Supplementary Information

# Tranylcypromine specificity for monoamine oxidase is limited by promiscuous protein labelling and lysosomal trapping

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# Content



**Important Note**

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE[1] partner repository with the dataset identifier PXD018580.

# 1. Supporting Figures

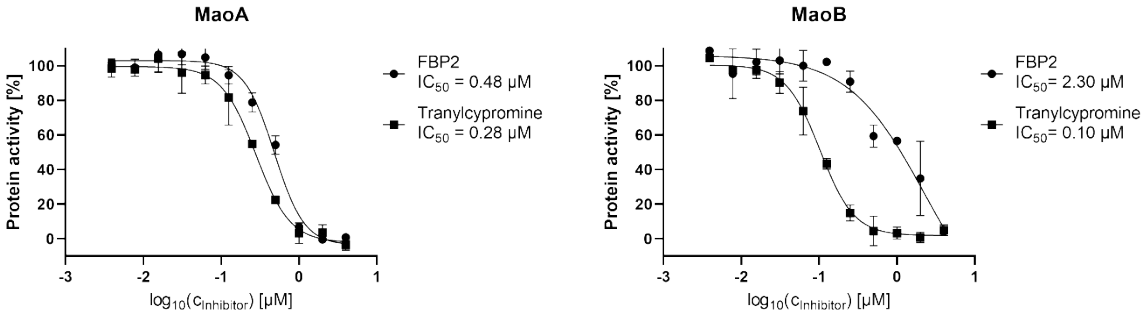



Figure S 1: Inhibition of MAOA (A) and MAOB (B) by probe **FBP2** and tranylcypromine. Apparent $IC_{50}$ values were determined using non-linear curve fittings by Origin Pro 8.3.1.

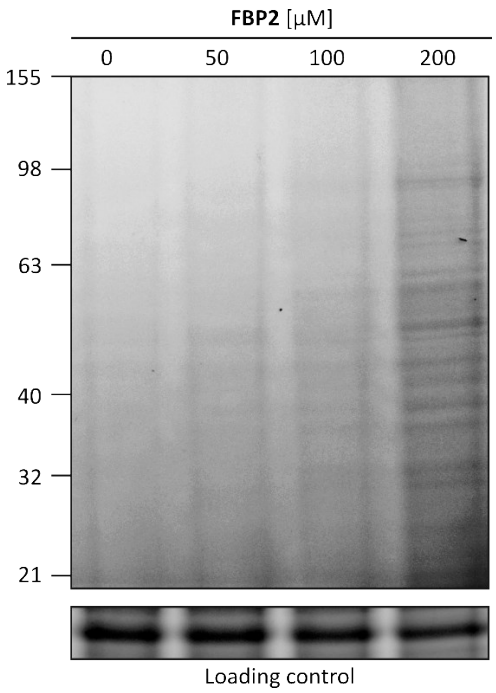



Figure S 2: Fluorescent SDS-gel of the concentration dependent labelling of **FBP2** in the human cancer cell line HeLa, to determine the optimal probe concentration for subsequent gel-free target identification

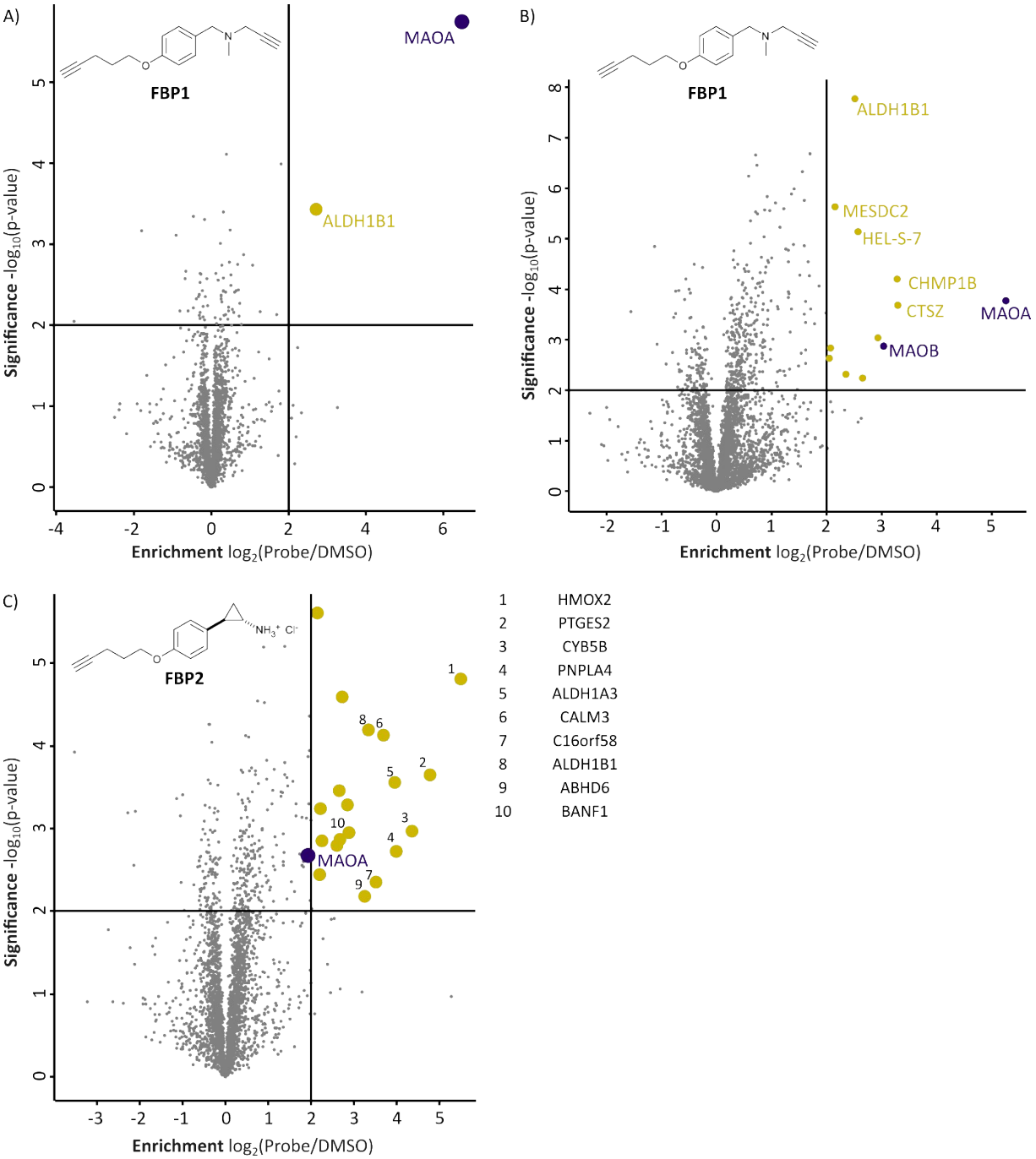



Figure S 3: A+B) Results of target enrichment experiments using **FBP1** in the human cancer cell lines A) SH-SY5Y and B) HeLa. The scatter plots show statistical significance of protein enrichment levels over protein enrichment ratios from probe treated to control cells. Cut offs are at a p value < 0.01 and 4-fold enrichment (indicated by solid lines). C) Results of the corresponding experiments using **FBP2** in SH-SY5Y cells.

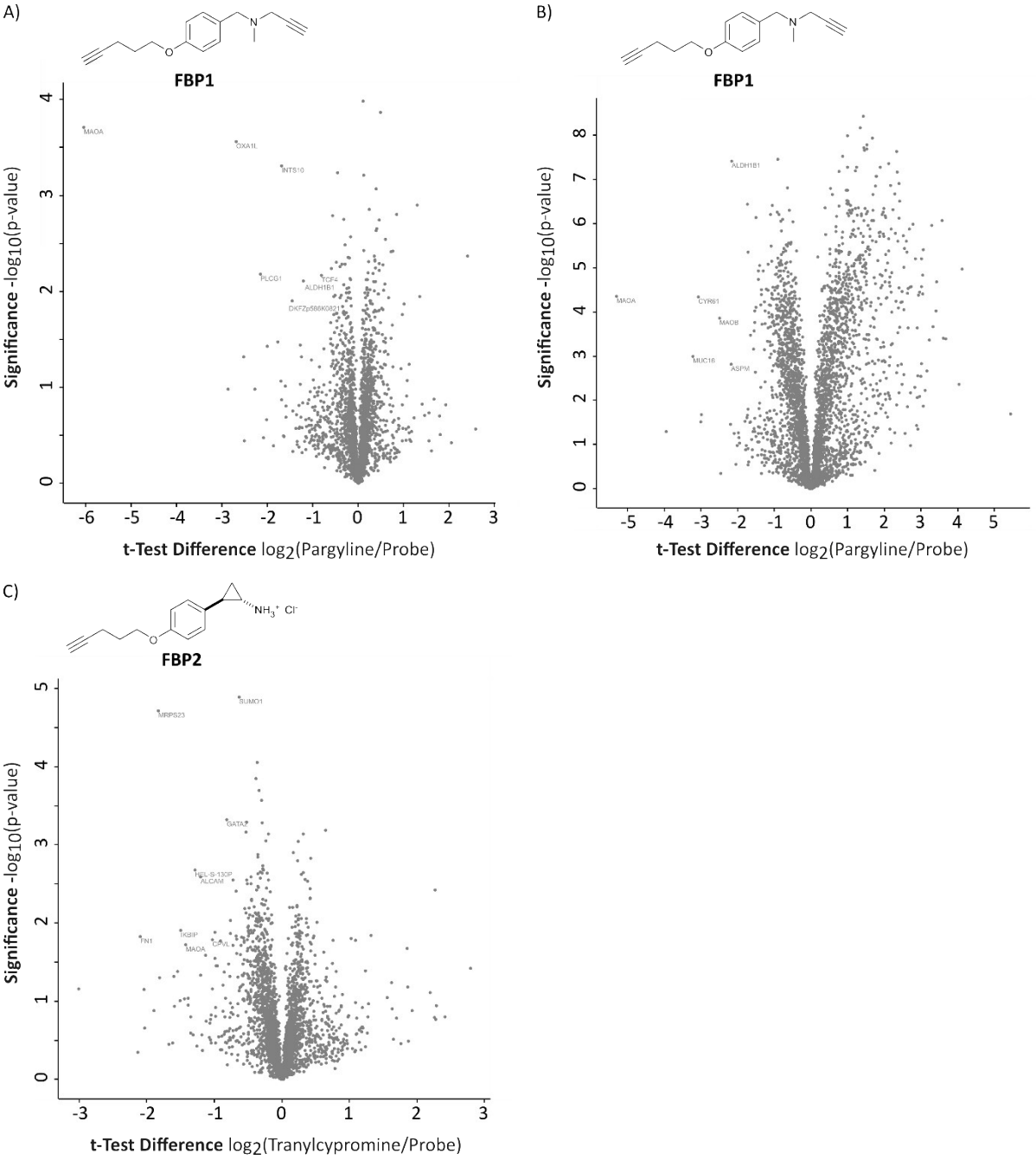



Figure S 4: A+B) Results of competition experiments using **FBP1** in the human cancer cell lines A) SH-SY5Y and B) HeLa. The scatter plots show statistical significance of protein enrichment levels over protein enrichment ratios from drug treated to control cells. C) Results of the corresponding experiments using **FBP2** in SH-SY5Y cells.

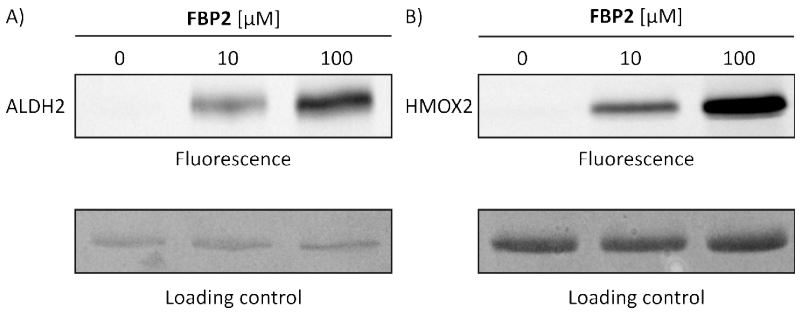



Figure S 5: Fluorescent SDS-gel of recombinant ALDH2 (A) and HMOX2 (B) labelled with either 10 or 100 eq. **FBP2** showing the covalent modification with the probe.

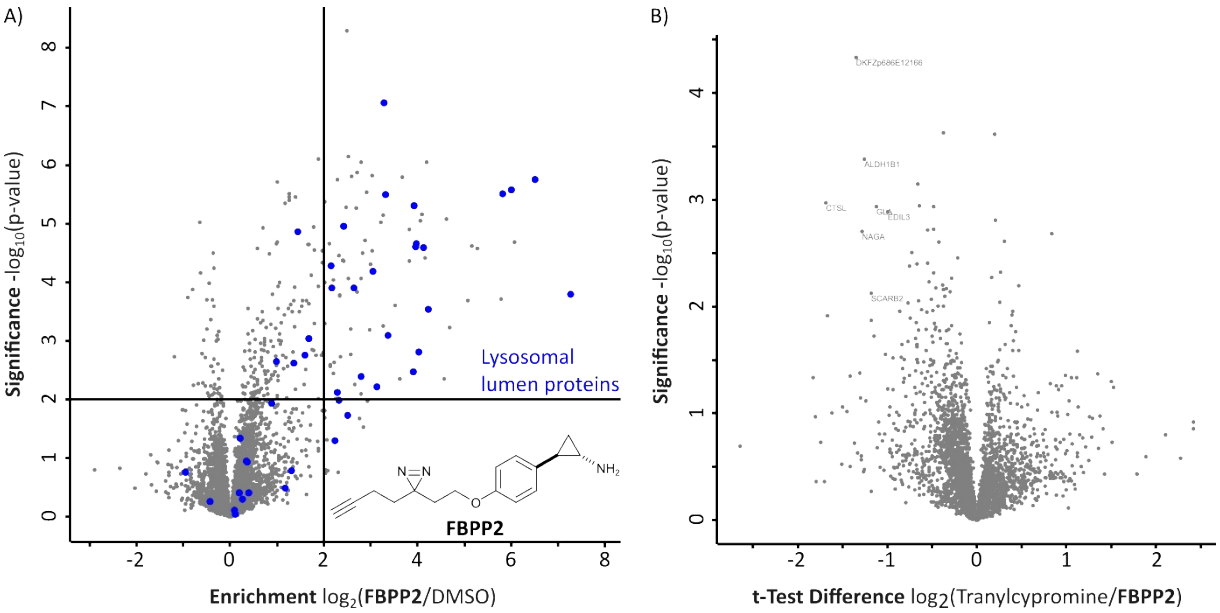



Figure S 6: A) Results of the photo-affinity labelling experiment in HeLa cells using **FBPP2**. The scatter plot shows statistical significance of protein enrichment levels over protein enrichment ratios from probe treated to control cells. Cut offs are at a p-value < 0.01 and 4-fold enrichment (indicated by solid lines). Proteins annotated to be located in the lysosomal lumen (GO:0043202) are highlighted in blue. B) Results of the corresponding competition experiment. The scatter plots show statistical significance of protein enrichment levels over protein enrichment ratios from tranylcypromine treated to control cells.

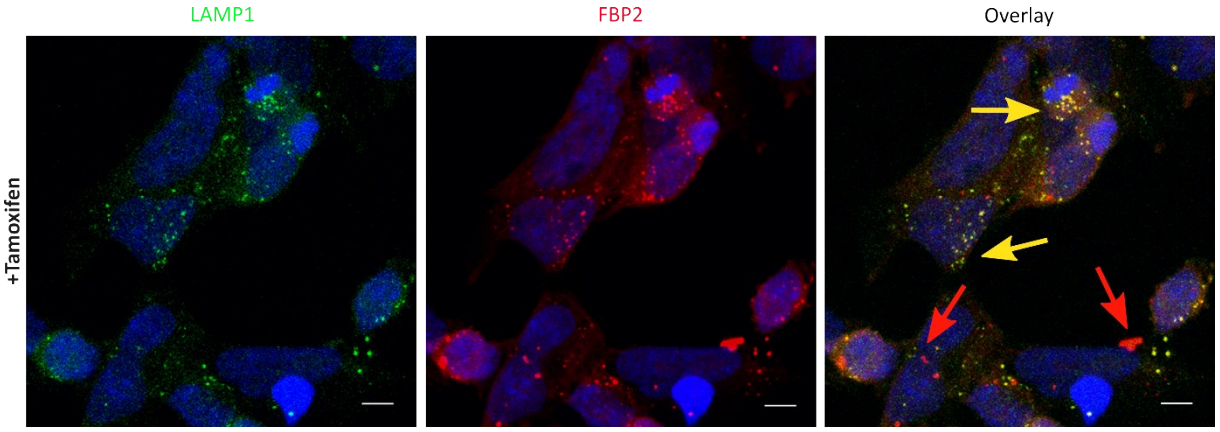



Figure S 7: Fluorescence imaging with LAMP1 (green) and FBP2 clicked to TAMRA-azide (red) in fixed SH-SY5Y cells. The colocalization (yellow) between lysosomes and probe is diminished by pre-treatment of cells with tamoxifen (scale bar: 5 µm).

# 2. Supporting Tables

Table S1. MS-Results of all experiments conducted in this work can be found as an additional data file named “Drechsel_et_al_Table_S1.xlsx”.

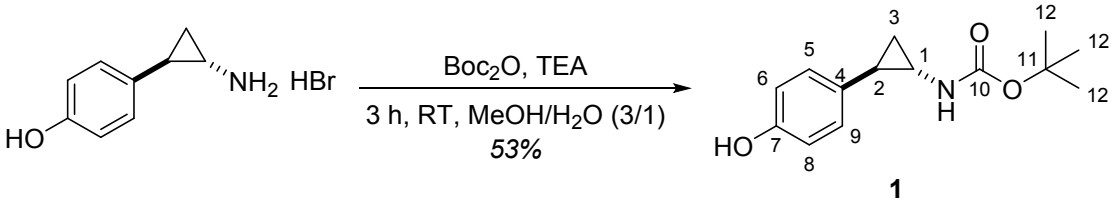
# 3. Chemical Synthesis

## 3.1. General remarks

All air or water sensitive reactions were carried out under argon in oven-dried glassware. Chemicals and solvents were purchased from Labseeker, Kumidas SA, Sigma-Aldrich, Alfa Aesar, Acros Organics, TCI Europe and Merck, were of reagent grade or better and were used without further purification. In all reactions, temperatures were measured externally. Solvents removed under reduced pressure were evaporated at 40 °C. Flash column chromatography was performed on silica gel (40-63 µm) by VWR, elution solvents were distilled prior to use. Analytical thin-layer chromatography was carried out on aluminium-baked TLC Silica gel plates by Merck. Components were visualized by UV detection (λ = 254 nm, 312 nm) or stained via aqueous $KMnO_4$ or aqueous cerium molybdate (Hanessian's stain). $^1H$ NMR and $^{13}C$ spectra of small molecules were recorded on Bruker instruments (300 MHz, 400 MHz or 500 MHz) and referenced to the residual proton signal of the deuterated solvent. ($CDCl_3$, DMSO-d6). Multiplets are described using following abbreviations: s - singlet, d - doublet, t - triplet, q - quartet, and m - multiplet. HR-MS-ESI spectra were recorded with a Thermo Scientific LTQ FT Ultra.

## 3.2. Probe synthesis

***tert*-Butyl ((*trans*)-2-(4-hydroxyphenyl)cyclopropyl)carbamate (1)**




**1**

To a solution of 100 mg of (*trans*)-2-(4-hydroxyphenyl)cyclopropanamine hydrobromide (435 µmol, 1.0 eq.) and 150 µL of di-*tert*-butyl dicarbonate (142 mg, 652 µmol, 1.5 eq.) in 8 mL of MeOH/$H_2O$ (3:1) were added 181 µL of $Et_3N$ (132 mg, 1.30 mmol, 3.0 eq.) and stirred for 3 h at RT. The reaction mixture was diluted with EtOAc and extracted with $H_2O$. The organic phase was dried over $Na_2SO_4$and the solvent removed under reduced pressure. The crude product was purified by flash chromatography to yield 57.0 mg of **1** (229 µmol, 53%) as a yellow oil.

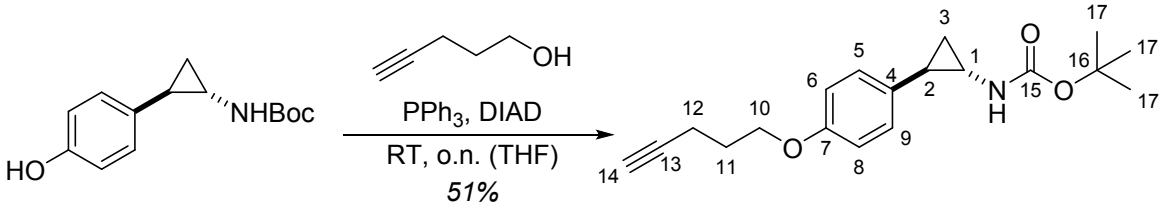
HRMS (ESI): m/z calcd for $C_{14}H_{19}NO_3$+$H^+$: 250.1443 $[M+H]^+$; found 250.1438.

$^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ [ppm] = 6.90 (d, $^3J$ = 8.1 Hz, 2H, H5/9), 6.69 (d, $^3J$ = 8.1 Hz, 2H, H6/8), 4.97 (s, 1H, NH), 2.72 – 2.57 (m, 1H, H1), 1.96 (ddd, $^3J$ = 9.5, 6.6, 3.2 Hz, 1H, H2), 1.46 (s, 9H, H17), 1.11 – 0.99 (m, 2H, H3).

$^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ [ppm] = 157.1, 154.8, 131.9, 127.7, 115.4, 80.3, 32.3, 28.6, 25.3, 15.8.

The analytical data obtained match those reported in the literature. [2]

***tert*-Butyl ((*trans*)-2-(4-(pent-4-yn-1-yloxy)phenyl)cyclopropyl)carbamate (2)**




**1** **2**

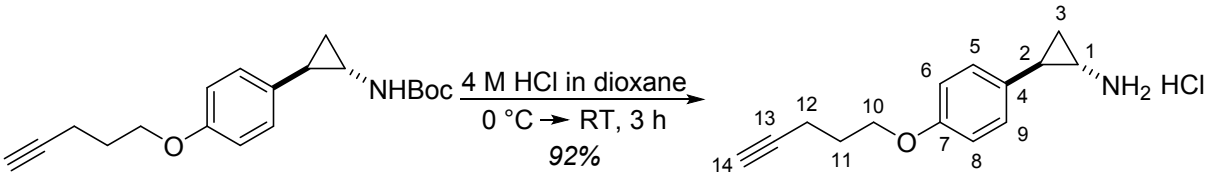
To a solution of 55.0 mg of **1** (221 µmol, 1.0 eq.) in 1.5 mL of dry THF were added 69.5 mg of triphenylphosphane (265 µmol, 1.2 eq.), 52.0 µL of diisopropyl azodicarboxylate (DIAD) (53.6 mg, 265 µmol, 1.2 Eq.) and 24.7 µL of 4-pentyn-1-ol (22.3 mg, 265 µmol, 1.2 eq.). The reaction was stirred over night at RT. Afterwards, the solvent was removed under reduced pressure. The crude product was purified by flash chromatography to yield 35.8 of **2** (114 µmol, 51%) as a light yellowish solid.

HRMS (ESI): m/z calcd for $C_{38}H_{50}N_2O_6$+H$^+$: 631.3747 [2M+H]$^+$; found 631.3738.

$^1$H NMR (500 MHz, $CDCl_3$) $\delta$ [ppm] = 7.06 (d, $^3J$ = 8.1 Hz, 2H, H5/9), 6.80 (d, $^3J$ = 8.1 Hz, 2H, H6/8), 4.86 (s, 1H, NH), 4.02 (t, $^3J$ = 6.1 Hz, 2H, H10), 2.69 – 2.56 (m, 1H, H1), 2.39 (td, $^{3/4}J$ = 7.0, 2.7 Hz, 2H, H12), 2.02 – 1.91 (m, 4H, H2/11/14), 1.45 (s, 9H, H17), 1.12 – 1.01 (m, 2H, H3).

$^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ [ppm] = 157.4, 132.9, 127.9, 114.6, 83.6, 68.9, 66.4, 60.5, 28.5, 28.3, 21.2, 16.0, 15.3, 14.3.

**(*trans*)-2-(4-(Pent-4-yn-1-yloxy)phenyl)cyclopropan-1-amine hydrochloride (FBP2)**




**2** **FBP2**

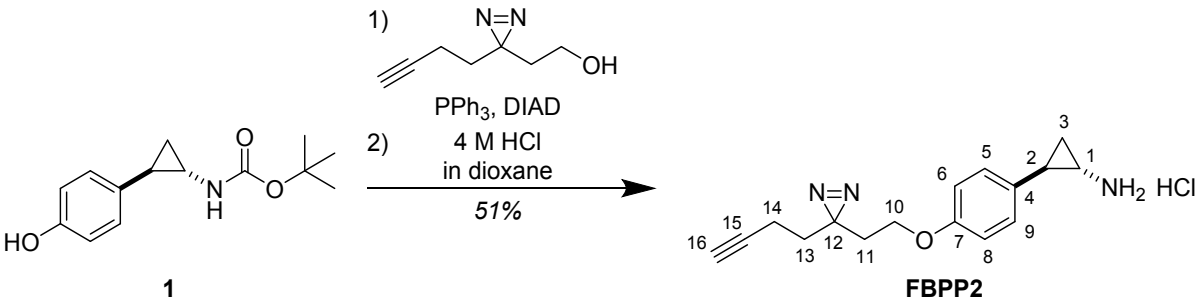
35.8 mg of **2** (114 µmol, 1.0 eq.) were cooled to 0 °C. Then, 1.0 mL of 4 M HCl in dioxane was added. The reaction was stirred for 2.5 h at 0 °C followed by another 30 min at RT. The solvent was removed under reduced pressure to give 26.4 mg of **FBP2** (105 µmol, 92%) as a white solid.

HRMS (ESI): m/z calcd for $C_{14}H_{17}NO$+H$^+$: 216.1388 [M+H]$^+$; found 216.1382.

$^1$H NMR (500 MHz, MeOD) $\delta$ [ppm] = 7.09 (d, $^3J$ = 8.5 Hz, 2H, H5/9), 6.86 (d, $^3J$ = 8.5 Hz, 2H, H6/8), 4.03 (t, $^3J$ = 6.1 Hz, 2H, H10), 2.78 – 2.73 (m, 1H, H1), 2.39 – 2.30 (m, 3H, H2/12), 2.24 (t, $^4J$ = 2.7 Hz, 1H, H14), 1.94 (tt, $^3J$ = 6.6, 6.6 Hz, 2H, H11), 1.37 (ddd, $^{2/3}J$ = 10.4, 6.5, 4.2 Hz, 1H, H3'), 1.26 – 1.22 (m, 1H, H3'').

$^{13}$C NMR (101 MHz, MeOD) $\delta$ [ppm] = 159.4, 131.7, 128.6, 115.7, 84.1, 70.0, 67.4, 31.8, 29.5, 21.9, 15.7, 13.4.

**(*trans*)-2-(4-(2-(3-(But-3-yn-1-yl)-3H-diazirin-3-yl)ethoxy)phenyl)cyclopropan-1-amine hydrochloride (FBPP2)**




To a solution of 57.0 mg of **1** (229 µmol, 1.0 eq.) in 2.0 mL of dry THF were added 72.0 mg of triphenylphosphane (274 µmol, 1.2 eq.), 53.9 µL of DIAD (55.5 mg, 274 µmol, 1.2 Eq.) and 37.9 mg of 2-(3-but-3-ynyl-3*H*-diazirin-3-yl)-ethanol (274 µmol, 1.2 eq.). The reaction was stirred for 4 h at RT. Afterwards, the solvent was removed under reduced pressure. The residue was purified by flash chromatography, intermediate containing fractions were pooled, the solvent evaporated under reduced pressure, and the residue taken up in 1.0 mL 4 M HCl in dioxane. The resulting precipitate was filtered and dried in high vacuum to yield 33.0 mg of **FBPP2** (108 µmol, 47%) as an orange solid.

HRMS (ESI): m/z calcd for $C_{16}H_{19}N_3O+H^+$: 270.1606 $[M+H]^+$; found 270.1603.

$^1$H NMR (300 MHz, MeOD) $\delta$ [ppm] = 7.10 (d, $^3J$ = 8.6 Hz, 2H, H5/9), 6.86 (d, $^3J$ = 8.6 Hz, 2H, H6/8), 3.82 (t, $^3J$ = 6.1 Hz, 2H, H10), 3.35 (s, 1H, NH), 2.76 (dt, $^3J$ = 7.8, 4.0 Hz, 1H, H1), 2.33 (ddd, $^3J$ = 10.2, 6.6, 3.5 Hz, 1H, H2), 2.26 (t, $^4J$ = 2.6 Hz, 1H, H16), 2.06 (td, $^{3/4}J$ = 7.4, 2.6 Hz, 2H, H14), 1.85 (t, $^3J$ = 6.1 Hz, 2H, H11), 1.67 (t, $^3J$ = 7.4 Hz, 2H, H13), 1.41 – 1.22 (m, 2H, H3).

$^{13}$C NMR (75 MHz, MeOD) $\delta$ [ppm] = 159.0, 131.9, 128.7, 115.8, 83.7, 70.3, 63.9, 33.9, 33.8, 31.8, 27.8, 22.0, 13.8, 13.4.

# 4. Biochemical Procedures

## 4.1. Cell Culture

SH-SY5Y cells were grown in DMEM/F12 medium, HeLa cells in DMEM medium. All media were supplemented with 10% (v/v) FBS and 2 mM glutamine. For splitting, the medium was removed, and cells were washed with 10 mL of PBS. Afterwards, the cells were incubated with 1 mL of Accutase® for 10 min at 37 °C until full detachment. Then, 10 mL of the respective medium were added, mixed thoroughly and 1 mL of this cell-solution was transferred into a new flask. The cell-solution was diluted with medium until a volume of 10 mL.

## 4.2. Apparent $IC_{50}$ Measurements

Activity of MaoA and MaoB was assigned in the continuous AzBTS – HRP coupled assay using kynuramine as substrate. The activity was assayed spectrophotometrically by monitoring the rate of the oxidation product formation at 420 nm. Apparent $IC_{50}$ values for the inhibition of MAOA and MAOB by probes **FBP2** as well as by tranylcypromine were determined by incubating 2.5 µg of the respective protein in 50 µL 50 mM potassium phosphate buffer (pH 7.5) with varying concentrations of probes for 1 h at 25 °C. Then 50 µL chromogenic solution (3 µL 100 mM stock of kynuramine dihydrobromide, 1 µL 100 U/mL stock horse radish peroxidase, 1 µL 5 mM stock AzBTS and 45 µL 50 mM potassium phosphate buffer) were added to give a total volume of 100 µL. The absorbance change was recorded at 420 nm at 25 °C by a TECAN Infinite M200pro. All measurements were performed in triplicates. Apparent $IC_{50}$ values were calculated from non-linear curve fittings by Origin Pro 8.3.1.

## 4.3. Gel-based labelling in HeLa cells

HeLa cells were seeded in 6 well plates and allowed to grow to 80 - 90% confluence. After removing the growth medium, 1 mL PBS containing 1 µL of **FBP2** (DMSO stock in various concentrations) was added. The cells were incubated for 1 h at 37 °C in a 5% $CO_2$ atmosphere, afterwards scraped of the dish, pelletized (800 g, 3 min, 4 °C), subsequently washed with 1 mL PBS and pelletized again (800 g, 3 min, 4 °C). For lysis, the cells were resuspended in 100 µL lysis buffer (1% NP40 (v/v), 1% sodium deoxycholate (w/v) in PBS), incubated for 15 min on ice and insoluble fragments were separated by centrifugation (max. rpm, 45 min, 4 °C). The supernatant was subjected to click-reaction by adding 2 µL rhodamine azide (10 mM in DMSO), 2 µL tris(2-carboxyethyl)phosphine (TCEP) (52 mM in $ddH_2O$), 6 µL 1 × tris(benzyltriazolylmethyl)amine (TBTA) ligand (1.67 mM) and 2 µL $CuSO_4$ (50 mM in $ddH_2O$) and incubating for 1 h at RT. After adding 100 µL of gel loading buffer, the samples were stored at −20 °C or directly loaded on a SDS-gel.

## 4.4. Labelling of Recombinant Proteins

ALDH2 and HMOX2(1-264) were purified as described previously.[3, 4] For labeling of recombinant proteins 100 µL protein solution in PBS (1 µM) were incubated with 1 µL of **FBP2** (100 eq., DMSO-stock) for 1 h at RT. Afterwards, the samples were subjected to click-reaction by adding 2 µL rhodamine azide (10 mM in DMSO), 2 µL tris(2-carboxyethyl)phosphine (TCEP) (52 mM in $ddH_2O$), 6 µL 1 × tris(benzyltriazolylmethyl)amine (TBTA) ligand (1.67 mM) and 2 µL $CuSO_4$ (50 mM in $ddH_2O$) and incubating for 1 h at RT. After adding 100 µL of gel loading buffer, the samples were stored at −20 °C or directly loaded on a SDS-gel.

## 4.5. Gel-Free ABPP

SH-SY5Y or HeLa cells were plated on a 15 cm dish and grown to 80-90% confluence. The cells were washed with 10 mL PBS and treated with 10 mL PBS containing the respective probe **FBP1/2** (final concentration 100 µM) and incubated for 1 h at 37 °C in a 5% CO2 atmosphere. For control experiments, cells were treated with DMSO. Then, the cells were scraped off the dish, subsequently pelletized (800 g, 3 min, 4 °C), washed with 10 mL PBS and pelletized again (800 g, 3 min, 4 °C). For

lysis, the cells were resuspended in 1 mL lysis buffer (1% NP40 (v/v), 1% sodium deoxycholate (w/v) in PBS) and incubated for 15 min on ice, followed by mild sonication (10% intensity, 15 sec on ice). Protein concentration of each sample was determined by BCA-assay and samples were adjusted to the same protein concentration using PBS. The total volume was then adjusted to 1880 µL with PBS and the samples were further subjected to the following click-reaction by adding 40 µL Biotin-PEG3-$N_3$ (10 mM in DMSO), 20 µL TCEP (52 mM in $ddH_2O$), 60 µL 1 × TBTA ligand (1.67 mM) and 20 µL $CuSO_4$ (50 mM in $ddH_2O$). After incubation for 1 h at RT, the proteins were precipitated by adding 8 mL of cold acetone and incubation overnight at -20 °C. After centrifugation (≥13000 rpm, 15 min, 4 °C) the pellet was washed twice with 200 µL cold methanol (-80 °C) and the proteins were resuspended in 500 µL PBS + 0.4% SDS (v/v) by sonication at RT. For enrichment, the protein solution was added to 50 µL pre-washed avidin-agarose bead suspension (1.1 mg/mL in glycerol, washed 3 times with 1 mL PBS + 0.4% SDS) and incubated for 1 h under continuous gentle mixing. To remove unbound proteins, the beads were washed three times with 1 mL PBS + 0.4% SDS, two times with urea (6 M in $ddH_2O$) and three times with 1 mL PBS. Afterwards, the beads were carefully resuspended in 200 µL capping-buffer (7 M urea, 2 M thiourea in 20 mM HEPES). Reduction was performed by adding 0.2 µL dithiothreitol (DTT) (1 M in $ddH_2O$) and shaking the reaction mixture (450 rpm) for 45 min at RT. On addition of 2 µL iodoacetamide (IAA) (550 mM in $ddH_2O$) the proteins were alkylated for 30 min under continuous shaking (450 rpm) at RT and exclusion of light. The reaction was stopped by adding 0.8 µL DTT (1 M in $ddH_2O$) and incubation for 30 min at RT. To digest the proteins, 1 µL of endoproteinase Lys-C (LysC) (1 M in $ddH_2O$) was added to each sample and incubated for 2 h at RT under continuous shaking (450 rpm) and exclusion of light. Afterwards, 600 µL of tetraethylammonium bromide (TEAB) (50 mM in $ddH_2O$) and 1.5 µL of Trypsin (0.5 µg/µL in $ddH_2O$) were added and the samples were incubated overnight at 37 °C under continuous shaking (450 rpm). The digestion was stopped by adding 8 µL formic acid (FA) and the suspension was then centrifuged (13000 rpm, 3 min, RT) to pelletize the beads. The supernatant was loaded on 50 mg SepPak C 18 columns (Waters) equilibrated with 0.1% trifluoroacetic acid (TFA). The peptides were washed three times with 1 mL 0.1% TFA and 250 µL 0.5% FA. Afterwards, the peptides were eluted three times with 250 µL elution buffer (80% acetonitrile (ACN), 0.5% FA), lyophilized and stored at -80 °C until further usage.

## 4.6. Competition Experiments

For competition experiments, cells were pre-treated with tenfold excess of parent drug (pargyline in case of **FBP1,** tranylcypromine in case of **FBP2** and **FBPP2**) for 1 h prior to probe treatment. Further sample handling was done as described in section 4.3.

## 4.7. Photo-Affinity Labelling

SH-SY5Y or HeLa cells were plated on a 15 cm dish and grown to 80-90% confluence. The cells were washed with 10 mL PBS and treated with 10 mL PBS containing **FBPP2** (final concentration 10 µM) and incubated for 1 h at 37 °C in a 5% CO2 atmosphere. For control experiments, cells were treated with DMSO. In case of chloroquine pre-treatment, cells were treated with twofold excess of drug for 1 h prior to adding the probe. Afterwards, cells were irradiated with UV light (365 nm, 2 x 5 min at 0 °C, *Philips* TL-DBLB18W) to induce photo-crosslinking. Further sample handling was done as described in section 4.3.

## 4.8. LC-MS/MS

Before MS measurements, the lyophilized peptides were resolved in 30 µL 1% FA and filtered through centrifugal filters (0.45 µm, VWR), which were equilibrated with 300 µL 1% FA. The filtrate was transferred into MS-vials and stored at -20 °C until the measurements were performed.

Samples were analyzed with an UltiMate 3000 nano HPLC system (Dionex) using an Acclaim C18 PepMap100 (75 µm ID x 2 cm) trap and an Aurora Series Emitter Column with Gen2 nanoZero fitting

(75 µm ID x 25 cm, 1.6 µm FSC C18) separation columns (both heated to 40 °C) in an EASY-spray setting coupled to a Q Exactive Plus mass spectrometer (ThermoFisher). 1-10 µL of peptide samples were loaded on the trap and washed with 0.1% TFA, then transferred to the analytical column (buffer A: $H_2O$ with 0.1% FA, buffer B: MeCN with 0.1% FA, flow 0.4 µL/min, gradient: 5% buffer B for 7 min, from 5% to 22% buffer B in 105 min, then to 32% buffer B in 10 min, to 90% buffer B in 10 min and hold at 90% buffer B for 10 min, then to 5% buffer B in 0.1 min and hold 5% buffer B for 9.9 min) and ionized at spray voltage of 2.0 kV and a capillary temperature of 275 °C. The Q Exactive Plus mass spectrometer was operated in a TOP12 data dependent mode with full scan acquisition in the orbitrap at a resolution of R = 140,000 and an AGC target of $3e^6$ in a scan range of 300 – 1500 m/z with a maximum injection time of 80 ms. Monoisotopic precursor selection as well as dynamic exclusion (dynamic exclusion duration: 60 s) was enabled. Precursors with charge states >1 and intensities greater than $1e^4$ were selected for fragmentation. Isolation was performed in the quadrupole using a window of 1.6 m/z. Precursors were analyzed in a scan range of 200 – 2000 m/z to an AGC target of $1e^5$ and a maximum injection time of 100 ms. Peptide fragments were generated by higher-energy collisional dissociation (HCD) with a normalized collision energy of 27% and detected in the orbitrap.

## 4.9. Data Evaluation

Raw files were analyzed using MaxQuant software (version 1.6.2.10).[5] The following settings were applied: fixed modification: carbamidomethylation (cysteine); variable modification: oxidation (methionine), acetylation (N-terminus), NH (aspartate); proteolytic enzyme: trypsin/P; missed cleavages: 2; main search tolerance: 4.5 ppm; MS/MS tolerance: 0.5 Da; false discovery rates: 0.01. The options “LFQ” and “match between runs” (0.7 min match and 20 min alignment time windows) were enabled.[6] Searches were performed against the Uniprot database for Homo sapiens. Statistical analysis of the data was performed using Perseus (version 1.6.2.3.).[7] Putative contaminants, reverse peptides and peptides only identified by site were deleted. Data was filtered for three valid values in at least one group and a missing value imputation was performed over the total matrix. LFQ intensities were $\log_2$-transformed and $-\log_{10}$ (p-values) were obtained by a two-sided two sample Student’s t-test.

## 4.10. Immunofluorescence Imaging

SH-SY5Y cells were cultured on glass coverslips and treated at 80% confluency with **FBP2** directly or following treatment with chloroquine or tamoxifen. In detail, cells were treated for 1 h with culture medium (DMEM/F12GlutaMAX supplemented with 10% FBS and 1:100 Antibiotic-Antimycotic) containing 20 µM of chloroquine or tamoxifen at 37 °C and 5% $CO_2$. Then, fresh medium was added to the cells containing 100 µM **FBP2** and cells were incubated for additional 1 h at 37 °C and 5% CO2. For control experiments, cells were treated with DMSO. For click chemistry and immunofluorescence, cells were fixed with 4% PFA at 4 °C and were permeabilized using 0.3% Triton X100 in 1x PBS for 5 min at RT. Cells were then incubated with click-chemistry staining mix (10 µM rhodamine-alkyne, 1 mM $CuSO_4$, 10 mM freshly prepared sodium ascorbate in 1x PBS) at RT for 2 h in the dark, followed by three washes with 1x PBS. Cells were subsequently blocked with 0.1% TWEEN, 10% Normal Goat Serum and 3% BSA diluted in 1x PBS.[8] Primary and secondary antibodies were diluted in blocking solution. Nuclei were visualized using 0.5 µg/mL 4,6-diamidino-2-phenylindole (DAPI, Sigma Aldrich). The antibodies used were mouse anti-LAMP1 1:50 (abcam ab25630) and goat anti-mouse Alexa Fluor 488 1:1000 (ThermoFisher Scientific, A28175). Immunostained cells were analysed using Leica SP8 confocal microscope. Different fields were taken using 63x objectives. Digital images were processed using Image J and Adobe Photoshop software.

# 5. Literature


1. Vizcaíno, J. A.; Csordas, A.; del-Toro, N.; Dianes, J. A.; Griss, J.; Lavidas, I.; Mayer, G.; Perez-Riverol, Y.; Reisinger, F.; Ternent, T.; Xu, Q.-W.; Wang, R.; Hermjakob, H. 2016 update of the PRIDE database and its related tools. *Nucleic acids research* **2016,** *44* (D1), D447-56.

2. Ortega-Munoz A.; Castro-Palomino Laria J.; Fyfe M. C. T. Lysine-specific demethylase 1 inhibitors and their use. WO2011035941 (A1), 2011.

3. Heydenreuter, W.; Kunold, E.; Sieber, S. A. Alkynol natural products target ALDH2 in cancer cells by irreversible binding to the active site. *Chemical communications (Cambridge, England)* **2015,** *51* (87), 15784–15787.

4. Bianchetti, C. M.; Yi, L.; Ragsdale, S. W.; Phillips, G. N. Comparison of apo- and heme-bound crystal structures of a truncated human heme oxygenase-2. *The Journal of biological chemistry* **2007,** *282* (52), 37624–37631.

5. Cox, J.; Mann, M. MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nature biotechnology* **2008,** *26* (12), 1367–1372.

6. Cox, J.; Hein, M. Y.; Luber, C. A.; Paron, I.; Nagaraj, N.; Mann, M. Accurate proteome-wide label-free quantification by delayed normalization and maximal peptide ratio extraction, termed MaxLFQ. *Molecular & cellular proteomics : MCP* **2014,** *13* (9), 2513–2526.

7. Tyanova, S.; Temu, T.; Sinitcyn, P.; Carlson, A.; Hein, M. Y.; Geiger, T.; Mann, M.; Cox, J. The Perseus computational platform for comprehensive analysis of (prote)omics data. *Nature methods* **2016,** *13* (9), 731–740.

8. Kielkowski, P.; Buchsbaum, I. Y.; Becker, T.; Bach, K.; Cappello, S.; Sieber, S. A. A Pronucleotide Probe for Live-Cell Imaging of Protein AMPylation. *Chembiochem : a European journal of chemical biology* **2020**, [early view].

# 6. NMR Spectra

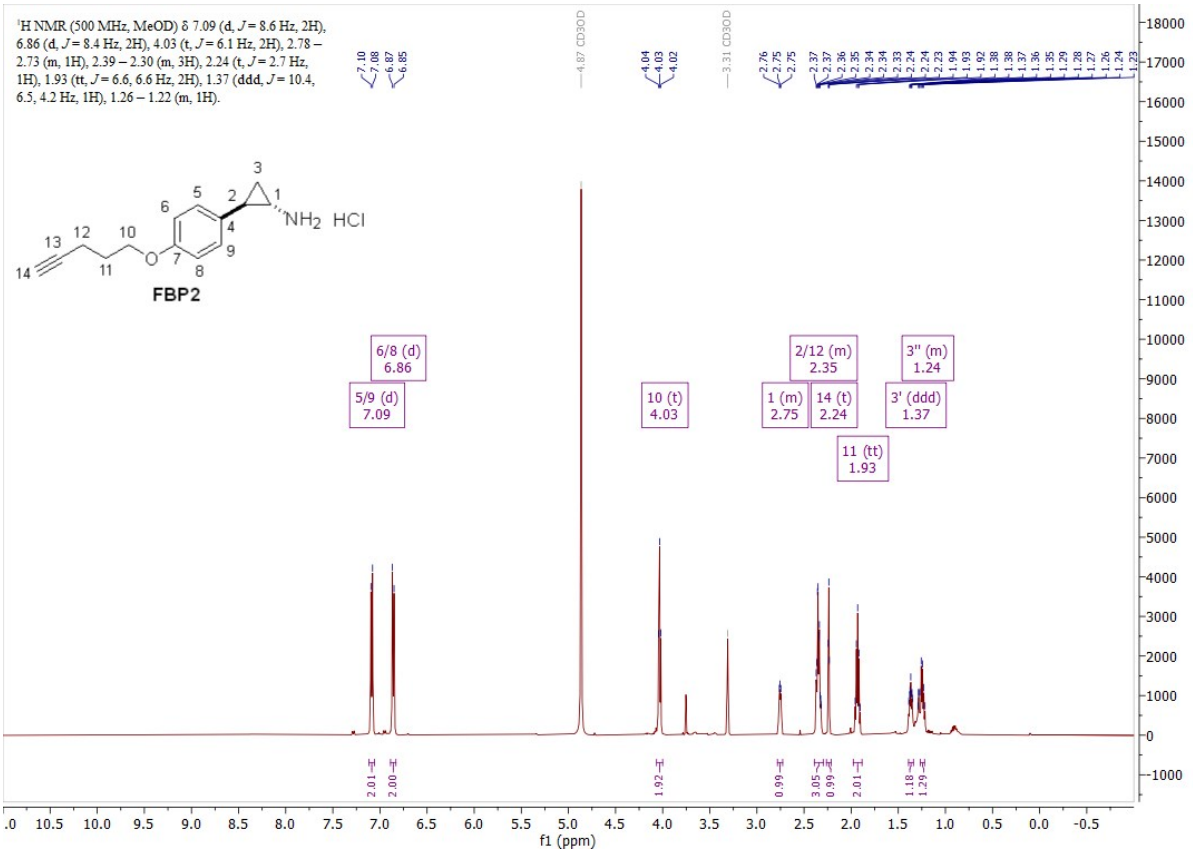



Figure S 8: $^1$H-NMR of **FBP2**.

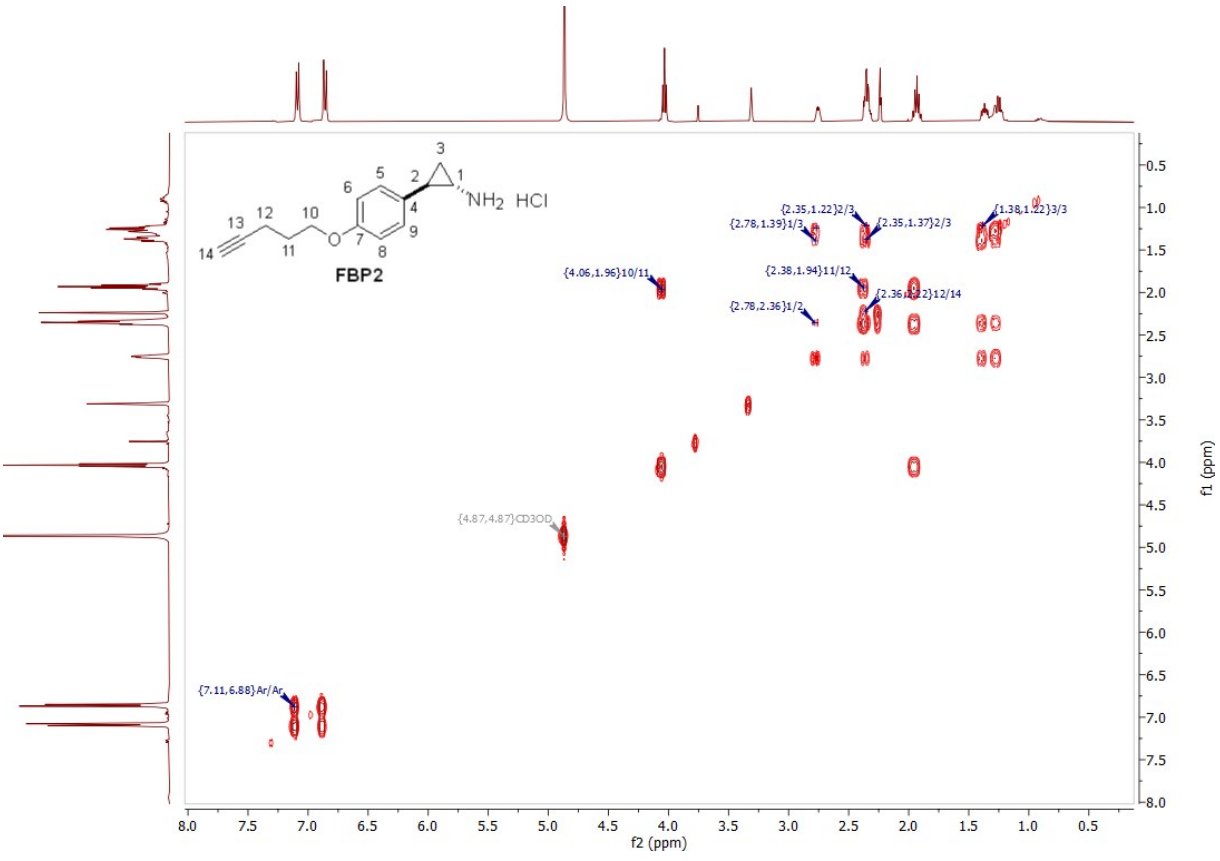



Figure S 9: COSY of **FBP2**.

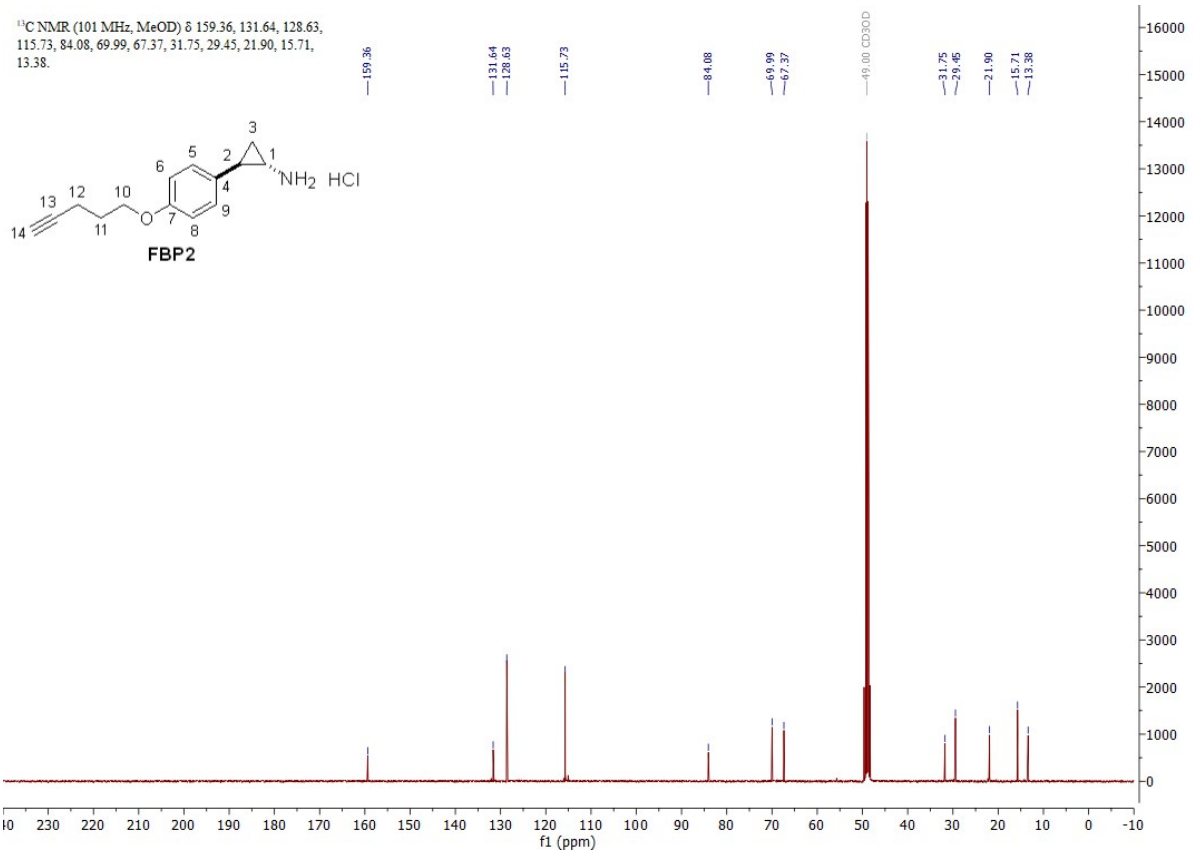



Figure S 10: $^{13}$C-NMR of **FBP2**.

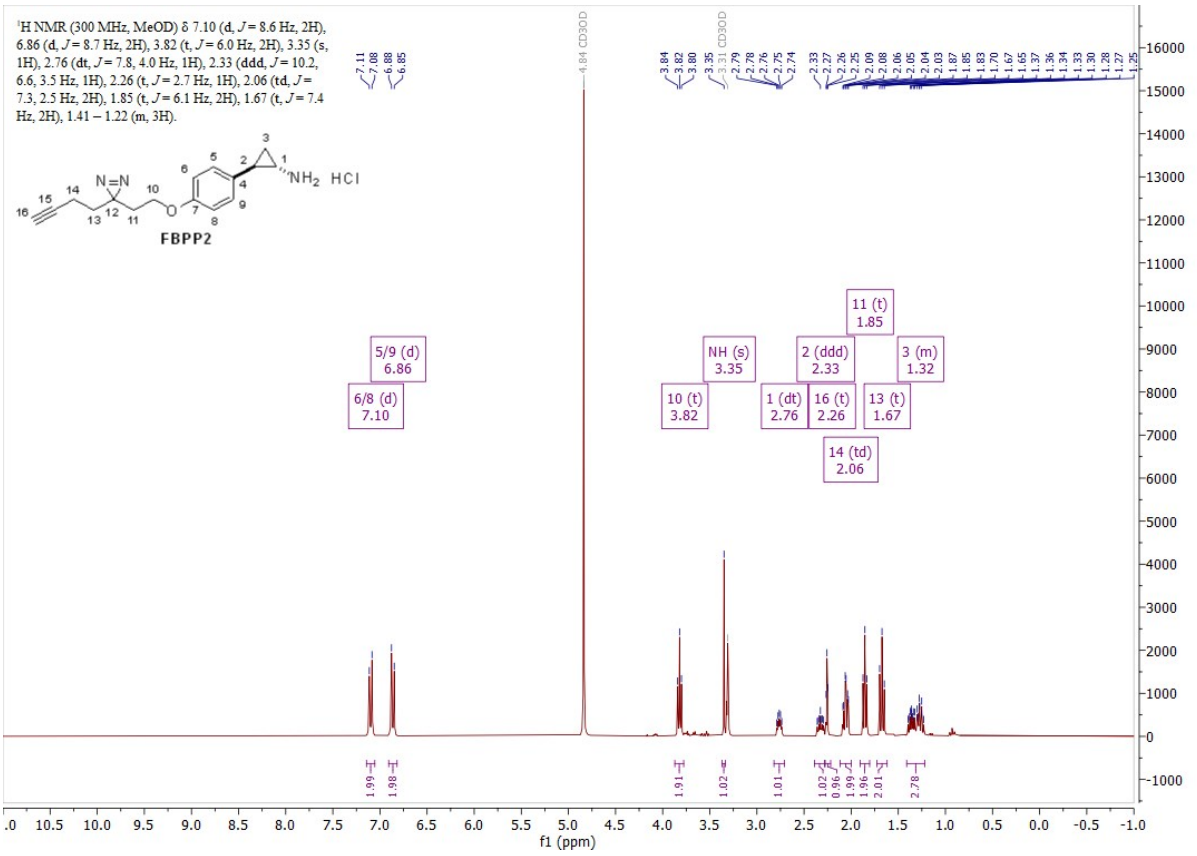



Figure S 11: $^{1}$H-NMR of **FBPP2**.

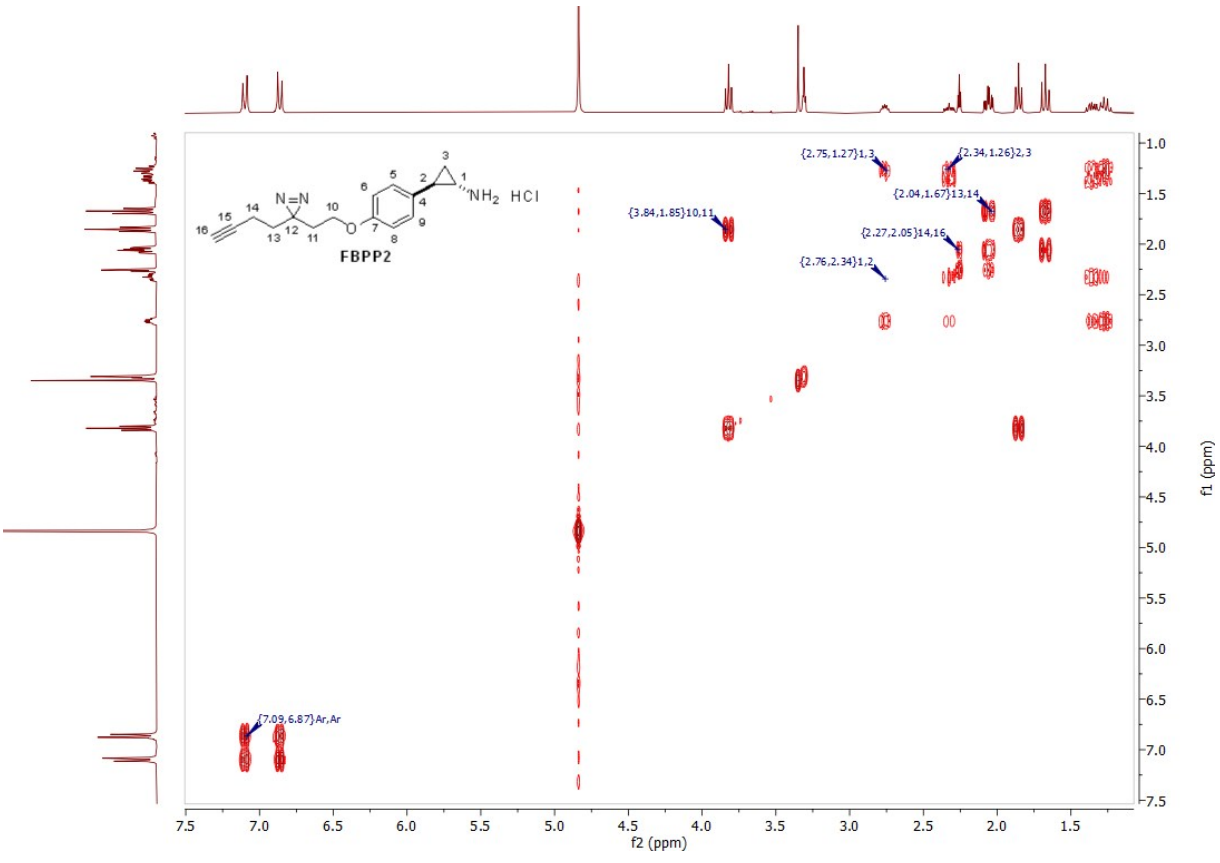



Figure S 12: COSY of **FBPP2**.

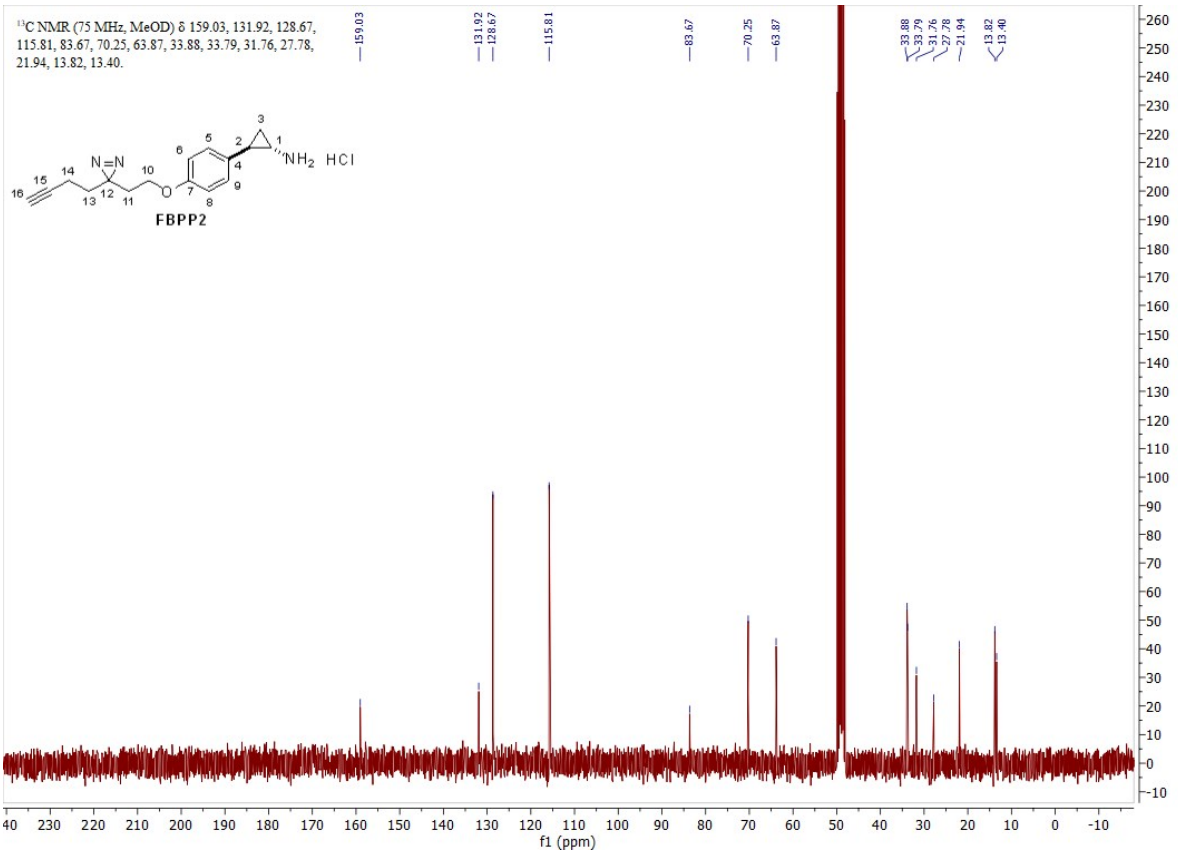



Figure S 13: $^{13}$C-NMR of **FBPP2**.