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Supporting Information

Targeting the IspD Enzyme in the MEP Pathway: Identification of a Novel Fragment Class

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1 LIST OF ABBREVIATIONS

Acetonitrile (CH₃CN), formic acid (HCOOH), dimethylsulfoxide (DMSO), ethyl acetate (EtOAc), sodium carbonate (Na₂CO₃), water (H₂O), magnesium sulfate (MgSO₄), hour (h), minutes (min), ON (overnight).

2 EXPERIMENTAL

2.1 CHEMISTRY

2.1.1 Materials and Methods

Starting materials and solvents were purchased from commercial suppliers, and used without further purification. All chemical yields refer to purified compounds and were not optimized. Reaction progress was monitored using TLC silica gel 60 F₂₅₄ aluminum sheets, and visualization was accomplished by UV at 254 nm. Column chromatography was performed using the automated flash chromatography system CombiFlash® Rf (Teledyne Isco) equipped with RediSepRf silica columns. Preparative RP-HPLC was performed using an UltiMate 3000 Semi-Preparative System (Thermo Fisher Scientific) with nucleodur® C18 Gravity (250 mm × 16 mm, 5 µm) column. ¹H and ¹³C NMR spectra were recorded as indicated on a Bruker Avance Neo 500 MHz (¹H, 500 MHz; ¹³C, 126 MHz) with prodigy cryoprobe system. Chemical shifts were recorded as δ values in ppm units and referenced against the residual solvent peak (DMSO-*d*₆, δ = 2.50, 39.52, acetone-*d*₆: δ = 2.05, 29.84). Splitting patterns describe apparent multiplicities and are designated as s (singlet), br s (broad singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), m (multiplet). Coupling constants (*J*) are given in hertz (Hz). Low resolution mass analytics and purity control of final compounds was carried out using an Ultimate 3000-MSQ LCMS system (Thermo Fisher Scientific) consisting of a pump, an autosampler, MWD detector and a ESI quadrupole mass spectrometer. Purity of all compounds used in biochemical assays was ≥ 95%. High resolution mass spectra were recorded on a ThermoFisher Scientific (TF, Dreieich, Germany) Q Exactive Focus system equipped with heated electrospray ionization (HESI)-II source. Final products were dried at high vacuum.

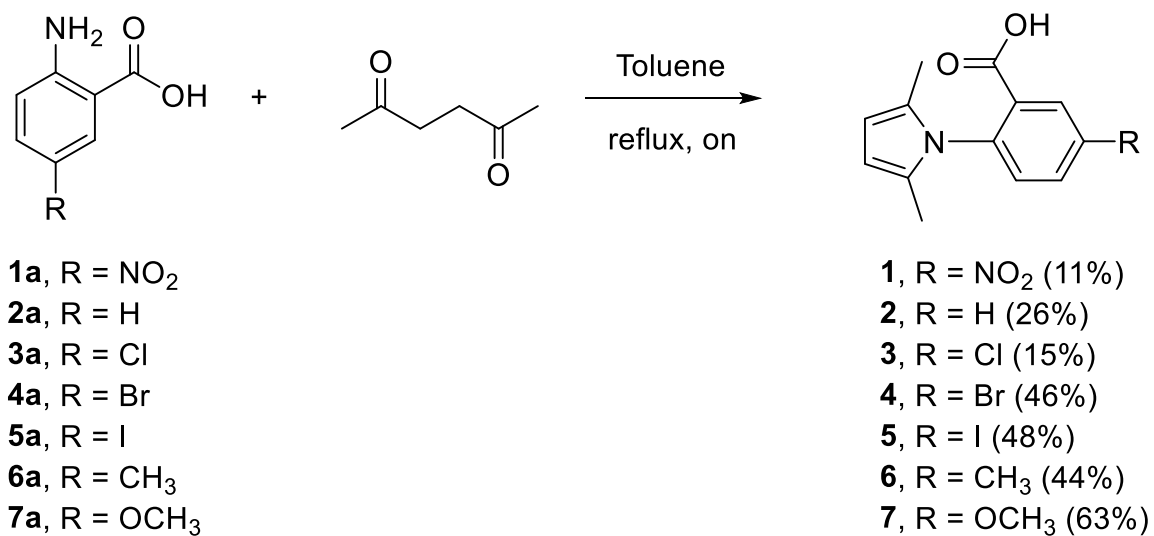
2.1.2 Preparative RP-HPLC purifications

Purifications via preparative RP-HPLC were carried out using the following condition:

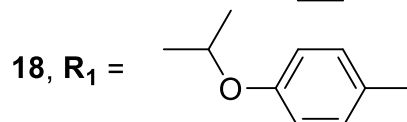
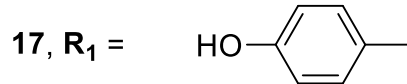
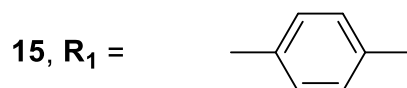
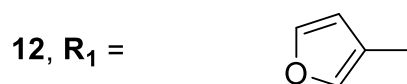
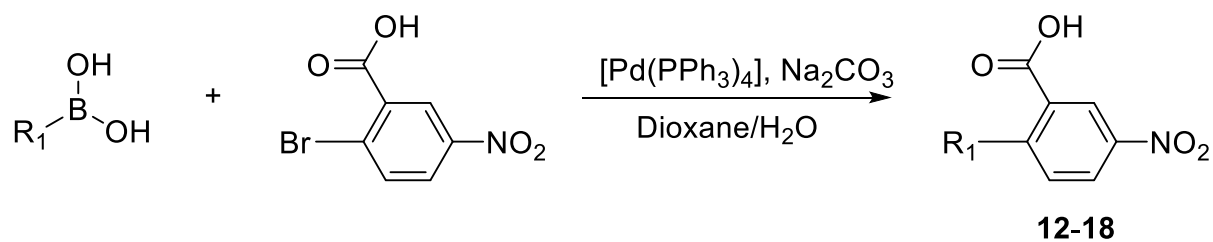
Gradient 5–100% CH₃CN + 0.05% HCOOH in water + 0.05% HCOOH in 53 min at a flow rate of 10 mL/min.

The sample was dissolved in DMSO and manually injected to the HPLC system.

2.1.3 Synthetic Schemes



Scheme S1: General procedure for the synthesis of compounds **1–7**.



Scheme S2: General procedure for the synthesis of compounds **12–18**.

2.1.4 Experimental procedures

Compounds **8**, **9**, **10** and **11** were commercially available and were purchased directly. Compound **8** (CAS: 332898-63-2) was purchased from abcr. Compounds **9** (CAS: 19555-48-7), **10** (CAS: 42106-50-3) and **11** (CAS: 4036-83-3) were purchased from Enamine.

General Procedure A (GPA): Paal-Knorr condensation.

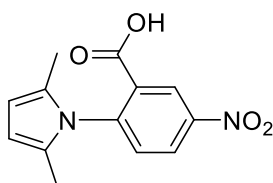
To a stirred solution of 5-substituted 2-amino benzoic acid in toluene (0.1 M), 1.2 eq of 2,5-hexanedione were added. The reaction mixture was stirred under reflux overnight in the presence

of molecular sieves. Once cooled, the solvent was evaporated, and the crude mixture was purified via preparative HPLC.

General Procedure B (GPB): Suzuki coupling.

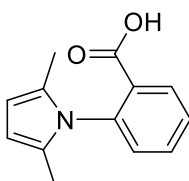
In a microwave vial containing compound 2-bromo-5-nitrobenzoic acid (1 mmol), boronic acid derivative (1.3 mmol) and $[\text{Pd}(\text{PPh}_3)_4]$ (0.05 mmol), was added the dioxane: H_2O mixture (3 mL, 4:1) and Na_2CO_3 (4 mmol). The reaction was heated in a microwave at 120°C for 1 h. H_2O (30 mL) was added followed by extraction with ethyl acetate (2 x 30 mL). The combined organic layers were dried over MgSO_4 , filtered and concentrated under reduced pressure. Purification was done using preparative HPLC to yield compounds (**12–18**).

2-(2,5-Dimethyl-1H-pyrrol-1-yl)-5-nitrobenzoic acid (**1**)



According to GPA, using 2-amino-5-nitrobenzoic acid (0.100 g, 0.55 mmol) and 2,5-hexanedione (0.077 mL, 0.66 mmol) in toluene (5.5 mL) to give, after purification by preparative HPLC (eluted at 40% CH_3CN), the product **1** as slightly red powder (0.015 g, 11%). $^1\text{H NMR}$ (500 MHz, $\text{DMSO}-d_6$) δ 8.59 (d, $J = 2.5$, 1H), 8.47 (dd, $J = 8.6$, 2.6, 1H), 7.60 (d, $J = 8.6$, 1H), 5.80 (s, 2H), 1.88 (s, 6H). $^{13}\text{C NMR}$ (126 MHz, $\text{DMSO}-d_6$) δ 165.5, 146.8, 142.7, 133.4, 132.3, 128.1, 126.9, 125.1, 106.5, 12.5. HRMS (ESI) m/z calcd for $\text{C}_{13}\text{H}_{11}\text{N}_2\text{O}_4$ ($M-H$): 259.07243, found: 259.07233.

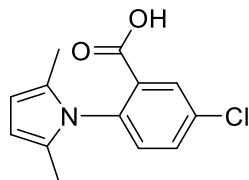
2-(2,5-Dimethyl-1H-pyrrol-1-yl)benzoic acid (**2**)



According to GPA, using 2-aminobenzoic acid (0.100 g, 0.73 mmol) and 2,5-hexanedione (0.103 mL, 0.87 mmol) in toluene (7.3 mL) to give, after purification by preparative HPLC (eluted at 55% CH_3CN) the product **2** as red powder (0.040 g, 26%). $^1\text{H NMR}$ (500 MHz, $\text{DMSO}-d_6$) δ 7.84 (dd, $J = 7.7$, 1.5, 1H), 7.66 (td, $J = 7.7$, 1.6, 1H), 7.56 (td, $J = 7.6$, 1.1, 1H), 5.72 (s, 2H), 5.72 (s, 2H), 1.85 (s, 6H). $^{13}\text{C NMR}$ (126

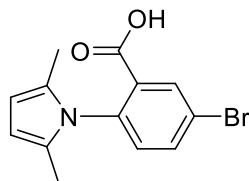
MHz, DMSO-*d*₆) δ 167.2, 137.05, 132.4, 132.1, 130.3, 129.9, 128.5, 127.8, 105.4, 12.5. **HRMS (ESI) *m/z*** calcd for C₁₃H₁₂NO₂ (*M-H*)⁻: 214.08735, found: 214.08678.

5-Chloro-2-(2,5-dimethyl-1*H*-pyrrol-1-yl)benzoic acid (**3**)



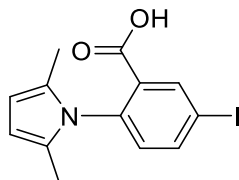
According to GPA, using 2-amino-5-chlorobenzoic acid (0.100 g, 0.58 mmol) and 2,5-hexanedione (0.08 mL, 0.7 mmol) in toluene (5.8 mL) to give, after purification by preparative HPLC (eluted at 55% CH₃CN) the product **3** as red powder (0.022g, 15%). **¹H NMR (500 MHz, DMSO-*d*₆)** δ 7.85 (d, *J* = 2.4, 1H), 7.72 (dd, *J* = 8.4, 2.5, 1H), 7.31 (d, *J* = 8.4, 1H), 5.73 (s, 2H), 1.85 (s, 6H). **¹³C NMR (126 MHz, DMSO-*d*₆)** δ 165.9, 135.8, 134.2, 132.9, 132.3, 131.9, 129.5, 128.0, 105.7, 12.5; **HRMS (ESI) *m/z*** calcd for C₁₃H₁₁ClNO₂ (*M-H*)⁻: 248.04838 found: 248.04796.

5-Bromo-2-(2,5-dimethyl-1*H*-pyrrol-1-yl)benzoic acid (**4**)



According to GPA, using 2-amino-5-bromobenzoic acid (0.100 g, 0.46 mmol) and 2,5-hexanedione (0.065 mL, 0.55 mmol) in toluene (4.6 mL) to give, after purification by preparative HPLC (eluted at 74% CH₃CN) the product **4** as red oil (0.060 g, 46%). **¹H NMR (500 MHz, DMSO-*d*₆)** δ 13.18 (s, 1H), 7.98 (d, *J* = 2.4, 1H), 7.86 (dd, *J* = 8.4, 2.4, 1H), 7.24 (d, *J* = 8.4, 1H), 5.73 (s, 2H), 1.85 (s, 6H). **¹³C NMR (126 MHz, DMSO-*d*₆)** δ 165.8, 136.3, 134.9, 134.4, 132.5, 132.3, 127.9, 121.3, 105.7, 12.5. **HRMS (ESI) *m/z*** calcd for C₁₃H₁₃BrNO₂ (*M+H*)⁺: 294.01242, found: 294.01175.

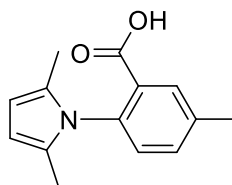
5-Iodo-2-(2,5-dimethyl-1*H*-pyrrol-1-yl)benzoic acid (**5**)



According to GPA, using 2-amino-5-iodobenzoic acid (0.100 g, 0.38 mmol), 2,5-hexanedione (0.053 mL, 0.45 mmol) in Toluene (3.8 mL) to give, after purification by preparative HPLC (eluted at 75% CH₃CN)

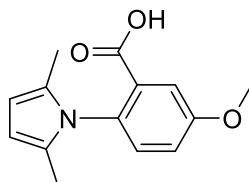
the product **6** as red powder (0.062 g, 48%). ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.12 (d, *J* = 2.1, 1H), 8.00 (dd, *J* = 8.2, 2.1, 1H), 7.06 (d, *J* = 8.2, 1H), 5.72 (s, 2H), 1.85 (s, 6H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 166.2, 141.2, 138.6, 137.1, 134.8, 132.8, 128.4, 106.13, 94.8, 12.9. HRMS (ESI) *m/z* calcd for C₁₃H₁₃INO₂ (*M*+H)⁺: 341.99855, found: 341.99792.

2-(2,5-Dimethyl-1*H*-pyrrol-1-yl)-5-methylbenzoic acid (**6**)



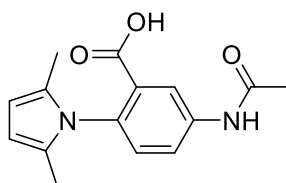
According to GPA, using 2-amino-5-methylbenzoic acid (0.100 g, 0.66 mmol), 2,5-hexanedione (0.09 mL, 0.8 mmol) in Toluene (6.6 mL) to give, after purification by preparative HPLC (eluted at 60% CH₃CN) the product **6** as red powder (0.065 g, 44%). ¹H NMR (500 MHz, DMSO-*d*₆) δ 7.65 (d, *J* = 1.6, 1H), 7.45 (dd, *J* = 8.0, 1.7, 1H), 7.12 (d, *J* = 7.9, 1H), 5.70 (s, 2H), 2.41 (s, 3H), 1.84 (s, 6H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 167.3, 138.1, 134.5, 132.6, 131.9, 130.3, 130.0, 127.9, 105.2, 20.5, 12.6. HRMS (ESI) *m/z* calcd for C₁₄H₁₆NO₂ (*M*+H)⁺: 230.11756, found: 230.11723.

2-(2,5-Dimethyl-1*H*-pyrrol-1-yl)-5-methoxybenzoic acid (**7**)



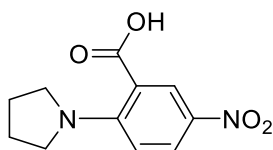
According to GPA, using 2-amino-5-methoxybenzoic acid (0.100 g, 0.59 mmol), 2,5-hexanedione (0.084 mL, 0.71 mmol) in toluene (5.9 mL) to give, after purification by preparative HPLC (eluted at 72% CH₃CN) the product **7** as red powder (0.090 g, 63%). ¹H NMR (500 MHz, DMSO-*d*₆) δ 7.32 (d, *J* = 2.8, 1H), 7.20 (dd, *J* = 8.6, 2.8, 1H), 7.16 (d, *J* = 8.6, 1H), 5.69 (s, 2H), 3.85 (s, 3H), 1.84 (s, 6H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 166.9, 158.5, 133.3, 131.5, 129.6, 128.1, 117.5, 114.4, 105.1, 55.6, 12.6. HRMS (ESI) *m/z* calcd for C₁₄H₁₄NO₃ (*M*-H)⁻: 244.09792, found: 244.09744.

5-Acetamido-2-(2,5-dimethyl-1*H*-pyrrol-1-yl)benzoic acid (**8**)



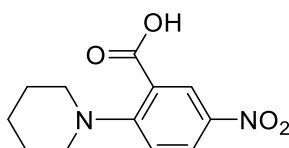
CAS: 332898-63-2

5-Nitro-2-(pyrrolidin-1-yl)benzoic acid (9)



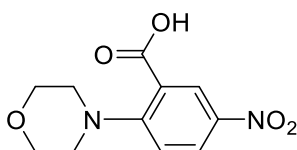
CAS: 19555-48-7

5-Nitro-2-(piperidin-1-yl)benzoic acid (10)



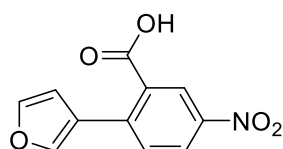
CAS: 42106-50-3

2-Morpholino-5-nitrobenzoic acid (11)



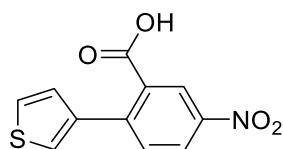
CAS: 4036-83-3

2-(Furan-3-yl)-5-nitrobenzoic acid (12)



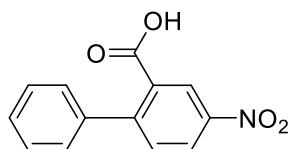
Compound **12** was synthesized according to GPB by reacting 2-bromo-5-nitrobenzoic acid with furan-3-ylboronic acid. The crude product was purified by preparative HPLC (eluted at 45% CH₃CN, pale yellow solid, 59 % yield). ¹H NMR (500 MHz, Acetone-*d*₆) δ 8.57 (d, *J* = 2.5, 1H), 8.38 (dd, *J* = 8.6, 2.5, 1H), 7.97 (s, 1H), 7.82 (d, *J* = 8.6, 1H), 7.67 (t, *J* = 1.5, 1H), 6.76 (d, *J* = 1.0, 1H). ¹³C NMR (126 MHz, Acetone-*d*₆) δ 168.1, 147.3, 144.4, 142.6, 139.4, 133.4, 132.3, 126.3, 125.2, 124.6, 112.0. HRMS (ESI) *m/z* calcd for C₁₁H₆NO₅ (*M*-H)⁻: 232.02515 found 232.02499.

5-Nitro-2-(thiophen-3-yl)benzoic acid (13)



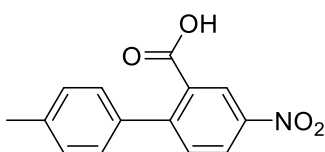
Compound **13** was synthesized according to GPB by reacting 2-bromo-5-nitrobenzoic acid with thiophen-3-ylboronic acid. The crude product was purified by preparative HPLC (eluted at 55% CH₃CN, pale yellow solid, 25 % yield). **¹H NMR (500 MHz, Acetone-*d*₆)** δ 8.58 (d, *J* = 2.4, 1H), 8.40 (dd, *J* = 8.5, 2.4, 1H), 7.80 (d, *J* = 8.5, 1H), 7.69 (d, *J* = 1.8, 1H), 7.57 (dd, *J* = 4.9, 3.0, 1H), 7.29 (d, *J* = 4.5, 1H). **¹³C NMR (126 MHz, Acetone-*d*₆)** δ 168.1, 147.5, 143.2, 140.2, 133.9, 132.8, 129.1, 126.8, 126.2, 125.5, 125.1. **HRMS (ESI)** *m/z* calcd for C₁₁H₆NO₄S (*M*-H): 248.00230 found 248.00226.

4-Nitro-[1,1'-biphenyl]-2-carboxylic acid (**14**)



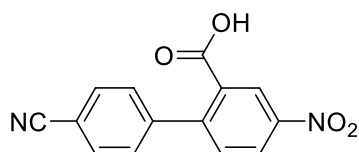
Compound **14** was synthesized according to GPB by reacting 2-bromo-5-nitrobenzoic acid with phenylboronic acid. The crude product was purified by preparative HPLC (eluted at 55% CH₃CN, white solid, 20% yield). **¹H NMR (500 MHz, Acetone-*d*₆)** δ 8.64 (d, *J* = 2.4, 1H), 8.44 (dd, *J* = 8.5, 2.5, 1H), 7.73 (d, *J* = 8.5, 1H), 7.49 – 7.43 (m, 5H); **¹³C NMR (126 MHz, Acetone-*d*₆)** δ 167.8, 149.1, 147.7, 140.3, 133.9, 133.2, 129.2, 129.2, 126.3, 125.41. **HRMS (ESI)** *m/z* calcd for C₁₃H₈NO₄ (*M*-H): 242.04588 found 242.04581.

4'-Methyl-4-nitro-[1,1'-biphenyl]-2-carboxylic acid (**15**)



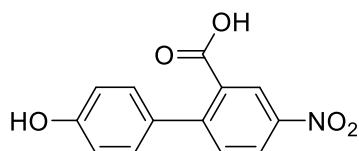
Compound **15** was synthesized according to GPB by reacting 2-bromo-5-nitrobenzoic acid with *p*-tolylboronic acid. The crude product was purified by preparative HPLC (eluted at 60% CH₃CN, white solid, 58 % yield). **¹H NMR (500 MHz, Acetone-*d*₆)** δ 8.61 (d, *J* = 2.3, 1H), 8.41 (dd, *J* = 8.5, 2.4, 1H), 7.70 (d, *J* = 8.5, 1H), 7.34 (d, *J* = 8.0, 2H), 7.27 (d, *J* = 7.9, 2H), 2.38 (s, 3H). **¹³C NMR (126 MHz, Acetone-*d*₆)** δ 168.0, 149.0, 147.5, 139.1, 137.3, 133.9, 133.1, 129.8, 129.2, 126.2, 125.3, 21.2. **HRMS (ESI)** *m/z* calcd for C₁₄H₁₀NO₄ (*M*-H): 256.06153 found 256.06161.

4'-Cyano-4-nitro-[1,1'-biphenyl]-2-carboxylic acid (**16**)



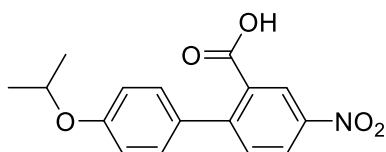
Compound **16** was synthesized according to GPB by reacting 2-bromo-5-nitrobenzoic acid with (4-cyanophenyl)boronic acid. The crude product was purified by preparative HPLC (eluted at 50% CH₃CN, white solid, 25 % yield). **¹H NMR (500 MHz, Acetone-*d*₆)** δ 8.74 (d, *J* = 2.1, 1H), 8.49 (dd, *J* = 8.4, 2.2, 1H), 7.88 (d, *J* = 8.1, 2H), 7.76 (d, *J* = 8.4, 1H), 7.66 (d, *J* = 8.1, 2H). **¹³C NMR (126 MHz, Acetone-*d*₆)** δ 166.9, 148.4, 147.8, 145.4, 133.4, 133.3, 132.8, 130.3, 126.7, 125.9, 119.2, 112.7. **HRMS (ESI)** *m/z* calcd for C₁₄H₇N₂O₄ (*M*-H)⁻: 267.04113 found 267.04128.

4'-Hydroxy-4-nitro-[1,1'-biphenyl]-2-carboxylic acid (**17**)



Compound **17** was synthesized according to GPB by reacting 2-bromo-5-nitrobenzoic acid with (4-hydroxyphenyl)boronic acid. The crude product was purified by preparative HPLC (eluted at 45% CH₃CN, beige solid, 62 % yield). **¹H NMR (500 MHz, Acetone-*d*₆)** δ 8.57 (d, *J* = 2.5, 1H), 8.38 (dd, *J* = 8.5, 2.5, 1H), 7.69 (d, *J* = 8.5, 1H), 7.34 – 7.29 (m, 2H), 6.96 – 6.91 (m, 2H). **¹³C NMR (126 MHz, Acetone-*d*₆)** δ 168.4, 158.9, 148.9, 147.1, 133.7, 132.8, 131.2, 130.7, 126.1, 125.3, 116.2. **HRMS (ESI)** *m/z* calcd for C₁₃H₈NO₅ (*M*-H)⁻: 258.04080 found 258.04082.

4'-Isopropoxy-4-nitro-[1,1'-biphenyl]-2-carboxylic acid (**18**)



Compound **18** was synthesized according to GPB by reacting 2-bromo-5-nitrobenzoic acid with (4-isopropoxyphenyl)boronic acid. The crude product was purified by preparative HPLC (eluted at 62% CH₃CN, pale yellow solid, 33 % yield). **¹H NMR (500 MHz, Acetone-*d*₆)** δ 8.59 (s, 1H), 8.40 (d, *J* = 7.6, 1H), 7.71 (d, *J* = 8.3 Hz, 1H), 7.38 (d, *J* = 8.0, 2H), 7.00 (d, *J* = 8.0, 2H), 4.77 – 4.64 (m, 1H), 1.34 (d, *J* = 5.6, 6H). **¹³C NMR (126 MHz, Acetone-*d*₆)** δ 168.2, 159.4, 148.7, 147.2, 133.7, 132.9, 131.9, 130.7, 126.2, 125.3, 116.3, 70.4, 22.3. **HRMS (ESI)** *m/z* calcd for C₁₆H₁₄NO₅ (*M*-H)⁻: 300.08775 found 300.08796.

3 Biochemistry

3.1 PflspD assay

Assays were conducted in 384-well plates (Corning) with a transparent flat bottom. Assay buffer A (total volume, 30 μ L) contained 200 mM Tris hydrochloride, pH 7.6, 20 mM KCl, 10 mM MgCl₂, 10 mM DTT, 1.2 mM NADH, 2 mM ATP, 2 mM phosphoenolpyruvic acid, 0.2 U of pyruvate kinase (PK), 0.2 U of lactate dehydrogenase (LDH), 0.2 U (1.8 μ M) IspE from *E. coli* (EclspE) and 0.004 U (0.050 μ M) of IspD protein from *Plasmodium falciparum* (PflspD). Dilution series were performed with dilution step 1:2 and approximately covered the concentration range of 200 μ M to 0.01 μ M of tested compounds. The reaction was started by addition of the assay buffer B that contained 200 mM Tris hydrochloride, pH 7.6, 1 mM MEP and 1 mM CTP.

The impact of the tested compounds on auxiliary enzymes in the photometric activity inhibition assay was tested as follows. Test buffer A (total volume, 30 μ L) contained 200 mM Tris hydrochloride, pH 7.6, 20 mM KCl, 10 mM MgCl₂, 10 mM DTT, 1.2 mM NADH, 2 mM ATP, 2 mM phosphoenolpyruvic acid, 0.2 U of pyruvate kinase (PK), 0.2 U of lactate dehydrogenase (LDH) and 0.004 U (0.02 μ M) EclspE. Dilution series were performed with dilution step 1:2 and approximately covered the concentration range of 200 μ M to 0.01 μ M of tested compounds. The reaction was started by addition of the assay buffer B that contained 200 mM Tris hydrochloride, pH 7.6, 1 mM CDP-ME. The reactions were monitored photometrically (room temperature) at 340 nm in a plate reader (SpectraMax5, Molecular Dynamics). Initial rate values were evaluated with a nonlinear regression method using the program Dynafit.^[1]

MEP and CDP-ME were used as starting substrate in the IspD and IspE assay, respectively, and were synthesised and purified as described earlier.^[2]

All the compounds tested (**1–18**) showed no activity against the auxiliary enzyme.

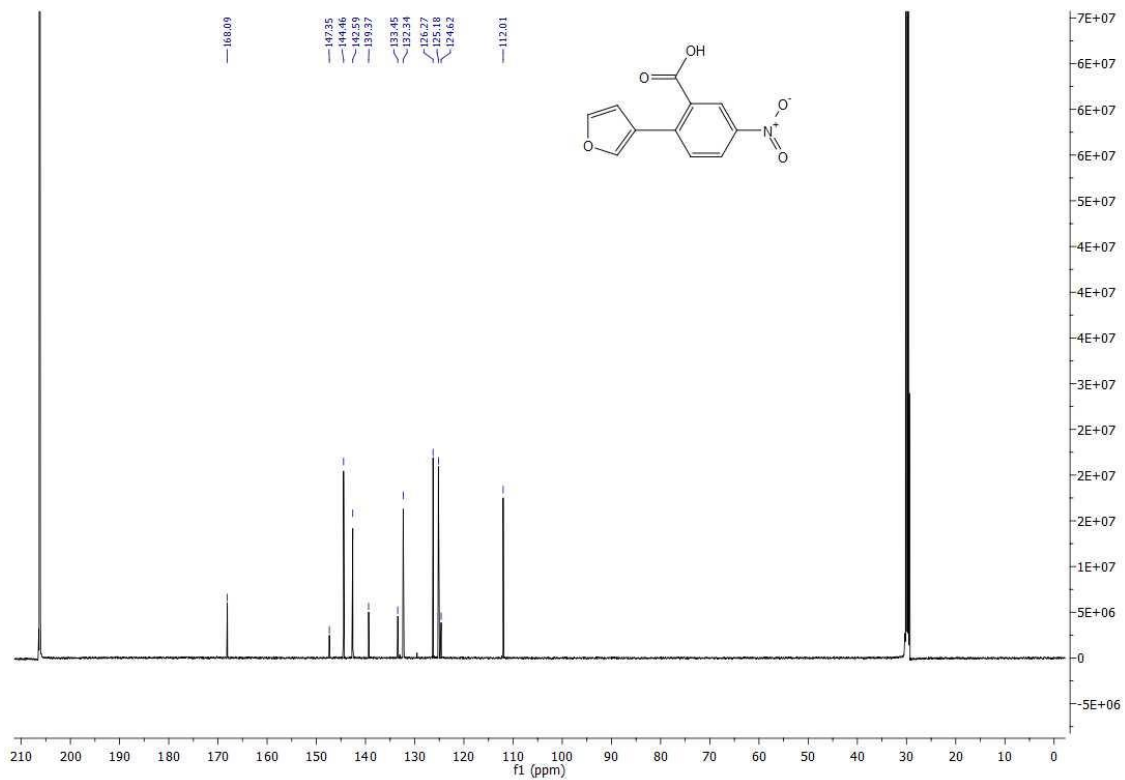
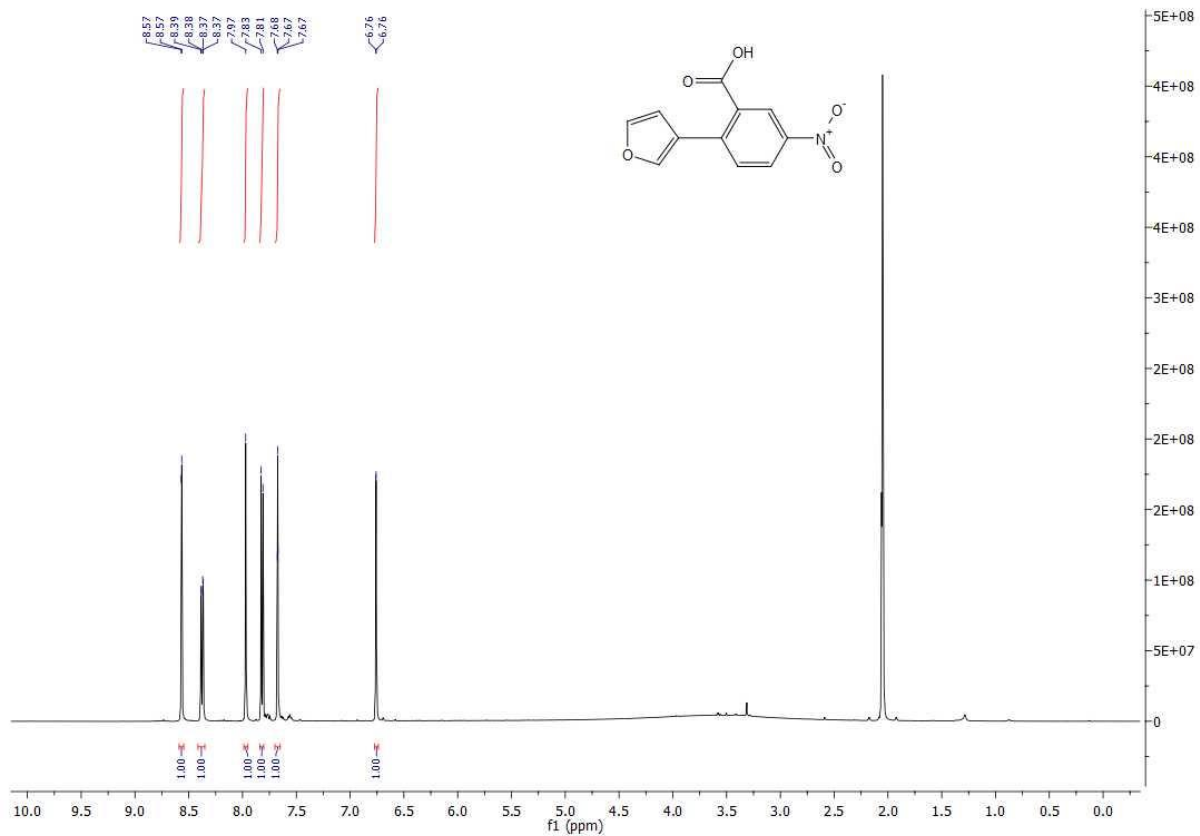
This assay has been used in the initial HTS and as with most enzyme-based approaches, IspD hits may fall under PAIN-subcluster (pan-assay interference compounds), which indicate risk of non-specific interference with the assay, but sometimes also exclude valuable leads. The control reaction via the auxiliary IspE-enzyme done for the most active hits confirm that the described inhibitors are, in fact, selectively inhibiting the target enzyme and not the auxiliary enzymes of the IspD assay. Still, in follow up optimization the potential of non-specific assay interaction should be taken into account for further analogues

4 Molecular modeling

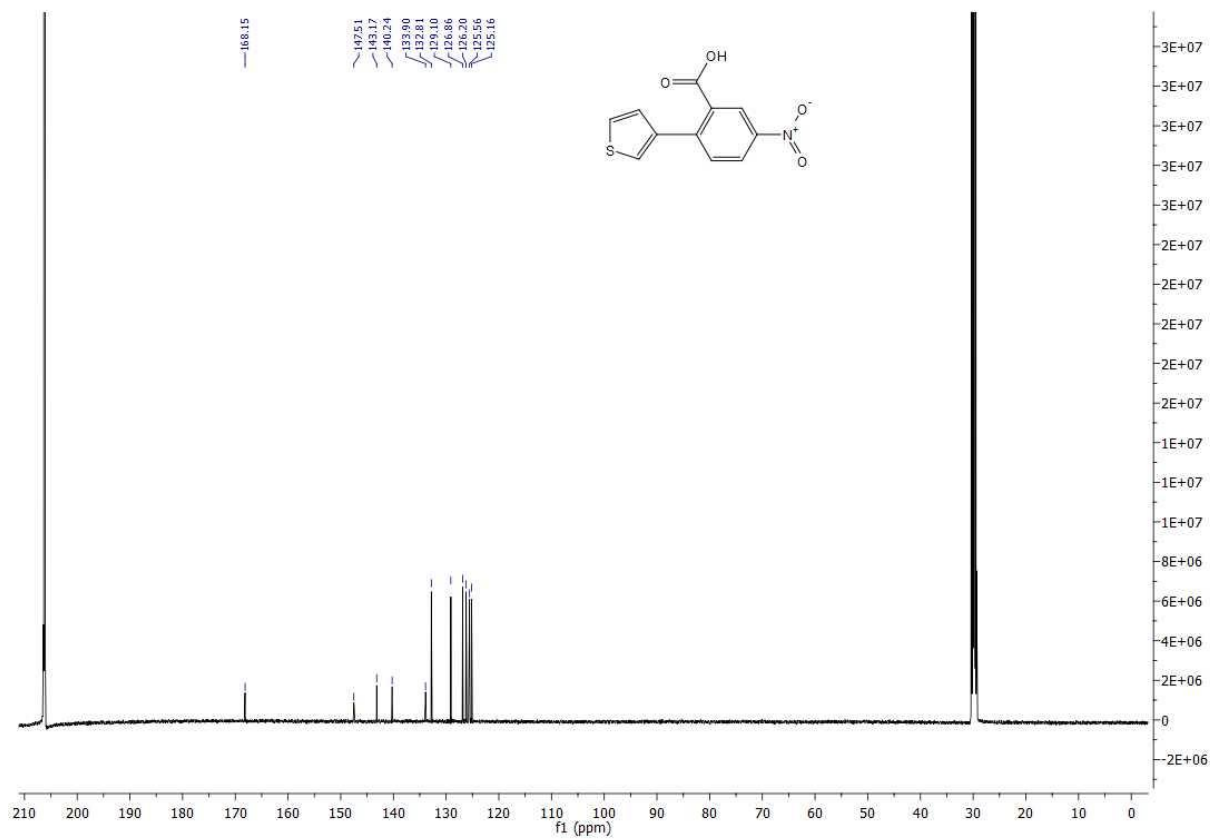
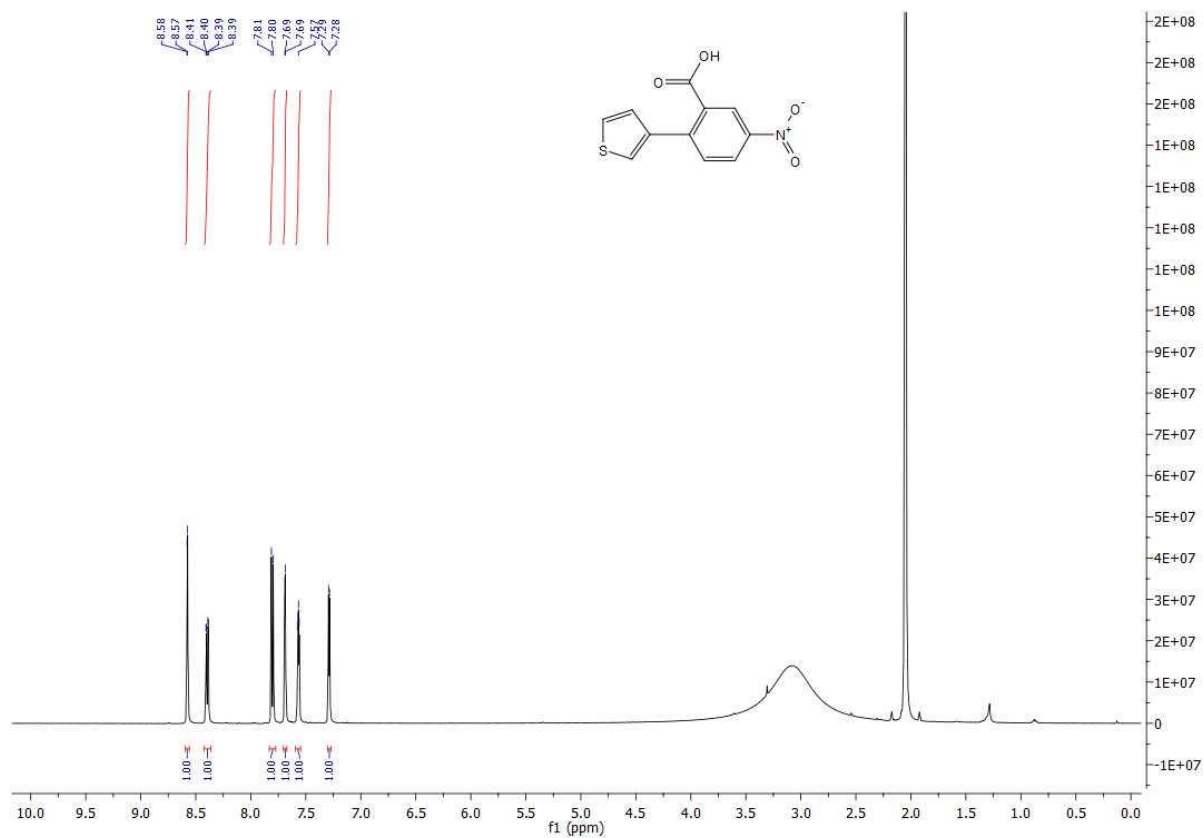
The *PflspD* homology model was constructed using the sequence from 2-C-methyl-D-erythritol 4-phosphate cytidyltransferase from *Plasmodium falciparum* 3D7 (obtained from the NCBI (www.ncbi.nlm.nih.gov, sequence ID: XP_001351000.2, accessed 11/10/2021) and the structure from *E. coli* IspD (accessed 11/10/2021). The model was built using the PHYRE2 online homology modeling program.^[3] Previous work showed the 1I52 *E. coli* IspD structure served as a good template.^[4] The model created in PHYRE2 using 1I52 as the template structure showed a 99.77% degree of confidence. Compounds of interest were docked into the homology model described above using the docking algorithm provided in SeeSAR 11.0 (BioSolveIT GPBH). Binding site definition was carried out manually from residues selected by SeeSAR's pocket finding function. The binding site selected is analogous to the CDP-ME binding site in the 1I52 *E. coli* IspD structure used as a template.

5 ¹H- AND ¹³C-NMR SPECTRA OF SOME REPRESENTATIVE COMPOUNDS

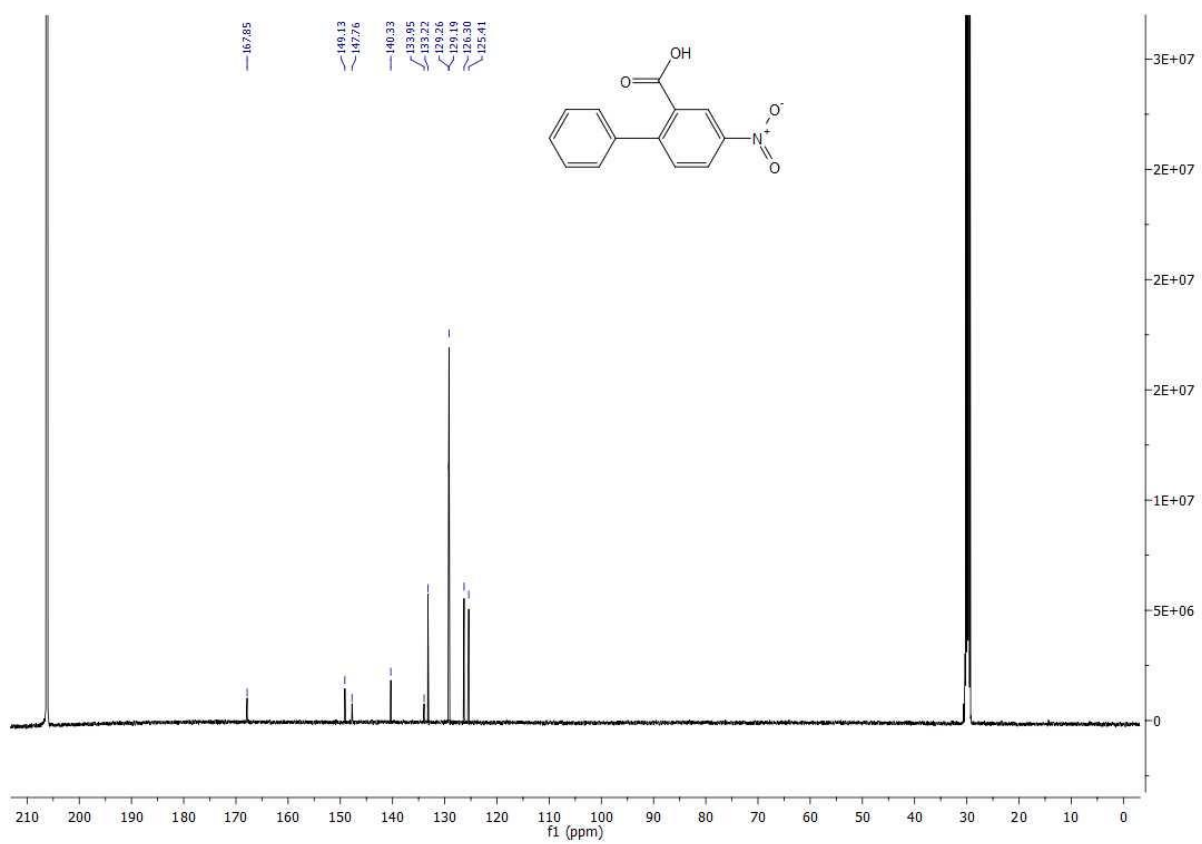
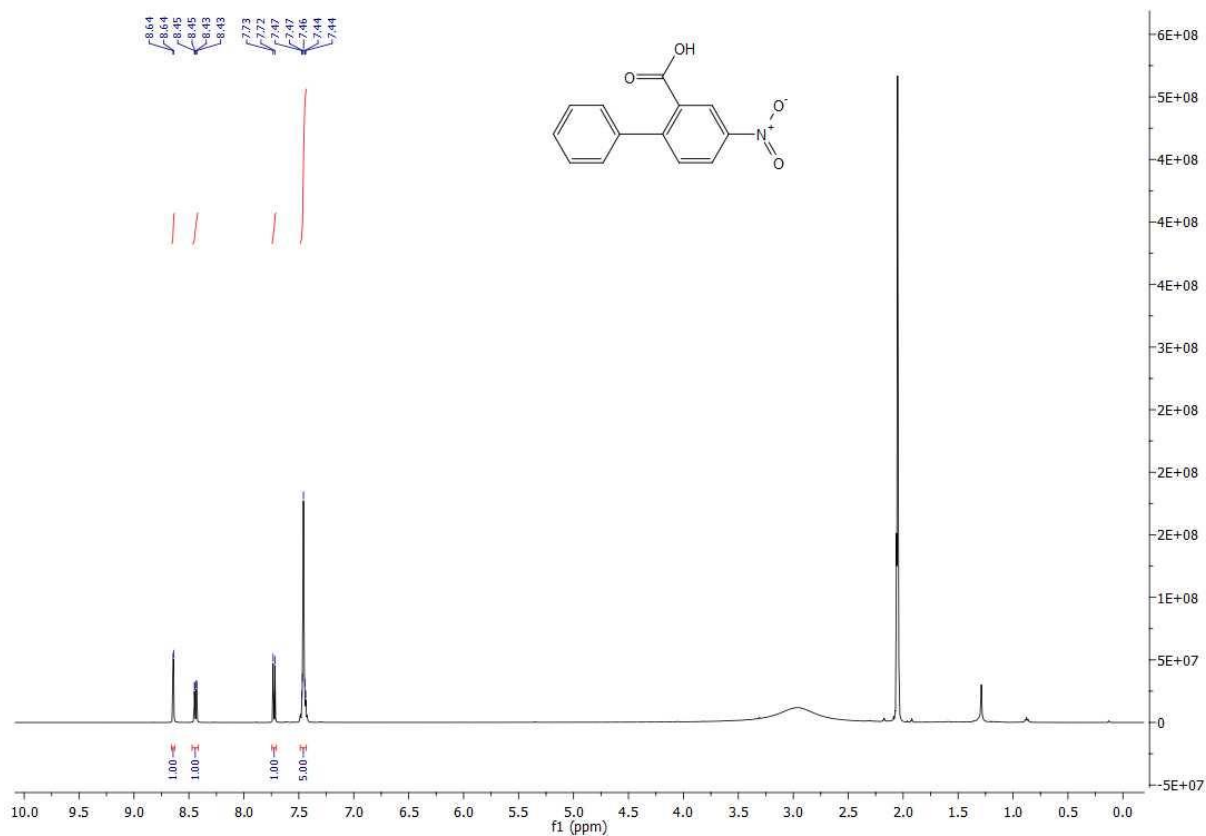
Compound **12**



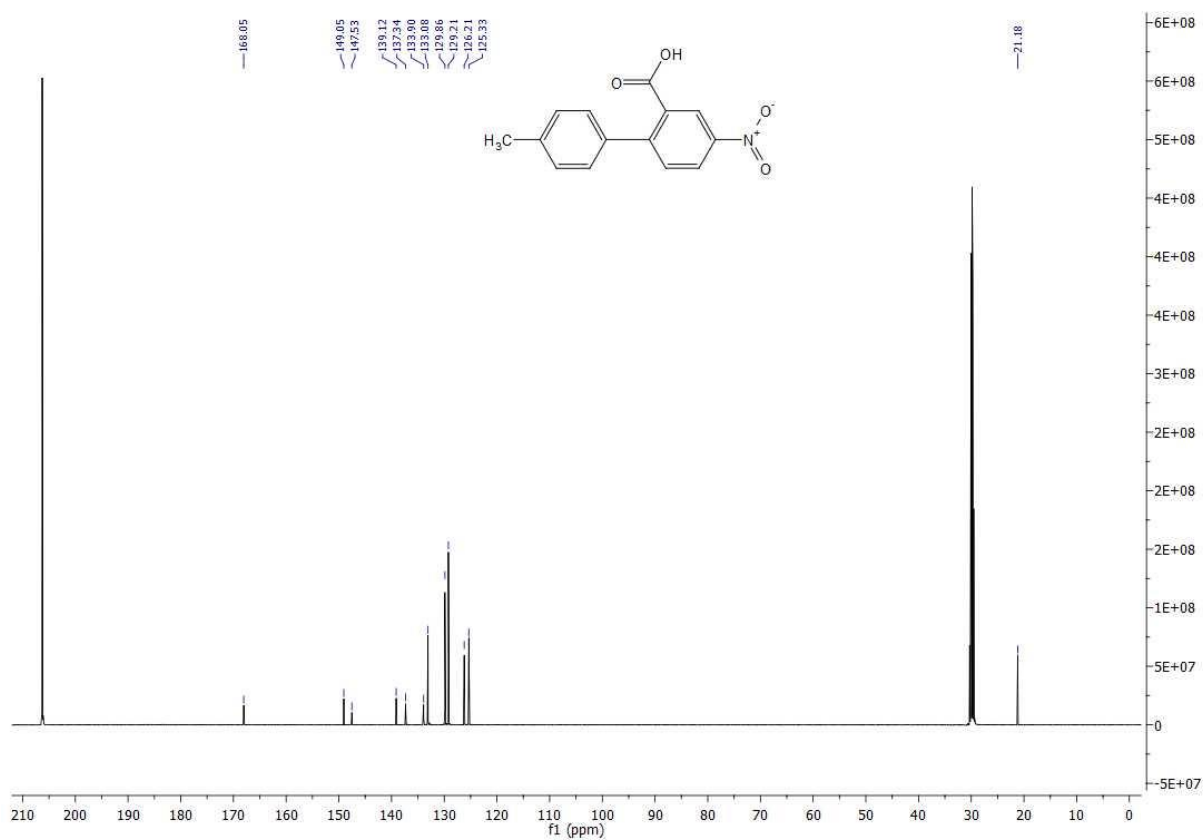
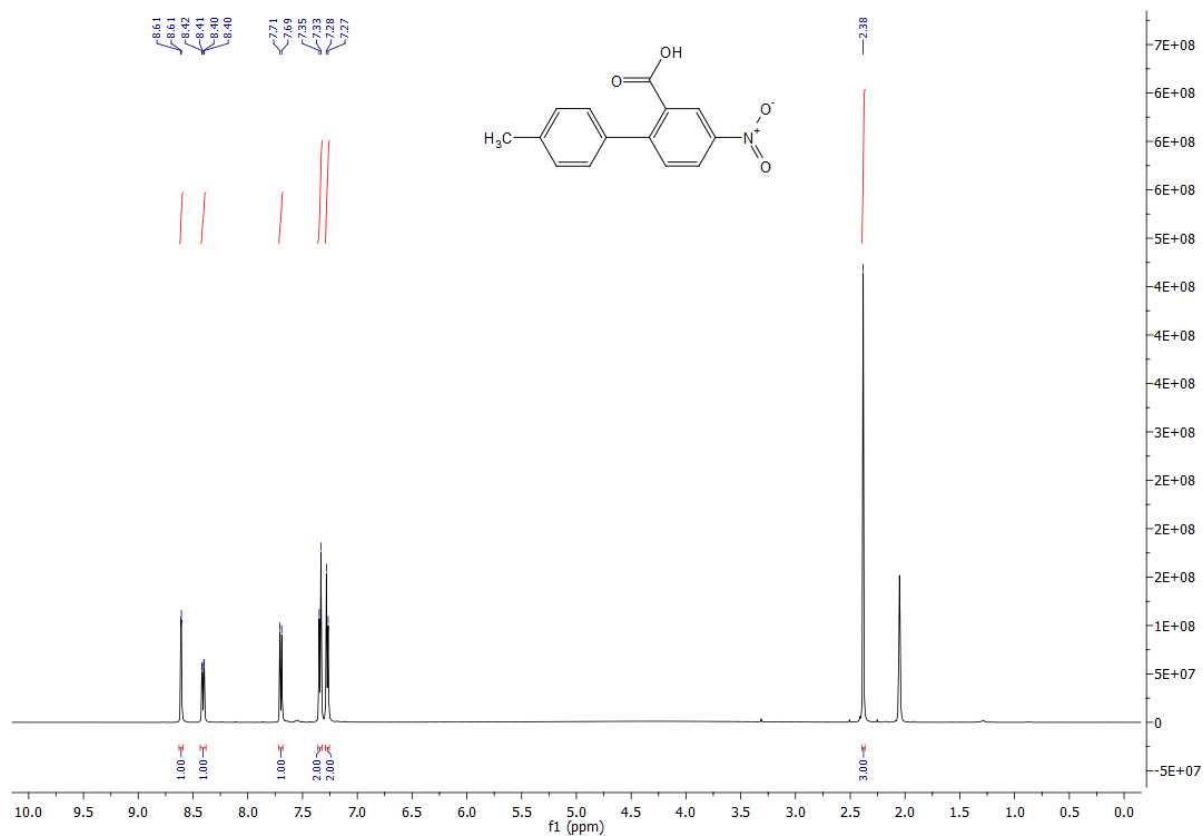
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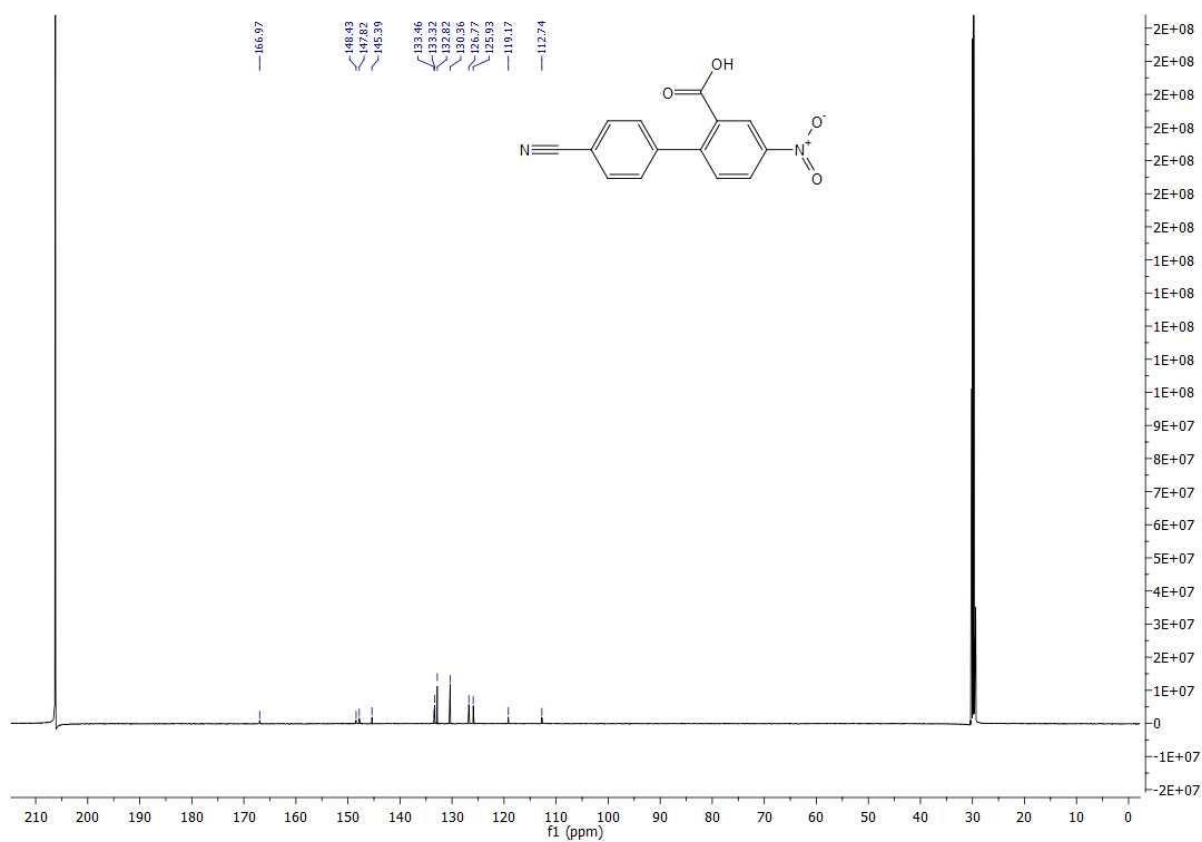
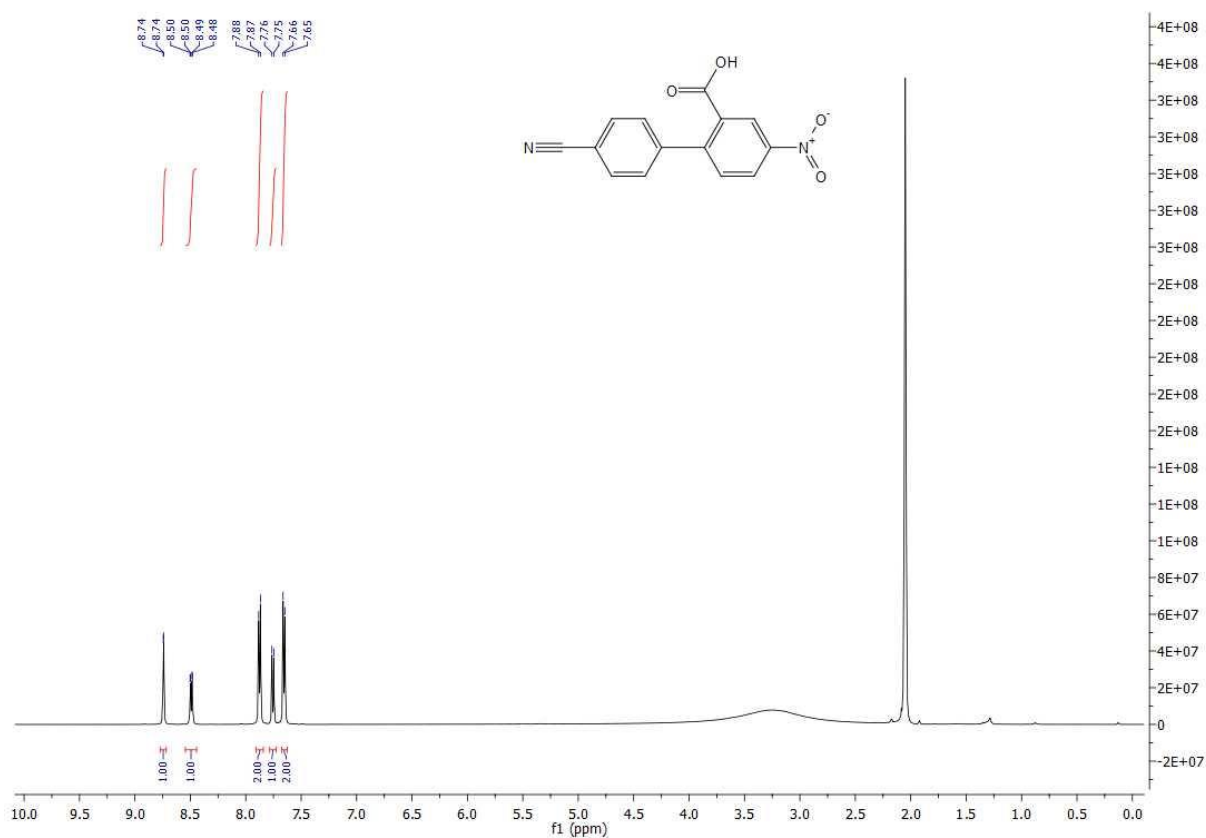
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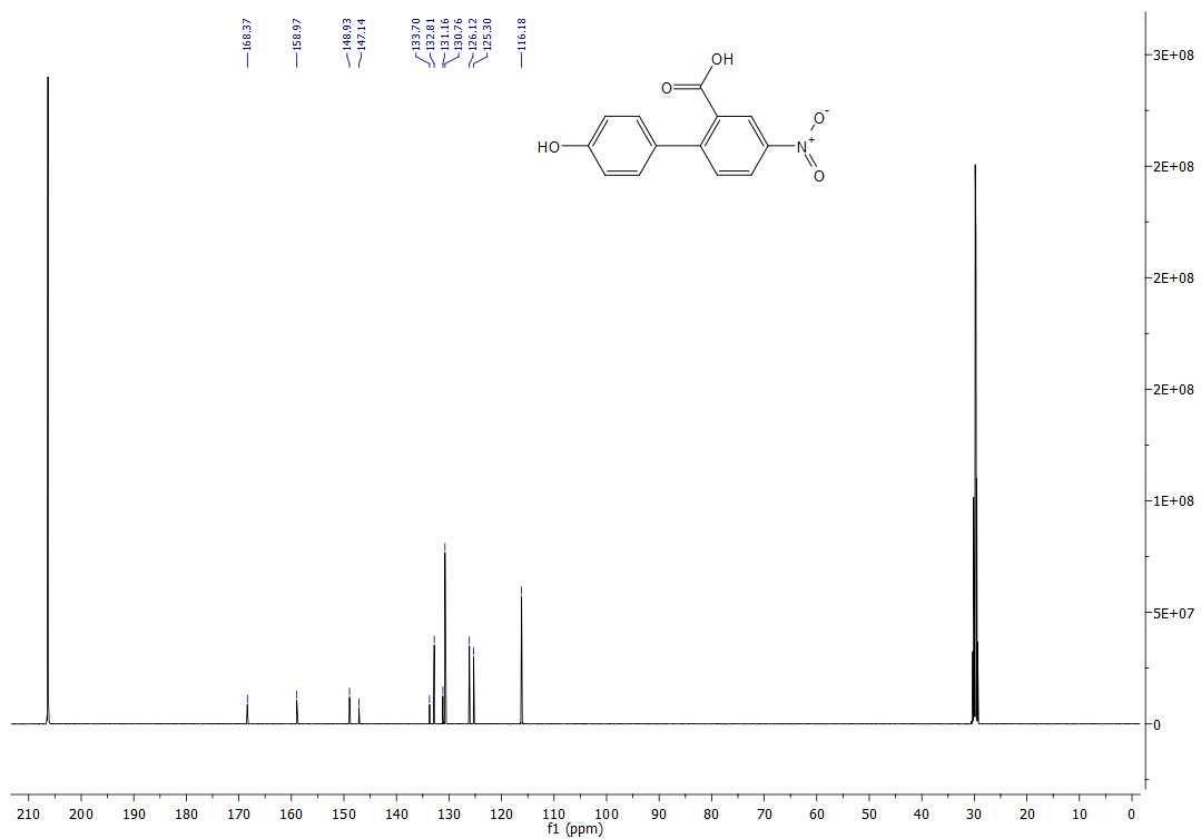
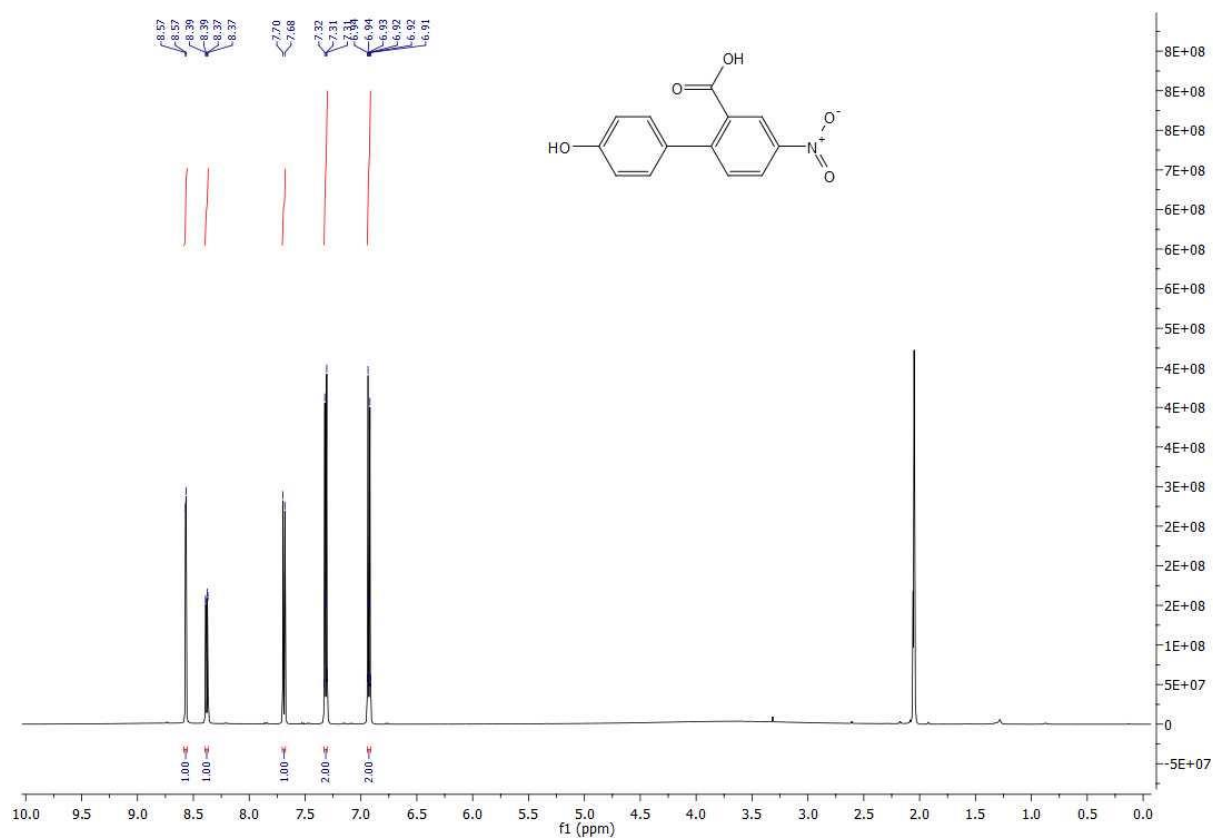
Compound 15



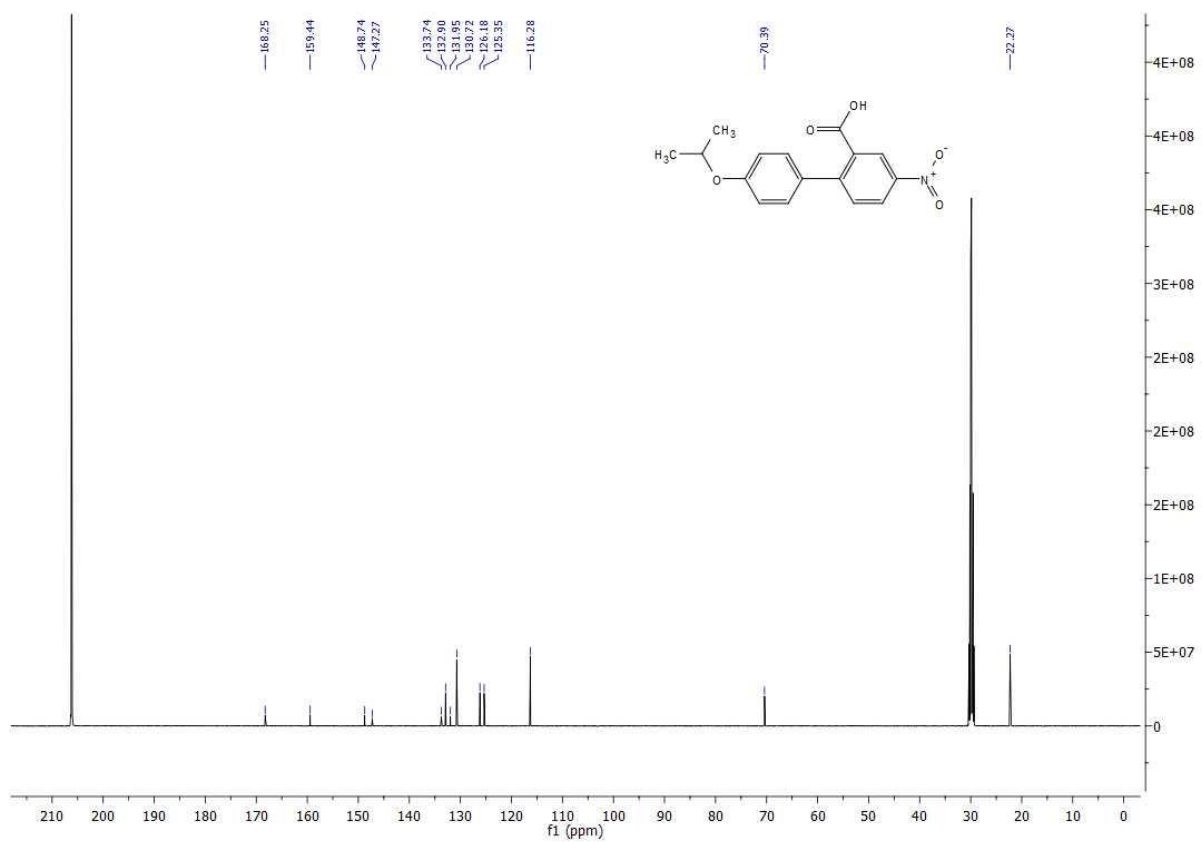
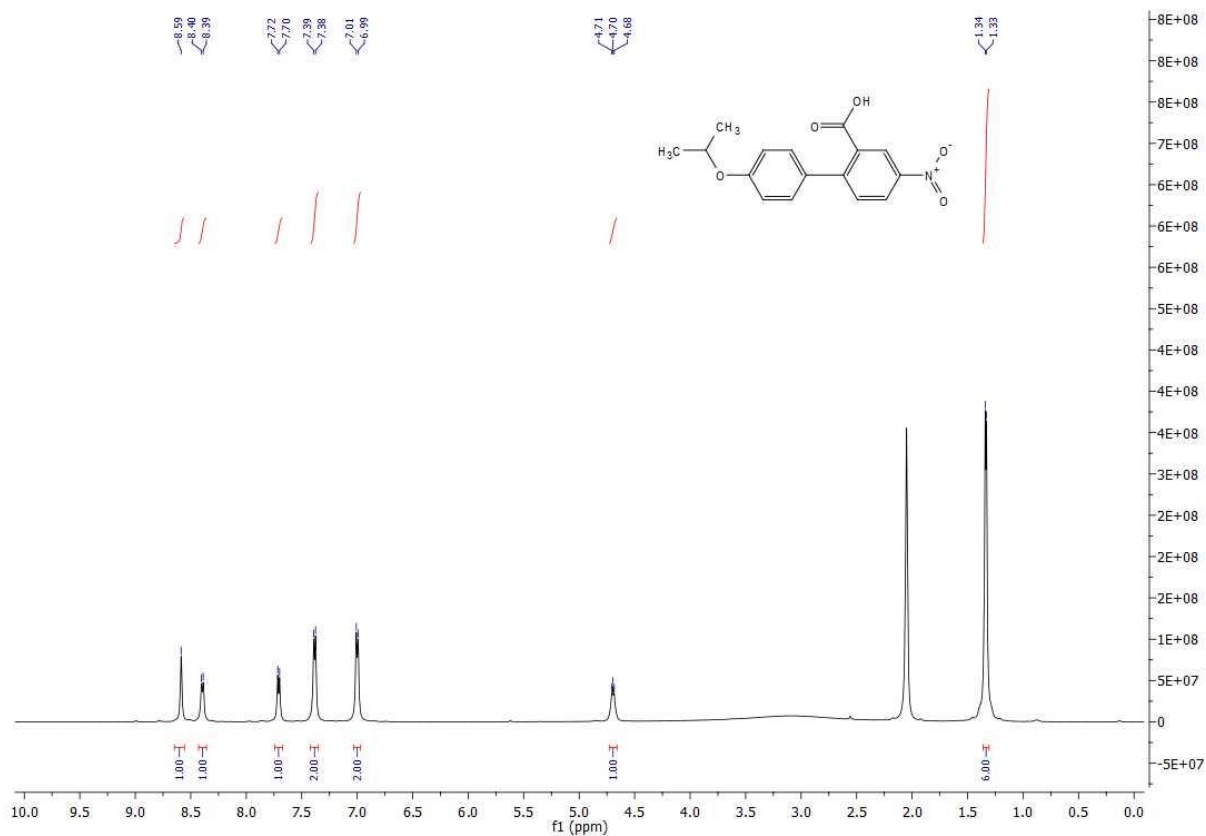
Compound 16



Compound 17



Compound 18



6 REFERENCES

- [1] P. Kuzmic, *Anal. Biochem.* **1996**, *237*, 260–273.
- [2] A. Honold, C. Lettl, F. Schindele, B. Illarionov, R. Haas, M. Witschel, A. Bacher, M. Fischer, *Helv. Chim.* **2019**, *102*.
- [3] L. A. Kelley, S. Mezulis, C. M. Yates, M. N. Wass, M. J. Sternberg, *Nat. Protoc.* **2015**, *10*, 845–858.
- [4] L. S. Imlay, C. M. Armstrong, M. C. Masters, T. Li, K. E. Price, R. L. Edwards, K. M. Mann, L. X. Li, C. L. Stallings, N. G. Berry, P. M. O'Neill, A. R. Odom, *ACS Infect. Dis.* **2015**, *1*, 157–167.