Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

-Supporting Information –

A Pd-labile fluoroquinolone prodrug efficiently prevents biofilm formation on coated surfaces

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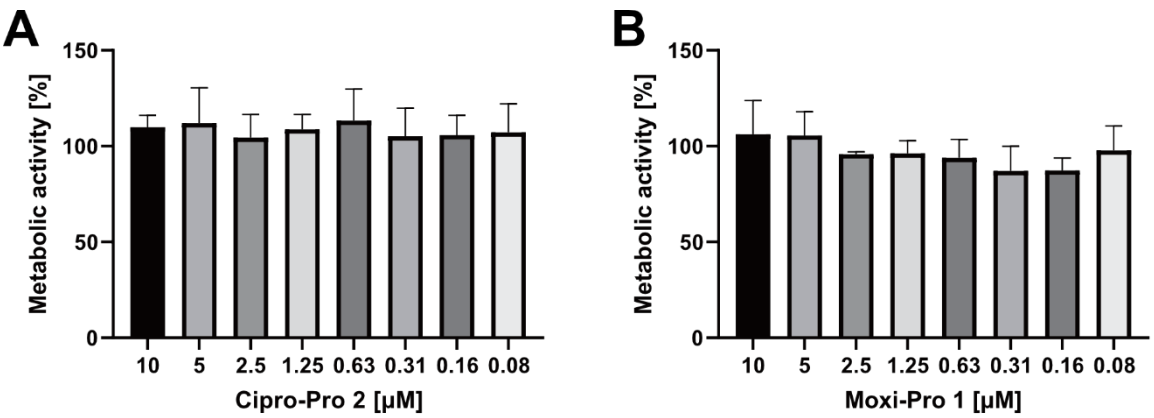
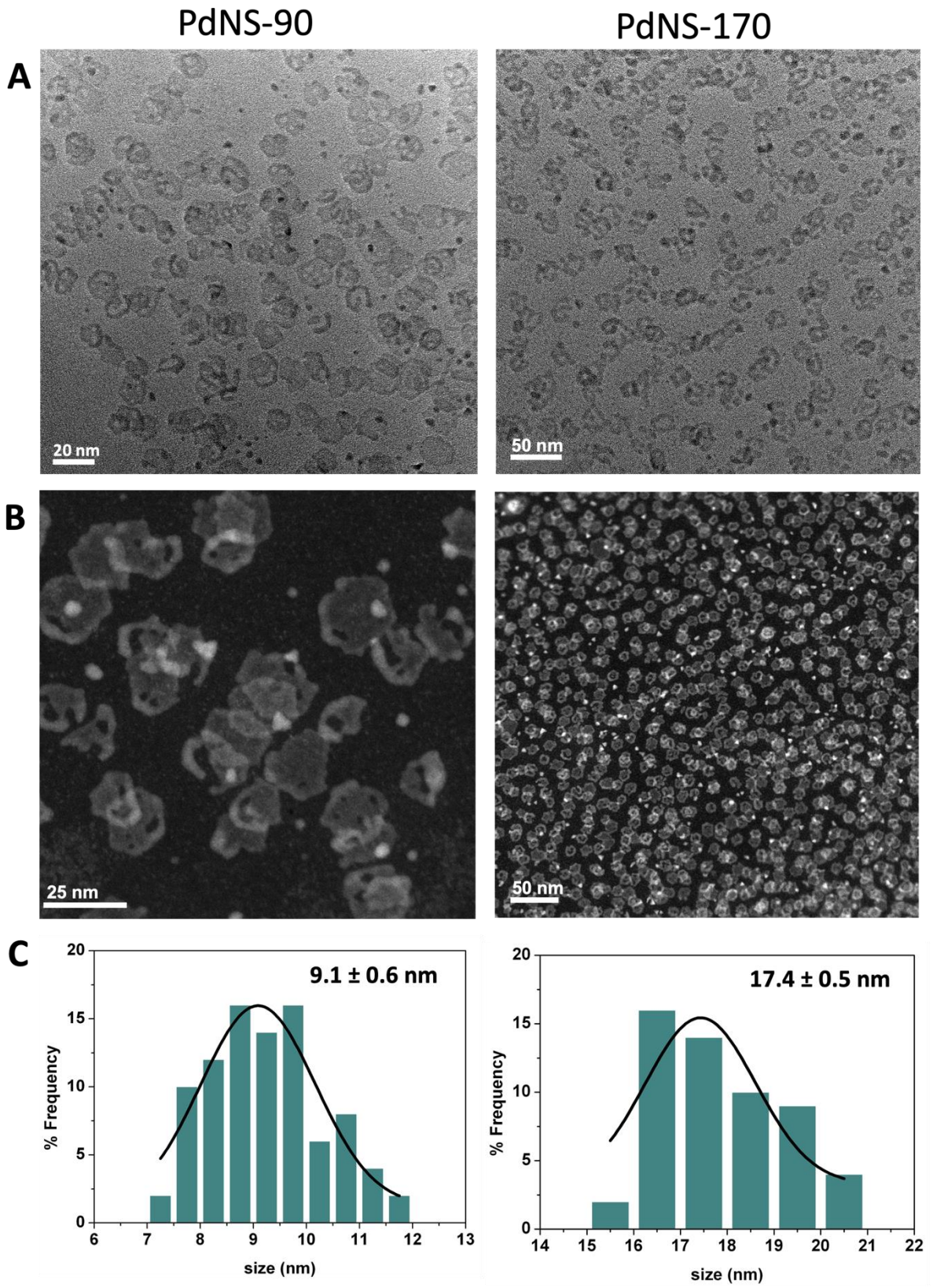


Figure S1. Prodrug toxicity on HepG2 cells determined by MTT assay after 24 h of compound treatment in FCS-free DMEM medium. (A) Toxicity of Cipro-Pro 2. (B) Toxicity of Moxi-Pro 1. Experiments were performed in two biological replicates, with three technical replicates each, which were in qualitative agreement. Error bars: ±SD from n = 3.



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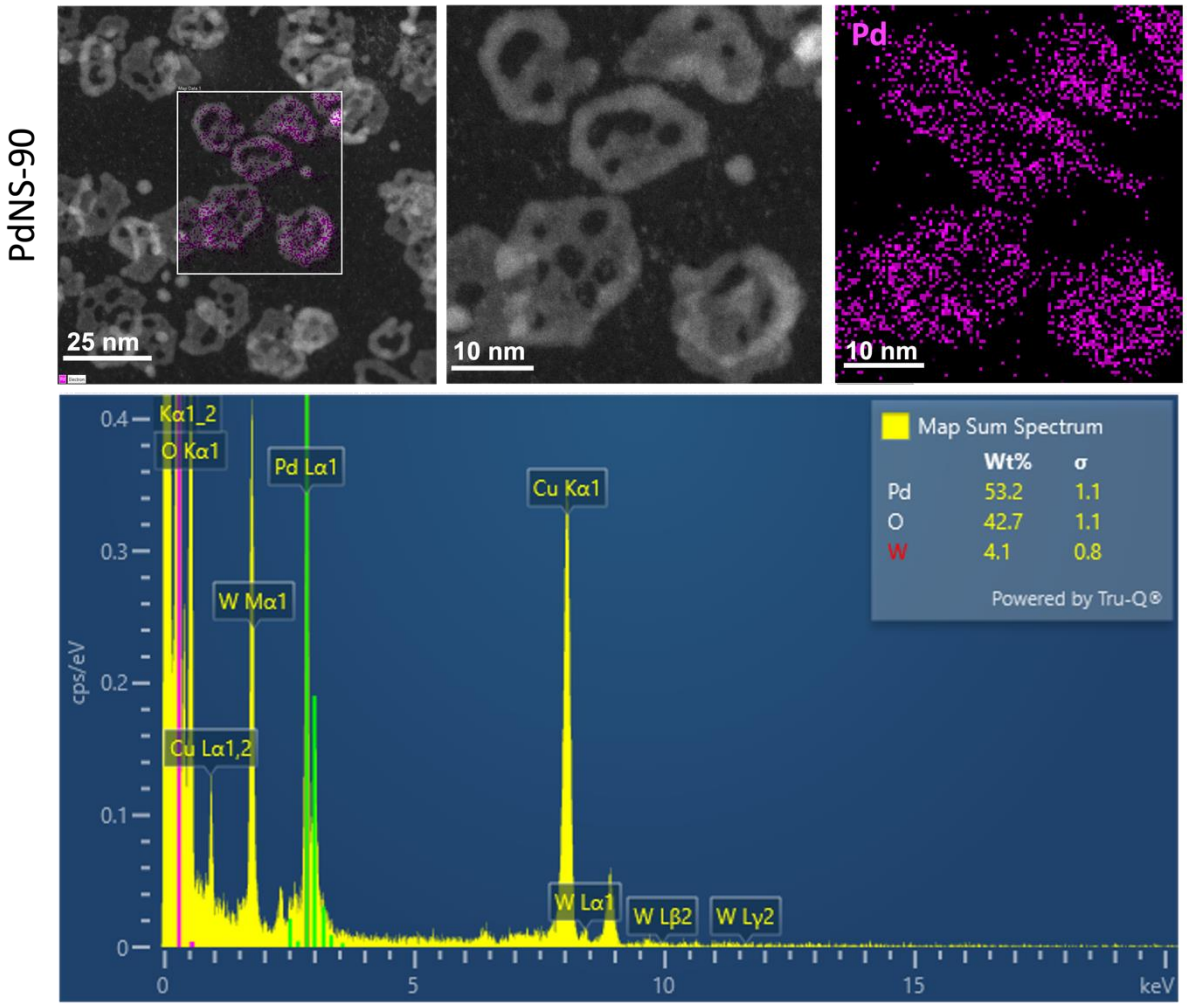

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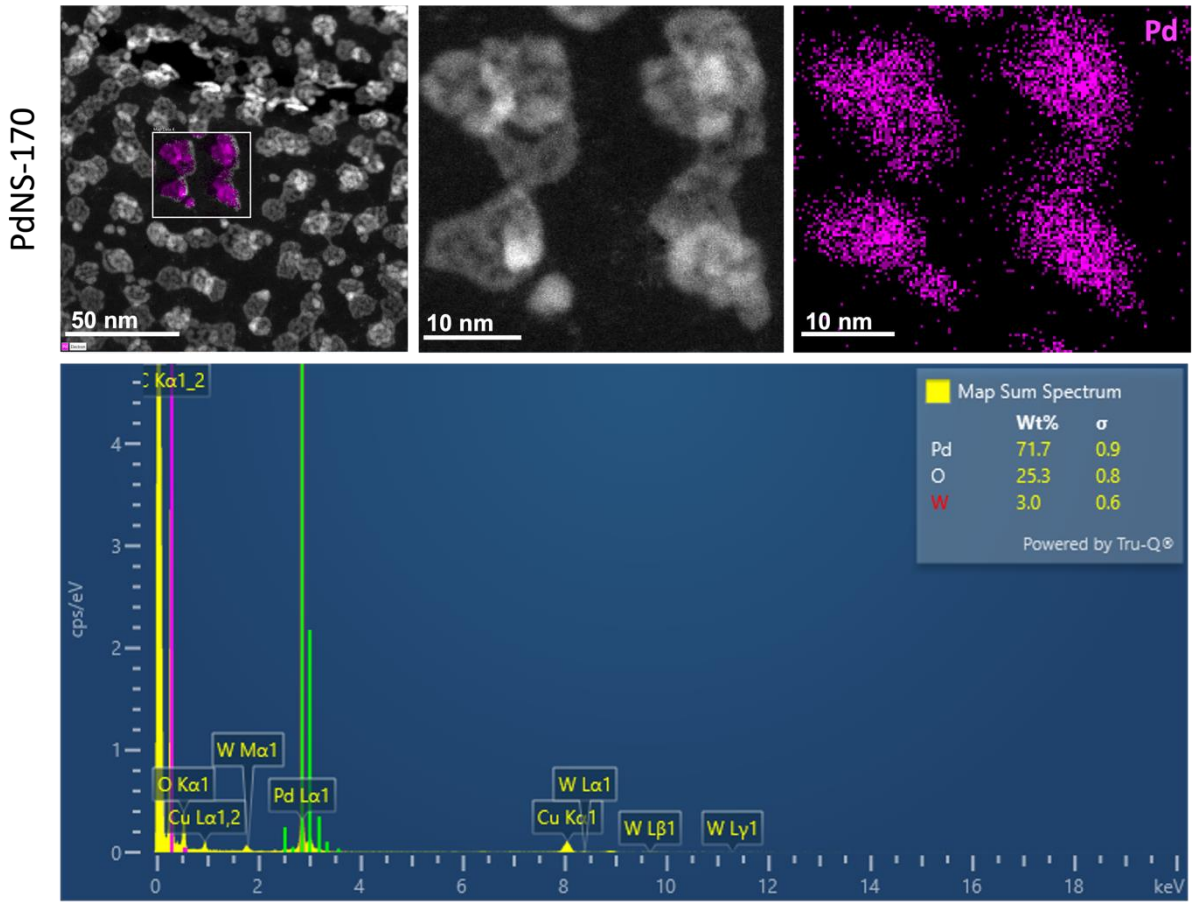

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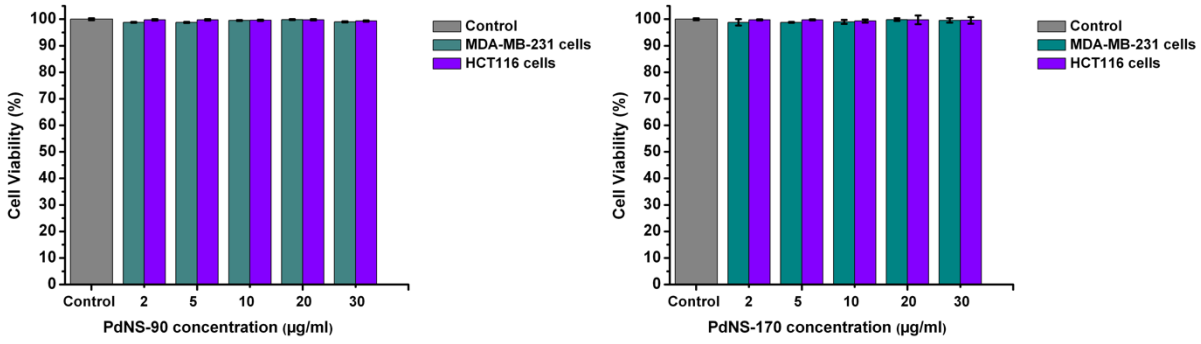

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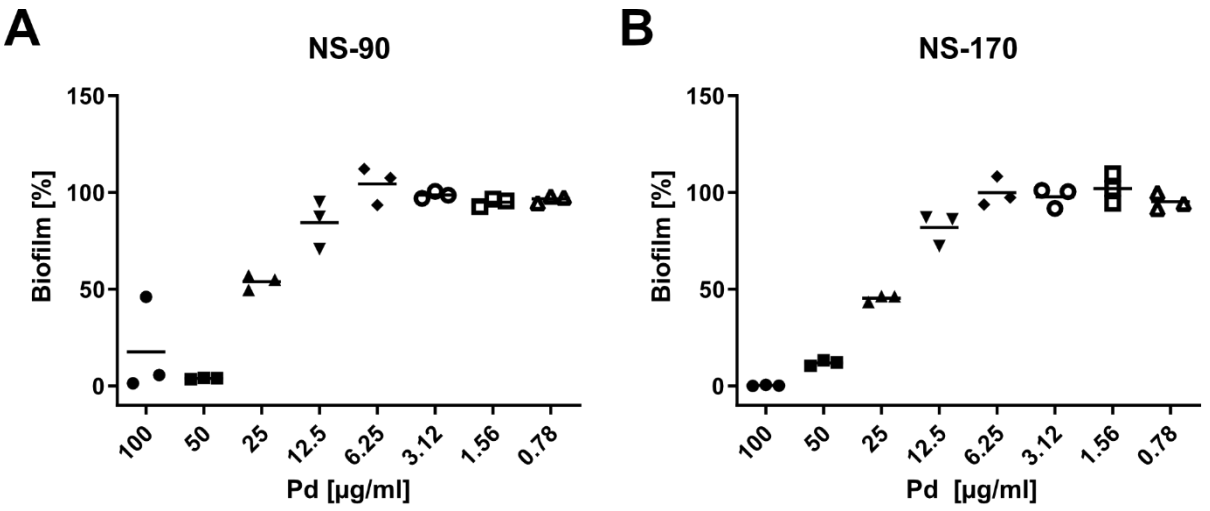

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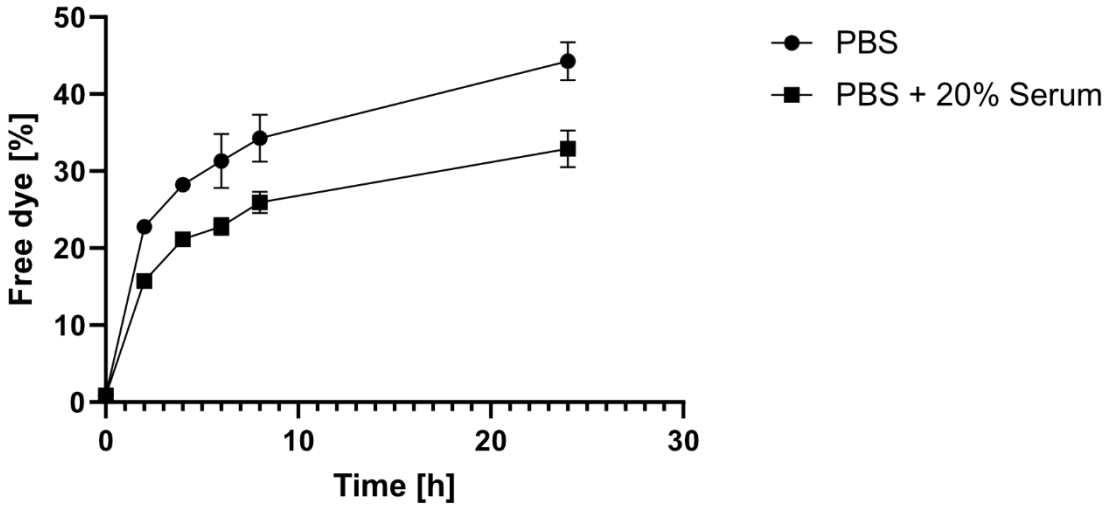

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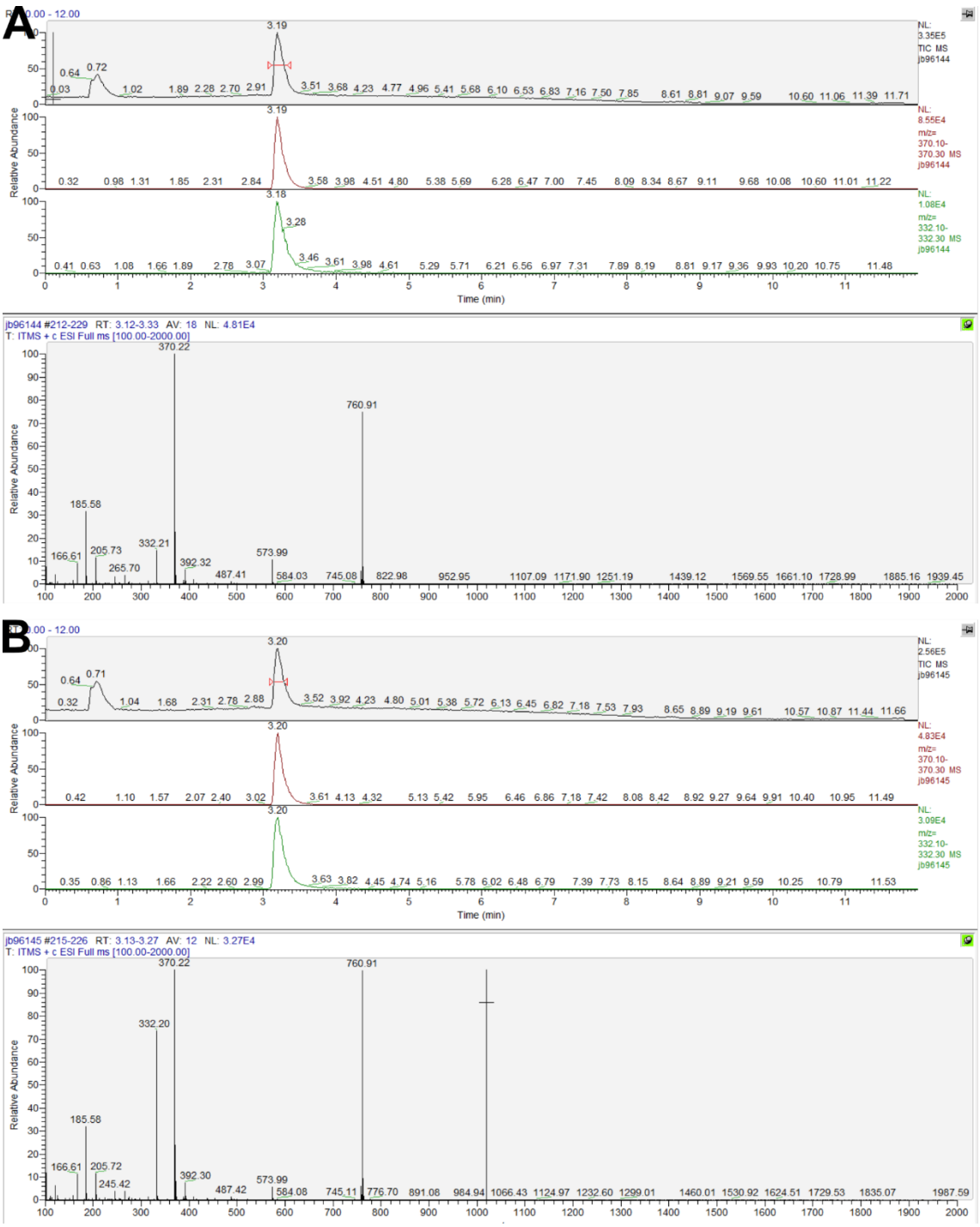

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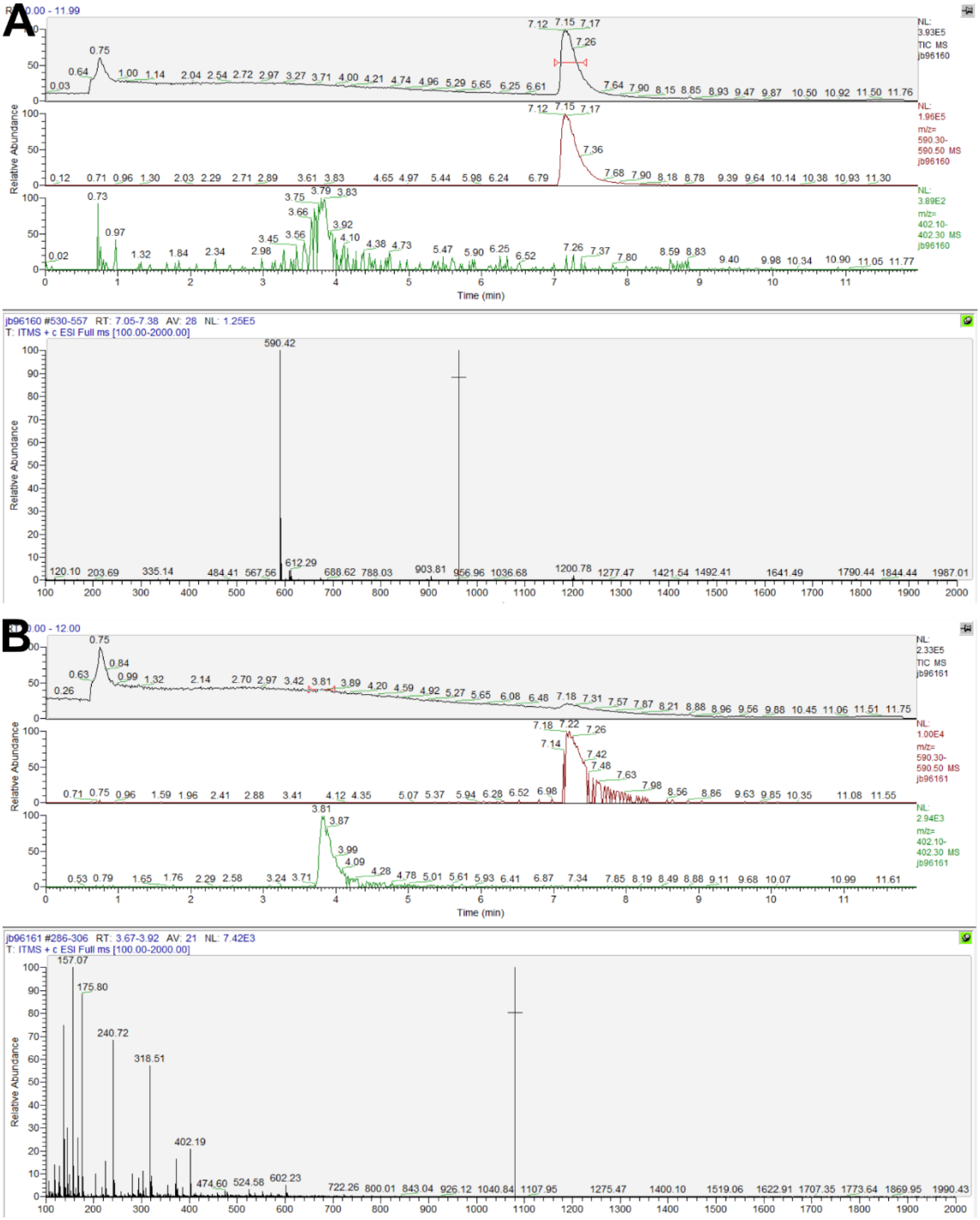

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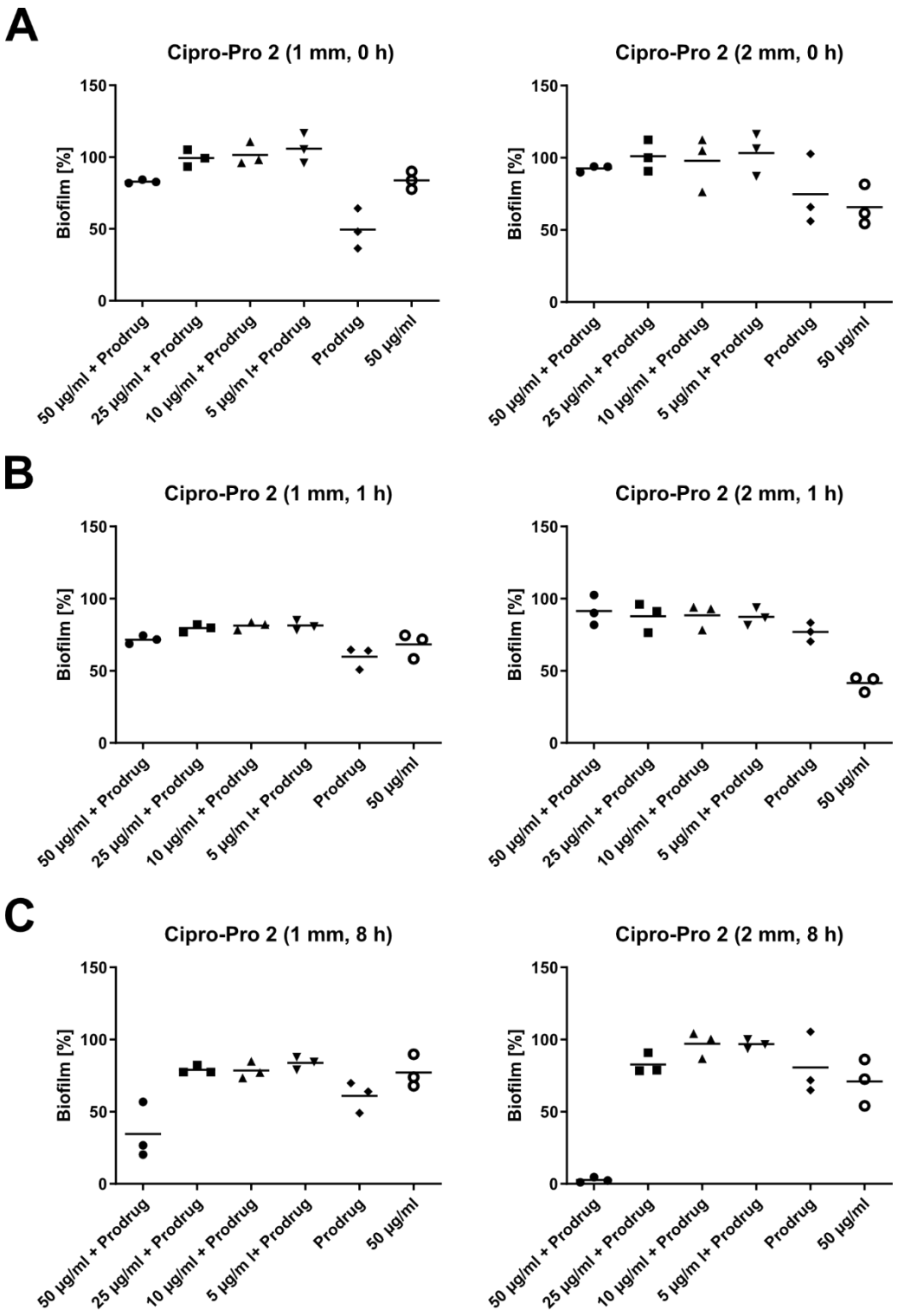

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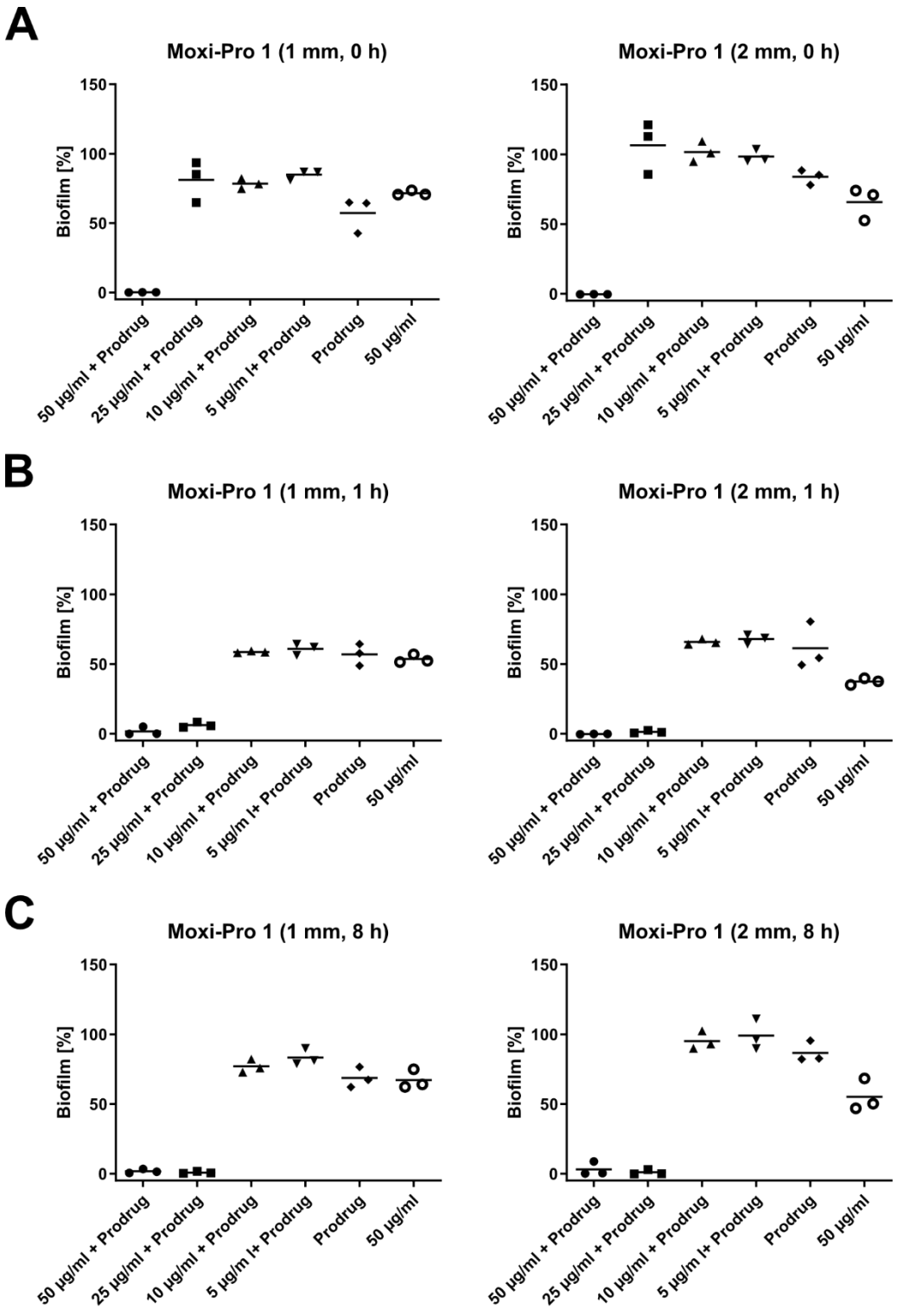


Figure S11. In vitro activity of agarose hydrogels loaded with different amounts of palladium nanosheets (PdNS-90) and Moxi-Pro 1 (5 µM) in the medium against *S. aureus* SA113 biofilm formation. (A) Biofilm growth on 1 (left) or 2 mm (right) thick hydrogels with no preincubation of prodrug. (B) Biofilm growth on 1 (left) or 2 mm (right) thick hydrogels with 1 h of preincubation of prodrug. (C) Biofilm growth on 1 (left) or 2 mm (right) thick hydrogels with 8 h of preincubation of prodrug. Experiments were performed in two biological replicates, with three technical replicates each, which were in qualitative agreement.

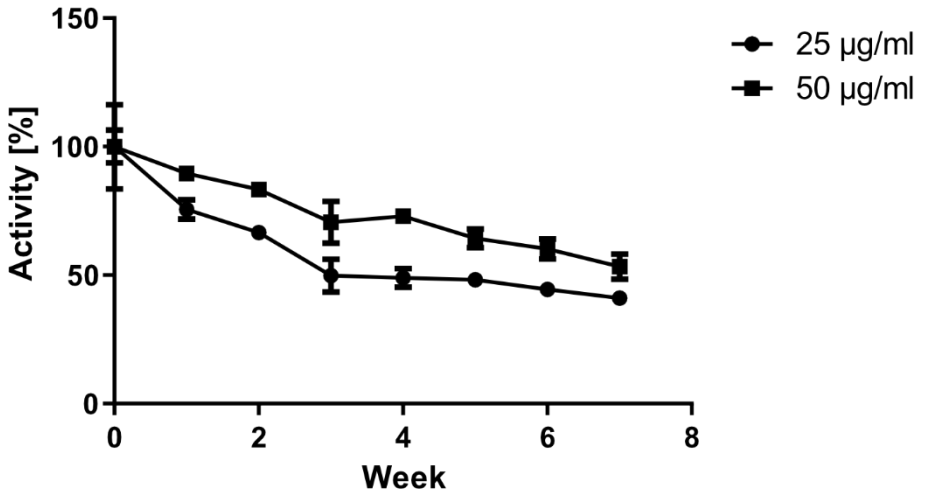


Figure S12. Longterm stability of the catalytic activity of PdNS-90 nanosheets in agarose hydrogels. Catalytic activity normalized to the initial activity. Error bars: ±SD from *n* = 3.

2. Supplementary Tables

Table S1. Bacterial strains and media

Species	Strain	Medium
Escherichia coli	UTI89	LB
Staphylococcus aureus	SA133	CASO

Table S2. Composition of the used media

Medium	Composition
LB	10.0 g peptone ex casein 5.00 g NaCl 5.00 g yeast extract in 1 l ddH_2O, pH = 7.5
CASO	17.0 g peptone ex casein 3.00 g peptone ex soybean 2.50 g K_2HPO_4 5.00 g NaCl 2.50 g glucose in 1 l ddH_2O, pH = 7.3

3. Methods

3.1 Compounds

Stocks of Ciprofloxacin, Moxifloxacin and the prodrug Cipro-Pro 2 were made with 0.1 M HCl_{aq}. Stocks of prodrug Cipro-Pro 1, Moxi-Pro 1 and compound **1** were made with DMSO.

3.2. Bacteria Based Assay

Caution: All bacterial strains used are risk group 2.

3.2.1. Minimal Inhibitory Concentration (MIC) Assay

The MICs were determined by the broth dilution method. Overnight cultures were diluted 1:10 000. Various dilutions of the compounds were prepared and 2 µl were added to 48 µl medium in a 96 well plate (transparent Nunc 96-well flat bottom, Thermo Fisher Scientific). A growth control, containing just vehicle, was included. To the wells containing compound or the growth control 50 µl of the diluted overnight culture were added (Final vehicle conc. 1%). A sterile control, containing only 100 µl medium, was included. The plates were incubated for 24 h at 37 °C and 200 rpm and the OD600 was measured on a microplate reader (Infinite® M Nano+, Tecan). The lowest concentration at which no bacterial growth could be observed was defined as the minimal inhibitory concentration (MIC). MIC values were determined in two experiments with three technical replicates each.

3.2.2. Minimal Biofilm Inhibitory Concentration (MBIC) Assay

Overnight cultures of *S. aureus* SA113 were diluted 1:100. Various dilutions of the compounds were prepared and 2 µl were added to 98 µl medium in a 96 well plate (transparent Nunc 96-well flat bottom, Thermo Fisher Scientific). A growth control, containing just vehicle, was included. To the wells containing compound or the growth control 100 µl of the diluted overnight culture were added (Final vehicle conc. 1%). A sterile control, containing only 200 µl medium, was included. The plates were incubated for 24 h at 37 °C without shaking. The supernatant was carefully removed and each biofilm was washed with 100 µl PBS. The biofilms were dried at 37 °C overnight and 50 µl crystal violet (1% in ddH_2O) were added. After 10 min incubation at room temperature the supernatant was removed and the biofilms were rinsed twice with 200 µl ddH_2O. The remaining crystal violet was dissolved in 10% acetic acid and the absorbance at 595 nm of the solution was determined with a microplate reader (Infinite® M Nano+, Tecan). The lowest concentration at which no biofilm growth could be observed was defined as the minimal biofilm inhibitory concentration (MBIC). MBIC values were determined in two experiments with three technical replicates each.

3.2.3. Minimal Biofilm Inhibitory Concentration of Pd-nanosheets

An overnight culture of *S. aureus* SA113 was diluted 1:100. To 50 µl of various dilutions of Pd-nanosheets in water in a 96 well plate (transparent Nunc 96-well flat bottom, Thermo Fisher Scientific) 50 µl of the diluted overnight culture were added. The plates were incubated at 37 °C, 200 rpm for 24 h. The medium was carefully aspirated and the biofilms were carefully rinsed with 200 µl PBS. 100 µl PBS and 20 µl CellTiter-Blue™ (Promega) were added to each well and the plates were incubated for 1 h at 37 °C. 100 µl were transferred into a new 96 well plate (black pure Grade™ 96-well flat bottom, Brand). Fluorescence (Ex: 560 nm, Em: 590 nm) was determined with a microplate reader (Infinite® M Nano+, Tecan). A growth control, containing just vehicle, and a negative growth control, containing just vehicle and no bacteria, were included and used to normalize the results. The experiment was conducted twice with three technical replicates each.

3.2.4 Prevention of Biofilms by Pd-labile Prodrugs

Agarose (for DNA electrophoresis, SERVA) hydrogels (20 mg/ml) with PdNS-90 nanosheets (5 – 50 µg/ml) were poured in a 96 well plate (transparent pure Grade™ 96-well flat bottom, Brand) to a thickness of 1 or 2 mm and allowed to solidify overnight at room temperature. The plates were sterilized by UV-radiation (1 h) and 50 µl of 5 µM Prodrug in CASO-Medium (1% vehicle) was added. The plates were incubated for 0, 1 or 8 h at 37 °C. An overnight culture of *S. aureus* SA113 was diluted 1:100 with CASO-medium and 50 µl were added to each well. The plates were incubated for 24 h at 37 °C, before the medium was carefully aspirated. The biofilms were washed with 200 µl PBS and 100 µl PBS and 20 µl CellTiter-Blue™ (Promega) were added to each well. The plates were incubated for 1 h at 37 °C and 100 µl were transferred into a new 96 well plate (black pure Grade™ 96-well flat bottom, Brand). Fluorescence (Ex: 560 nm, Em: 590 nm) was determined with a microplate reader (Infinite® M Nano+, Tecan). Controls containing only Pd-nanosheets or prodrug were included. A growth control, containing just vehicle, and a negative growth control, containing just vehicle and no bacteria, were included and used to normalize the results. Inhibition of biofilm growth was determined in two experiments with three technical replicates each.

3.3 Cell Culture

3.3.1 General

HepG2 cells were obtained from DSMZ and cultured in Dulbecco´s Modified Eagle´s Medium high glucose (DMEM) supplemented with heat-inactivated 10% (v/v) fetal bovine serum (FBS, Sigma-Aldrich) and 2 mM L-glutamine (Sigma-Aldrich). Cells were grown at 37 °C and 5% CO_2.

Human breast adenocarcinoma MDA-MB-231 cells, and human colon carcinoma HCT116 cells (purchased from ECACC) were cultured in culture media supplemented with serum (10 % of FBS) and L-glutamine (2 mM). HCT116 cells were cultured in McCoy's 5A medium, and MDA-MB-231 cells were cultured in DMEM media supplemented both with serum (10 % of FBS) and L-glutamine (2 mM). Each cell line was checked for mycoplasma before use and maintained in normoxic conditions at 37 ºC and 5% CO_2.

3.3.2 Study of the biocompatibility of Pd nanosheets

The tolerability of cells to PdNS-90 and PdNS-170 were tested by performing dose-response studies in HCT116 and MDA-MB-231 cells. Cells were seeded in a 96-well plate at a density of 1.500 cells/well for MDA-MB-231 cells, and 3.000 cell/wells for HCT116 cells; then incubated for 24 h before treatment. Each well was then replaced with 100 µL of fresh media containing PdNS-90 or PdNS-170 at 2, 5, 10, 20 and 30 µg/mL for all cells. After 1 week, PrestoBlue™ cell viability reagent (10 % *v*/*v*) was added to each well and the plate incubated for 90 min. Fluorescence emission was detected using a PerkinElmer EnVision 2101 multilabel reader (Ex/Em: 540/590 nm). Experiments were performed in triplicate. All conditions were normalised to the untreated cells (100 %).

3.3.3 Compound Toxicity Assay

The MTT-Assay was used to determine compound toxicity. HepG2 cells (12000 in 200 µl medium per well) were seeded into a 96-well plate (BioLite™, Thermo Fisher Scientific) and were grown for 1 d at 37 °C and 5%. The medium was aspirated and 100 µl of FCS-free medium with various concentration of the compound (1% final conc. of vehicle) or vehicle (1% final conc.) were added. The cells were incubated with the compound or controls for 24 h and 20 µl of thiazolyl blue tetrazolium bromide (MTT, 5 mg/ml in PBS, Sigma-Aldrich) were added. The plates were incubated for 3 h at 37 °C, 5% CO_2 and 80 µl of supernatant was carefully

removed. The formazan crystals were dissolved in 200 µl of DMSO and the absorbance at 570 nm was measured on a microplate reader (Infinite® M Nano+, Tecan) and the background at 630 nm was subtracted. Metabolic activity was normalized to the vehicle control. Metabolic activity was determined in two experiments with three technical replicates each.

3.4 Conversion Studies

3.4.1 Fluorophore Activation Studies

Per time point 100 µl of 100 µM compound **1** in PBS or PBS + 20% mouse serum (Sigma-Aldrich) were incubated with 5 µg/ml Pd-nanosheets at 37 °C and 200 rpm (Final DMSO conc. 1%). Time point t=0 never contained any Pd. At the respective time points samples were spun down (13 000 rpm, 15 min) and 75 µl of the supernatant were transferred to a 96 well plate (black pure Grade™ 96-well flat bottom, Brand). Fluorescence (Ex: 350 nm, Em: 450 nm) was determined with a microplate reader (Infinite® M Nano+, Tecan). An external calibration curve was used to quantify the conversion. All time points were done in technical triplicates.

3.4.2 MS-based Prodrug Conversion Studies

Per time point 100 µl of 100 µM of the respective prodrug in PBS were incubated with 5 µg/ml PdNS-90 at 37 °C and 200 rpm (Final vehicle conc. 1%). Time point t=0 never contained any Pd. At the respective time points samples were spun down (13 000 rpm, 15 min) and 75 µl of the supernatant were transferred into MS-vials. The samples were measured on a LCQ-Fleet (Thermo Fisher Scientific) coupled to an UltiMate 3000 HPLC (Thermo Fisher Scientific). An external calibration curve of the prodrug was used to quantify the conversion.

3.4.3 Longterm Stability Assay

Agarose (for DNA electrophoresis, SERVA) hydrogels (20 mg/ml) with PdNS-90 nanosheets (25 or 50 µg/ml) were poured in a 96 well plate (transparent pure Grade™ 96-well flat bottom, Brand) to a thickness of 1 mm and allowed to solidify overnight. The plates were sterilized by UV-radiation (1 h) and 200 µl PBS were added. The plates were incubated at 37 °C for various intervals. Each week the PBS was aspirated and 200 µl of fresh PBS was added. For the wells of the respective time point the PBS was removed again and replaced by 100 µM 100 µM compound **1** in PBS (1% DMSO). The plates were incubated at 37 °C and 50 µl of the compound **1** solution were transferred to a new 96 well plate (black pure Grade™ 96-well flat bottom, Brand). Fluorescence (Ex: 350 nm, Em: 450 nm) was determined with a microplate reader (Infinite® M Nano+, Tecan). An external calibration curve was used to quantify the conversion. All time points were done in technical triplicates.

4. Synthesis and Characterization of Pd Nanosheets

4.1 Chemical Reagents

Palladium (II) acetylacetonate ($Pd(acac)_2$, 99%), poly-(vinylpyrrolidone) (PVP, MW=29000), citric acid (CA, 99.5 %,), cetyltrimethylammonium bromide (CTAB, 98%), and N,N-dimethylformamide (DMF, anhydrous, 99.8%) were all purchased form Sigma-Aldrich. Tungsten hexacarbonyl ($W(CO)_6$, 97%) was purchased from Acros Organic. The Milli-Q water obtained from the Milli-Q System was used in all experiments. All the chemicals were used as received without further purification.

4.2 Synthesis of Pd Nanosheets of different Sizes

Pd nanosheets were prepared as previously described[1] with slight modifications. To obtain Pd nanosheets with an average edge length of 17.4 nm, 16 mg of $Pd(acac)_2$, 90 mg of CA, 60 mg of CTAB, and 30 mg of PVP successively in 5 min addition intervals were dissolved in DMF (10 mL total volume) and stirred in a 50 mL three-neck round-bottom flask for 1 h. N_2 was bubbled into the solution to remove dissolved oxygen. Then, a certain amount (100 mg) of $W(CO)_6$ were added into the homogeneous orange-red solution. Subsequently, the flask was

capped placed into a hot oil bath at 80 °C. The temperature was maintained at 80 °C for 1 h, as was the N_2 flow in the reaction. After the reaction, the Pd nanosheets were isolated by centrifugation using a sufficient amount of acetone (15 mL), and then re-dispersed in dH_2O. This process was repeated three times, at 7500 rpm for 5 min. To achieve Pd nanosheets with edge lengths of 17.4 (labelled as PdNS-90) to 9.1 nm (labelled as PdNS-170), the amount of CA fed in the synthesis was increased from 90 to 170 mg, respectively, with all other parameters being the same as in the aforementioned procedure.

4.3 Morphological, structural and elemental Characterization

The optical properties of Pd NSs were analysed by a NanoDrop™ 2000c spectrophotometer (Thermo Scientific™). Transmission electron microscopy (TEM) images were obtained using a Titan (Thermofisher Scientic, formerly FEI) with a Field Emission Gun operating at 300 kV. The microscope spherical aberration corrector (CESCOR-CEOS) allows a point resolution of 0.8 Å. The microscope is fitted with a High-Angle Annular Dark Field (HAADF) detector (Fischione) to operate in Scanning Transmission Electron Microscopy (STEM) mode with Z-contrast imaging.

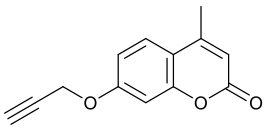
5. Synthetic Procedures

5.1 General Methods and Materials

All reagents and solvents were purchased in reagent grade or higher from commercial vendors (Sigma-Aldrich, Thermo Fisher Scientific Inc., Merck KGaA, Alfa Aesar, Roth, VWR International, Acros Chemicals) and were used as delivered without further purification. All air and/or water sensitive reactions were conducted under argon atmosphere using flame dried glassware using standard Schlenk-techniques. Merck silica-gel 60 F254 plates were used for analytical thin-layer chromatography (TLC). The spots were visualized using short wave UV light (λ=254 nm and 366 nm) or a $KMnO_4$-stain (1.50 g $KMnO_4$, 10.0 g K_2CO_3, 1.25 mL $NaOH_{aq}$ (10 wt-%), 200 mL ddH_2O). Flash chromatography was performed using Silica gel 60 (particle size = 40–63 µM) from Merck KGaA with compressed air. Proton-NMR spectra were recorded on Avance-III (AV-HD300, AV-HD400 or AV-HD500) NMR systems (Bruker Co.) at room temperature in deuterated Chloroform ($CDCl_3$) or Dimethylsulfoxid (DMSO-d_6). Spectra were referenced to the residual proton signal of the corresponding deuterated solvent ($CDCl_3$: δ = 7.26 ppm, DMSO-d_6: δ = 2.50 ppm). Chemical shifts are reported in parts per million (ppm). Coupling constants (*J*) are reported in hertz (Hz). For the assignment of multiplicity to the signals the following abbreviations were used: virt. = vitual, s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet or unresolved. ^{13}C-NMR spectra were collected on Avance-III (AV-HD300, AV-HD400) NMR systems (Bruker Co.) at 75, 101 MHz with $CDCl_3$ or DMSO-d_6 as solvents. Chemical shifts were referenced to the residual solvent peak as an internal standard ($CDCl_3$: δ = 77.16 ppm, DMSO-d_6: δ = 39.52 ppm). High resolution mass spectra were recorded using an LTQ-FT Ultra (Thermo Fisher Scientific) coupled with a Dionex UltiMate 3000 HPLC system and an ESI or APCI ion source.

5.2 Synthesis

O-Propargyl-4-methyl-umbelliferone (1)



Chemical Formula: $C_{13}H_{10}O_3$
Molecular Weight: 214.22 g/mol

O-Propargyl-4-methyl-umbelliferone was synthesized as previously reported.[2]

4-Methyl-umbelliferone (1.00 g, 5.68mmol, 1.0 eq) was dissolved in acetone (20 ml) and K_2CO_3 (1.57 g, 11.4 mmol, 2.0 eq) was added. Propargyl bromide (860 µl, 1.35 g, 11.4 mmol, 2.0 eq) was added dropwise. The reaction was heated to 50 °C and stirred at this temperature overnight. The reaction was allowed to reach room temperature and the volatile components were removed under reduced pressure. Water (100 ml) was added to the residue and the resulting solid was filtered of. Purification by recrystallization from ethanol (20 ml) yielded the desired product (917 mg, 4.26 mmol, 75%) as an off white solid.

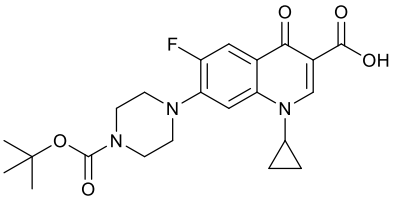
HRMS ESI calcd. for $C_{13}H_{11}O_3$ $[M+H^+]^+$: 215.0703, found 215.0694.

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 7.66 – 7.41 (m, 1H), 7.00 – 6.81 (m, 2H), 6.16 (q, *J* = 1.2 Hz, 1H), 4.76 (d, *J* = 2.4 Hz, 2H), 2.57 (t, *J* = 2.4 Hz, 1H), 2.41 (d, *J* = 1.2 Hz, 3H).

^{13}C-NMR (101 MHz, $CDCl_3$): δ [ppm] = 161.2, 160.5, 155.2, 152.5, 125.8, 114.4, 112.9, 112.6, 102.6, 76.6, 56.3, 18.8.

The spectroscopic data is in accordance with the literature.[3]

***N*-Boc-Ciprofloxacin (2)**



Chemical Formula: $C_{22}H_{26}FN_3O_5$
Molecular Weight: 431.46 g/mol

N-Boc-Ciprofloxacin was synthesized as previously reported.[4]

Ciprofloxacin (500 mg, 1.51 mmol, 1.0 eq) was dissolved in dioxane/water (50:50, 9 ml) and $NaOH_{aq}$ (1 M, 2.26 ml, 90.5 mg, 2.26 mmol, 1.5 eq) and Boc anhydride (494 mg, 2.26 mmol, 1.5 eq) were added. The reaction was stirred at room temperature overnight and the volatile components were removed under reduced pressure. Purification by flash chromatography (DCM/MeOH = 95:5) yielded the desired product (486 mg, 1.13 mmol, 75%) as a white solid.

TLC: R_f = 0.39 (DCM/MeOH = 95:5) [UV]

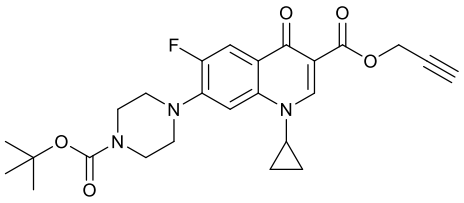
HRMS ESI calcd. for $C_{22}H_{27}FN_3O_5$ $[M+H^+]^+$: 432.1924, found 432.1926.

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 14.93 (s, 1H), 8.78 (s, 1H), 8.05 (d, *J* = 12., 1H), 7.36 (d, *J* = 7.1 Hz, 1H), 3.69 – 3.65 (m, 4H), 3.60 – 3.48 (m, 1H), 3.34 – 3.26 (m, 4H), 1.50 (s, 9H), 1.43 – 1.37 (m, 2H), 1.24 – 1.18 (m, 2H).

^{13}C-NMR (101 MHz, $CDCl_3$): δ [ppm] = 167.1, 154.7, 147.7, 113.0, 112.7, 108.5, 105.1, 80.5, 35.4, 28.6, 8.4.

The spectroscopic data is in accordance with the literature.[4]

***N*-Boc-*O*-Propargyl-Ciprofloxacin (3)**



Chemical Formula: $C_{25}H_{28}FN_3O_5$
Molecular Weight: 469.51 g/mol

N-Boc-*O*-Propargyl-Ciprofloxacin was synthesized analogous to previous reports.[5]

Compound **2** (468 mg, 1.08 mmol, 1.0 eq) was dissolved in dry DCM (15 ml). HBTU (514 mg, 1.36 mmol, 1.3 eq), DMAP (13.5 mg, 108 µmol, 0.1 eq), trimethylamine (166 µl, 121 mg, 1.19 mmol, 1.1 eq) and propargyl alcohol (256 µl, 243 mg, 4.34 mmol, 4.0 eq) were added sequentially. The reaction was stirred at room temperature overnight and the volatile components were removed under reduced pressure. The crude product was purified by flash chromatography (DCM/MeOH = 97:3) to yielded the desired product (208 mg, 443 µmol, 41%) as a white solid.

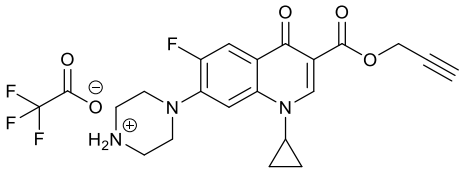
TLC: R_f = 0.26 (DCM/MeOH = 95:5) [UV]

HRMS ESI calcd. for $C_{25}H_{29}FN_3O_5$ $[M+H^+]^+$: 470.2086, found 470.2082.

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 8.55 (s, 1H), 8.02 (d, *J* = 13.1 Hz, 1H), 7.27 (s, 1H), 4.92 (d, *J* = 2.4 Hz, 2H), 3.69 – 3.61 (m, 4H), 3.51 – 3.38 (m, 1H), 3.24 – 3.17 (m, 4H), 2.49 (t, *J* = 2.4 Hz, 1H), 1.50 (s, 9H), 1.38 – 1.30 (m, 2H), 1.19 – 1.13 (m, 2H).

^{13}C-NMR (101 MHz, $CDCl_3$): δ [ppm] = 173.0, 164.8, 154.8, 154.8, 152.3, 148.7, 144.7, 144.6, 138.1, 123.4, 123.4, 113.7, 113.5, 109.6, 105.2, 105.2, 80.4, 78.3, 74.9, 52.3, 34.8, 28.6, 8.3.

O-Propargyl-Ciprofloxacin trifluoroacetate (Cipro-Pro 2)



Chemical Formula: $C_{22}H_{21}F_4N_3O_5$
Molecular Weight: 483.42 g/mol

O-Propargyl-Ciprofloxacin trifluoroacetate was synthesized analogous to previous reports.[5]

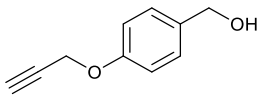
Compound **3** (200 mg, 426 µmol, 1.0 eq) was dissolved in a mixture of DCM (6 ml) and trifluoroacetic acid (3 ml) and the solution was stirred at room temperature for 2 h. Diethylether (20 ml) was added and the product (96.5 mg, 200 µmol, 47%) was filtered of as a white solid.

HRMS ESI calcd. for $C_{20}H_{21}FN_3O_3$ $[M+H^+]^+$: 370.1561, found 370.1559.

^{1}H-NMR (500 MHz, DMSO-d_6): δ [ppm] = 8.91 (br s, 2H), 8.48 (s, 1H), 7.81 (d, *J* = 13.1 Hz, 1H), 7.49 (d, *J* = 7.4 Hz, 1H), 4.84 (d, *J* = 2.4 Hz, 2H), 3.72 – 3.66 (m, 1H), 3.57 (t, *J* = 2.4 Hz, 1H), 3.33 (s, 4H), 1.31 – 1.21 (m, 2H), 1.14 – 1.07 (m, 2H).

^{13}C-NMR (126 MHz, DMSO): δ [ppm] = 171.5, 163.6, 158.2, 157.9, 153.6, 151.6, 148.9, 142.9, 142.8, 138.1, 122.6, 112.0, 111.8, 108.3, 107.0, 79.0, 77.6, 51.5, 46.7, 42.8, 35.1, 7.7.

(4-(Prop-2-yn-1-yloxy)phenyl)methanol (4)



Chemical Formula: $C_{10}H_{10}O_2$
Molecular Weight: 162.19 g/mol

Compound **4** was synthesized as reported in the literature.[6]

4-Hydroxybenzyl alcohol (2.50 g, 20.1 mmol, 1.0 eq) was dissolved in acetonitrile (50 ml) and K_2CO_3 (4.70 g, 34.0 mmol, 1.7 eq) was added. The mixture was stirred for 1 h at room temperature and propargyl bromide (80%, 2.56 ml, 3.21 g, 27.0 mmol, 1.4 eq) was added. The reaction was heated to 80 °C and stirred at this temperature for 2 days. The reaction was allowed to reach room temperature, the solids were filtered off and the volatile components were removed under reduced pressure. Purification by flash chromatography (Hex/EtOAc = 1:0 → 1:1) yielded the desired product (2.72 g, 16.7 mmol, 83%) as a yellow oil.

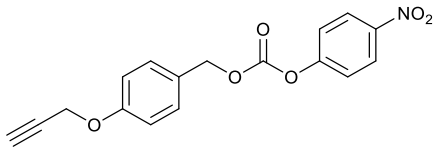
TLC: R_f = 0.35 (Hex/EtOAc = 2:1) [UV]

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 7.55 – 7.25 (m, 2H), 7.09 – 6.78 (m, 2H), 4.70 (d, *J* = 2.4 Hz, 2H), 4.63 (s, 2H), 2.52 (s, 1H).

^{13}C-NMR (101 MHz, $CDCl_3$): δ [ppm] = 157.3, 134.2, 128.7, 115.2, 78.6, 75.7, 65.1, 56.0.

The spectroscopic data is in accordance with the literature.[6]

4-Nitrophenyl 4-propargyloxybenzyl carbonate (5)



Chemical Formula: $C_{17}H_{13}NO_6$
Molecular Weight: 327.29 g/mol

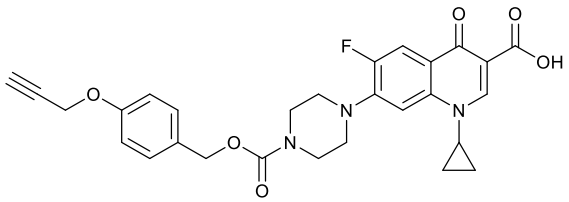
4-Nitrophenyl 4-propargyloxybenzyl carbonate was synthesized as reported in the literature.[6]

(4-(Prop-2-yn-1-yloxy)phenyl)methanol (4, 1.00 g, 6.17 mmol, 1.0 eq) and pyridine (54 µl, 53.7 mg, 678 µmol, 0.1 eq) were dissolved in dichloromethane (20 ml). The solution was cooled to 0 °C and 4-nitrophenyl chloroformate (1.37 g, 6.78 mmol, 1.1 eq) dissolved in dichloromethane (20 ml) was slowly added. The reaction was stirred overnight and allowed to reach room temperature. The volatile components were removed under reduced pressure. The residue was dissolved in ethyl acetate (100 ml) and washed with water (2 × 100 ml) and brine (2 × 100 ml). The organic phase was dried over Na_2SO_4 and the volatile organic compounds were removed under reduced pressure to yield the desired product (1.65 g, 5.04 mmol, 82%) as a white solid.

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 8.27 (d, *J* = 9.1 Hz, 2H), 7.60 – 7.33 (m, 4H), 7.01 (d, *J* = 8.7 Hz, 2H), 5.24 (s, 2H), 4.72 (d, *J* = 2.3 Hz, 2H), 2.53 (t, *J* = 2.3 Hz, 1H).

^{13}C-NMR (101 MHz, $CDCl_3$): δ [ppm] = 158.3, 155.7, 152.6, 130.8, 127.4, 125.4, 121.9, 115.3, 78.4, 75.9, 70.9, 56.0.
The spectroscopic data is in accordance with the literature.[6]

***O*-(4-Propargyloxybenzyl)-*N*-ciprofloxacin carbamate (Cipro-Pro 1)**



Chemical Formula: $C_{28}H_{26}FN_3O_6$
Molecular Weight: 519.53 g/mol

Ciprofloxacin (605 mg, 1.82 mmol, 1.1 eq) and triethylamine (463 µl, 336 mg, 3.32 mmol, 2.0 eq) were dissolved in DMF (40 ml). Compound **5** (543 mg, 1.66 mmol, 1.0 eq), dissolved in DMF (10 ml), was added dropwise. The reaction was stirred at room temperature over the weekend and the volatile components were removed under reduced pressure. Purification by flash chromatography (DCM/MeOH = 97:3) yielded the desired product (457 mg, 880 µmol, 53%) as a white solid.

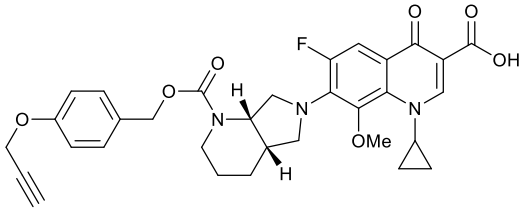
TLC: R_f = 0.22 (DCM/MeOH = 95:5) [UV]

HRMS ESI calcd. for $C_{28}H_{27}FN_3O_6$ $[M+H^+]^+$: 520.1873, found 520.1875.

^{1}H-NMR (500 MHz, DMSO-d_6): δ [ppm] = 8.67 (s, 1H), 7.94 (d, *J* = 13.1 Hz, 1H), 7.59 (d, *J* = 7.5 Hz, 1H), 7.35 (d, *J* = 8.7 Hz, 2H), 6.99 (d, *J* = 8.7 Hz, 2H), 5.05 (s, 2H), 4.80 (d, *J* = 2.4 Hz, 2H), 3.86 – 3.76 (m, 1H), 3.58 (t, *J* = 2.4 Hz, 1H), 1.37 – 1.28 (m, 2H), 1.20 – 1.14 (m, 2H).

^{13}C-NMR (126 MHz, DMSO-d_6): δ [ppm] = 166.0, 157.0, 154.5, 148.2, 129.7, 114.8, 79.3, 78.4, 66.3, 55.40, 49.3, 36.0, 7.7.

***O*-(4-Propargyloxybenzyl)-*N*-moxifloxacin carbamate (Moxi-Pro-1)**



Chemical Formula: $C_{32}H_{32}FN_3O_7$
Molecular Weight: 589.62 g/mol

Moxifloxacin hydrochloride (294 mg, 672 µmol, 1.1 eq) was dissolved in DMF (15 ml) and triethylamine (264 µl, 192 mg, 1.89 mmol, 3.1 eq) was added. Compound **5** (200 mg, 611 µmol, 1.0 eq), dissolved in DMF (3 ml), was added dropwise. The reaction was stirred at room temperature for 4 d and the volatile components were removed under reduced pressure. The crude product was purified by flash chromatography (DCM/MeOH = 1:0 → 98:2) and preparative HPLC (40 → 98%, 17 min) to yield the desired product (136 mg, 226 µmol, 37%) as an off white solid.

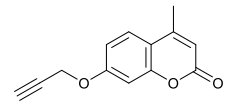
TLC: R_f = 0.15 (DCM/MeOH = 98:2) [UV]

HRMS ESI calcd. for $C_{32}H_{33}FN_3O_7$ $[M+H^+]^+$: 560.2297, found 560.2294.

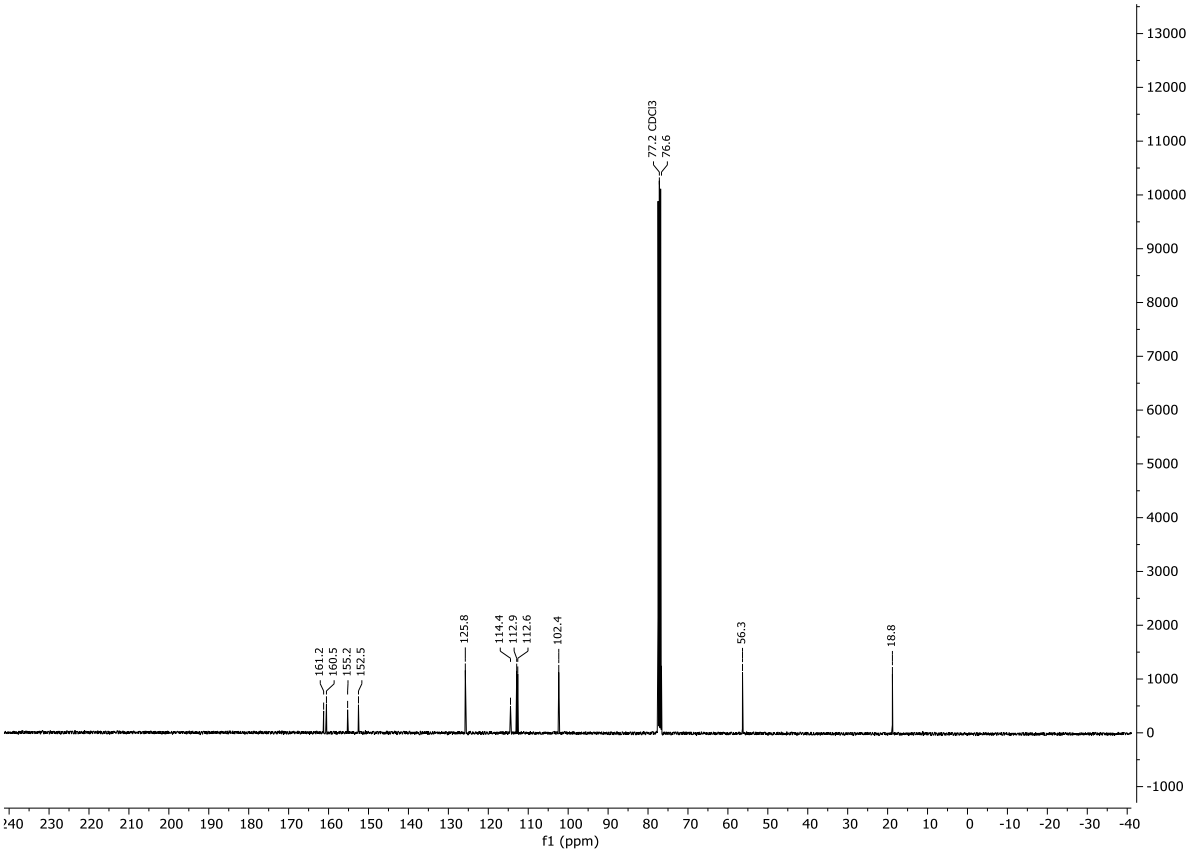
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 8.75 (s, 1H), 7.75 (d, *J* = 13.9 Hz, 1H), 7.32 (d, *J* = 8.7 Hz, 2H), 6.95 (d, *J* = 8.6 Hz, 2H), 5.09 (s, 2H), 4.83 (br s, 1H), 4.68 (d, *J* = 2.4 Hz, 2H), 4.18 – 4.04 (m, 2H), 4.02 – 3.93 (m, 1H), 3.91 – 3.84 (m, 1H), 3.56 (s, 3H), 3.40 (br s, 1H), 3.27 (d, *J* = 10.6 Hz, 1H), 2.94 (t, *J* = 11.6 Hz, 1H), 2.51 (t, *J* = 2.4 Hz, 1H), 2.39 – 2.16 (m, 1H), 1.90 – 1.74 (m, 2H), 1.63 – 1.39 (m, 2H), 1.37 – 1.21 (m, 1H), 1.19 – 0.99 (m, 2H), 0.90 – 0.75 (m, 1H).

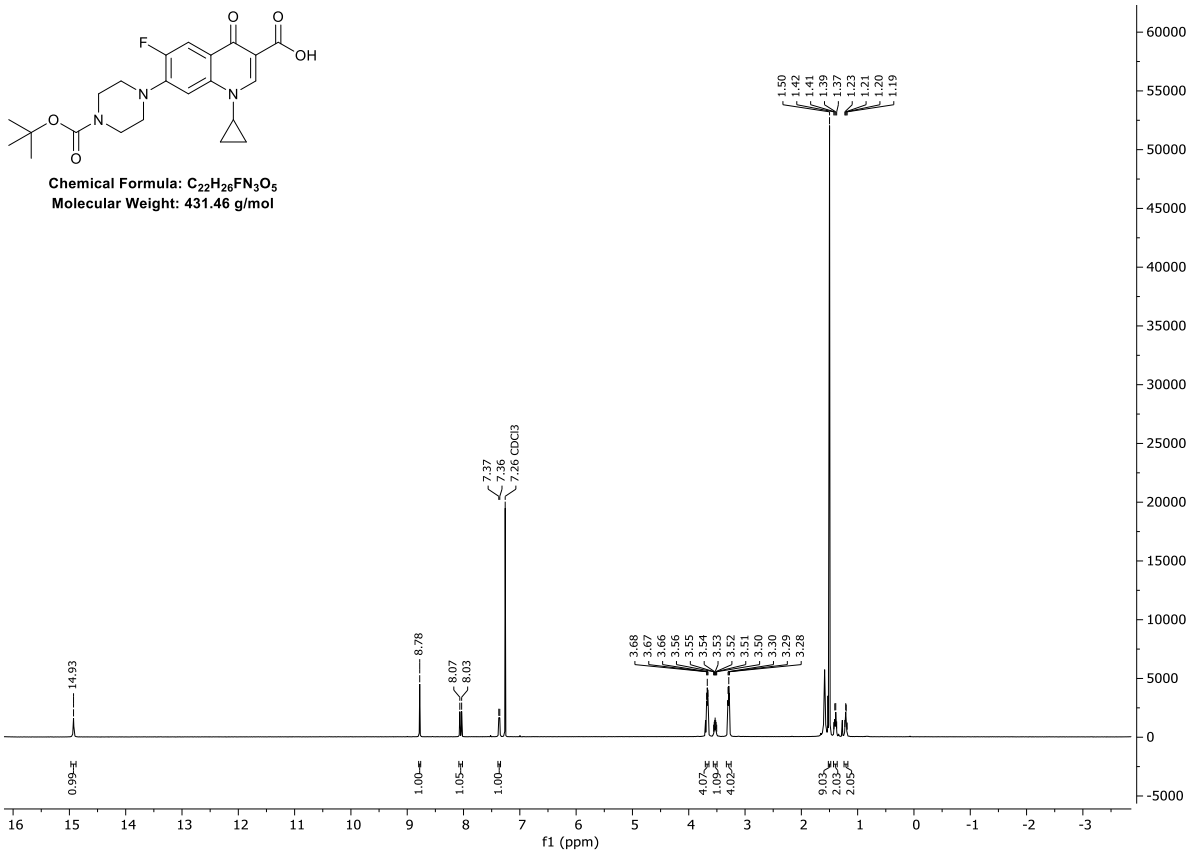
^{13}C-NMR (101 MHz, $CDCl_3$): δ [ppm] = 176.8, 167.1, 157.6, 156.0, 155.0, 152.5, 149.8, 141.0, 137.4, 137.3, 134.5, 130.0, 129.7, 118.8, 115.0, 108.2, 108.0, 107.7, 78.5, 75.8, 67.2, 61.3, 56.6, 56.5, 55.9, 52.7, 40.5, 39.7, 35.6, 25.3, 24.1, 10.7, 8.6.

6. NMR Spectra



Chemical Formula: $C_{13}H_{10}O_3$
Molecular Weight: 214.22 g/mol




Chemical Formula: $C_{22}H_{26}FN_3O_5$
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8.03
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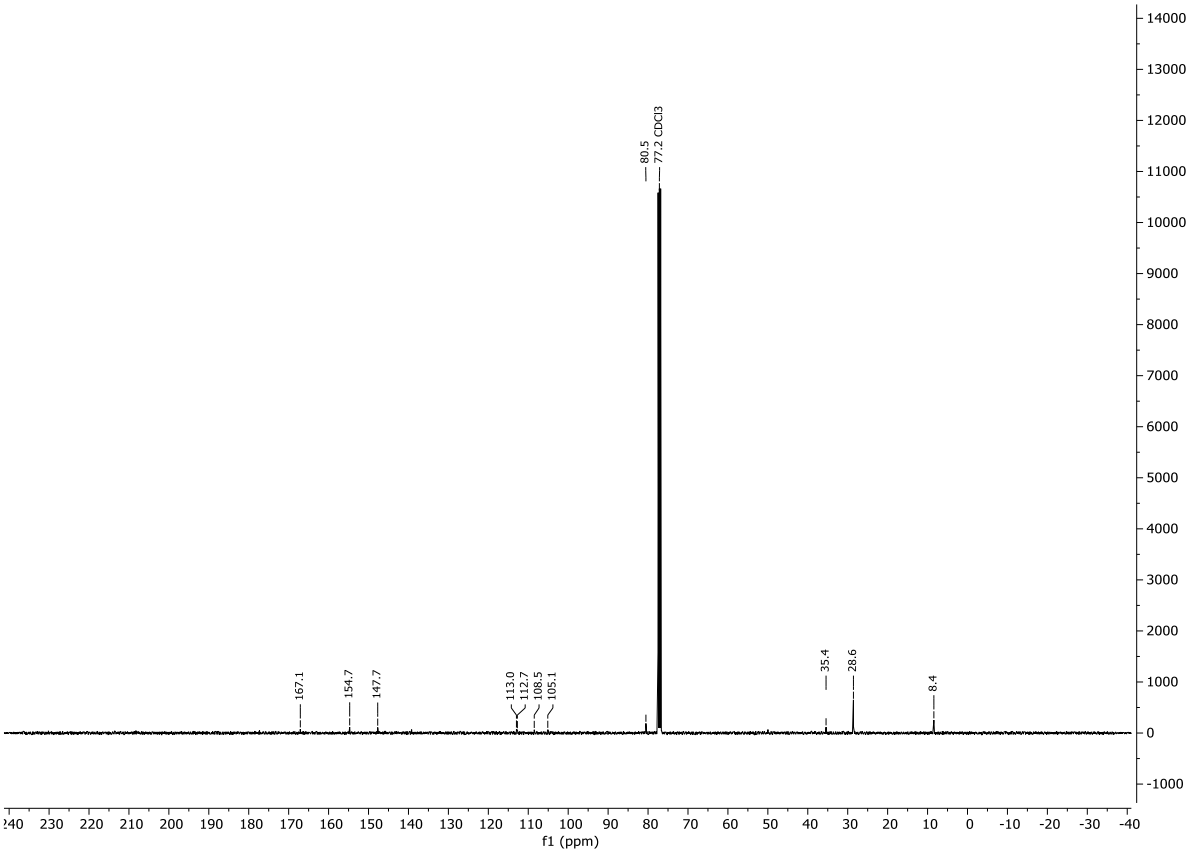
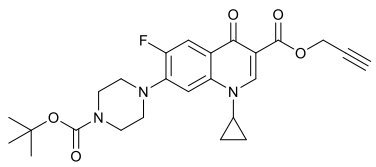
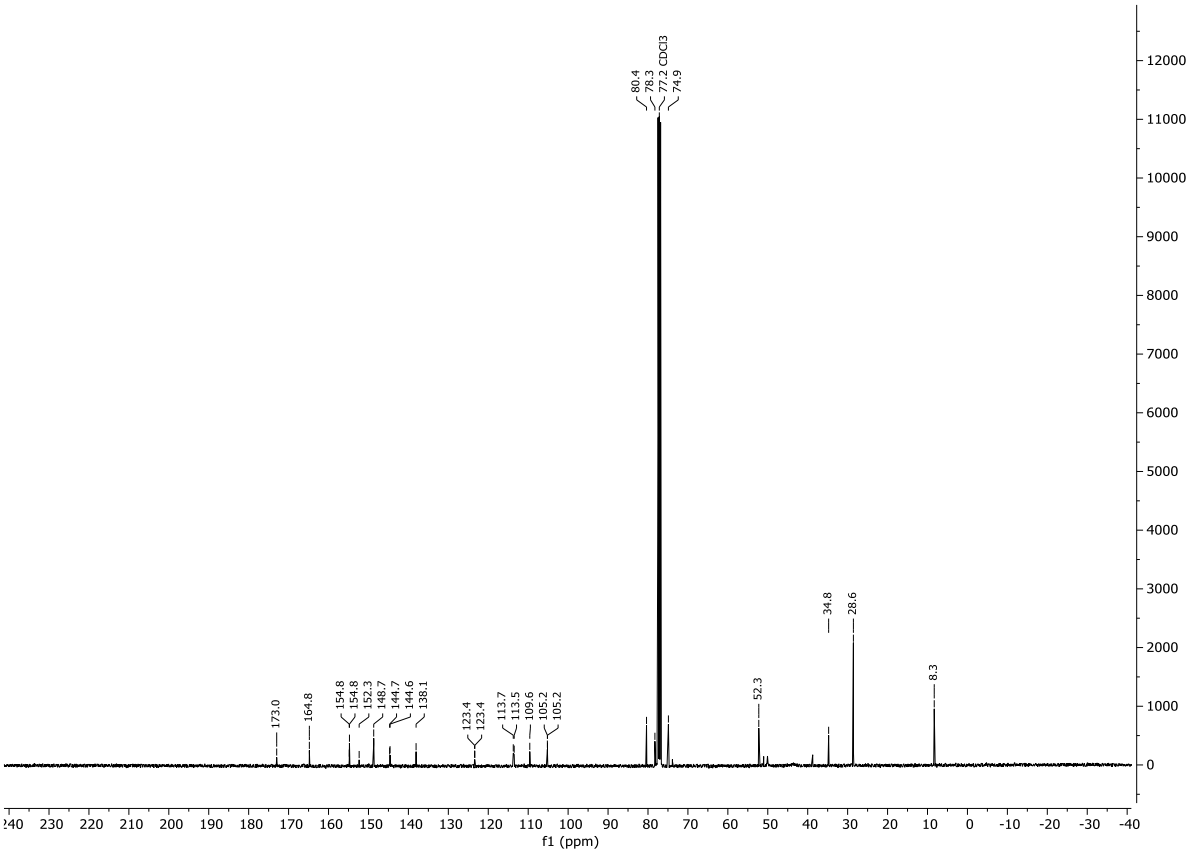
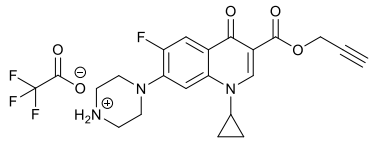
7.26 CDCl3
3.68
3.67
3.66
3.56
3.55
3.54
3.53
3.52
3.51
3.50
3.30
3.29
3.28
1.50
1.42
1.41
1.39
1.37
1.23
1.21
1.20
1.19
0.99
1.00
1.05
1.00
4.07
1.09
4.02
9.03
2.03
2.05
f1 (ppm)


80.5
77.2 CDCl3
167.1
154.7
147.7
113.0
112.7
108.5
105.1
35.4
28.6
8.4
f1 (ppm)

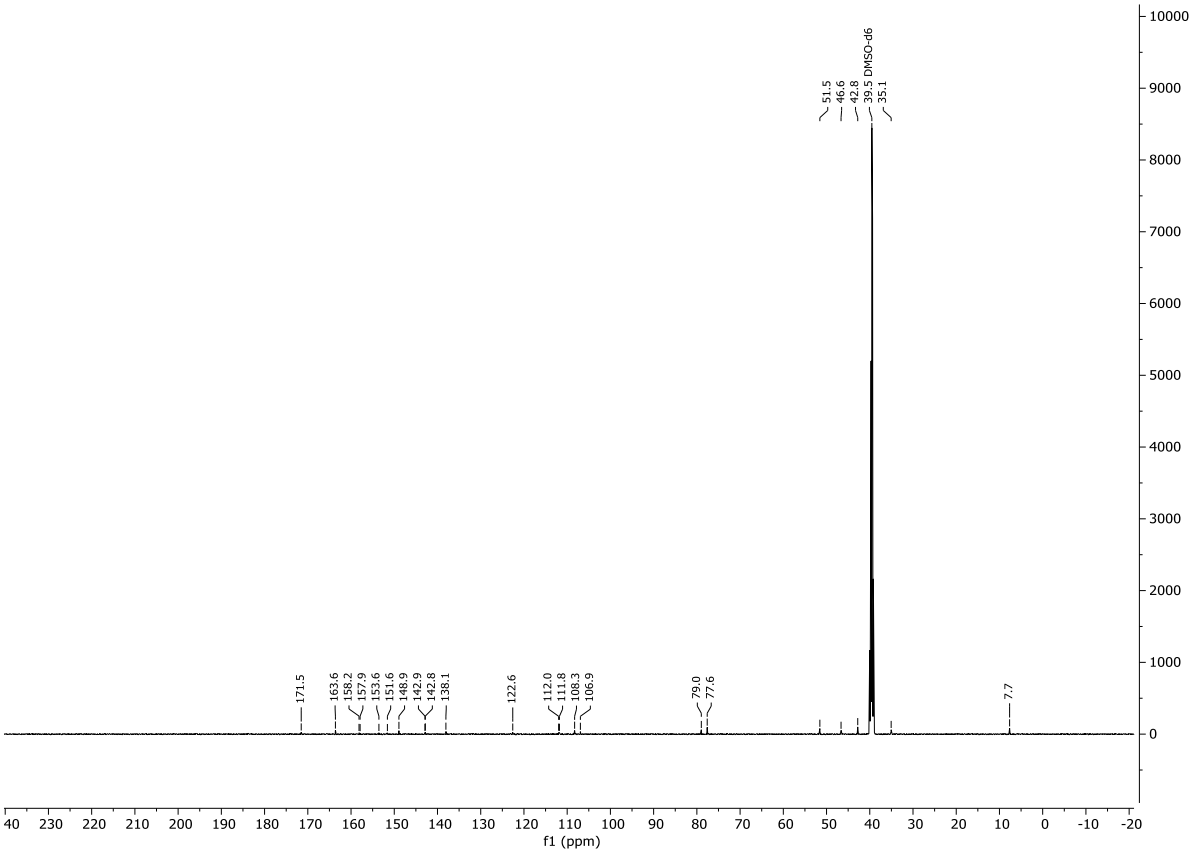


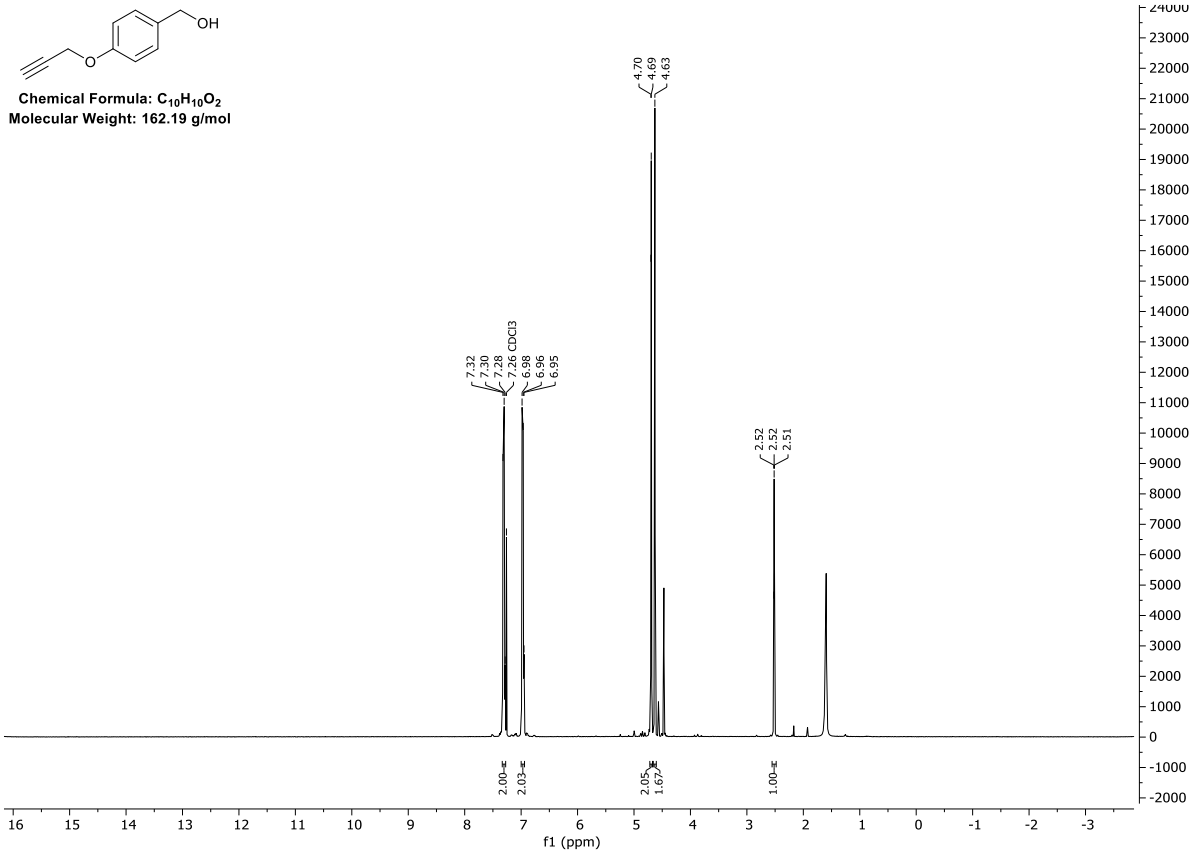
Chemical Formula: $C_{25}H_{28}FN_3O_5$
Molecular Weight: 469.51 g/mol





Chemical Formula: $C_{22}H_{21}F_4N_3O_5$
Molecular Weight: 483.42 g/mol




OH
O
Chemical Formula: $C_{10}H_{10}O_2$
Molecular Weight: 162.19 g/mol
4.70
4.69
4.63
7.32
7.30
7.28
7.26 CDCl3
6.98
6.96
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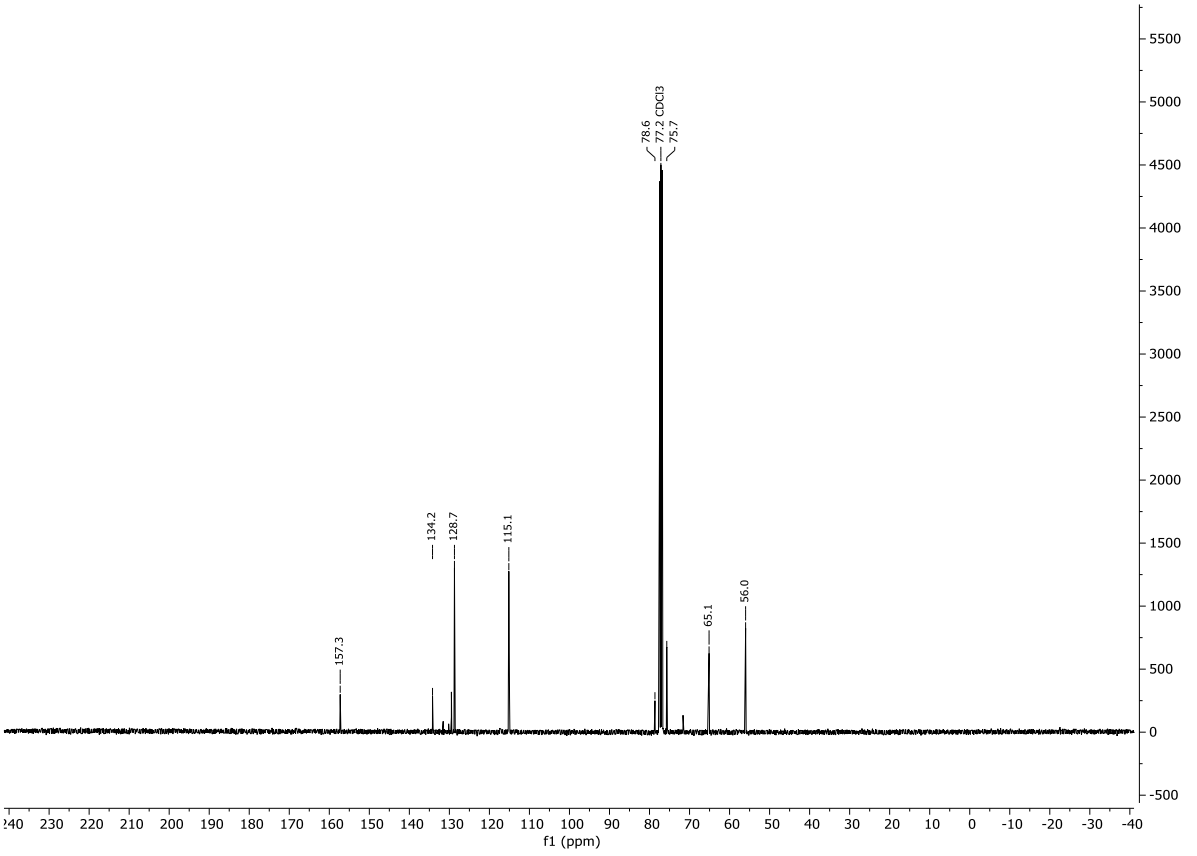
2.51
2.00
2.03
2.05
1.67
1.00
f1 (ppm)


78.6
77.2 CDCl3
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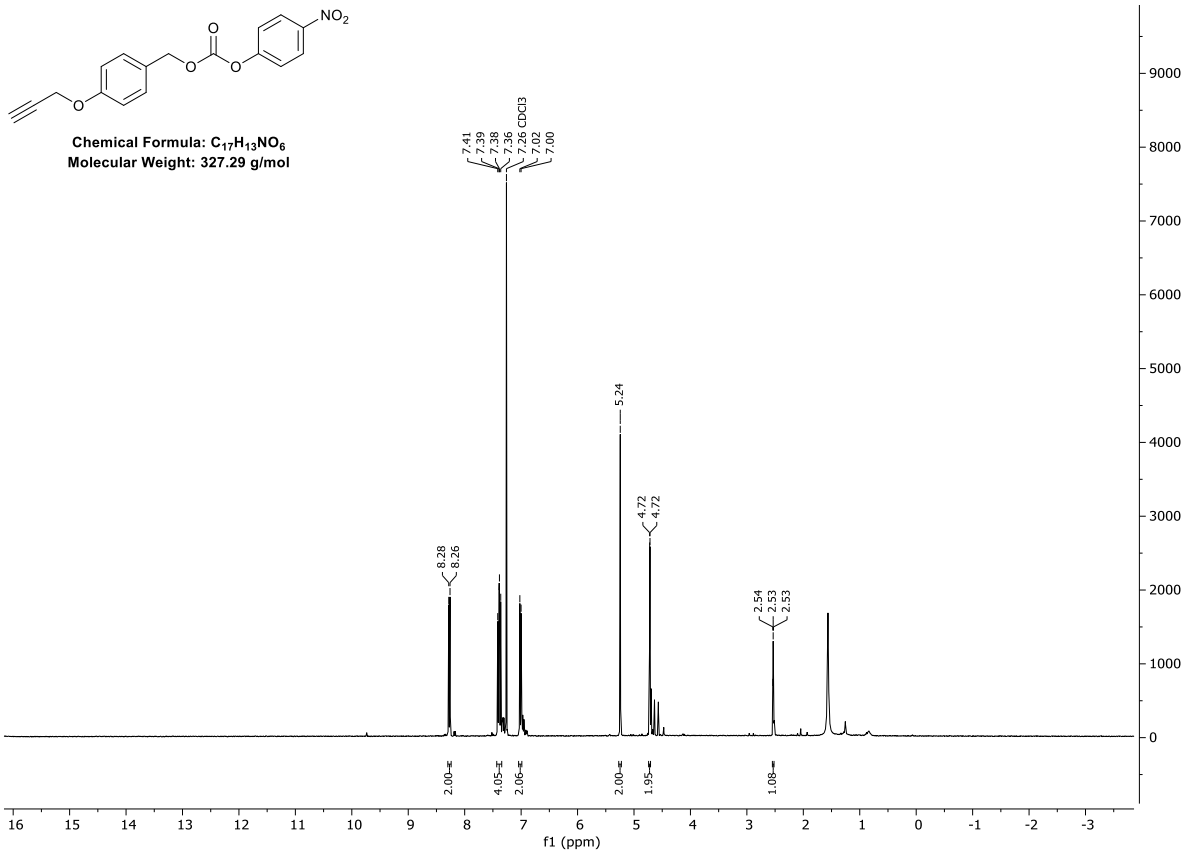
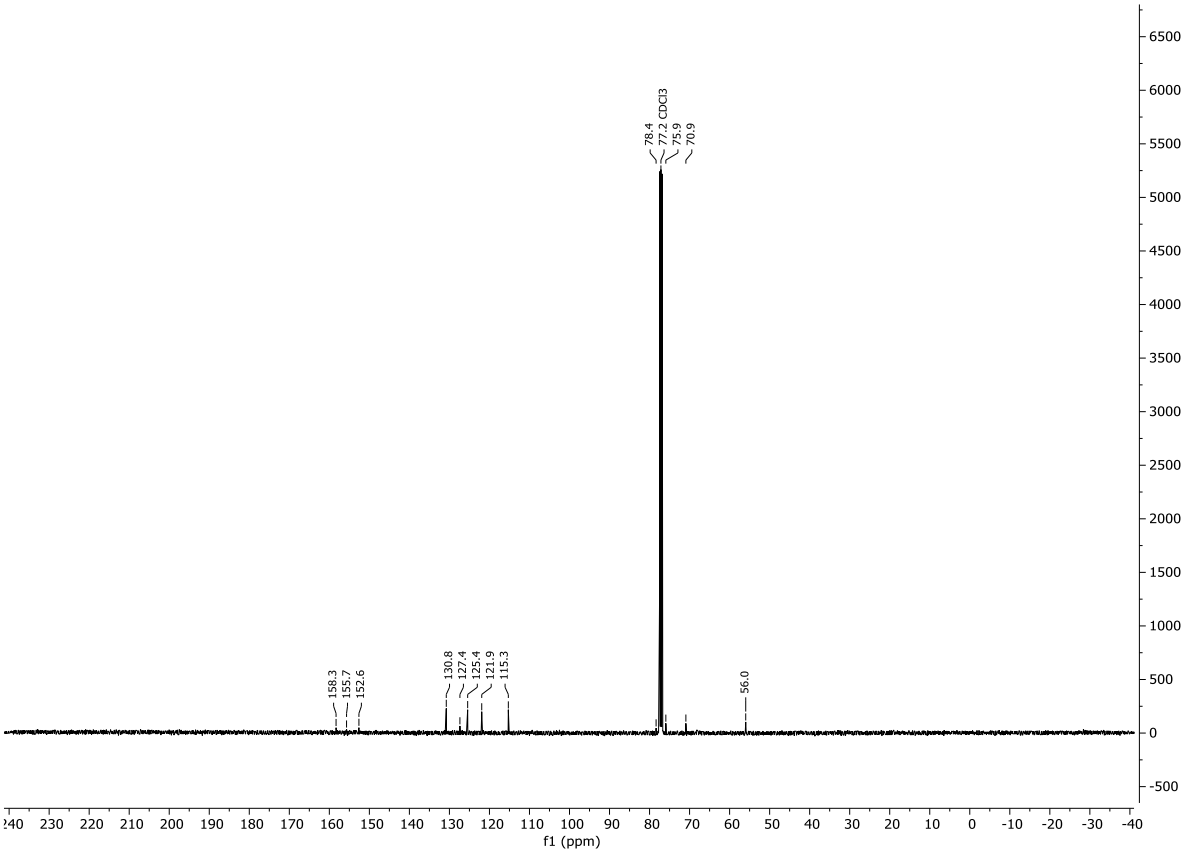
115.1
157.3
65.1
56.0
f1 (ppm)


Chemical Formula: C17H13NO6
Molecular Weight: 327.29 g/mol
7.41
7.39
7.38
7.36
7.26 CDCl3
7.02
7.00
8.28
8.26
5.24
4.72
4.72
2.54
2.53
2.53
2.00
4.05
2.06
2.00
1.95
1.08
f1 (ppm)


78.4
77.2 CDCl3
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70.9
158.3
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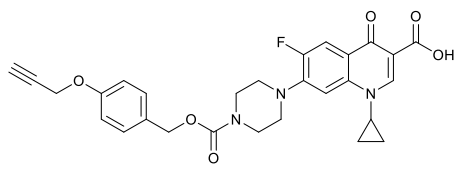
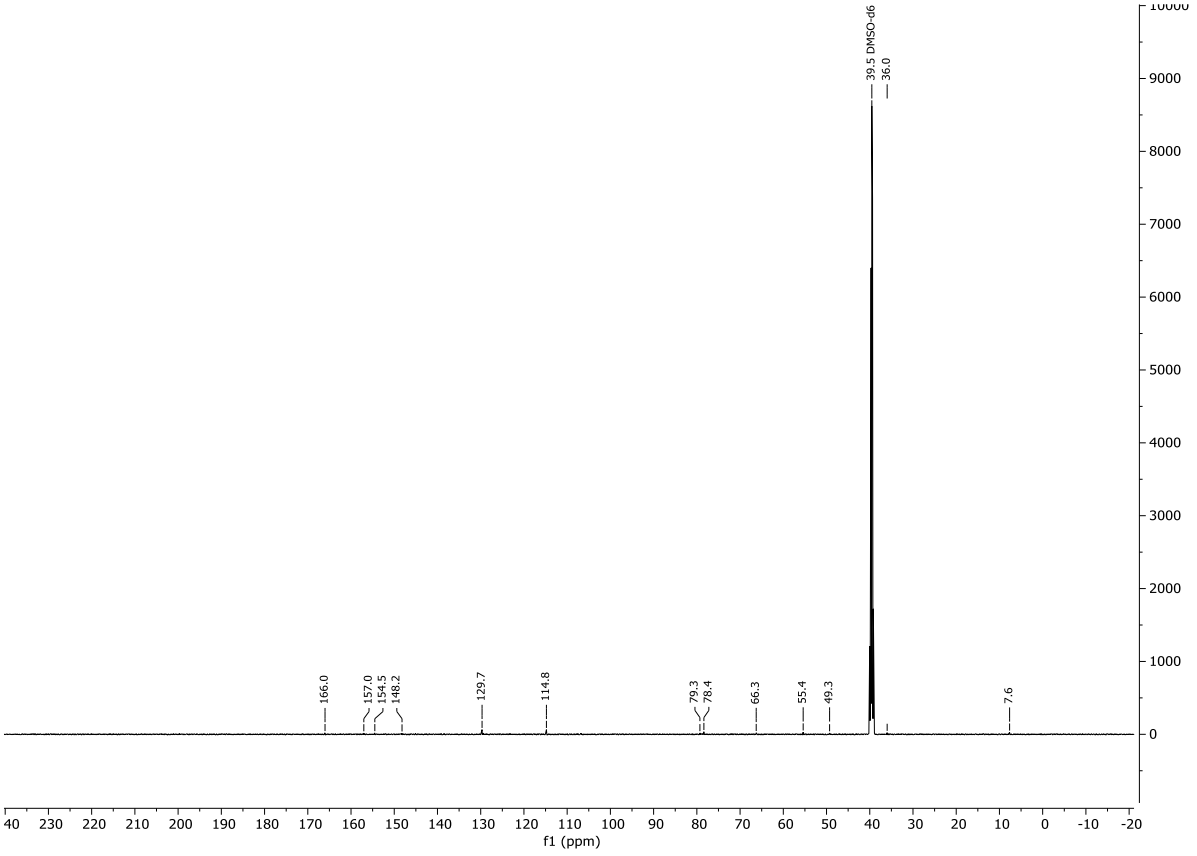
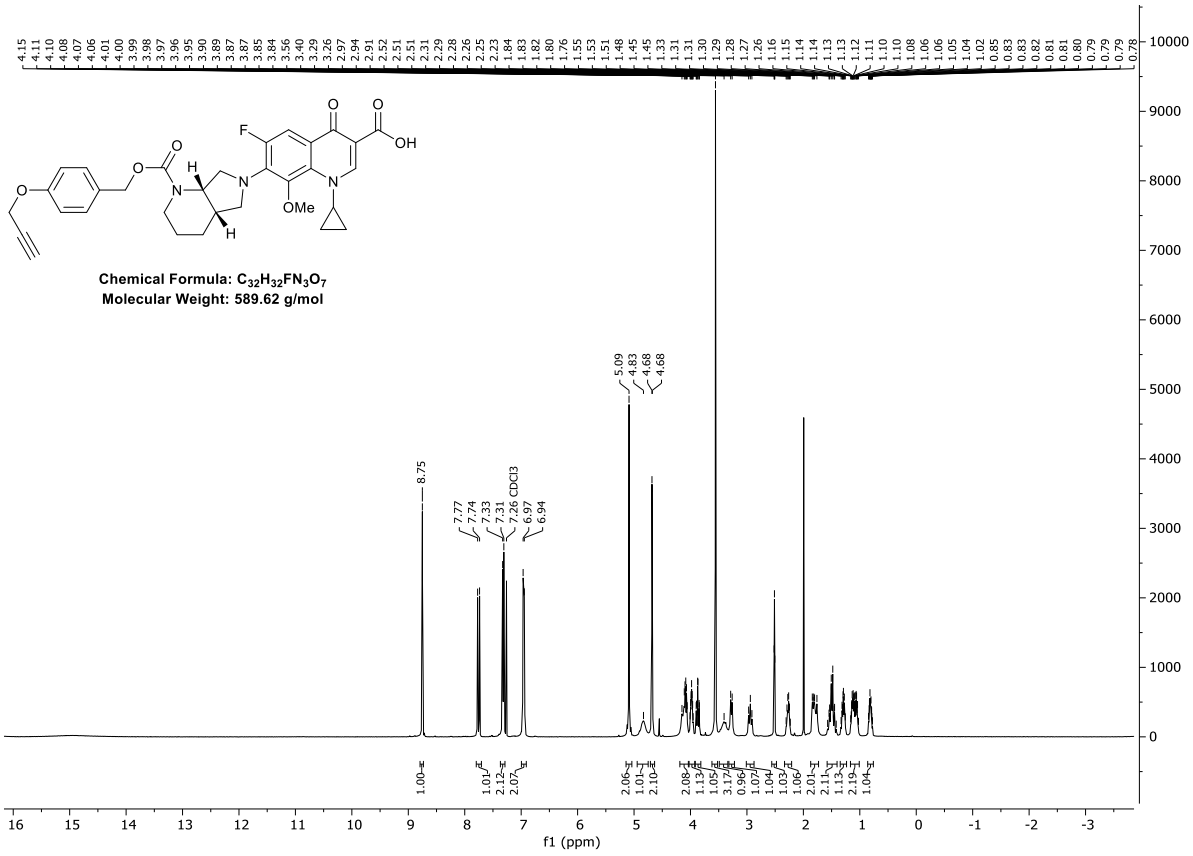
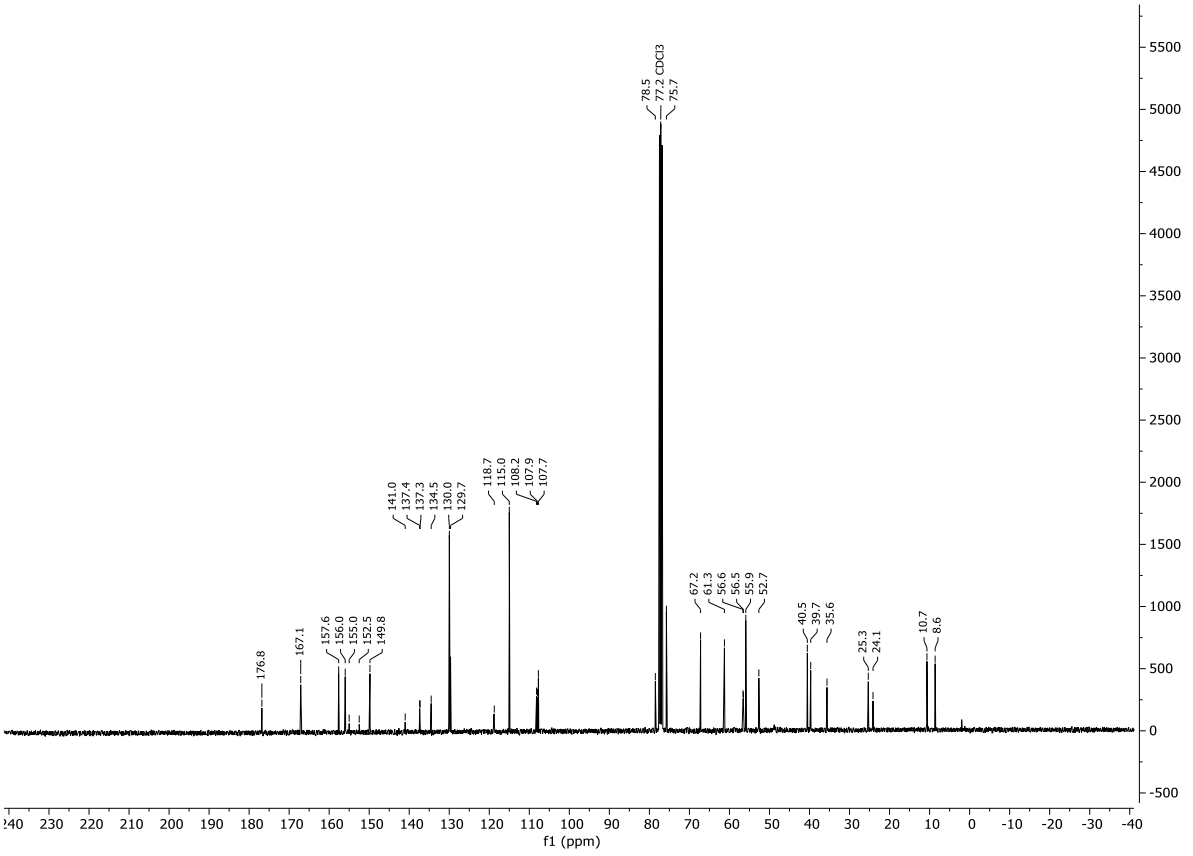
152.6
130.8
127.4
125.4
121.9
115.3
56.0
f1 (ppm)



Chemical Formula: $C_{28}H_{26}FN_3O_6$
Molecular Weight: 519.53 g/mol




Chemical Formula: C32H32FN3O7
Molecular Weight: 589.62 g/mol
f1 (ppm)


f1 (ppm)

7. Supplementary References

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