Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information (I)

Proteomic Profiling and Potential Cellular Target Identification of K11777, a Clinical Cysteine Protease Inhibitor, in *Trypanosoma brucei*

Peng-Yu Yang,[a] Min Wang,[b] Cynthia Y. He,[b] and Shao Q. Yao[a]*

[a]*Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543;* [b]*Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Singapore 117543.*

Corresponding author (S.Q.Y.: chmyaosq@nus.edu.sg)

1. General Procedures.

All chemicals were purchased as reagent grade and used without further purification, unless otherwise noted. Tetrahydrofuran (THF) was distilled over sodium benzophenone and used immediately. Dichloromethane (CH_2Cl_2) was distilled over CaH_2. All non-aqueous reactions were carried out under nitrogen atmosphere in oven-dried glassware. Reaction progress was monitored by TLC on pre-coated silica plates (Merck 60 F254, 250 μm thickness) and spots were visualized by basic $KMnO_4$, UV light or iodine. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker model Avance 300 MHz or DPX-300 MHz or DPX-500 MHz NMR spectrometer. Chemical shifts are reported in parts per million relative to internal standard tetramethylsilane ($Si(CH_3)_4$ = 0.00 ppm) or residual solvent peaks ($CHCl_3$ = 7.26 ppm).

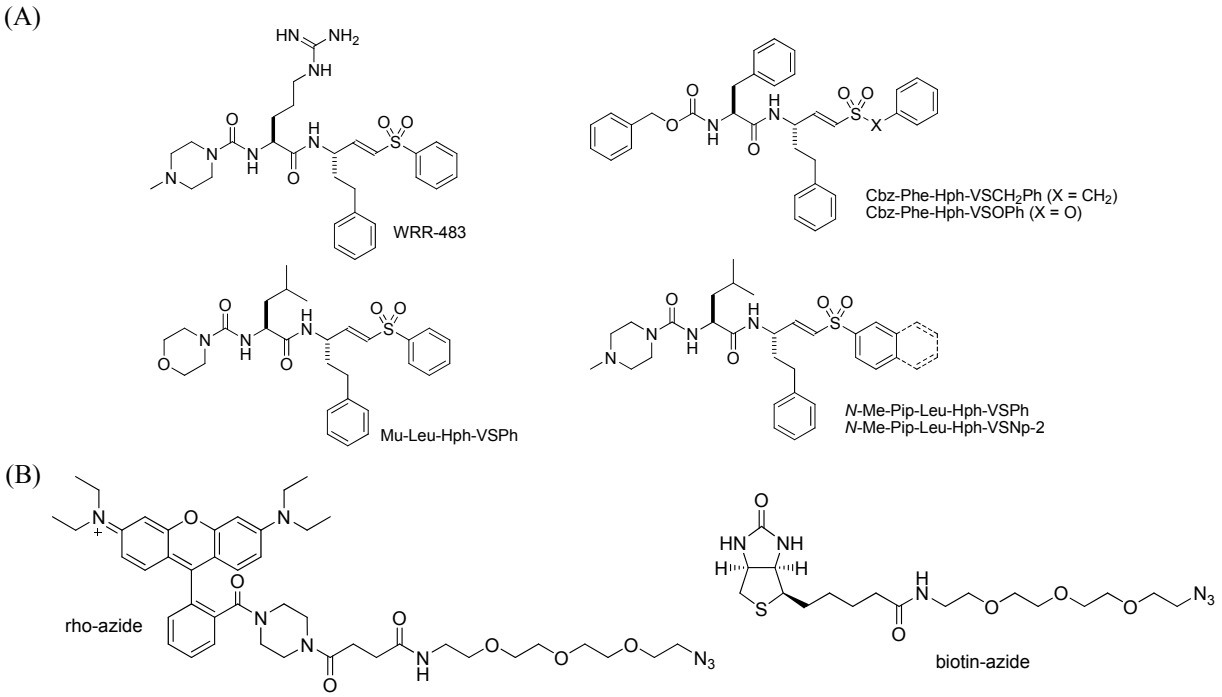
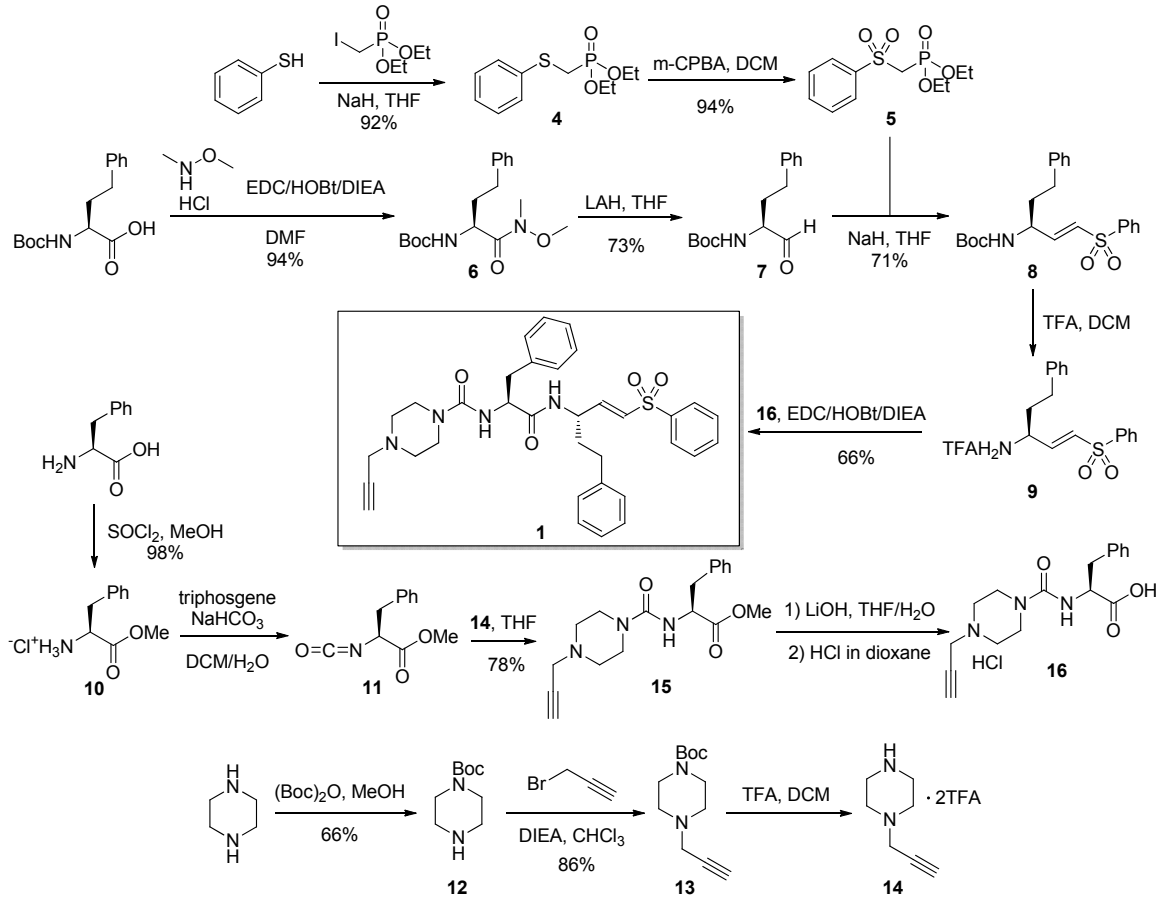


Fig. S1. (A) Structural representatives of vinyl sulfones which are anti-Trypanosomal agents (WRR-483,[1] Cbz-Phe-Hph-VSCH$_2$Ph[2] and Cbz-Phe-Hph-VSOPh[3]), or anti-malarial agents (Mu-Leu-Hph-VSPh,[4a,5] *N*-Me-Pip-Leu-Hph-VSPh,[4b,5] and *N*-Me-Pip-Leu-Hph-VSNp-2[4c,5]). (B) Structures of the two azide-containing reporter tags used in current study.[6]

2. Synthesis and Characterizations

2.1 Synthesis of compound **1** (**VS-1**).



Scheme S1. Synthesis of probe **1** (**VS-1**).

Diethyl phenylthiomethylphosphonate (4)

To a cooled (0 °C) suspension of hexane-washed NaH (60% in mineral oil; 1.0 g, 24 mmol) in dry THF (100 mL) was added benzenethiol (2.0 mL, 20 mmol) drop-wise via syringe. The mixture was stirred for an additional 30 min at 0 °C until effervescence ceased. Diethyl iodomethylphosphonate (4.0 mL, 22 mmol) was added and the mixture was stirred for 12 h. A cold HCl solution (1 M) was added to break up the gelatinous emulsion until *p*H 6~7 was reached. Upon concentration *in vacuo*, the reaction was diluted with H_2O (150 mL) and extracted with EtOAc (3 × 50 mL). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash column chromatography (silica gel; using 20 to 50% EtOAc in hexanes) gave the product **4** as a colorless liquid (4.79 g, 92%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.30 (t, *J* = 7.1, 6H), 3.20 (d, *J* = 14.0, 2H), 4.09-4.20 (m, 4H), 7.20-7.33 (m, 3H), 7.42-7.46 (m, 2 H).

Diethyl phenylsulfonylmethylphosphonate (5)

To a solution of compound **4** (5.0 g, 19.2 mmol) in CH_2Cl_2 (100 mL) at 0 °C was added *m*-chloroperbenzoic acid (12.9 g of 77% *m*-CPBA, 57.2 mmol) over 1 h. The mixture was stirred overnight while being warmed to room temperature. The solution was then cooled to 0 °C and treated with NaOH (2 M) until *p*H 8~9. The organic phase was separated, dried over Na_2SO_4, filtered, and concentrated to dryness, giving the product **5** as a colorless oil (5.6 g, 94%). ^{1}H NMR (300

MHz, $CDCl_3$): δ 1.30 (t, *J* = 7.1 Hz, 6H), 3.77 (d, *J* =17.0 Hz, 2H), 4.11-4.21(m, 4 H), 7.55-7.61 (m, 2 H), 7.65-7.71 (m, 1 H), 7.98-8.01 (m, 2 H).

(*S*)-tert-butyl [1-(methoxymethylcarbamoyl)-3-phenylpropyl]carbamate (6)

To a solution of (*S*)-Boc-Homophenylalanine (5.59 g, 20 mmol) in dry THF (100 mL) at 0 °C was added EDC (4.60 g, 24 mmol), HOBt (3.24 g, 12 mmol), *N*,*O*-dimethylhydroxylamine hydrochloride (2.34 g, 24 mmol) and DIPEA (5.2 mL, 30 mmol). The reaction was stirred at room temperature for 12 h, and concentrated *in vacuo*. Upon dilution with H_2O (150 mL) and extraction with EtOAc (3 × 50 mL), the combined organic extracts were washed with 1 wt% HCl, 20 wt% Na_2CO_3 and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (silica gel; using 20 to 50% EtOAc in hexanes), giving Boc-Hph-N(Me)OMe **(6)** as a white solid (6.20 g, 96%). ^{1}H NMR (300 MHz, $CDCl_3$) δ:1.45 (s, 9H), 1.80-2.02 (m, 1H), 2.72 (m, 1H), 3.16 (s, 3H), 3.62 (s, 3H), 4.68 (br s, 1H), 5.23 (m, 1H), 7.15-7.31 (m, 5H).

(*S*)-tert-butyl (1-formyl-3-phenylpropyl)carbamate (Boc-Homophenylalaninal, Boc-HphH, 7)

To a solution of **6** (3.2 g, 10 mmol) in dry THF (50 mL) at 0 °C was added $LiAlH_4$ (0.45 g, 12 mmol) over 10 min, with vigorous stirring. The mixture was stirred for an additional 20 min at 0 °C, whereupon cold water was carefully added until effervescence ceased. A cold HCl solution (1 M) was added to break up the gelatinous emulsion until *p*H 6~7. Upon dilution with H_2O (150 mL) and extraction with EtOAc (3 × 50 mL), the combined organic extracts were washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash column chromatography (silica gel; using 20 to 50% EtOAc in hexanes) provided the product **7** as a white solid (1.92 g, 73%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46 (s, 9H), 1.83-1.95 (m, 1H), 2.22 (m, 1H), 2.67 (t, *J* = 7.6 Hz, 2H), 4.24 (m, 1H), 5.09 (br s, 1H), 7.17-7.32 (m, 5H), 9.55 (s, 1H).

(*S*)-tert-butyl (3-benzenesulfonyl-1-phenethylallyl)carbamate (Boc-HphVSPh, 8)

To a cooled (0°C) suspension of hexane-washed NaH (60% in mineral oil; 0.24 g, 6 mmol) in dry THF (50 mL) was added drop-wise **5** (1.61 g, 5.5 mmol) in dry THF (10 mL) via syringe. The mixture was stirred for an additional 30 min at 0 °C and **7** (1.32 g, 5 mmol) in dry THF (10 mL) was added drop-wise. The stirring was continued for 1 h, before a cold 5 wt% $NaHSO_4$ solution was added to break up the gelatinous emulsion until *p*H 6~7. The solution was concentrated *in vacuo*, diluted with water (100 mL) and extracted with EtOAc (3 × 25 mL). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated under vacuum. Purification by flash column chromatography (silica gel; using 20 to 50% EtOAc in hexanes) provided the product **8** as a white foam (1.4 g, 70%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.40 (s, 9H), 1.83-1.94 (m, 2H), 2.62-2.70 (m, 2H), 4.36 (br s, 1H), 4.52 (br s, 1H), 6.43 (br d, *J* = 14.5 Hz, 1H), 6.87-6.90 (m, 1H), 7.13-7.30 (m, 5H), 7.51-7.61 (m, 3H), 7.61 (d, *J* = 6.0 Hz, 2H); LC-IT-TOF/MS (m/z) calcd for $C_{22}H_{27}NO_4S$ $[M+Na]^+$: 424.1661, Found: 424.1575.

(*S*)-3-benzenesulfonyl-1-phenethylallylamine trifluoroacetate (TFA•HphVSPh, 9)

To a cooled (0°C) solution of **8** (1.2 g, 3 mmol) in CH_2Cl_2 (15 mL) was added drop-wise TFA (5 mL) *via* syringe. After stirring for 2 h, the reaction was added Et_2O (100 mL). The precipitate was filtered off, washed twice with Et_2O, and finally dried *in vacuo* to give **9** (0.95 g; 76%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.34 (br s, 2H), 1.76-1.86 (m, 2H), 2.68 (t, *J* = 7.9 Hz, 2H), 3.53 (m, 1H), 6.49 (m, 1H), 6.98 (dd, *J* = 5.6, 14.9 Hz, 1H), 7.12-7.30 (m, 5H), 7.51-7.56 (m, 3H), 7.86 (d, *J* = 7.3 Hz, 2H). This material was pure enough to be used in the next step without further purification.

(*S*)-*N*-(4-chlorobenzylidene)phenylalanine methyl ester (HCl•Phe-OMe, 10)

To a cooled (0 °C) suspension of phenylalanine (16.5 g, 100 mmol) in dry MeOH (150 mL) was added drop-wise $SOCl_2$ (9 mL, 120 mmol) over 1 h. The mixture was kept cool in an ice-bath throughout the whole duration in order to

keep the temperature < 5 °C. The clear solution was stirred for 12 h and subsequently heated at 50 °C for 2 h. Upon evaporation of the solvent under reduced pressure, Et_2O (100 mL) was added with stirring. The precipitate was filtered off, washed twice with ether, and finally dried *in vacuo* to give **10** (21.6 g; 100 %) as a white solid. This material was pure enough to be used in the next step without further purification.

Methyl (*S*)-2-isocyanato-3-phenylpropanoate (OCN-PheOMe, 11)

To a solution of **10** (5.5 g, 25.5 mmol) in CH_2Cl_2 (50 mL) at 0 °C was added saturated aqueous $NaHCO_3$ (50 mL) and triphosgene (2.52 g, 8.42 mmol) in a single portion with vigorous stirring. The reaction mixture was stirred at 0 °C for 15 min and then poured into a 250-mL separatory funnel. The organic layer was collected, and the aqueous layer is extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), vacuum filtered, and concentrated at reduced pressure using a rotary evaporator to give the product **11** as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.03 (dd, J = 7.8, 13.8 Hz, 1H), 3.16 (dd, J = 4.8, 13.6 Hz, 1H), 3.81 (s, 3 H), 4.27 (dd, J = 4.61, 7.8 Hz, 1H), 7.18-7.21 (m, 2H), 7.27-7.36 (m, 3H). This material was used in the next step without further purification, assuming a quantitative yield.

***tert*-Butyl 1-piperazinecarboxylate (12)**

To a solution of di-*tert*-butyl dicarbonate (5.80 g, 25.54 mmol) in 50 mL of dry MeOH was added drop-wise a solution of piperazine (4.0 g, 46.44 mmol) in 100 mL of dry MeOH at 0 °C. After 30 min, the mixture was warmed to room temperature and the reaction was continued for 2 d. Upon concentration under reduced pressure, the crude solid was dissolved in 200 mL of Et_2O, and the left-over white precipitate was filtered off. The aqueous solution obtained by extracting the organic solution with 1 M citric acid (aq) (3 × 100 mL) was washed with EtOAc (3 × 100 mL) and brought to *p*H ~ 11 by adding solid K_2CO_3. The turbid solution was extracted with EtOAc (3 × 100 mL) and dried over Na_2SO_4. The solution was concentrated under reduced pressure at 40 °C and stripped with CH_2Cl_2 to yield a clear oil which was recrystallized into a white solid upon drying under reduced pressure. Yield: 71%; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.45-3.33 (m, 4H), 2.88-2.74 (m, 4H), 1.57 (s, 1H), 1.46 (s, 9H).

***tert*-Butyl 4-propargylpiperazine-1-carboxylate (13)**

To a solution of **12** (1.86 g, 10 mmol) and diisopropylethylamine (1.9 mL, 11 mmol) in $CHCl_3$ (50 mL) at 0 °C was added drop-wise a solution of propargyl bromide (80% in toluene, 1.2 mL, 10mmol) in $CHCl_3$ (50 mL). After the mixture was stirred for 24 h at room temperature, the solution obtained was washed with 5% $NaHCO_3$ (3 × 50 mL), brine (2 × 50 mL), and then dried over Na_2SO_4. The solution was filtered and evaporated to provide a brown oil. Purification by flash column chromatography (silica gel; using 50% EtOAc in hexanes) provided the product **13** as a yellow oil (1.4 g, 86%), which ultimately crystallized upon standing. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.49 (s, 9H), 2.26 (t, J = 2.5 Hz, 1H), 2.51 (t, J = 5.0 Hz, 4H), 3.32 (d, J = 2.55 Hz, 2H), 3.47 (t, J = 5.0 Hz, 4H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 29.10, 47.67, 52.32, 74.10, 79.10, 80.40, 155.39.

***N*-Propargylpiperazine•TFA salt (14)**

To a solution of **13** (1.1 g, 5 mmol) in CH_2Cl_2 (25 mL) at 0 °C was added trifluoroacetic acid (25 mL). The solution was stirred at room temperature overnight, and then evaporated to dryness *in vacuo*. The residue was suspended in 20 mL of THF and used immediately in the next step without further purification.

(S)-methyl 3-phenyl-2-(4-(prop-2-yn-1-yl)piperazine-1-carboxamido)propanoate (15)

To a solution of **14** (0.7 g, 2 mmol) in dry THF (10 mL) at 0 °C was added drop-wise a solution of DIEA (0.7 mL, 4 mmol) in 10 mL of dry THF. After 10 min, a solution of **11** (0.68 g, 2.4 mmol) in dry THF (10 mL) was added. The mixture was stirred for 12 h and concentrated *in vacuo* to give a brown oil, which was subsequently diluted with water

(100 mL) and extracted with EtOAc (3 × 50 mL). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash column chromatography (silica gel; using 5 to 10% methanol in CH_2Cl_2) provided the product **15** as a white solid (0.53 g, 80%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.49-2.56 (m, 4H), 3.08-3.16 (m, 2H), 3.31-3.43 (m, 6H), 3.72 (s, 3H), 4.77-4.80 (m, 1H), 4.81-4.91 (m, 1H), 7.10-7.11 (m, 2H), 7.23-7.30 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 38.97, 44.24, 47.53, 52.02, 52.87, 55.00, 74.23, 78.88, 127.68, 129.17, 129.96, 136.87, 157.07, 173.74; LC-IT-TOF/MS (m/z) calcd for $C_{18}H_{23}N_3O_3$ $[M+Na]^+$: 352.1739, Found: 352.1738.

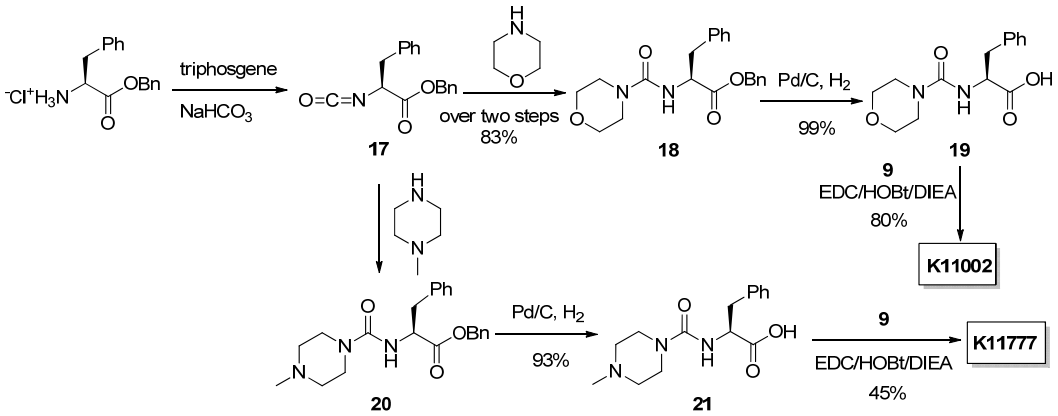
(*S*)-3-phenyl-2-(4-(prop-2-yn-1-yl)piperazine-1-carboxamido)propanoic acid hydrochloride (16)

To a solution of **15** (0.6 g, 1.8 mmol) in THF (30 mL) at 0 °C was added drop-wise a solution of LiOH•H_2O (0.23 g, 5.5 mmol) in 10 mL of H_2O. The mixture was stirred for 4 h, and 4 N HCl in dioxane was then added slowly to adjust the *p*H of the mixture to ~ 2 at 0 °C. The resulting solution was evaporated *in vacuo*. The residue was washed with Et_2O (2 × 25 mL), dried *in vacuo*, and then lyophilized overnight to give the crude product **16**, along with a small amount of LiCl, which was used directly in the following reaction without further purification, assuming a quantitative yield.

***N*-((*S*)-1-oxo-3-phenyl-1-(((*S*,*E*)-5-phenyl-1-(phenylsulfonyl)pent-1-en-3-yl)amino)propan-2-yl)-4-(prop-2-yn-1-yl)piperazine-1-carboxamide (1)**

To a solution of **16** (215 mg, 0.6 mmol) in DMF (5 mL) was added EDC/HCl (115 mg, 0.6 mmol), HOBt (81 mg, 0.6 mmol) and DIEA (0.4 mL, 2.4 mmol). After 10 min, TFA•HphVSPh (**9**; 208 mg, 0.5 mmol) in DMF (5 mL) was added drop-wise. The reaction was stirred at rt for 21 h. The resulting solution was evaporated *in vacuo* to give a brown oil, which was diluted with water (50 mL) and extracted with DCM (3 × 50 mL). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash column chromatography (silica gel; using 5 to 10% methanol in CH_2Cl_2) provided the product **1** as a white solid (165 mg, 55%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.72-1.75 (m, 1H), 1.82-1.84 (m, 1H), 2.44-2.56 (m, 7H), 3.01 (d, *J* = 7.6 Hz, 2H), 3.27-3.37 (m, 6H), 4.54-4.60 (m, 2H), 5.13 (d, *J* = 7.6 Hz, 1H), 6.09 (dd, *J* = 1.2, 15.1 Hz, 1H), 6.78 (dd, *J* = 4.9, 15.1 Hz, 1H), 6.87 (d, *J* = 8.3 Hz, 1H), 7.02 (d, *J* = 7.3 Hz, 2H), 7.11-7.24 (m, 8H), 7.54 (t, *J* = 7.6 Hz, 2H), 7.62 (t, *J* = 7.6 Hz, 1H), 7.84 (d, J = 7.6 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 31.77, 35.70, 36.49, 38.48, 43.71, 46.82, 49.10, 51.26, 56.01, 73.66, 78.14, 126.25, 127.14, 127.66, 128.37, 128.56, 128.72, 129.26, 129.30, 130.48, 133.47, 136.66, 140.23, 140.44, 145.66, 156.90, 171.86; LC-IT-TOF/MS (m/z) calcd for $C_{34}H_{38}N_4O_4S$ $[M+H]^+$: 599.2614, Found: 599.2545.

2.2 Synthesis of compounds **K11002** and **K11777**



Scheme S2. Synthesis of **K11002** and **K11777**.

(*S*)-benzyl-2-isocyanato-3-phenylpropionate (OCN-PheOBzl, 17)[7]

To a solution of (*S*)-Benzyl-2-amino-3-phenylpropionate hydrochloride (HCl•PheOBzl) (3.72 g, 12.75 mmol) in CH_2Cl_2 (50 mL) at 0 °C was added saturated aqueous $NaHCO_3$ (50 mL) and triphosgene (1.25 g, 4.21 mmol) in a single portion with vigorous stirring. The reaction mixture was stirred at 0 °C for 15 min and then poured into a 250-mL separatory funnel. The organic layer was collected, and the aqueous layer is extracted with CH_2Cl_2 (3 × 15 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), vacuum filtered, and concentrated at reduced pressure using a rotary evaporator to give a colorless oil. The product, OCN-PheOBzl was used in the next step without further purification, assuming a quantitative yield.

(*S*)-benzyl 2-(morpholine-4-carboxamido)-3-phenylpropanoate (18)[7]

To a solution of **19** (3.59 g, 12.75 mmol) in dry THF (50 mL) at 0 °C was added morpholine (1.1 mL, 12.75 mmol). The mixture was stirred for 1 h and was concentrated in vacuo to a pale orange oil, and diluted with water (100 mL) and extracted with EtOAc (3 × 50 mL). The combined organic extracts were washed with HCl (1 M), saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated under vacuum. Purification by flash column chromatography (silica gel) using 10 to 20% EtOAc in hexanes to give the product (*S*)-benzyl 2-(morpholine-4-carboxamido)-3-phenylpropanoate (Mu-PheOBzl, **18**) as a white solid (3.9 g, 83% over two steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.11 (d, *J* = 5.3 Hz, 2H), 3.27-3.31 (m, 4H), 3.62-3.65 (m, 4H), 4.81-4.90 (m, 2H), 5.15 (dd, *J* = 12.3, 27.8 Hz, 2H), 6.99 (dd, *J* = 3.5, 7.0 Hz, 2H), 7.19-7.22 (m, 3H), 7.29-7.38 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.22, 43.92, 54.28, 66.41, 67.20, 127.01, 128.51, 128.58, 129.34, 135.19, 136.01, 156.66, 172.43.

(*S*)-2-(morpholine-4-carboxamido)-3-phenylpropanoic acid (19)[7]

A solution of Mu-PheOBzl (**18**) (3.9 g, 10.6 mmol) in 1% HOAc/ethanol (100 mL) was charged with 10% palladium on active charcoal (Aldrich: 0.4 g). The solution in the Parr bottle was exposed to hydrogen on a Parr shaker (50 psi) for 12 h, filtered through Celite, and concentrated *in vacuo*. The residue was triturated with ether (100 mL) to remove residual ethanol and was reprecipitated from CH_2Cl_2/ether to give 2.94 g (99%) of (*S*)-2-(morpholine-4-carboxamido)-3-phenylpropanoic acid (Mu-PheOH, **19**). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.86-2.94 (m, 1H), 3.00-3.16 (m, 1H), 3.18-3.28 (m, 4H), 3.41-3.48 (m, 4H), 4.19-4.27 (m, 1H), 6.72 (d, *J* = 8.2 Hz, 1H), 7.17-7.30 (m, 5H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 36.60, 43.94, 65.87, 126.24, 128.08, 129.15, 138.44, 157.37, 174.24. Without further purification, the mixture was used directly in the next step.

***N*-((*S*)-1-oxo-3-phenyl-1-(((*S*,*E*)-5-phenyl-1-(phenylsulfonyl)pent-1-en-3-yl)amino)propan-2-yl)morpholine-4-carboxamide (K11002)**

Prepared according to the similar procedure mentioned above by using **19** (290 mg, 1.04 mmol), **9** (420 mg, 1.0 mmol), EDC/HCl (190 mg, 1.0 mmol), HOBt (140 mg, 1.0 mmol) and DIEA (0.34 mL, 2 mmol) in DMF (5 mL). Purification by flash column chromatography (silica gel) using 25 to 50% EtOAc in hexanes to give K11002 as a white solid (450 mg, 80%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.74-1.90 (m, 2H), 2.55-2.60 (m, 2H), 3.25 (d, *J* = 4.1 Hz, 2H), 3.25-3.34 (m, 4H), 3.59-3.65 (m, 4H), 4.51 (m, 1H), 4.62 (m, 1H), 5.06 (m, 1H), 6.10 (dd, *J* = 1.65, 15.1 Hz, 1H), 6.79 (dd, *J* = 4.85, 15.1 Hz, 1H), 7.07 (d, *J* = 7.65 Hz, 2H), 7.15-7.28 (m, 8H), 7.57 (t, *J* = 7.8 Hz, 2H), 7.65 (t, *J* = 7.4 Hz, 1H), 7.87 (d, *J* = 7.5 Hz, 2H); LC-IT-TOF/MS (m/z) calcd for $C_{31}H_{35}N_3O_5S$ $[M+H]^+$: 562.2297, Found: 562.2629.

(*S*)-benzyl 2-(4-methylpiperazine-1-carboxamido)-3-phenylpropanoate (20)[7]

Prepared according to the similar procedure mentioned above by using (*S*)-benzyl-2-amino-3-phenylpropionate hydrochloride (HCl•PheOBzl) (5.84 g, 20 mmol), triphosgene (1.98 g, 6.67 mmol), and *N*-methylpiperazine (2.2 mL, 20 mmol). Purification by flash column chromatography (silica gel) using 5 to 10% methanol in DCM to give the

product [(*S*)-benzyl-2-[(4-methylpiperazine-1-carbonyl)amino]-3-phenylpropionate, MePip-PheOBzl, **20**) as a pale orange oil.

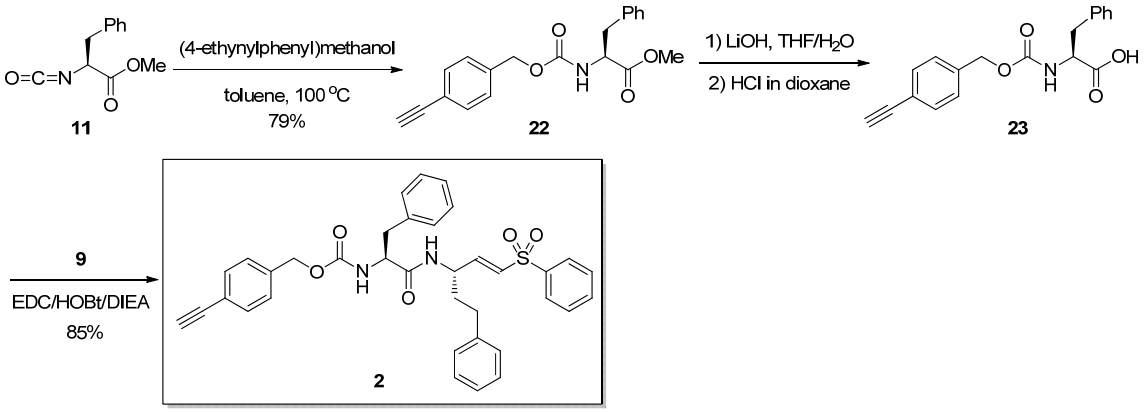
(*S*)-2-(4-methylpiperazine-1-carboxamido)-3-phenylpropanoic acid (21)[7]

Prepared according to the similar procedure mentioned above by using MePip-PheOBzl (**20**) (7.5 g, 19.7 mmol), 10% Pd/C (0.75 g) in 1% HOAc/ethanol (50 mL) under 50 psi for 12 h. The compound was obtained as white solid (5.61 g, 98%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.15 (s, 3H), 2.18 (m, 4H), 2.84-2.94 (dd, J = 10.8, 15.1 Hz, 1H), 2.95-3.04 (dd, J = 5.0, 15.1 Hz, 1H), 3.14-3.30 (m, 4H), 4.17 (m, 1H), 6.65 (d, J = 8.0 Hz, 1H), 7.17-7.27 (m, 5H).

4-Methyl-*N*-((*S*)-1-oxo-3-phenyl-1-(((*S*,*E*)-5-phenyl-1-(phenylsulfonyl)pent-1-en-3-yl)amino)propan-2-yl)piperazine-1-carboxamide (K11777)[7]

Prepared according to the similar procedure mentioned above by using **21** (291 mg, 1.0 mmol), **9** (420 mg, 1.0 mmol), EDC/HCl (190 mg, 1.0 mmol), HOBt (140 mg, 1.0 mmol) and DIEA (0.34 mL, 2 mmol) in DMF (5 mL). Purification by flash column chromatography (silica gel) using 5 to 10% methanol in DCM to give K11777 as a white solid (260 mg, 45%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.76-1.88 (m, 2H), 2.27-2.35 (m, 7H), 2.55-2.60 (m, 2H), 3.05 (d, J = 7.5 Hz, 2H), 3.29-3.37 (m, 4H), 4.51 (m, 1H), 4.63 (m, 1H), 5.02 (m, 1H), 6.12 (dd, J = 1.55, 15.2 Hz, 1H), 6.79 (dd, J = 4.95, 15.1 Hz, 1H), 7.07 (d, J = 7.6 Hz, 2H), 7.15-7.30 (m, 8H), 7.57 (t, J = 7.9 Hz, 2H), 7.65 (t, J = 7.25 Hz, 1H), 7.87 (d, J = 1.3 Hz, 2H); LC-IT-TOF/MS (m/z) calcd for $C_{32}H_{38}N_4O_4S$ $[M+H]^+$: 575.2614, Found: 575. 2600.

2.3 Synthesis of compound **2** (**VS-2**)



Scheme S3. Synthesis of probe **2** (**VS-2**).

(*S*)-methyl 2-((((4-ethynylbenzyl)oxy)carbonyl)amino)-3-phenylpropanoate (22)

To a solution of **11** (2.46 g, 12 mmol) in anhydrous toluene (25 mL) was added (4-ethynylphenyl)methanol (1.32 g, 10 mmol). The resulting solution was heated to 100 °C for 6 h and concentrated *in vacuo* to give a pale orange oil, which was diluted with water (100 mL) and extracted with ether (3 × 50 mL). The combined organic extracts were washed with HCl (1 M), saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash column chromatography (silica gel; using 10% EtOAc in hexanes) provided **22** as a white solid (2.65 g, 79%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.03-3.16 (m, 2H), 3.72 (s, 3H), 4.62-4.69 (m, 1H), 5.03-5.12 (m, 2H), 5.34 (br d, J = 12.65 Hz, 2H), 7.08-7.11 (m, 2H), 7.21-7.31 (m, 5H), 7.46 (d, J = 13.7 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.90, 53.01, 55.49, 67.02, 78.19, 83.98, 122.60, 127.85, 128.46, 129.30, 129.92, 132.93, 136.32, 137.71, 156.12, 172.59.

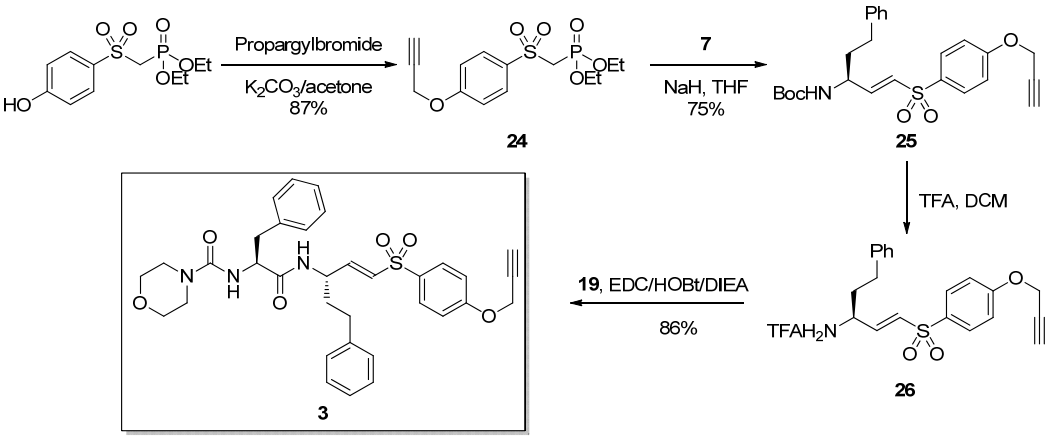
(*S*)-2-((((4-ethynylbenzyl)oxy)carbonyl)amino)-3-phenylpropanoic acid (23)

To a solution of **15** (3.4 g, 10 mmol) in THF (60 mL) at 0 °C was added drop-wise an aqueous solution of LiOH (0.72 g, 30 mmol) in 20 mL of H_2O. The reaction was stirred for 2 h) of the starting ester, then acidified with 2 M HCl (to *p*H 2) and extracted with EtOAc (3 × 50 mL). Upon drying over Na_2SO_4, filtration and evaporation of the organic phase, the compound was used directly in the following reaction without further purification (assuming quantitative yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.84 (m, 1H), 2.88-3.11 (m, 1H), 4.17-4.23 (m, 2H), 4.99 (s, 2H), 7.20-7.31 (m, 2H), 7.45 (d, J = 13.45 Hz, 2H), 7.68 (d, J = 13.95 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 36.49, 55.52, 64.73, 80.86, 83.27, 121.01, 126.39, 127.54, 128.18, 129.09, 131.65, 137.86, 138.00, 155.88, 173.26.

4-Ethynylbenzyl((*S*)-1-oxo-3-phenyl-1-(((*S*,*E*)-5-phenyl-1-(phenylsulfonyl)pent-1-en-3-yl)amino)propan-2-yl)carbamate (2)

Prepared according to the same procedure mentioned above by using **23** (162 mg, 0.5 mmol), **9** (208 mg, 0.5 mmol), EDC/HCl (115 mg, 0.6 mmol), HOBt (81 mg, 0.6 mmol) and DIEA (0.2 mL, 1.2 mmol) in DMF (5 mL). Purification by flash column chromatography (silica gel; using 20% EtOAc in hexanes) provided **2** as a white solid (258 mg, 85%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.74-1.79 (m, 1H), 1.86-1.89 (m, 1H), 2.52-2.57 (m, 2H), 2.95-3.03 (m, 2H), 3.09 (s, 1H), 4.27 (m, 1H), 4.64 (dd, J = 3.5, 5.0 Hz, 1H), 5.05 (s, 2H), 5.23 (br d, J = 6.25 Hz, 1H), 5.79 (br d, J = 7.55 Hz, 1H), 6.04 (dd, J = 0.9, 15.1 Hz, 1H), 6.75 (dd, J = 4.7, 15.1 Hz, 1H), 7.03 (d, J = 7.2 Hz, 2H), 7.11 (d, J = 7.1 Hz, 2H), 7.16-7.28 (m, 8H), 7.44 (d, J = 8.1 Hz, 2H), 7.55 (t, J = 7.8 Hz, 2H), 7.63 (t, J = 7.35 Hz, 1H), 7.85 (d, J = 7.65 Hz, 2H); LC-IT-TOF/MS (m/z) calcd for $C_{36}H_{34}N_2O_5S$ $[M+H]^+$: 607.2188, Found: 607. 2078.

2.4 Synthesis of compound **3** (**VS-3**)



Scheme S4. Synthesis of probe **3** (**VS-3**)

Diethyl (((4-(prop-2-yn-1-yloxy)phenyl)sulfonyl)methyl)phosphonate (24)

A mixture of 4-hydroxy-thiophenyl-methyl-diethylphosphonate sulfone[8] (3.08 g, 10 mmol) and anhydrous K_2CO_3 (1.66 g, 12 mmol) in dry acetone (50 mL) was stirred at rt for 2 h. 80% of propargyl bromide in toluene (1.25 mL, 11 mmol) was added drop-wise. The mixture was then stirred for 12 h, and TLC analysis indicated all the starting materials had been consumed. Upon removal of acetone under reduced pressure, the reaction mixture was poured into water (50 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layer was washed successively with 1 M HCl, water, and brine. Upon drying over Na_2SO_4, filtration and evaporation of the organic phase, the compound was purified by flash column chromatography (silica gel; using 50% EtOAc in hexanes) provided **24** as a white solid (3.01 g, 87%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.30 (t, J = 7.0 Hz, 6H), 2.57 (t, J = 2.4 Hz, 1H), 3.74 (d, J = 16.8 Hz, 2H), 4.15 (m, 4H), 4.78 (d, J = 2.4 Hz, 2H), 7.10-7.23 (m, 2H), 7.93-7.95 (m, 2H).

(*S*,*E*)-tert-butyl (5-phenyl-1-((4-(prop-2-yn-1-yloxy)phenyl)sulfonyl)pent-1-en-3-yl)carbamate (25)

Prepared according to the same procedure mentioned above by using **24** (3.0 g, 8.67 mmol), **7** (2.1 g, 7.88 mmol), and NaH (60% in oil, 0.38 g, 9.5 mmol) in anhydrous THF (100 mL). Purification by flash column chromatography (silica gel; using 20% EtOAc in hexanes) provided **25** as a white solid (2.67 g, 75%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.40 (s, 9H), 1.78-1.86 (m, 1H), 1.89-1.96 (m, 1H), 2.55 (t, *J* = 2.5 Hz, 1H), 2.62-2.70 (m, 2H), 4.35 (br s, 1H), 4.52 (br s, 1H), 4.76 (d, *J* = 1.9 Hz, 2H), 6.40 (d, *J* = 15.1 Hz, 1H), 6.84 (dd, *J* = 3.8, 14.5 Hz, 1H), 7.08 (t, *J* = 3.15 Hz, 2H), 7.14 (d, *J* = 6.95 Hz, 2H), 7.18-7.30 (m, 3H), 7.81 (dd, *J* = 2.55, 11.35 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 28.23, 31.89, 35.96, 50.62, 56.03, 76.53, 77.30, 80.23, 115.44, 126.32, 128.32, 128.61, 129.84, 131.09, 132.67, 140.75, 145.23, 154.88, 161.42, 184.26.

(*S*,*E*)-5-phenyl-1-((4-(prop-2-yn-1-yloxy)phenyl)sulfonyl)pent-1-en-3-amine trifluoroacetate (26)

Prepared according to the same procedure mentioned above by using **25** (2.28 g, 5.0 mmol) in 100 mL of TFA/DCM (1/1). Upon completion of the reaction, the mixture was precipitated with Et_2O, filtered off, washed twice with Et_2O, and finally dried *in vacuo* to give 2.3 g (98%) of **26**. This material was pure enough to be used in the next step without further purification.

***N*-((*S*)-1-oxo-3-phenyl-1-(((*S*,*E*)-5-phenyl-1-((4-(prop-2-yn-1-yloxy)phenyl)sulfonyl)pent-1-en-3-yl)amino)propan-2-yl)morpholine-4-carboxamide (3)**

Prepared according to the same procedure mentioned above by using **19** (139 mg, 0.5 mmol), **26** (234 mg, 0.5 mmol), EDC/HCl (115 mg, 0.6 mmol), HOBt (81 mg, 0.6 mmol) and DIEA (0.2 mL, 1.2 mmol) in DMF (5 mL). Purification by flash column chromatography (silica gel; using 20% EtOAc in hexanes) provided **3** as a white solid (265 mg, 86%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.64-1.85 (m, 2H), 2.41 (t, *J* = 7.55 Hz, 2H), 2.59 (t, *J* = 1.8 Hz, 1H), 3.03-3.11 (m, 2H), 3.22-3.31 (m, 4H), 3.60-3.66 (m, 4H), 4.46 (dd, *J* = 7.6, 15.2 Hz, 1H), 4.60-4.62 (m, 1H), 4.78 (d, *J* = 2.55 Hz, 2H), 5.03 (d, *J* = 7.55 Hz, 1H), 6.39 (br s, 1H), 6.50 (br d, *J* = 15.15 Hz, 1H), 6.80 (dd, *J* = 5.0, 15.1 Hz, 1H), 7.05 (d, *J* = 6.95 Hz, 2H), 7.08 (dd, *J* = 1.9, 6.95 Hz, 2H), 7.20-7.30 (m, 8H), 7.80 (dd, *J* = 1.85, 6.9 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 31.68, 35.48, 37.97, 43.92, 49.15, 56.04, 56.20, 66.27, 76.62, 77.32, 115.41, 126.25, 127.15, 128.32, 128.53, 128.79, 129.22, 129.84, 131.16, 132.63, 136.68, 140.37, 144.48, 157.23, 161.41, 171.64; LC-IT-TOF/MS (m/z) calcd for $C_{34}H_{37}N_3O_6S$ $[M+H]^+$: 616.2403, Found: 616. 2293.

3. Biological and Other Experiments.

3.1 General.

Anti-cathepsin L (ab6314) was from Abcam. Anti-rhodesain, and anti-TbCatB were generous gifts from James H. McKerrow (University of California, San Francisco). Other reagents are from commercial sources, unless otherwise indicated. For Cell Cultures, *T. brucei* procyclic cells YTAT 1.1 were grown at 28 °C and 5% CO_2 in Cunningham's medium supplemented with 15% heat-inactivated fetal bovine serum (FBS). *T. brucei* BSF cells were grown at 37 °C and 5% CO_2 in HMI-9 medium supplemented with 20% heat-inactivated fetal bovine serum (FBS). HepG2 cells were grown in DMEM containing 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin and maintained in a humidified 37 °C incubator with 5% CO_2.

3.2 Molecular modeling.

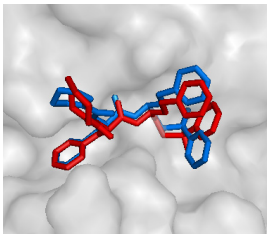


Fig. S2. Molecular docking experiments were carried out as previously described.[6] Superimposition of rhodesain•K11777 (PDB entry 2P7U) and rhodesain•K11002 (PDB entry 2P86) are shown. Images were generated with PyMOL.

3.2 Guava ViaCount anti-trypanocidal assay.

Parasite number and percentage viability were determined in 96-well plate format using the Guava ViaCount assay on a Guava PCA-96 system (Guava Technologies, USA) following the manufacturer's instructions. Briefly, BSF and PCF trypanosomes were harvested in exponential growth phase and adjusted to a concentration of 1×10^5 cells/mL in complete growth medium. Diluted trypanosomes were dispensed manually using a multichannel pipette. After 24 h of incubation with compounds, the final DMSO concentration in the assay never exceeded 1% in cultivation medium, and medium containing 1% DMSO was used as a negative control. Cell density and viability were evaluated using ViaCount assay on the Guava PCA-96 system. ED_{50} values were calculated by sigmoid curve fitting with GraphPad Prism 5.0 software (San Diego, USA). All data were collected in triplicate.

3.3 *In situ* proteomic profiling and in-gel fluorescence scanning.

T. brucei parasites were plated into 6-well plates (PCF, 2 mL at ~1×10^7cells/mL) or 25-mL cell culture flasks (BSF, 10 mL at ~2×10^6cells/mL), and incubated with probe for 2 h at culture temperature with or without a competing inhibitor, K11777. All compounds were solubilized in DMSO. To avoid adverse effects on parasite growth, the final DMSO concentration in the assay never exceeded 1% in cultivation medium. After incubation, the parasite cells were pelleted at 2,000 rpm for 10 min, washed twice with PBS and re-suspended in PBS (100 µL). Cells were homogenized by sonication, and diluted to ~1 mg/mL with PBS. To initiate the click chemistry reaction, 20 µL of freshly premixed solution containing rho-azide (100 µM final concentration), TCEP (1 mM final concentration), ligand (100 µM final concentration), and $CuSO_4$ (1 mM final concentration) was added. The reaction was incubated at 10 °C for 4 h with gentle mixing. Termination of the reaction was done by addition of pre-chilled acetone (0.5 mL). The resulting solution was then placed at -20 °C for 30 min, followed by centrifugation (13000 rpm × 10 min) at 4 °C. The supernatant was discarded and the precipitated protein pellets were washed with pre-chilled methanol (2 × 200 µL), air-dried for 10 min, resuspended in 1 × standard reducing SDS-loading buffer (25 µL) then heated for 10 min at 95 °C. Finally, the protein sample (~20 µg/lane) was loaded onto 12% SDS-PAGE gel, separated followed by in-gel fluorescence scanning with a Typhoon 9410 Variable Mode Imager scanner (GE Amersham).

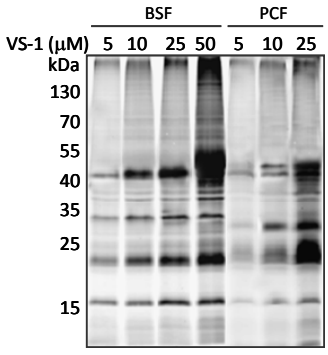


Fig. S3 Dose-dependent *in situ* proteome profiling of *T. brucei* in BSF and PCF with **VS-1**.

3.4 Affinity pull-down and LC/MS-MS experiments.

For proteomic experiments, BSF and PCF trypanosomes (~2 × 10^9 cells, ~5 mg each), labeled in Cunningham's media (1 × 10^7 cells/mL) with **VS-1** (25 μM) or DMSO (negative control), were harvested, washed and homogenized in PBS. CuAAC reagents were added at the same concentrations as described above, except that biotin-azide was substituted for rho-azide. Acetone-precipitated and methanol-washed protein pellets were solubilized in PBS containing 0.1% (w/v) SDS by brief sonication. Insoluble materials were precipitated by centrifugation (13,000g × 10 min) at 4 °C. The supernatants were then incubated with gentle shaking at 4 °C overnight with Neutravidin agarose beads (50 μL/mg protein, Prod # 29204, Thermo Scientific, USA) which have been pre-washed twice with PBS. After centrifugation, the bead/complexes were washed extensively 8 times with 1% (w/v) SDS in PBS, three times with PBS and twice with 250 mM of ammonium bicarbonate (ABC). Elution of bound proteins from beads was then performed twice using the boiling buffer (200 mM Tris *p*H 6.8, 400 mM DTT, 8% (w/v) SDS), then pooled. Protein samples were concentrated using an YM-10 Centricon spin column (Millipore, USA). Following SDS-PAGE separation, protein bands were visualized by Coomassie blue staining. Gel lanes corresponding to both DMSO- and VS-1-treated samples were then each cut into 10 slices. Subsequent trypsin digestion (using In-Gel Trypsin Digestion Kit, Pierce Co., USA) and peptide extraction (with 50% acetonitrile and 1% formic acid) generated a total of 10 LCMS samples for each pull-down experiment. All samples were dried *in vacuo* and stored at -20 ^{o}C until future LCMS analysis.

Each LCMS sample was resuspended in 0.1% formic acid for mass spectrometry analysis as previously described.[9] Briefly, peptides were separated and analyzed on a Shimadzu UFLC system (Shimadzu, Kyoto, Japan) coupled to an LTQ-FT Ultra (Thermo Electron, Germany). Mobile phase A (0.1% formic acid in H_2O) and mobile phase B (0.1% formic acid in acetonitrile) were used to establish the 60 min gradient comprised of 45 min of 5-35% B, 8 min of 35-50% B and 2 min of 80% B followed by re-equilibrating at 5% B for 5 min. Peptides were then analyzed on LTQ-FT with an ADVANCE™ CaptiveSpray™ Source (Michrom BioResources, USA) at an electrospray potential of 1.5 kV. A gas flow of 2 L/min, ion transfer tube temperature of 180°C and collision gas pressure of 0.85 mTorr were used. The LTQ-FT was set to perform data acquisition in the positive ion mode as previously described except that the m/z range of 350-1600 was used in the full MS scan.[10] The raw data were converted to mgf format as previously described.[9] The database (76708 sequences, 33362815 residues) used for Mascot search was a concatenated *T. brucei* protein database. The database search was performed using an in-house Mascot server (version 2.2.07, Matrix Science, UK) with MS tolerance of 10 ppm and MS/MS tolerance of 0.8 Da. Two missed cleavage sites of trypsin were allowed. Carbamidomethylation (C) was set as a fixed modification, and oxidation (M) and phosphorylation (S, T and Y) were set as variable modifications.

LCMS results obtained from above experiments (with **VS-1** as well as with DMSO as a negative control) were processed as above. As in the case of most large-scale LCMS experiments, a large number of proteins were identified from each LCMS run, many of which were "sticky" and/or highly abundant proteins. These proteins were excluded. For those proteins that appeared in the "negative" run (i.e. pull-down/LCMS experiments with DMSO in place of **VS-1**), they were automatically removed from the list as well. The final list was shown in SI_2. From this list, we placed our focus on those proteins that might be potential K11777 targets, and they were shown in Table S1 (in ESI) and Table 1 in the maintext.

3.5 Pull-down and western blotting analysis.

Pull-down samples from *in situ* labeling with **VS-1** (25 μM) were separated on 12% SDS-PAGE gel together with pull-down sample from DMSO-treated (negative control). After SDS-PAGE gel separation, proteins were then transferred to a PVDF membrane and subsequently blocked with 3% (w/v) BSA/PBST overnight at 4^{o}C. Membranes were incubated for 1 h at room temperature with the respective antibodies (anti-cathepsin L for HepG2; anti-rhodesain, or anti-TbcatB for *T. brucei*), and washed with PBST (3 × 15 min with gentle agitation), then followed by incubation

with an anti-mouse-IgG conjugated secondary antibody in the blocking buffer mentioned above. After washing with PBST (3 × 15 min with gentle agitation), the SuperSignal West Pico kit (Pierce) was used to develop the blot.

3.6 Fluorescence Microscopy.

For drug uptake analysis, trypanosomes (1×10^5 cells/mL for both forms) were incubated in growth medium containing different concentrations of **VS-1** at culture temperature and 5% CO_2 for 2 h. Medium containing 1% DMSO was used as a negative control. The parasites were then washed twice with PBS, and fixed with 4% paraformaldehyde in PBS for 15 min at room temperature and washed with PBS (2 × 5 min with gentle agitation), and then sedimented to poly-L-lysine-coated coverslips. Fixed cells were permeabilized with 0.25% Triton-X 100 in PBS for 15 min at room temperature, and washed with PBS (2 × 5 min with gentle agitation). The cells were blocked with 3% BSA in PBS for 30 min at room temperature, and washed with PBS (2 × 5 min with gentle agitation). The cells were then treated with a freshly pre-mixed click chemistry reaction solution [rhodamine-azide (10 μM final concentration from a 10 mM stock solution in DMSO), TCEP (1 mM final concentration from a 50 mM freshly prepared stock solution in deionized water), TBTA (100 μM final concentration from a 10 mM stock solution in DMSO), and $CuSO_4$ (1 mM final concentration from a 100 mM freshly prepared stock solution in deionized water)] in PBS for 1 h at room temperature. The cells were washed with PBS (1 × 5 min with gentle agitation), and cold methanol (1 × 5 min with gentle agitation), followed by 1% Tween-20 and 0.5 mM of EDTA in PBS (3 × 2 min with gentle agitation), and with PBS (2 × 5 min with gentle agitation). The cells were then incubated in PBS containing 2 μg/mL of DAPI for 15 min at room temperature to stain the kinetoplast and nuclear DNA, and washed with PBS (2 × 5 min with gentle agitation) and a final wash with deionized water (1 × 5 min with gentle agitation) before mounting onto the Fluoromount G (Emsdiasum, USA). For immunofluorescence (IF) analysis, cells were then incubated for l h in PBS with anti-rhodesain and washed with PBS (3 × 5 min with gentle agitation), followed labeled with FITC-conjugated anti-rabbit IgG (1:500) and a final wash with PBS (3 × 5 min with gentle agitation) before mounting. Confocal images were taken on a Leica TCS SP5X Confocal Microscope System equipped with Leica HCX PL APO 100×/1.40 oil objective, 405 nm Diode laser, White laser (470 nm to 670 nm, with 1 nm increments, with 8 channels AOTF for simultaneous control of 8 laser lines, each excitation wavelength provides 1.5 mV, PMT detector range from 420 nm to 700 nm for steady state fluorescence. DAPI, FITC and rhodamine were excited with a krypton/argon laser at 405, 488 nm and 554 nm, respectively, and the emission was collected through a 420-470, 500-550 and 565-650 nm filters, respectively. Images were processed with Leica Application Suite Advanced Fluorescence (LAS AF).

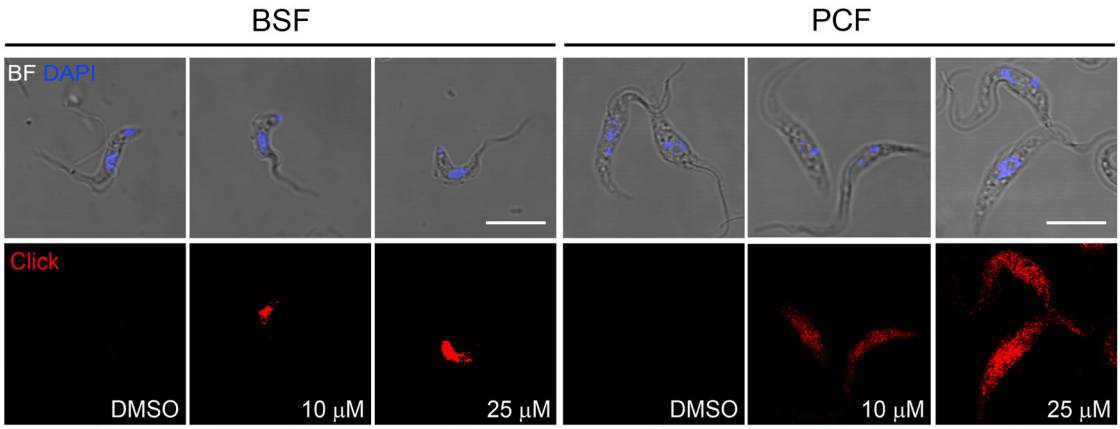


Fig. S4 Cellular uptake of **VS-1** within *T. brucei*. Parasites (2×10^5 cells) were incubated with **VS-1** (at 0, 10 and 25 μM, respectively) for 2 h, reacted with 10 μM of rho–azide under CuAAC conditions, and then imaged. DAPI stained (with nucleus and/or kinetoplast pseudocolored in Blue); Rhodamine channel showing cellular distribution of VS-1 (pseudocolored in Red). Scale bar represents 10 μm.

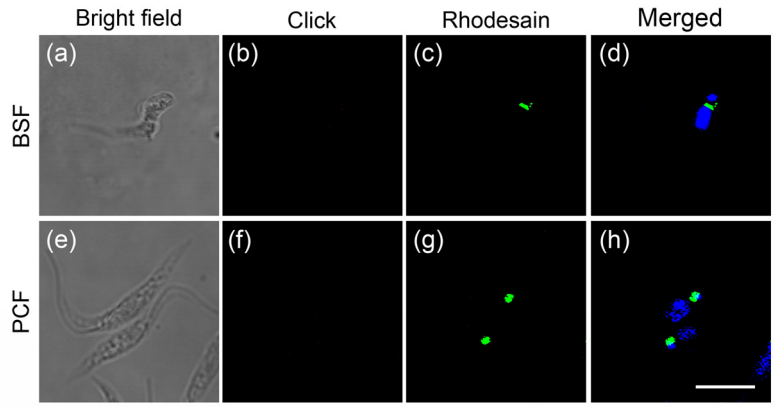


Fig. S5 Confocal microscope images of rhodesain in BSF (top) and PCF (bottom) treated with DMSO and immunofluorescence staining. Panel (a) and (e): Bright field images of the corresponding parasites. Panel (b) and (f): 554 nm channel (pseudocolored in red). Panel (c) and (g): immunofluorescence staining at 488 nm channel (pseudocolored in green) using anti-rhodesain primary antibody and FITC-conjugated anti-rabbit IgG secondary antibody detecting cellular localization of rhodesain. Panel (d) and (g): merged images of panels (b) and (c), (f) and (g) together with stained nuclei (with DAPI; pseudocolored in blue). All images were acquired under the same settings. Scale bar = 10 μm.

4. *In Situ* Proteomic Profiling and Fluorescence Microscopy in HepG2 Mammalian Cells.

For *in situ* proteomic profiling and cellular imaging of HepG2 live cells using **VS-1**, our previous published procedures were followed.[6] Briefly, cells were grown to 80-90% confluence in 24-well plates, and medium was removed, washed twice with cold PBS, then treated with 0.5 mL of DMEM-containing probe for 2 h (the final DMSO concentration in the assay never exceeded 1% in cultivation medium) as previously described.[6] After incubation, the growth medium was aspirated, and cells were washed twice with PBS to remove the excessive probe, trypsined, and pelleted at 1,000 rpm for 10 min, washed twice with PBS and re-suspended in PBS (100 μL). Cells were homogenized by sonication, and diluted to ~1 mg/mL with PBS, then followed by click chemistry, SDS-PAGE gel analysis, and in-gel fluorescence scanning (Fig. S6). For cellular imaging, cells were grown to ~50% confluence in 24-well plates containing sterile glass coverslips, and medium was removed, washed twice with cold PBS, then treated with 0.5 mL of DMEM-containing probe for 2 h. After incubation, the growth medium was aspirated, and cells were washed twice with PBS. Cells were fixed, permeabilized, and blocked, then followed by click chemistry, washing, staining (for IF, using mouse anti-cathepsin L; 1:100) and mounting mentioned above. Confocal images were taken as above described using a Leica TCS SP5X Confocal Microscope System equipped with Leica HCX PL APO 63x/1.20 W CORR CS (Fig. S7).

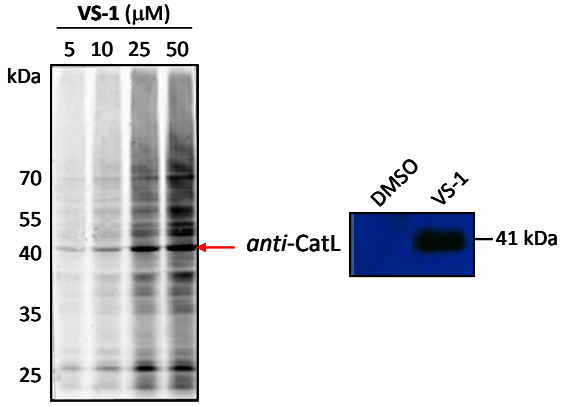


Fig. S6 *In situ* proteome-profiling of **VS-1** against HepG2 live cells and Western blotting analysis of pulled-down fractions treated with **VS-1** (25 μM), or DMSO as negative controls with anti-cathepsin L antibody.

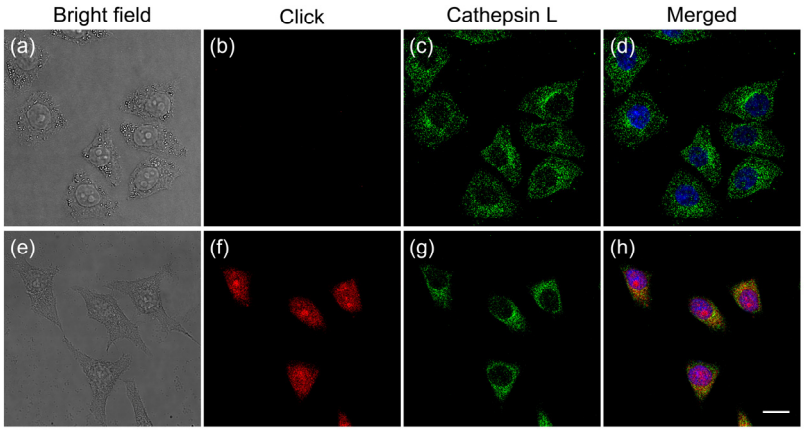


Fig. S7 Confocal microscope images of cathepsin L in HepG2 cells treated with DMSO (top) or **VS-1** (bottom) and immunofluorescence staining. Panel (a) and (e): Bright field images of the corresponding cells. Panel (b) and (f): 554 nm channel (pseudocolored in red) detecting cellular localization of **VS-1**. Panel (c) and (g): immunofluorescence staining at 488 nm channel (pseudocolored in green) using anti-cathepsin L primary antibody and FITC-conjugated anti-rabbit IgG secondary antibody detecting cellular localization of cathepsin L. Panel (d) and (g): merged images of panels (b) and (c), (f) and (g) together with stained nuclei (with Hoechest; pseudocolored in blue). All images were acquired under the same settings. Scale bar = 10 μm.

5. Affinity Pull-Down and LC/MS-MS Results

Details are described in the maintext and key proteins (i.e., putative drug targets) were summarized in Table 1. Table S1 provides a list of the rest of functional proteins (some of them are also putative drug targets), many of which are high-abundance proteins (such as proteins involved in carbohydrate metabolism), and some are sensitive to RNA interference. Though they only appeared in our positive pull-downs, some of them could be due to non-specific bindings (as a result of high abundance).The complete list is shown in SI_2.

Table S1. Representative proteins identified in *Trypanosoma brucei*[a]

T. brucei gene	protein name	M_w / kDa	location	detection
Carbohydrate metabolism				
Tb10.70.5820	hexokinase1 (HK1)**	51.3	G	both
Tb10.70.1370	fructose-biphosphate aldolase (ALD)**	41.07	G	both
Tb927.3.3270	ATP-dependent phosphofructokinase (PFK)**	53.52	G	both
Tb11.02.3210	trisephosphate isomerase (TIM)**	26.82	G	both
Tb927.6.4280	glyceraldehyde 3-phosphate dehydrogenase (GAPDH)**	39.05	G	both
Tb927.8.3530	glycerol-3-phosphate dehydrogenase [NAD+]**	37.81	G	both
Tb927.1.700	phosphoglycerate kinase (PGK)**	47.25	G	BSF
Tb09.211.3550	glycerol kinase	56.37	G	both
Tb10.70.4740	enolase**	46.59	G	PCF
Amino acid metabolism				
Tb927.6.4840	S-adenosylhomocysteine hydrolase	43.54	n/a	PCF
Tb09.160.4560	arginine kinase	44.72	G	PCF
Tb927.8.6060	2-amino-3-ketobutyrate coenzyme A ligase	43.74	M	PCF
Protein synthesis				

Tb10.05.0220	60S ribosomal protein L10a	24.6	ribosome	PCF
Tb10.70.3510	60S ribosomal protein L18a	20.91	ribosome	PCF
Tb10.70.7010	60S ribosomal protein L9	21.86	ribosome	PCF
Tb11.46.0001	60S acidic ribosomal subunit protein	34.63	ribosome	PCF
Tb09.160.4450	40S ribosomal protein S3	30.72	ribosome	PCF
Tb10.61.1960	40S ribosomal protein S2	28.8	ribosome	PCF
Tb10.70.1670	40S ribosomal protein S10	19.33	ribosome	PCF
Tb09.160.4450	40S ribosomal protein S3	30.4	ribosome	PCF
Tb10.70.7695	40S ribosomal protein S11	20.30	ribosome	PCF
Cytoskeletal proteins				
Tb927.1.2340	alpha tubulin	49.79	cytoskeleton	both
Tb927.1.2330	beta tubulin	49.71	cytoskeleton	both
Tb927.8.4970	69 kDa paraflagellar rod protein (PFR2)*	69.6	flagellum	BSF
Tb927.3.4290	73 kDa paraflagellar rod protein (PFR1)*	68.68	flagellum	both

[a] G, M and n/a represent, respectively, glycosomal, mitochondrial and not available. Symbols in the protein name column: *, sensitive to RNA interference; **, putative drug target.

6. References

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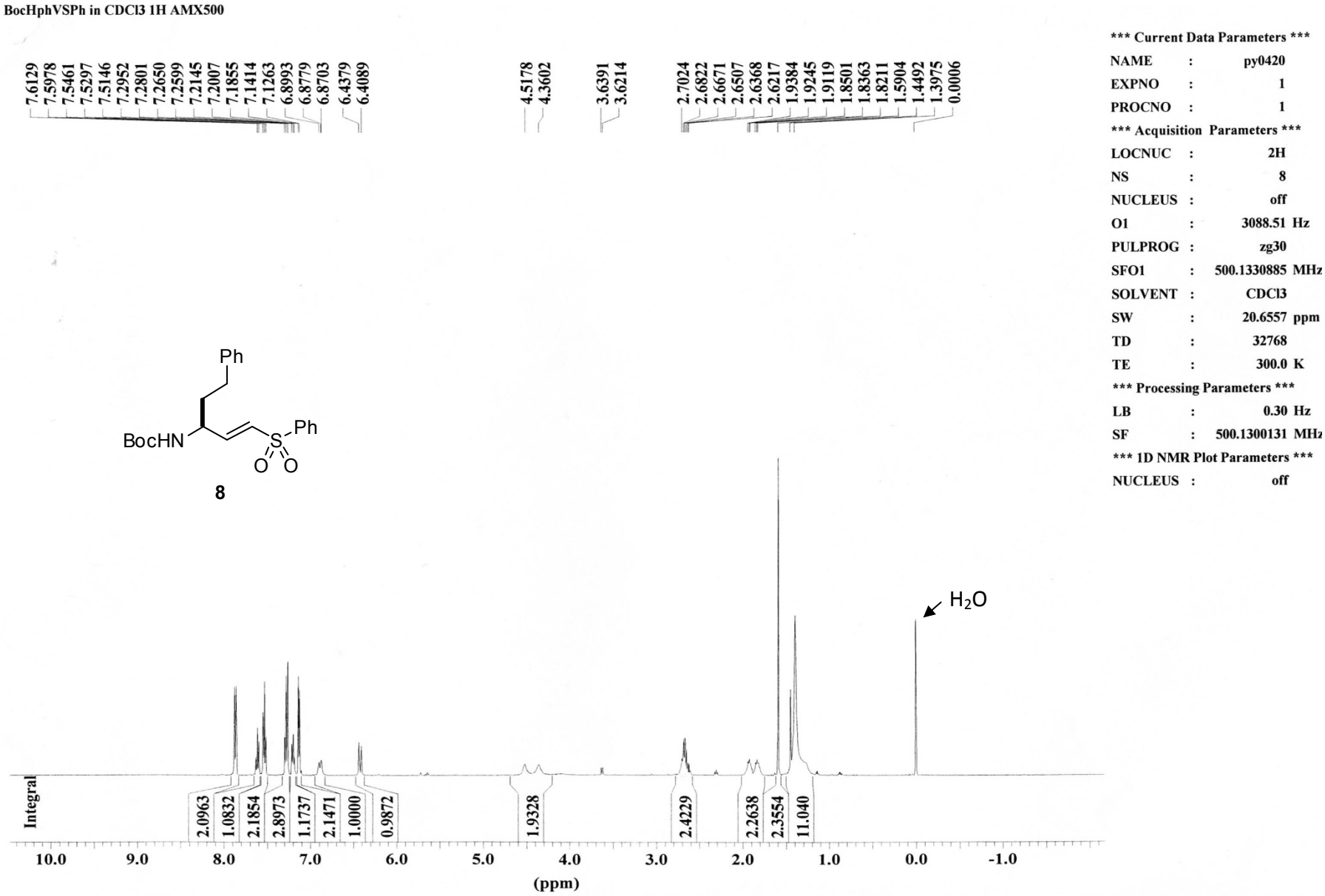
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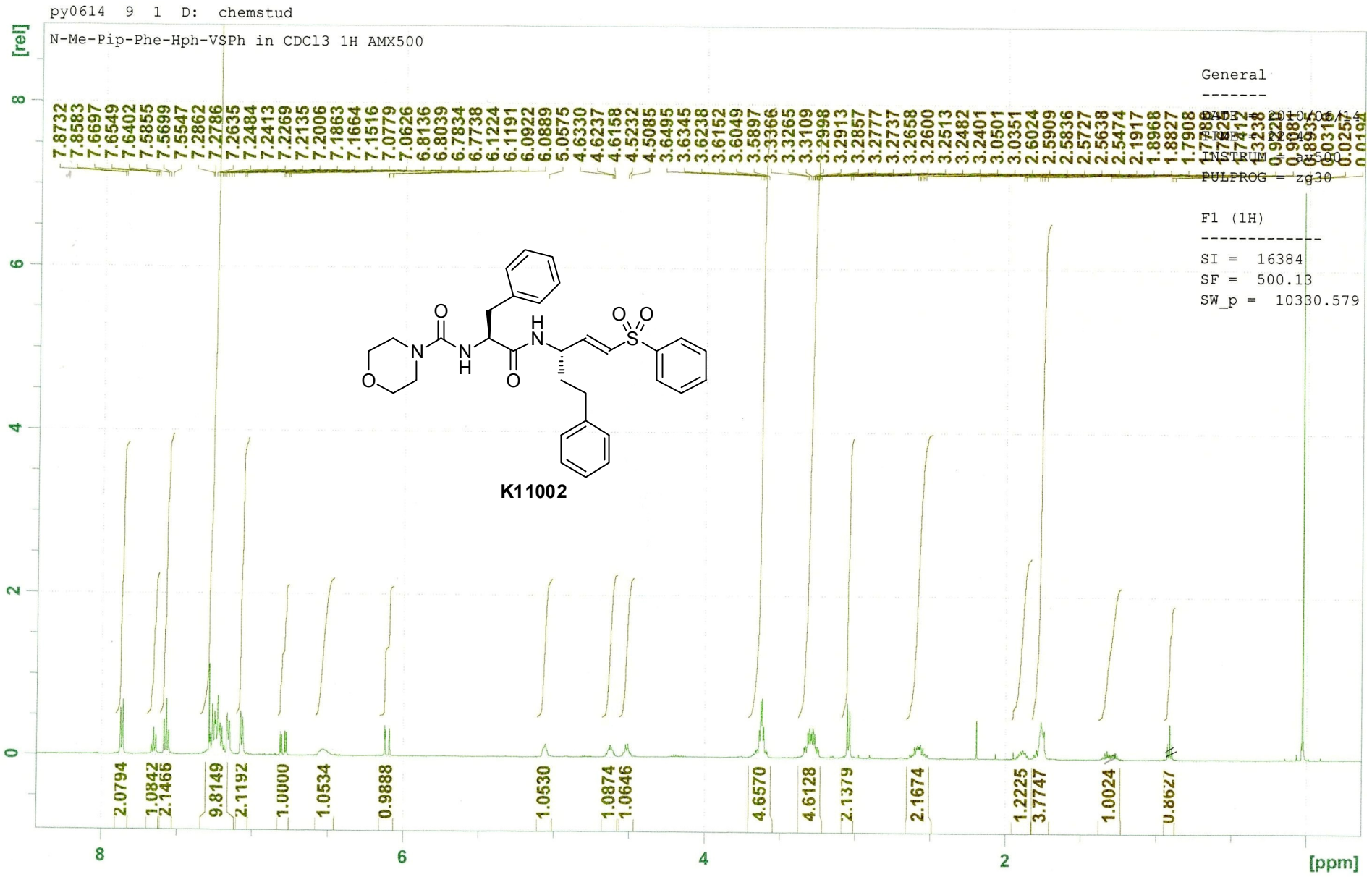
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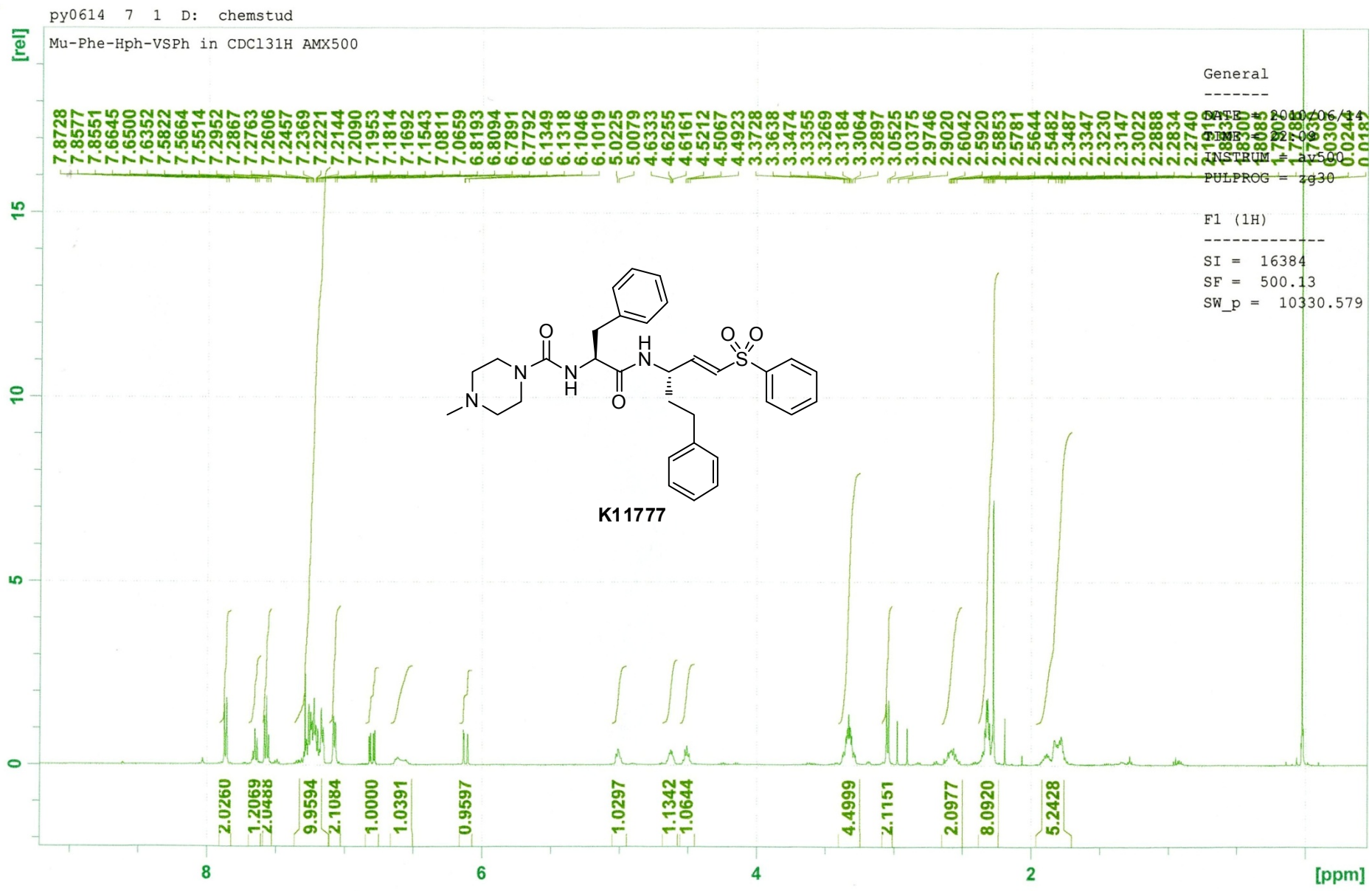
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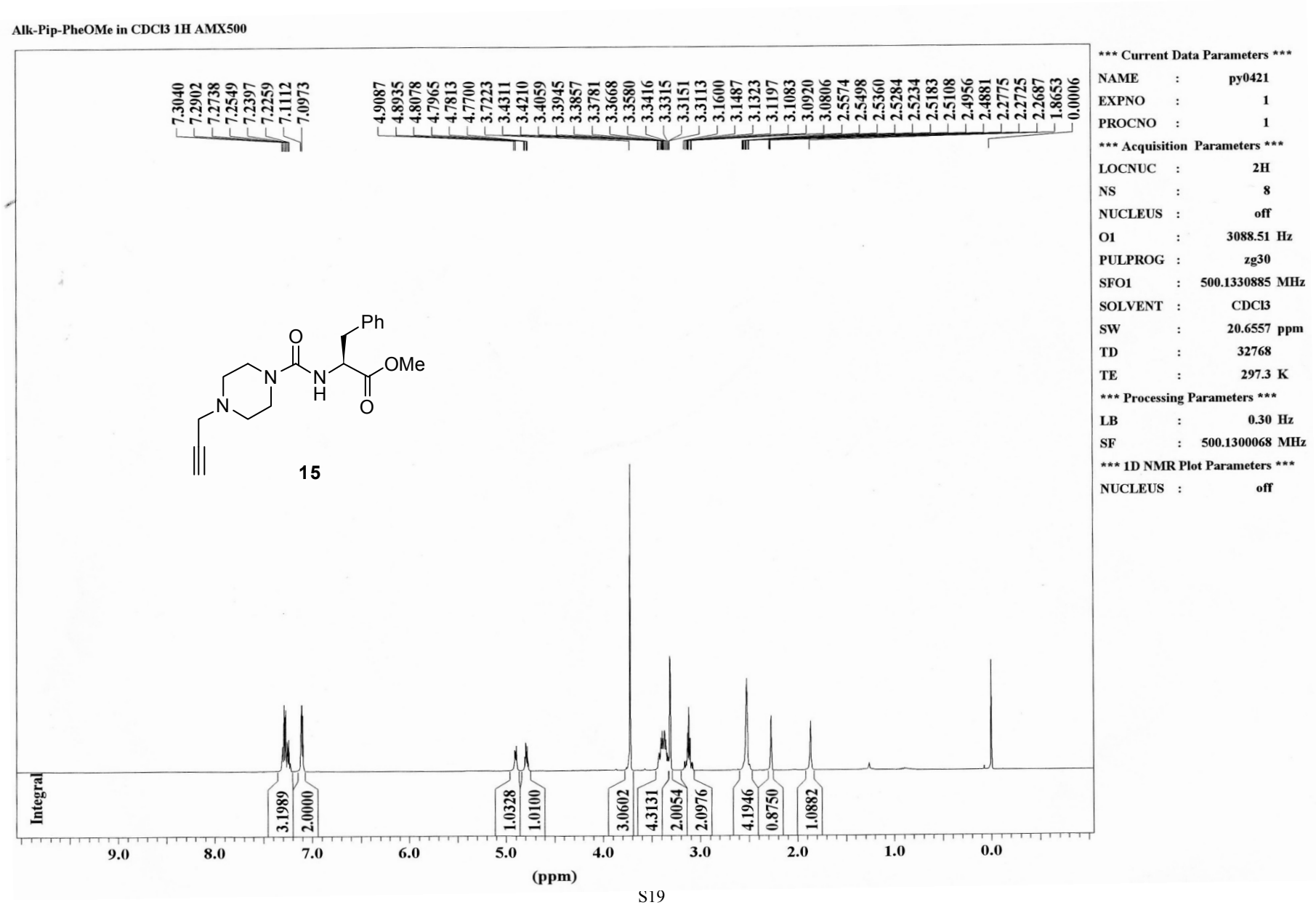
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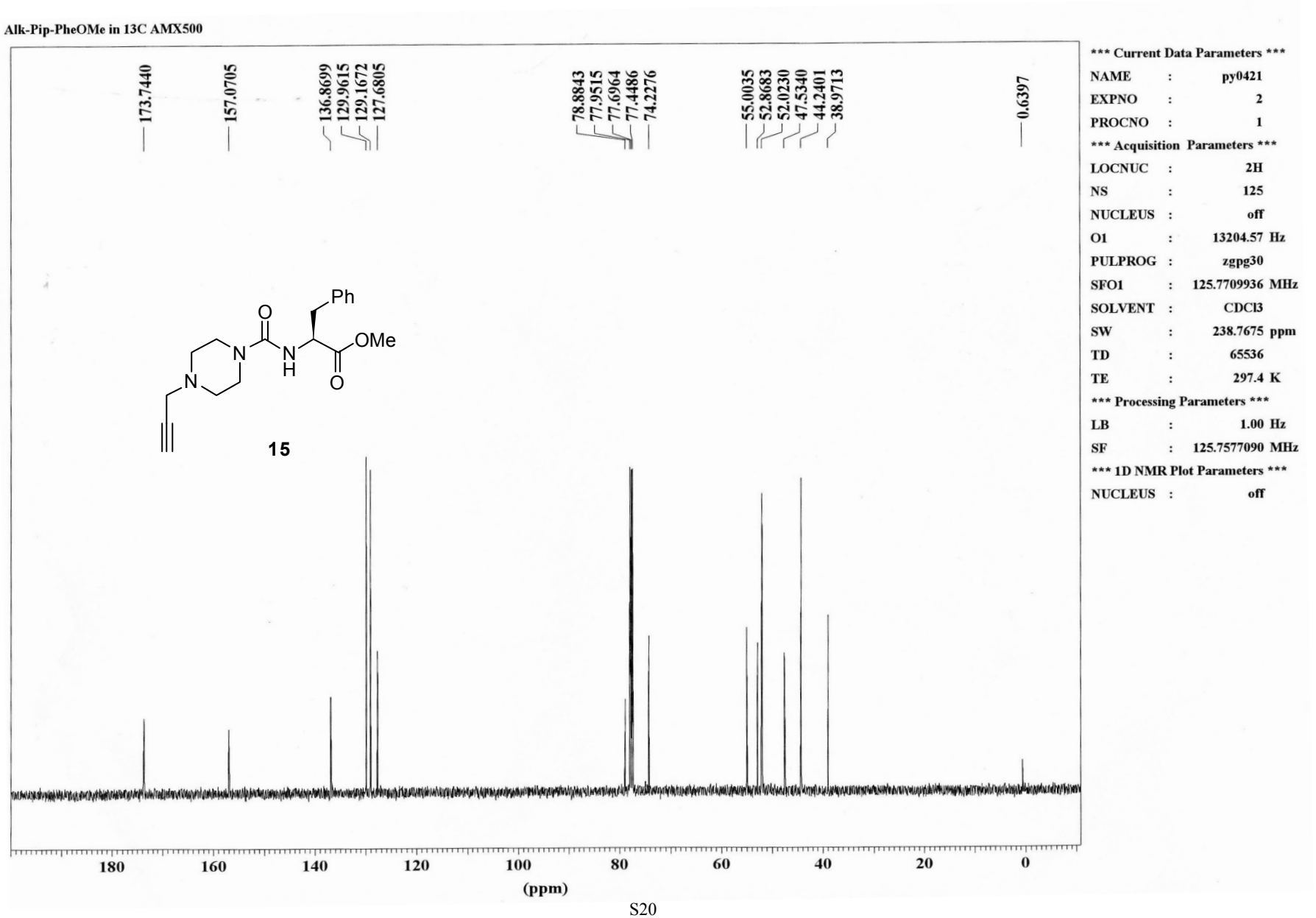

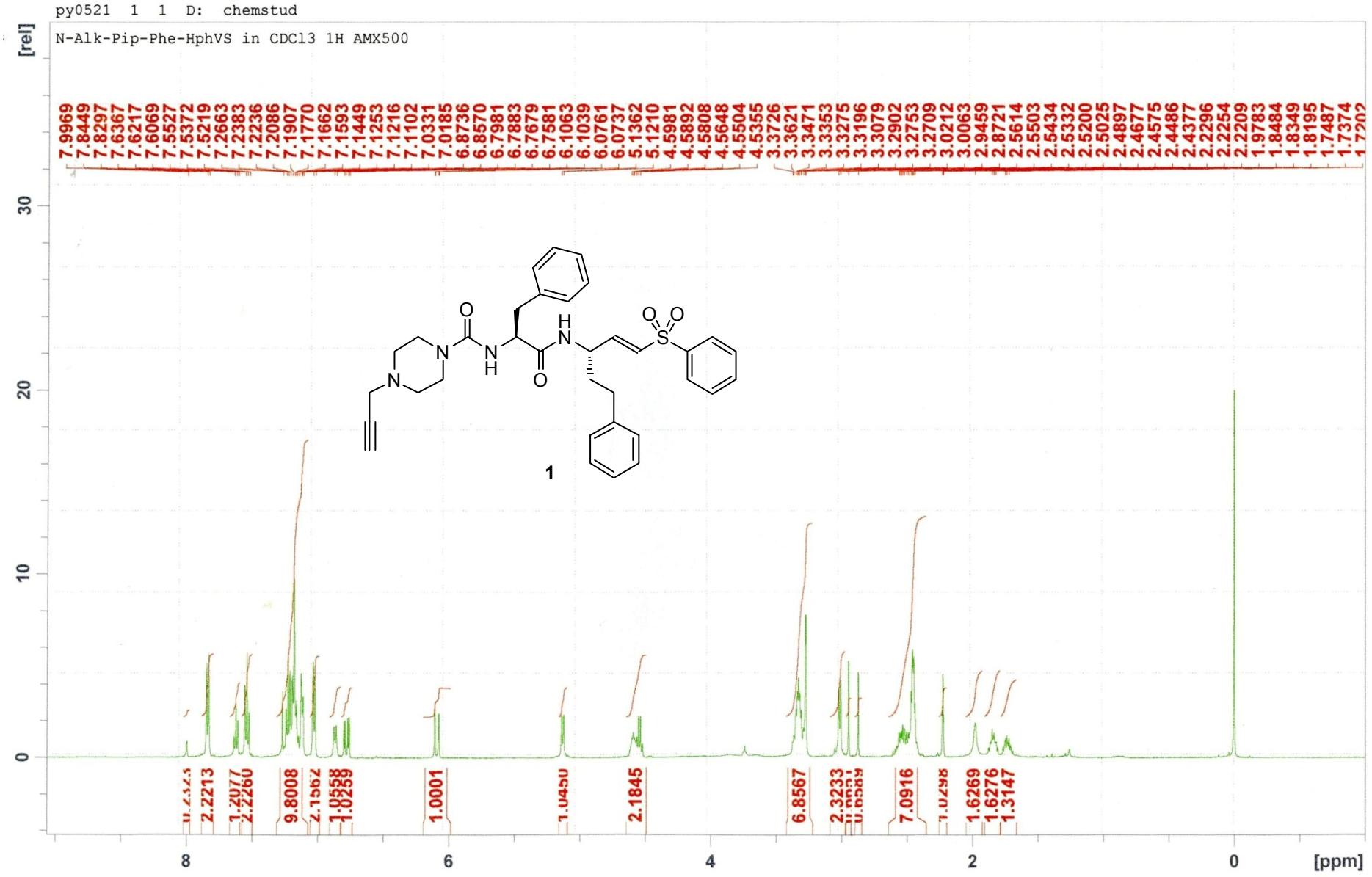

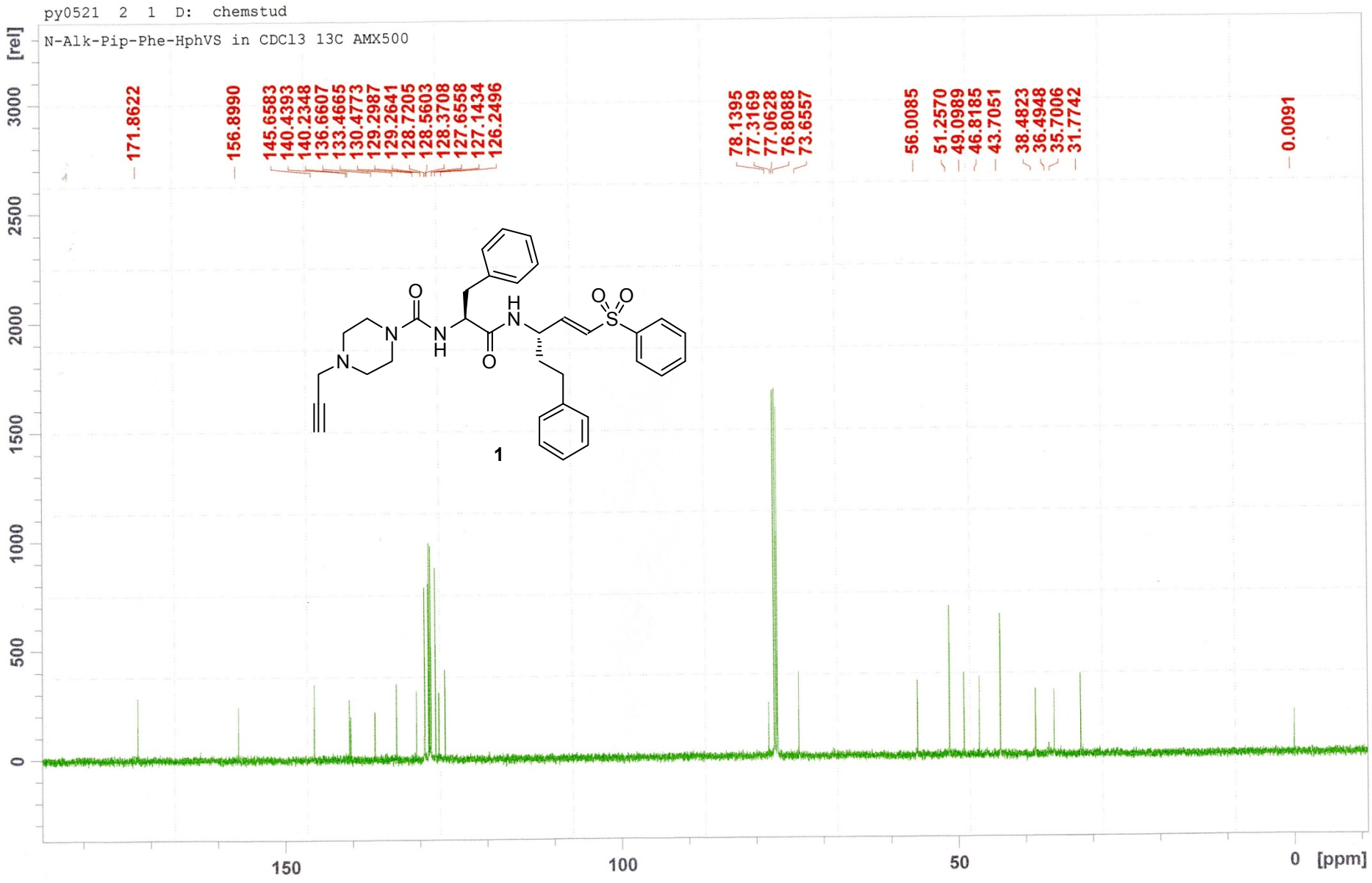

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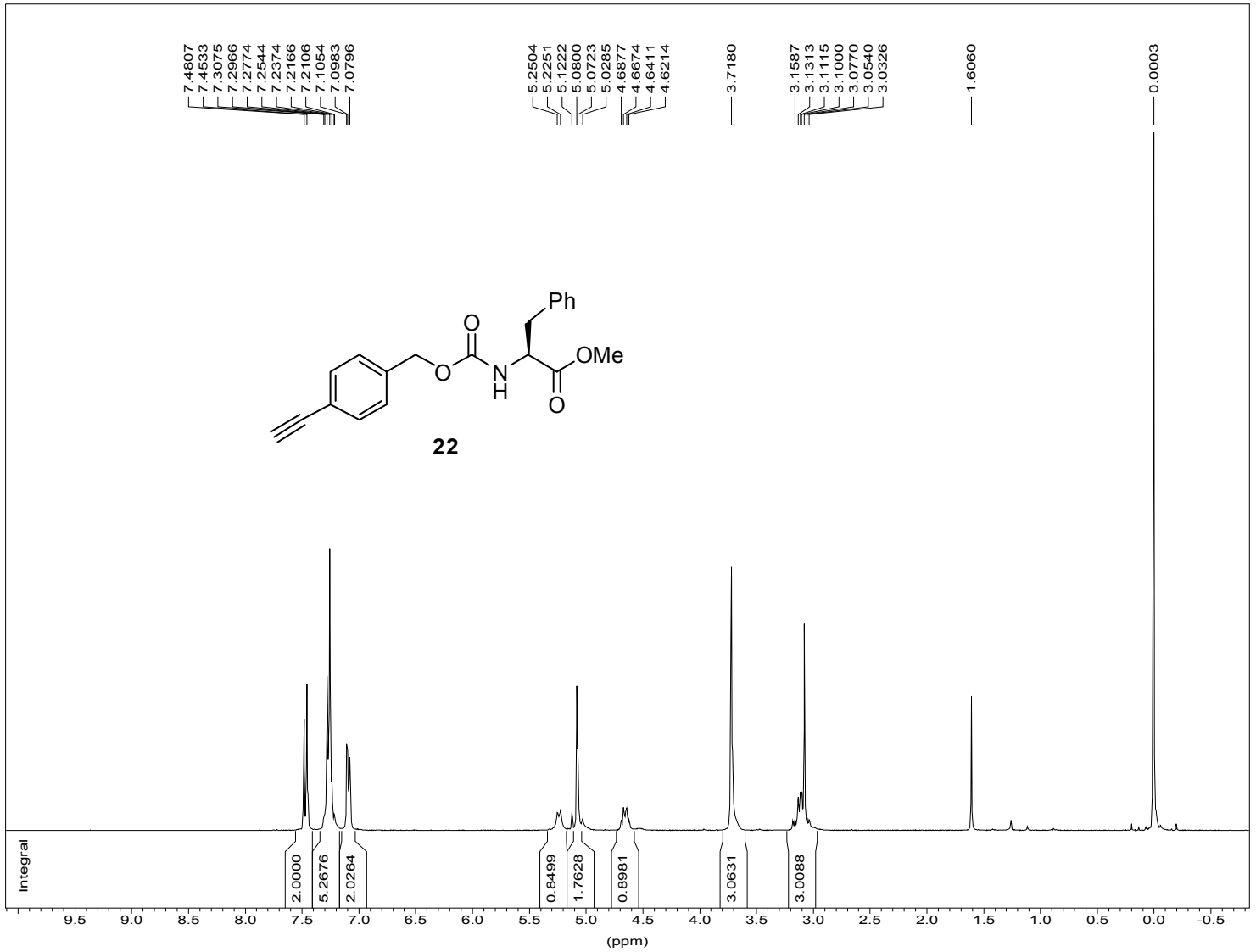

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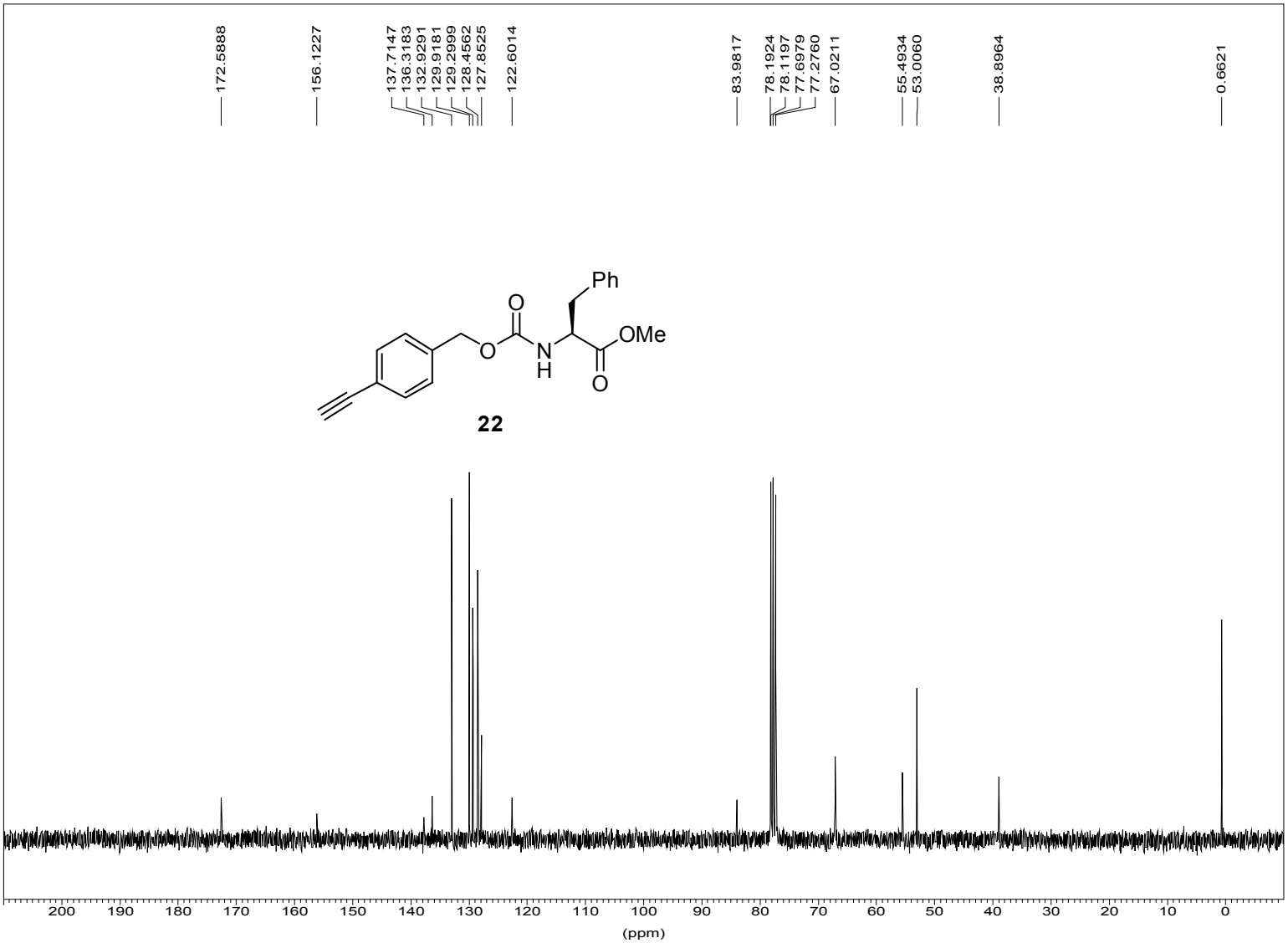

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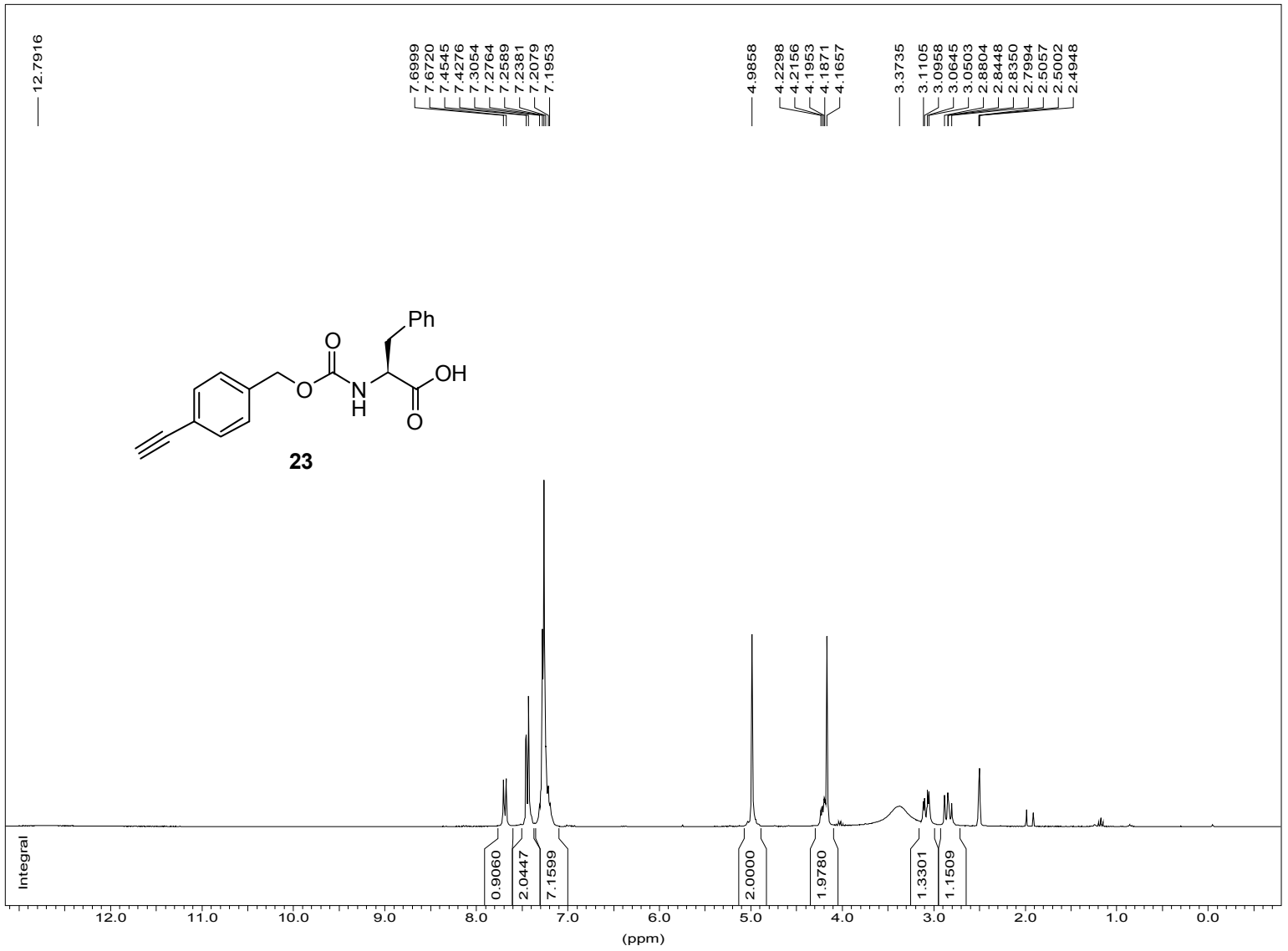

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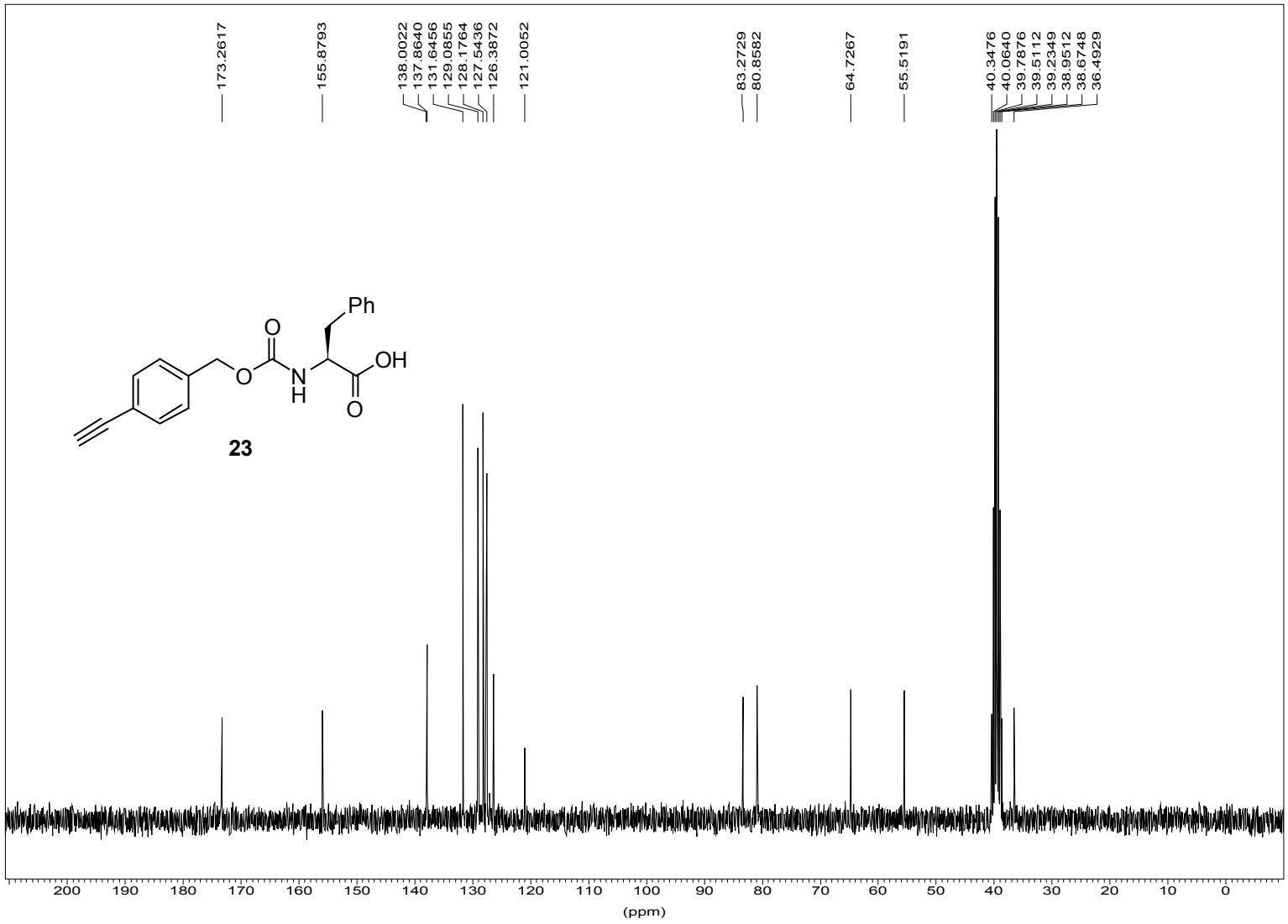

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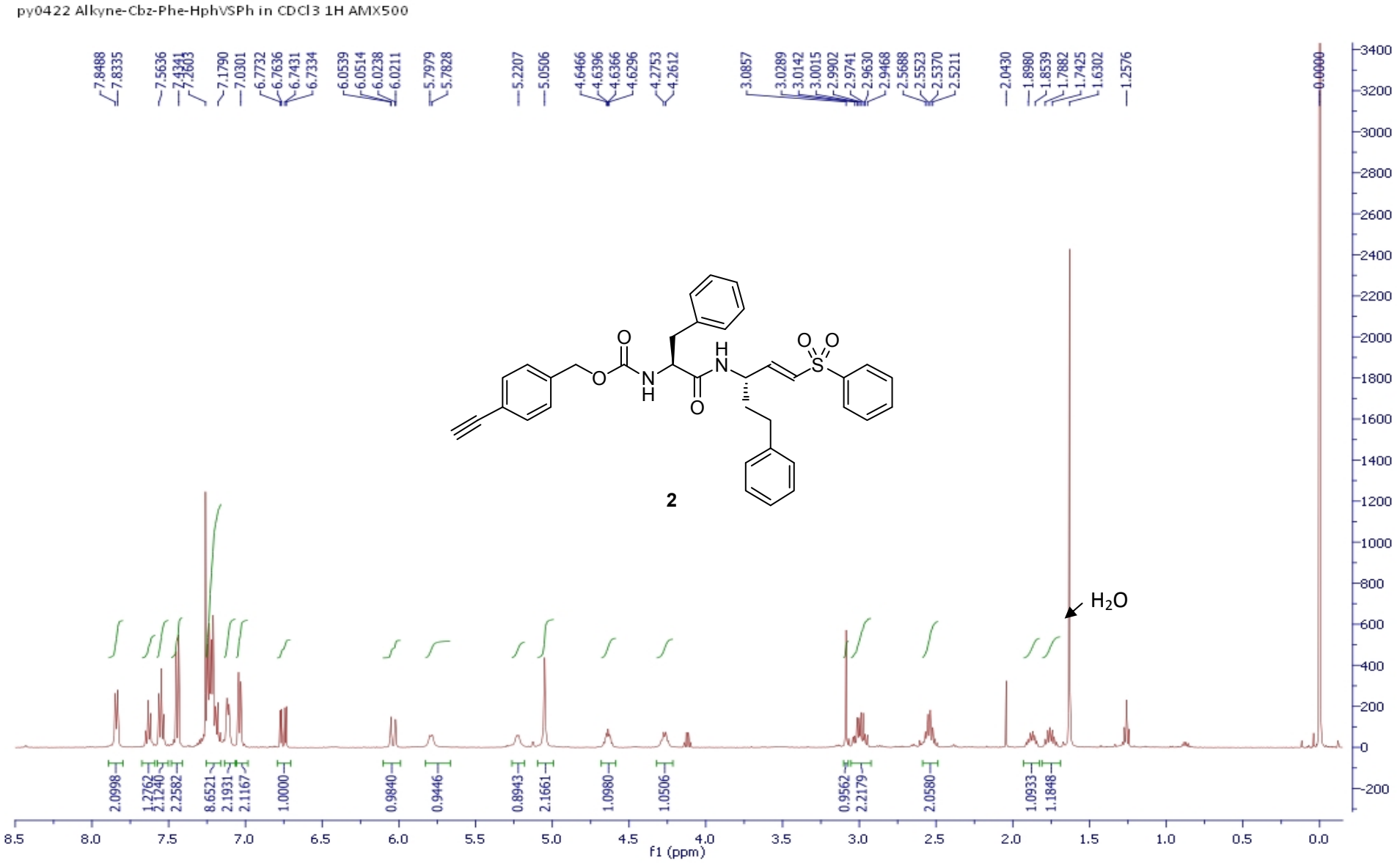

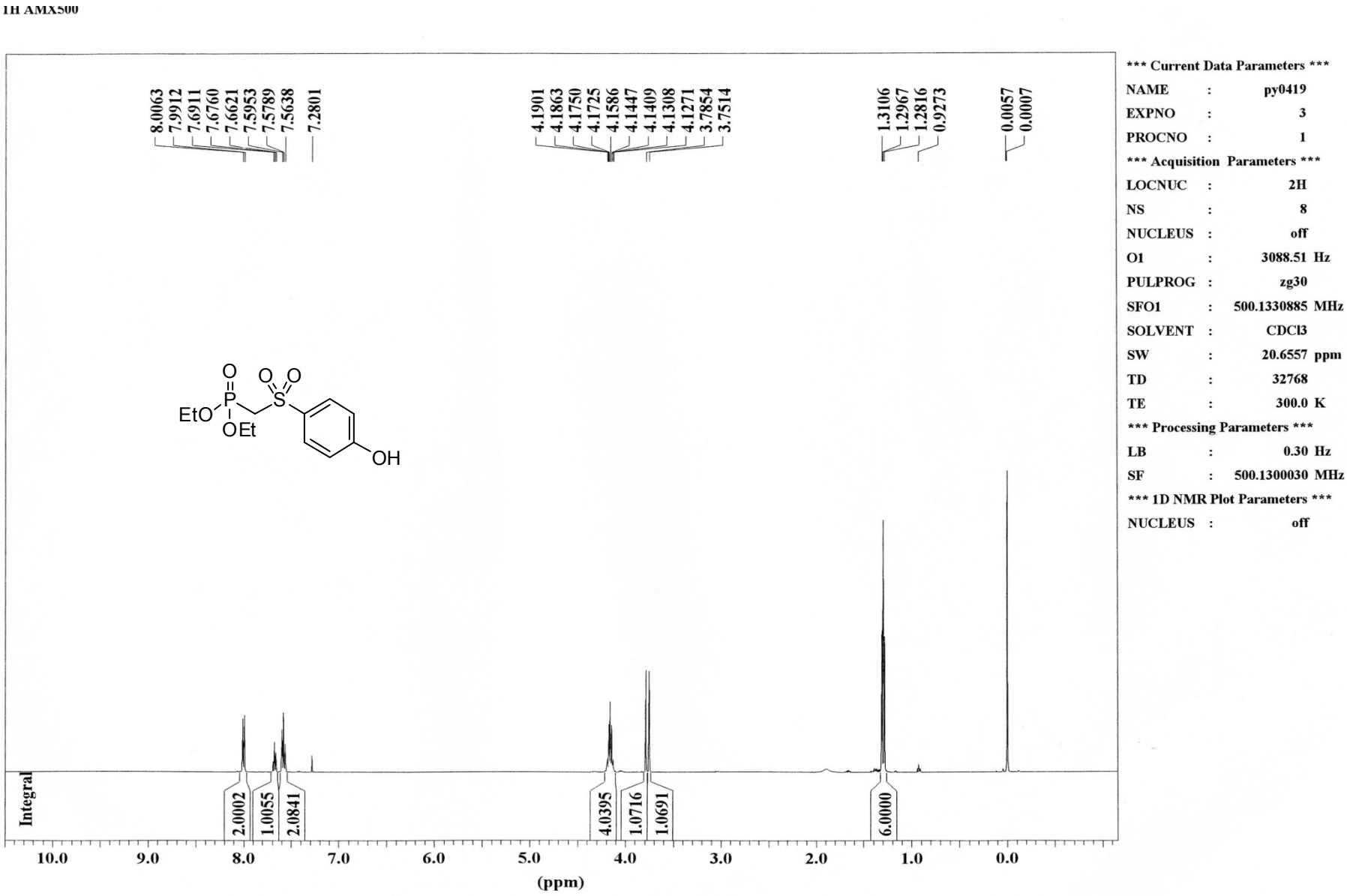
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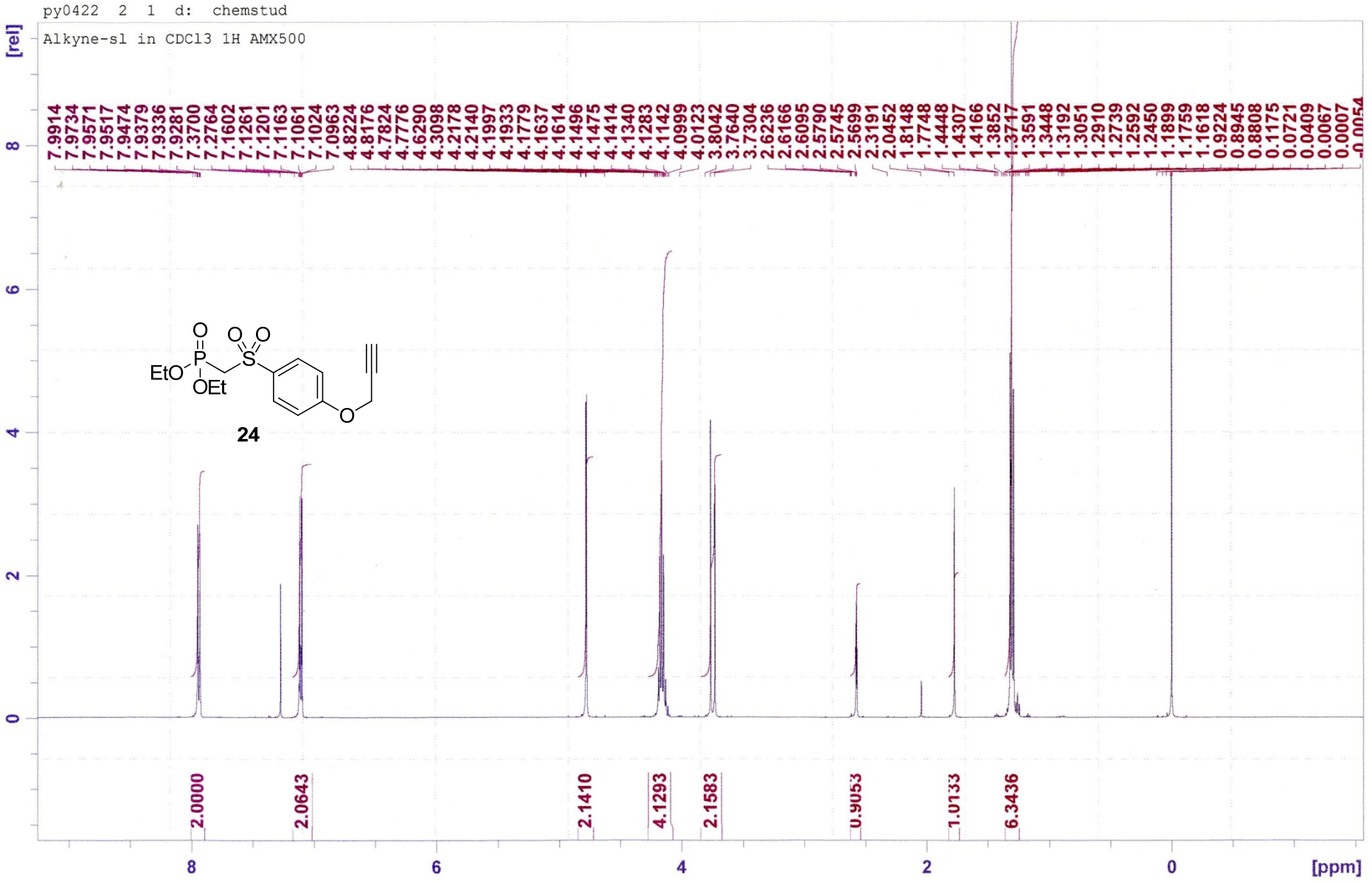
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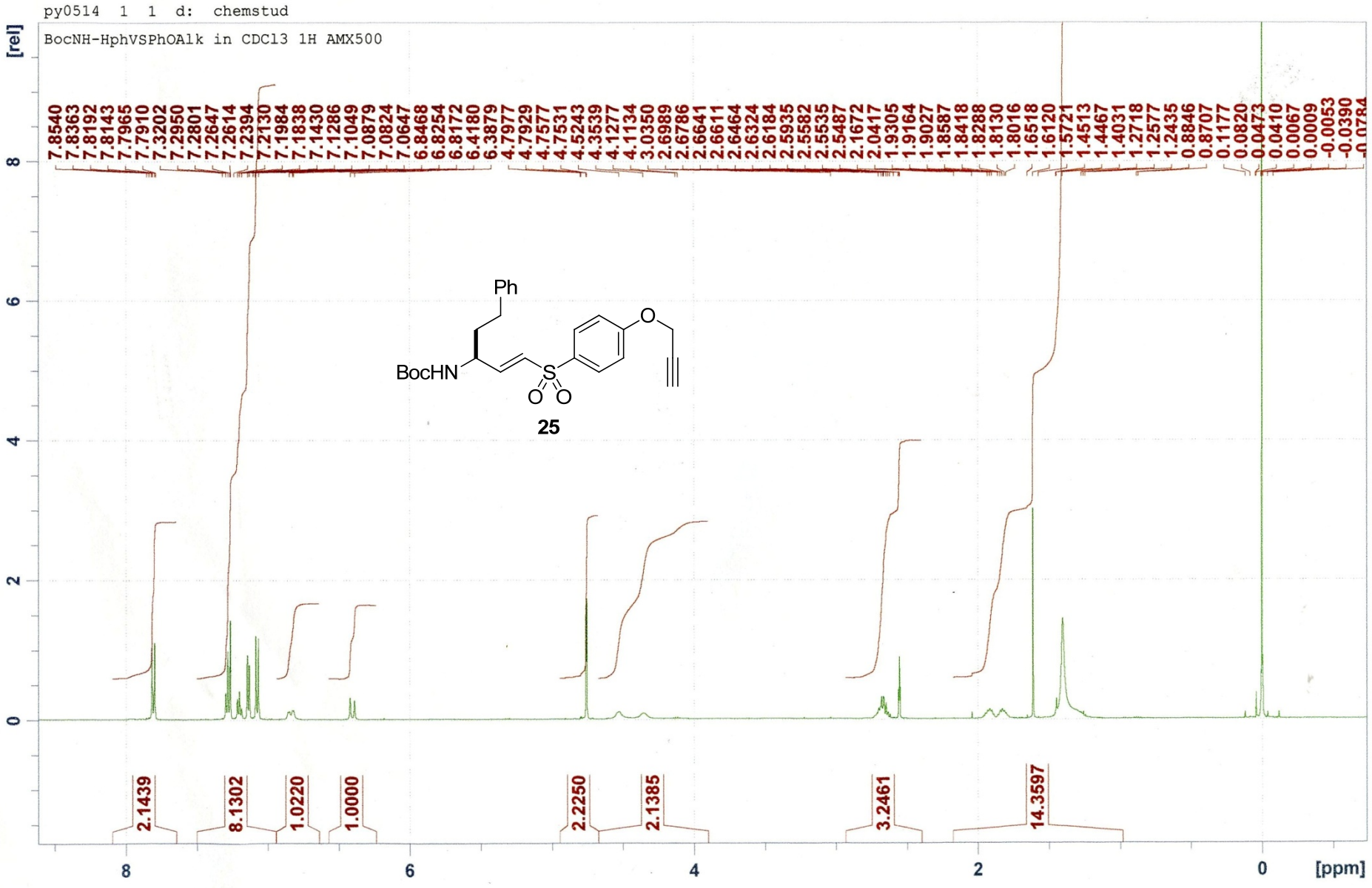
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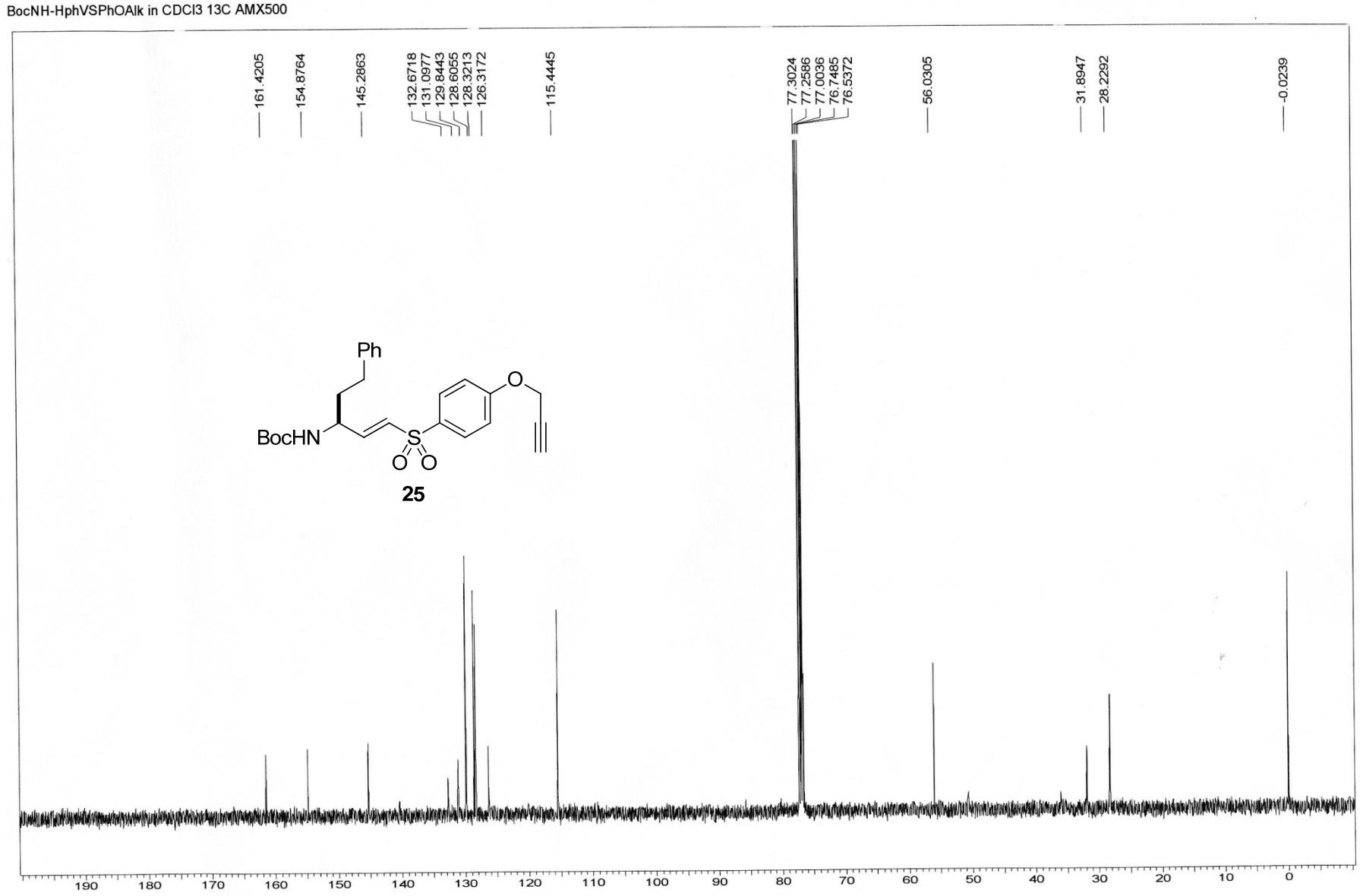
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130
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110
100
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70
60
50
40
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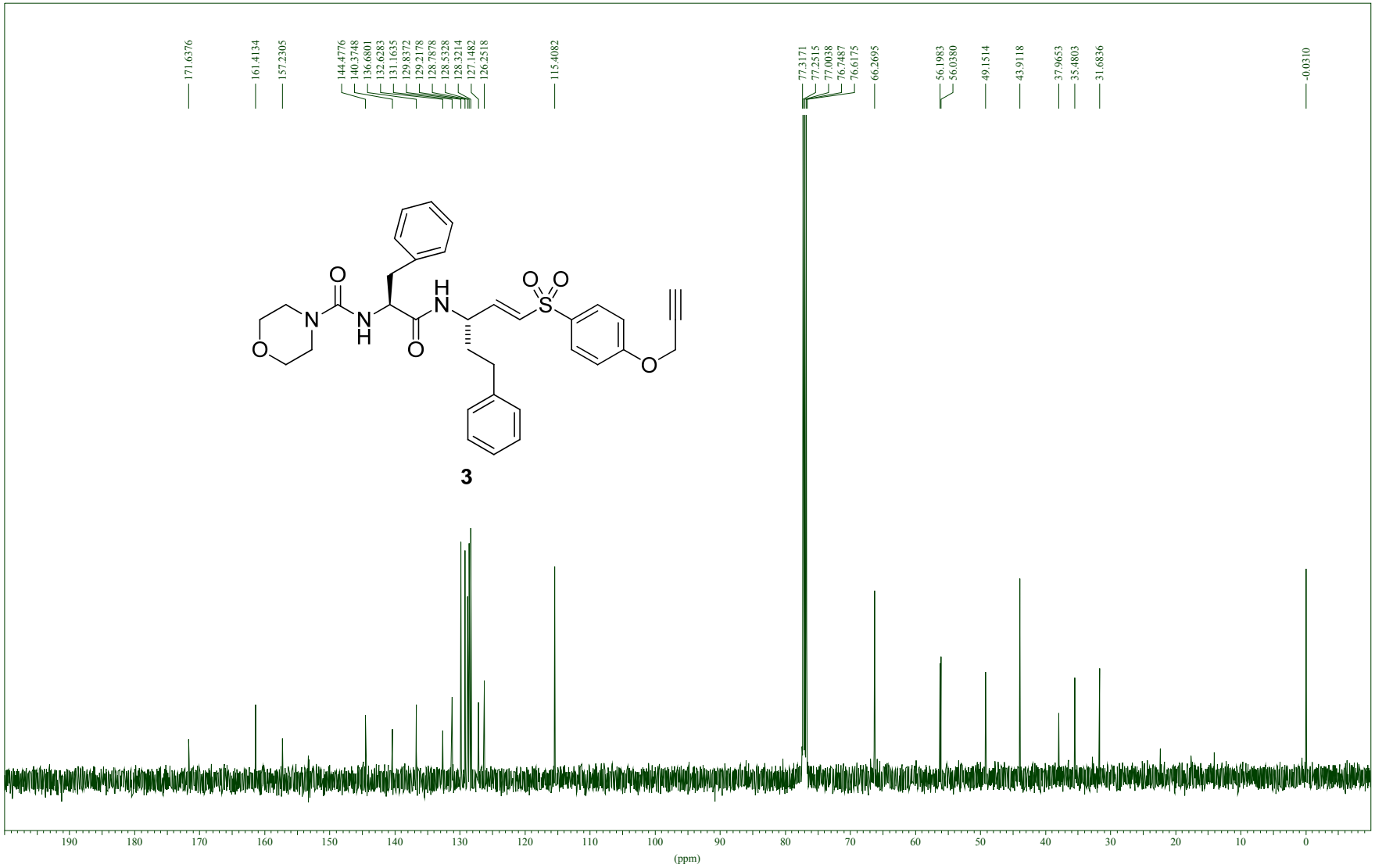
Mu-Phe-Hph-VS-OAlk in CDCl31H AMX500

3

Mu-Phe-Hph-VS-OAlk in CDCl3 13C AMX50



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3	Tb927.8.3530	glycerol-3-phosphate dehydrogenase [NAD+], glycosomalTrypanosoma bruceichr 8Manual	1051	38408	27.32	4	
4	Tb10.70.4740	enolaseTrypanosoma bruceichr 10Manual	1042	47133	60.38	8	RNAi:lethal
5	Tb927.6.2790	L-threonine 3-dehydrogenase, putativeTrypanosoma bruceichr 6Manual	919	37333	36.31	6	
6	Tb09.211.2730	gim5AGim5A proteinTrypanosoma bruceichr 9Manual	897	26790	30.97	5	
7	Tb11.02.3210	TIMtriosephosphate isomeraseTrypanosoma bruceichr 11Manual	792	26973	70.27	8	drug target
8	Tb10.70.5650	TEF1elongation factor 1-alphaTrypanosoma bruceichr 10Manual	766	49474	23.07	7	
9	Tb11.01.3110	heat shock protein 70Trypanosoma bruceichr 11Manual	613	75719	89.97	11	
10	Tb927.6.3740	heat shock 70 kDa protein, mitochondrial precursor, putativeTrypanosoma bruceichr 6Manual	606	72000	23.37	6	
11	Tb10.61.0980	gMDHglycosomal malate dehydrogenaseTrypanosoma bruceichr 10Manual	604	33917	52.61	7	
12	Tb10.70.0280	HSP60chaperonin Hsp60, mitochondrial precursorTrypanosoma bruceichr 10Manual	496	59751	27.11	13	
13	Tb09.211.3550	glk1gkglycerol kinase, glycosomalTrypanosoma bruceichr 9Manual	468	57071	62.22	10	
14	Tb10.70.1370	ALDfructose-bisphosphate aldolase, glycosomalTrypanosoma bruceichr 10Manual	396	41558	33.28	6	
15	Tb10.26.1080	heat shock protein 83Trypanosoma bruceichr 10Manual	373	81169	22.81	5	
16	Tb927.6.4280	GAPDHglyceraldehyde 3-phosphate dehydrogenase, glycosomalTrypanosoma bruceichr 6Manual	327	39251	25.31	4	drug target
17	Tb10.70.5110	mMDHmitochondrial malate dehydrogenaseTrypanosoma bruceichr 10Manual	319	33567	63.43	9	
18	Tb927.2.4210	28H13.455glycosomal phosphoenolpyruvate carboxykinaseTrypanosoma bruceichr 2Manual	290	58927	28.47	11	
19	Tb10.v4.0053	hypothetical proteinTrypanosoma bruceichr 10PartialManual	277	483570	26.83	12	
20	Tb927.3.1380	ATP synthase beta chain, mitochondrial precursorTrypanosoma bruceichr 3Manual	248	55969	21.28	6	drug target
21	Tb927.6.4840	S-adenosylmethionine synthetase, putativeTrypanosoma bruceichr 6Manual	241	43855	21.89	9	
22	Tb927.6.4440	hypothetical protein, conservedTrypanosoma bruceichr 6Manual	218	37923	64.13	13	
23	Tb09.160.4250	TRYP1TXNPx, 28G16.415tryparedoxin peroxidaseTrypanosoma bruceichr 9Manual	217	22752	52.67	8	drug target
24	Tb09.160.4560	AKarginine kinaseTrypanosoma bruceichr 9Manual	216	44973	51.29	7	drug target
25	Tb10.70.2650	elongation factor 2Trypanosoma bruceichr 10Manual	202	95300	25.21	6	
26	Tb11.03.0090	ribokinase, putativeTrypanosoma bruceichr 11Manual	199	35779	32.29	6	
27	Tb927.7.1780	adenine phosphoribosyltransferase, putativeTrypanosoma bruceichr 7Manual	176	26185	87.25	11	
28	Tb927.2.470	3B10.190retrotransposon hot spot (RHS) protein, putativeTrypanosoma bruceichr 2Manual	176	98769	39.95	8	
29	Tb11.46.0001	60S acidic ribosomal subunit protein, putativeTrypanosoma bruceichr 11Manual	171	34891	45.15	8	
30	Tb927.3.4500	fumarate hydratase, putativeTrypanosoma bruceichr 3Manual	151	62907	21.59	7	
31	Tb927.6.1000	CPcysteine peptidase precursor, Clan CA, family C1, Cathepsin L-likeTrypanosoma bruceichr 6Manual	139	49224	25.46	7	drug target
32	Tb10.61.1810	mitochondrial carrier protein, putativeTrypanosoma bruceichr 10Manual	135	34338	47.27	8	
33	Tb927.8.6060	2-amino-3-ketobutyrate coenzyme A ligase, putativeTrypanosoma bruceichr 8Manual	133	44049	20.61	7	
34	Tb927.6.3840	reticulon domain proteinTrypanosoma bruceichr 6Manual	132	21285	59.47	7	
35	Tb11.03.0410	eIF-5Aeukaryotic translation initiation factor 5a, putativeTrypanosoma bruceichr 11Manual	129	17923	64.64	10	
36	Tb927.8.1990	TRYP2tryparedoxin peroxidaseTrypanosoma bruceichr 8Manual	127	25786	42.5	5	
37	Tb927.2.2510	25N14.10hypothetical protein, conservedTrypanosoma bruceichr 2Manual	126	29684	25.45	9	
38	Tb11.01.3550	2-oxoglutarate dehydrogenase E2 component, putativeTrypanosoma bruceichr 11Manual	126	41516	72.51	10	
39	Tb10.70.3360	40S ribosomal protein S3a, putativeTrypanosoma bruceichr 10Manual	126	29632	41	7	
40	Tb11.01.1820	biotin--acetyl-CoA-carboxylase ligase, putativeTrypanosoma bruceichr 11Manual	125	26642	70.05	17	
41	Tb10.70.5820	HK1hexokinaseTrypanosoma bruceichr 10Manual	123	51776	54	7	drug target
42	Tb927.7.2980	hypothetical protein, conservedTrypanosoma bruceichr 7Manual	123	21457	22.57	5	
43	Tb09.160.3270	1L12.525eukaryotic initiation factor 4a, putativeTrypanosoma bruceichr 9Manual	119	45447	48.21	8	
44	Tb927.3.4290	PFR1PFRC73 kDa paraflagellar rod proteinTrypanosoma bruceichr 3Manual	110	69096	62.96	11	
45	Tb10.05.0220	60S ribosomal protein L10aTrypanosoma bruceichr 10Manual	109	25037	49.59	17	
46	Tb09.211.2570	TCP-1-etat-complex protein 1, eta subunit, putativeTrypanosoma bruceichr 9Manual	103	62231	28.52	16	
47	Tb927.8.5440	Tb-24flagellar calcium-binding proteinTrypanosoma bruceichr 8Manual	93	24580	75.11	16	
48	Tb927.8.5470	Tb-17flagellar calcium-binding proteinTrypanosoma bruceichr 8Manual	93	25728	75.11	16	
49	Tb927.8.5600	transaldolase, putativeTrypanosoma bruceichr 8Manual	92	36832	31.81	7	
50	Tb927.5.1210	short-chain dehydrogenase, putativeTrypanosoma bruceichr 5Manual	86	34083	30.79	6	
51	Tb10.70.5380	pyruvate dehydrogenase complex E3 binding protein, putativeTrypanosoma bruceichr 10Manual	86	27489	27.88	7	
52	Tb09.160.4450	RPS340S ribosomal protein S3, putativeTrypanosoma bruceichr 9Manual	81	30724	80.15	9	
53	Tb11.01.3170	TRACKguanine nucleotide-binding protein beta subunit-like proteinTrypanosoma bruceichr 11Manua	80	35181	43.67	10	
54	Tb10.70.3510	60S ribosomal protein L18a, putativeTrypanosoma bruceichr 10Manual	77	21119	63.68	10	
55	Tb927.8.6750	translationally controlled tumor protein (TCTP), putativeTrypanosoma bruceichr 8Manual	76	19367	50.54	8	
56	Tb927.10.290	proteasome alpha 2 subunit, putativeTrypanosoma bruceichr 10Manual	75	25567	74.74	10	RNAi:lethal
57	Tb11.02.1070	aminopeptidase, putativeTrypanosoma bruceichr 11Manual	73	98494	40.91	8	
58	Tb927.8.3750	nucleolar protein, putativeTrypanosoma bruceichr 8Manual	72	54723	72.26	10	
59	Tb10.6k15.1220	IleRSisoleucyl-tRNA synthetase, putativeTrypanosoma bruceichr 10Manual	72	131490	72.11	11	
60	Tb11.03.0250	CYPAcyclophilin aTrypanosoma bruceichr 11Manual	72	18933	26.02	7	
61	Tb10.389.0880	heat shock protein, putativeTrypanosoma bruceichr 10Manual	72	91491	43.11	9	
62	Tb927.3.3270	TbPFKATP-dependent phosphofructokinaseTrypanosoma bruceichr 3Manual	71	53997	71.14	11	drug target
63	Tb927.3.3750	hypothetical protein, conservedTrypanosoma bruceichr 3Manual	70	20018	58.87	8	
64	Tb927.3.2230	succinyl-CoA synthetase alpha subunit, putativeTrypanosoma bruceichr 3Manual	68	31844	40.23	16	
65	Tb11.v4.0004	RNR2ribonucleoside-diphosphate reductase small chainTrypanosoma bruceichr 11Manual	64	39506	64.28	8	
66	Tb10.61.1960	RPS240S ribosomal protein S2, putativeTrypanosoma bruceichr 10Manual	64	28795	63.67	7	
67	Tb11.22.0001	hypothetical protein, conservedTrypanosoma bruceichr 11Manual	63	24736	37.02	7	
68	Tb927.7.7040	methylthioadenosine phosphorylase, putativeTrypanosoma bruceichr 7Manual	63	33536	22.63	6	
69	Tb927.7.1790	adenine phosphoribosyltransferase, putativeTrypanosoma bruceichr 7Manual	62	25779	62.27	21	
70	Tb09.160.1820	COXV3C4.225cytochrome oxidase subunit VTrypanosoma bruceichr 9Manual	61	22329	41.07	7	
71	Tb927.8.7040	hypothetical protein, conservedTrypanosoma bruceichr 8Manual	59	21523	58.72	9	
72	Tb927.7.4570	nucleoside hydrolase, putativeTrypanosoma bruceichr 7Manual	59	39708	58.63	7	
73	Tb10.70.1130	hypothetical protein, conservedTrypanosoma bruceichr 10Manual	58	48342	55.3	8	
74	Tb927.10.7410	succinyl-CoA ligase [GDP-forming] beta-chain, putativeTrypanosoma bruceichr 10Manual	58	55571	58.02	8	RNAi:lethal
75	Tb10.70.5050	hypothetical protein, conservedTrypanosoma bruceichr 10Manual	56	21966	56.09	10	
76	Tb10.70.7190	hypothetical protein, conservedTrypanosoma bruceichr 10Manual	56	66417	32.41	6	
77	Tb10.70.6540	HGPRThypoxanthine-guanine phosphoribosyltransferaseTrypanosoma bruceichr 10Manual	54	23585	54.25	9	
78	Tb11.02.2960	mitochondrial carrier protein, putativeTrypanosoma bruceichr 11Manual	54	30206	53.71	7	
79	Tb927.10.6080	PRCEproteasome beta 5 subunit, putativeTrypanosoma bruceichr 10Manual	54	34850	53.7	6	RNAi:lethal
80	Tb10.70.1670	40S ribosomal protein S10, putativeTrypanosoma bruceichr 10Manual	53	19331	45.26	6	
81	Tb10.6k15.2330	TCP-1-thetat-complex protein 1, theta subunit, putativeTrypanosoma bruceichr 10Manual	52	58501	52.47	9	
82	Tb10.70.4280	delta-1-pyrroline-5-carboxylate dehydrogenase, putativeTrypanosoma bruceichr 10Manual	52	62624	25.3	7	
83	Tb10.70.7010	60S ribosomal protein L9, putativeTrypanosoma bruceichr 10Manual	51	21901	51.36	10	
84	Tb10.70.7695	40S ribosomal proteins S11, putativeTrypanosoma bruceichr 10Manual	51	20303	50.18	7	
85	Tb927.3.1120	rtb2GTP-binding nuclear protein rtb2, putativeTrypanosoma bruceichr 3Manual	50	24732	50.67	12	
86	Tb09.211.0740	p21 antigen protein, putativeTrypanosoma bruceichr 9Manual	50	21104	49.57	9	
87	Tb927.3.1790	pyruvate dehydrogenase E1 beta subunit, putativeTrypanosoma bruceichr 3Manual	50	37998	49.52	8	
88	Tb11.01.5100	paraflagellar rod component, putativeTrypanosoma bruceichr 11Manual	49	68934	49.37	9	
89	Tb09.244.2600	ankyrin-repeat protein, putativeTrypanosoma bruceichr 9Manual	48	338870	21.15	6	
90	Tb927.5.3350	iron superoxide dismutase, putativeTrypanosoma bruceichr 5Manual	48	27148	28.77	8	drug target
91	Tb11.02.4150	PPDKpyruvate phosphate dikinaseTrypanosoma bruceichr 11Manual	47	101300	35.8	6	
92	Tb10.6k15.3820	sterol 24-c-methyltransferase, putativeTrypanosoma bruceichr 10Manual	47	40673	46.61	10	
93	Tb927.5.940	NADH-dependent fumarate reductase, putativeTrypanosoma bruceichr 5Manual	47	95546	22.82	5	
94	Tb927.6.2420	p22 protein precursorTrypanosoma bruceichr 6Manual	46	25391	46.32	8	
95	Tb11.01.3370	PEX11glycosomal membrane protein, putativeTrypanosoma bruceichr 11Manual	45	24240	44.89	12	
96	Tb11.01.8510	TCP-1-alphat-complex protein 1, alpha subunit, putativeTrypanosoma bruceichr 11Manual	44	54875	20.61	6	
97	Tb09.160.3710	28G16.165proliferative cell nuclear antigen (PCNA), putativeTrypanosoma bruceichr 9Manual	43	32750	43.4	7	
98	Tb927.3.2960	IAGNHinosine-adenosine-guanosine-nucleosidehydrolaseTrypanosoma bruceichr 3Manual	43	36509	43.2	13	
99	Tb11.02.4300	hypothetical protein, conservedTrypanosoma bruceichr 11Manual	43	48868	32.96	6	
100	Tb927.7.3590	hypothetical protein, conservedTrypanosoma bruceichr 7Manual	43	17122	36.34	9	
101	Tb11.01.3040	cytosolic malate dehydrogenase, putativeTrypanosoma bruceichr 11Manual	40	35528	40.11	17	
102	Tb927.5.1060	mitochondrial processing peptidase, beta subunit, putativeTrypanosoma bruceichr 5Manual	40	54773	39.83	6	
103	Tb10.70.7050	TCP-1-deltat-complex protein 1, delta subunit, putativeTrypanosoma bruceichr 10Manual	40	59009	33.5	9	
104	Tb10.6k15.3850	GAPglyceraldehyde 3-phosphate dehydrogenase, cytosolicTrypanosoma bruceichr 10Manual	39	35760	25.31	4	
105	Tb11.02.4700	14-3-3-like protein, putativeTrypanosoma bruceichr 11Manual	38	29463	23.77	8	
106	Tb927.3.2100	hypothetical protein, conservedTrypanosoma bruceichr 3Manual	37	34260	37.3	9	
107	Tb11.02.5450	glucose-regulated protein 78, putativeTrypanosoma bruceichr 11Manual	37	71505	37.29	11	
108	Tb927.3.4740	hypothetical protein, conservedTrypanosoma bruceichr 3Manual	37	46361	37.14	5	
109	Tb927.4.1300	hypothetical protein, conservedTrypanosoma bruceichr 4Manual	37	42444	36.97	7	
110	Tb927.1.4100	COXIVcytochrome oxidase subunit IVTrypanosoma bruceichr 1Manual	37	40680	21.05	7	
111	Tb09.v1.0380	spermidine synthase, putativeTrypanosoma bruceichr 9Manual	36	33304	24.12	5	drug target

VS_BSF

prot_hit_num	prot_acc	prot_desc	prot_sc	prot_ma	pep_sc	pep_num_match			
1	Tb927.6.1000	CPcysteine peptidase precursor, Clan CA, family C1, Cathepsin L-likeTrypanosoma bruceichr 6Manua	2627	49224	50.89	6			
2	Tb10.70.1370	ALDfructose-bisphosphate aldolase, glycosomalTrypanosoma bruceichr 10Manual	2134	41558	50.76	6			
3	Tb927.1.2330	beta tubulinTrypanosoma bruceichr 1Manual	932	50413	68.14	8			
4	Tb11.02.5450	glucose-regulated protein 78, putativeTrypanosoma bruceichr 11Manual	695	71505	37.91	6			
5	Tb927.3.3270	TbPFKATP-dependent phosphofructokinaseTrypanosoma bruceichr 3Manual	528	53997	27.83	8	drug target		
6	Tb09.211.3550	glk1gkglycerol kinase, glycosomalTrypanosoma bruceichr 9Manual	498	57071	43.95	9			
7	Tb10.70.5820	HK1hexokinaseTrypanosoma bruceichr 10Manual	475	51776	46.85	7	drug target		
8	Tb927.1.2340	alpha tubulinTrypanosoma bruceichr 1Manual	395	50383	26.04	6			
9	Tb10.6k15.2290	BS2protein disulfide isomeraseTrypanosoma bruceichr 10Manual	369	55887	58.41	13			
10	Tb927.5.1210	short-chain dehydrogenase, putativeTrypanosoma bruceichr 5Manual	257	34083	39.14	5			
11	Tb11.02.3210	TIMtriosephosphate isomeraseTrypanosoma bruceichr 11Manual	214	26973	60.51	8	drug target		
12	Tb927.6.4280	GAPDHglyceraldehyde 3-phosphate dehydrogenase, glycosomalTrypanosoma bruceichr 6Manual	202	39251	23.81	6	drug target		
13	Tb927.8.5440	Tb-24flagellar calcium-binding proteinTrypanosoma bruceichr 8Manual	198	24580	67.02	9			
14	Tb927.8.5470	Tb-17flagellar calcium-binding proteinTrypanosoma bruceichr 8Manual	198	25728	67.02	9			
15	Tb09.211.2730	gim5AGim5A proteinTrypanosoma bruceichr 9Manual	179	26790	39.83	19			
16	Tb10.70.5650	TEF1elongation factor 1-alphaTrypanosoma bruceichr 10Manual	141	49474	26.92	5			
17	Tb927.8.3530	glycerol-3-phosphate dehydrogenase [NAD+], glycosomalTrypanosoma bruceichr 8Manual	136	38408	54.8	8			
18	Tb927.6.560	TbcatBcysteine peptidase C (CPC)Trypanosoma bruceichr 6Manual	124	38112	50.98	10			
19	Tb11.01.2000	hslVU complex proteolytic subunit, putativeTrypanosoma bruceichr 11Manual	123	22898	37.84	7			
20	Tb10.70.0280	HSP60chaperonin Hsp60, mitochondrial precursorTrypanosoma bruceichr 10Manual	113	59751	44.12	7			
21	Tb11.01.3110	heat shock protein 70Trypanosoma bruceichr 11Manual	95	75719	24.37	9			
22	Tb927.4.5010	calreticulin, putativeTrypanosoma bruceichr 4Manual	90	45242	60.7	19			
23	Tb927.4.2450	thioredoxin, putativeTrypanosoma bruceichr 4Manual	83	44748	60.64	8	null=viable		
24	Tb927.6.3740	heat shock 70 kDa protein, mitochondrial precursor, putativeTrypanosoma bruceichr 6Manual	79	72000	63.05	14			
25	Tb927.5.1810	lysosomal/endosomal membrane protein p67Trypanosoma bruceichr 5Manual	78	73028	22.64	6			
26	Tb927.3.1380	ATP synthase beta chain, mitochondrial precursorTrypanosoma bruceichr 3Manual	76	55969	75.6	11	drug target		
27	Tb10.26.1080	heat shock protein 83Trypanosoma bruceichr 10Manual	72	81169	57.37	8			
28	Tb927.3.4290	PFR1PFRC73 kDa paraflagellar rod proteinTrypanosoma bruceichr 3Manual	71	69096	53.86	11			
29	Tb11.01.3550	2-oxoglutarate dehydrogenase E2 component, putativeTrypanosoma bruceichr 11Manual	70	41516	45.05	16			
30	Tb927.4.1610	hypothetical protein, conservedTrypanosoma bruceichr 4Manual	67	39892	67.4	16			
31	Tb927.7.7420	ATP synthase alpha chain, mitochondrial precursorTrypanosoma bruceichr 7Manual	64	63862	32.6	5	drug target		
32	Tb927.1.700	PGKCgPGKphosphoglycerate kinaseTrypanosoma bruceichr 1Manual	63	47558	62.64	9	drug target		
33	Tb927.1.120	retrotransposon hot spot (RHS) protein, putativeTrypanosoma bruceichr 1Manual	61	98534	61.38	8			
34	Tb10.61.1810	mitochondrial carrier protein, putativeTrypanosoma bruceichr 10Manual	60	34338	48.12	16			
35	Tb927.8.4970	PFR2PFR69 kDa paraflagellar rod proteinTrypanosoma bruceichr 8Manual	59	69953	42.17	8			
36	Tb927.2.2510	25N14.10hypothetical protein, conservedTrypanosoma bruceichr 2Manual	55	29684	54.83	8			
37	Tb927.3.3580	LPG3lipophosphoglycan biosynthetic protein, putativeTrypanosoma bruceichr 3Manual	54	87712	54.09	11			
38	Tb927.2.4210	28H13.455glycosomal phosphoenolpyruvate carboxykinaseTrypanosoma bruceichr 2Manual	53	58927	52.75	11			
39	Tb927.6.3840	reticulon domain proteinTrypanosoma bruceichr 6Manual	50	21285	49.75	12			
40	Tb10.70.5250	MCA4metacaspase MCA4, cysteine peptidase, Clan CD, family C13Trypanosoma bruceichr 10Manua	48	39628	49.05	7	drug target		
41	Tb927.7.4180	fatty acid elongase, putativeTrypanosoma bruceichr 7Manual	48	34077	26.17	10	null=viable		
42	Tb10.70.7190	hypothetical protein, conservedTrypanosoma bruceichr 10Manual	46	66417	26.5	5			
43	Tb10.v4.0053	hypothetical proteinTrypanosoma bruceichr 10PartialManual	44	483570	44.38	10			
44	Tb927.7.3330	hypothetical protein, conservedTrypanosoma bruceichr 7Manual	44	504918	43.65	14			
45	Tb09.160.4560	AKarginine kinaseTrypanosoma bruceichr 9Manual	42	44973	41.94	12			
46	Tb11.03.0230	IDHisocitrate dehydrogenase, putativeTrypanosoma bruceichr 11Manual	41	47140	40.8	6			
47	Tb10.6k15.3640	AOXTAOalternative oxidaseTrypanosoma bruceichr 10Manual	39	37738	38.88	7	drug target		
48	Tb927.6.2420	p22 protein precursorTrypanosoma bruceichr 6Manual	39	25391	38.8	10			
49	Tb10.70.6640	hypothetical protein, conservedTrypanosoma bruceichr 10Manual	38	28302	37.85	8			
	Proteins who were also detected in procyclic forms are shaded in Turquoise.								