Supplementary Information

Synthesis of plasmodione metabolites and ^{13}C-enriched plasmodione as chemical tools for drug metabolism investigation

Liwen Feng,[a] Don Antoine Lanfranchi,[a] Leandro Cotos-Munoz,[a] Elena Cesar-Rodo,[a] Katharina Ehrhardt,[b] Alice-Anne Goetz,[b] Herbert Zimmermann,[c] François Fenaille,[d] Stephanie Blandin[b]* and Elisabeth Davioud-Charvet[a]*

[a.] *a. Université de Strasbourg, Université de Haute-Alsace, CNRS, LIMA, UMR 7042, Team Bioorganic and Medicinal Chemistry, ECPM 25 Rue Becquerel, 67087 Strasbourg, France. E-mail: elisabeth.davioud@unistra.fr*

[b.] *Université de Strasbourg, CNRS, Inserm, MIR UPR9022/U1257, F-67000 Strasbourg, France. E-mail: sblandin@unistra.fr*

[c.] *Max Planck Institut für medizinische Forschung, Department of Biomolecular Mechanisms, Jahnstraße 29, D-69120 Heidelberg.*

[d.] *CEA Paris-Saclay, Laboratoire d'Etudes du Métabolisme des Médicaments (LEMM), Bât. 136, 91191 Gif-Sur-Yvette cedex France.*

† Electronic Supplementary Information (ESI) available: ^{1}H, ^{13}C and ^{19}F NMR spectrum, mass spectrum of compounds **1-17** and UHPLC-MS/MS spectrum of drug metabolites from mice urine. See DOI: 10.1039/x0xx00000x

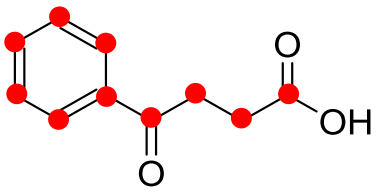
Synthesis of 4-oxo-4-phenylbutanoic acid (1a/1b): Dihydrofuran-2,5-dione-2,3,4,5-$^{13}C_4$ (1 equiv., 500 mg, 4.81 mmol) was refluxed in sulfuryl chloride (24.2 mL) for 3 hours under argon atmosphere. Excess of sulfuryl chloride was removed under vacuum and the residue suspended in tetrachloroethane (20 mL) in an ice-water bath. Addition of benzene-1,2,3,4,5,6-$^{13}C_6$ (1 equiv., 404 mg, 0.43 mL, 6.71 mmol) was followed by portion-wise addition of fresh aluminium chloride (4.71 equiv., 3015 mg, 22.6 mmol) with stirring. The resulting mixture was stirred overnight at room temperature under argon atmosphere, gradually deepening in color. The reaction was poured into the mixture of ice (50 g) and concentrated HCl (5 ml, 37%), the solvent was removed under reduced pressure. The remaining mixture was extracted with ether (5×25 ml). The combined organic layers were washed with water (2×25 ml), brine, dried over $MgSO_4$ and concentrated under reduced pressure to give a yellowish solid (887 mg, 98%). **4-oxo-4-phenylbutanoic acid (1a):** ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.99 (d, 2H, *J* = 7.2 Hz, phenylH), 7.58 (t, 1H, *J* = 7.6 Hz, phenylH), 7.47 (t, 2H, *J* = 7.6 Hz, phenylH), 3.32 (t, 2H, *J* = 6.6 Hz, CH_2), 2.82 (t, 2H, *J* = 6.6 Hz, CH_2) ppm (see Fig. S1, ESI†). ^{13}C NMR (100 MHz, $CDCl_3$): δ 197.9, 178.4, 136.5, 133.5, 128.8, 128.2, 33.3, 28.1 ppm (see Fig. S2, ESI†). HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{10}H_{11}O_3$: 179.0703; found 179.0702. **4-oxo-4-(phenyl-$^{13}C_6$)butanoic-1,2,3,4-$^{13}C_4$ acid (1b):** ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98 (dm, 2H, *J* = 164.0 Hz, phenylH), 7.56 (dt, 1H, *J* = 150.2 Hz, *J* = 7.7Hz, phenylH), 7.47 (dt, 2H, *J* = 161.3 Hz, *J* = 7.9 Hz phenylH), 3.32 (dm, 2H, *J* = 126.8 Hz, *J* = 5.6 Hz, CH_2), 2.82 (ddm, 2H, *J* = 129.3 Hz, *J* = 7.5 Hz, *J* = 3.5 Hz, CH_2) ppm(see Fig. S1, ESI†). ^{13}C NMR (100 MHz, $CDCl_3$): δ 198.0 (dd, *J* = 52.5 Hz, *J* = 39.8 Hz), 177.7 (d, *J* = 56.3 Hz), 136.5 (qm, *J* = 49.5 Hz), 133.5 (tm, *J* = 50.3 Hz), 128.5 (td, *J* = 39.1 Hz, *J* = 3.5 Hz), 128.5 (dtt, *J* = 76.7 Hz, *J* = 53.3 Hz, *J* = 3.5 Hz), 33.4 (td, *J* = 40.0 Hz, *J* = 13.1 Hz), 28.0 (dd, *J* = 56.8 Hz, *J* = 38.3 Hz) ppm (see Fig. S2, ESI†). ESI-MS *m/z*: $[M+H]^+$ calcd for $^{13}C_{10}H_{11}O_3$: 189.15; found 189.10.

Synthesis of 4-phenylbutanoic acid (2a/2b): To a solution of 4-oxo-4-(phenyl-$^{13}C_6$)butanoic-1,2,3,4-$^{13}C_4$ acid **1b** (1 equiv., 800 mg, 4.25 mmol) in diethylene glycol (12.9 mL) was added hydrazine monohydrate (5.2 equiv., 1108 mg, 1.08 mL, 22.1 mmol). After stirring at room temperature for 30 min, KOH (4.57 equiv., 1282 mg, 19.4 mmol) was added and the reaction mixture was heated at 120°C for 1.5 hours. Then the reaction mixture was heated (T = 215 °C) to distilled low boiling material; when diethylene glycol started to be distilled, the heating was stopped. The reaction mixture was then refluxed for 3 hours. After cooling down, the reaction mixture was poured into ice (25 g), acidified to pH = 2 and extracted with Et_2O (5×20 mL). The combined organic layers were washed with water (2×25 mL) and brine, dried over $MgSO_4$ and concentrated under reduce pressure to give a brownish visqueous oil (699.2 mg, 94%). **4-phenylbutanoic acid (2a):** ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28 (m, 2H, phenylH), 7.19 (m, 3H, phenylH), 2.67 (t, 2H, *J* = 7.5 Hz, CH_2), 2.37 (t, 2H, *J* = 7.5 Hz, CH_2), 1.97 (p, 2H, *J* = 7.5 Hz, CH_2) ppm (see Fig. S3, ESI†). **^{13}C** NMR (100 MHz, $CDCl_3$): δ 179.6, 141.5, 128.8, 128.7, 126.4, 35.3, 33.6, 26.6 ppm (see Fig. S4, ESI†). HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{10}H_{13}O_2$ 165.0910; found 165.0908. **4-(phenyl-$^{13}C_6$)butanoic-1,2,3,4-$^{13}C_4$ acid (2b):** ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45 (m, 2H, phenylH), 7.07 (m, 3H, phenylH), 2.68 (dm, 2H, *J* = 121.3 Hz, CH_2), 2.37 (dm, 2H, *J* = 122.1 Hz, CH_2), 1.98 (dm, 2H, *J* = 131.2 Hz, CH_2) ppm (see Fig. S3, ESI†). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.23 (d, *J* = 54.1Hz), 142.4-140.61 (m), 129.0 (t, *J* = 39.3 Hz), 128.5 (t, *J* = 39.3 Hz), 127.3-125.5 (m), 35.3 (t, *J* = 38.6 Hz), 33.7 (dd, *J* = 55.2 Hz, *J* = 34.5 Hz), 26.5 (t, *J* = 34.4 Hz) ppm (see Fig. S4, ESI†). ESI-MS *m/z*: $[M+H]^+$ calcd for $^{13}C_{10}H_{13}O_2$: 175.13; found 175.09.

Synthesis of 3,4-dihydronaphthalen-1(*2H*)-one (3a/3b): 4-(phenyl-$^{13}C_6$)butanoic-1,2,3,4-$^{13}C_4$ acid **2b** (1 equiv., 660 mg, 3.79 mmol) was added to methanesulfonic acid (41.3 equiv., 15036 mg, 10.15 mL, 156.5 mmol) and the mixture was heated at 90 °C for 30 min under argon atmosphere. The reaction mixture was then poured into ice-water and extracted with ether (3×40 mL). The combined organic layers were washed with diluted sodium bicarbonate (20 mL), water (2×15 mL) and brine, dried over $MgSO_4$ and concentrated under reduce pressure to give a yellowish liquid (554.7 mg, 94%). **3,4-dihydronaphthalen-1(*2H*)-one (3a):** ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.03 (d, 1H, *J* = 7.9 Hz, ArH), 7.47 (td, 1H, *J* = 7.6 Hz, *J* = 1.1 Hz, ArH), 7.31 (t, 1H, *J* = 7.7 Hz, ArH), 7.25 (m, 1H, ArH), 2.97 (t, 2H, *J* = 5.9 Hz, CH_2), 2.66 (t, 2H, *J* = 6.6 Hz, CH_2), 2.14 (tt, 2H, *J* = 5.9 Hz, *J* = 6.6 Hz, CH_2) ppm (see Fig. S5, ESI†). ^{13}C NMR (100 MHz, $CDCl_3$): δ 198.7, 144.8, 133.7,132.9, 129.1, 127.5, 126.9, 39.5, 30.1, 23.6 ppm (see Fig. S6, ESI†). HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{10}H_{11}O_1$: 147.0804; found 147.0812. **3,4-dihydro-naphthalen-1(*2H*)-one-$^{13}C_{10}$ (3b):** ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.03 (d, 1H, *J* = 106.1 Hz, ArH), 7.47 (m, 1H, *J* = 157.4 Hz, ArH), 7.31 (m, 1H, *J* = 162.4 Hz, ArH), 7.25 (m, 1H, *J* = 158.3 Hz, ArH), 2.96 (d, 2H, *J* = 132.3 Hz, CH_2), 2.66 (dm, 2H, *J* = 127.7 Hz, *J* = 5.7 Hz, CH_2), 2.14 (d, 2H, *J* = 131.3 Hz, CH_2) ppm (see Fig. S5, ESI†). ^{13}C NMR (100 MHz, $CDCl_3$): δ 198.5 (dd, *J* = 50.1 Hz, *J* = 41.0 Hz), 144.6 (dd, *J* = 102.7 Hz, *J* = 50.4 Hz), 133.3 (q, *J* = 51.9 Hz), 132.9 (dd, *J* = 140.3 Hz, *J* = 57.8 Hz), 128.9 (tq, *J* = 56.3 Hz, *J* = 3.0 Hz), 127.5 (m), 126.9 (m), 39.5 (ddd, *J* = 42.1 Hz, *J* = 31.8 Hz, *J* = 12.2 Hz), 30.1 (dd, *J* = 39.8 Hz, *J* = 34.5 Hz), 23.6 (t, *J* = 33.2 Hz) ppm (see Fig. S6, ESI†). ESI-MS *m/z*: $[M+H]^+$ calcd for $^{13}C_{10}H_{11}O_1$: 157.16; found 157.12.

4-oxo-4-phenylbutanoic acid (1a/1b):

a=^{12}C
b=^{13}C



4-oxo-4-(phenyl-$^{13}C_6$)butanoic-1,2,3,4-$^{13}C_4$ acid

^{1}H NMR

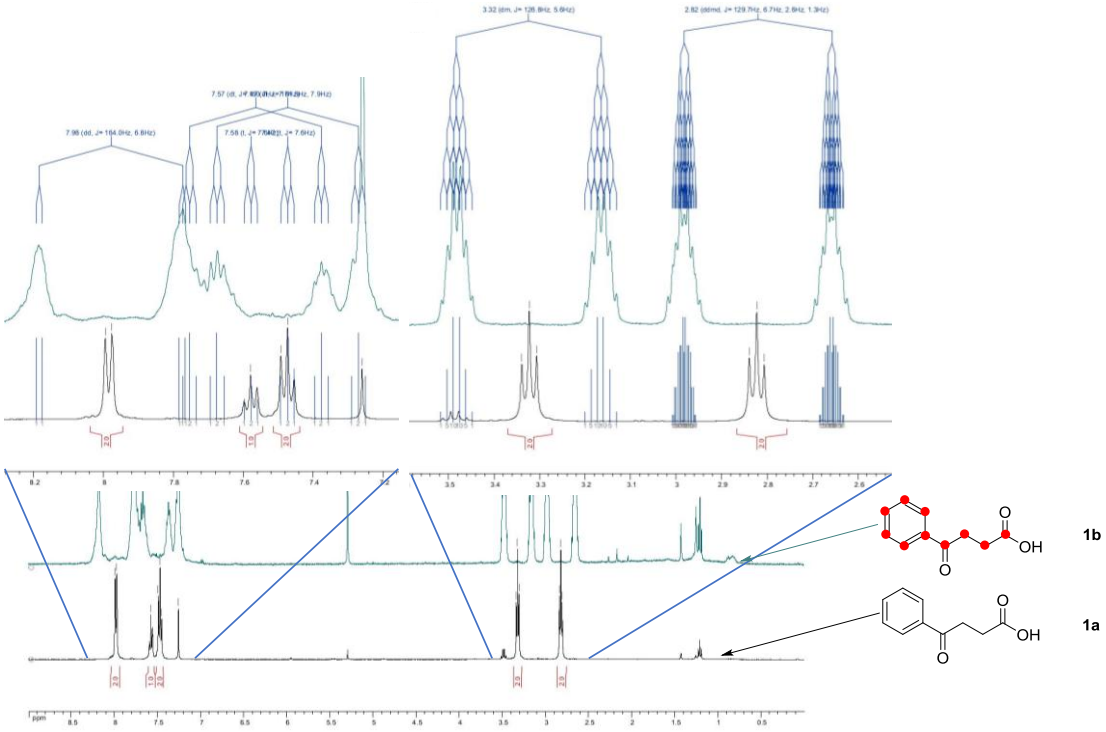


Figure S1. ^{1}H NMR (400 MHz, $CDCl_3$) of 4-oxo-4-phenylbutanoic acid **1a** and 4-oxo-4-(phenyl-$^{13}C_6$)butanoic-1,2,3,4-$^{13}C_4$ acid **1b**. The black line represents **1a** and the green line represents **1b**.

^{13}C NMR:

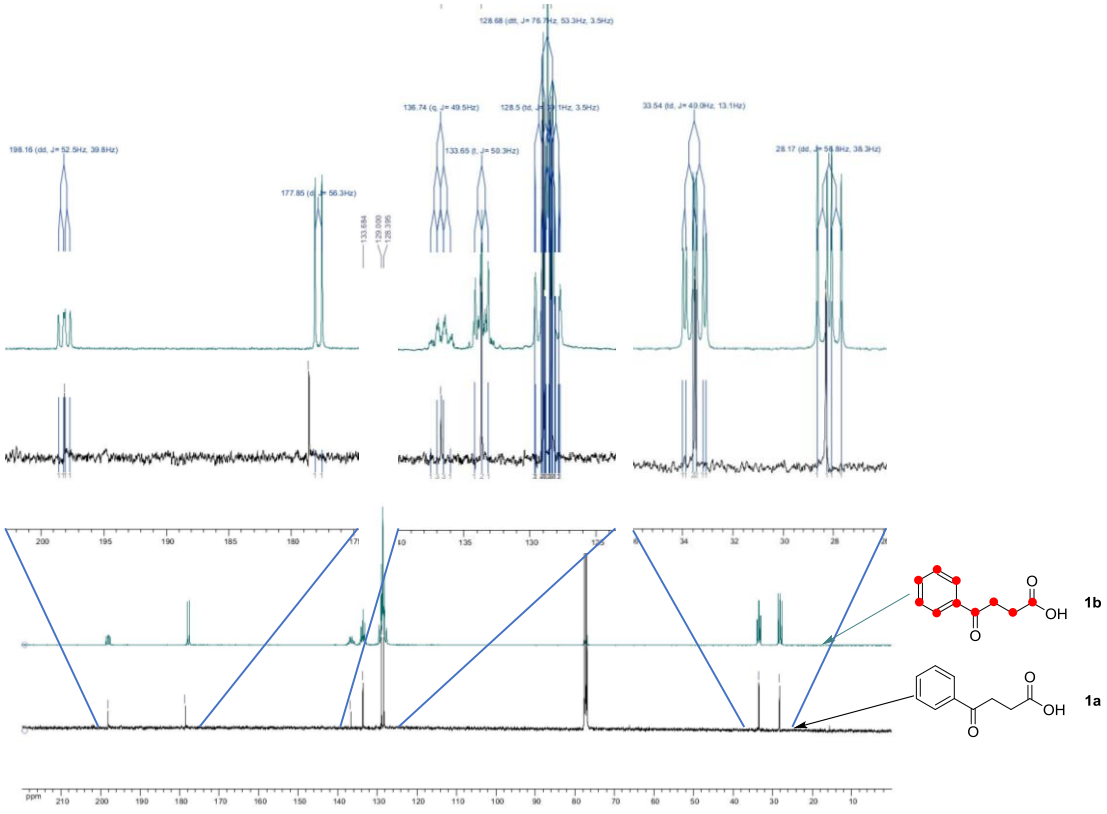
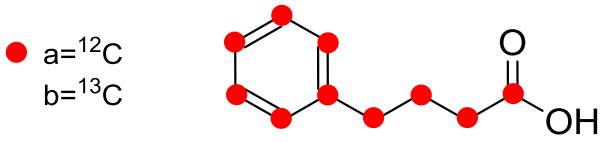


Figure S2. ^{13}C NMR (100 MHz, $CDCl_3$) of 4-oxo-4-phenylbutanoic acid **1a** and 4-oxo-4-(phenyl-$^{13}C_6$)butanoic-1,2,3,4-$^{13}C_4$ acid **1b**. The black line represents **1a** and the green line represents **1b**.

4-phenylbutanoic acid (2a/2b):



4-(phenyl-$^{13}C_6$)butanoic-1,2,3,4-$^{13}C_4$ acid

^{1}H NMR

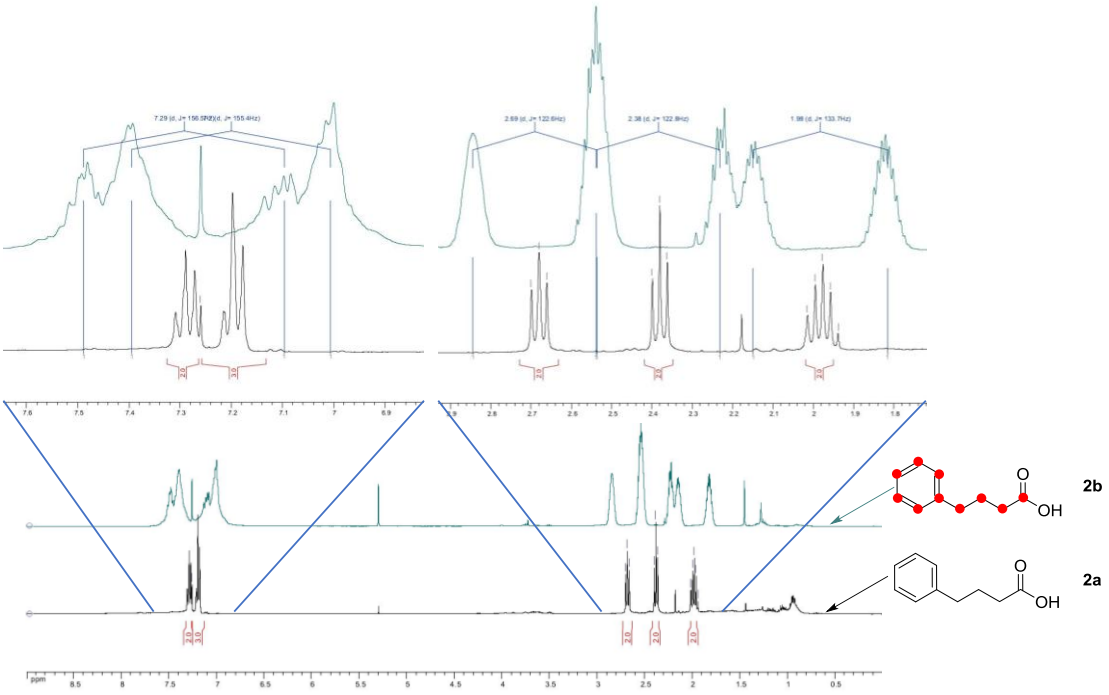


Figure S3. ^{1}H NMR (400 MHz, $CDCl_3$) of 4-phenylbutanoic acid **2a** and 4-(phenyl-$^{13}C_6$)butanoic-1,2,3,4-$^{13}C_4$ acid **2b**. The black line represents **2a** and the green line represents **2b**.

^{13}C NMR

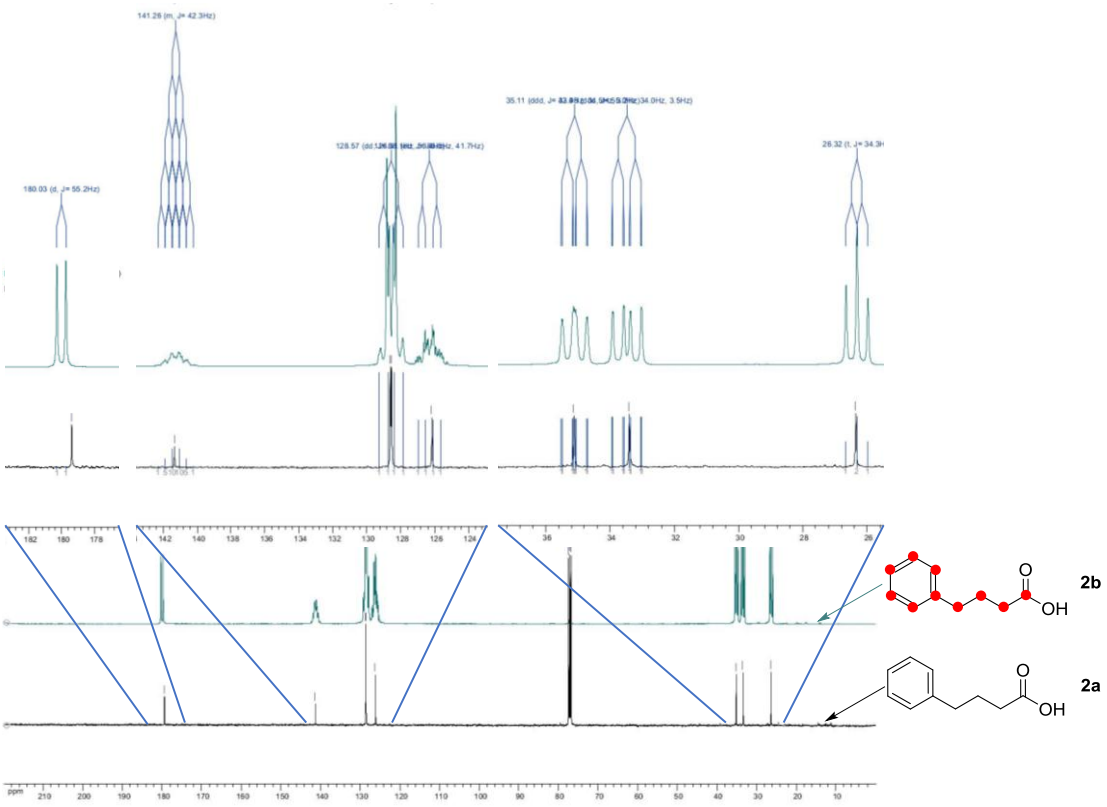
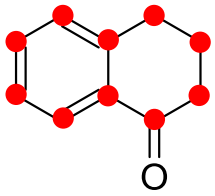


Figure S4. ^{13}C NMR (100 MHz, $CDCl_3$) of 4-phenylbutanoic acid **2a** and 4-(phenyl-$^{13}C_6$)butanoic-1,2,3,4-$^{13}C_4$ acid **2b**. The black line represents **2a** and the green line represents **2b**.

3,4-dihydronaphthalen-1(*2H*)-one (3a/3b):

a=^{12}C
b=^{13}C



3,4-dihydronaphthalen-1(*2H*)-one-$^{13}C_{10}$

^{1}H NMR

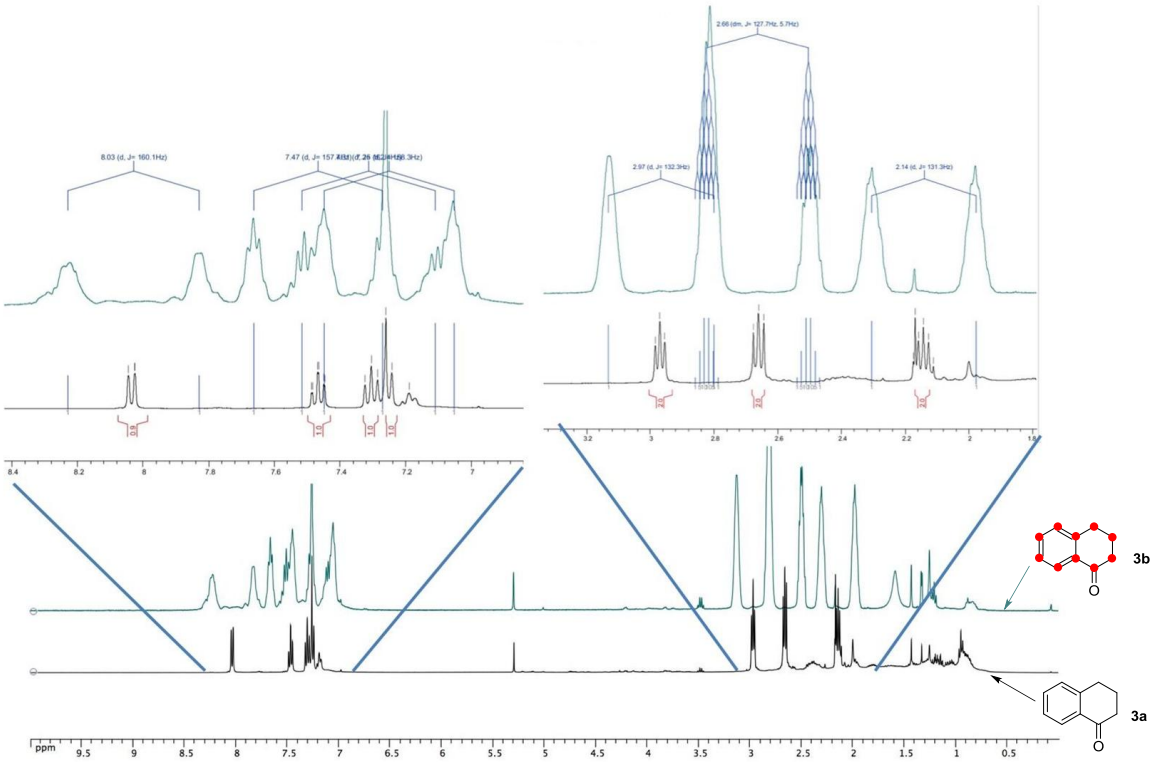


Figure S5. ^{1}H NMR (400 MHz, $CDCl_3$) of tetralone **3a** and $^{13}C_{10}$-enriched tetralone **3b**. The black line represents **3a** and the green line represents **3b**.

^{13}C NMR

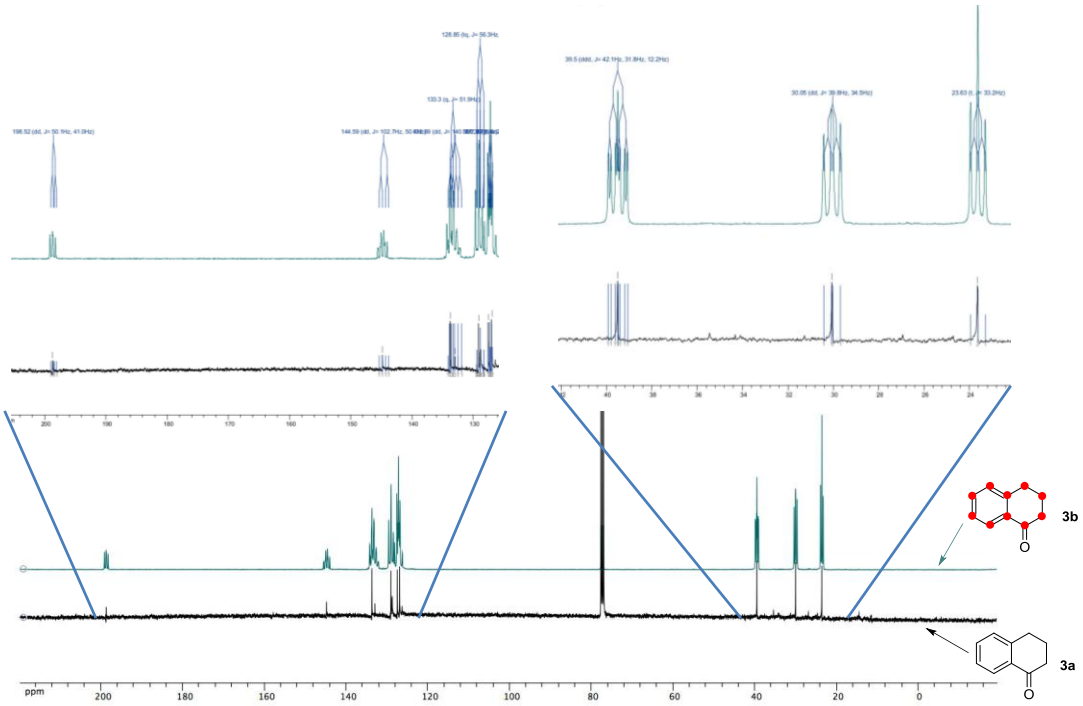
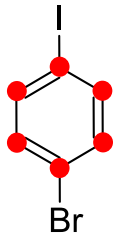


Figure S6. ^{13}C NMR (100 MHz, $CDCl_3$) of tetralone **3a** and $^{13}C_{10}$-enriched tetralone **3b**. The black line represents **3a** and the green line represents **3b**.

1-bromo-4-iodobenzene (4a/4b):

a=^{12}C
b=^{13}C



1-bromo-4-iodobenzene-1,2,3,4,5,6-$^{13}C_6$

^{1}H NMR

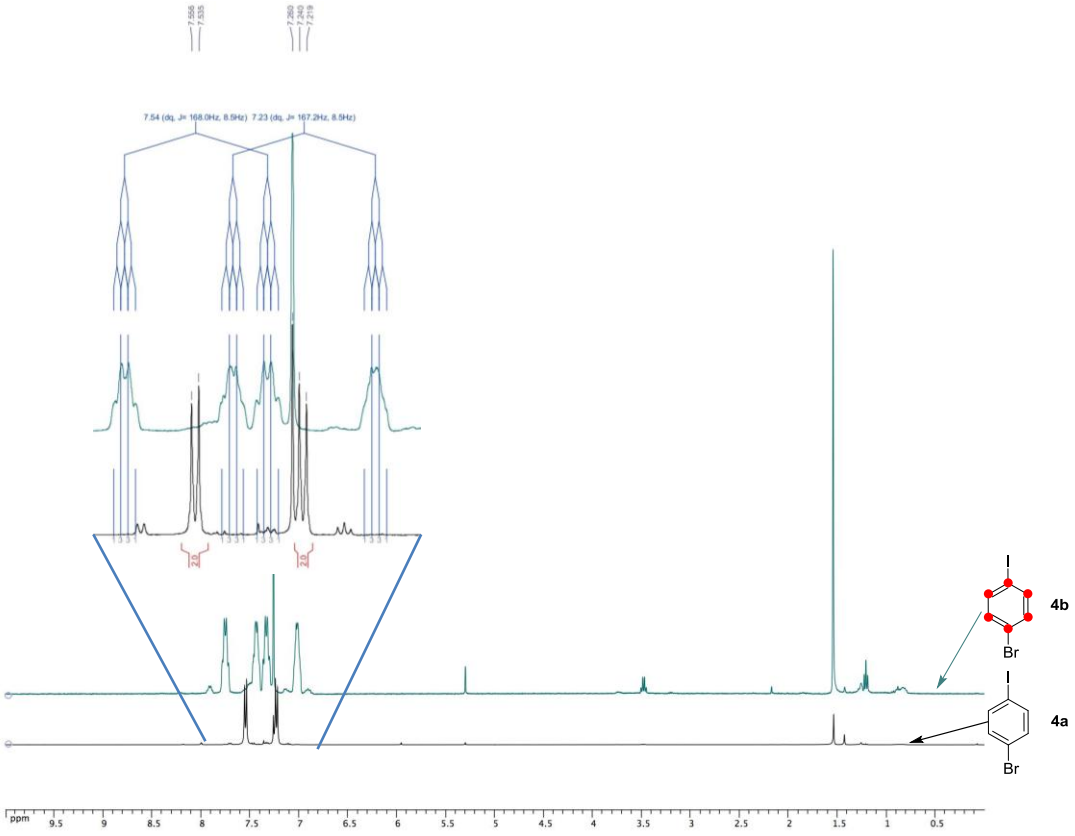


Figure S7. ^{1}H NMR (400 MHz, $CDCl_3$) of 1-bromo-4-iodobenzene **4a** and 1-bromo-4-iodobenzene-1,2,3,4,5,6-$^{13}C_6$ **4b**. The black line represents **4a** and the green line represents **4b**.

^{13}C NMR

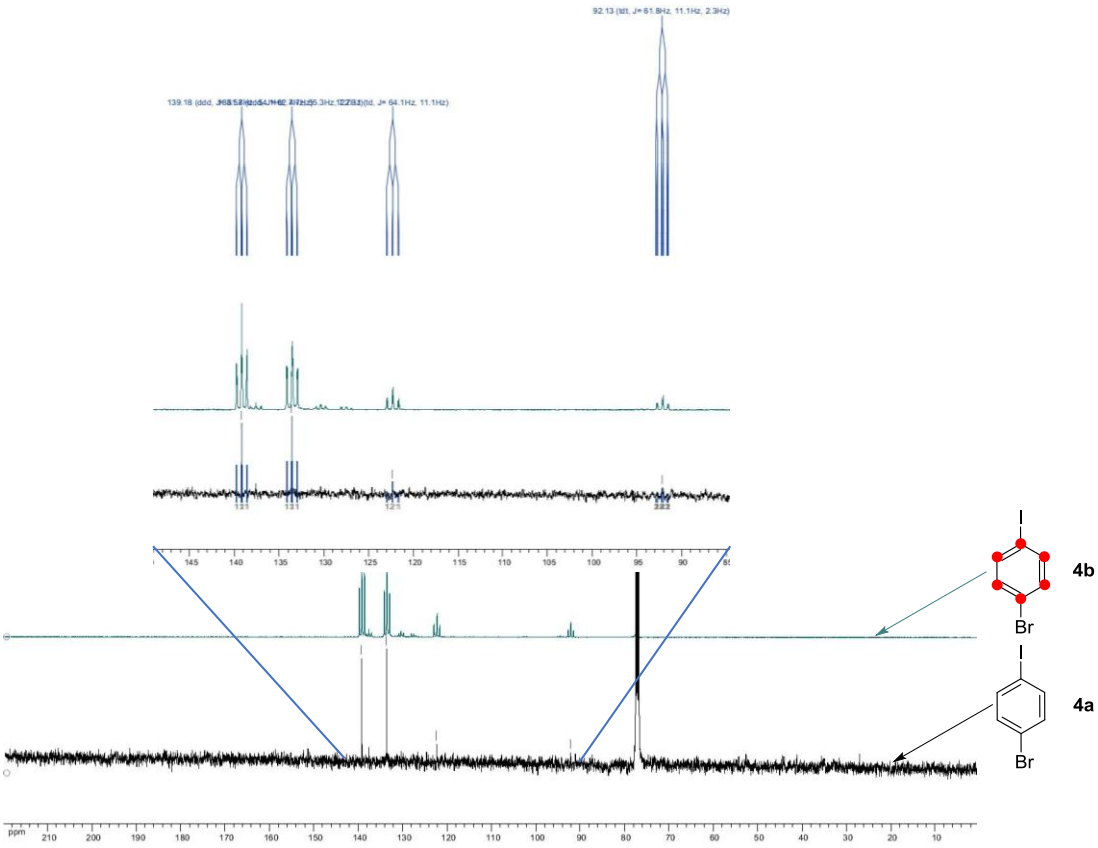
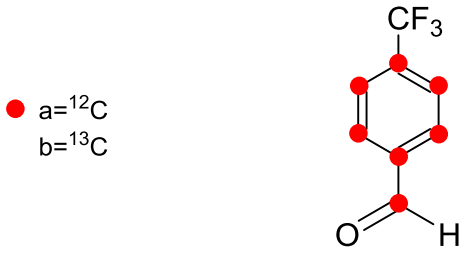


Figure S8. ^{13}C NMR (100 MHz, $CDCl_3$) of 1-bromo-4-iodobenzene **4a** and 1-bromo-4-iodobenzene-1,2,3,4,5,6-$^{13}C_6$ **4b**. The black line represents **4a** and the green line represents **4b**.

4-(trifluoromethyl)benzaldehyde (6a/6b):



4-(trifluoromethyl)benzaldehyde-1,2,3,4,5,6-$^{13}C_6$

^{1}H NMR

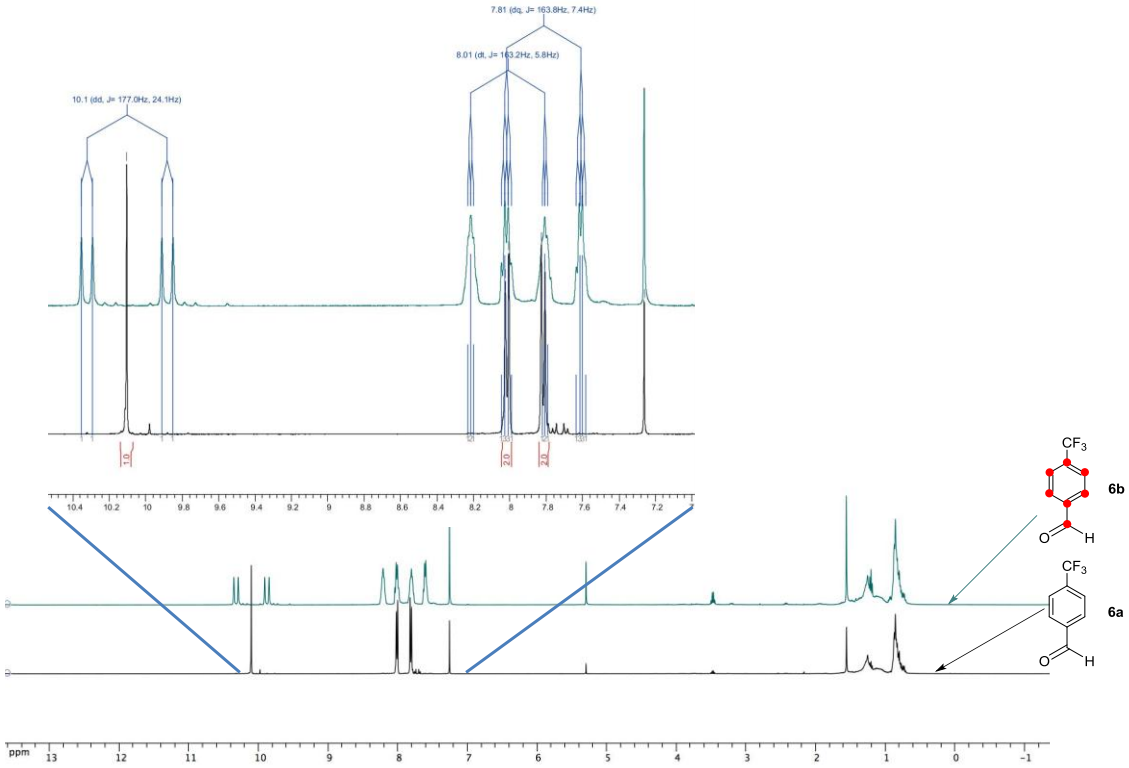


Figure S9. ^{1}H NMR (400 MHz, $CDCl_3$) of *p*-trifluoromethylbenzaldehyde **6a** and $^{13}C_7$-*p*-trifluoromethylbenzaldehyde **6b**. The black line represents **6a** and the green line represents **6b**.

^{13}C NMR

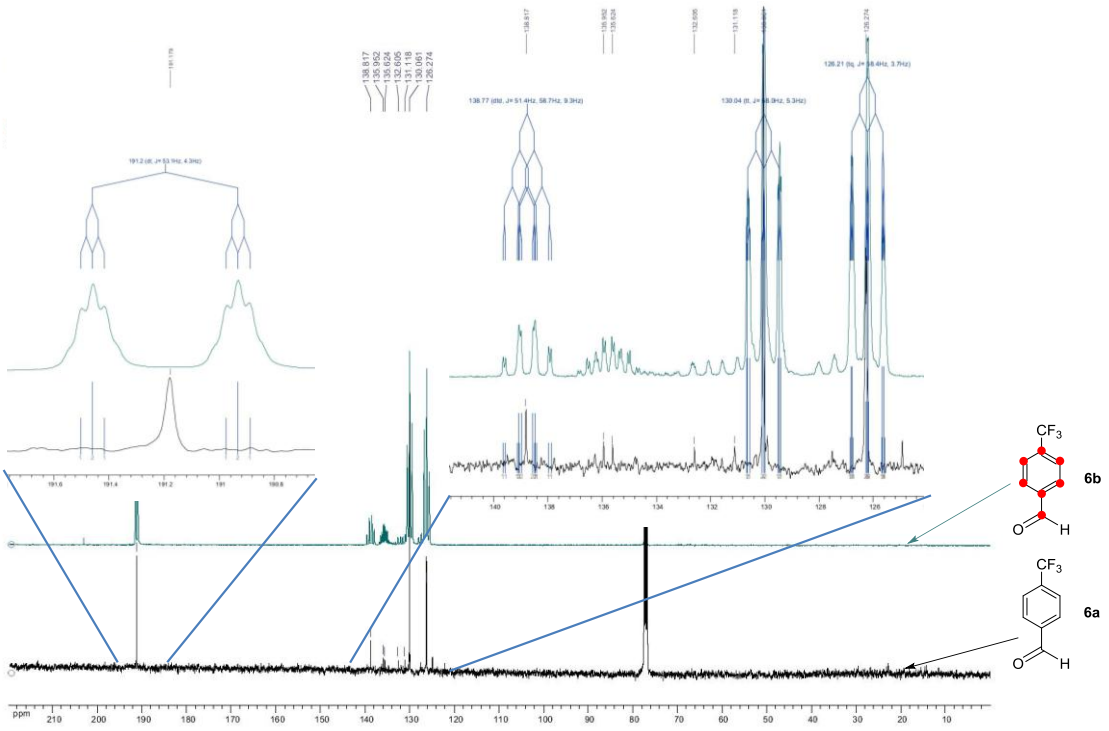


Figure S10. ^{13}C NMR (100 MHz, $CDCl_3$) of *p*-trifluoromethylbenzaldehyde **6a** and $^{13}C_{7}$-*p*-trifluoromethylbenzaldehyde **6b**. The black line represents **6a** and the green line represents **6b**.

^{19}F NMR

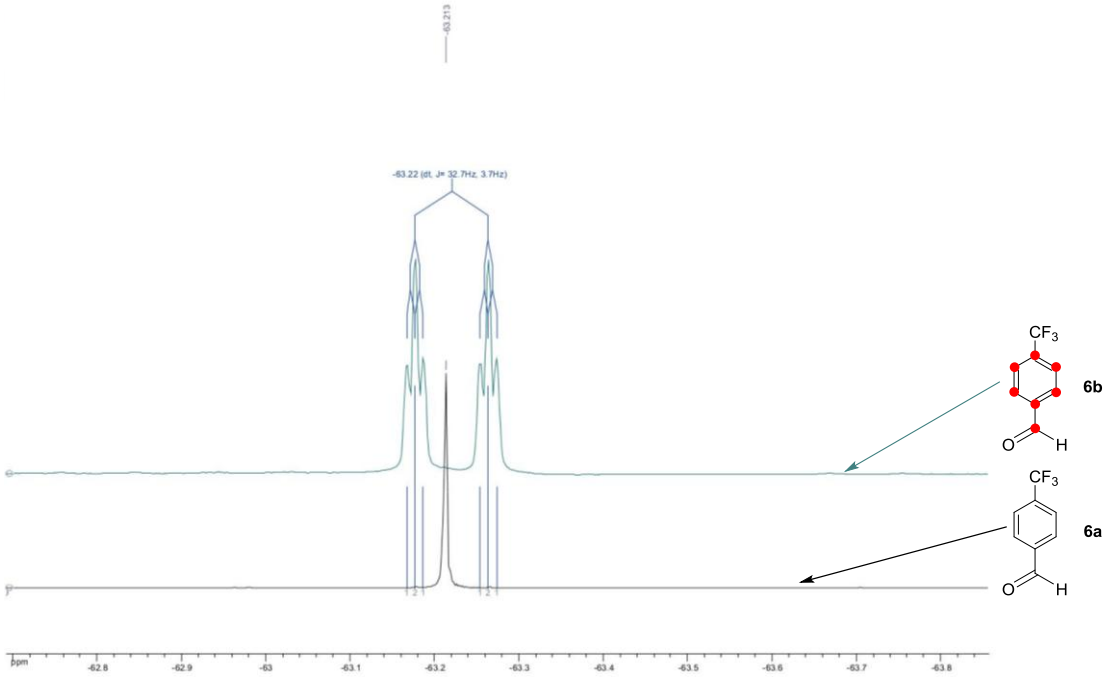
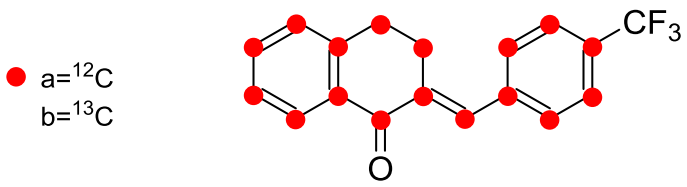


Figure S11. ^{19}F NMR (376 MHz, $CDCl_3$) of *p*-trifluoromethylbenzaldehyde **6a** and $^{13}C_7$-*p*-trifluoromethylbenzaldehyde **6b**. The black line represents **6a** and the green line represents **6b**.

2-(4-(trifluoromethyl)benzylidene)-3,4-dihydronaphthalen-1(2H)-one (7a/7b):



2-((4-(trifluoromethyl)phenyl-1,2,3,4,5,6-$^{13}C_6$)methylene-^{13}C)-3,4-dihydronaphthalen-1(*2H*)-one-$^{13}C_{10}$

^{1}H NMR

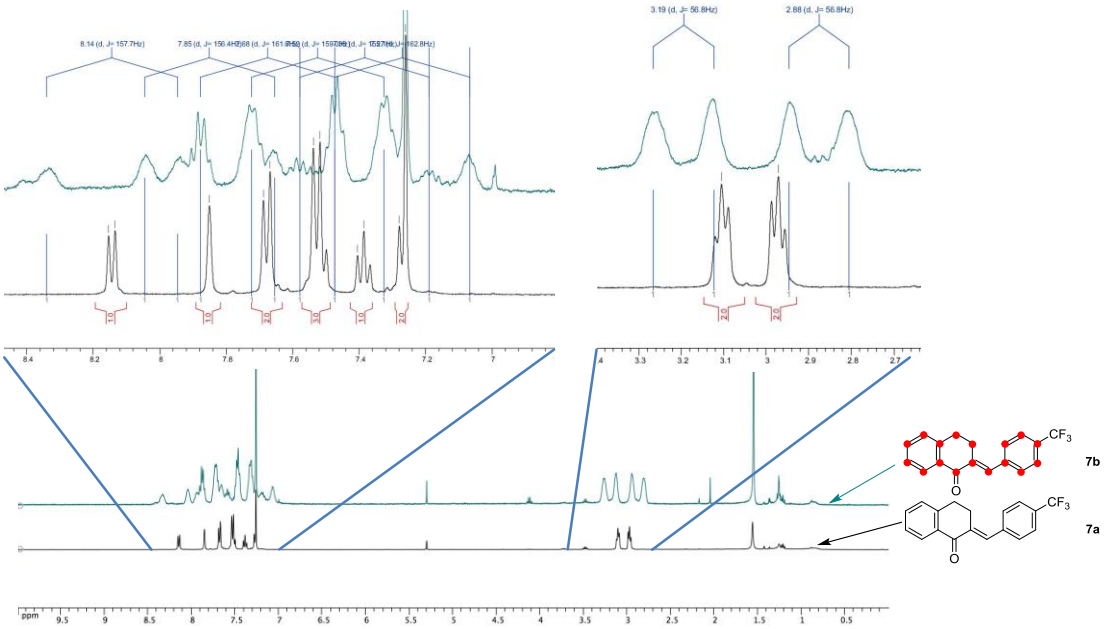


Figure S12. ^{1}H NMR (400 MHz, $CDCl_3$) of 2-(4-(trifluoromethyl)benzylidene)-3,4-dihydronaphthalen-1(2H)-one **7a** and 2-((4-(trifluoromethyl)phenyl-1,2,3,4,5,6-$^{13}C_6$)methylene-^{13}C)-3,4-dihydronaphthalen-1(*2H*)-one-$^{13}C_{10}$ **7b**. The black line represents **7a** and the green line represents **7b**.

^{13}C NMR

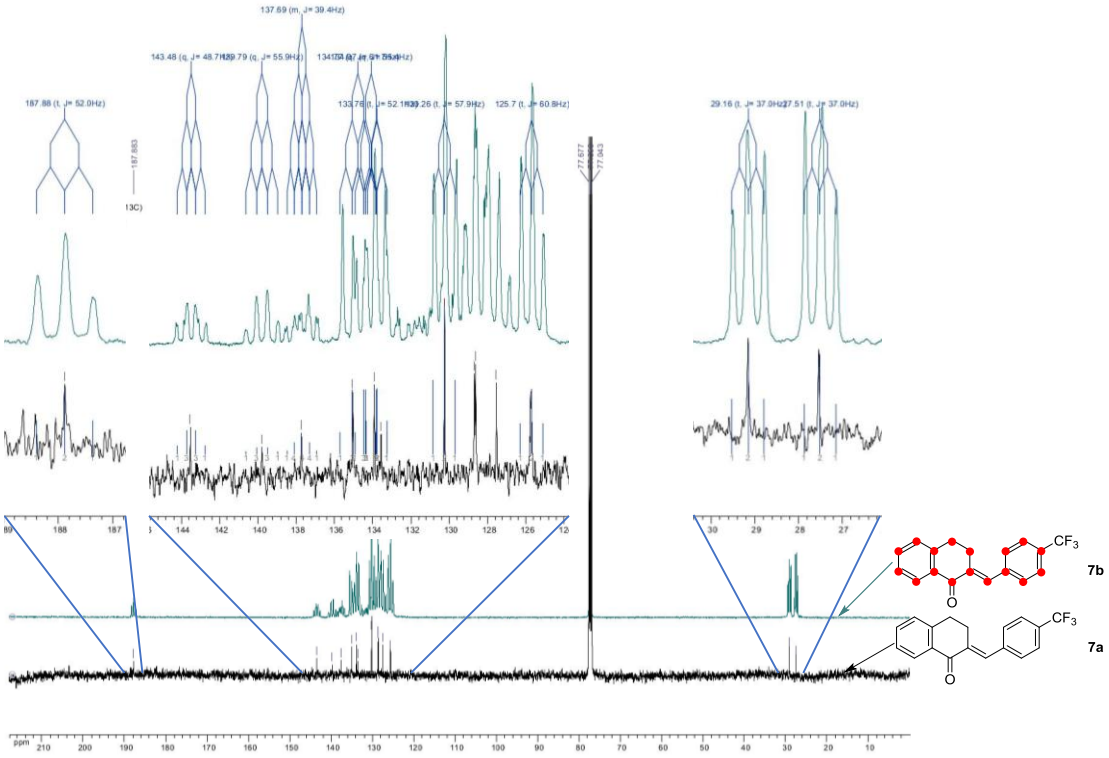


Figure S13. ^{13}C NMR (100 MHz, $CDCl_3$) of 2-(4-(trifluoromethyl)benzylidene)-3,4-dihydronaphthalen-1(2H)-one **7a** and 2-((4-(trifluoromethyl)phenyl-1,2,3,4,5,6-$^{13}C_6$)methylene-^{13}C)-3,4-dihydronaphthalen-1(*2H*)-one-$^{13}C_{10}$ **7b**. The black line represents **7a** and the green line represents **7b**.

^{19}F NMR

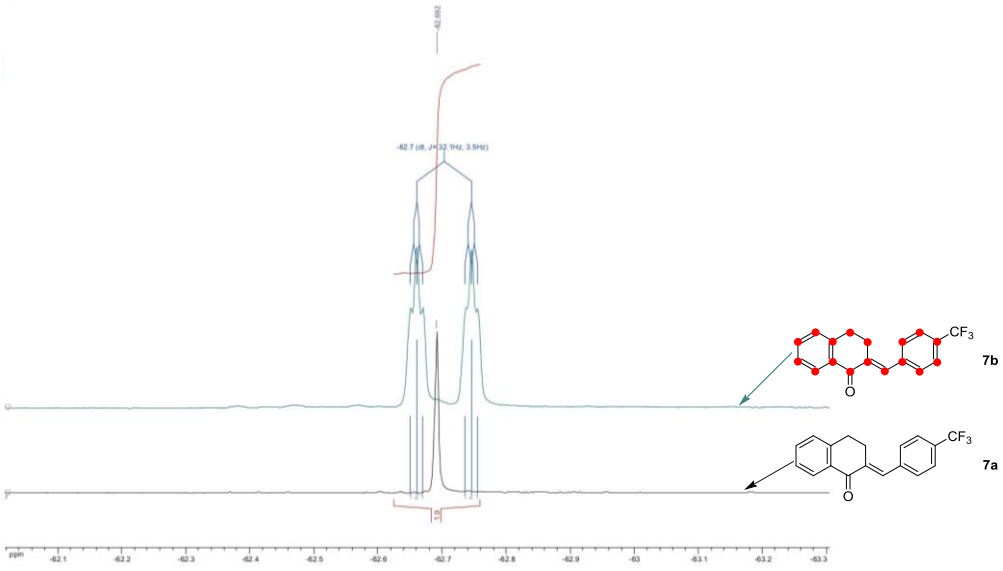
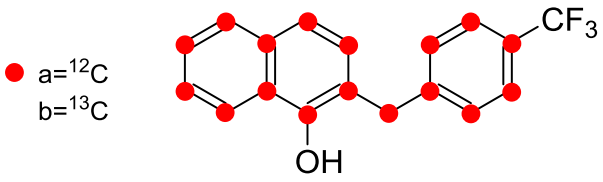


Figure S14. ^{19}F NMR (376 MHz, $CDCl_3$) of 2-(4-(trifluoromethyl)benzylidene)-3,4-dihydronaphthalen-1(2H)-one **7a** and 2-((4-(trifluoromethyl)phenyl-1,2,3,4,5,6-$^{13}C_6$)methylene-^{13}C)-3,4-dihydronaphthalen-1(*2H*)-one-$^{13}C_{10}$ **7b**. The black line represents **7a** and the green line represents **7b**.

2-(4-(trifluoromethyl)benzyl)naphthalen-1-ol (8a/8b):



2-((4-(trifluoromethyl)phenyl-1,2,3,4,5,6-$^{13}C_6$)methyl-^{13}C)naphthalen-1-ol-$^{13}C_{10}$

^{1}H NMR

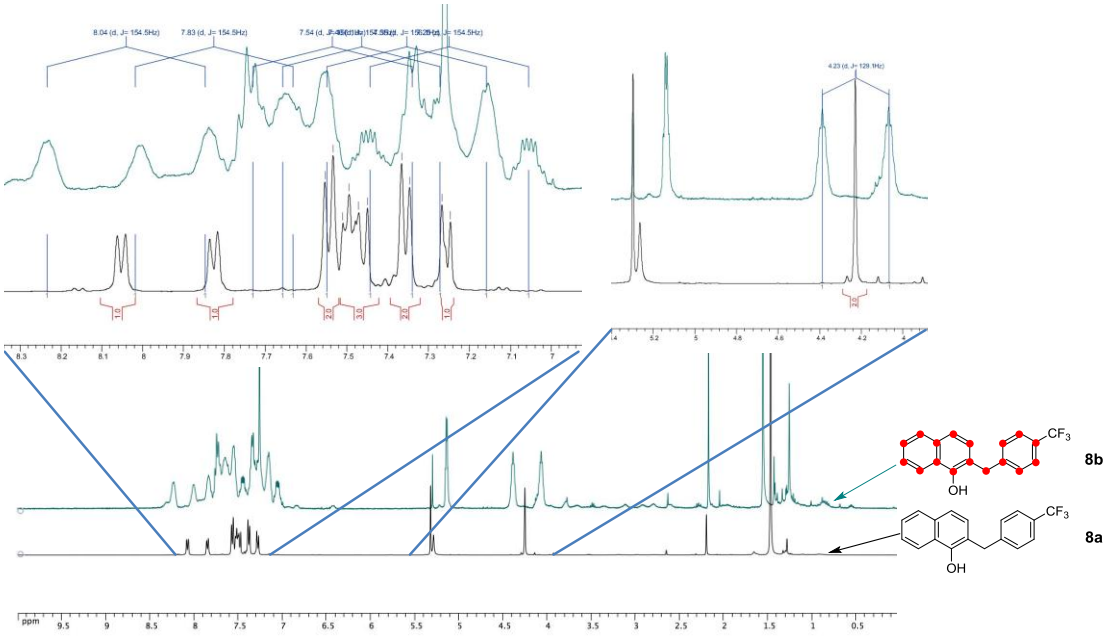


Figure S15. ^{1}H NMR (400 MHz, $CDCl_3$) of 2-(4-(trifluoromethyl)benzyl)naphthalen-1-ol **8a** and 2-((4-(trifluoromethyl)phenyl-1,2,3,4,5,6-$^{13}C_6$)methyl-^{13}C)naphthalen-1-ol-$^{13}C_{10}$ **8b**. The black line represents **8a** and the green line represents **8b**.

^{13}C NMR

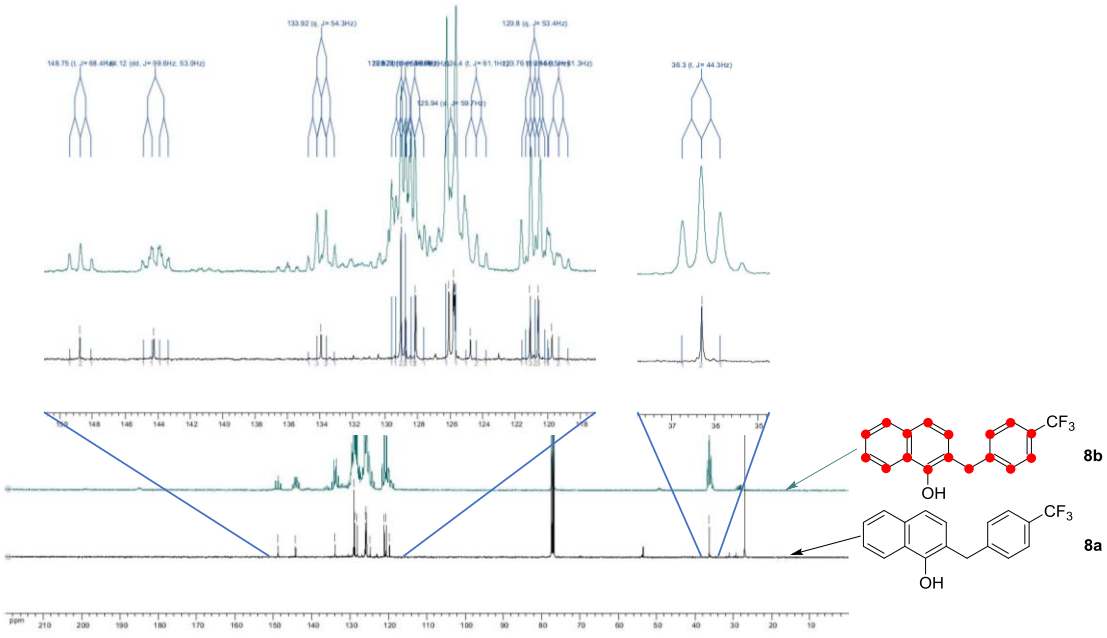


Figure S16. ^{13}C NMR (100 MHz, $CDCl_3$) of 2-(4-(trifluoromethyl)benzyl)naphthalen-1-ol **8a** and 2-((4-(trifluoromethyl)phenyl-1,2,3,4,5,6-$^{13}C_6$)methyl-^{13}C)naphthalen-1-ol-$^{13}C_{10}$ **8b**. The black line represents **8a** and the green line represents **8b**.

^{19}F NMR

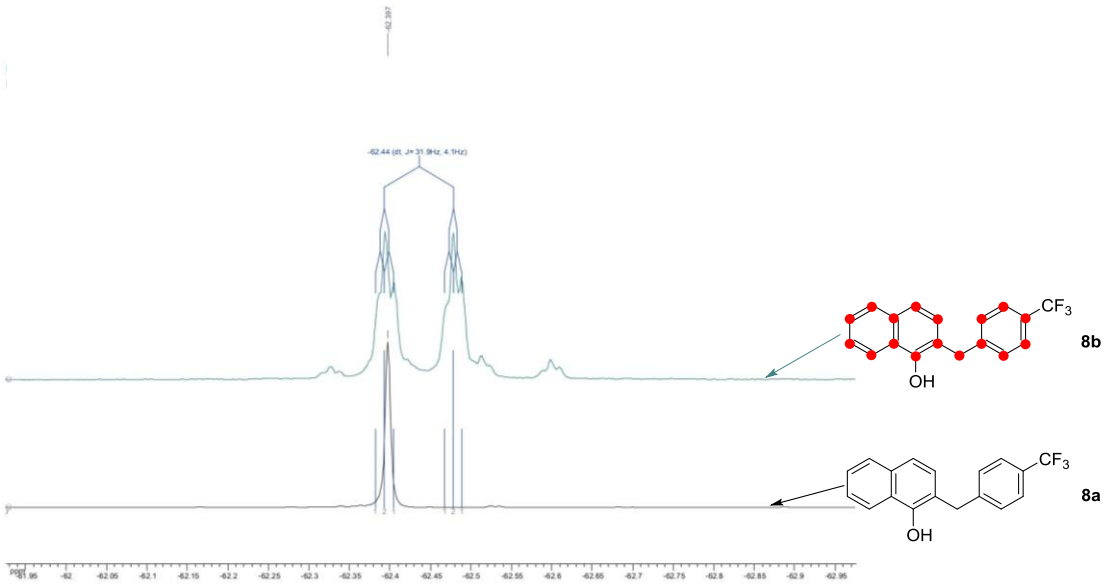
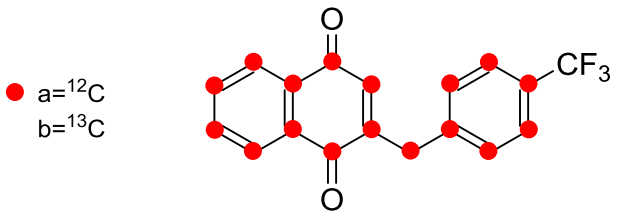


Figure S17. ^{19}F NMR (376 MHz, $CDCl_3$) of 2-(4-(trifluoromethyl)benzyl)naphthalen-1-ol **8a** and 2-((4-(trifluoromethyl)phenyl-1,2,3,4,5,6-$^{13}C_6$)methyl-^{13}C)naphthalen-1-ol-$^{13}C_{10}$ **8b**. The black line represents **8a** and the green line represents **8b**.

2-(4-(trifluoromethyl)benzyl)naphthalene-1,4-dione (9a/9b):



2-((4-(trifluoromethyl)phenyl-1,2,3,4,5,6-$^{13}C_6$)methyl-^{13}C)naphthalene-1,4-dione-$^{13}C_{10}$

^{1}H NMR

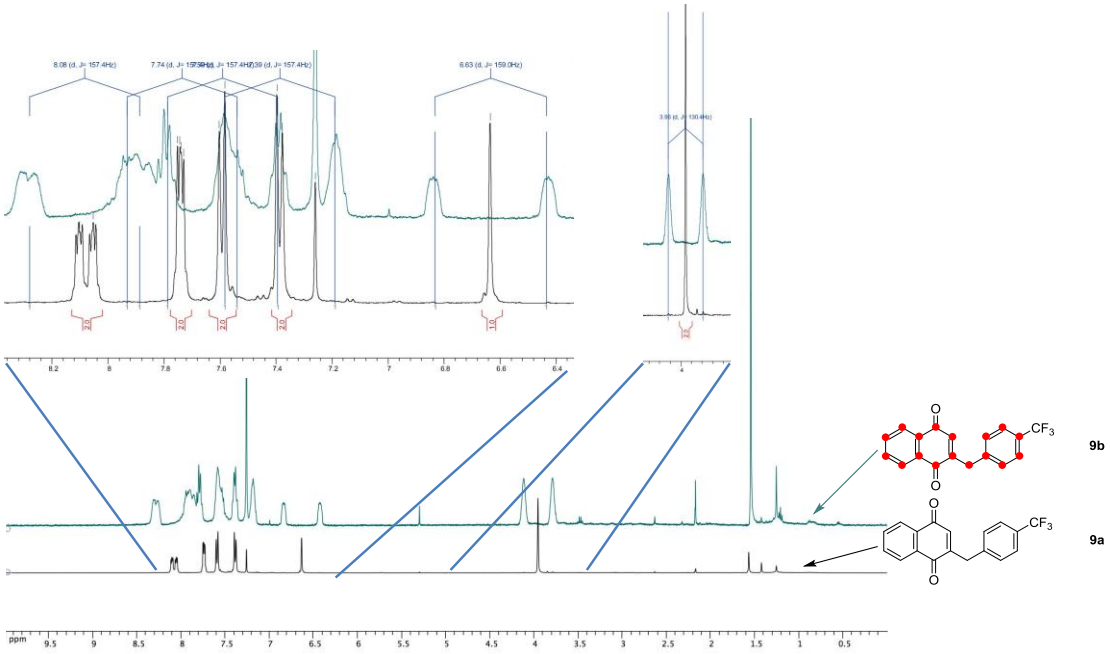


Figure S18. ^{1}H NMR (400 MHz, $CDCl_3$) of 2-(4-(trifluoromethyl)benzyl)naphthalene-1,4-dione **9a** and 2-((4-(trifluoromethyl)phenyl-1,2,3,4,5,6-$^{13}C_6$)methyl-^{13}C)naphthalene-1,4-dione-$^{13}C_{10}$ **9b**. The black line represents **9a** and the green line represents **9b**.

^{13}C NMR

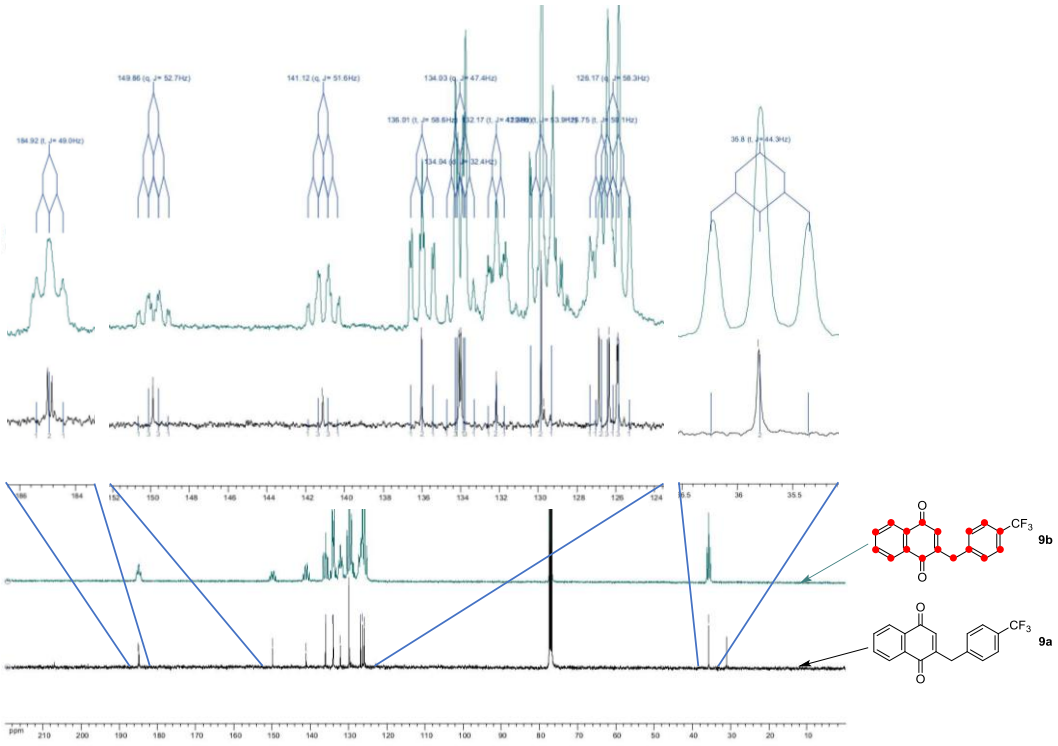


Figure S19. ^{13}C NMR (100 MHz, $CDCl_3$) of 2-(4-(trifluoromethyl)benzyl)naphthalene-1,4-dione **9a** and 2-((4-(trifluoromethyl)phenyl-1,2,3,4,5,6-$^{13}C_6$)methyl-^{13}C)naphthalene-1,4-dione-$^{13}C_{10}$ **9b**. The black line represents **9a** and the green line represents **9b**.

^{19}F NMR

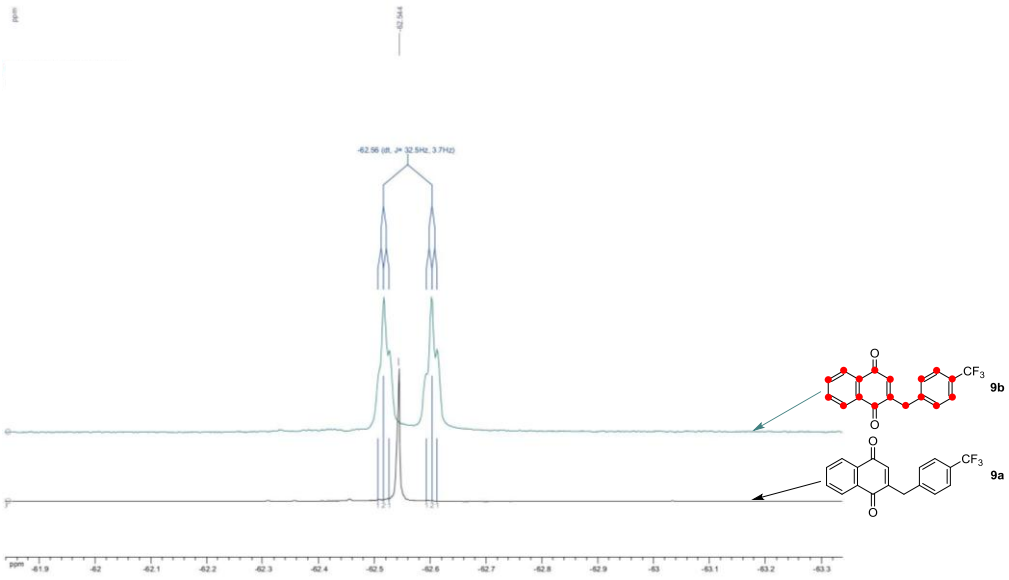
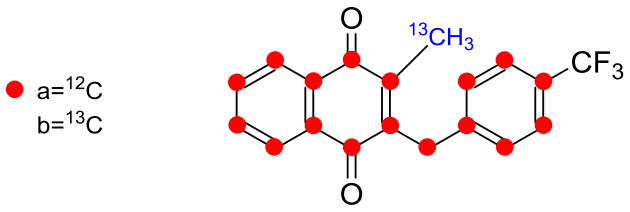


Figure S20. ^{19}F NMR (376 MHz, $CDCl_3$) of 2-(4-(trifluoromethyl)benzyl)naphthalene-1,4-dione **9a** and 2-((4-(trifluoromethyl)phenyl-1,2,3,4,5,6-$^{13}C_6$)methyl-^{13}C)naphthalene-1,4-dione-$^{13}C_{10}$ **9b**. The black line represents **9a** and the green line represents **9b**.

2-(methyl-^{13}C)-3-(4-(trifluoromethyl)benzyl)naphthalene-1,4-dione (10a/10b):



2-(methyl-^{13}C)-3-((4-(trifluoromethyl)phenyl-1,2,3,4,5,6-$^{13}C_6$)methyl-^{13}C)naphthalene-1,4-dione-$^{13}C_{10}$

^{1}H NMR

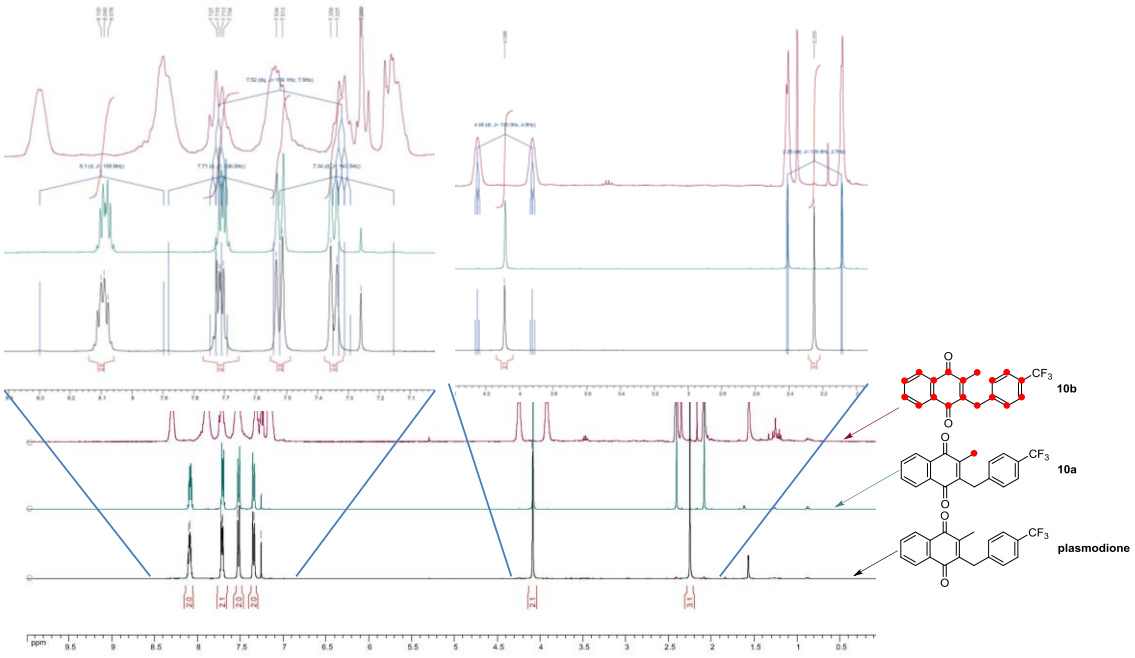


Figure S21. ^{1}H NMR (400 MHz, $CDCl_3$) of plasmodione and $^{13}C_{18}$-plasmodione **10b** and $^{13}C_1$-plasmodione **10a**. The black line represents plasmodione, the red line represents **10b** and the green line represents **10a**.

^{13}C NMR

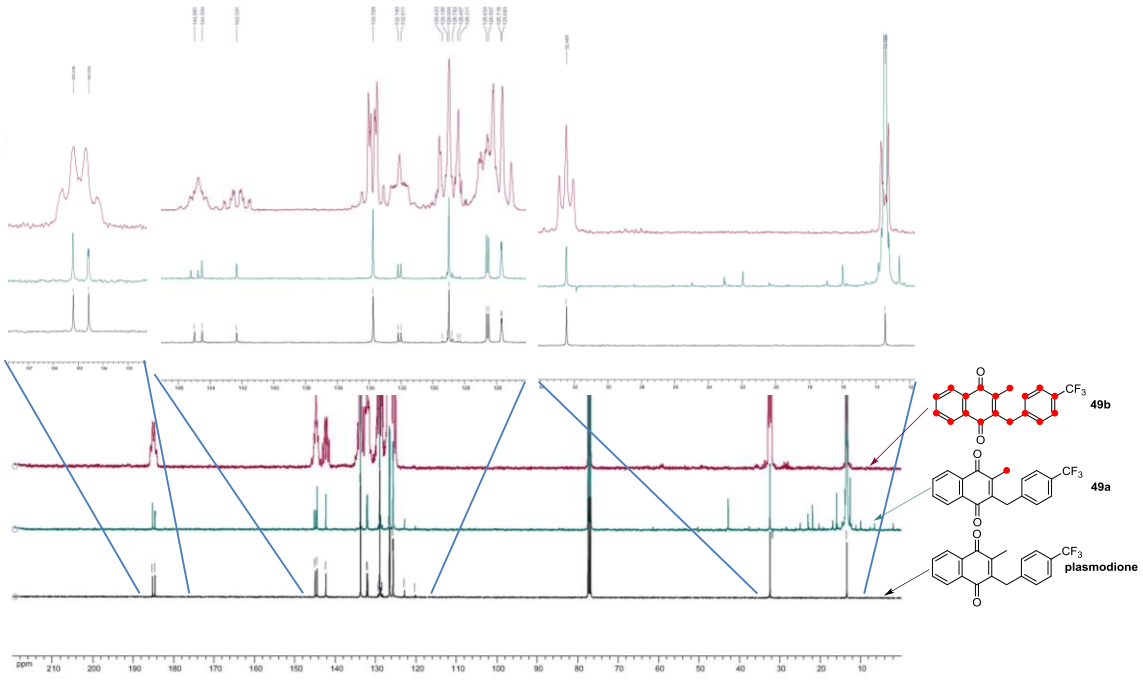


Figure S22. ^{13}C NMR (100 MHz, $CDCl_3$) of plasmodione and $^{13}C_{18}$-plasmodione **10b** and $^{13}C_{1}$-plasmodione **10a**. The black line represents plasmodione, the red line represents **10b** and the green line represents **10a**.

^{19}F NMR

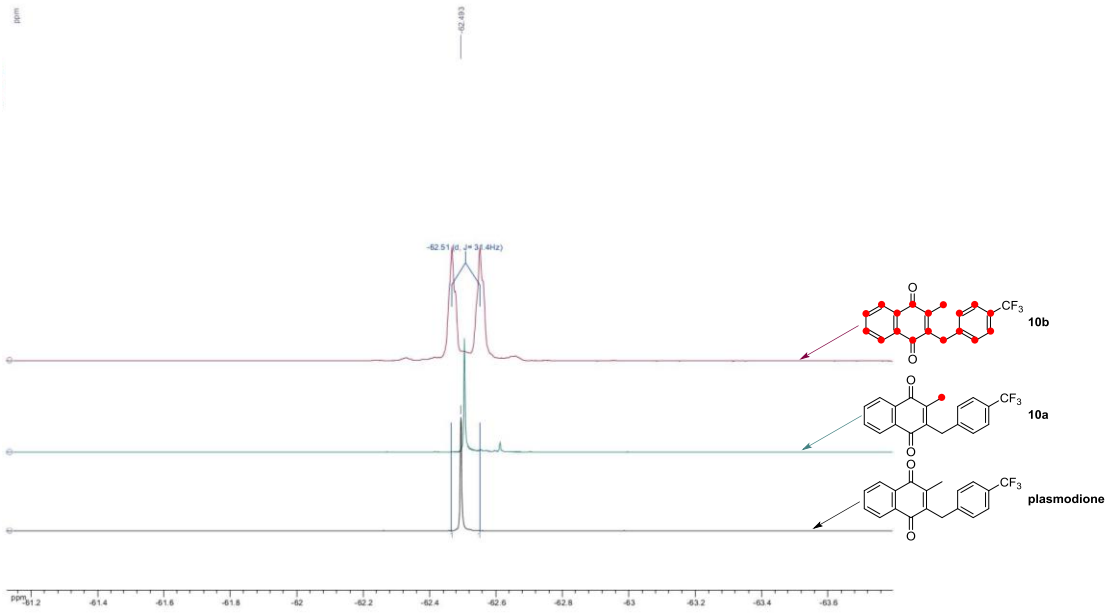


Figure S23. ^{19}F NMR (376 MHz, $CDCl_3$) of plasmodione and $^{13}C_{18}$-plasmodione **10b** and $^{13}C_{1}$-plasmodione **10a**. The black line represents plasmodione, the red line represents **10b** and the green line represents **10a**.

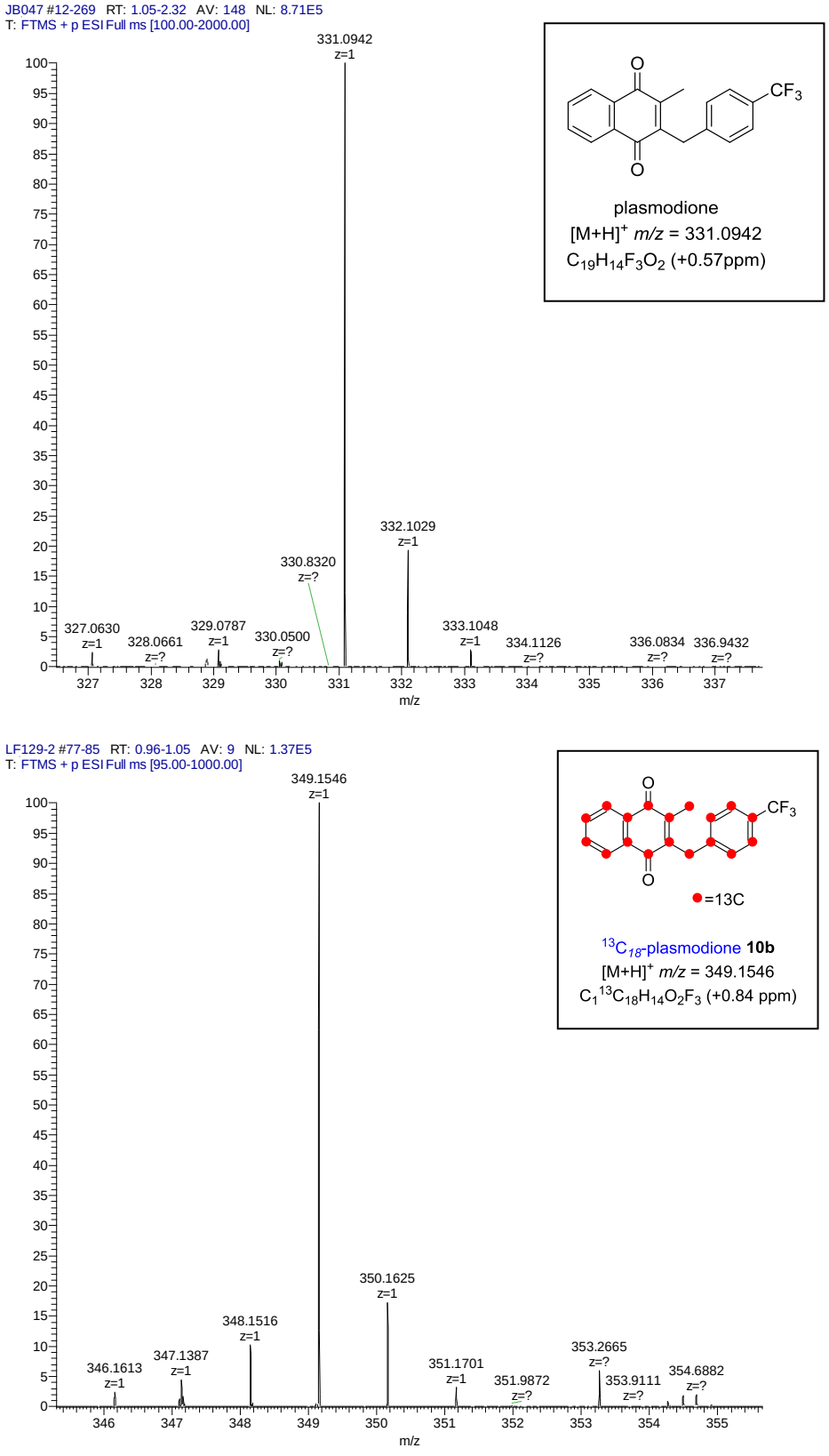
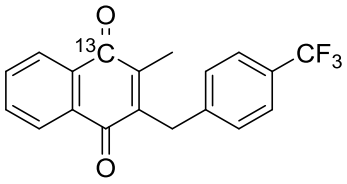


Figure S24. ESI-MS mass spectra of $^{13}C_{18}$-plasmodione **10b**. Analyses were performed on an LTQ-Orbitrap Discovery instrument (R=30,000 at m/z 400).

2-methyl-3-(4-(trifluoromethyl)benzyl)naphthalene-1,4-dione-1-^{13}C (10c):



2-methyl-3-(4-(trifluoromethyl)benzyl)naphthalene-1,4-dione-1-^{13}C

^{1}H NMR

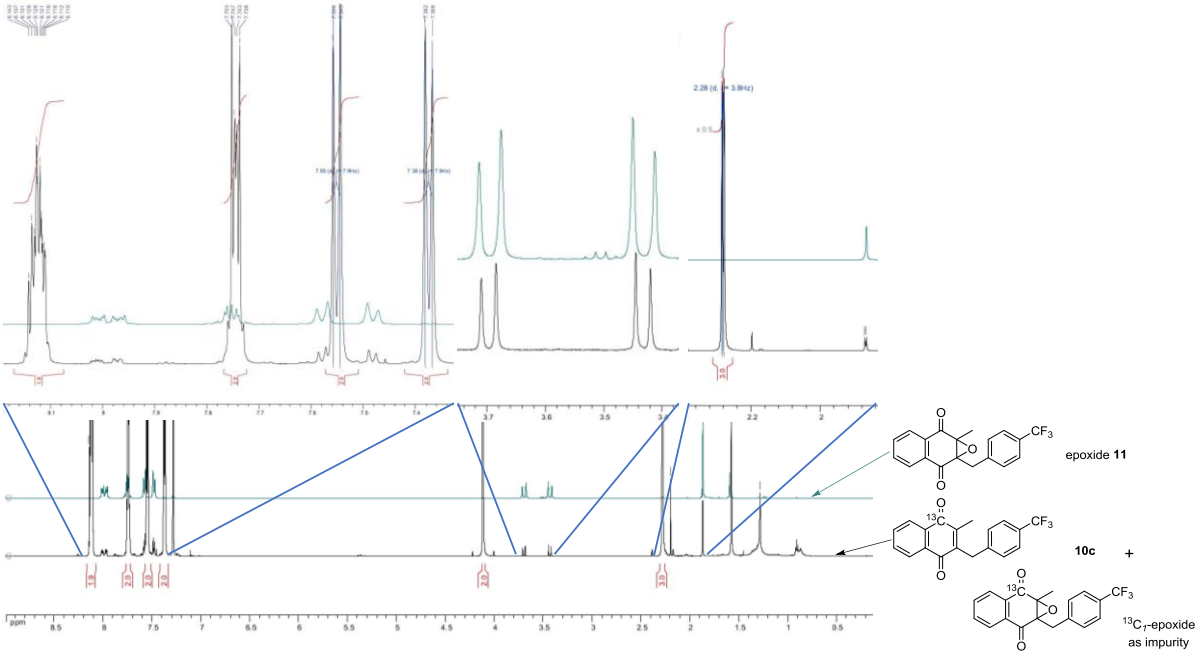


Figure S25. ^{1}H NMR (400 MHz, $CDCl_3$) of $^{13}C_1$-plasmodione **10c** and its impurity. The black line represents $^{13}C_1$-plasmodione **10c** contaminated by the $^{13}C_1$-epoxide as an impurity < 4% and the green line represents the pure epoxide **11**.

^{13}C NMR

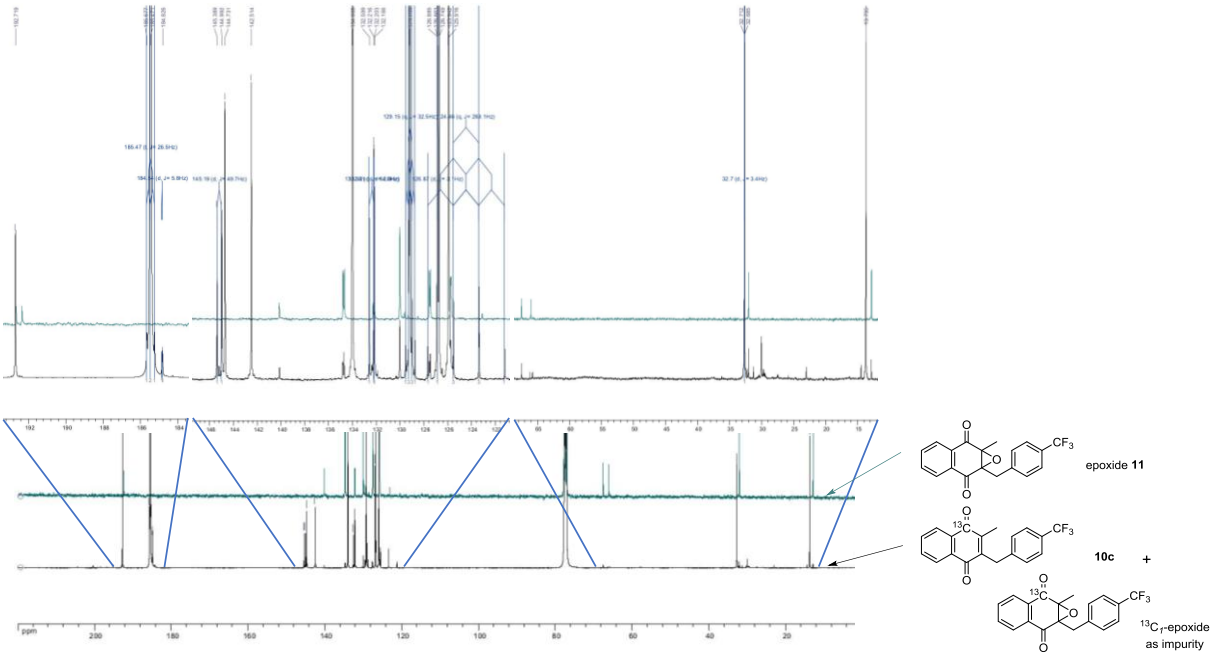
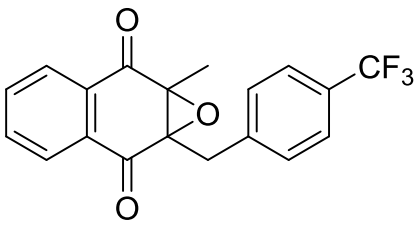


Figure S26. ^{13}C NMR (100 MHz, $CDCl_3$) of $^{13}C_1$-plasmodione **10c** and its impurity. The black line represents $^{13}C_1$-plasmodione **10c** contaminated by the $^{13}C_1$-epoxide as an impurity < 4% and the green line represents the pure epoxide **11**.

1a-methyl-7a-(4-(trifluoromethyl)benzyl)-1a,7a-dihydronaphtho[2,3-*b*]oxirene-2,7-dione (11):



1a-methyl-7a-(4-(trifluoromethyl)benzyl)-
1a,7a-dihydronaphtho[2,3-*b*]oxirene-2,7-dione

^{1}H NMR

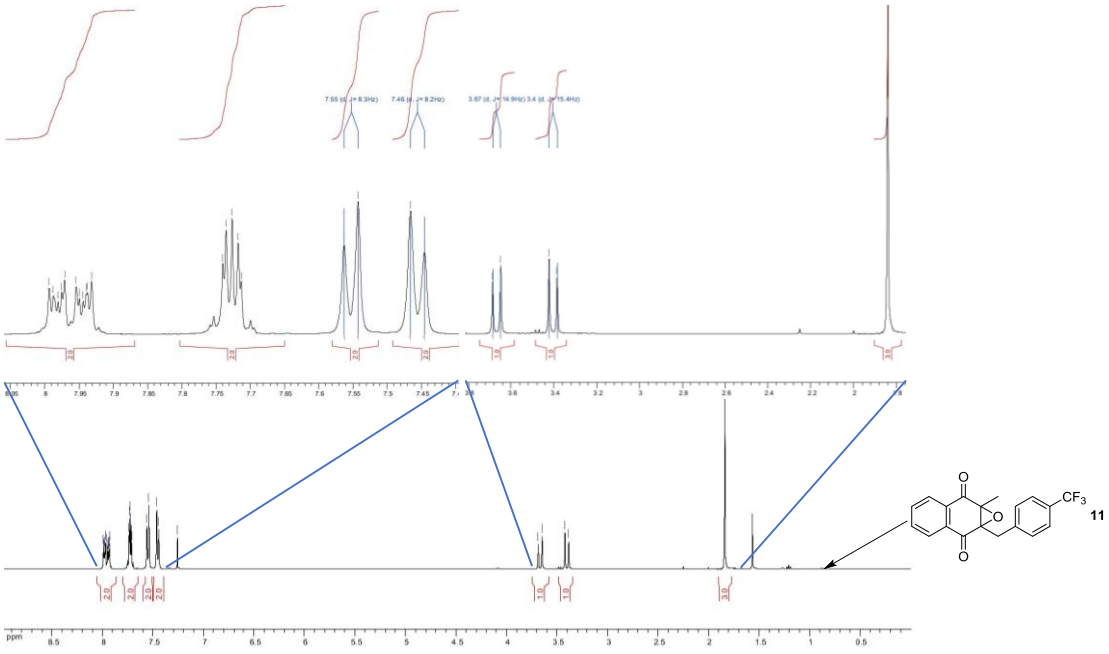


Figure S27. ^{1}H NMR (400 MHz, $CDCl_3$) of 1a-methyl-7a-(4-(trifluoromethyl)benzyl)-1a,7a-dihydronaphtho[2,3-b]oxirene-2,7-dione **11**.

^{13}C NMR

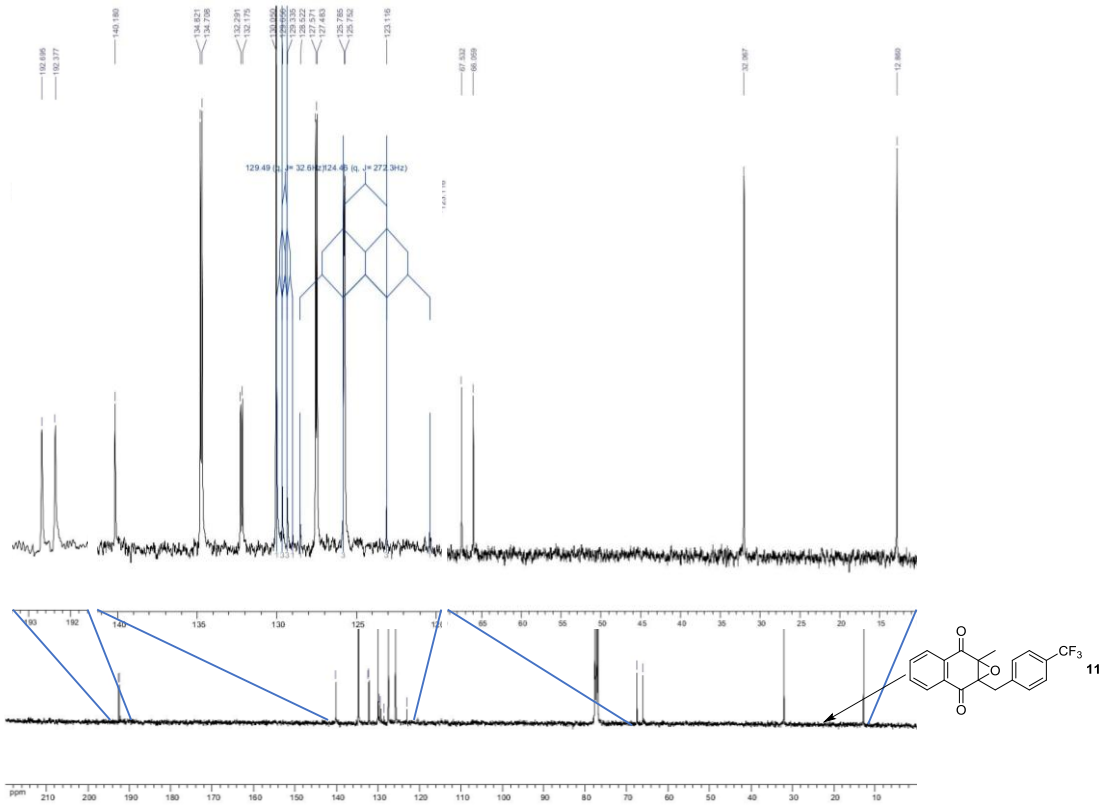


Figure S28. ^{13}C NMR (100 MHz, $CDCl_3$) of 1a-methyl-7a-(4-(trifluoromethyl)benzyl)-1a,7a-dihydronaphtho[2,3-b]oxirene-2,7-dione **11**.

^{19}F NMR

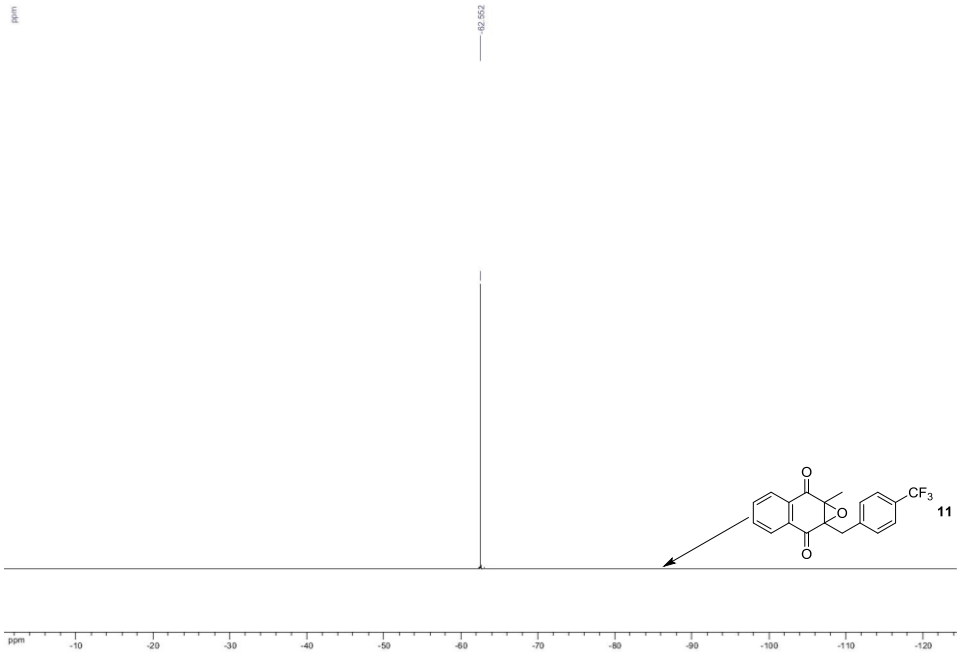
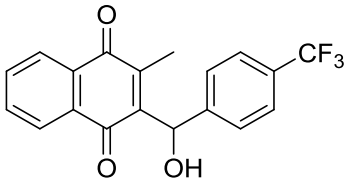


Figure S29. ^{19}F NMR (376 MHz, $CDCl_3$) of 1a-methyl-7a-(4-(trifluoromethyl)benzyl)-1a,7a-dihydronaphtho[2,3-b]oxirene-2,7-dione **11**.

2-(hydroxy(4-(trifluoromethyl)phenyl)methyl)-3-methylnaphthalene-1,4-dione (14):



2-(hydroxy(4-(trifluoromethyl)phenyl)methyl)-3-methylnaphthalene-1,4-dione

^{1}H NMR

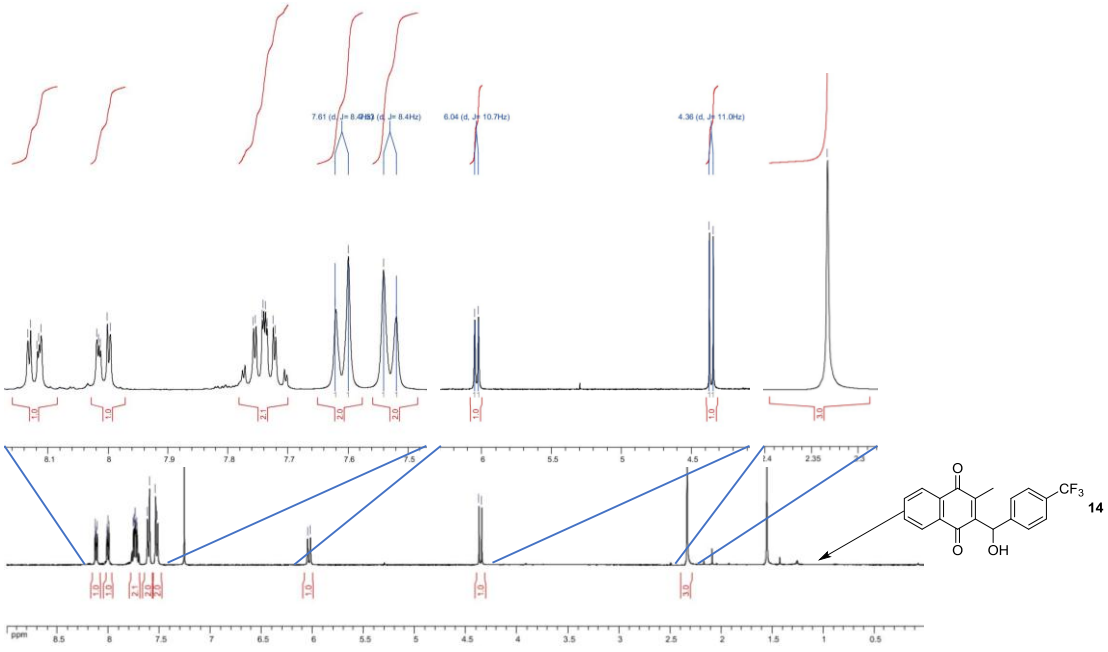


Figure S30. ^{1}H NMR (400 MHz, $CDCl_3$) of 2-(hydroxy(4-(trifluoromethyl)phenyl)methyl)-3-methylnaphthalene-1,4-dione **14**.

^{13}C NMR

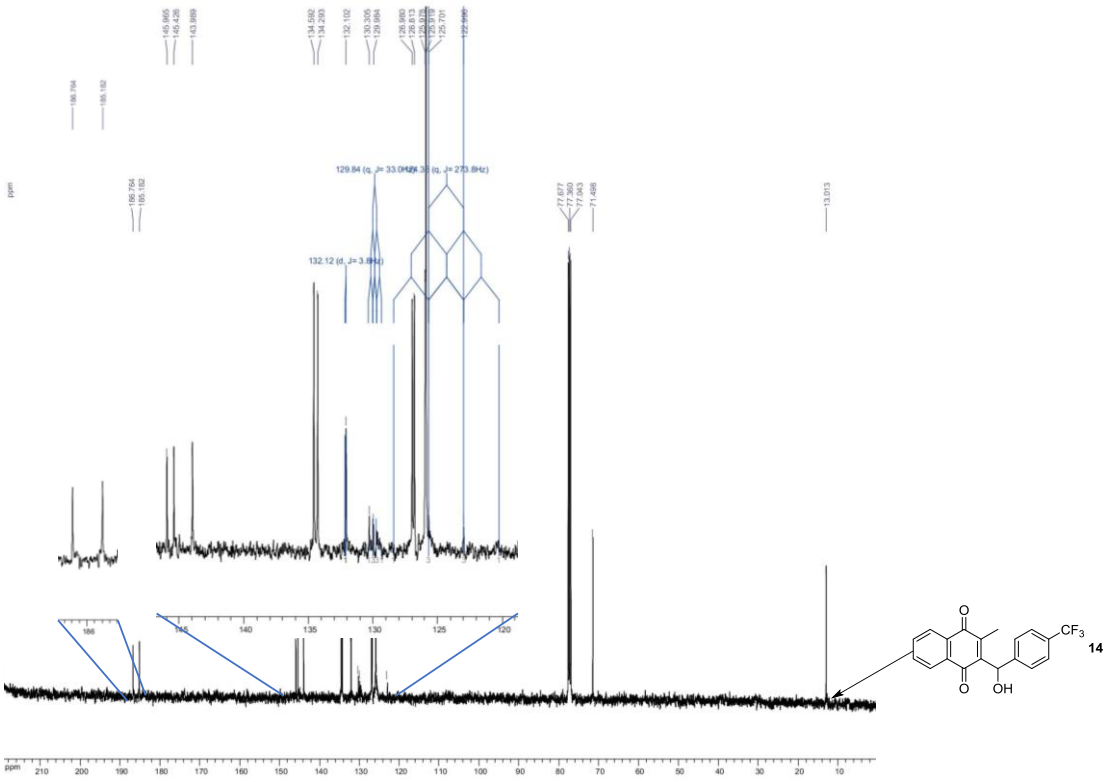


Figure S31. ^{13}C NMR (100 MHz, $CDCl_3$) of 2-(hydroxy(4-(trifluoromethyl)phenyl)methyl)-3-methylnaphthalene-1,4-dione **14**.

^{19}F NMR

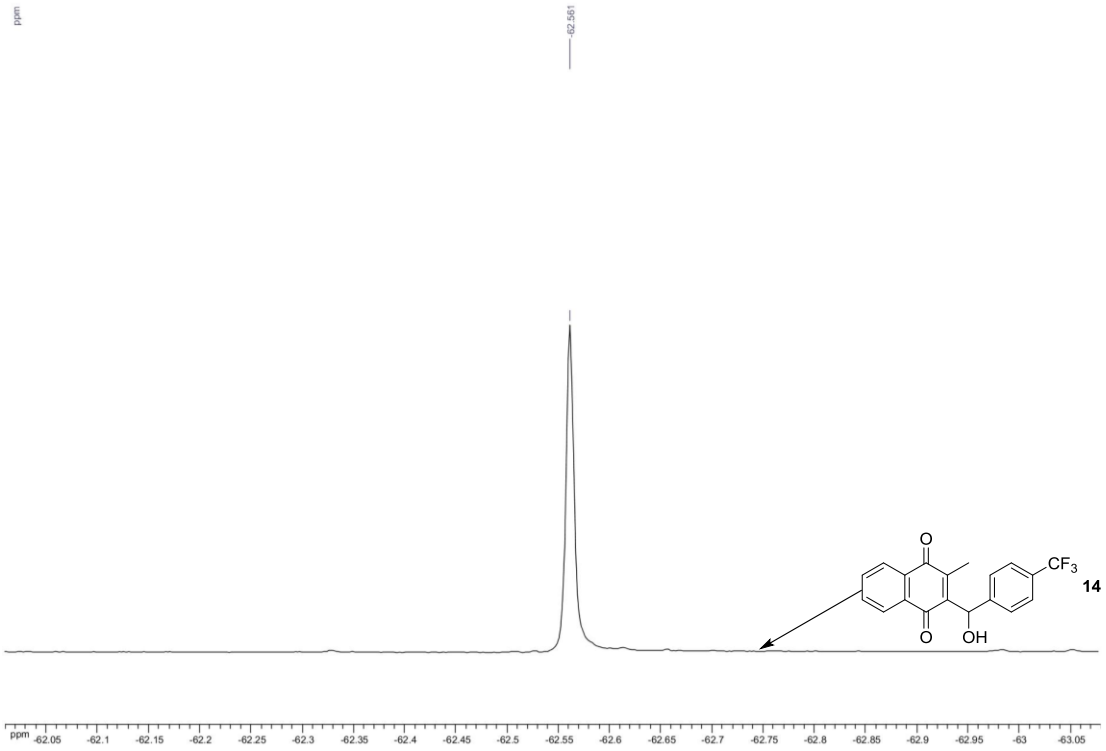
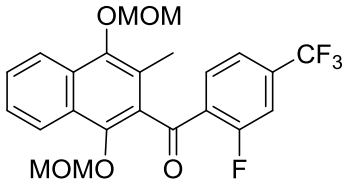


Figure S32. ^{19}F NMR (376 MHz, $CDCl_3$) of 2-(hydroxy(4-(trifluoromethyl)phenyl)methyl)-3-methylnaphthalene-1,4-dione **14**.

(1,4-bis(methoxymethoxy)-3-methylnaphthalen-2-yl)(2-fluoro-4-(trifluoromethyl)phenyl)methanone (22):



(1,4-bis(methoxymethoxy)-3-methylnaphthalen-2-yl)(2-fluoro-4-(trifluoromethyl)phenyl)methanone

^{1}H NMR

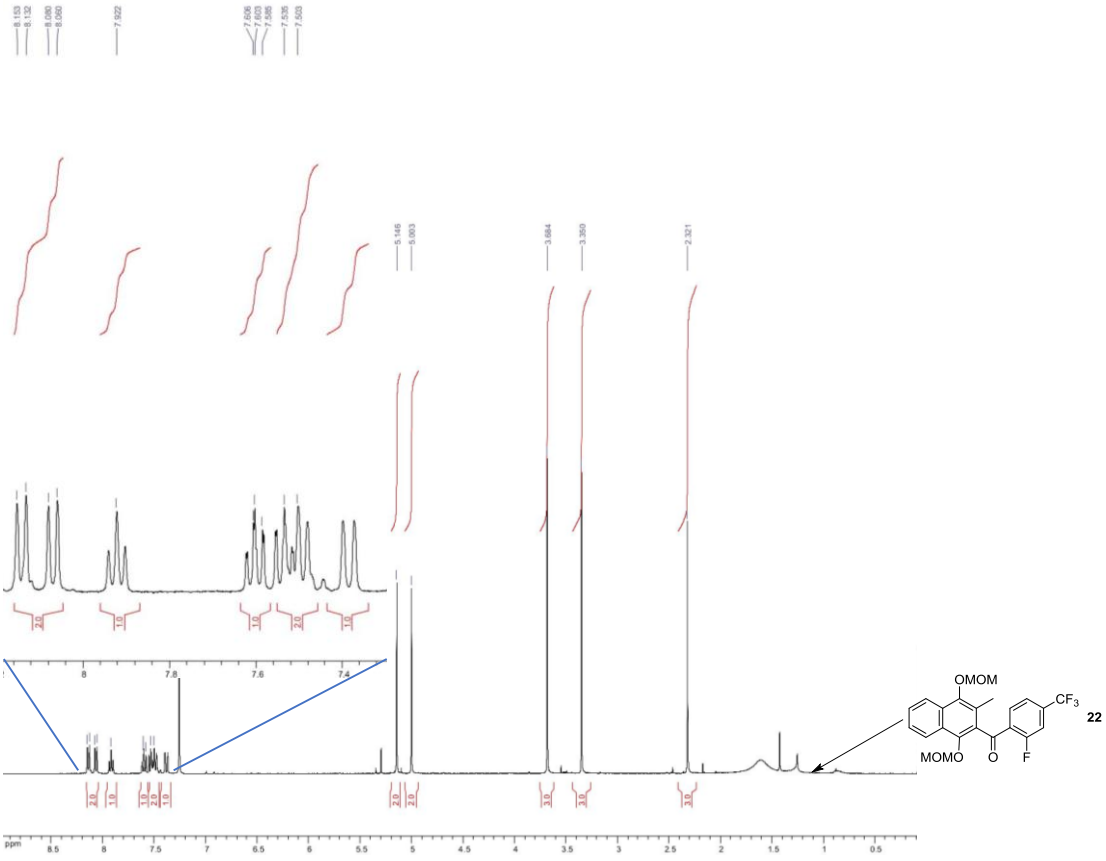
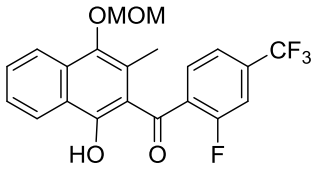


Figure S33. ^{1}H NMR (400 MHz, $CDCl_3$) of (1,4-bis(methoxymethoxy)-3-methylnaphthalen-2-yl)(2-fluoro-4-(trifluoromethyl)phenyl)methanone **22**.

(2-fluoro-4-(trifluoromethyl)phenyl)(1-hydroxy-4-(methoxymethoxy)-3-methylnaphthalen-2-yl)methanone (23):



(2-fluoro-4-(trifluoromethyl)phenyl)(1-hydroxy-4-(methoxymethoxy)-3-methylnaphthalen-2-yl)methanone

^{1}H NMR

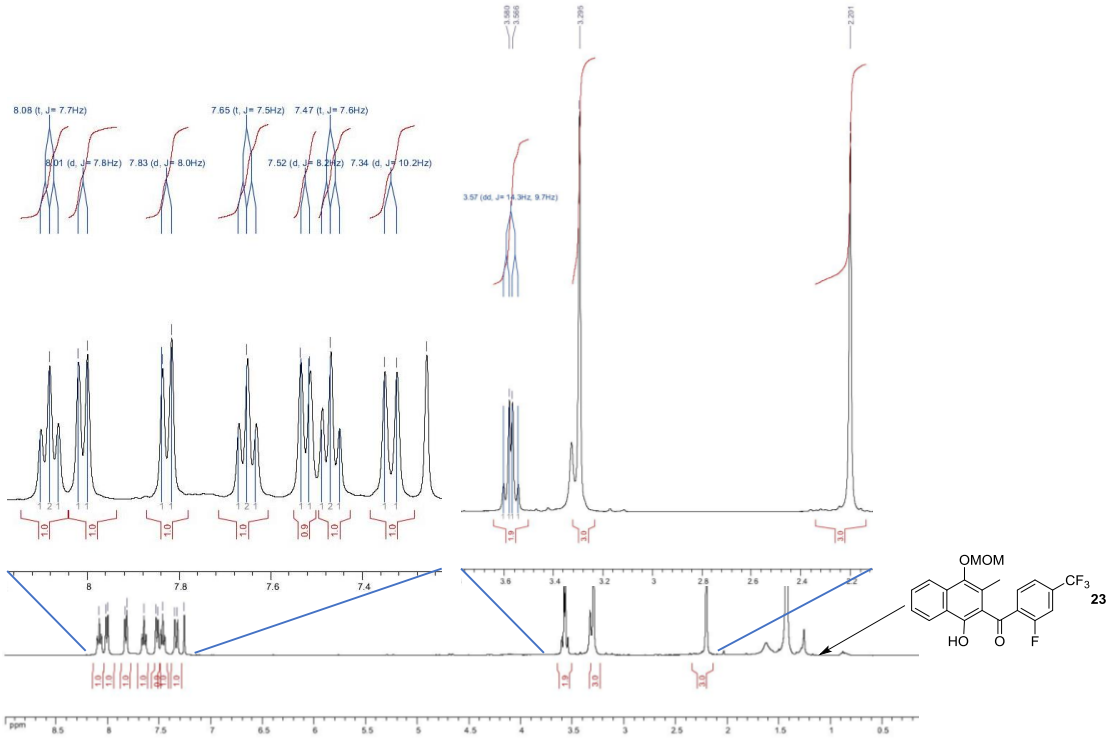
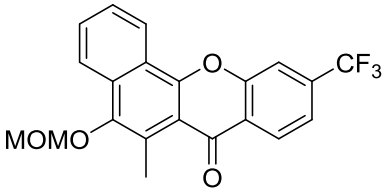


Figure S34. ^{1}H NMR (400 MHz, $CDCl_3$) of (2-fluoro-4-(trifluoromethyl)phenyl)(1-hydroxy-4-(methoxymethoxy)-3-methylnaphthalen-2-yl)methanone **23**.

5-(methoxymethoxy)-6-methyl-10-(trifluoromethyl)-7H-benzo[c]xanthen-7-one (24):



5-(methoxymethoxy)-6-methyl-10-(trifluoromethyl)-7*H*-benzo[*c*]xanthen-7-one

^{1}H NMR

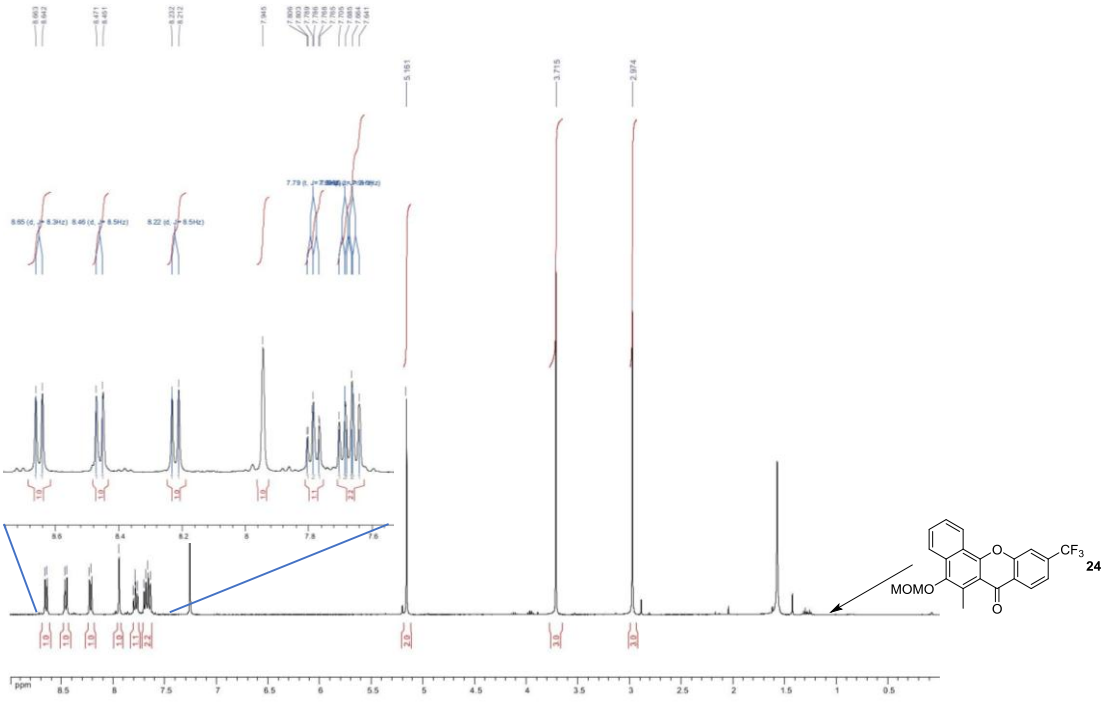


Figure S35. ^{1}H NMR (400 MHz, $CDCl_3$) of 5-(methoxymethoxy)-6-methyl-10-(trifluoromethyl)-7H-benzo[*c*]xanthen-7-one **24**.

^{13}C NMR

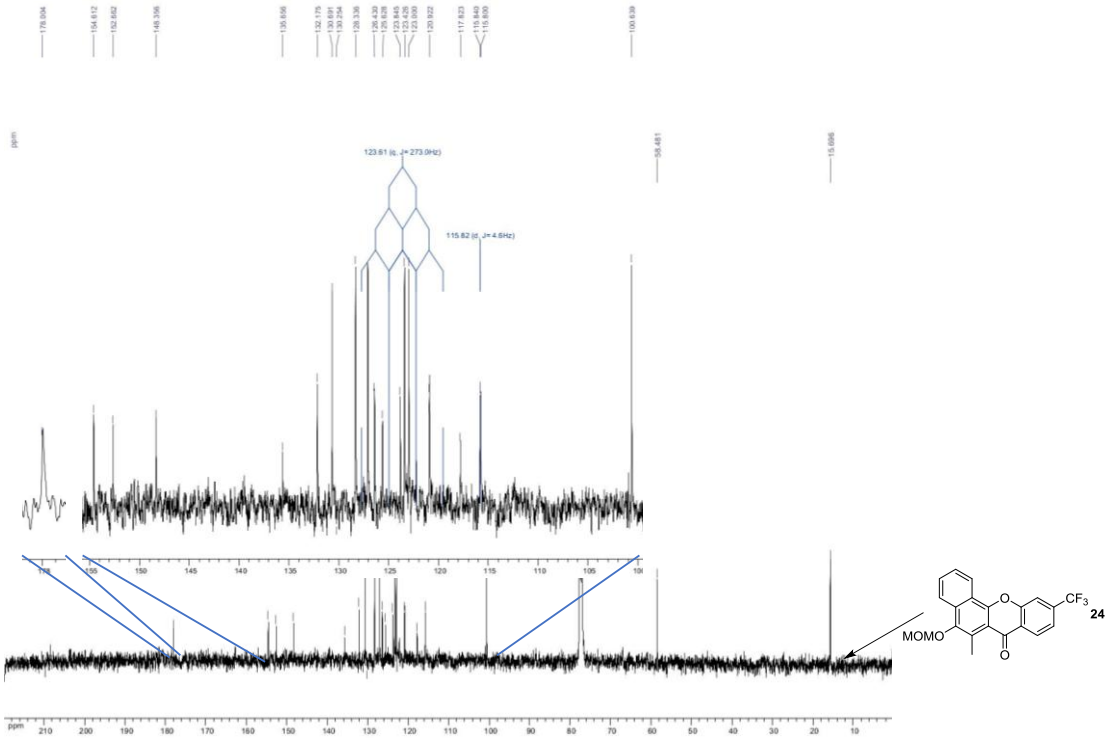


Figure S36. ^{13}C NMR (100 MHz, $CDCl_3$) of 5-(methoxymethoxy)-6-methyl-10-(trifluoromethyl)-7H-benzo[*c*]xanthen-7-one **24**.

^{13}C NMR and DEPT

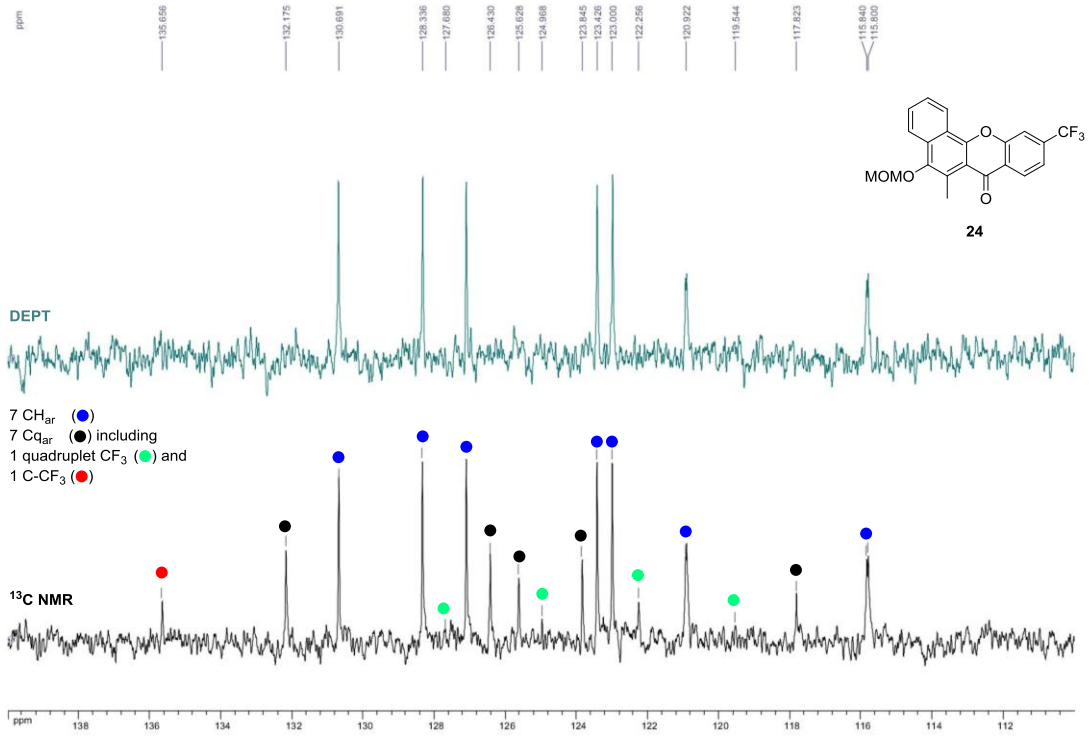


Figure S37. NMR spectrum of 5-(methoxymethoxy)-6-methyl-10-(trifluoromethyl)-7H-benzo[*c*]xanthen-7-one (**24**): zoom of ^{13}C and DEPT spectrum.

^{19}F NMR

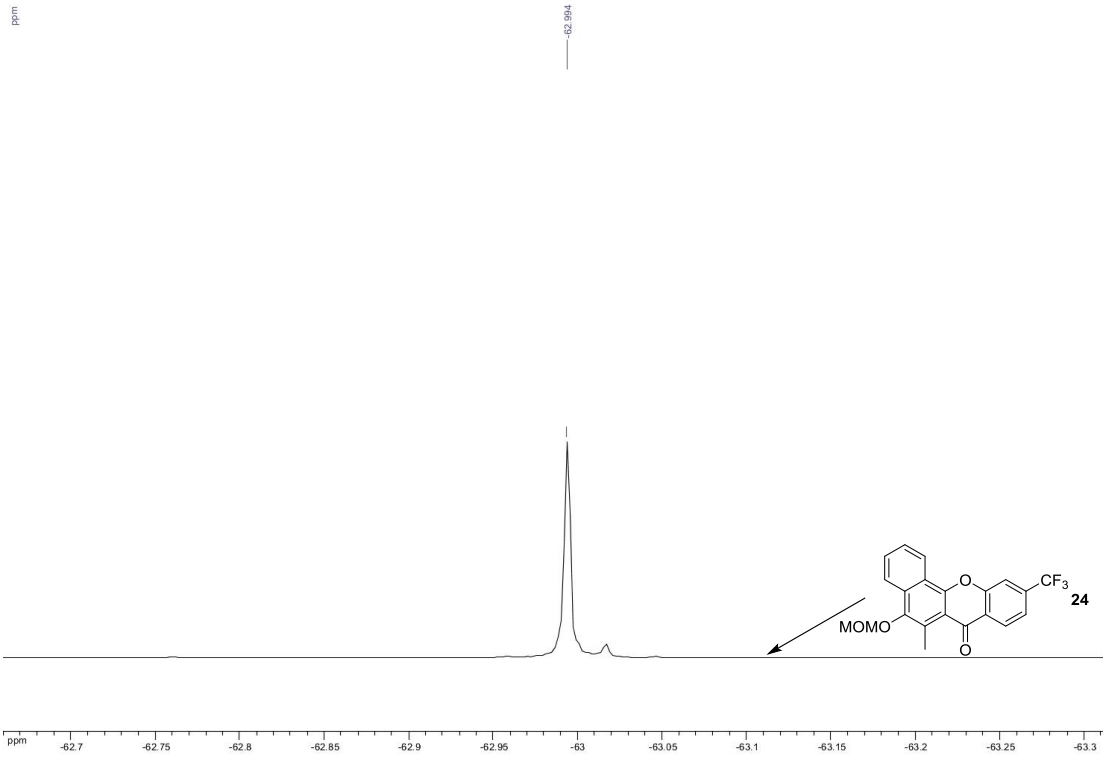
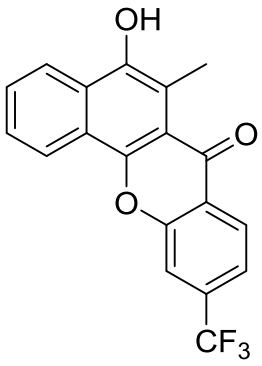


Figure S38. ^{19}F NMR (376 MHz, $CDCl_3$) of 5-(methoxymethoxy)-6-methyl-10-(trifluoromethyl)-7H-benzo[*c*]xanthen-7-one **24**.

5-hydroxy-6-methyl-10-(trifluoromethyl)-7H-benzo[c]xanthen-7-one (15):



5-hydroxy-6-methyl-10-(trifluoromethyl)-7*H*-benzo[*c*]xanthen-7-one

^{1}H NMR

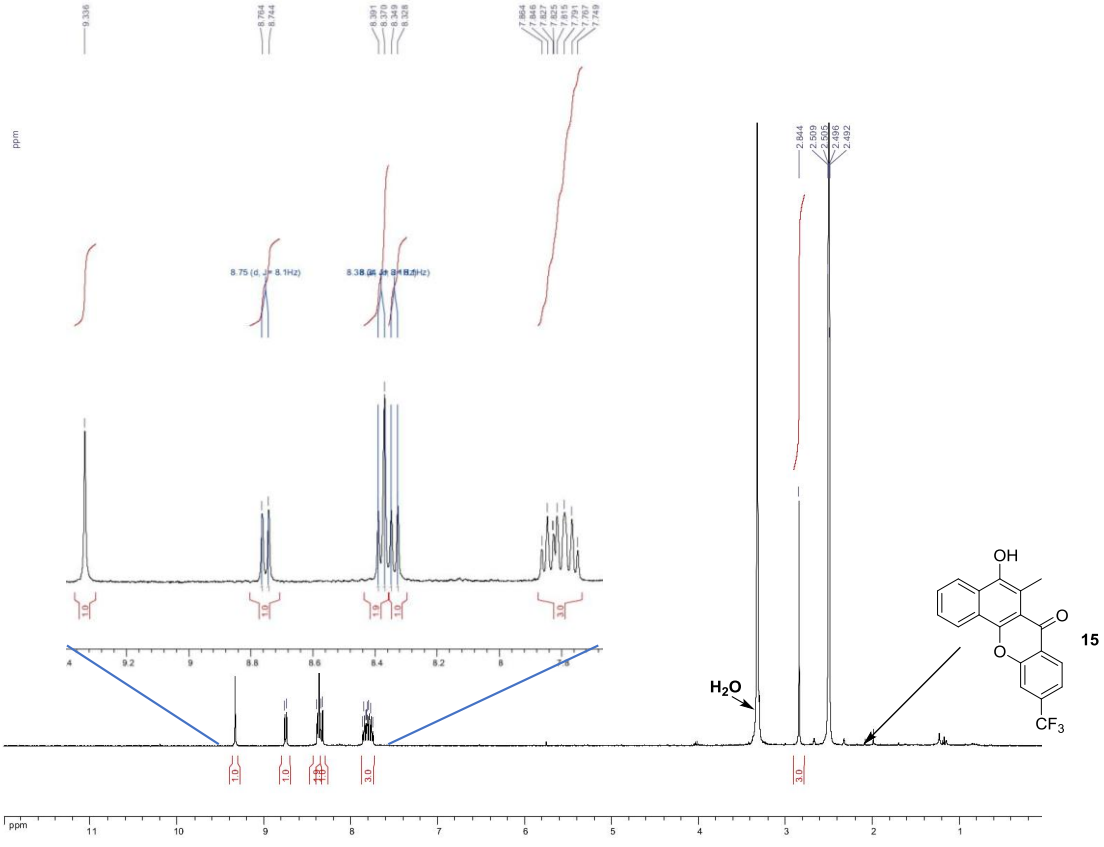


Figure S39. ^{1}H NMR (400 MHz, DMSO-d_6) of 5-hydroxy-6-methyl-10-(trifluoromethyl)- 7H-benzo[*c*]xanthen-7-one **15**.

^{19}F NMR

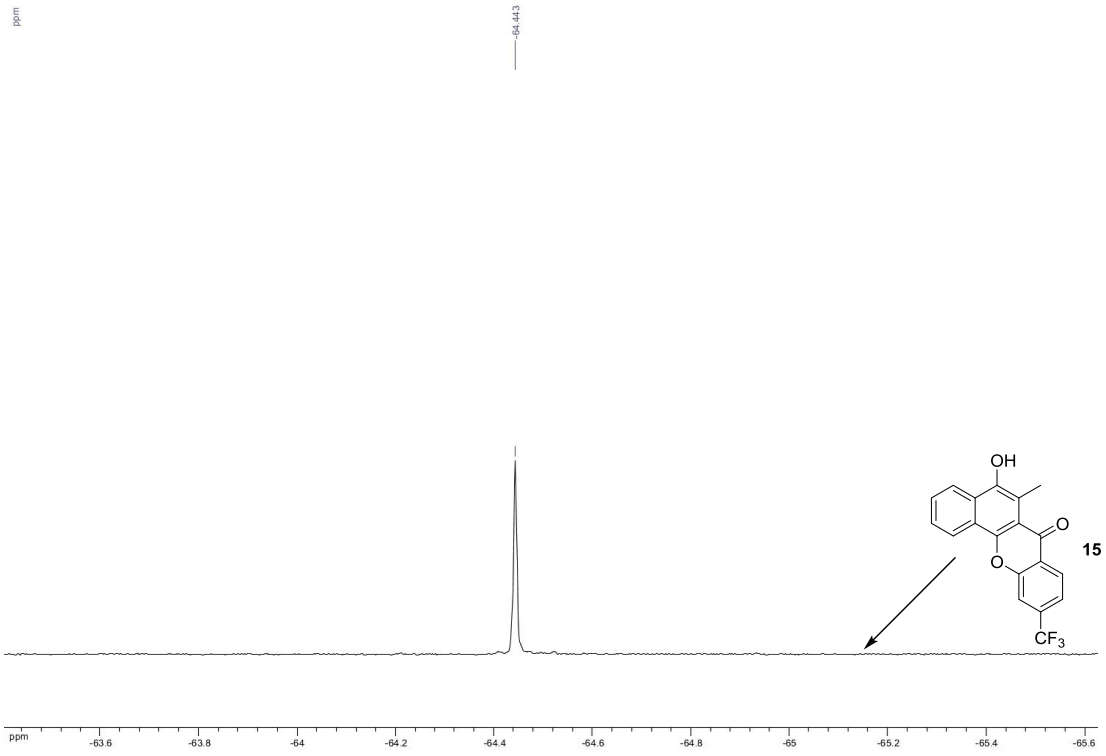
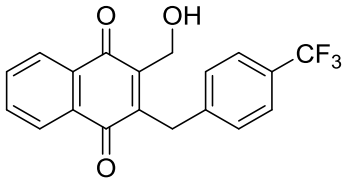


Figure S40. ^{1}H NMR (376 MHz, DMSO-d_6) of 5-hydroxy-6-methyl-10-(trifluoromethyl)- 7H-benzo[*c*]xanthen-7-one **15**.

2-(hydroxymethyl)-3-(4-(trifluoromethyl)benzyl)naphthalene-1,4-dione (17):



2-(hydroxymethyl)-3-(4-(trifluoromethyl)benzyl)naphthalene-1,4-dione

^{1}H NMR

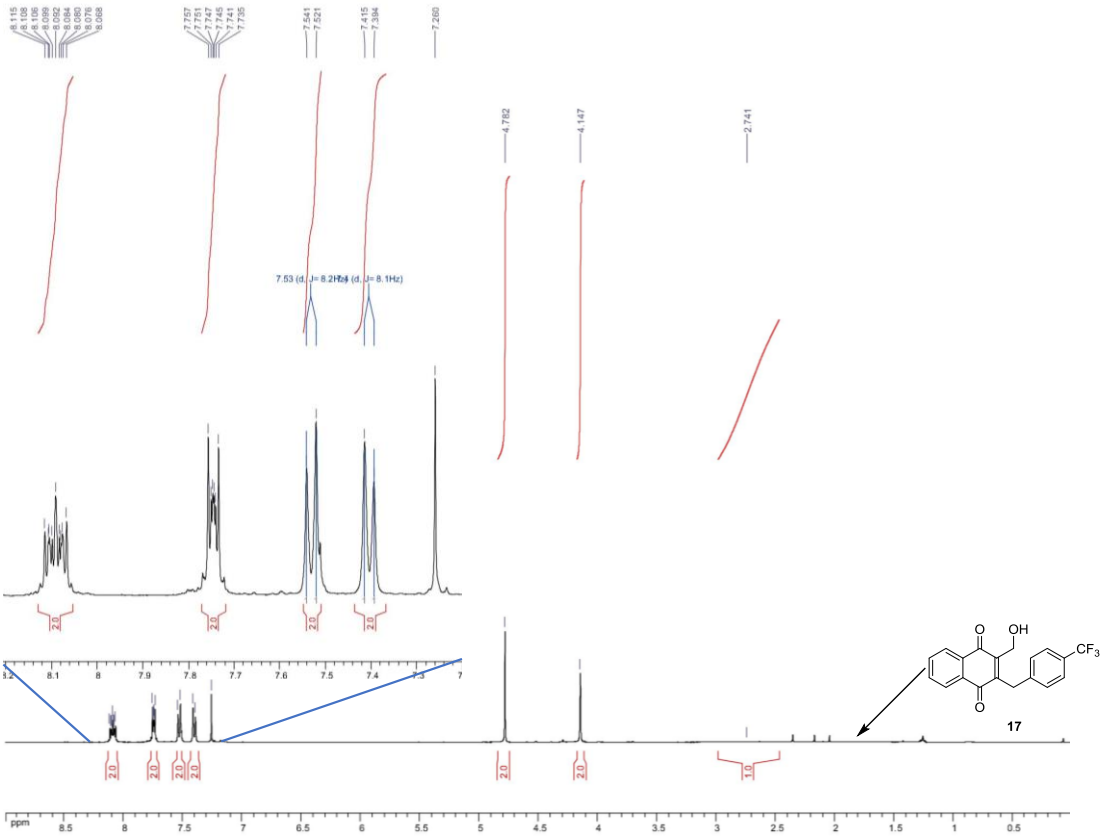


Figure S41. ^{1}H NMR (400 MHz, $CDCl_3$) of 2-(hydroxymethyl)-3-(4-(trifluoromethyl)benzyl)naphthalene-1,4-dione **17**.

^{13}C NMR

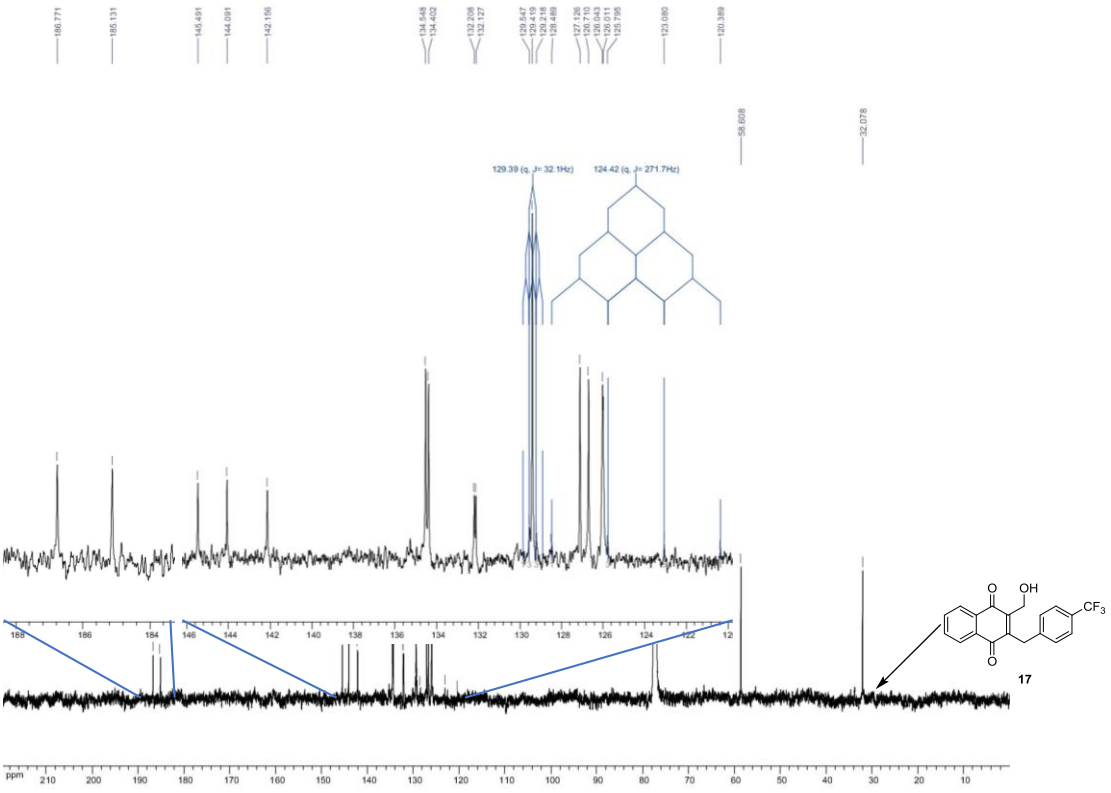


Figure S42. ^{13}C NMR (100 MHz, $CDCl_3$) of 2-(hydroxymethyl)-3-(4-(trifluoromethyl)benzyl)naphthalene-1,4-dione **17**.

^{19}F NMR

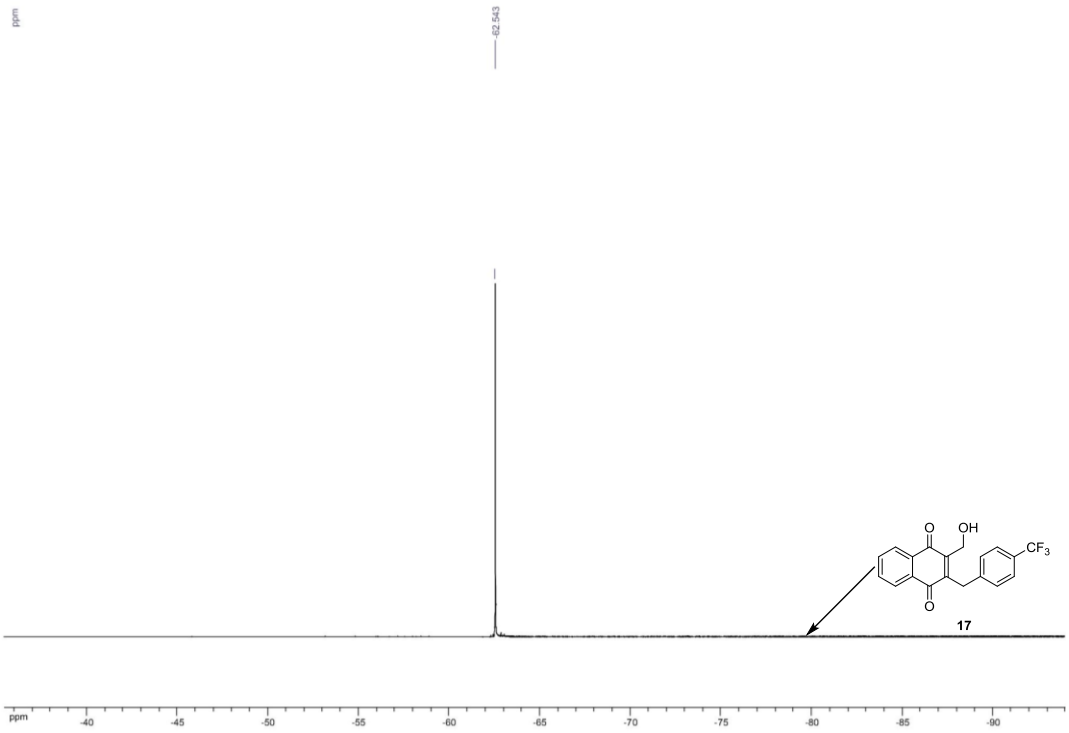
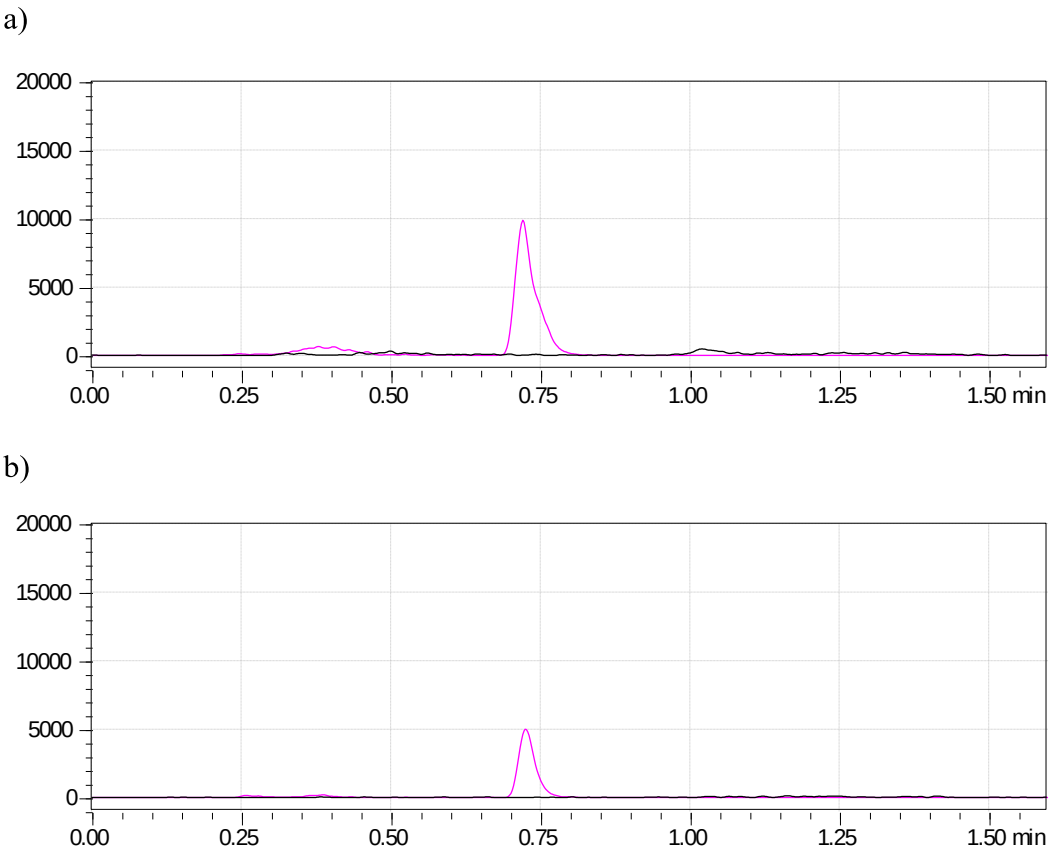


Figure S43. ^{19}F NMR (376 MHz, $CDCl_3$) of 2-(hydroxymethyl)-3-(4-(trifluoromethyl)benzyl)naphthalene-1,4-dione **17**.

MS data acquisition of drug metabolites

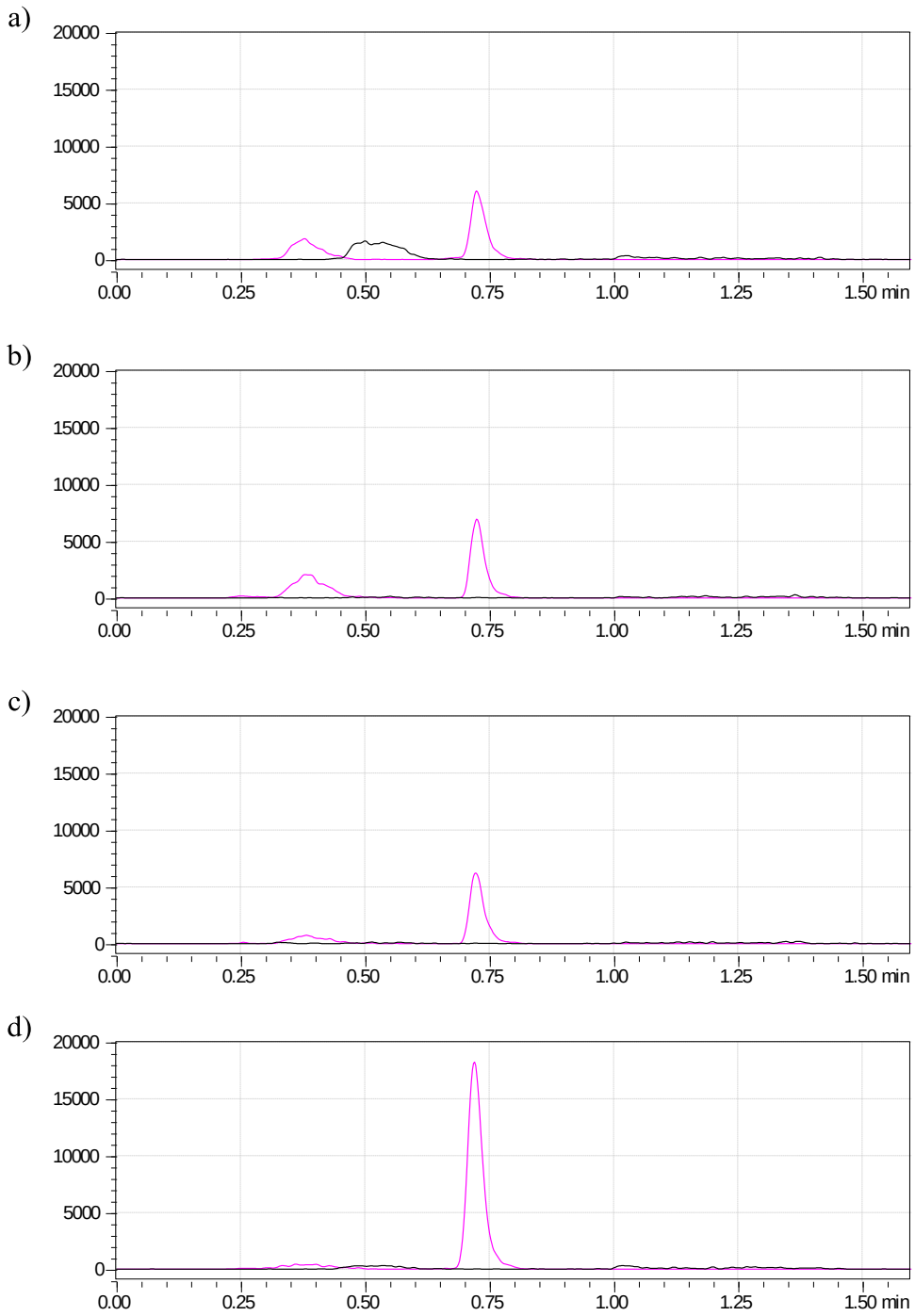
The experiment was performed by using an UHPLC coupling to a triple quadripole Shimadzu LC-MS 8030 operating in the negative ion mode at Plate-forme de Chimie Biologique Integrative de Strasbourg (TechMed^{ILL}, Patrick Gizzi, Strasbourg).

The urine samples were loaded and separated on kinetex 2.6µ C8 100A 50x2.1 mm maintained at 50°C, with a flow rate set at 500 µL/min. The gradient condition was described as follows: Mobile phases were water containing 0.05% formic acid (mobile phase A) and acetonitrile (ACN) (mobile phase B). The gradient conditions implied first a from 0 to 0.1 min equilibration at 40% B, then the proportion of B was raised to 100% from 0.6 to 0.9 min. The proportion of B was decreased to 40% from 0.92 to 2.3 min (total runtime: 2.3 min).



Aire sous le pic à 0.72 min
4a 23605
4b 9902

Figure S44. UHPLC spectrum of hydroxyl plasmodione metabolites. a) Naive mouse 24 h post 1st injection of plasmodione. b) Naive mouse 24 h post 2nd injection of plasmodione



Aire sous le pic à 0.72 min

5a	12787
5b	14090
5c	13312
5d	39639

Figure S45. UHPLC spectrum of hydroxyl plasmodione metabolites. a) Infected mouse 1 day post 1st injection of plasmodione. b) Infected mouse 1 day post 2nd injection of plasmodione. c) Infected mouse 1 day post 3rd injection of plasmodione. d) Infected mouse 2 days post 3rd injection of plasmodione.

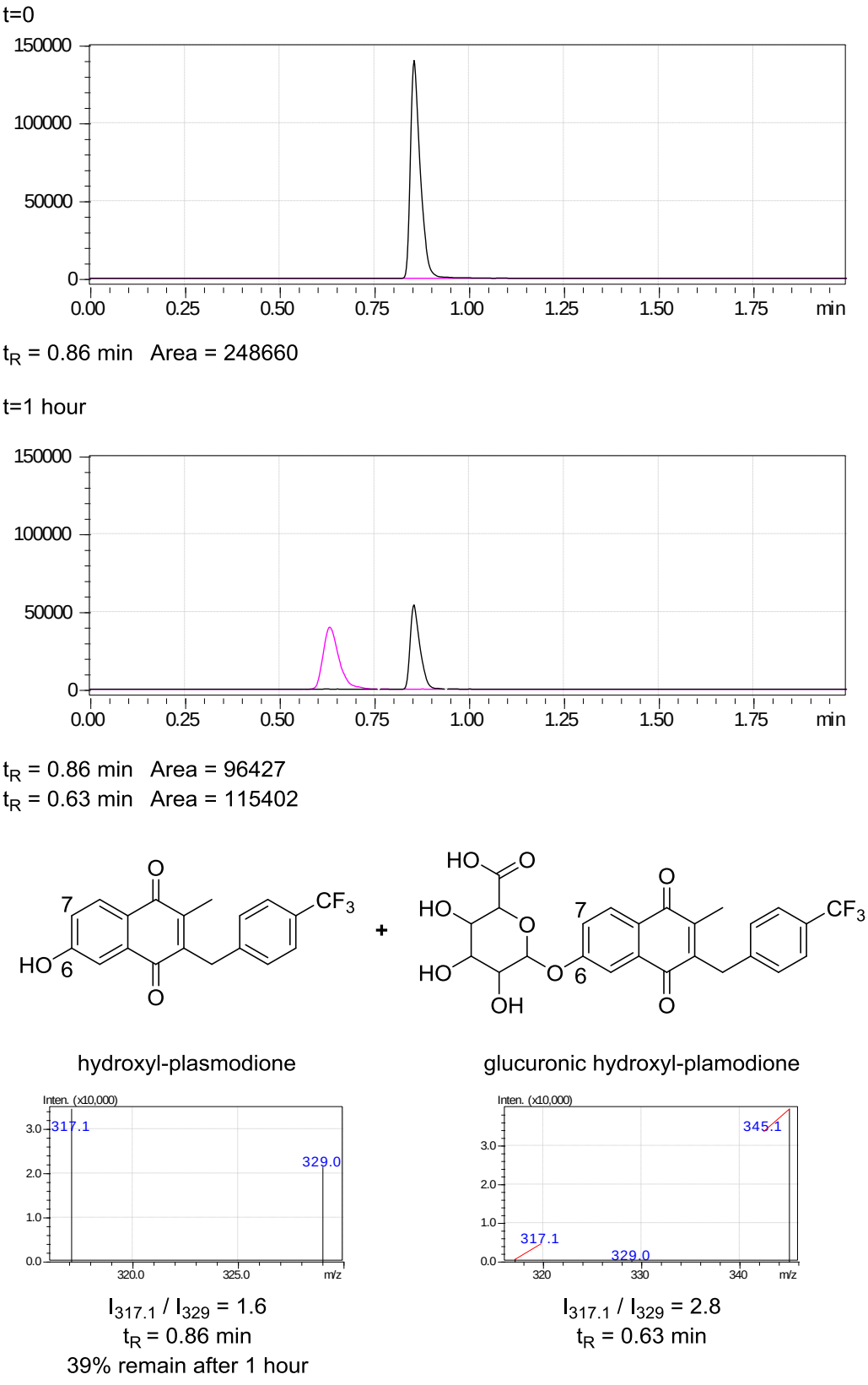


Figure S46. UHPLC/MS-MS spectrum of 6-hydroxyl plasmodione **16a** which have incubated 0 or 1 hour with mice liver microsomes and 6-glucuronic plasmodione metabolites.

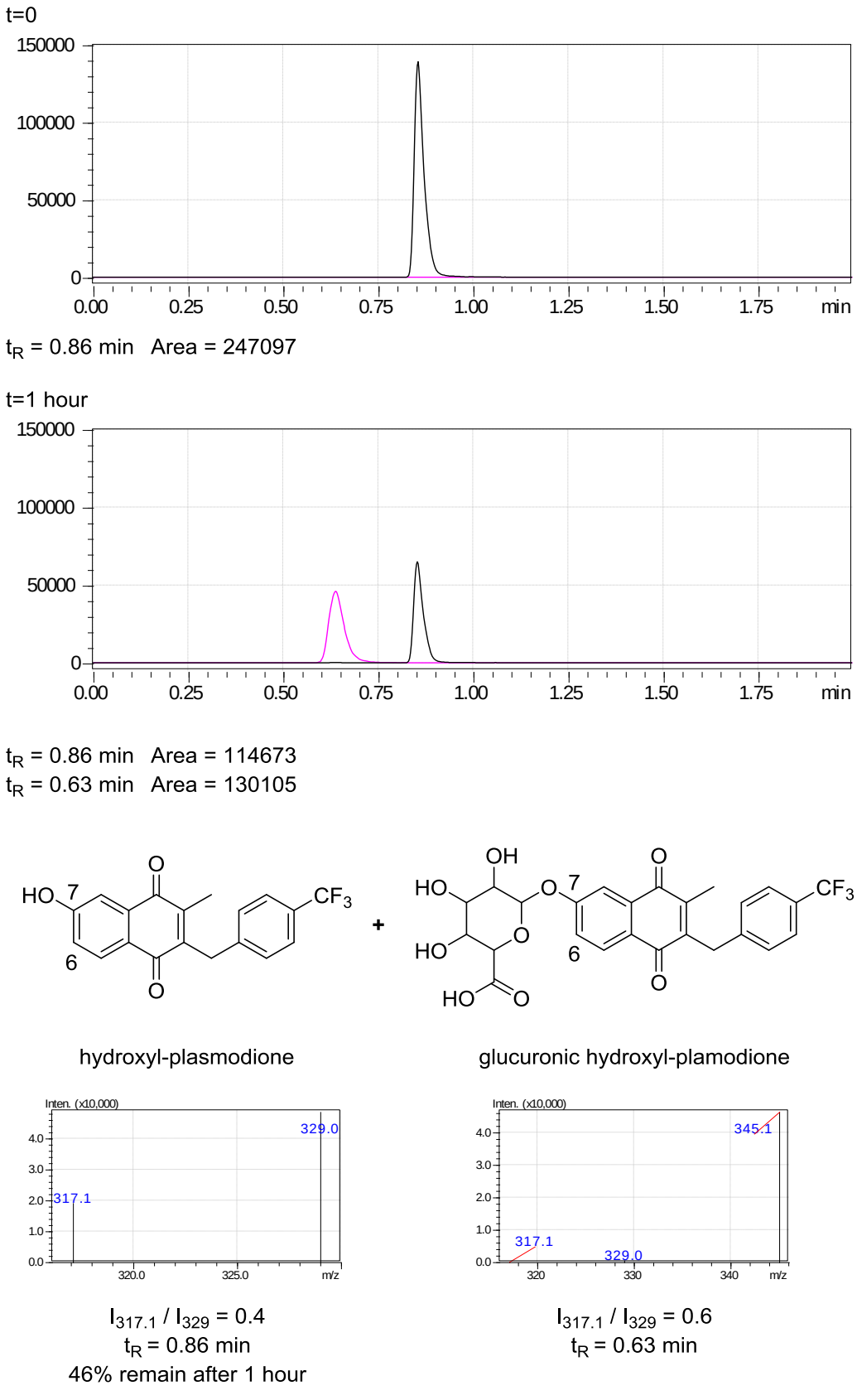


Figure S47. UHPLC/MS-MS spectrum of 7-hydroxyl plasmodione **16b** which have incubated 0 or 1 hour with mice liver microsomes and 7-glucuronic plasmodione metabolites.

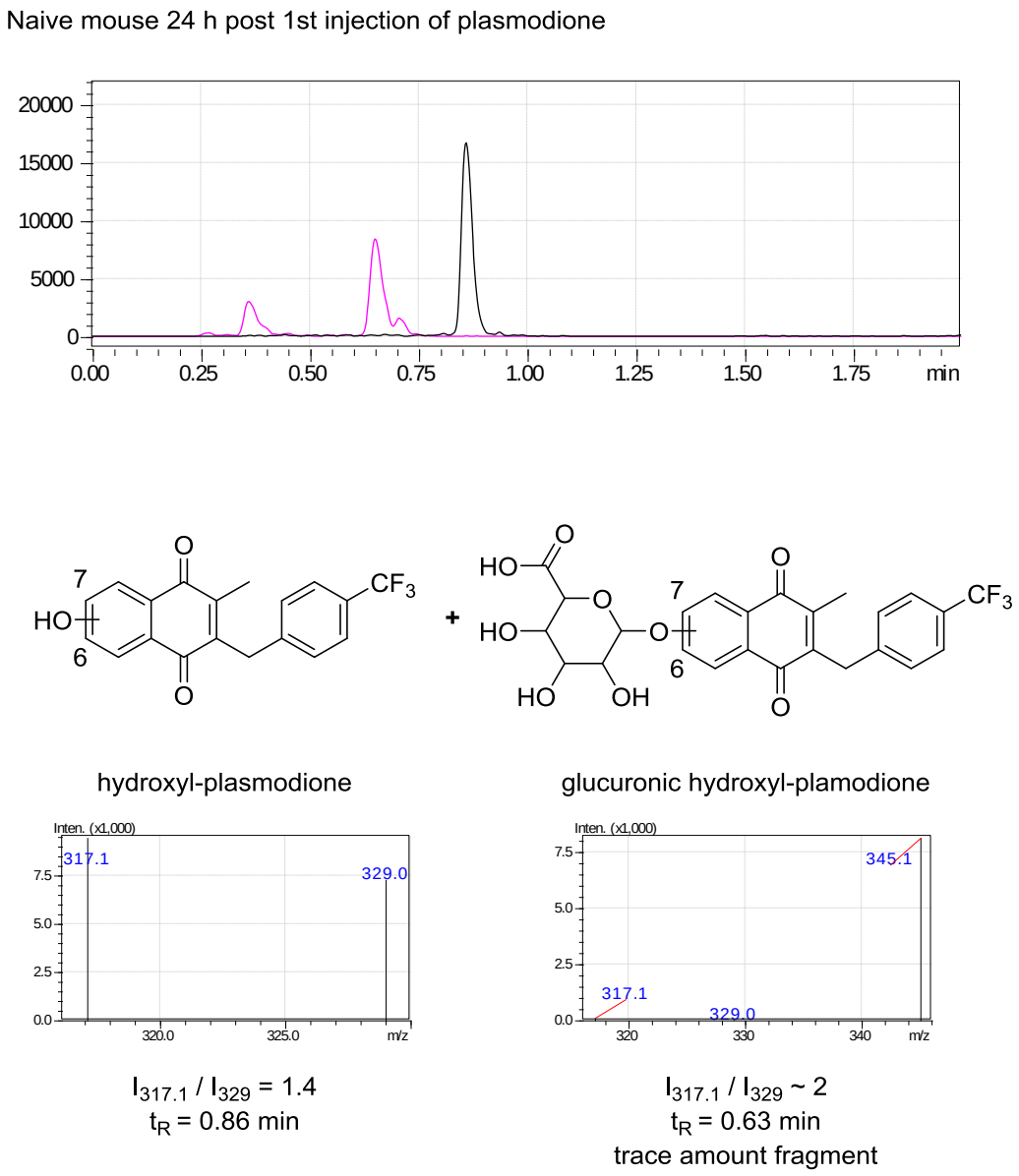


Figure S48. UHPLC/MS-MS spectrum of mice urine: Naive mouse 24 h post 1st injection of plasmodione.

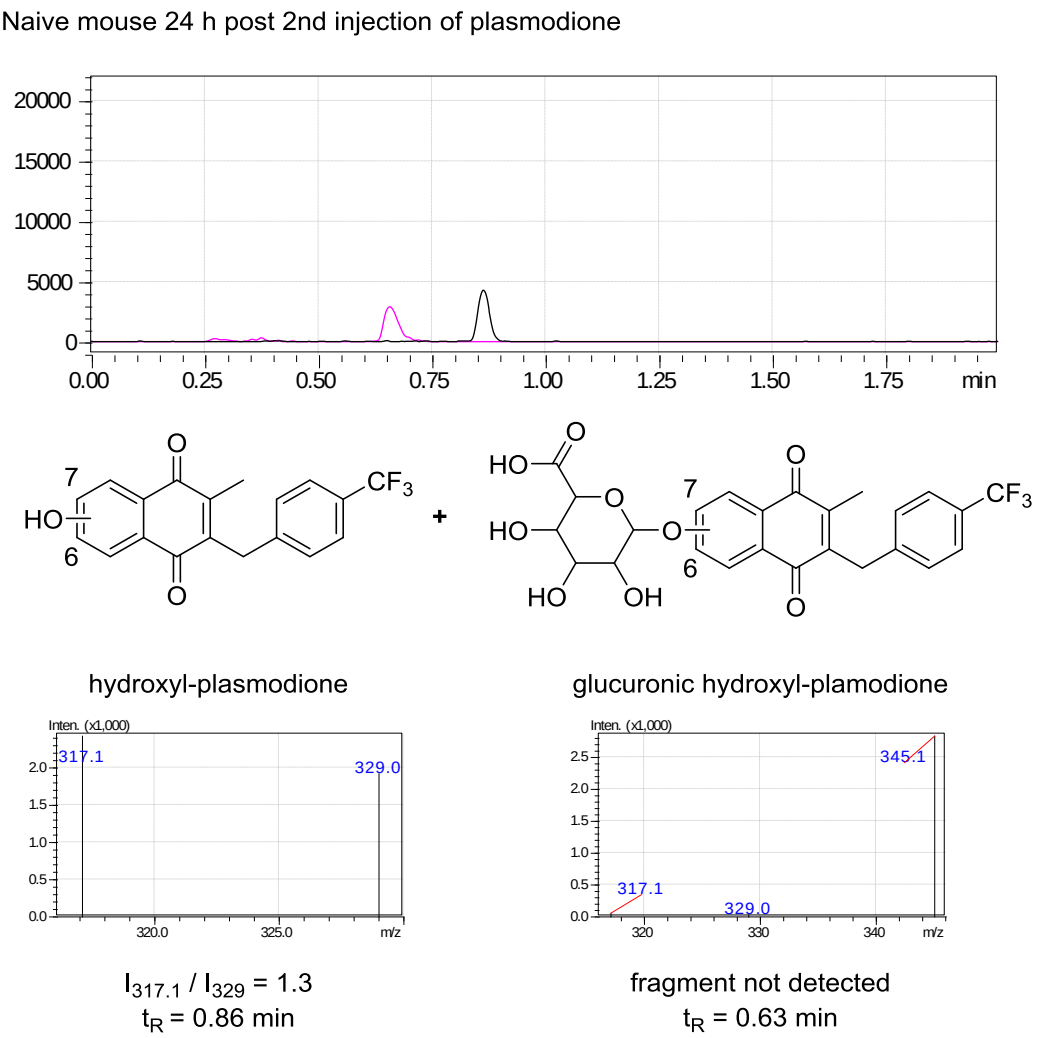


Figure S49. UHPLC/MS-MS spectrum of mice urine: Naive mouse 24 h post 2nd injection of plasmodione.

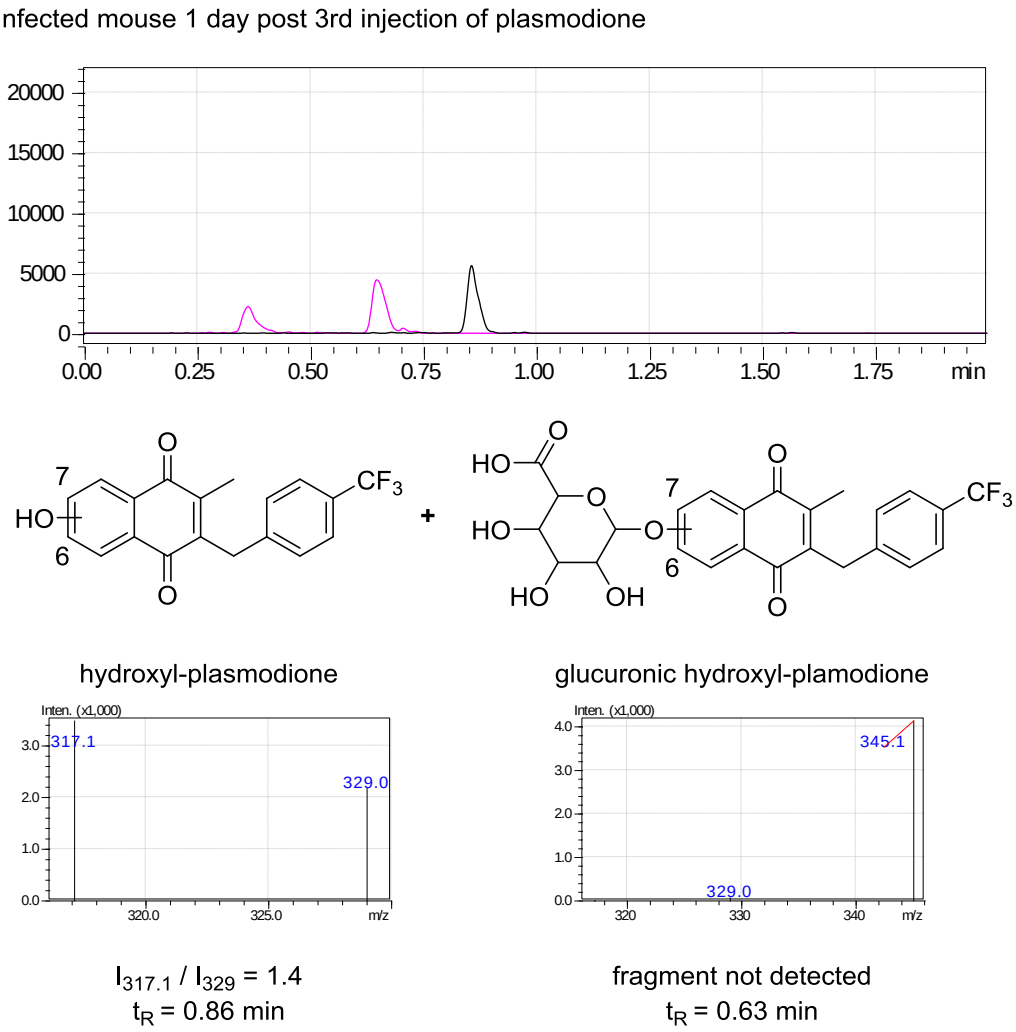


Figure S50. UHPLC/MS-MS spectrum of mice urine: Infected mouse 1 day post 3rd injection of plasmodione.

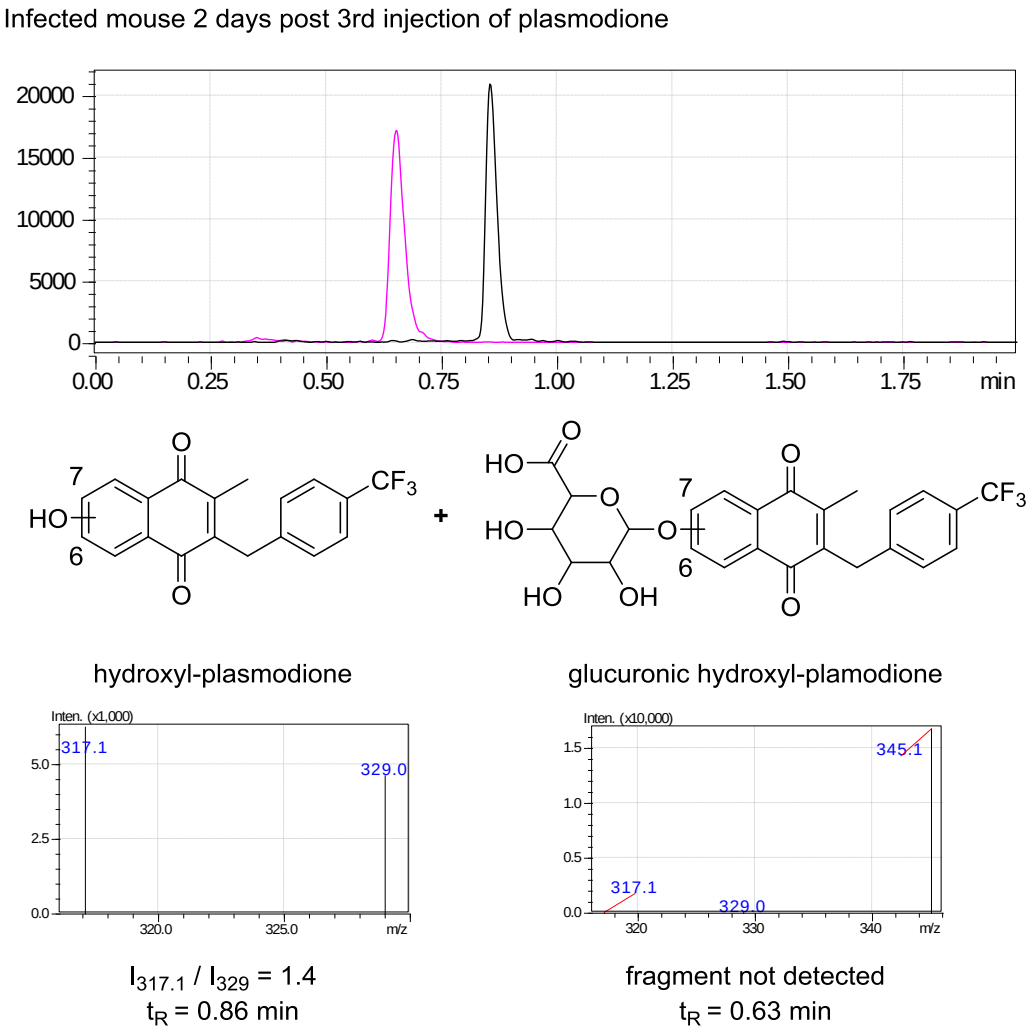


Figure S51. UHPLC/MS-MS spectrum of mice urine: Infected mouse 2 day post 3rd injection of plasmodione.