatin sulfonamides.[a]

Compound No.	Retention Time (mm:ss)	Purity (%)
10b	7.50	97
10c	8.16	98
10d	8.07	99
10e	9.11	98
10f	2.39	99
10g	1.54	98
10h	2.56	99
12	9.00	97
13	9.36	99

[a]Conditions: Phenomenex Luna 50 × 4.6 mm (3 μm) HPLC column attached and a mobile phase of 0.1 M ammonium formate and methanol/acetonitrile (1.8:1 v/v), gradient (50% organic for 1 min; 50 → 90% organic in 14 min; 90% organic for 4 min; 90 → 50% organic phase for 4 min) flow rate 1 mL/min and wavelength 254 nm.

Table S2. HPLC purity analysis of final isatin sulfonamides.[a]

Compound No.	Retention Time (mm:ss)	Purity (%)
2	12.00	97
4	14.07	98
5	12.08	98
6	6.50	98
11b	12.15	99
11c	12.34	98
11d	12.30	98
11e	13.00	99
11f	8.35	97
11g	7.27	99
11h	8.48	99
14	7.56	99
15	8.27	98
16	6.35	98

[a]Conditions: Phenomenex Luna 50 × 4.6 mm (3 μm) HPLC column attached and a mobile phase of 0.1 M ammonium formate and methanol/acetonitrile (1.8:1 v/v), gradient (50% organic for 1 min; 50 → 90% organic in 14 min; 90% organic for 4 min; 90 → 50% organic phase for 4 min) flow rate 1 mL/min and wavelength 254 nm.

.

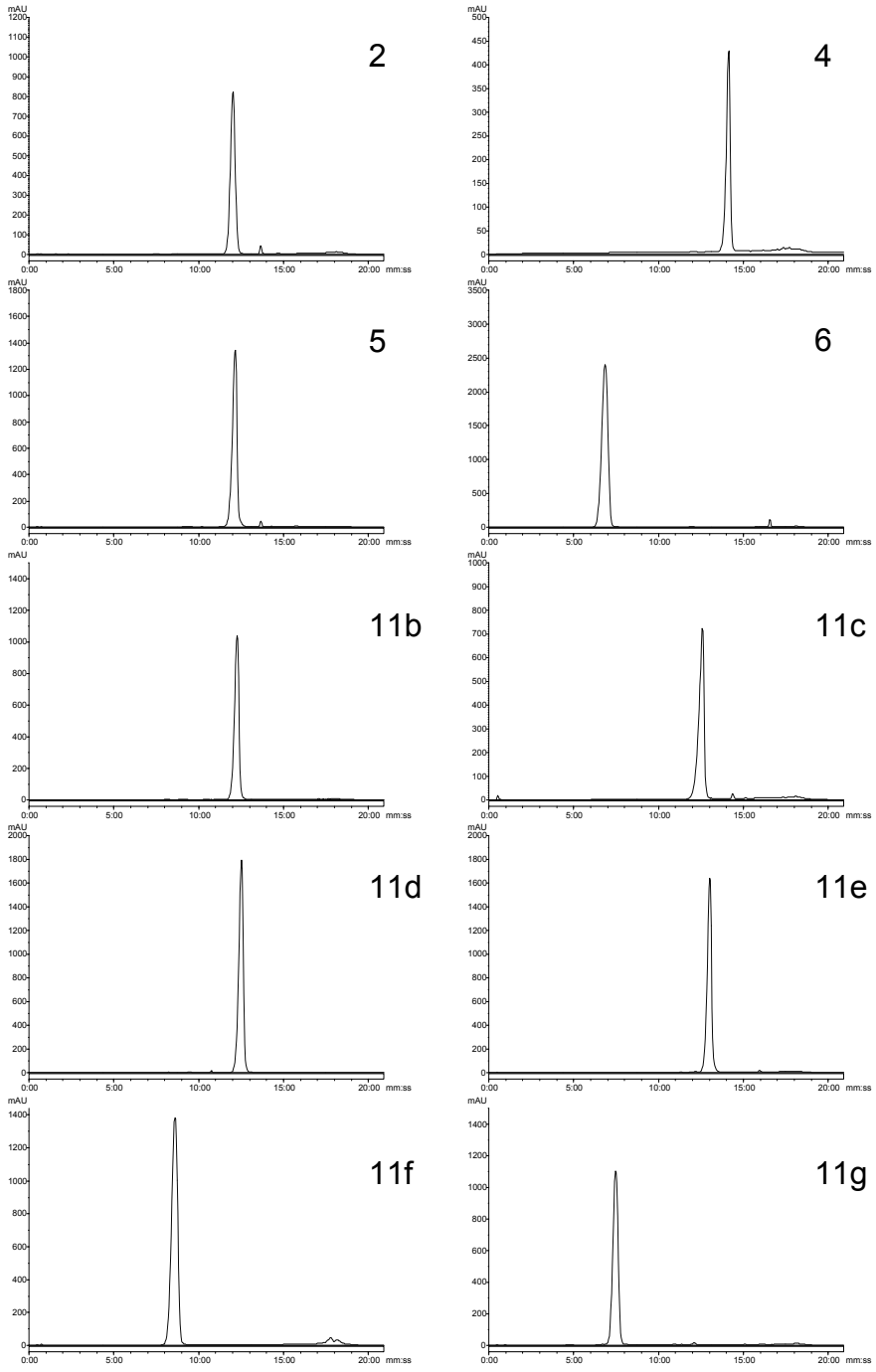


Figure S4. HPLC chromatograms for compounds **2-11g** screened for caspase affinity. Conditions: Phenomenex Luna 50 × 4.6 mm (3 μm) HPLC column attached and a mobile phase of 0.1 M ammonium formate and methanol/acetonitrile (1.8:1 v/v), gradient (50% organic for 1 min; 50 → 90% organic in 14 min; 90% organic for 4 min; 90 → 50% organic phase for 4 min) flow rate 1 mL/min and wavelength 254 nm.

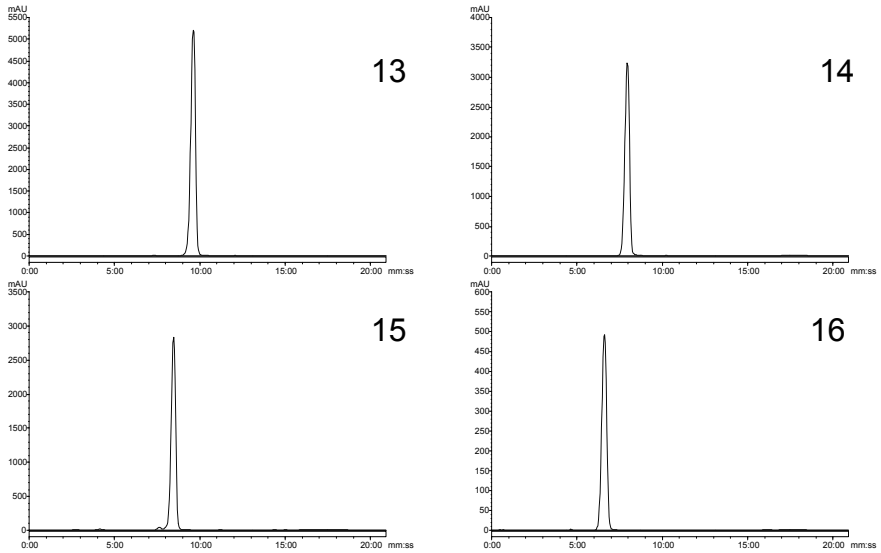
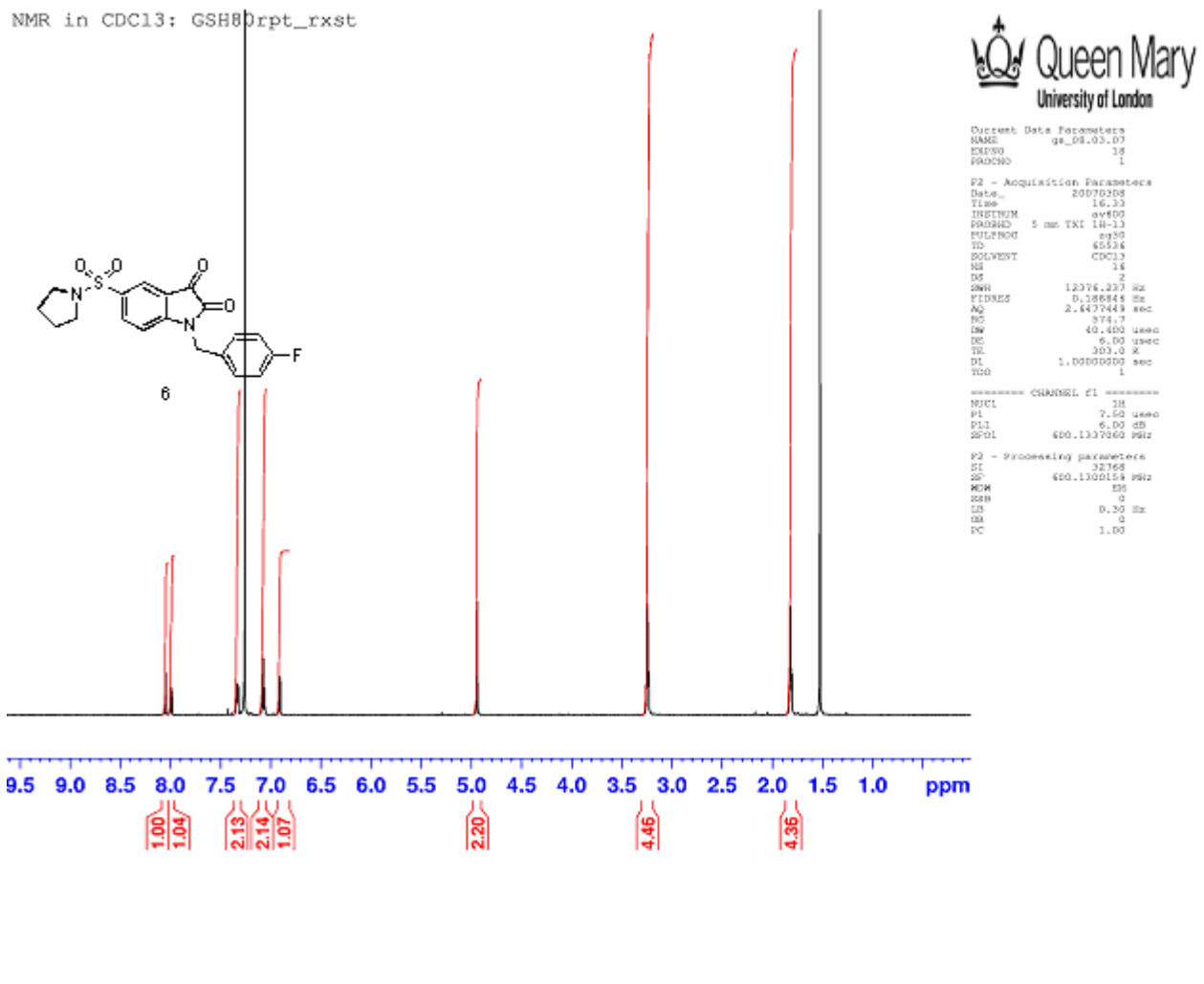


Figure S5. HPLC chromatograms for compounds **13-16** screened for caspase affinity. Conditions: Phenomenex Luna 50 × 4.6 mm (3 μm) HPLC column attached and a mobile phase of 0.1 M ammonium formate and methanol/acetonitrile (1.8:1 v/v), gradient (50% organic for 1 min; 50 → 90% organic in 14 min; 90% organic for 4 min; 90 → 50% organic phase for 4 min) flow rate 1 mL/min and wavelength 254 nm.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6
Queen Mary
University of London
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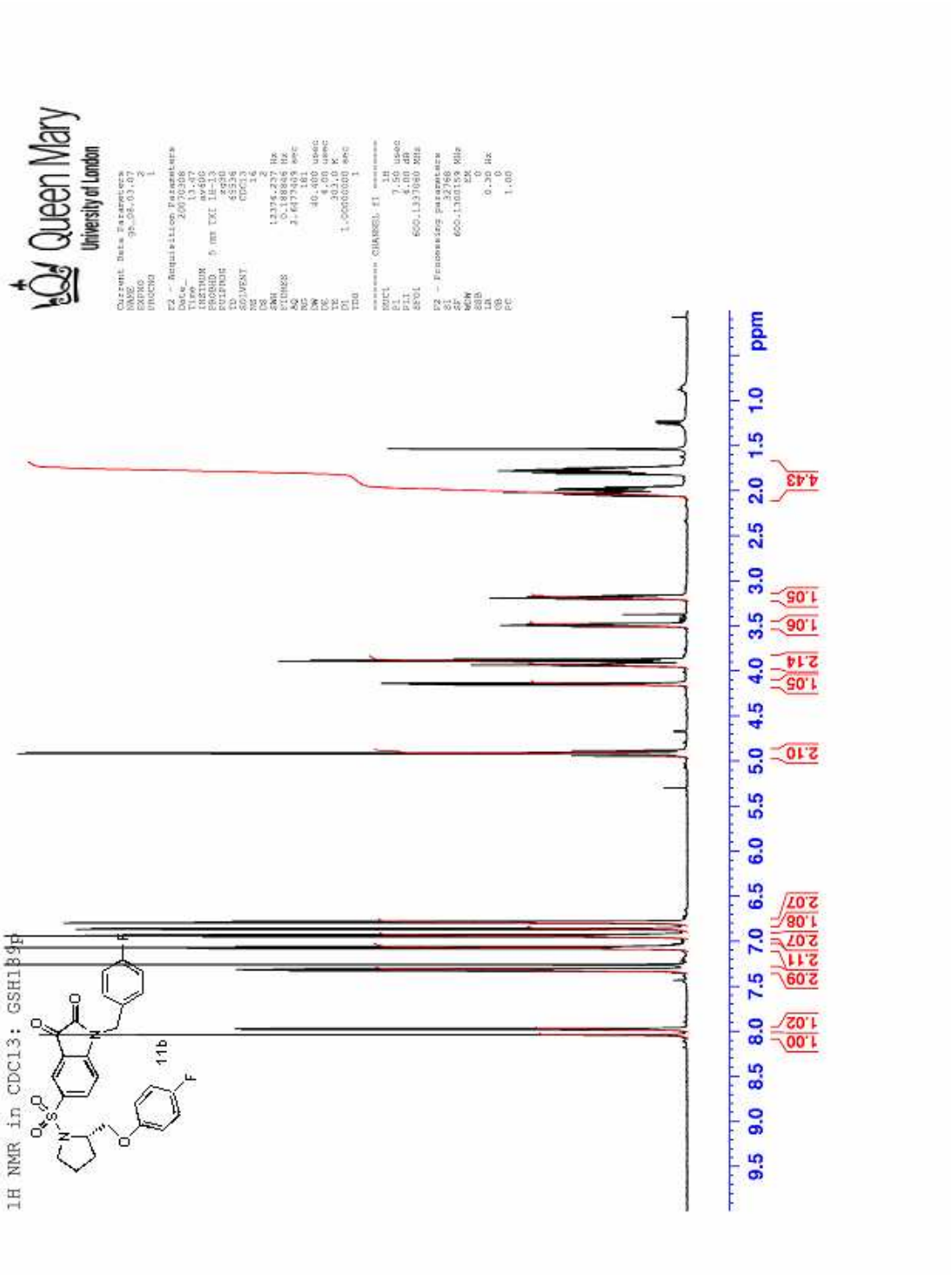
2.13
2.14
1.07
2.20
4.46
4.36


Queen Mary
University of London
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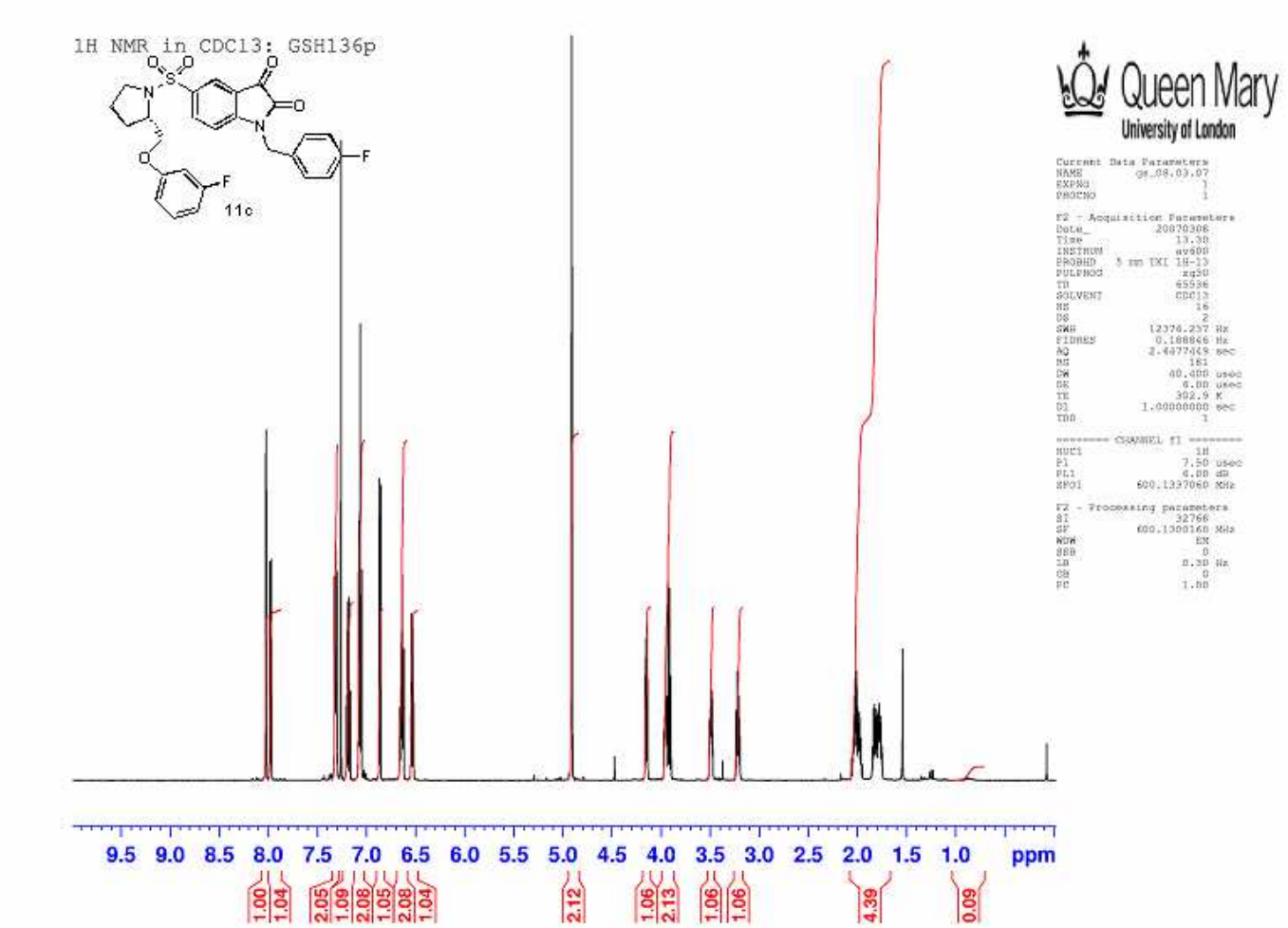
1.00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Queen Mary
University of London
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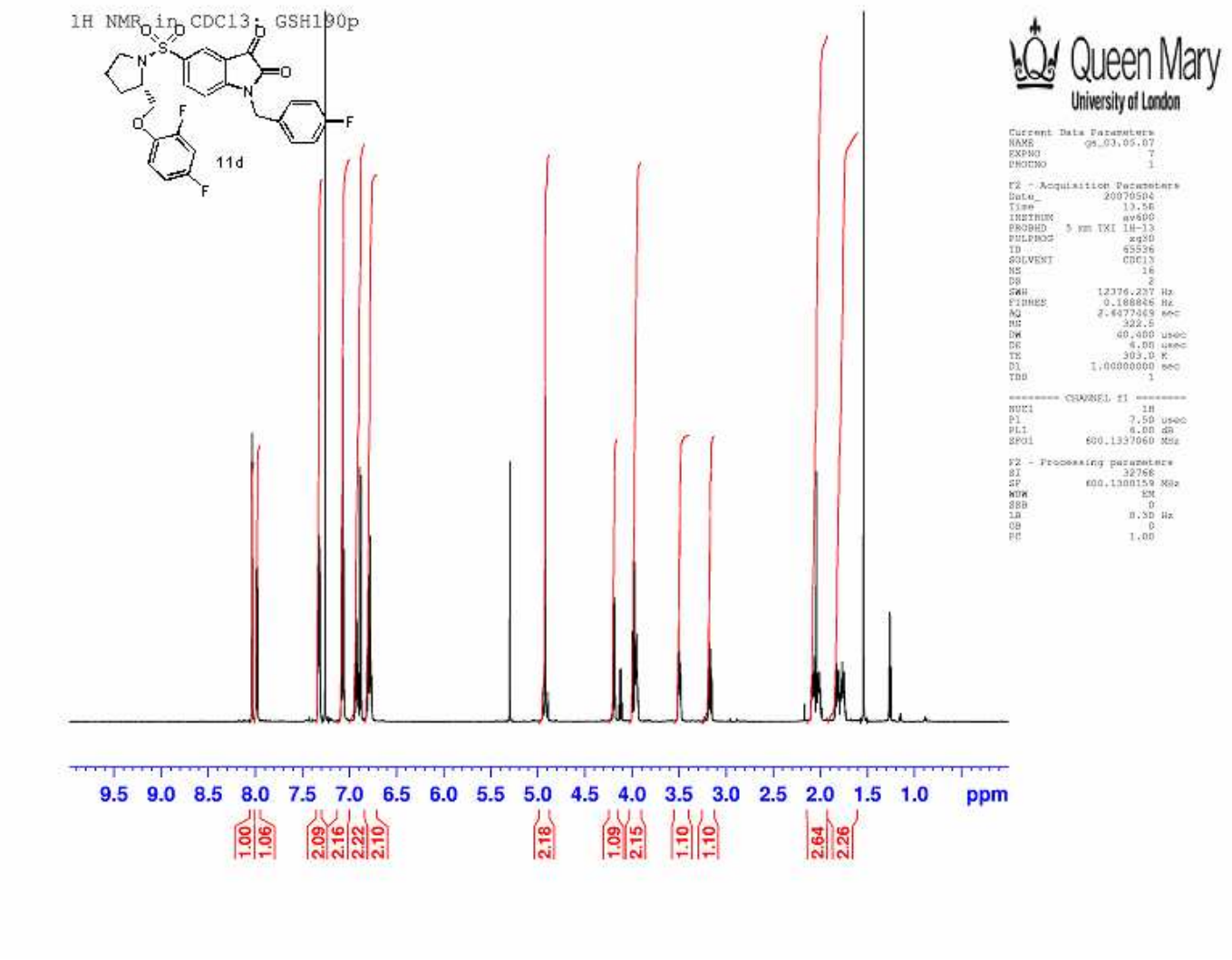
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Queen Mary
University of London
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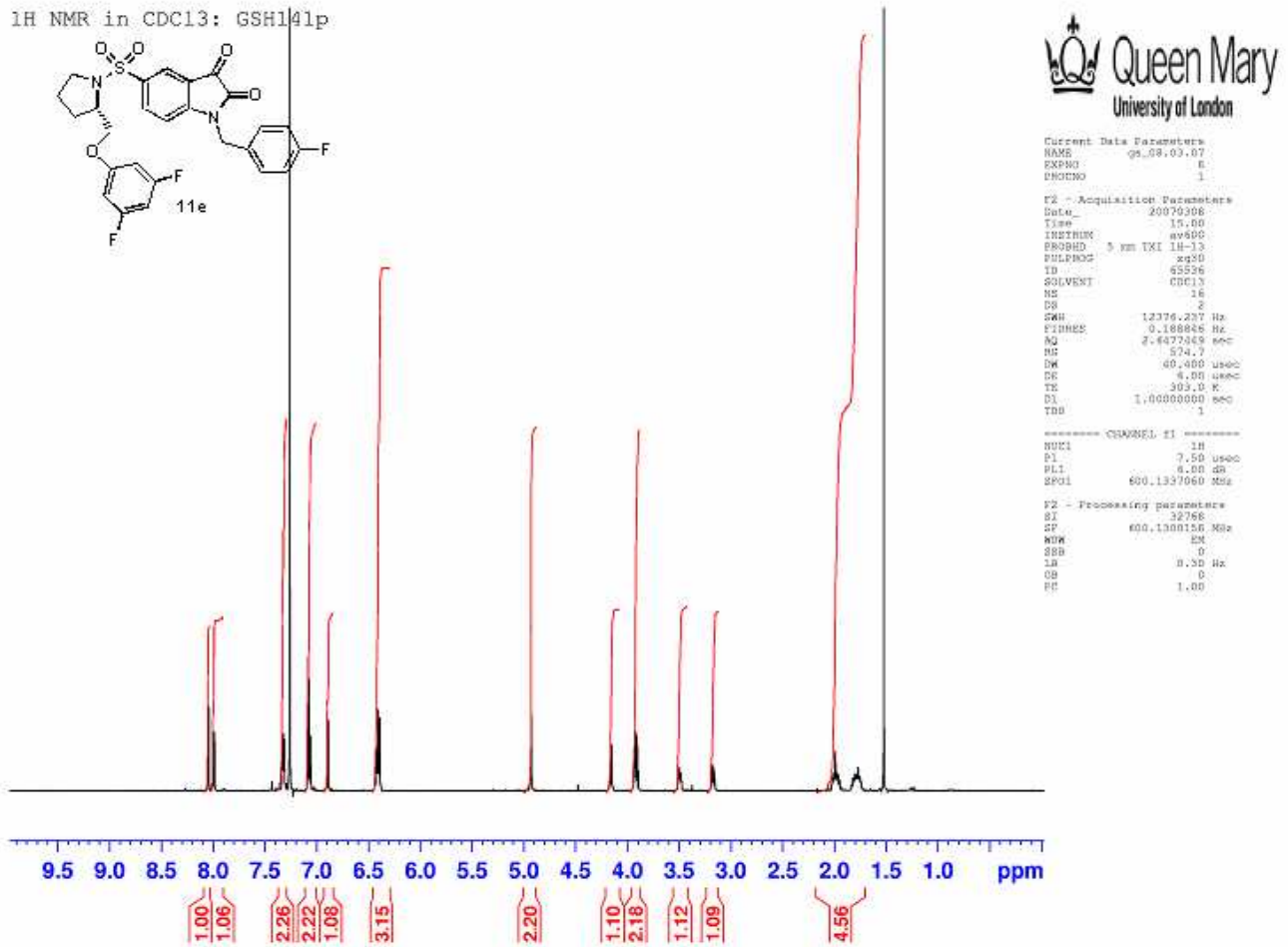
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Queen Mary
University of London
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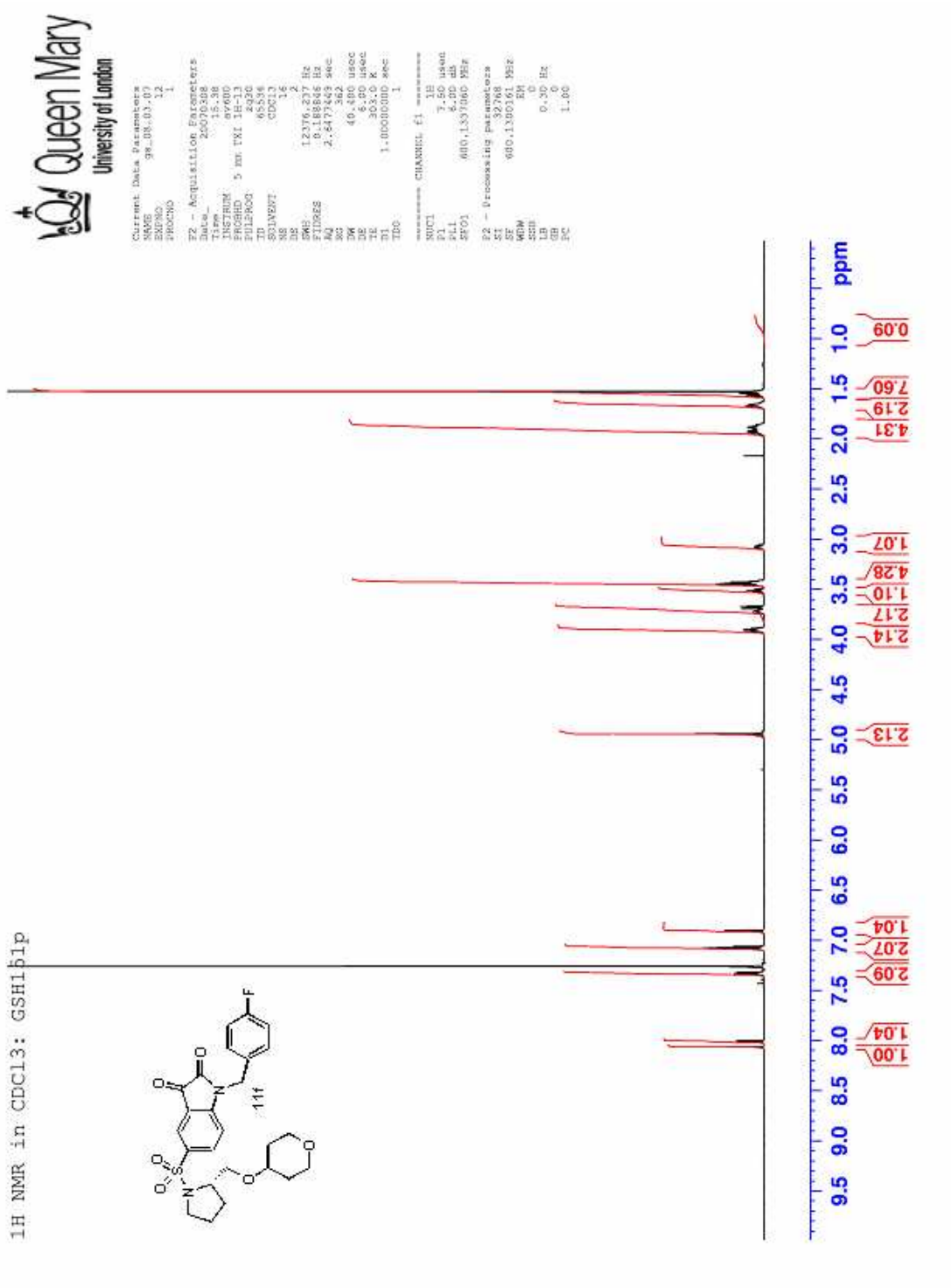
Queen Mary
University of London
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1.00
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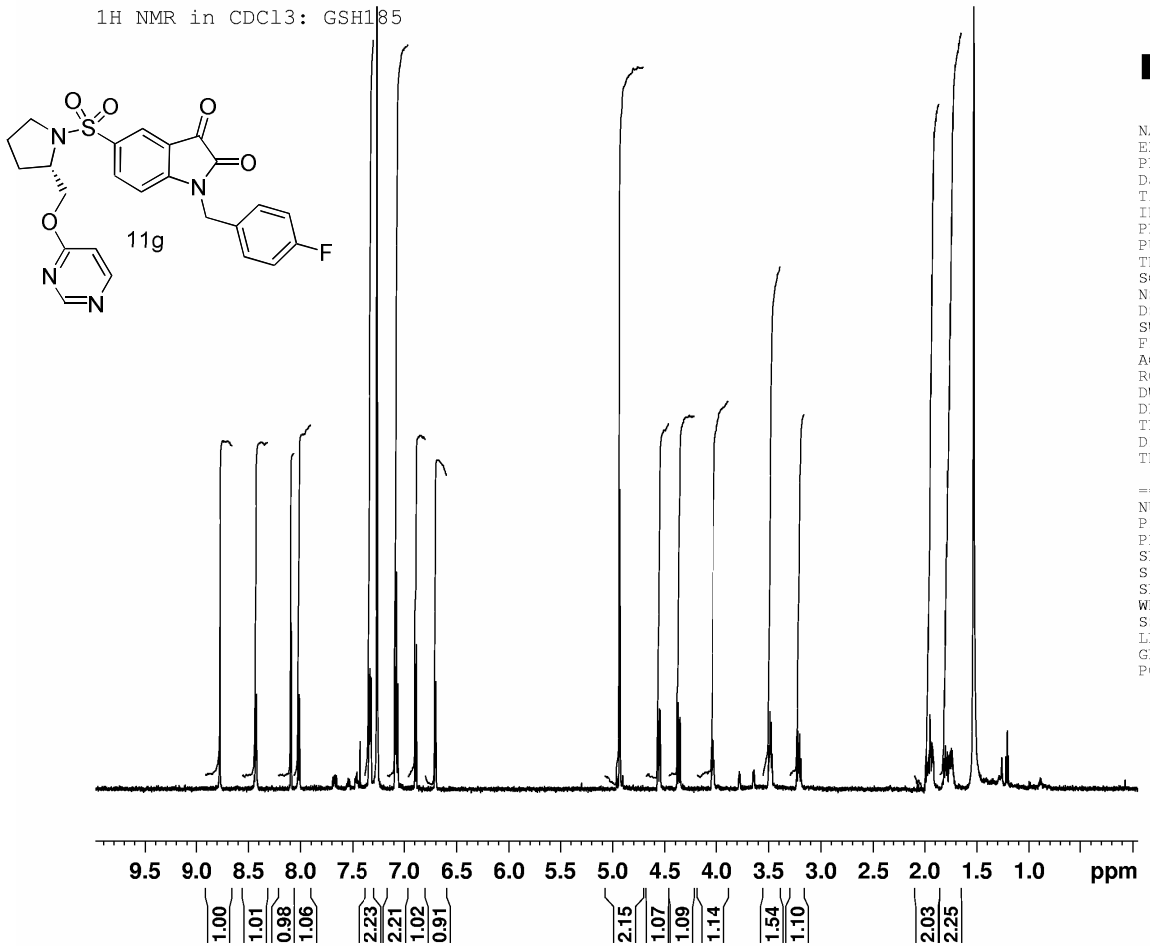

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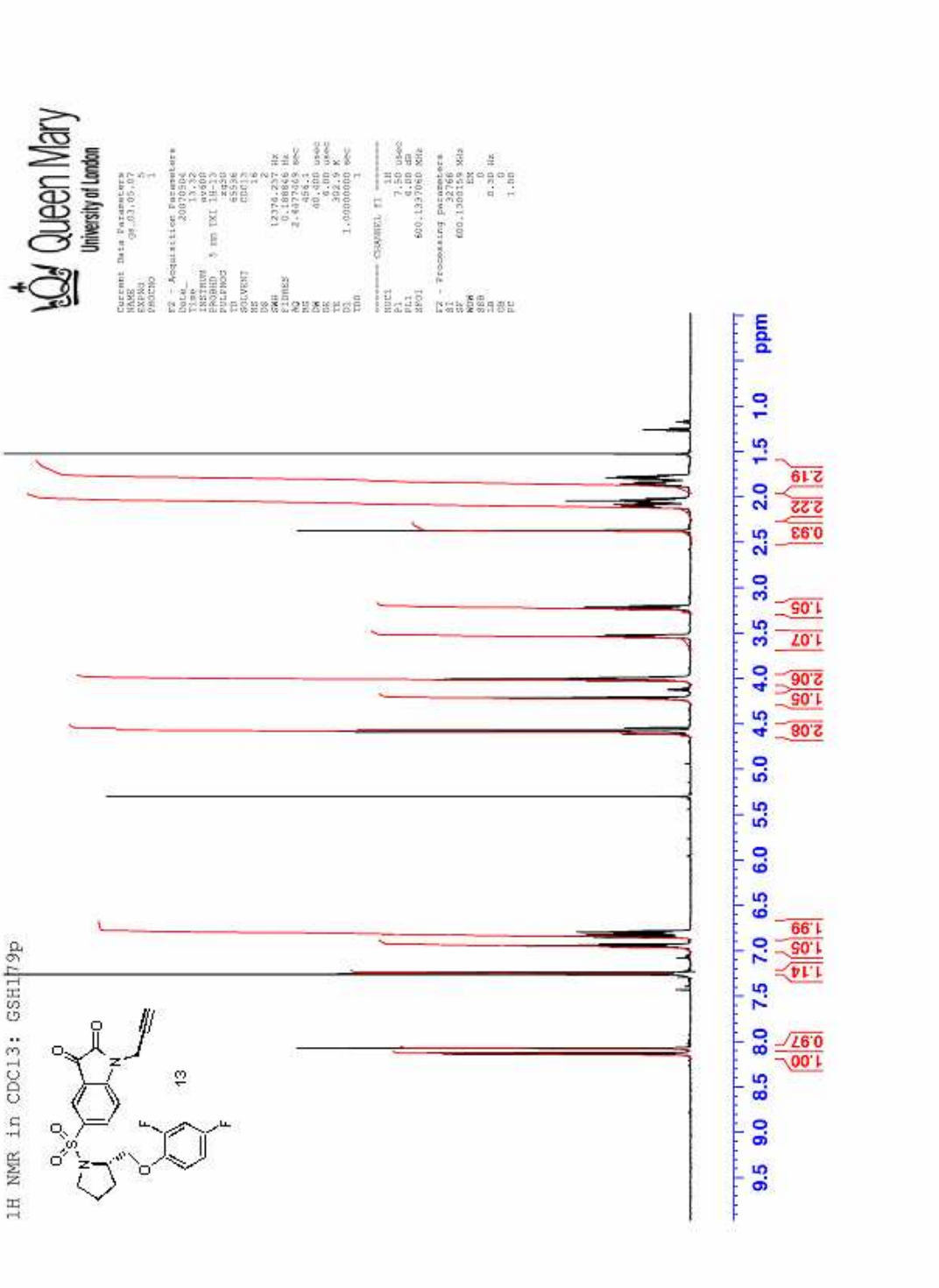
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Queen Mary
University of London
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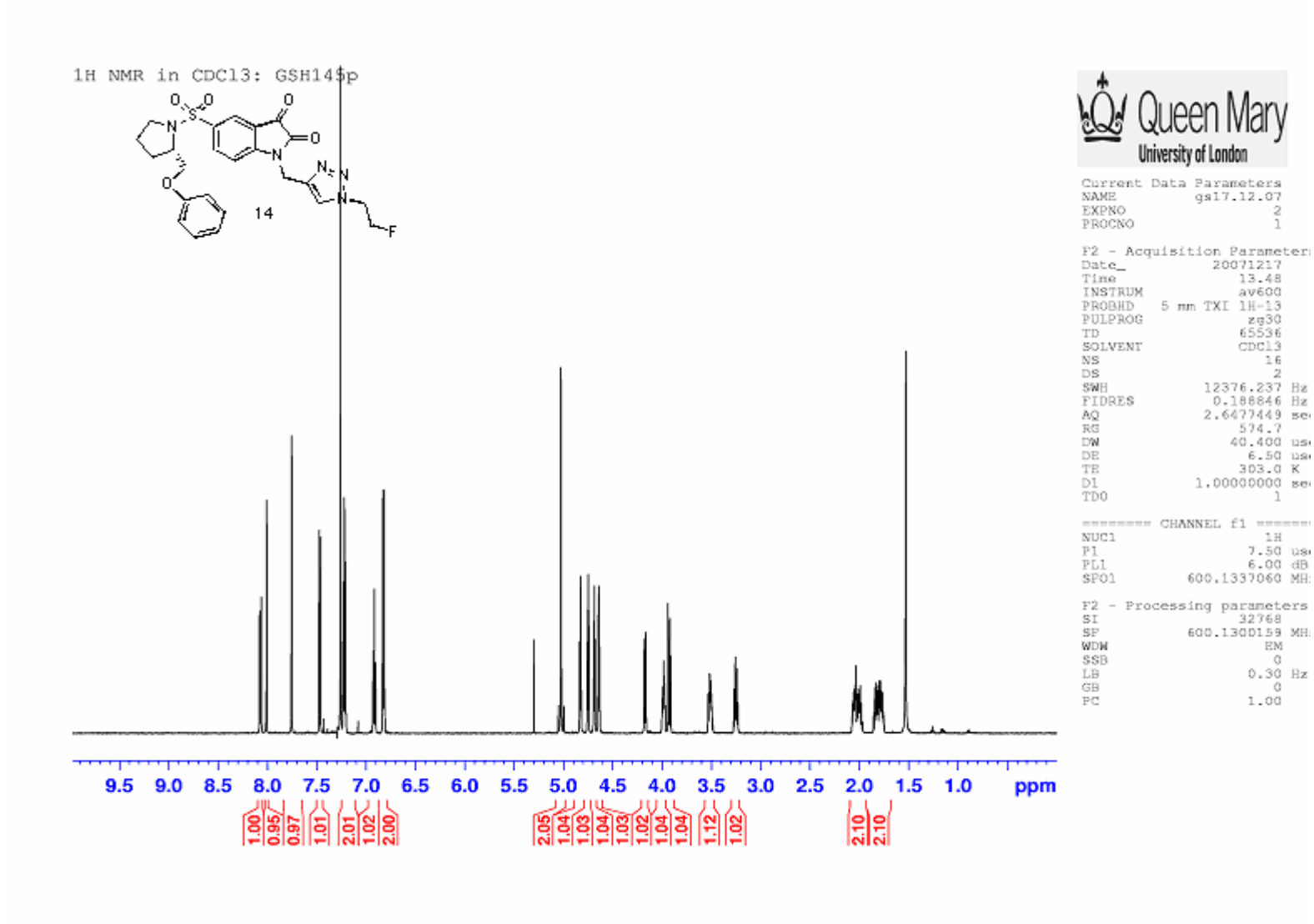
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University of London
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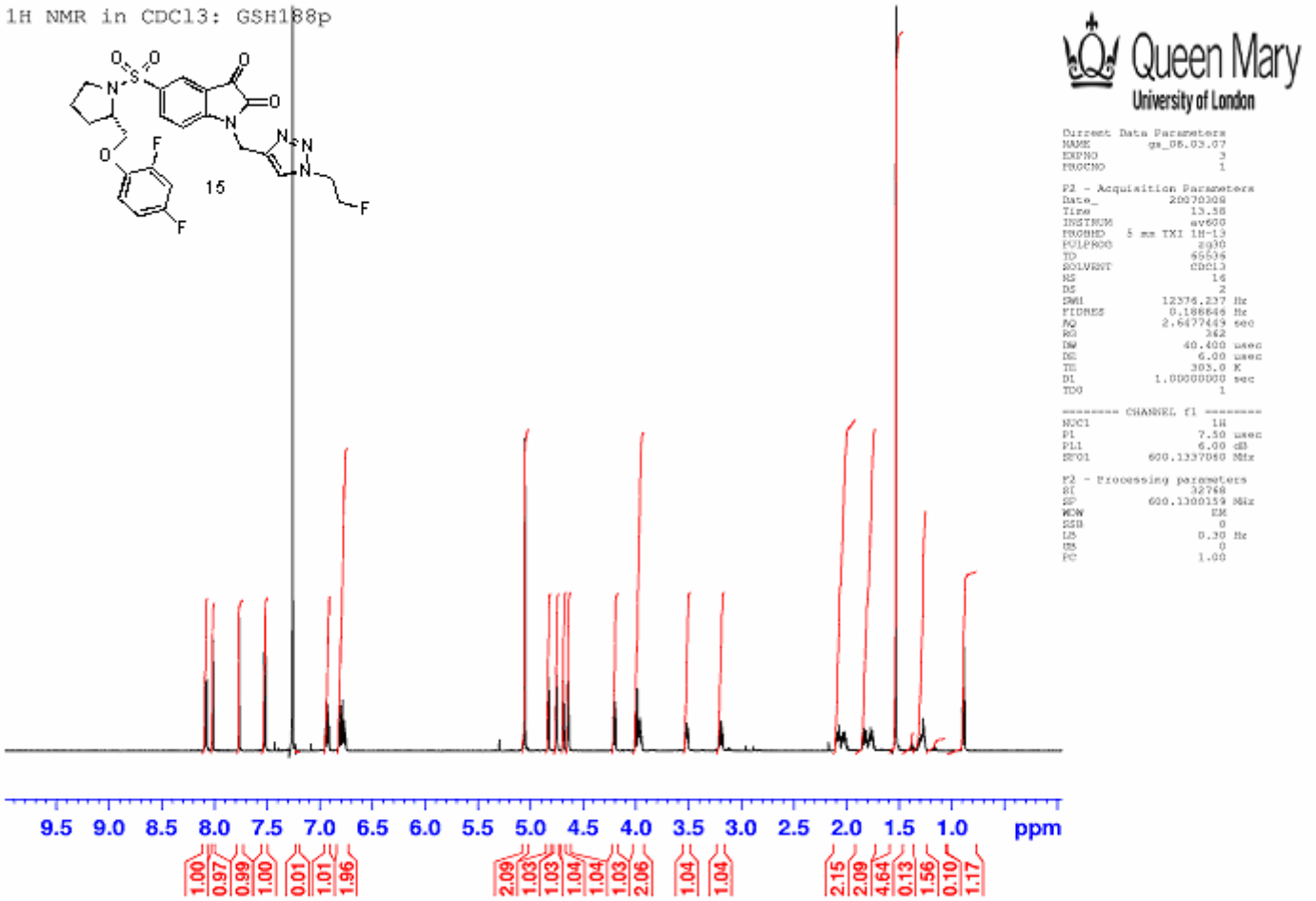
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Queen Mary
University of London
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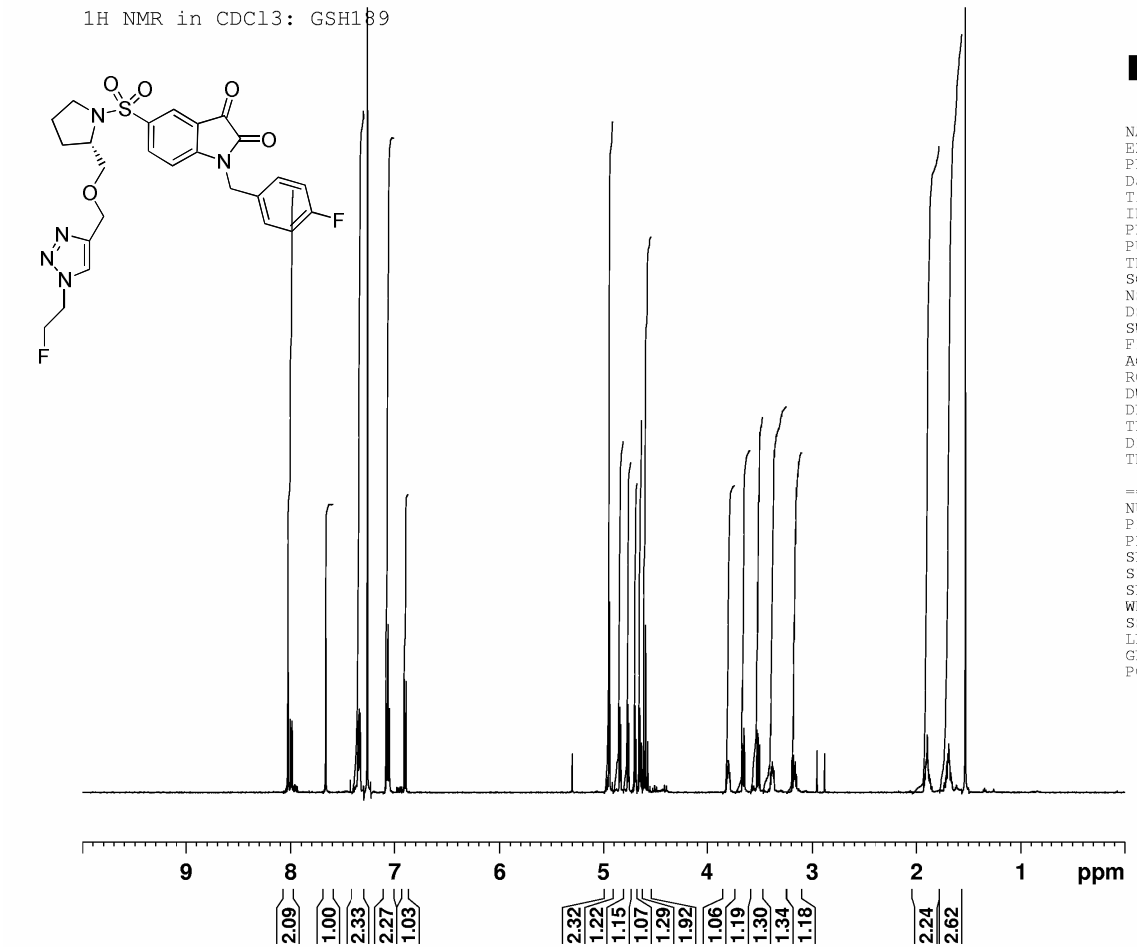

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