Radiosynthesis of [^{18}F]Brequinar for *in vivo* PET Imaging of *h*DHODH for Potential Studies of Acute Myeloid Leukemia and Cancers

Vinay Kumar[1†], Stefano Sainas[3†], Elena Martino[3], Jiacheng Wang[1], Marco Lucio Lolli [3‡], Yu-Shin Ding, PhD[1,2, ‡*]

[1]Department of Radiology, New York University School of Medicine, New York, NY, USA.
[2]Department of Psychiatry, New York University School of Medicine, New York, NY, USA.
[3]Department of Drug Science and Technology (DSTF), University of Torino, Turin, Italy.

† equal contribution as the 1st author
‡ equal contribution as the senior author
*corresponding author

Supporting Information

Table of Content	
Page S2 – S4	Chemical Synthesis
Page S5 -S15	^{1}H-NMR and ^{13}C-NMR for Compounds 1-5
Page S16	HPLC analysis of borono-precursor (Compound **4**)
Page S17	HPLC analysis of Sn-precursor (Compound **5**)
Page S18	Figure S1. UPLC gradient method
Page S19	Figure S2. GE Healthcare Tracerlab FX2N automodule instrument design and software flow diagram
Page S20	Figure S3. Analytical QC HPLC (analysis was performed using the gradient method) chromatogram of protected [^{18}F]brequinar (intermediate).
Page S21	Figure S4: Analytical QC HPLC (analysis was performed using the isocratic method) chromatogram of [^{18}F]brequinar.
Page S22	Figure S5. PET/CT dynamic images_video.pptx
Page S23	Table S1. Preparation of reagents for the GE Tracerlab FX2N synthesis module (should be right after Figure S2)

Chemical Synthesis

Procedures for the synthesis of compounds 1-5 are described in the Supporting Information (SI)

1-(2'-Fluoro-[1,1'-biphenyl]-4-yl)propan-1-one (**1**). 1-(4-Bromophenyl)propan-1-one (1.00 g, 4.69 mmol), K_2CO_3 (1.95 g, 14.08 mmol), and $Pd[P(Ph)_3]_4$ (0.54 g, 0.94 mmol) was dissolved in 100 mL of a solution dioxane/water (8:2 v / v) kept under nitrogen atmosphere. The mixture was stirred for 85 minutes, then (2-fluorophenyl)boronic acid (1.31 g, 9.39 mmol) was added, and the reaction mixture was stirred at 100 °C for 5 hours. Upon completion, the reaction mixture was cooled to room temperature, water (500 mL) was added, and the resulting mixture was extracted using EtOAc (3 x 100 mL). The combined organic layers were washed with brine, dried over anhyd. Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash chromatography (eluent: *petroleum ether* / EtOAc 90/10 v / v) to afford the title compound as a white solid. Yield 95 %. ^{1}H NMR (600 MHz, *Chloroform-d*): δ 1.25 (*t*, 3H, J = 7.2 Hz, -CH_2CH_3), 3.04 (*q*, 2H, J = 7.2 Hz, -CH_2CH_3), 7.17 (*ddd*,1H, J = 10.7, 8.3, 1.0 Hz), 7.24 (*td*, 1H, J = 7.5, 1.1 Hz), 7.33 – 7.40 (*m*,1H), 7.46 (*td*, 1H, J = 7.7, 1.7 Hz), 7.65 (*dd*, 2H, J = 8.4, 1.6 Hz), 8.04 (*d*, 2H, J = 8.4 Hz). ^{13}C NMR (151 MHz, *Chloroform-d*): δ 8.4 (-CH_2CH_3), 32.0 (-CH_2CH_3), 116.4 (*d*, J = 22.4 Hz), 124.7 (*d*, J = 3.8 Hz), 128.1 (*d*, J = 13.1 Hz), 128.3, 129.3 (*d*, J = 2.5 Hz), 130.0 (*d*, J = 8.3 Hz), 130.7 (*d*, J = 2.5 Hz), 136.0, 140.4, 159.9 (*d*, J = 249.1 Hz, C-F), 200.5. (MS) (ES^+) 229 (M+1).

***General procedure for the synthesis of compounds* 2a-d**:

KOH (6.0 eq.) was added to a solution of appropriate isatin (1.5 eq.) in EtOH/H_2O (2 : 1 v / v). The resulting dark solution was stirred at room temperature for 15 min then 4'-(2-fluorophenyl)propiophenone (1.0 eq.) was added and the mixture was heated at reflux overnight. Upon completion of the reaction, checked by TLC, the mixture was cooled to room temperature and concentrated under reduced pressure to remove EtOH, then diluted with 1M KOH solution. The basic mixture was then acidified until pH 5 by adding acetic acid and observing precipitation. The solid precipitate was isolated by filtration and recrystallized with EtOH to afford the title compound as a solid.

2-(2'-Fluoro-[1,1'-biphenyl]-4-yl)-3-methylquinoline-4-carboxylic acid (**2a**). White solid. Yield 88 %. ^{1}H NMR (600 MHz, *DMSO-d_6*): δ 2.46 (*s*, 3H, -CH_3), 7.33 – 7.39 (*m*, 2H), 7.44 – 7.49 (*m*, 1H), 7.64 (*td*, 1H, J = 7.9, 1.4 Hz), 7.66 – 7.83 (*m*, 7H), 8.06 (*d*, 1H, J = 8.3 Hz). ^{13}C NMR (151 MHz, *DMSO-d_6*): δ 17.6 (-CH_3), 116.2 (*d*, J = 22.3 Hz), 122.5, 123.9, 124.4, 125.1 (*d*, J = 2.6 Hz), 127.6, 127.8 (*d*, J = 12.9 Hz), 128.6 (*d*, J = 1.8 Hz), 129.2, 129.4, 129.5, 129.9 (*d*, J = 8.2 Hz), 130.8 (*d*, J = 3.2 Hz), 135.1, 139.6, 141.3, 145.7, 159.2 (*d*, J = 245.5 Hz, C-F), 160.0, 168.9. (MS) (ES^-) 356 (M-1).

6-Fluoro-2-(2'-fluoro-[1,1'-biphenyl]-4-yl)-3-methylquinoline-4-carboxylic acid (**2b**). White solid. Yield 88 %. ^{1}H NMR (600 MHz, *DMSO-d_6*): δ 2.47 (*s*, 3H, -CH_3), 7.33 – 7.39 (*m*, 2H), 7.43 – 7.49 (*m*, 1H), 7.50 (*dd*, 1H, J = 9.7, 2.7 Hz), 7.63 (*td*, 1H, J = 7.8, 1.5 Hz), 7.69 – 7.76 (*m*, 5H), 8.15 (*dd*, 1H, J = 9.2, 5.6 Hz). ^{13}C NMR (151 MHz, *DMSO-d_6*): δ 17.8 (-CH_3), 107.7 (*d*, J = 23.5 Hz), 116.2 (*d*, J = 22.1 Hz), 119.7 (*d*, J = 25.9 Hz), 123.2 (*d*, J = 10.0 Hz), 125.1 (*d*, J = 2.6 Hz), 125.4, 127.7 (*d*, J = 13.0 Hz), 128.6, 129.4, 129.9 (*d*, J = 8.4 Hz), 130.8 (*d*, J = 2.4 Hz), 132.4 (*d*, J = 9.7 Hz), 135.2, 139.3, 140.4 (*d*, J = 5.2 Hz), 143.0, 159.2 (*d*, J = 246.4 Hz, C-F), 159.6, 160.4 (*d*, J = 246.7 Hz, C-F), 168.4, (MS) (ES^-) 374 (M-1).

6-Bromo-2-(2'-fluoro-[1,1'-biphenyl]-4-yl)-3-methylquinoline-4-carboxylic acid (**2c**). White solid. Yield 63 %. ^{1}H NMR (600 MHz, *DMSO-d_6*): δ 2.46 (*s*, 3H, -CH_3), 7.32 – 7.38 (*m*, 2H), 7.44 – 7.50 (*m*, 1H), 7.63 (*dd*, 1H, J = 8.6, 7.5 Hz), 7.70 (*d*, 2H, J = 7.7 Hz), 7.75 (*d*, 2H, J = 8.2 Hz), 7.92 (*dd*, 1H, J = 8.9, 2.2 Hz), 7.95 (*d*, 1H, J = 1.9 Hz), 8.01 (*d*, 1H, J = 8.9 Hz). ^{13}C NMR (151 MHz, *DMSO-*

d$_6$): δ 17.8 (-*C*H$_3$), 116.2 (*d*, J = 22.5 Hz), 120.7, 123.7, 125.1 (*d*, J = 2.5 Hz), 125.6, 126.2, 127.7 (*d*, J = 13.1 Hz), 128.6, 129.40, 129.9 (*d*, J = 8.3 Hz), 130.8 (*d*, J = 2.1 Hz), 131.6, 132.7, 135.3, 139.2, 140.1, 144.3, 159.2 (*d*, J = 245.8 Hz, C-F), 160.4, 168.4. (MS) (ES$^-$) 434/436 (M-1).

2-(2'-Fluoro-[1,1'-biphenyl]-4-yl)-6-iodo-3-methylquinoline-4-carboxylic acid (**2d**). White solid. Yield 86 %. ^{1}H NMR (600 MHz, *DMSO-d*$_6$): *δ* 2.46 (*s*, 3H, -C*H*$_3$), 7.33 – 7.38 (*m*, 2H), 7.44 – 7.49 (*m*, 1H), 7.63 (*td*, 1H, J = 7.9, 1.4 Hz), 7.71 (*d*, 2H, J = 8.1 Hz), 7.75 (*d*, 2H, J = 8.3 Hz,), 7.92 (*dd*, 1H, J = 8.9, 2.1 Hz), 7.95 (*d*, 1H, J = 2.0 Hz), 8.02 (*d*, 1H, J = 8.9 Hz). ^{13}C NMR (151 MHz, *DMSO-d*$_6$): *δ* 17.8 (-*C*H$_3$), 116.2 (*d*, J = 22.1 Hz), 120.7, 123.7, 125.1 (*d*, J = 3.1 Hz), 125.6, 126.2, 127.7 (*d*, J = 13.0 Hz), 128.6, 129.4, 129.9 (*d*, J = 8.2 Hz), 130.8 (*d*, J = 3.3 Hz), 131.6, 132.7, 135.3, 139.2, 140.1, 144.3, 159.2 (*d*, J = 245.7 Hz, C-F), 160.4, 168.4. (MS) (ES$^-$) 482 (M-1).

***General procedure for the synthesis of compounds* 3a-d**:
Cs_2CO_3 (3.0 eq.) was added to a solution of the corresponding brequinar analogue **2a - d** (1.0 eq.) in dry DMF (25 mL). The resulting suspension was stirred for 15 min at room temperature, then 4-nitrobenzylbromide (1.2 eq.) was added, and the reaction mixture was stirred at room temperature for 3 hours. The pale pink suspension was diluted with water (150 mL), observing precipitation. The precipitate was isolated by filtration, dried, and then triturated with hexane. The obtained solid was crystallized from ethanol to afford the final compound as a solid.

4-Nitrobenzyl 2-(2'-fluoro-[1,1'-biphenyl]-4-yl)-3-methylquinoline-4-carboxylate (**3a**). White solid. Yield 88 %. ^{1}H NMR (600 MHz, *Chloroform-d*): δ 2.44 (*s*, 3H, Ar-C*H*$_3$), 5.64 (*s*, 2H, -OC*H*$_2$Ar), 7.19 (*ddd*, 1H, J = 10.7, 8.2, 1.1 Hz), 7.23 – 7.27 (*m*, 1H), 7.33 – 7.38 (*m*, 1H), 7.49 (*dt*, 1H, J = 7.7, 1.8 Hz), 7.57 (*ddd*, 1H, J = 8.3, 6.9, 1.2 Hz), 7.64 - 7.71 (*m*, 7H), 7.73 (*ddd*, 1H, J = 8.4, 6.9, 1.3 Hz), 8.18 (*d*, 1H, J = 8.3 Hz), 8.28 (*d*, 2H, J = 8.8 Hz). ^{13}C NMR (151 MHz, *Chloroform-d*): δ 18.2 (Ar-*C*H$_3$), 66.2 (-O*C*H$_2$Ar), 116.3 (*d*, J = 22.9 Hz), 123.4, 123.9, 124.2, 124.6 (*d*, J = 3.2 Hz), 125.8, 127.9, 128.6 (*d*, J=13.2 Hz), 129.2 (2 x C), 129.3 (*d*, J = 2.5 Hz), 129.4 (*d*, J = 8.4 Hz), 129.7, 130.0, 130.9 (*d*, J = 3.6 Hz), 136.4, 138.8, 142.2, 146.3, 148.2, 159.9 (*d*, J = 248.3 Hz), 160.4, 167.7. (MS) (ES$^+$) 493 (M+1).

4-Nitrobenzyl-6-fluoro-2-(2'-fluoro-[1,1'-biphenyl]-4-yl)-3-methylquinoline-4-carboxylate (**3b**). White solid. Yield 86 %. ^{1}H NMR (600 MHz, *Chloroform-d*): δ 2.44 (s, 3H, Ar-CH$_3$), 5.64 (s, 2H, -OCH$_2$Ar), 7.16 – 7.22 (m, 1H), 7.23 – 7.27 (m, 1H), 7.31 (d, 1H, J = 9.2 Hz), 7.33 – 7.39 (m, 1H), 7.47 – 7.53 (m, 2H), 7.64 (d, 2H, J = 7.5 Hz), 7.66 – 7.72 (m, 4H), 8.17– 8.23 (m, 1H), 8.29 (d, 2H, J = 7.8 Hz). ^{13}C NMR (151 MHz, *Chloroform-d*): δ 18.4 (Ar-*C*H$_3$), 66.4 (-O*C*H$_2$Ar), 107.7 (*d*, J = 23.7 Hz), 116.4 (*d*, J = 23.0 Hz), 120.1 (*d*, J = 25.4 Hz), 120.2, 123.8, 124.2, 124.6 (*d*, J = 2.5 Hz), 127.1, 128.5 (*d*, J = 13.5 Hz), 129.2, 129.3, 129.5 (*d*, J = 8.1 Hz), 130.9 (*d*, J = 2.0 Hz), 132.6 (*d*, J = 9.7 Hz), 136.5, 137.6, 142.0, 148.3, 159.8, 160.0 (*d*, J = 248.7 Hz, C-F), 161.3 (*d*, J = 249.8 Hz, C-F), 167.3. (MS) (ES$^+$) 511 (M+1).

4-Nitrobenzyl-6-bromo-2-(2'-fluoro-[1,1'-biphenyl]-4-yl)-3-methylquinoline-4-carboxylate (**3c**). White solid. Yield 91 %. ^{1}H NMR (600 MHz*, Chloroform-d*): *δ* 2.44 (*s*, 3H, Ar-C*H*$_3$), 5.65 (*s*, 2H, -OC*H*$_2$Ar), 7.17 – 7.21 (*m*, 1H), 7.23 – 7.27 (*m*, 1H), 7.33 – 7.39 (*m*, 1H), 7.49 (*td*, 1H, J = 7.7, 1.3 Hz), 7.64 (*d*, 2H, J = 8.1 Hz), 7.67 – 7.73 (*m*, 4H), 7.75 (*d*, 1H, J = 1.8 Hz), 7.78 (*dd*, 1H, J = 8.9, 1.9 Hz), 8.03 (*d*, 1H, J = 8.9 Hz), 8.31 (*d*, 2H, J = 8.6 Hz). ^{13}C NMR (151 MHz, *Chloroform-d*): δ 18.3 (Ar-*C*H$_3$), 66.5 (-O*C*H$_2$Ar), 116.4 (*d*, J = 22.7 Hz), 122.1, 124.3, 124.5, 124.6 (*d*, J = 2.5 Hz), 126.2, 127.1, 128.5 (*d*, J = 12.9 Hz), 129.2, 129.3 (*d*, J = 1.9 Hz), 129.5, 129.6, 130.9 (*d*, J = 2.0 Hz), 131.5, 133.3, 136.6, 137.9, 138.8, 141.9, 144.8, 148.3, 160.0 (*d*, J = 248.4 Hz), 160.7, 167.1. (MS) (ES$^+$) 571/573 (M+1).

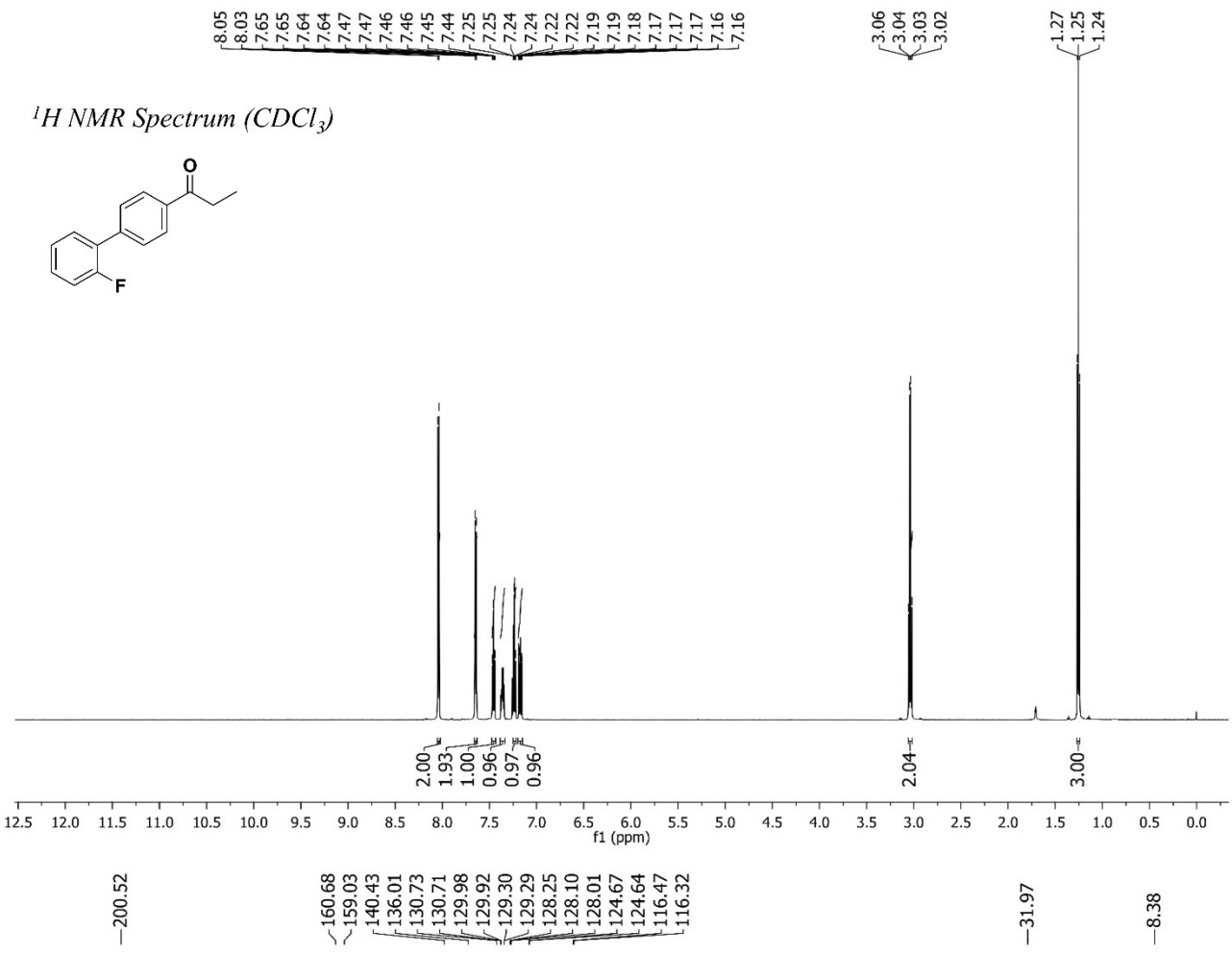
4-Nitrobenzyl-2-(2'-fluoro-[1,1'-biphenyl]-4-yl)-6-iodo-3-methylquinoline-4-carboxylate (**3d**). White solid. Yield 84%. ^{1}H NMR (600 MHz*, Chloroform-d*): δ 2.45 (*s*, 3H, Ar-CH_3), 5.64 (*s*, 2H, -OCH_2Ar), 7.16 - 7.21 (*m*, 1H), 7.22 – 7.27 (*m*, 1H), 7.33 – 7.38 (*m*, 1H), 7.49 (*td*, 1H, J = 7.8, 1.2 Hz), 7.64 (*d*, 2H, J = 8.1 Hz), 7.67 – 7.73 (*m*, 4H), 7.87 (*d*, 1H, J = 8.7 Hz), 7.91 – 7.95 (*m*, 2H), , 8.33 (*d*, 2H, J = 8.6 Hz). ^{13}C NMR (151 MHz, *Chloroform-d*): δ 18.3 (Ar-CH_3), 66.4 (-OCH_2Ar), 93.7, 116.4 (*d*, J = 22.3 Hz), 124.3, 124.6 (*d*, J = 3.7 Hz), 125.0, 126.8, 128.47, 128.3 (*d*, J = 13.2 Hz), 129.1, 129.3 (*d*, J = 2.1 Hz), 129.5, 129.6, 130.9 (*d*, J = 2.8 Hz), 131.7, 132.8, 136.5, 137.4, 138.4, 139.2, 142.0, 145.3, 148.3, 159.9 (*d*, J = 248.4 Hz), 161.0, 167.2. (MS) (ES^+) 619 (M+1).

4-Nitrobenzyl-2-(2'-fluoro-[1,1'-biphenyl]-4-yl)-3-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline-4-carboxylate (**4**). NaOAc (86.3 mg, 1.06 mmol), 4-nitrobenzyl 6-bromo-2-(2'-fluoro-[1,1'-biphenyl]-4-yl)-3-methylquinoline-4-carboxylate (200 mg, 0.35 mmol) and [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (Pd(dppf)Cl_2, 7.68 mg, 0.01 mmol) were dissolved in dioxane (5 mL). The mixture, kept under nitrogen atmosphere, was stirred for 20 minutes, then bis(pinacolato)diboron (98 mg, 0.385 mmol) was added and the mixture was stirred at 100 °C for 48 hours. Upon completion of the reaction, checked by TLC, the solution was cooled at room temperature and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (eluent: from *petroleum ether* to *petroleum ether* / EtOAc 95/5 v / v) to afford the title compound as white solid. Yield 30 %. ^{1}H NMR (600 MHz*, Chloroform-d*): δ 1.38 (s, 12H, -OC(CH_3)$_2$), 2.46 (*s*, 3H, Ar-CH_3), 5.67 (*s*, 2H, -OCH_2Ar), 7.19 (*ddd*, 1H, J = 10.5, 8.2, 0.7 Hz), 7.23 – 7.27 (*m*, 1H), 7.33 – 7.39 (*m*, 1H), 7.50 (*t*, 1H, J = 7.2 Hz), 7.64 – 7.71 (*m*, 4H), 7.73 (*d*, 2H, J = 8.4 Hz), 8.10 (*d*, 1H, J = 7.9 Hz), 8.13 – 8.18 (*m*, 1H), 8.19 (*s*, 1H), 8.30 (*d*, 2H, J = 8.3 Hz). ^{13}C NMR (151 MHz, *Chloroform-d*): δ 18.3 (Ar-CH_3), 25.1 (-OC(CH_3)$_2$), 66.4 (-OCH_2Ar), 84.1(-OC(CH_3)$_2$), 116.3 (*d*, J = 22.9 Hz), 122.7, 124.2, 124.6 (*d*, J = 2.9 Hz), 125.8, 128.6 (*d*, J = 13.1 Hz), 129.0, 129.28, 129.30, 129.4 (*d*, J = 8.2 Hz), 130.9 (*d*, J = 2.4 Hz), 131.8, 134.7, 136.4, 137.9, 139.1, 139.3, 139.4, 142.4, 147.7, 148.1, 160.0 (*d*, J = 248.2 Hz), 161.2, 167.8. (MS) (ES^+) 619 (M+1).

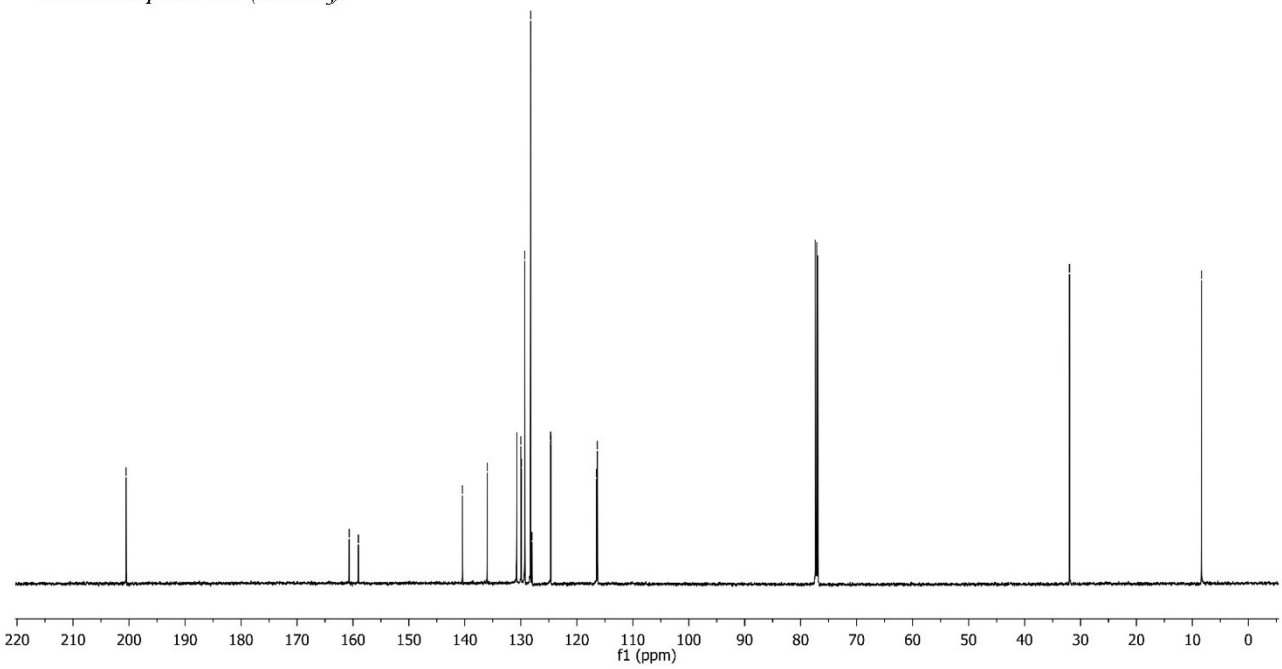
4-Nitrobenzyl2-(2'-fluoro-[1,1'-biphenyl]-4-yl)-3-methyl-6-(tributylstannyl)quinoline-4-carboxylate (**5**). A solution of 4-nitrobenzyl-2-(2'-fluoro-[1,1'-biphenyl]-4-yl)-6-iodo-3-methylquinoline-4-carboxylate (200 mg, 1.62 mmol), LiCl (68.6 mg, 1.62 mmol) and Pd[(PPh_3)]$_4$ (18.7 mg, 0.016 mmol) in 10 mL of dioxane under nitrogen atmosphere was stirred for 20 minutes, then hexabutyldistannane (0.326 mL, 0.64 mmol) was added and the resulting mixture was stirred at 100 °C for 10 hours. The reaction mixture was filtered on silica gel (silica gel and 10 % K_2CO_3, eluent: *petroleum ether*) to remove the insoluble precipitate, then concentrated under reduced pressure. The pale-yellow oil was treated with MeOH to precipitate the final compound as a white solid. Yield 35 %. ^{1}H NMR (600 MHz*, Chloroform-d*): δ 0.88 (*t*, 9H, J = 7.3 Hz, -Sn($CH_2CH_2CH_2CH_3$)$_3$), 1.05 – 1.11(*m*, 6H, -Sn($CH_2CH_2CH_2CH_3$)$_3$), 1.28 – 1.36 (*m*, 6H, -Sn($CH_2CH_2CH_2CH_3$)$_3$), 1.50 – 1.57 (*m*, 6H, -Sn($CH_2CH_2CH_2CH_3$)$_3$), 2.44 (*s*, 3H, Ar-CH_3), 5.64 (*s*, 2H, -OCH_2Ar), 7.19 (*dd*, 1H, J = 10.5, 8.4 Hz), 7.23 – 7.27 (*m*, 1H), 7.33 – 7.38 (*m*, 1H), 7.49 (*td*, 1H, J = 7.7, 1.6 Hz), 7.64 (*d*, 2H, J = 8.2 Hz), 7.66 – 7.70 (*m*, 4H), 7.74 (*s*, 1H), 7.81 (*d*, 1H, J = 8.2 Hz), 8.10 (*d*, 1H, J = 8.2 Hz), 8.28 (*d*, 2H, J = 8.8 Hz). ^{13}C NMR (151 MHz, *Chloroform-d*): δ 10.0 (-Sn($CH_2CH_2CH_2CH_3$)$_3$), 13.8 (-Sn($CH_2CH_2CH_2CH_3$)$_3$), 18.20 (Ar-CH_3) 27.5 (-Sn($CH_2CH_2CH_2CH_3$)$_3$), 29.2 (-Sn($CH_2CH_2CH_2CH_3$)$_3$), 66.0 (-OCH_2Ar), 116.3 (*d*, J = 22.6 Hz), 123.0, 124.2, 124.6 (*d*, J = 2.5 Hz), 125.5, 128.7(*d*, J = 13.0 Hz), 128.72, 129.1, 129.2, 129.3 (*d*, J = 1.8 Hz), 129.4 (*d,* J = 8.1 Hz), 130.9 (*d*, J = 2.3 Hz), 132.0, 136.2, 137.0, 138.2, 139.7, 142.3, 143.3, 146.4, 148.1, 160.0 (*d*, J = 248.3 Hz), 160.1, 168.0. (MS) (ES^+) 781 (^{116}Sn), 783 (^{118}Sn), 784 (^{119}Sn), 785 (^{120}Sn) (M+1).

1-(2'-Fluoro-[1,1'-biphenyl]-4-yl)propan-1-one (**1**)

^{1}H NMR Spectrum ($CDCl_3$)

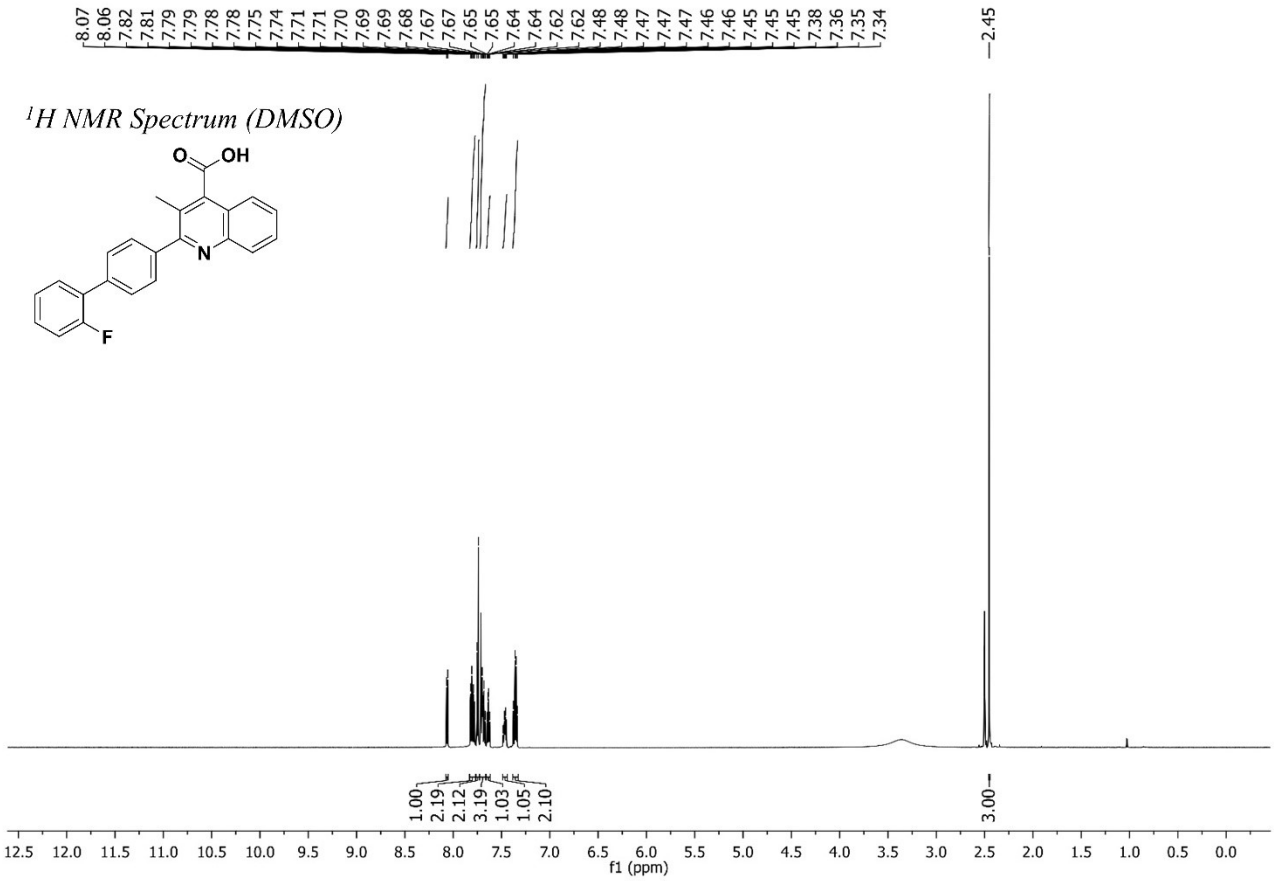


^{13}C NMR Spectrum ($CDCl_3$)

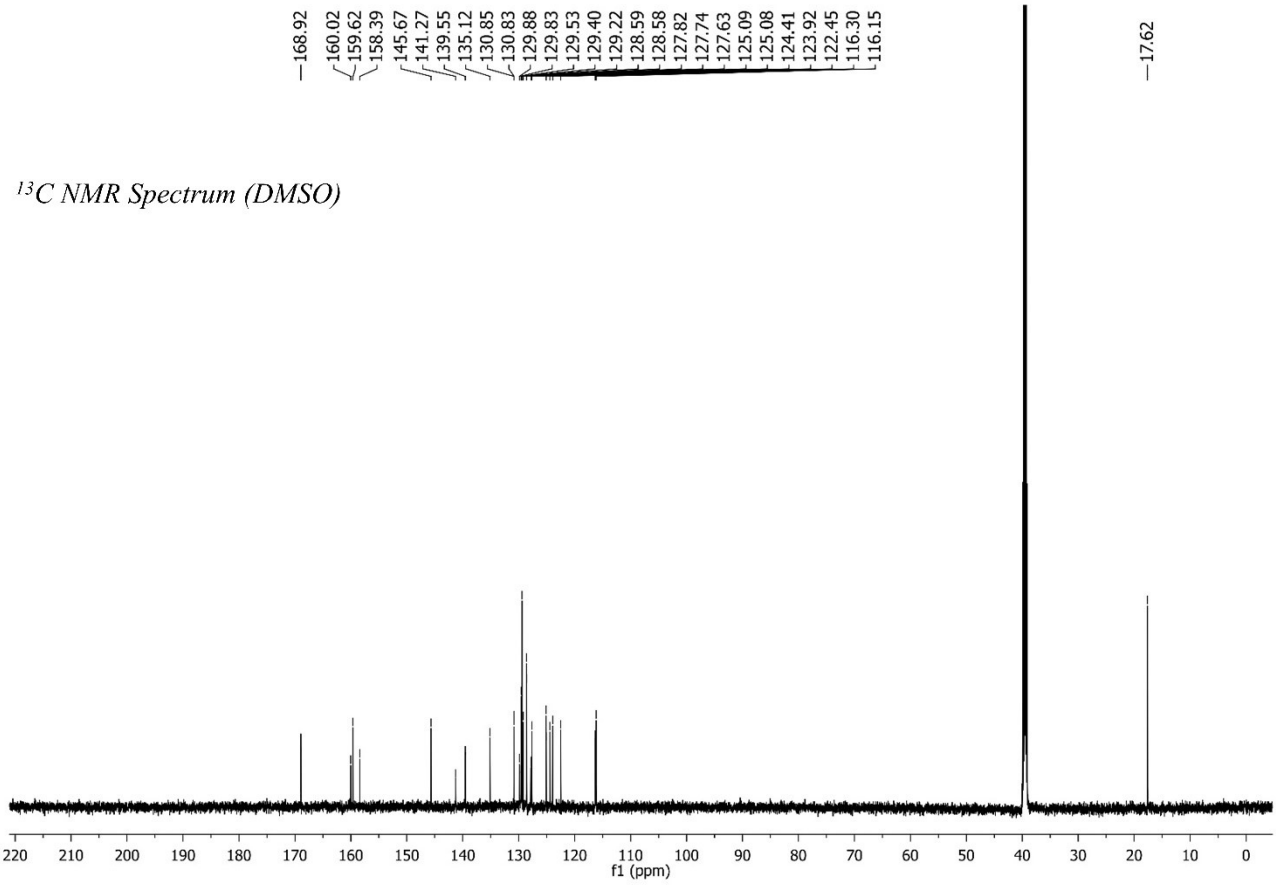


2-(2'-Fluoro-[1,1'-biphenyl]-4-yl)-3-methylquinoline-4-carboxylic acid (**2a**)

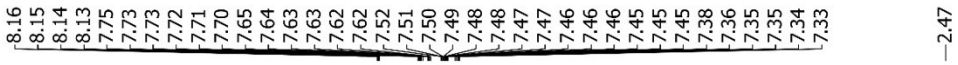
^{1}H NMR Spectrum (DMSO)



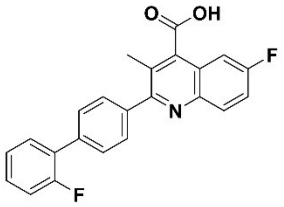
^{13}C NMR Spectrum (DMSO)

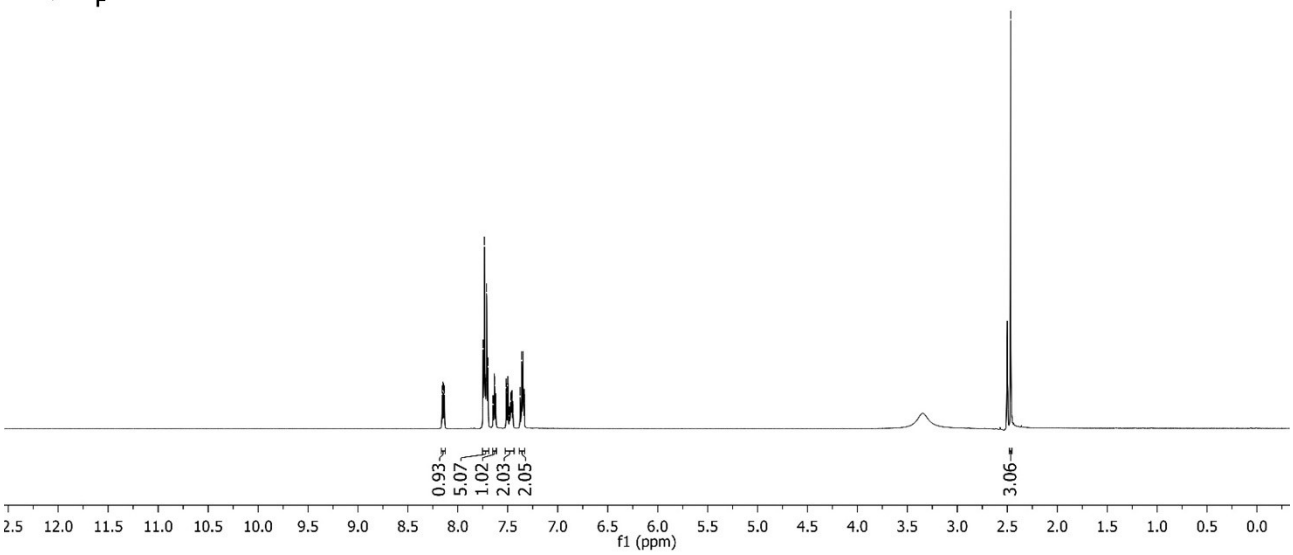


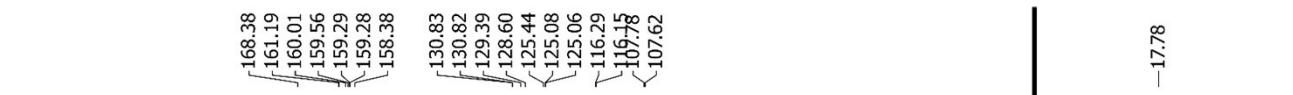
6-Fluoro-2-(2'-fluoro-[1,1'-biphenyl]-4-yl)-3-methylquinoline-4-carboxylic acid (**2b**)



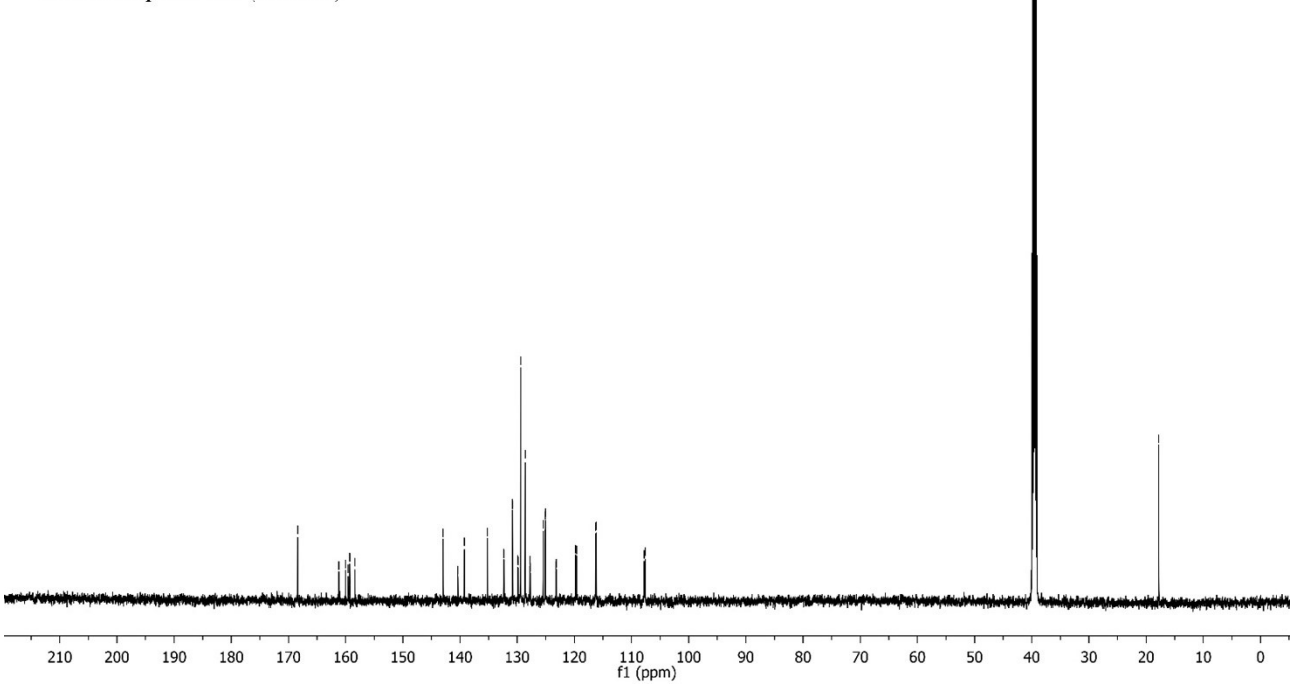
^{1}H NMR Spectrum (DMSO)





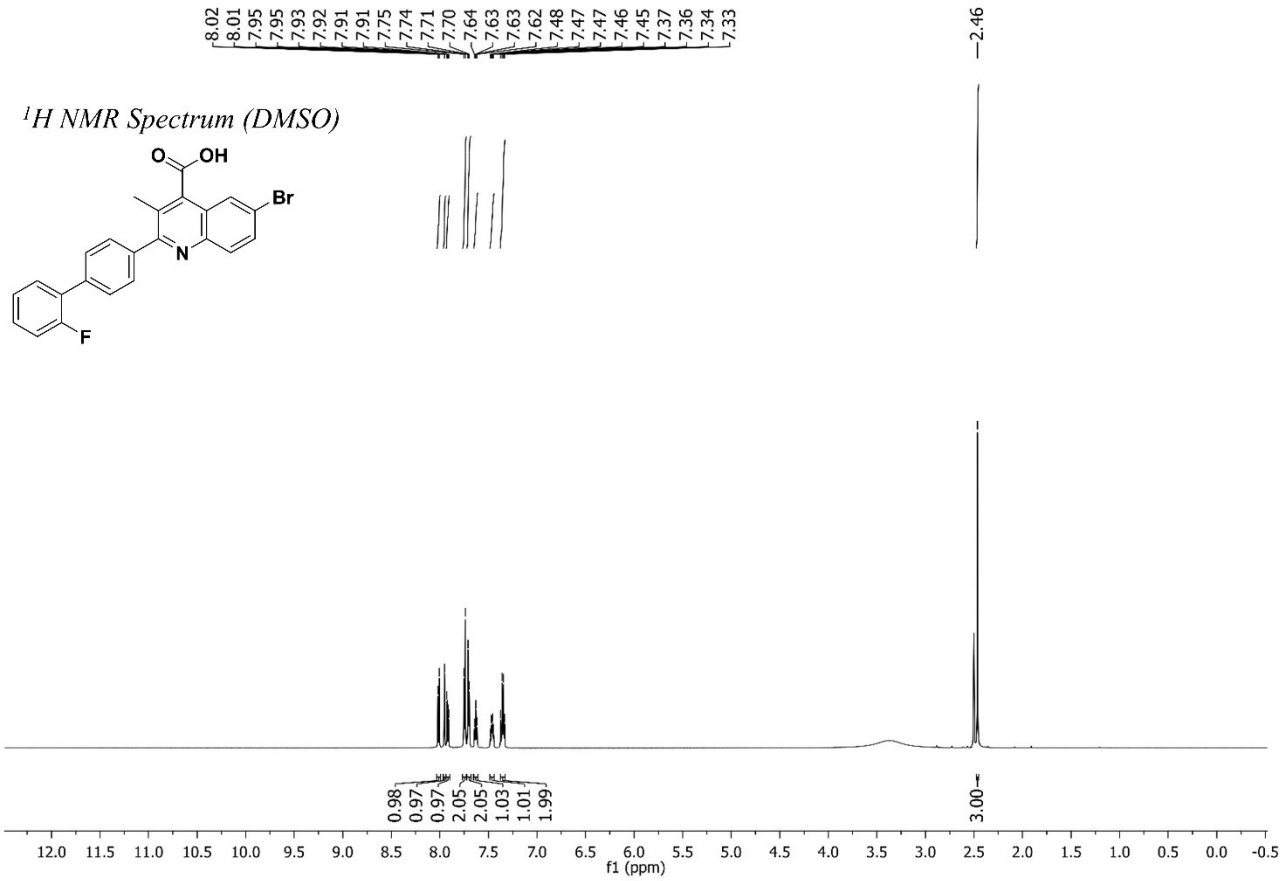


^{13}C NMR Spectrum (DMSO)

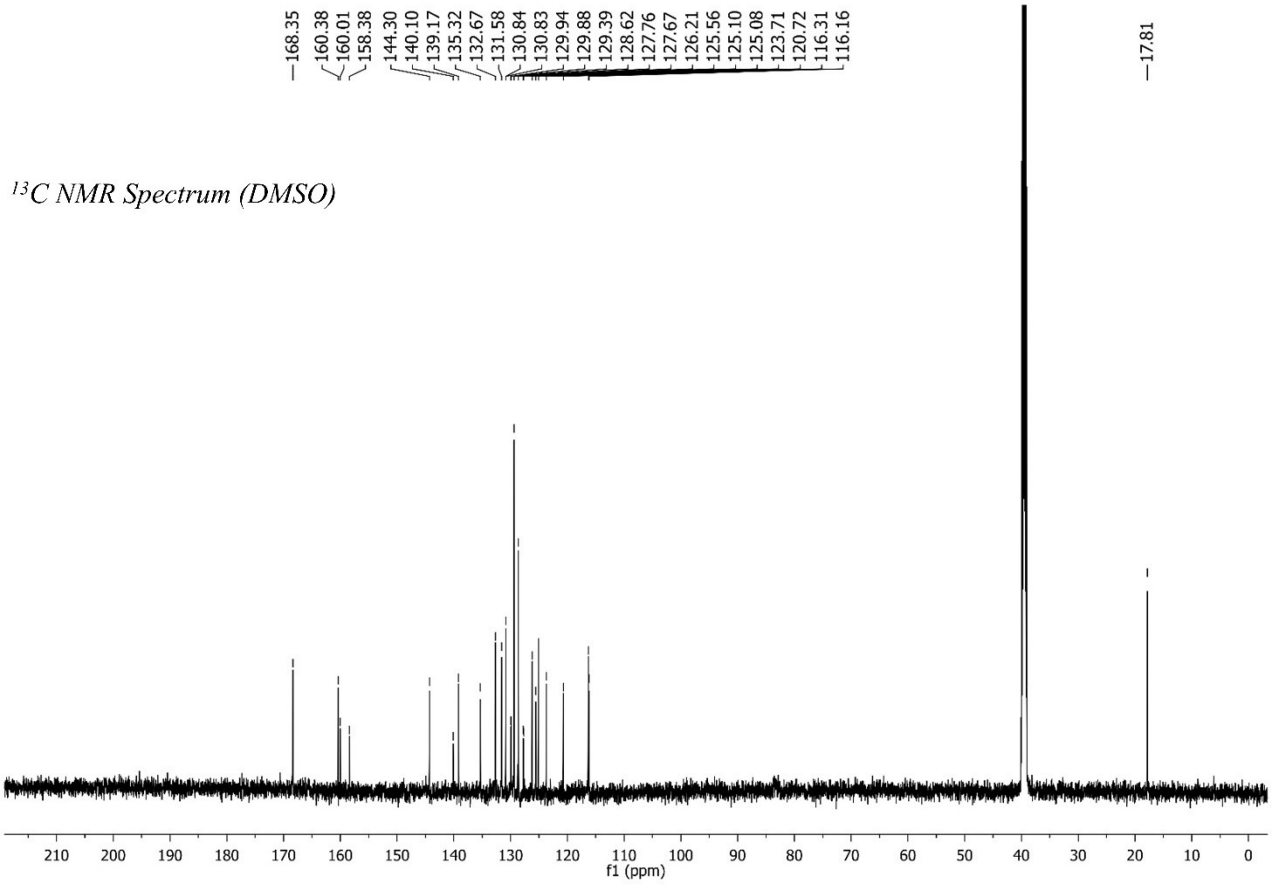


6-Bromo-2-(2'-fluoro-[1,1'-biphenyl]-4-yl)-3-methylquinoline-4-carboxylic acid (**2c**)

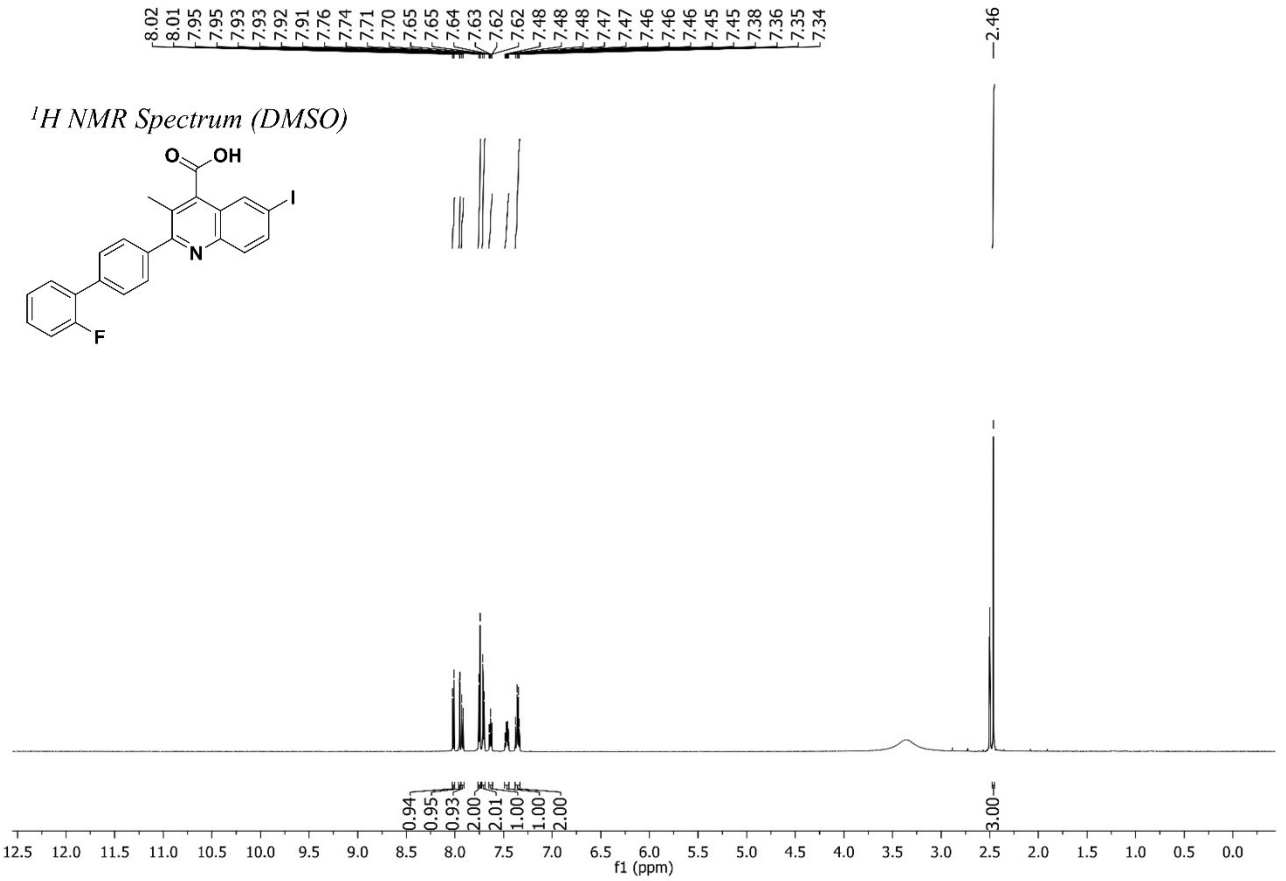
^{1}H NMR Spectrum (DMSO)



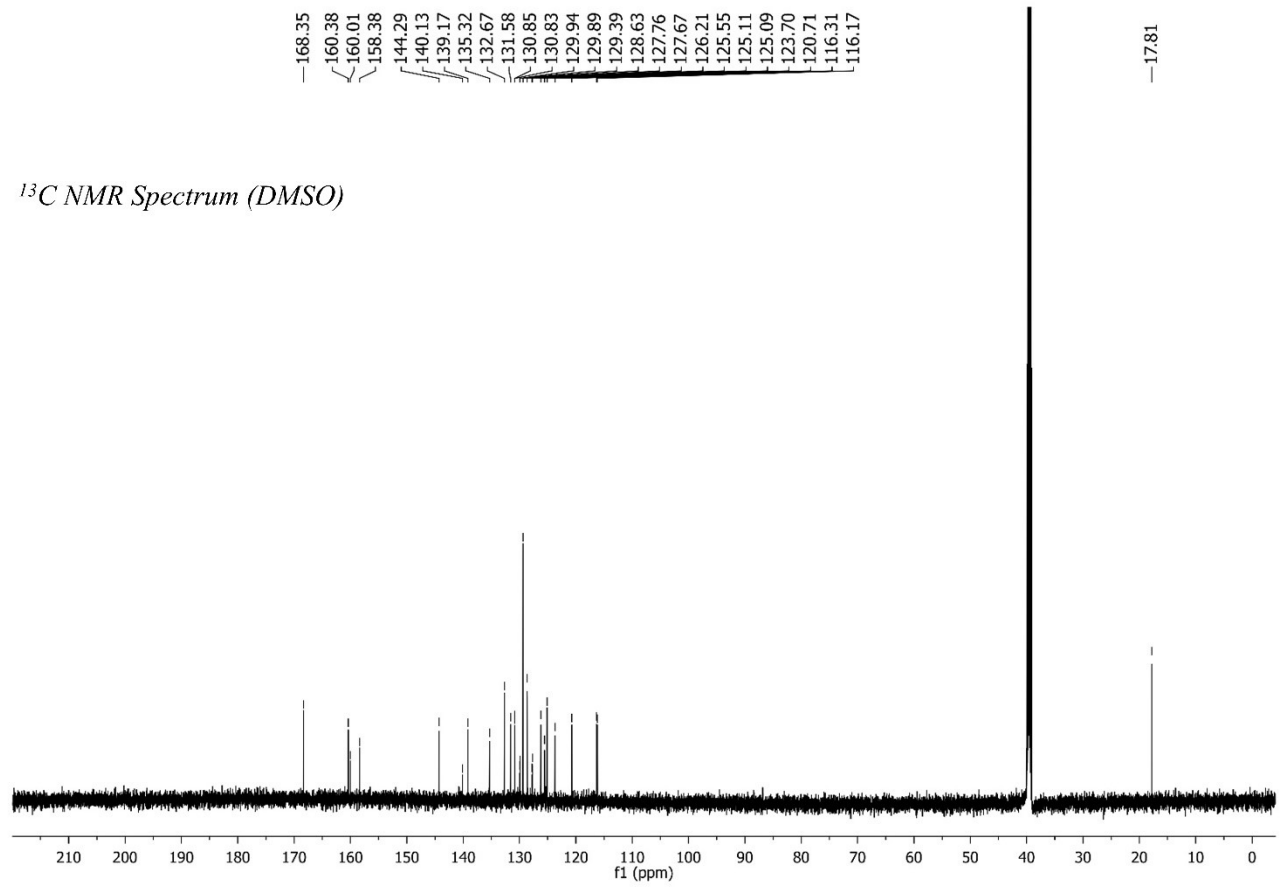
^{13}C NMR Spectrum (DMSO)



2-(2'-Fluoro-[1,1'-biphenyl]-4-yl)-6-iodo-3-methylquinoline-4-carboxylic acid (**2d**)



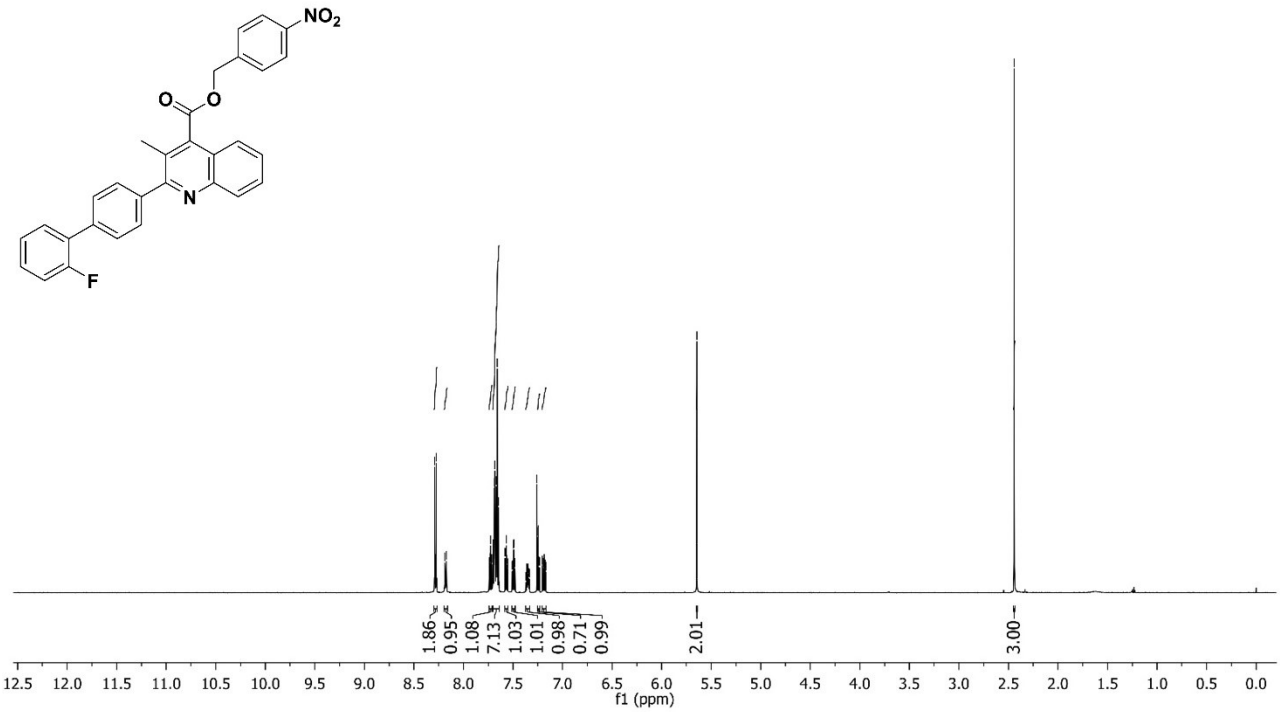
^{1}H NMR Spectrum (DMSO)

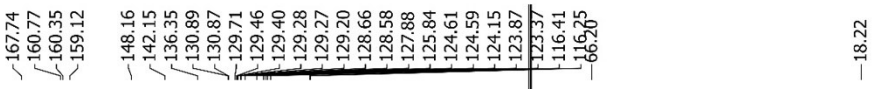


^{13}C NMR Spectrum (DMSO)

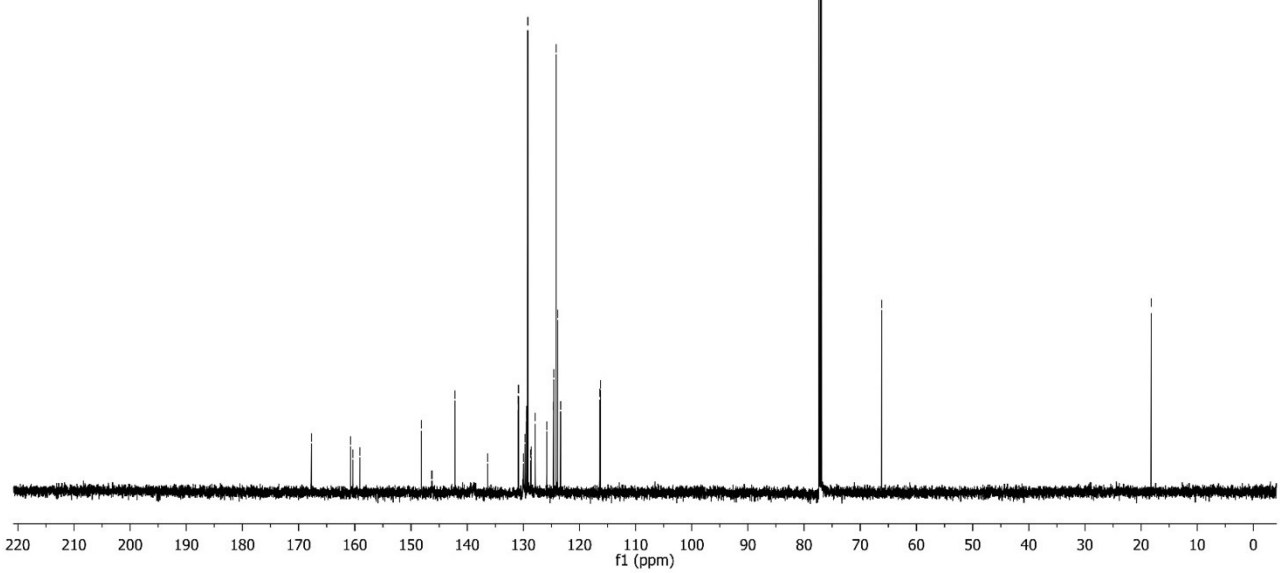
4-Nitrobenzyl 2-(2'-fluoro-[1,1'-biphenyl]-4-yl)-3-methylquinoline-4-carboxylate (**3a**)

^{1}H NMR Spectrum ($CDCl_3$)



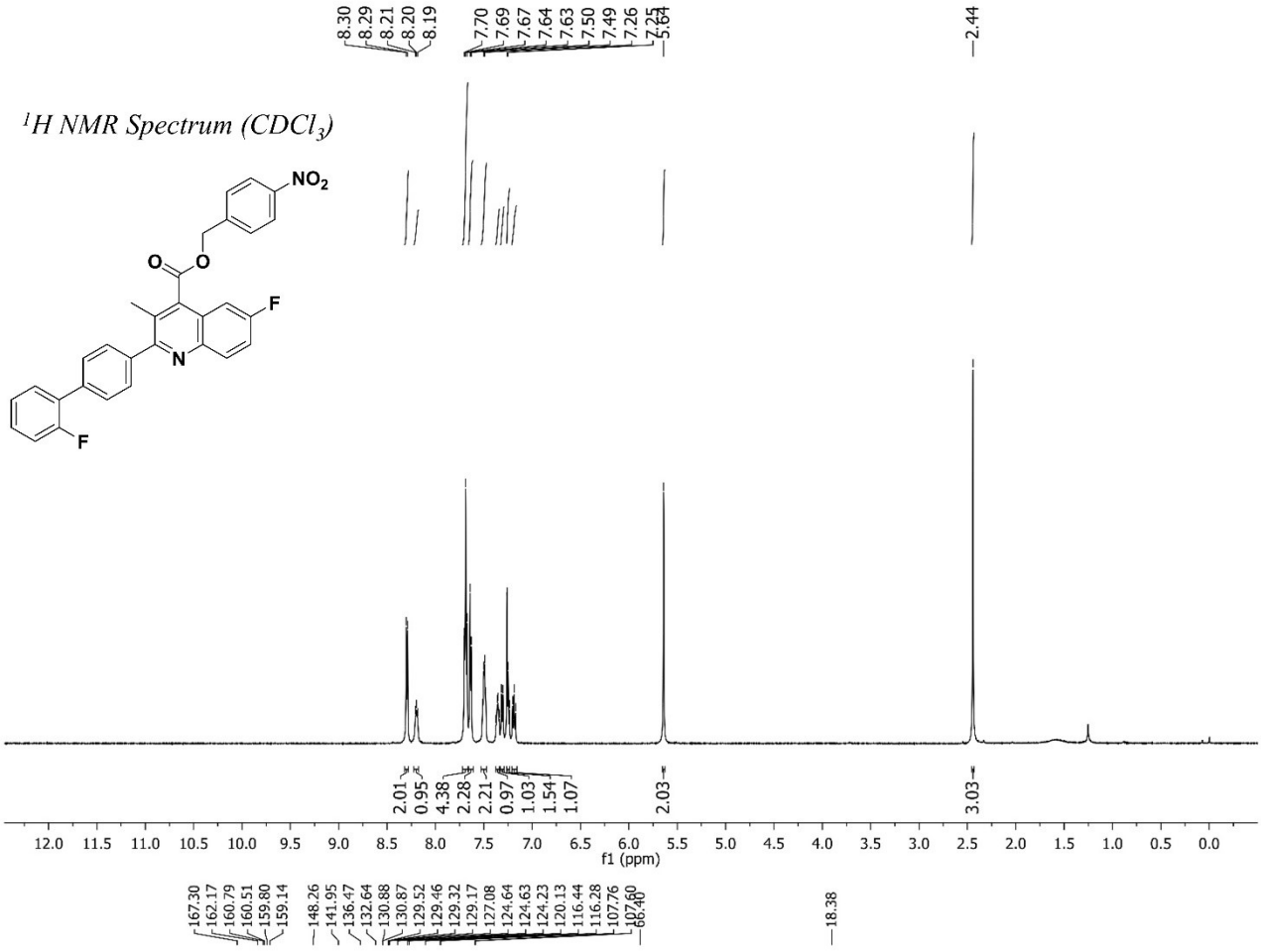


^{13}C NMR Spectrum ($CDCl_3$)

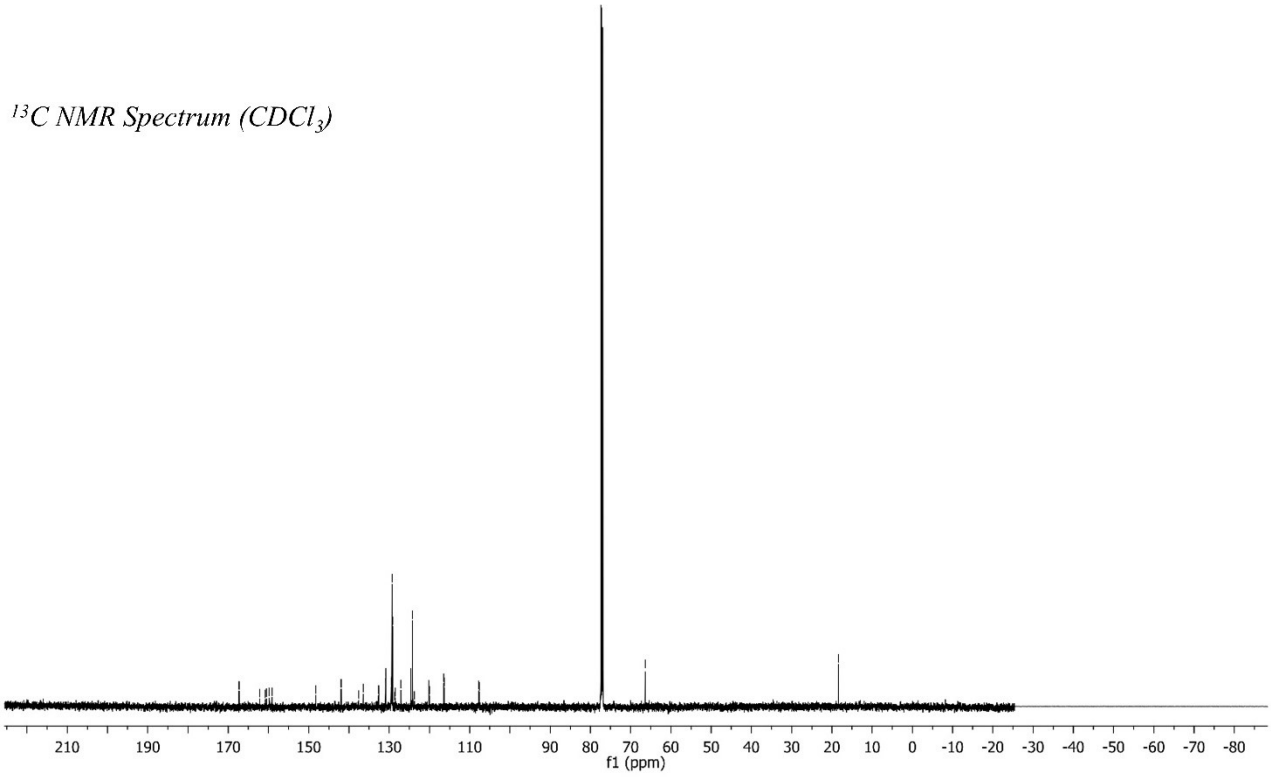


4-Nitrobenzyl-6-fluoro-2-(2'-fluoro-[1,1'-biphenyl]-4-yl)-3-methylquinoline-4-carboxylate (**3b**)

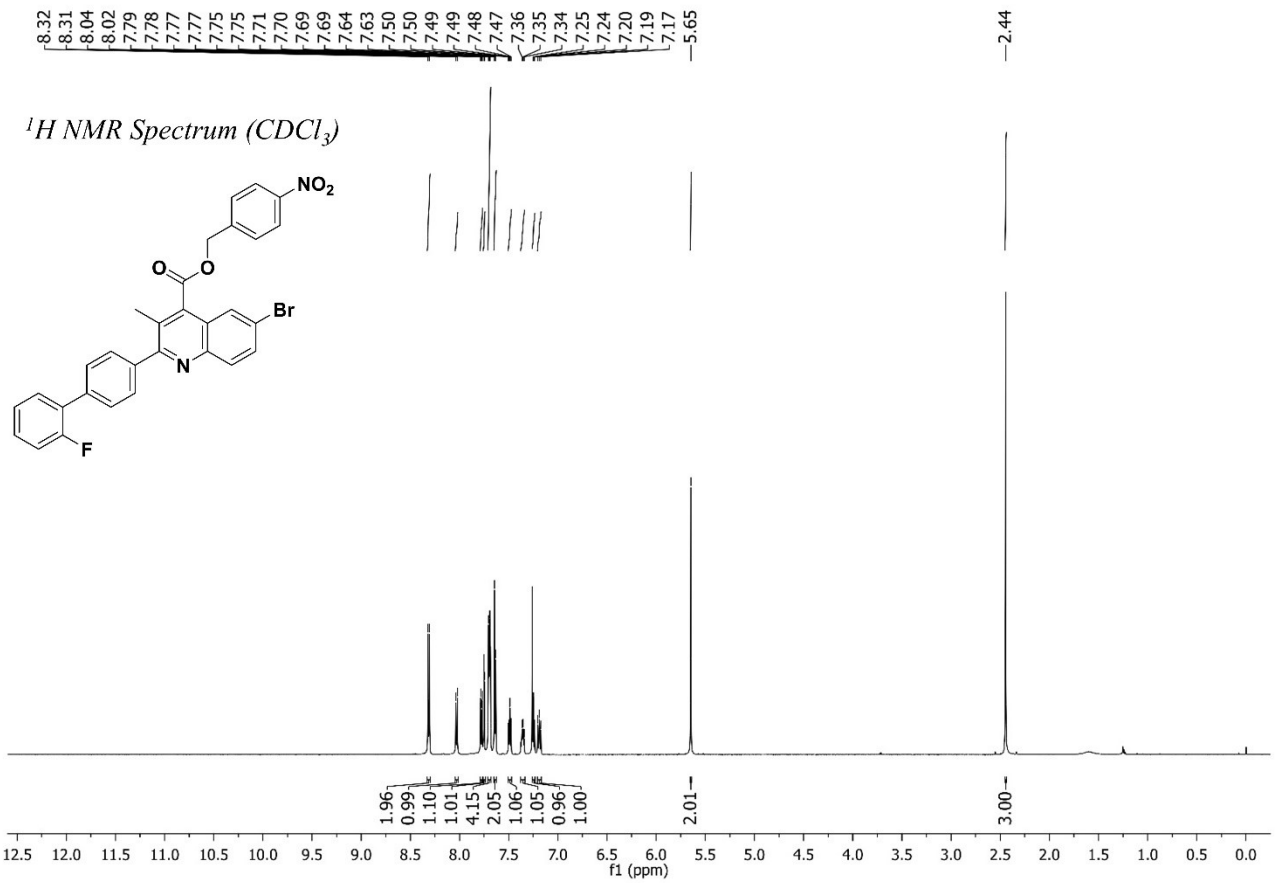
^{1}H NMR Spectrum ($CDCl_3$)



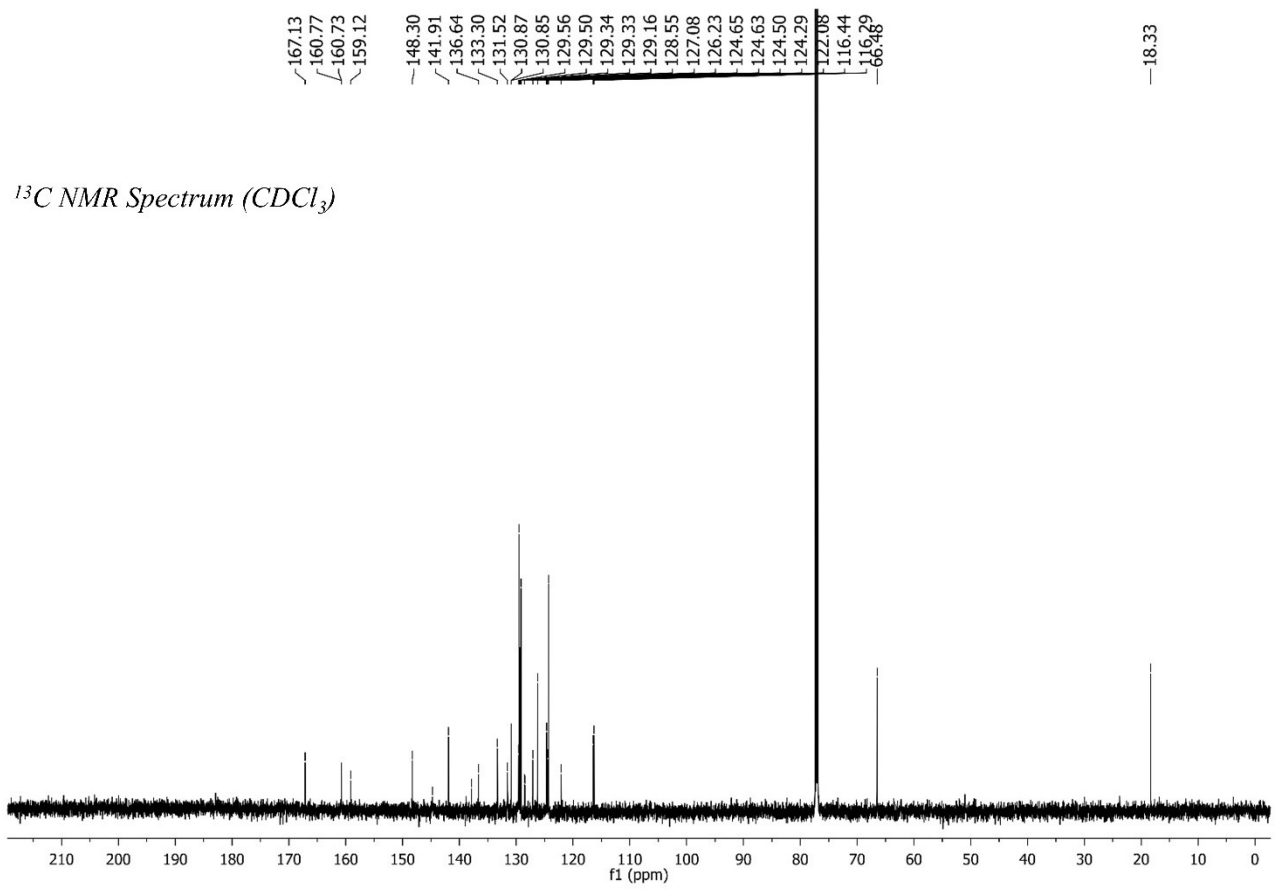
^{13}C NMR Spectrum ($CDCl_3$)



4-Nitrobenzyl-6-bromo-2-(2'-fluoro-[1,1'-biphenyl]-4-yl)-3-methylquinoline-4-carboxylate (**3c**)

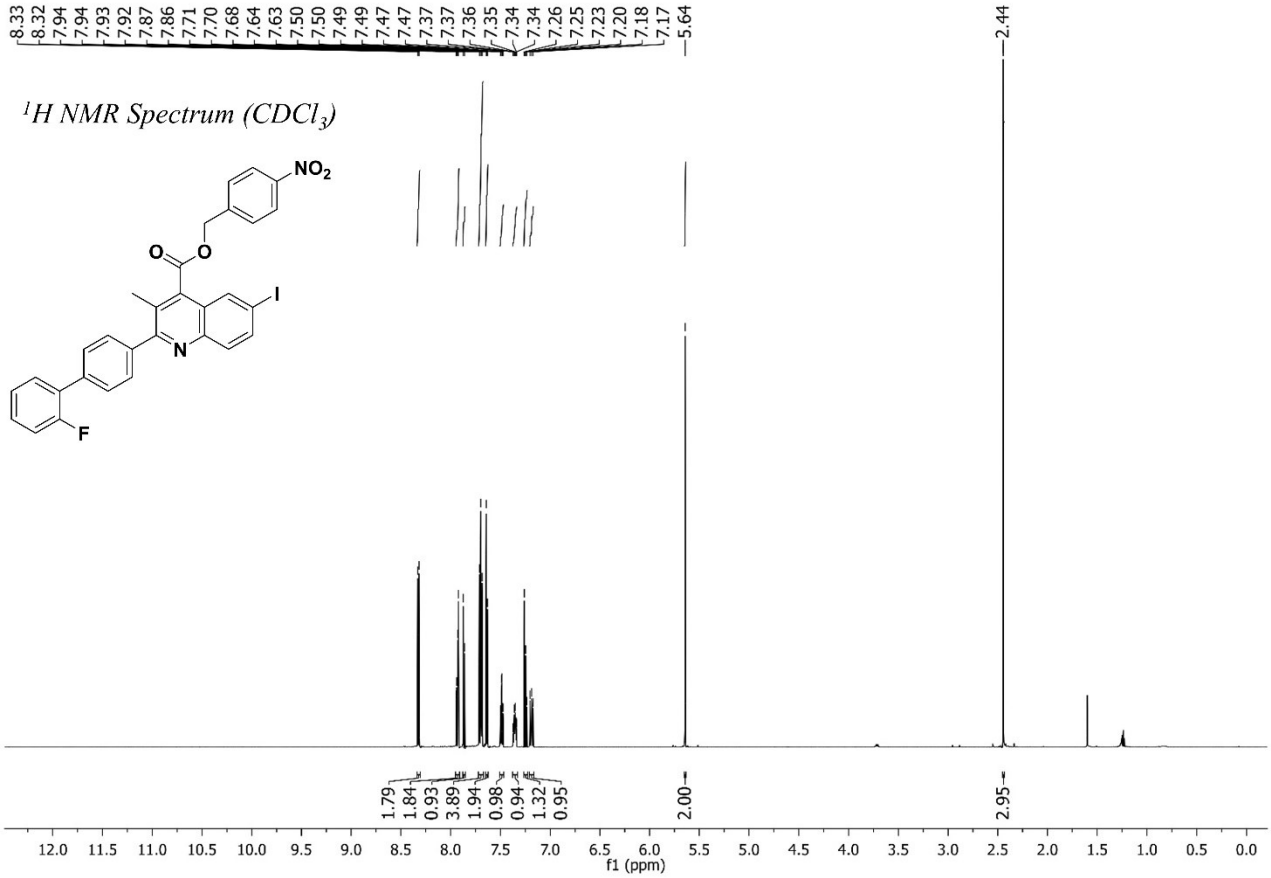


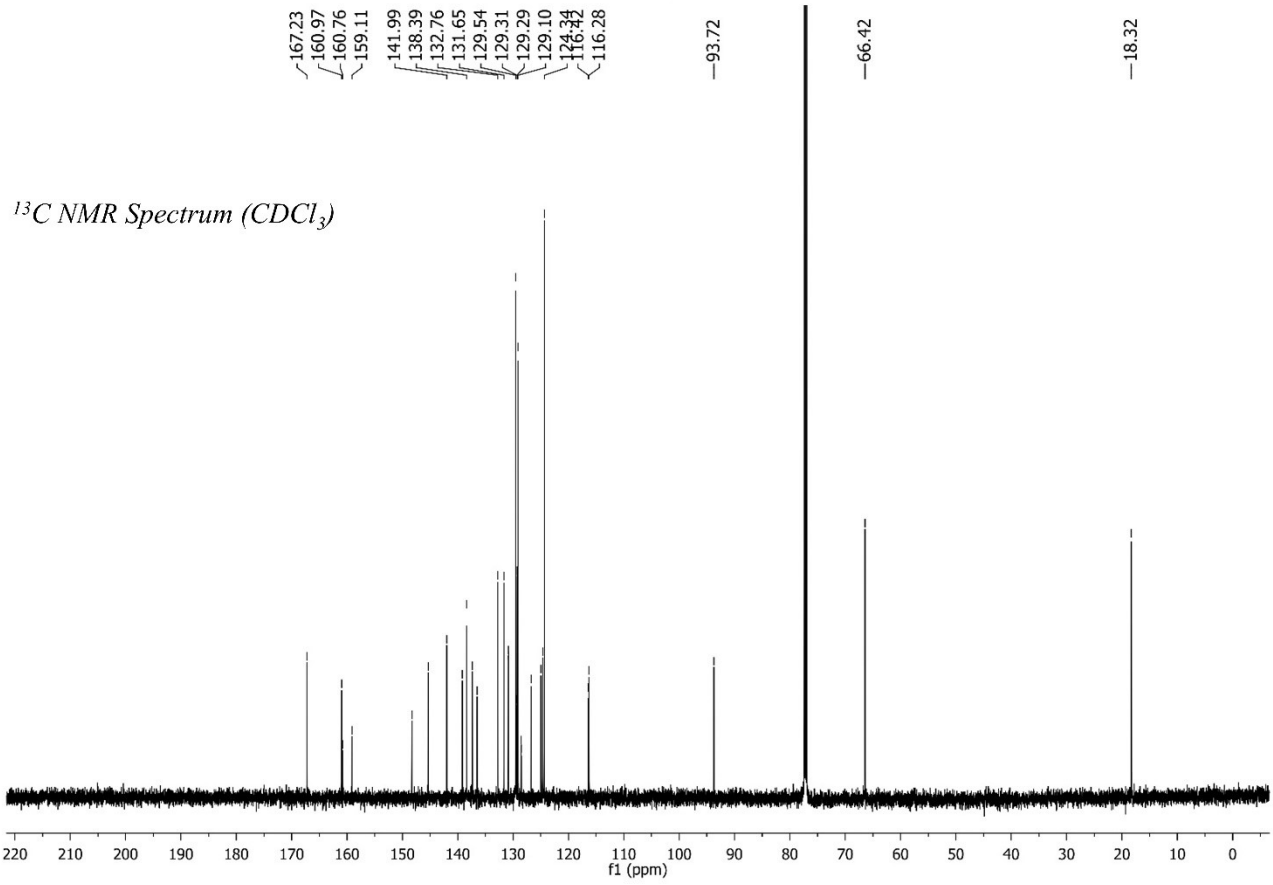
^{1}H NMR Spectrum ($CDCl_3$)



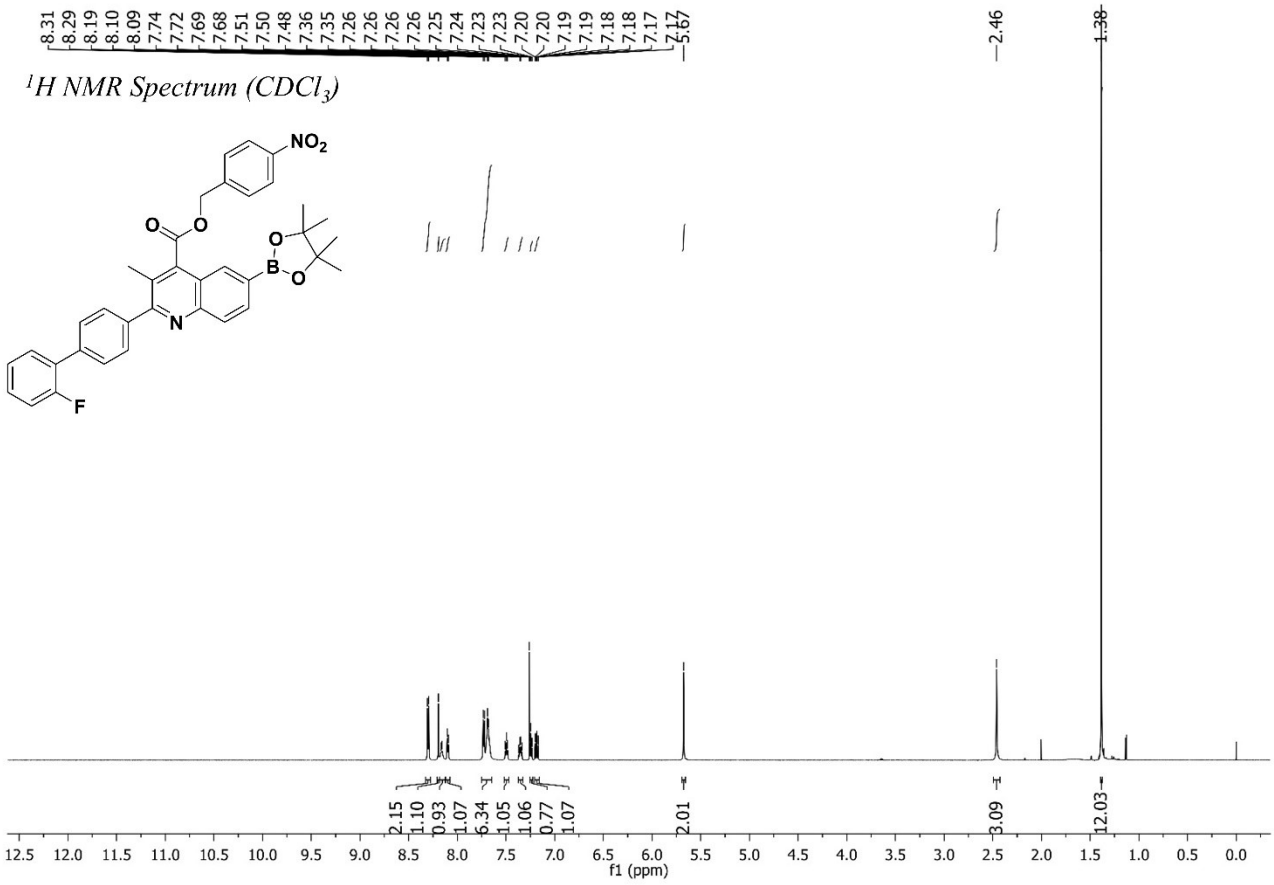
^{13}C NMR Spectrum ($CDCl_3$)

4-Nitrobenzyl-2-(2'-fluoro-[1,1'-biphenyl]-4-yl)-6-iodo-3-methylquinoline-4-carboxylate (**3d**)

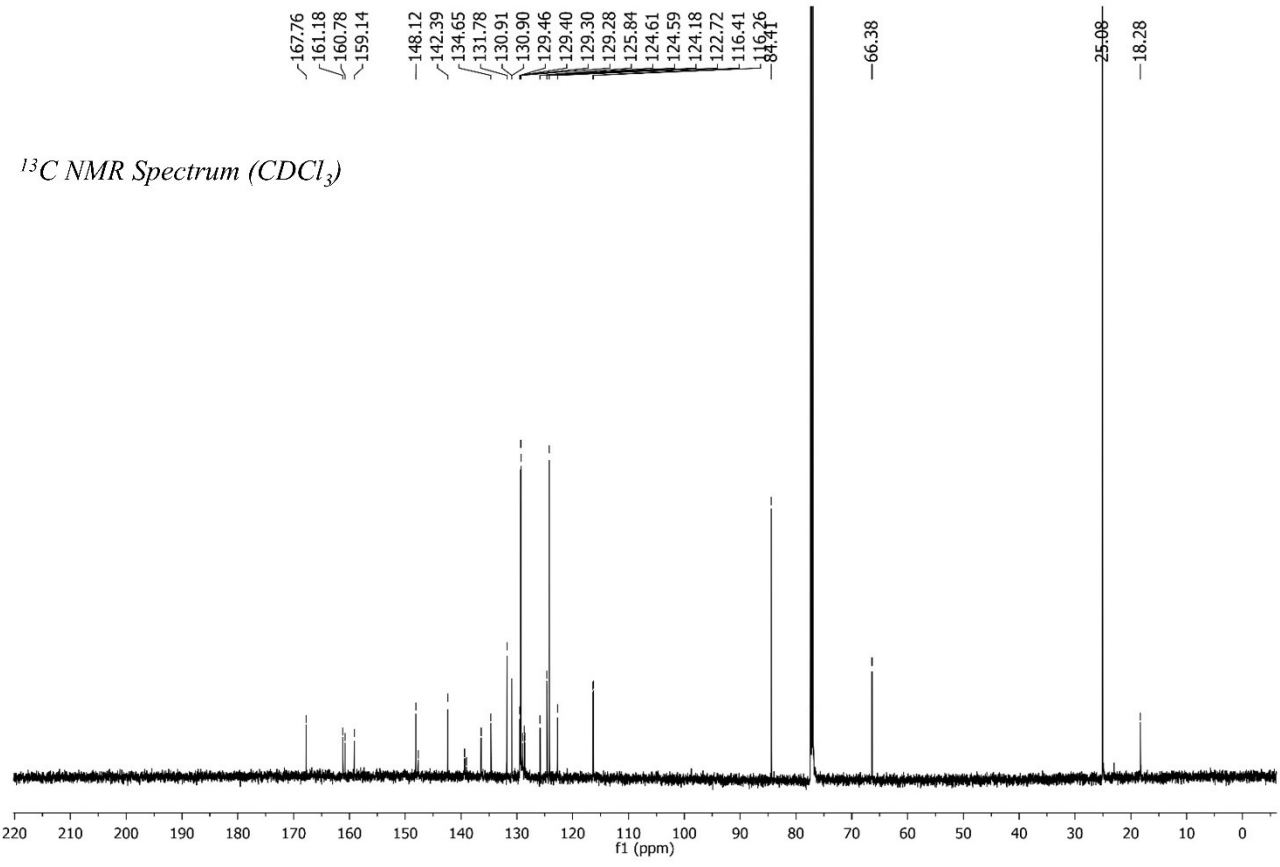




4-Nitrobenzyl-2-(2'-fluoro-[1,1'-biphenyl]-4-yl)-3-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline-4-carboxylate (**4**)

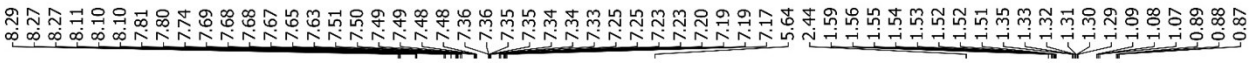


^{1}H NMR Spectrum ($CDCl_3$)

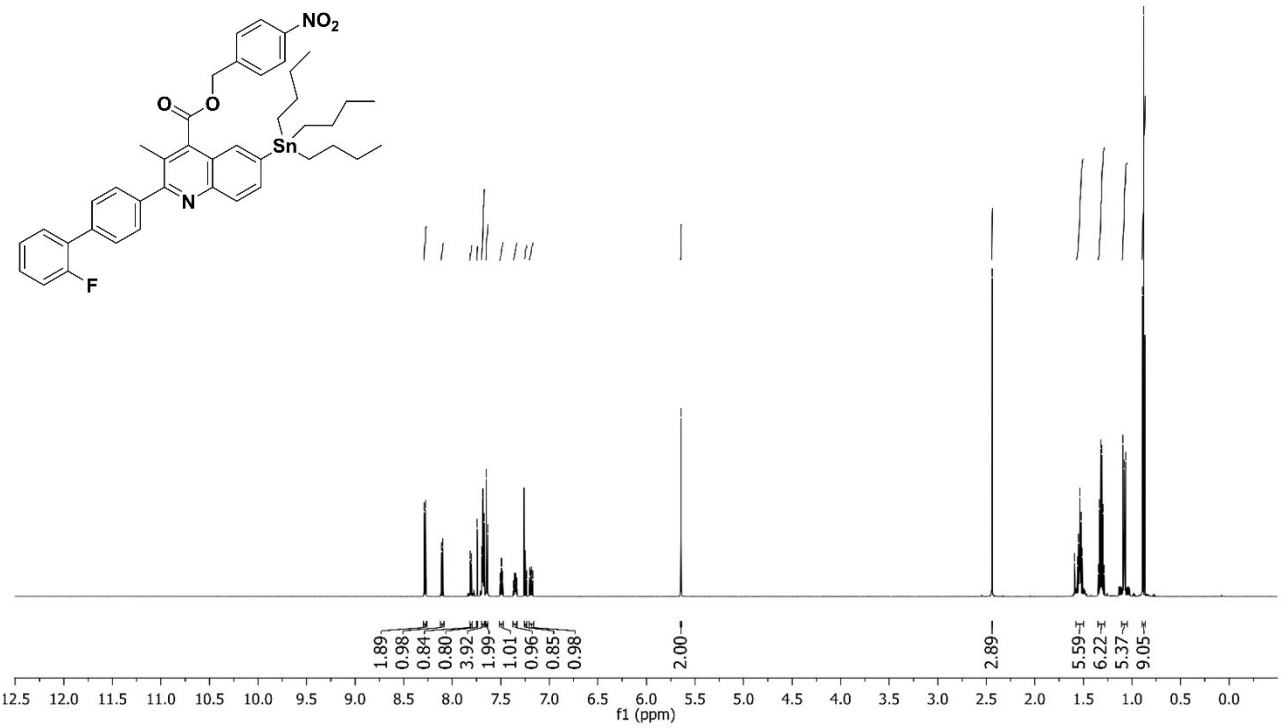


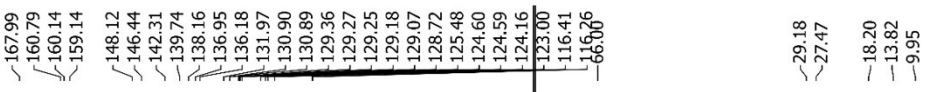
^{13}C NMR Spectrum ($CDCl_3$)

4-Nitrobenzyl2-(2'-fluoro-[1,1'-biphenyl]-4-yl)-3-methyl-6-(tributylstannyl)quinoline-4-carboxylate (**5**)

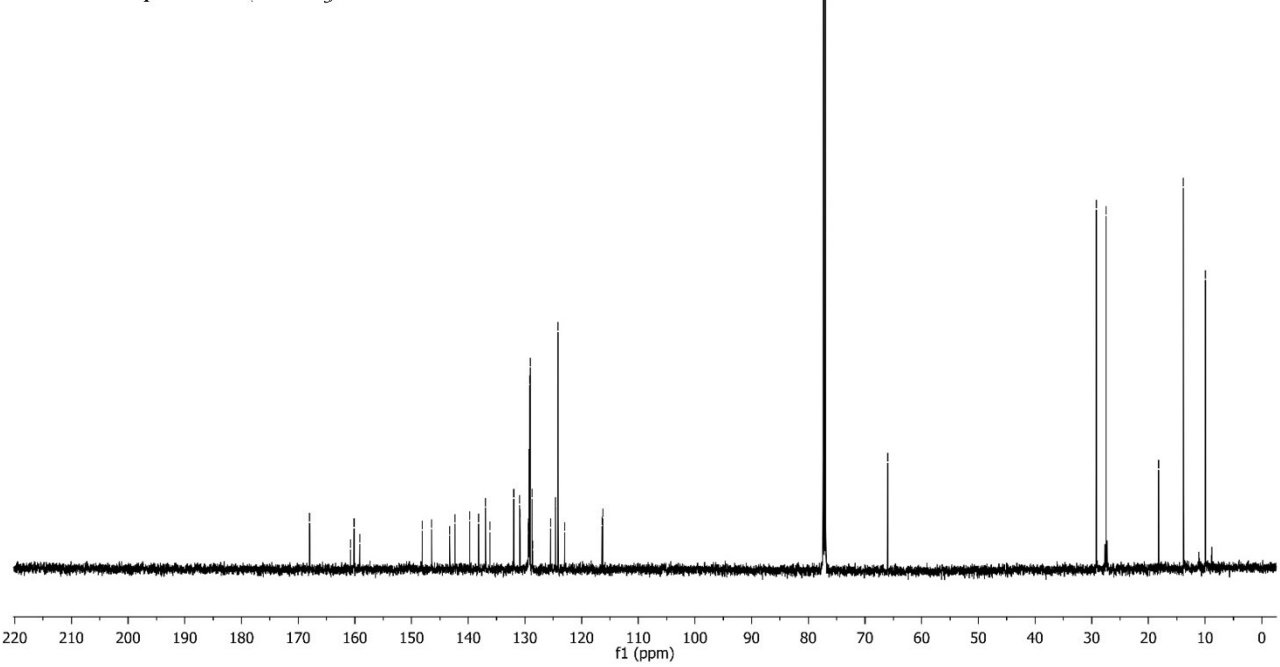


^{1}H NMR Spectrum ($CDCl_3$)



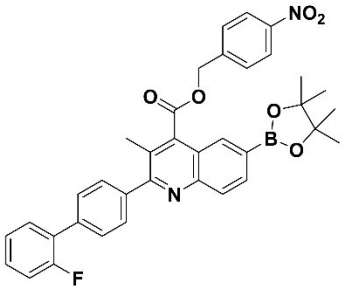


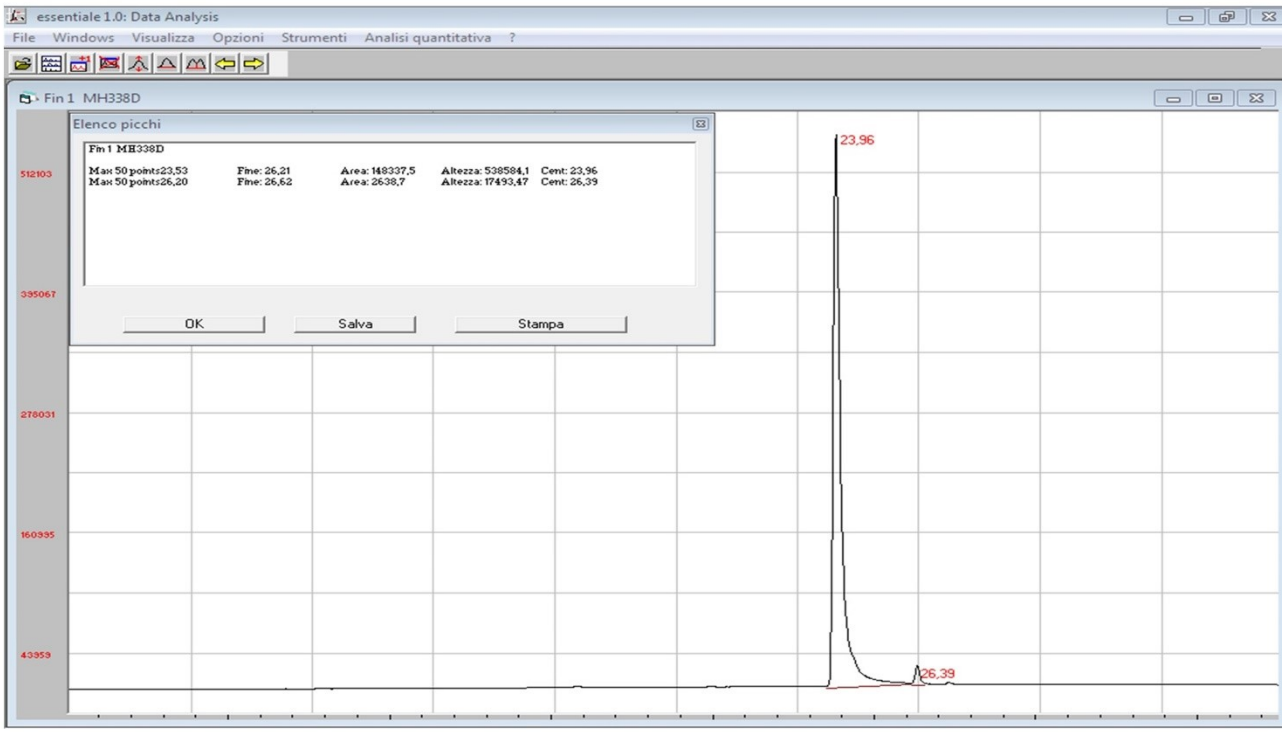
^{13}C NMR Spectrum ($CDCl_3$)



4-Nitrobenzyl-2-(2'-fluoro-[1,1'-biphenyl]-4-yl)-3-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline-4-carboxylate (**4**)

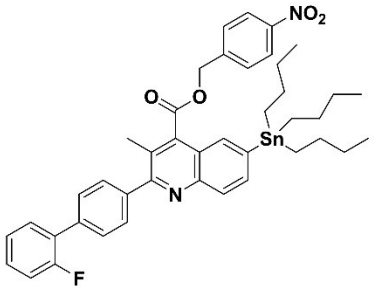
HPLC_Analysis

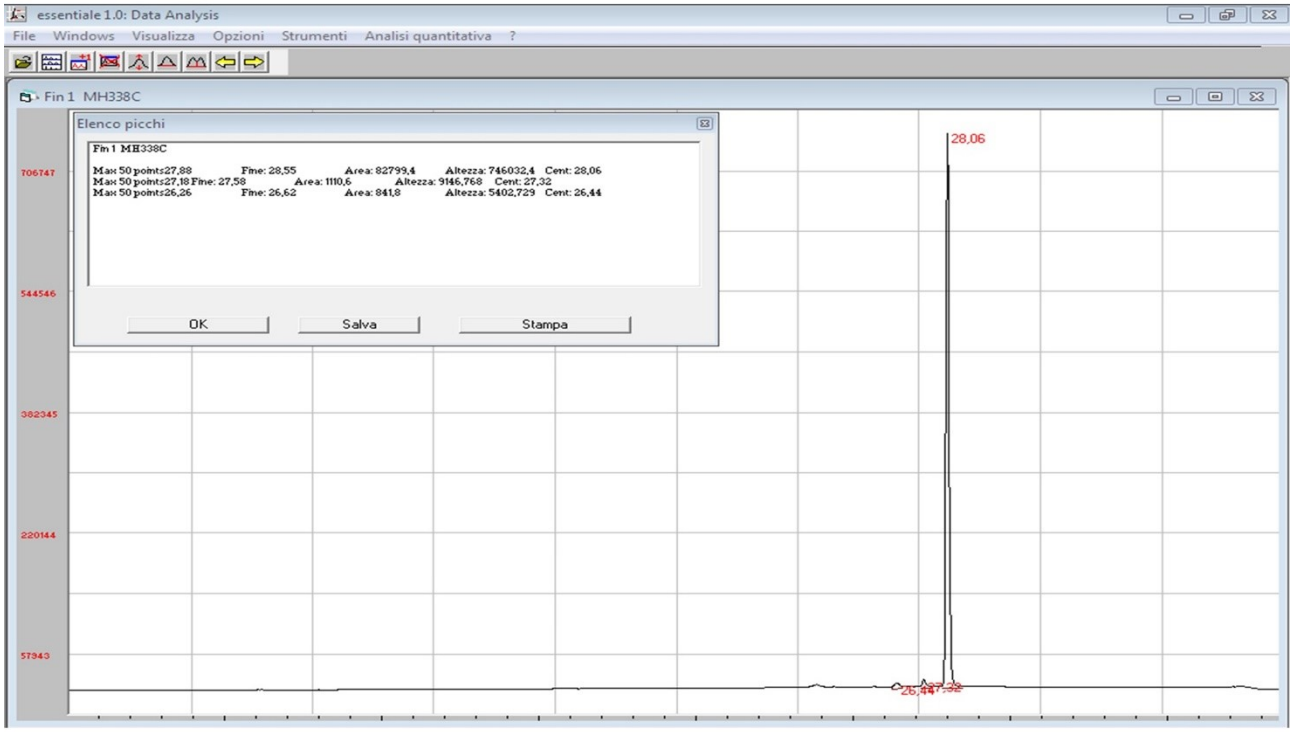




4-Nitrobenzyl2-(2'-fluoro-[1,1'-biphenyl]-4-yl)-3-methyl-6-(tributylstannyl)quinoline-4-carboxylate (**5**)

HPLC_Analysis





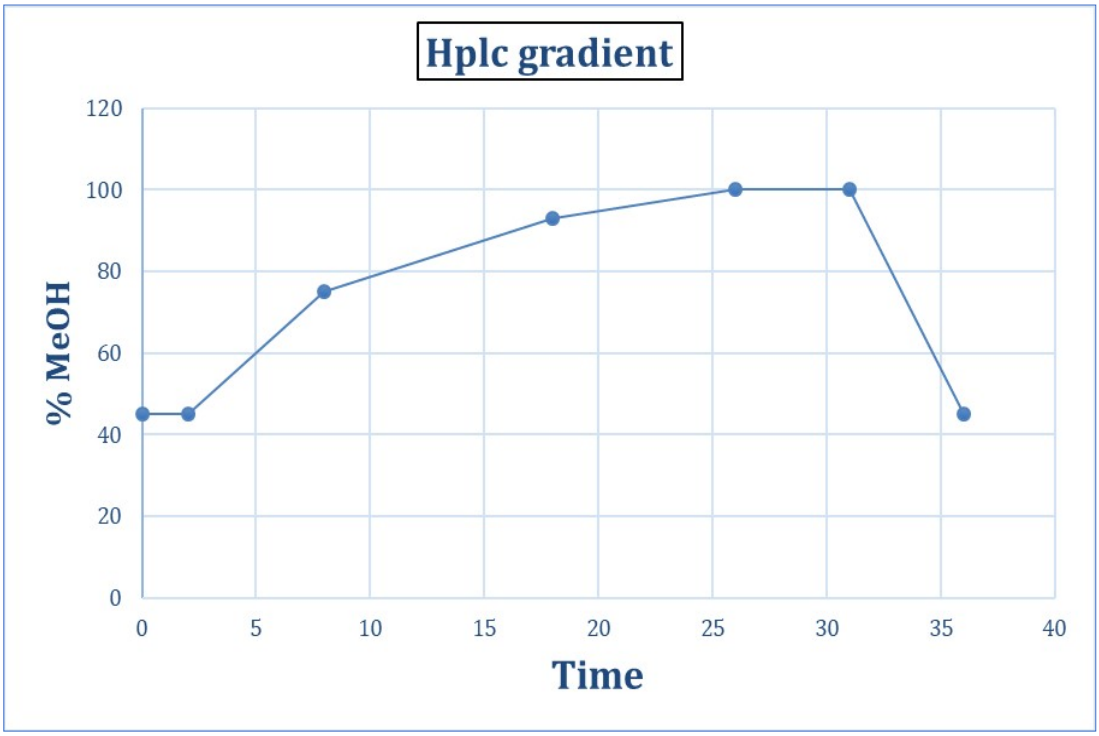


Figure S1: HPLC gradient method showing the percentage of MeOH + 0.1% trifluoroacetic acid **(B)** at various time points. LiChrosorb RP-18 column (250x4mm, 5µm particle size, Merck); Mobile phase gradient method: **A** (aqueous DW + 0.1% trifluoroacetic acid); **B** (MeOH + 0.1% trifluoroacetic acid); 0-2 min (isocratic 45% **B**), 2.0-8.0 min (45 → 75% **B**), 8.0-18.0 min (75 → 93% **B**), 18-26 min (93 → 100% **B**), 26.0-31.0 min (100% **B**), 10 (isocratic 100% **B**), 31.0-36.0 min (100 → 45% **B**), 36.0 min (Stop); 1.5 mL/min; UV 254 nm.

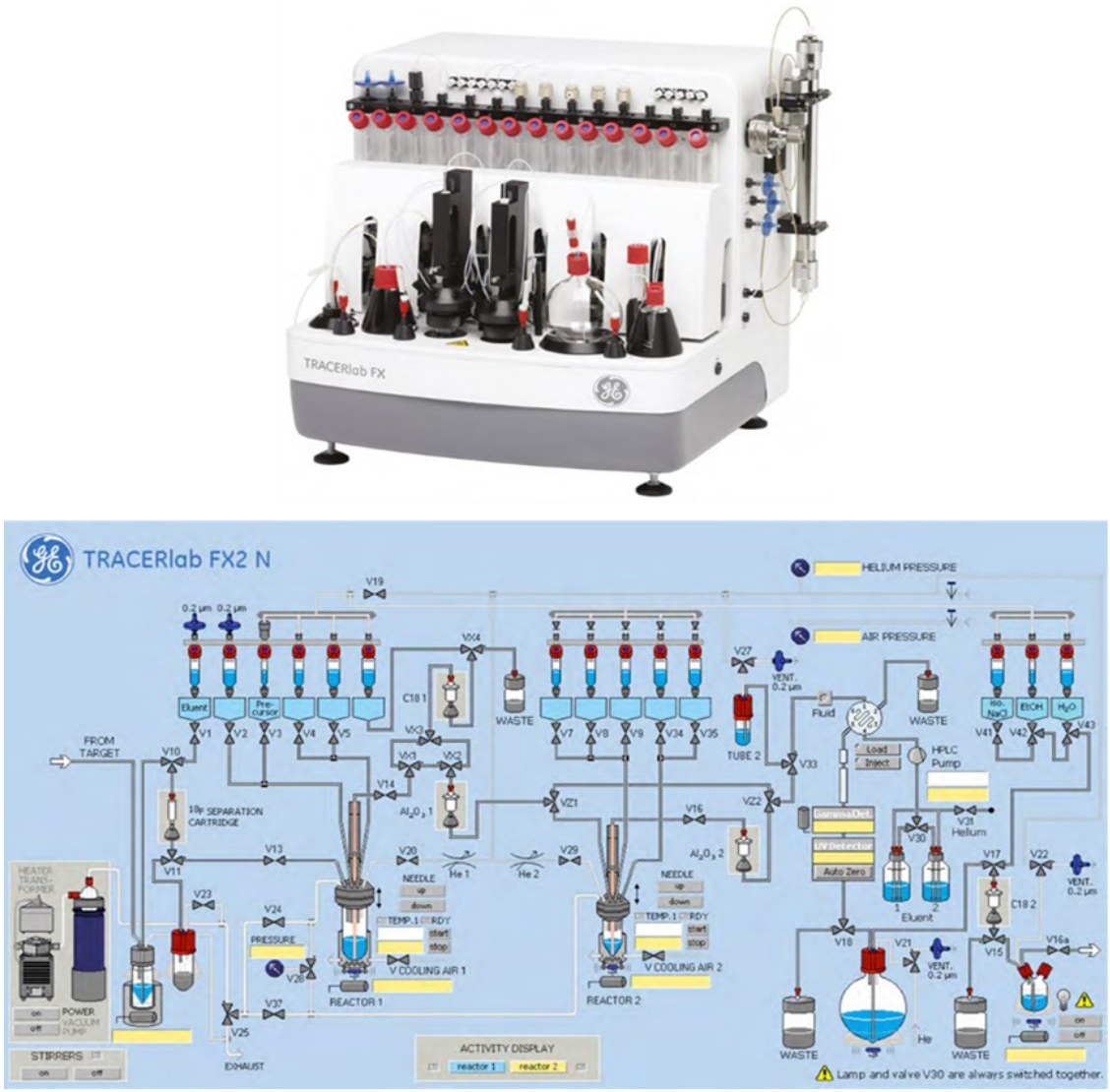
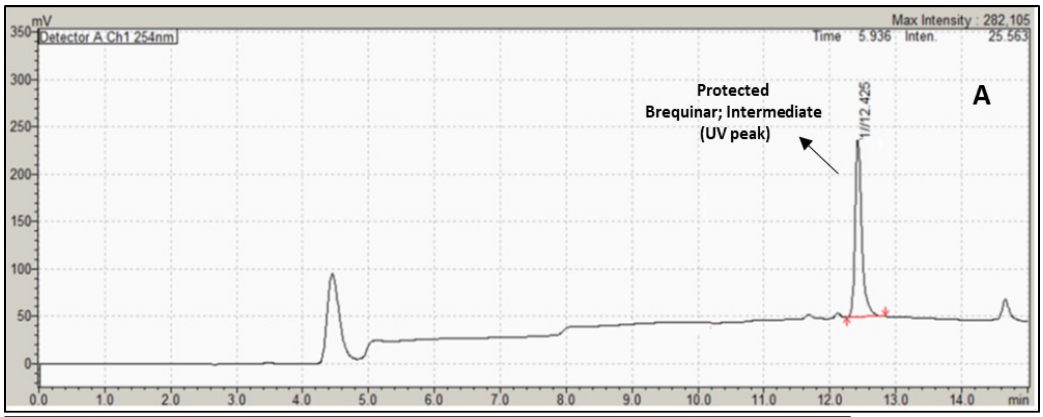
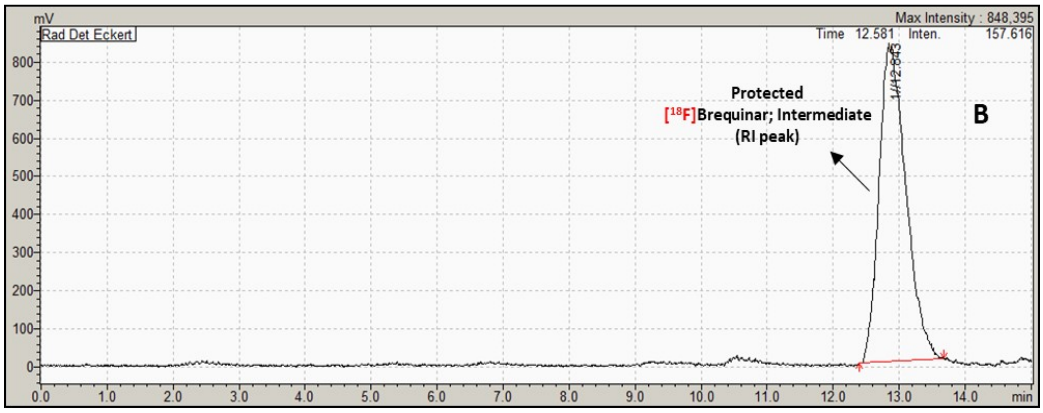


Figure S2. GE Healthcare Tracerlab FX2N (second generation) auto-module instrument design and software flow diagram.

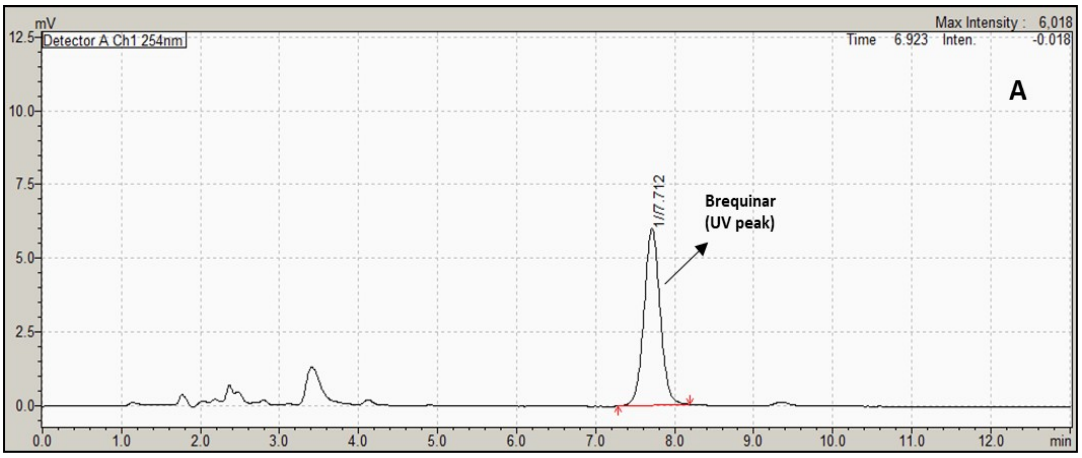


Peak#	Ret. Time	Area	Height	Mark	Conc.
1	12.425	1331062	188260	M	44.298

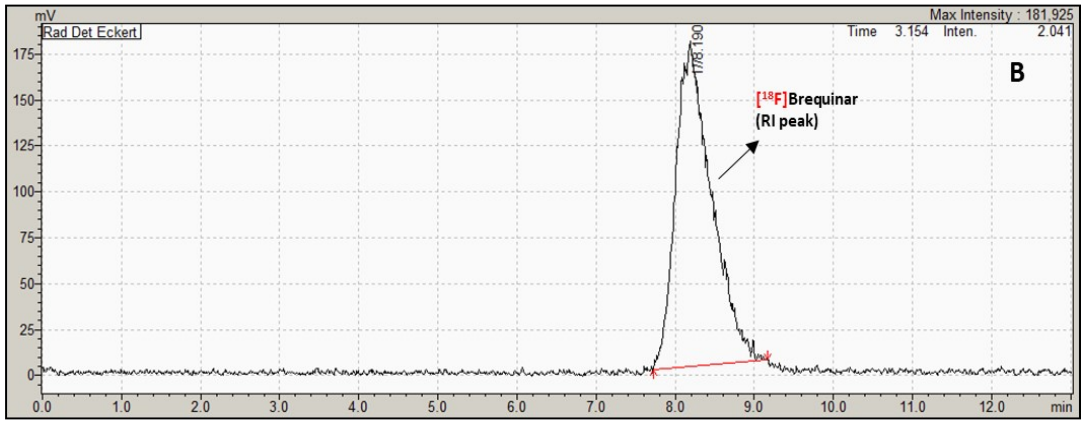


Peak#	Ret. Time	Area	Height	Mark	Conc.
1	12.843	23527378	832782	M	100.000

Figure S3. Analytical QC HPLC (analysis was performed using the gradient method) chromatogram of protected [^{18}F]brequinar (intermediate). UV peak **(A)**; RI Peak **(B)**



Peak#	Ret. Time	Area	Height	Mark	Conc.
1	7.712	84767	5991	M	100.000



Peak#	Ret. Time	Area	Height	Mark	Conc.
1	8.190	5563630	176810	M	100.000

Figure S4: Analytical QC HPLC (analysis was performed using the isocratic method) chromatogram of [^{18}F]brequinar. UV peak **(A)**; RI Peak **(B)**

Figure S5: PET/CT dynamic images_video.pptx

Table S1. Preparation of reagents for the GE Tracerlab FX2N synthesis module

Entry	Positions	Reagents or materials	Quantities
1	Vial V1	K222 in ACN + K_2CO_3 in H_2O	4 mg in 900 µL + 0.7 mg in 100 µL
2	Vial V2	$Cu(OTf)_2(py)_4$ in anhydrous ACN	0.3 mL
3	Vial V3	Precursor in ACN	3.9 mg in 0.3 mL
4	Vial V4, V5	ACN solvent (3 times rinse)	1.5 mL
5	Vial V8	ACN + DW + 8N NaOH	0.2 mL +0.4 mL+0.2 mL
6	Vial V9	3 N HCL	0.545 mL
7	Vial V34	DW	0.454 mL
8	RBF (Collection bulb)	DW	30 mL
9	Vail 43	DW	10 mL
10	Vail 42	Ethanol	0.5 mL
11	Vail 41	Saline	4.5 mL
12	V16	Connect product vial including 0.22 µm GS sterile filter and venting needle	1
13	V10-V11 cartridge	Sep-Pak QMA light Preconditioned with 1M K_2CO_3 (5mL) followed by water (5mL)	1
11	V15-V17	Sep-Pak C-18 Plus Preconditioned with Ethanol (5mL) followed by water (5mL)	1
12		Sep-Pak Silica plus Long Cartridge 690 mg Preconditioned with Acetonitrile (9mL)	1