Methods S1

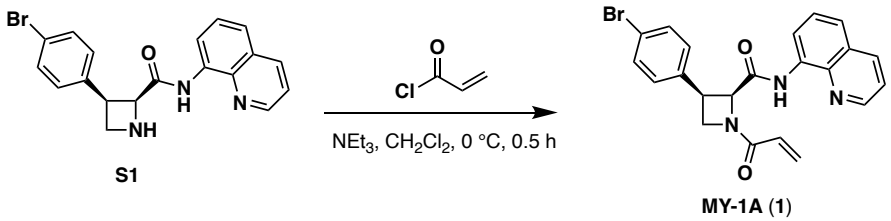
Contents

General considerations

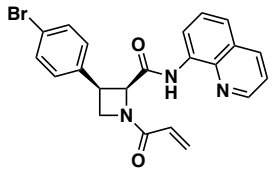
All reagents and solvents were purchased and used as received from commercial vendors or synthesized according to cited procedures. Yields refer to chromatographically and spectroscopically pure compounds, unless otherwise stated. Flash chromatography was performed using 20-40 μm silica gel (60-Å mesh) on a Teledyne Isco Combiflash Rf or a Biotage Isolera Prime, alternatively in a glass column using SiliaFlash® F60 40-63 μm silica gel (60-Å mesh). Preparative high-pressure liquid chromatography (prep-HPLC) was performed on a Gilson GX-281 instrument equipped with a Phenomenex Gemini C18 column (150 mm × 25 mm × 10 μM) eluting with a mixture of acetonitrile and a buffered aqueous phase. Aqueous buffers are denoted as follows: BASE (0.05% ammonia v/v), TFA (0.075% trifluoroacetic acid v/v), FA (0.225% formic acid v/v), HCL (0.05% concentrated hydrochloric acid, v/v), NEU (10 mmol ammonium bicarbonate). Analytical thin layer chromatography (TLC) was performed on 0.2 mm or 0.25 mm silica gel 60-F plates and visualized by UV light (254 nm). Preparative thin layer chromatography (prep-TLC) was performed on GF254 plates (acrylic adhesive, 0.5×200×200 mm, 5–20 μM particle size, 250 μM thickness). NMR spectra were recorded on Bruker Avance III 400, Avance III HD 400, Avance Neo 400 spectrometers (^{1}H, 400 MHz) at 300 K unless otherwise noted. Data for ^{1}H NMR are reported as follows: chemical shift (δ), multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet; br = broad), coupling constants, and integration. Chemical shifts are reported in parts per million (ppm) using the appropriate solvent as reference. Analytical supercritical fluid chromatography (SFC) was performed on a Shimadzu LC system (flow rate: 3 mL/min, back pressure: 100 Bar, column temperature: 35 ºC) equipped with a polydiode array detector unless otherwise noted. Tandem liquid chromatography/mass spectrometry (LC-MS) was performed on an Agilent 1200 series LC/MSD system equipped with an Agilent G6110A mass detector, alternatively a Shimadzu LC-20AD or AB series LC-MS system equipped with Shimadzu SPD-M20A or SPDM40 mass detectors, alternatively a Waters H-Class LC with equipped with diode array and QDa mass detector.

Synthesis of azetidine probes

Synthesis of MY-1A



(2*S*,3*R*)-1-acryloyl-3-(4-bromophenyl)-*N*-(quinolin-8-yl)azetidine-2-carboxamide (MY-1A) (1)



To a precooled (0 ºC) solution of **S1** (50.0 mg, 131 μmol) (Maetani et al., 2017) in dichloromethane (1 mL) were added triethylamine (26.5 mg, 262 μmol) and acryloyl chloride (14.2 mg, 157 μmol). The mixture was stirred at 0 °C for 0.5 hours. Upon completion, the reaction mixture was concentrated under reduced pressure to obtain a residue, which was purified by prep-TLC (SiO_2, petroleum ether/EtOAc = 2:1) to give **MY-1A** (51.0 mg, 89% yield) as a white solid.

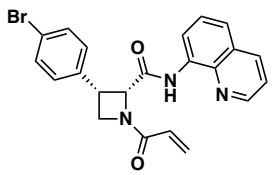
HRMS ESI-TOF m/z calculated for $C_{22}H_{19}BrN_3O_2$ $[M+H]^+$ 436.0655. Found 436.0646.

^{1}H NMR (400 MHz, $CDCl_3$): δ 10.47 (br s, 1H), 8.81 (d, *J* = 3.6 Hz, 1H), 8.43 (d, *J* = 4.0 Hz, 1H), 8.16 (d, *J* = 8.0 Hz, 1H), 7.54-7.42 (m, 3H), 7.29-7.25 (m, 4H + $CHCl_3$), 6.53 (d, *J* = 16.3 Hz, 1H), 6.48-6.18 (m, 1H), 5.98-5.62 (m, 1H), 5.38 (d, *J* = 8.0 Hz, 1H), 4.64 (t, *J* = 9.3 Hz, 1H), 4.59-4.40 (m, 1H), 4.33-4.24 (m, 1H).

Synthesis of MY-1B

Prepared in analogous fashion from *ent*-**S1** (Maetani et al., 2017).

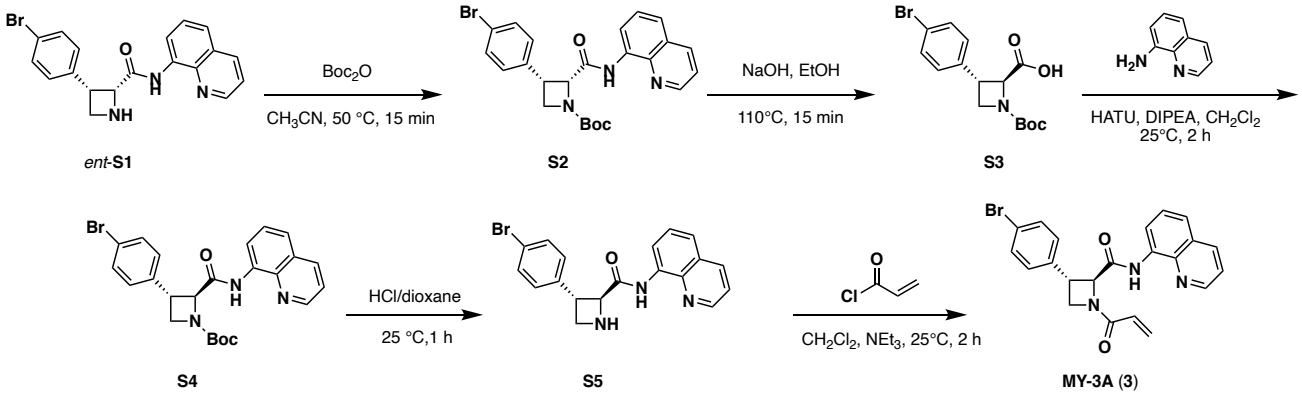
(2*R*,3*S*)-1-acryloyl-3-(4-bromophenyl)-*N*-(quinolin-8-yl)azetidine-2-carboxamide (MY-1B) (2)



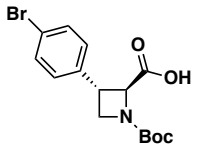
HRMS ESI-TOF m/z calculated for $C_{22}H_{19}BrN_3O_2$ $[M+H]^+$ 436.0655. Found 436.0649.

^{1}H NMR (400 MHz, $CDCl_3$): δ 10.47 (s, 1H), 8.80 (d, *J* = 4.0 Hz, 1H), 8.42 (d, *J* = 4.0 Hz, 1H), 8.15 (d, *J* = 8.0 Hz, 1H), 7.52-7.40 (m, 3H), 7.30-7.25 (m, 4H + $CHCl_3$), 6.64-6.15 (m, 2H), 5.90-5.64 (m, 1H), 5.37 (d, *J* = 8.0 Hz, 1H), 4.63 (t, *J* = 9.3 Hz, 1H), 4.57-4.38 (m, 1H), 4.33-4.22 (m, 1H).

Synthesis of MY-3A



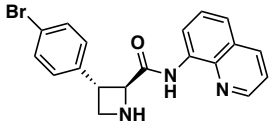
(2*S*,3*S*)-3-(4-bromophenyl)-1-(*tert*-butoxycarbonyl)azetidine-2-carboxylic acid (S3)



To a solution of *ent*-**S1** (450 mg, 1.18 mmol) in acetonitrile (3 mL) was added Boc_2O (308 mg, 1.41 mmol), and the resulting mixture was stirred at 50 °C for 15 min. Upon completion, the reaction mixture was filtered over Celite and concentrated under reduced pressure to give **S2** (600 mg, crude) as a yellow oil. To a solution of **S2** (450 mg, 933 μmol) in ethanol (3 mL) was added sodium hydroxide (373 mg, 9.33 mmol), and the resulting mixture was stirred at 110 °C for 15 min. Upon completion, the mixture was diluted with water and washed with dichloromethane (2×100 mL). The resulting aqueous solution was acidified with HCl (1 M) to adjust pH to 5~6, exhaustively extracted with i-PrOH/$CHCl_3$ (3:7, 5×60 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give **S3** (330 mg, 99% yield over two steps) as a white solid.

LC-MS m/z calculated for $C_{15}H_{19}BrNO_4$ $[M+H]^+$ 356.0. Found 356.0.

(2*S*,3*S*)-3-(4-bromophenyl)-*N*-(quinolin-8-yl)azetidine-2-carboxamide (S5)

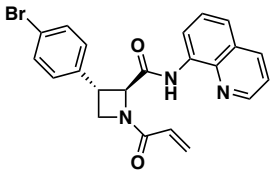


To a solution of **S3** (330 mg, 926 μmol) in dichloromethane (4 mL) were added diisopropylethylamine (239 mg, 1.85 mmol) and 8-aminoquinoline (23.7 mg, 262 μmol), followed by HATU (705 mg, 1.85 mmol). The resulting mixture was stirred at 25 °C for 2 hours. Upon completion, the reaction mixture was concentrated under reduced pressure to give a residue, which was purified by prep-TLC (SiO_2, petroleum ether/EtOAc = 2:1) to give **S4** (200 mg) as a yellow solid used directly in the next step. To a solution

of **S4** (100 mg) in dichloromethane (1 mL) was added HCl/dioxane (4 M, 1 mL). and the resulting mixture was stirred at 25 °C for 1 hour. Upon completion, the reaction mixture was partitioned between ethyl acetate (40 mL) and brine (30 mL). The water layer was extracted with i-PrOH/$CHCl_3$ (3:7, 5×20 mL), then the organic phase was dried over sodium sulfate, filtered, and concentrated under reduced pressure to give **S5** (90.0 mg, 51% yield over two steps) as an off-white solid.

LC-MS m/z calculated for $C_{19}H_{17}BrN_3O$ $[M+H]^+$ 382.1. Found 382.1.

(2*S*,3*S*)-1-acryloyl-3-(4-bromophenyl)-*N*-(quinolin-8-yl)azetidine-2-carboxamide (MY-3A) (3)



To a solution of **S5** (80.0 mg, 209 μmol) in dichloromethane (1 mL) were added triethylamine (42.4 mg, 419 μmol) and acryloyl chloride (37.9 mg, 419 μmol). The mixture was stirred at 25 °C for 2 hours. Upon completion, the reaction mixture was concentrated under reduced pressure. The residue was purified by preparative TLC (SiO_2, petroleum ether/EtOAc = 2:1) to give **MY-3A** (34.0 mg, 37% yield) as a white solid.

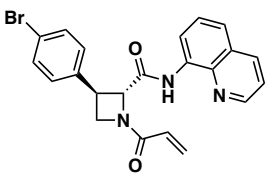
HRMS ESI-TOF m/z calculated for $C_{22}H_{19}BrN_3O_2$ $[M+H]^+$ 436.0655. Found 436.0649.

^{1}H NMR (400 MHz, $CDCl_3$): δ 11.13-10.67 (m, 1H), 8.95-8.84 (m, 1H), 8.83-8.75 (m, 1H), 8.16 (d, *J* = 8.0 Hz, 1H), 7.60-7.50 (m, 4H), 7.45 (dd, *J* = 8.2, 4.2 Hz, 1H), 7.33-7.28 (m, 2H), 6.54 (br d, *J* = 16.9 Hz, 1H), 6.42-6.25 (m, 1H), 5.91-5.75 (m, 1H), 5.18-4.95 (m, 1H), 4.76-4.55 (m, 1H), 4.43-4.14 (m, 2H).

Synthesis of MY-3B

Prepared in analogous fashion from **S1**.

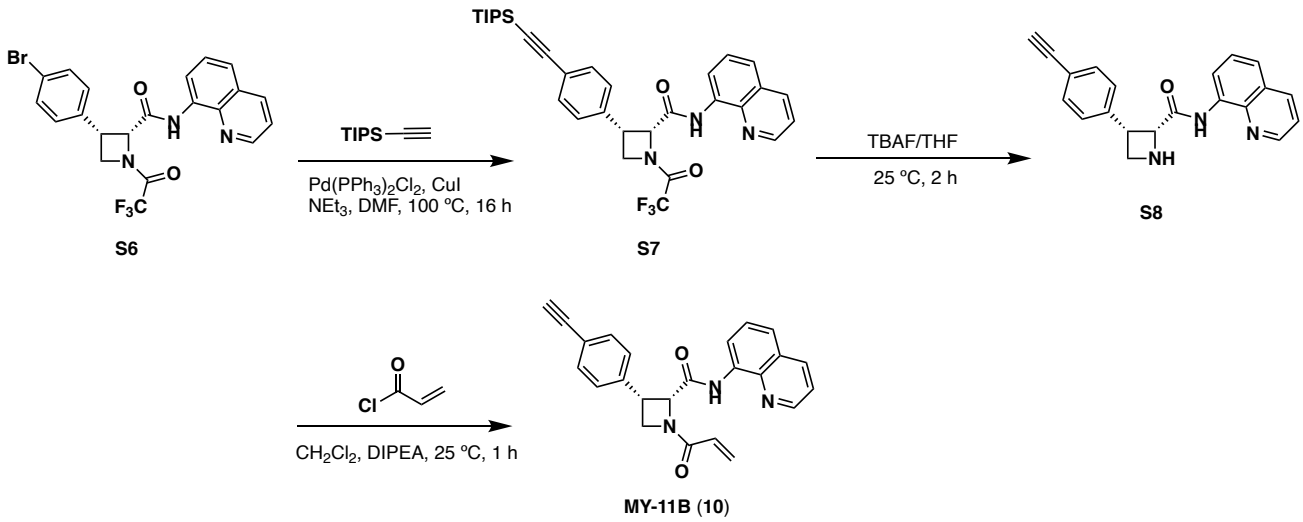
(2*S*,3*S*)-1-acryloyl-3-(4-bromophenyl)-*N*-(quinolin-8-yl)azetidine-2-carboxamide (MY-3B) (4)



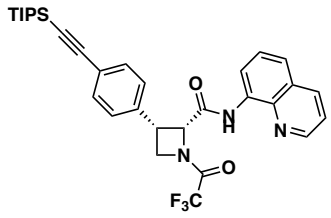
HRMS ESI-TOF m/z calculated for $C_{22}H_{19}BrN_3O_2$ $[M+H]^+$ 436.0655. Found 436.0643.

^{1}H NMR (400 MHz, $CDCl_3$): δ 10.95 (br s, 1H), 8.97-8.69 (m, 2H), 8.15 (d, *J* = 8.2 Hz, 1H), 7.55 (d, *J* = 4.7 Hz, 2H), 7.52 (d, *J* = 8.1 Hz, 2H), 7.45 (dd, *J* = 8.3, 4.2 Hz, 1H), 7.28 (d, *J* = 8.2 Hz, 2H), 6.53 (d, *J* = 16.9 Hz, 1H), 6.41-6.27 (m, 1H), 5.90-5.75 (m, 1H), 5.15-5.02 (m, 1H), 4.73-4.59 (m, 1H), 4.37-4.21 (m, 2H).

Synthesis of MY-11B



(2*R*,3*S*)-*N*-(quinolin-8-yl)-1-(2,2,2-trifluoroacetyl)-3-(4-((triisopropylsilyl)ethynyl)phenyl)azetidine-2-carboxamide (S7)

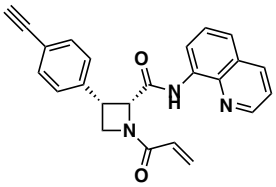


To a solution of **S6** (690 mg, 1.44 mmol) and (triisopropylsilyl)acetylene (789 mg, 4.33 mmol) in *N,N*-dimethyl formamide (2 mL) were added copper(I) iodide (27.5 mg, 144 μmol), $Pd(PPh_3)_2Cl_2$ (101 mg, 144 μmol) and triethylamine (292 mg, 2.89 mmol). The mixture was stirred at 100 °C for 16 hours under nitrogen atmosphere. Upon completion, the reaction mixture was partitioned between ethyl acetate (60 mL) and brine (40 mL). The water layer was extracted with ethyl acetate (40 mL×3). The organic layers were combined, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give a residue, which was purified by column chromatography (SiO_2, petroleum ether/EtOAc = 100:1 to 1:1) to give **S7** (500 mg, 59% yield) as a white solid.

LC-MS m/z calculated for $C_{32}H_{37}F_3N_3O_2Si$ $[M+H]^+$ 580.3. Found 580.4.

^{1}H NMR (400 MHz, CD_3OD, mixture of rotamers): δ 10.18-9.69 (m, 1H), 8.84-8.65 (m, 1H), 8.52-8.33 (m, 1H), 8.14 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.53-7.39 (m, 3H), 7.34-7.22 (m, 3H + $CHCl_3$), 7.17 (d, *J* = 8.0 Hz, 1H), 5.66-5.38 (m, 1H), 4.91-4.39 (m, 3H), 1.11-0.91 (m, 21H).

(2*R*,3*S*)-1-acryloyl-3-(4-ethynylphenyl)-*N*-(quinolin-8-yl)azetidine-2-carboxamide (MY-11B) (10)



To a solution of **S7** (500 mg, 862 μmol) in tetrahydrofuran (8.6 mL) was added tetrabutylammonium fluoride (1 M in THF, 8.6 mL). The mixture was stirred at 25 °C for 2 hours. Upon completion, the reaction mixture was concentrated in vacuo to give the residue. The residue was purified by prep-TLC (SiO_2, petroleum ether/ethyl acetate = 0:1) to obtain **S8** (250 mg) as a white solid. To a solution of **S8** (100 mg, 305 μmol) in dichloromethane (2 mL) were added diisopropylethylamine (79.0 mg, 611 μmol) and acryloyl chloride (55.3 mg, 611 μmol). The mixture was stirred at 25 °C for 1 hour. Upon completion, the reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by preparative TLC (SiO_2, petroleum ether/EtOAc = 1:2) and prep-HPLC (column: Waters Xbridge 150mm×25mm×5μm; mobile phase: [water (10mM NH_4HCO_3)-CH_3CN]; B%: 35%-68%, 8min). It was further separated by SFC (Column: Chiralpak AD-3 50×4.6mm I.D., 3μm Mobile phase: Phase A: CO_2, and Phase B: i-PrOH (0.05% diethylamine); Gradient elution: 40% i-PrOH (0.05% diethylamine) in CO_2; Flow rate: 3mL/min; Column Temp: 35 ºC; Back Pressure: 100 Bar) to obtain **MY-11B** (52.0 mg, 53% yield over two steps) as a white solid.

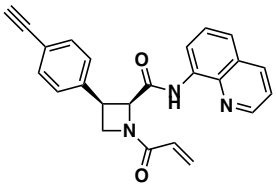
HRMS ESI-TOF m/z calculated for $C_{24}H_{20}N_3O_2$ $[M+H]^+$ 382.1550. Found 382.1542.

^{1}H NMR (400 MHz, DMSO-d_6, mixture of rotamers): δ 10.4-10.2 (m, 1H), 8.90 (d, *J* = 4.0 Hz, 1H), 8.37 (d, *J* = 8.0 Hz, 1H), 8.21-8.12 (m, 1H), 7.65-7.56 (m, 2H), 7.45 (t, *J* = 8.0 Hz, 1H), 7.40-7.34 (m, 2H), 7.26-7.16 (m, 2H), 6.62-6.10 (m, 2H), 5.84 (d, *J* = 9.9 Hz, 1H), 5.70-5.40 (m, 1H), 4.65-4.55 (m, 1H), 4.50-4.22 (m, 2H), 4.10-4.00 (m, 1H).

Synthesis of MY-11A

Prepared in analogous fashion from *ent*-**S6**.

(2*S*,3*R*)-1-acryloyl-3-(4-bromophenyl)-*N*-(quinolin-8-yl)azetidine-2-carboxamide (MY-11A) (9)

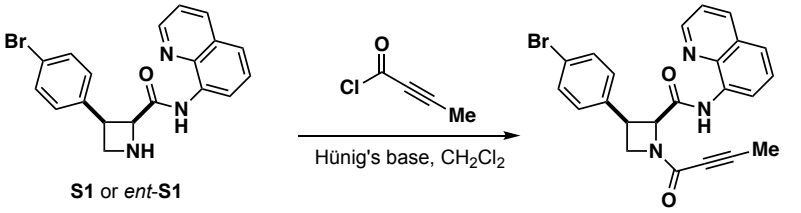


HRMS ESI-TOF m/z calculated for $C_{24}H_{20}N_3O_2$ $[M+H]^+$ 382.1550; Found 382.1540.

^{1}H NMR (400 MHz, CD_3OD): δ 8.85 (dd, *J* = 4.3, 1.7 Hz, 1H), 8.29 (dd, *J* = 8.3,

1.7 Hz, 1H), 8.26-8.11 (m, 1H), 7.60 (dd, *J* = 8.3, 1.3 Hz, 1H), 7.55 (dd, *J* = 8.3, 4.2 Hz, 1H), 7.44 (t, *J* = 8.0 Hz, 1H), 7.39 (d, *J* = 7.9 Hz, 2H), 7.21 (d, *J* = 7.8 Hz, 2H), 6.83-6.23 (m, 2H), 6.09-5.36 (m, 2H), 4.80-4.40 (m, 4H), 3.36 (s, 1H).

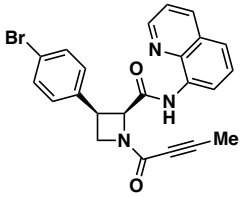
Synthesis of MY-45A and MY-45B



General procedure A: preparation of but-2-ynoyl chloride solution. PCl_5 (115 mg, 0.55 mmol, 1.1 equiv) was added to an ice-cold suspension of but-2-ynoic acid (42.0 mg, 0.50 mmol, 1.0 equiv) in dichloromethane (1 mL). The ice bath was removed and the reaction mixture was stirred at room temperature until it turned into a clear solution (typically 30 minutes to 1 hour).

General procedure B: butynamide formation. But-2-ynoyl chloride (0.5 M solution in dichloromethane, 1.5 equiv), prepared according to general procedure A, was slowly added to a solution of the corresponding amine (1.0 equiv) and Hünig's base (3.0 equiv) in dichloromethane (0.05 M) at 0 °C. The reaction was allowed to warm to room temperature and stirred until complete consumption of starting material (as monitored by TLC). The reaction was quenched by addition of sat. aq. $NaHCO_3$ and was extracted with EtOAc (3×). The combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography and preparative TLC as indicated.

(2*S*,3*R*)-3-(4-bromophenyl)-1-(but-2-ynoyl)-*N*-(quinolin-8-yl)azetidine-2-carboxamide (MY-45A) (11)

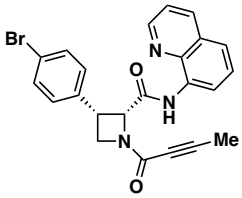


Following general procedure B using **S1** (16.9 mg, 0.044 mmol, 1.0 equiv).(Maetani et al., 2017) Purification by flash column chromatography (SiO_2, dichloromethane/acetone = 100:0 to 10:1) followed by preparative TLC (SiO_2, $CHCl_3$/acetone = 9:1) provided **MY-45A** as a white foam (9.9 mg, 50% yield).

HRMS ESI-TOF m/z calculated for $C_{23}H_{19}BrN_3O_2$ $[M+H]^+$ 448.0655. Found 448.0648.

^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers): δ 10.54 (s, 0.6H), 10.35 (s, 0.4H), 8.95-8.77 (m, 1H), 8.40 (d, *J* = 7.4 Hz, 1H), 8.21-8.09 (m, 1H), 7.57-7.38 (m, 3H), 7.32-7.20 (m, 4H), 5.40 (d, *J* = 9.7 Hz, 0.6H), 5.29 (d, *J* = 9.7 Hz, 0.4H), 4.69-4.53 (m, 1H), 4.53-4.32 (m, 1H), 4.32-4.15 (m, 1H), 2.10 (s, 1H), 1.79 (s, 2H).

(2*R*,3*S*)-3-(4-bromophenyl)-1-(but-2-ynoyl)-*N*-(quinolin-8-yl)azetidine-2-carboxamide (MY-45B) (12)



Following general procedure B using *ent*-**S1** (18.7 mg, 0.049 mmol, 1.0 equiv).(Maetani et al., 2017) Purification by flash column chromatography (SiO_2, dichloromethane/acetone = 100:0 to 10:1) followed by preparative TLC (SiO_2, $CHCl_3$/acetone = 9:1) provided **MY-45B** as a white foam (10.3 mg, 47% yield).

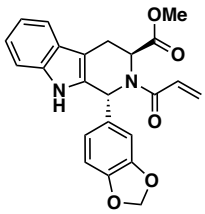
HRMS ESI-TOF m/z calculated for $C_{23}H_{19}BrN_3O_2$ $[M+H]^+$ 448.0655. Found 448.0644.

^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers): δ 10.55 (s, 0.6H), 10.35 (s, 0.4 H), 8.98-8.74 (m, 1H), 8.40 (d, *J* = 7.4 Hz, 1H), 8.22-7.98 (m, 1H), 7.73-7.40 (m, 3H), 7.30-7.19 (m, 4H), 5.40 (d, *J* = 9.7 Hz, 0.6H), 5.29 (d, *J* = 9.7 Hz, 0.4H), 4.72-4.50 (m, 1H), 4.52-4.32 (m, 1H), 4.32-4.08 (m, 1H), 2.10 (s, 1H), 1.80 (s, 2H).

Synthesis of tryptoline probes

EV-96, EV-97, EV-98, EV-99 were prepared as reported previously (Vinogradova et al., 2020).

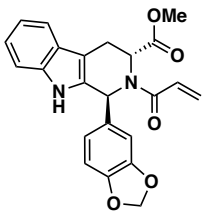
methyl (1*R*,3*S*)-2-acryloyl-1-(benzo[*d*][1,3]dioxol-5-yl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*] indole-3-carboxylate (EV-96) (5)



HRMS ESI-TOF m/z calculated for $C_{23}H_{21}N_2O_5$ $[M+H]^+$ 405.1445. Found 405.1441.

^{1}H NMR (400 MHz, CD_3OD): δ 7.44 (d, *J* = 7.8 Hz, 1H), 7.33-7.17 (m, 1H), 7.12-7.03 (m, 1H), 7.00 (t, *J* = 7.4 Hz, 1H), 6.95-6.85 (m, 2H), 6.84-6.65 (m, 2H), 6.30-6.04 (m, 2H), 6.00-5.79 (m, 2H), 5.70 (dd, *J* = 10.6, 1.8 Hz, 1H), 5.50-4.95 (m, 1H), 3.73-3.51 (m, 3H), 3.50-3.39 (m, 1H), 3.26-3.14 (m, 1H), 1 exchangeable proton not observed.

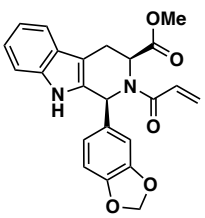
methyl (1*S*,3*R*)-2-acryloyl-1-(benzo[*d*][1,3]dioxol-5-yl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*] indole-3-carboxylate (EV-97) (6)



HRMS ESI-TOF m/z calculated for $C_{23}H_{21}N_2O_5$ $[M+H]^+$ 405.1445. Found 405.1450.

^{1}H NMR (400 MHz, CD_3OD): δ 7.44 (d, *J* = 8.0 Hz, 1H), 7.32-7.15 (m, 1H), 7.11-7.04 (m, 1H), 7.00 (t, *J* = 7.4 Hz, 1H), 6.95-6.86 (m, 2H), 6.85-6.68 (m, 2H), 6.30-6.05 (m, 2H), 6.00-5.79 (m, 2H), 5.70 (dd, *J* = 10.6, 1.8 Hz, 1H), 5.55-4.95 (m, 1H), 3.70-3.51 (m, 3H), 3.50-3.37 (m, 1H), 3.28-3.10 (m, 1H), 1 exchangeable proton not observed.

methyl (1*S*,3*S*)-2-acryloyl-1-(benzo[*d*][1,3]dioxol-5-yl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*] indole-3-carboxylate (EV-98) (7)

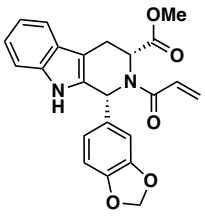


HRMS ESI-TOF m/z calculated for $C_{23}H_{21}N_2O_5$ $[M+H]^+$ 405.1445. Found 405.1444.

^{1}H NMR (400 MHz, CD_3OD): δ 7.52 (d, *J* = 7.8 Hz, 1H), 7.28 (d, *J* = 8.0 Hz, 1H), 7.15-7.09 (m, 1H), 7.08-7.03 (m, 1H), 7.02-6.81 (m, 2H), 6.80 (s, 1H), 6.73-6.39 (m,

2H), 6.49-6.20 (m, 1H), 5.90 (s, 2H), 5.82 (d, *J* = 8.0 Hz, 1H), 5.69-5.23 (m, 1H), 3.66-3.50 (m, 1H), 3.13 (s, 3H), 3.07-2.96 (m, 1H), 1 exchangeable proton not observed.

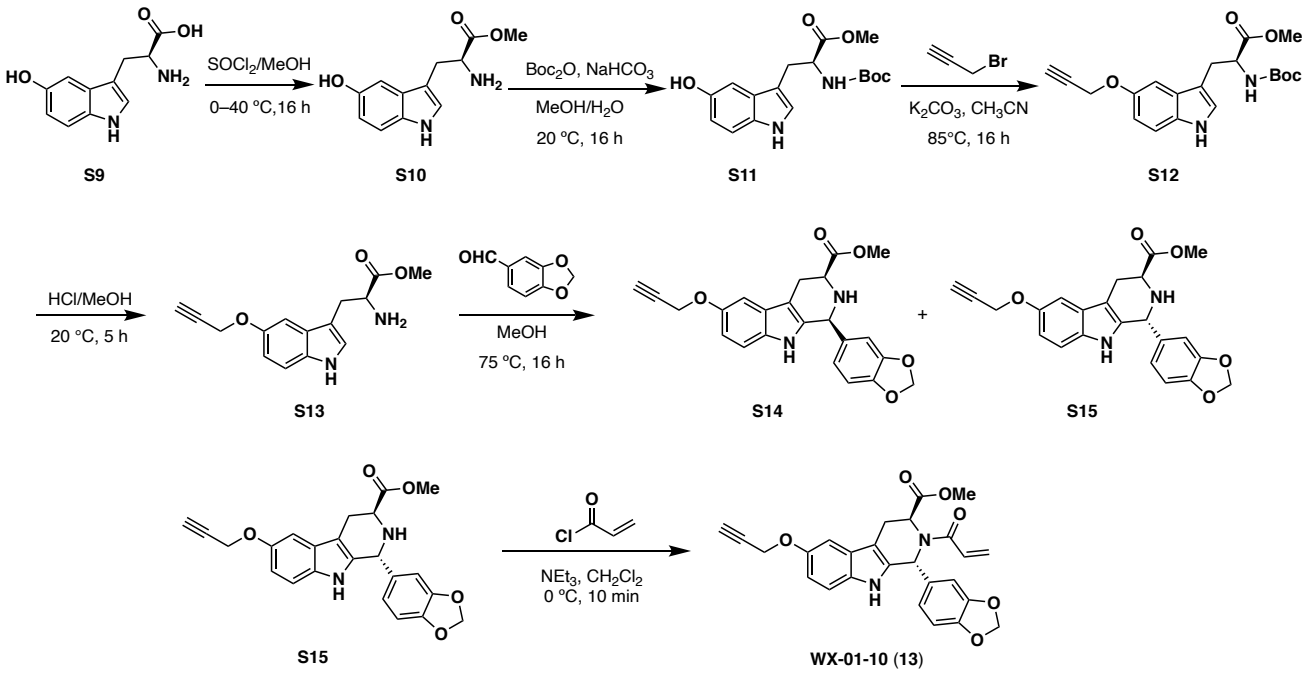
methyl (1*R*,3*R*)-2-acryloyl-1-(benzo[*d*][1,3]dioxol-5-yl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*] indole-3-carboxylate (EV-99) (8)



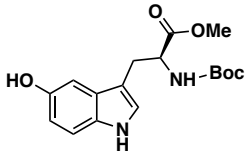
HRMS ESI-TOF m/z calculated for $C_{23}H_{21}N_2O_5$ $[M+H]^+$ 405.1445. Found 405.1442.

^{1}H NMR (400 MHz, CD_3OD): δ 7.52 (d, *J* = 8.0 Hz, 1H), 7.28 (d, *J* = 8.0 Hz, 1H), 7.15-7.09 (m, 1H), 7.08-7.02 (m, 1H), 7.02-6.84 (m, 2H), 6.80 (s, 1H), 6.73-6.65 (m, 1H), 6.62-6.53 (m, 1H), 6.38-6.21 (m, 1H), 5.90 (s, 2H), 5.87-5.78 (m, 1H), 5.68-5.25 (m, 1H), 3.66-3.56 (m, 1H), 3.13 (s, 3H), 3.09-2.97 (m, 1H), 1 exchangeable proton not observed.

Synthesis of WX-01-10



methyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(5-hydroxy-1*H*-indol-3-yl)propanoate (S11)

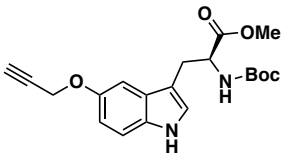


To a solution of **S9** (2.00 g, 9.08 mmol) in methanol (20 mL) was added dropwise thionyl chloride (2.00 g, 16.8 mmol, 1.22 mL) at 0 °C. The mixture was warmed and stirred at 40 °C for 16 hours. The reaction was monitored by LC-MS. The reaction mixture was concentrated under reduced pressure to give **S10** (1.90 g, crude) as a yellow oil, which was used for next step without purification. To a solution of **S10** (1.90 g, crude) in methanol (20 mL) and water (5 mL) were added Boc_2O (3.54 g, 16.2 mmol, 3.73 mL) and sodium bicarbonate (2.04 g, 24.3 mmol). The mixture was stirred at 20 °C for 16 hours and the reaction was monitored by LC-MS. Upon completion, the reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3×40 mL). The organic layers were combined, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give **S11** (3.00 g, crude) as a yellow solid, which was used in the next step without further purification.

LC-MS m/z calculated for $C_{17}H_{23}N_2O_5$ $[M+H]^+$ 335.2. Found 335.2.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93 (br s, 1H), 7.21 (d, *J* = 8.7 Hz, 1H), 6.97 (dd, *J* = 7.9, 2.4 Hz, 2H), 6.77 (dd, *J* = 8.7, 2.4 Hz, 1H), 5.13-5.00 (m, 1H), 4.69-4.55 (m, 1H), 3.68 (s, 3H), 3.49 (d, *J* = 4.5 Hz, 1H), 3.25-3.17 (m, 2H), 1.57 (s, 9H).

methyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(5-(prop-2-yn-1-yloxy)-1*H*-indol-3-yl)propanoate (S12)



To a solution of **S11** (2.00 g, crude) in acetonitrile (30 mL) were added potassium carbonate (2.48 g, 17.9 mmol) and propargyl bromide (934 mg, 6.28 mmol, 677 μL, 80% w/w in toluene). The mixture was stirred at 85 °C for 16 hours. The reaction was monitored by LC-MS. The reaction mixture was extracted with EtOAc (3×40 mL). The organic layers were combined, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give a residue, which was purified by column chromatography (SiO_2, petroleum ether/EtOAc = 10:1 to 5:1) to give **S12** (2.30 g, quant.) as a white solid.

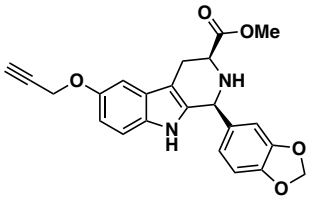
LC-MS m/z calculated for $C_{15}H_{17}N_2O_3$ $[M–Boc+H]^+$ 273.1. Found 273.1.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00 (br s, 1H), 7.29-7.24 (m, 1H + $CHCl_3$), 7.11 (d, *J* = 2.4 Hz, 1H), 7.01 (d, *J* = 2.5 Hz, 1H), 6.92 (dd, *J* = 8.8, 2.4 Hz, 1H), 5.13-5.05 (m, 1H), 4.73 (d, *J* = 2.4 Hz, 2H), 4.68-4.61 (m, 1H), 3.68 (s, 3H), 3.24 (d, *J* = 5.6 Hz, 2H), 2.52 (t, *J* = 2.4 Hz, 1H), 1.42 (s, 9H).

Compounds S14 and S15

To a solution of **S12** (1.30 g, 3.49 mmol) in methanol (13 mL) was added HCl (4 M in MeOH, 9 mL). The mixture was stirred at 20 °C for 5 hours. The reaction was monitored by LC-MS. Upon completion, the reaction mixture was concentrated under reduced pressure to give **S13** (1.00 g, crude) as a yellow oil, which was used in the next step without purification. To a solution of **S13** (900 mg, 3.31 mmol) in methanol (15 mL) was added piperonal (595 mg, 3.97 mmol). The mixture was stirred at 75 °C for 16 hours. The reaction was monitored by LC-MS. Upon completion, the reaction mixture was diluted with water (20 mL) to adjust the pH to 8-9, then extracted with EtOAc (3×20 mL). The organic layers were combined, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, petroleum ether/EtOAc = 1:0 to 2:1) to give **S14** (130 mg, 7.7% yield) and **S15** (130 mg, 7.2% yield) as yellow solids.

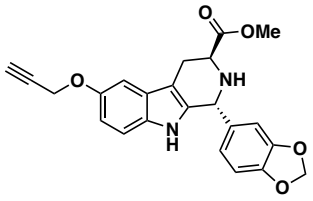
methyl (1*S*,3*S*)-1-(benzo[*d*][1,3]dioxol-5-yl)-6-(prop-2-yn-1-yloxy)-2,3,4,9-tetrahydro-1*H*-pyrido [3,4-*b*]indole-3-carboxylate (S14)



LC-MS m/z calculated for $C_{23}H_{21}N_2O_5$ $[M+H]^+$ 405.1. Found 405.2.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34 (br s, 1H), 7.13 (d, *J* = 8.7 Hz, 1H), 7.09 (d, *J* = 2.5 Hz, 1H), 6.87 (ddd, *J* = 8.4, 5.6, 2.1 Hz, 2H), 6.84-6.75 (m, 2H), 5.95 (s, 2H), 5.21-5.10 (m, 1H), 4.79-4.68 (m, 2H), 3.95 (dd, *J* = 11.1, 4.3 Hz, 1H), 3.82 (s, 3H), 3.17 (ddd, *J* = 15.0, 4.2, 1.8 Hz, 1H), 2.97 (ddd, *J* = 15.0, 11.1, 2.5 Hz, 1H), 2.52 (t, *J* = 2.4 Hz, 1H), 1 exchangeable proton not observed.

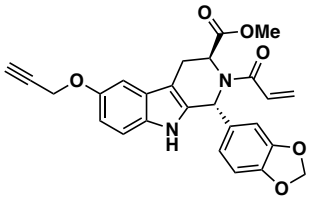
methyl (1*R*,3*S*)-1-(benzo[*d*][1,3]dioxol-5-yl)-6-(prop-2-yn-1-yloxy)-2,3,4,9-tetrahydro-1*H*-pyrido [3,4-*b*]indole-3-carboxylate (S15)



LC-MS m/z calculated for $C_{23}H_{21}N_2O_5$ $[M+H]^+$ 405.1. Found 405.2.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.46 (br s, 1H), 7.15 (d, *J* = 8.7 Hz, 1H), 7.14-7.08 (m, 1H), 6.93-6.78 (m, 1H), 6.75 (s, 3H), 5.99-5.89 (m, 2H), 5.35-5.29 (m, 1H), 4.74 (dd, *J* = 3.5, 2.4 Hz, 2H), 3.98 (t, *J* = 6.0 Hz, 1H), 3.72 (s, 3H), 3.22 (ddd, *J* = 15.3, 5.5, 1.3 Hz, 1H), 3.09 (ddd, *J* = 15.4, 6.6, 1.5 Hz, 1H), 2.52 (t, *J* = 2.4 Hz, 1H), 1 exchangeable proton not observed.

methyl (1*R*,3*S*)-2-acryloyl-1-(benzo[*d*][1,3]dioxol-5-yl)-6-(prop-2-yn-1-yloxy)-2,3,4,9-tetra-hydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (WX-01-10) (13)

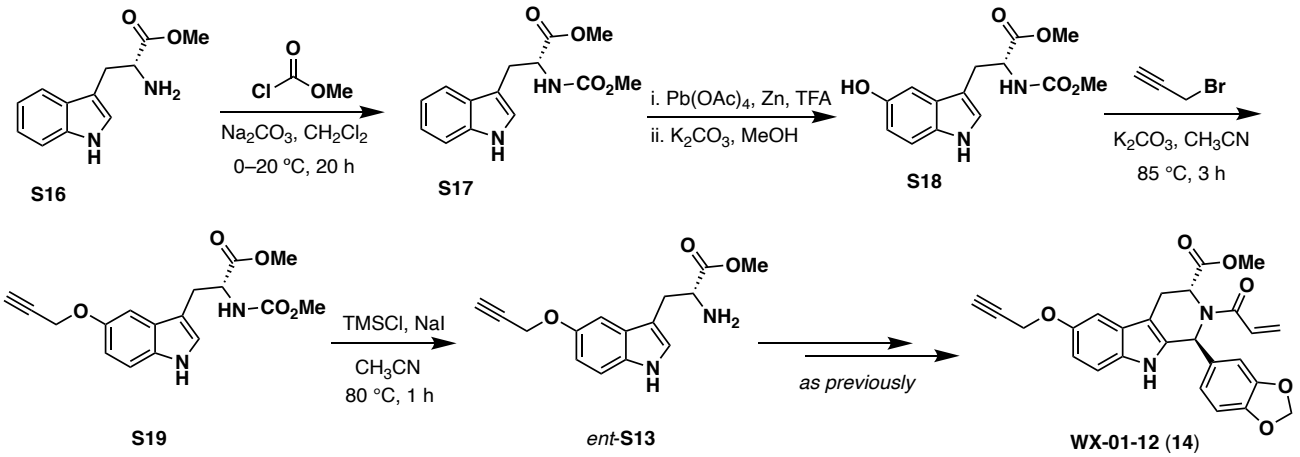


To a solution of **S15** (80.00 mg, 198 μmol) in dichloromethane (2 mL) were added triethylamine (40.0 mg, 396 μmol, 55.1 uL) and acryloyl chloride (17.9 mg, 198 μmol, 16.1 uL). The mixture was stirred at 0 °C for 10 min and the reaction was monitored by LC-MS. Upon completion, the reaction mixture was concentrated under reduced pressure to give a residue, which was purified by prep-HPLC (column: Phenomenex Luna C18 150*25mm*10um; mobile phase: [water (0.225%FA)-ACN]; B%: 39%-69%, 10min) and preparatory TLC (SiO_2, petroleum ether/EtOAc = 1:1) to give **WX-01-10** (18.0 mg, 20% yield) as a white solid.

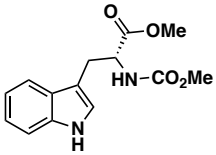
HRMS ESI-TOF m/z calculated for $C_{26}H_{23}N_2O_6$ $[M+H]^+$ 459.1551. Found 459.1551.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.64 (br s, 1H), 7.17 (d, *J* = 8.8 Hz, 1H), 7.08 (d, *J* = 2.4 Hz, 1H), 6.89 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.84 (br s, 1H), 6.82-6.74 (m, 2H), 6.57 (br dd, *J* = 16.6, 10.8 Hz, 1H), 6.30 (dd, *J* =1 6.6, 1.6 Hz, 1H), 6.10 (s, 1H), 5.93 (br d, *J* = 6.4 Hz, 2H), 5.66 (br d, *J* = 10.6 Hz, 1H), 5.10 (br s, 1H), 4.73 (d, *J* = 2.4 Hz, 2H), 3.66 (s, 3H), 3.54 (br d, *J* = 15.2 Hz, 1H), 3.25 (br s, 1H), 2.52 (t, *J* = 2.4 Hz, 1H).

Synthesis of WX-01-12



methyl (methoxycarbonyl)-*D*-tryptophanate (S17)



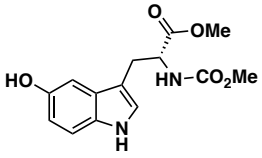
To a precooled (0 ºC) solution of **S16** (1.05 g, 4.12 mmol, HCl) in dichloromethane (20 mL) were added sodium carbonate (0.584 g, 6.18 mmol) and methyl chloroformate (0.655 g, 6.18 mmol, 0.48 mL). The mixture was allowed to gradually warm to 20 °C overnight (20 h). The reaction mixture was diluted with water (100 mL) and dichloromethane (50 mL). The organic layer was washed sequentially with water, sat. aq. sodium bicarbonate, and brine. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give **S17** (1.14 g, 4.12 mmol, quant.), which was used in the next step without further purification.

LC-MS m/z calculated for $C_{14}H_{17}N_2O_4$ $[M+H]^+$ 277.1. Found 277.1.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (br s, 1H), 7.54 (d, *J* = 7.9 Hz, 1H), 7.36 (dt, *J* = 8.1, 0.9 Hz, 1H), 7.20 (ddd, *J* = 8.2, 7.0, 1.3 Hz, 1H), 7.13 (ddd, *J* = 8.0, 7.0, 1.1 Hz, 1H), 7.01 (d, *J* = 2.2 Hz, 1H), 5.23

(d, *J* = 8.3 Hz, 1H), 4.71 (td, *J* = 5.8, 5.8 Hz, 1H), 3.68 (s, 3H), 3.67 (s, 3H), 3.31 (d, *J* = 5.5 Hz, 2H).

methyl (*R*)-3-(5-hydroxy-1*H*-indol-3-yl)-2-((methoxycarbonyl)amino)propanoate (S18)

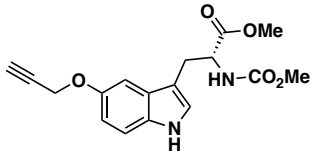


S17 (1.14 g, 4.12 mmol) was dissolved in trifluoroacetic acid (12 mL) and the solution was stirred at 20 °C for 2.5 h. Next, the reaction mixture was cooled to 12 ºC (1,4 dioxane dry ice bath) and a solution of lead tetraacetate (4.02 g, 9.08 mmol; best results were obtained using fresh Strem Chemicals batch) in dichloromethane (80 mL) was added over 10 min. The brown mixture was stirred for 1.5 h at the same temperature before adding zinc (1.35 g, 20.6 mmol) and warming the reaction to 20 °C over 45 min (the reaction becomes amber in color). The reaction was diluted with water (100 mL) and stirred vigorously over 30 min before extracting with dichloromethane (3×50 mL). The combined organic layers were filtered through a silica plug and concentrated under reduced pressure. The resulting brown oil was dissolved in methanol (20 mL) and treated with potassium carbonate (0.375 g, 2.71 mmol) overnight (18 h) at 20 °C to solvolyze any trifluoroacetate ester formed over previous steps. The resulting solution was diluted in 50% sat. aq. NaCl and extracted with dichloromethane (3×50 mL). The combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, hexanes/EtOAc = 1:1 with 0.1% acetic acid) to give **S18** as a tan oil/foam (0.605 g, 4.13 mmol, 50%).

LC-MS m/z calculated for $C_{14}H_{17}N_2O_5$ $[M+H]^+$ 293.3. Found 293.0.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (br s, 1H), 7.17 (dd, *J* = 8.8, 2.6 Hz, 1H), 6.99-6.92 (m, 2H), 6.77 (dd, *J* = 8.6, 2.4 Hz, 1H), 5.43-5.31 (m, 1H), 4.67 (ddd, *J* = 6.4, 6.3, 6.3 Hz, 1H), 3.67 (s, 3H), 3.65 (s, 3H), 3.20 (d, *J* = 5.7 Hz, 2H), 1 exchangeable proton not observed.

methyl (*R*)-2-((methoxycarbonyl)amino)-3-(5-(prop-2-yn-1-yloxy)-1*H*-indol-3-yl)propanoate (S19)

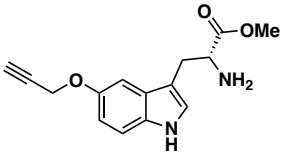


To a solution of **S18** (690 mg, 2.36 mmol) in acetonitrile (20 mL) were added potassium carbonate (979 mg, 7.08 mmol) and propargyl bromide (351 mg, 2.36 mmol, 254 µL, 80% w/w in toluene), and the mixture was stirred at 85 °C for 3 hours. The reaction was monitored by TLC and LC-MS. The reaction mixture

was diluted with water (100 mL) and extracted with ethyl acetate (50 mL × 3). The combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (SiO_2, petroleum ether/EtOAc = 1 : 1 to 1 : 1) to give **S19** (550 mg, 1.66 mmol, 71% yield) as a yellow oil.

LC-MS m/z calculated for $C_{17}H_{19}N_2O_5$ $[M+H]^+$ 331.1. Found 331.1.

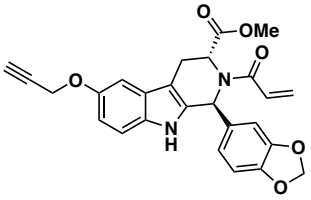
methyl (*R*)-2-amino-3-(5-(prop-2-yn-1-yloxy)-1*H*-indol-3-yl)propanoate (*ent*-S13)



To a solution of **S19** (400 mg, 1.21 mmol) in acetonitrile (4 mL) was added trimethylchlorosilane (263 mg, 2.42 mmol, 307 μL) and sodium iodide (363 mg, 2.42 mmol). The mixture was stirred at 80 °C for 1 hour. This reaction was monitored by LC-MS. The reaction mixture was diluted with water (100 mL) and extracted with EtOAc (3×30 mL). The combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give *ent*-**S13** (510 mg, crude) as a yellow oil. It was used in next step directly without purification.

The remaining transformations were performed as described previously for **WX-01-10**.

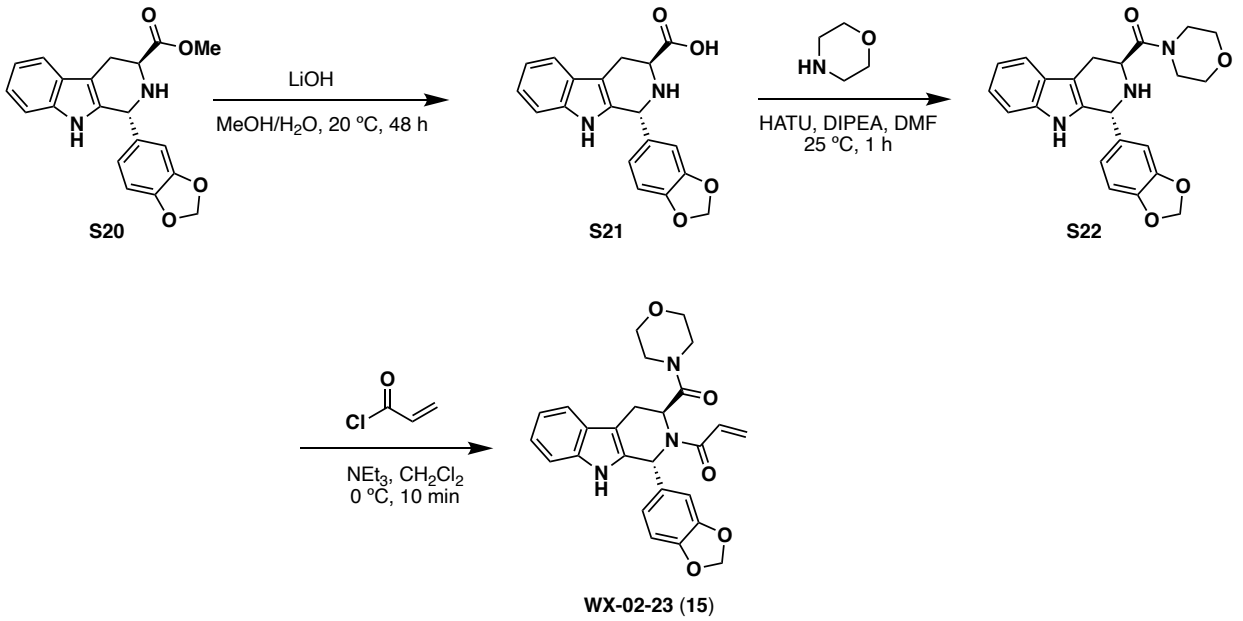
methyl (1*S*,3*R*)-2-acryloyl-1-(benzo[*d*][1,3]dioxol-5-yl)-6-(prop-2-yn-1-yloxy)-2,3,4,9-tetra-hydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (WX-01-12) (14)



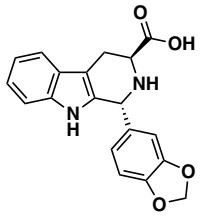
HRMS ESI-TOF m/z calculated for $C_{26}H_{23}N_2O_6$ $[M+H]^+$ 459.1551. Found 459.1552.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74 (br s, 1H), 7.15 (d, *J* = 8.8 Hz, 1H), 7.07 (d, *J* = 2.4 Hz, 1H), 6.87 (dd, *J* = 8.8, 2.5 Hz, 1H), 6.85-6.70 (m, 3H), 6.56 (dd, *J* = 16.7, 10.5 Hz, 1H), 6.29 (dd, *J* = 16.7, 1.8 Hz, 1H), 6.09 (s, 1H), 5.99-5.86 (m, 2H), 5.64 (d, *J* = 10.5 Hz, 1H), 5.17-5.00 (m, 1H), 4.72 (d, *J* = 2.4 Hz, 2H), 3.64 (s, 3H), 3.59-3.45 (m, 1H), 3.39-3.03 (m, 1H), 2.51 (t, *J* = 2.4 Hz, 1H).

Synthesis of WX-02-23



(1*R*,3*S*)-1-(benzo[*d*][1,3]dioxol-5-yl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylic acid (S21)

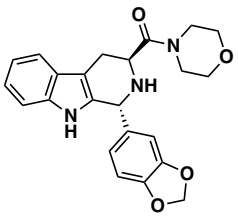


To a solution of **S20** (500 mg, 1.43 mmol) (Vinogradova et al., 2020) in methanol (10 mL) were added lithium hydroxide monohydrate (71.9 mg, 1.71 mmol) and water (257 mg, 14.3 mmol, 257 µL). The mixture was stirred at 20 °C for 48 hours. The reaction was monitored by LC-MS. The reaction mixture was concentrated under reduced pressure to give a residue, which was suspended in toluene (10 mL) and concentrated under reduced pressure to remove residual water. **S21** (500 mg, 97% yield), obtained as a white solid was used in the next step without further purification.

LC-MS m/z calculated for $C_{19}H_{17}N_2O_4$ $[M+H]^+$ 337.1. Found 337.1.

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.40 (d, *J* = 8.0 Hz, 1H), 7.30-7.10 (m, 3H), 7.05-6.89 (m, 2H), 6.80 (d, *J* = 8.0 Hz, 1H), 6.74 (m, 1H), 6.60-6.54 (m, 1H), 5.96 (s, 2H), 5.15 (m, 1H), 3.15-3.06 (m, 1H), 2.95-2.85 (m, 1H), 2.65-2.54 (m, 2H).

((1*R*,3*S*)-1-(benzo[*d*][1,3]dioxol-5-yl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-3-yl)(morpholino)methanone (S22)

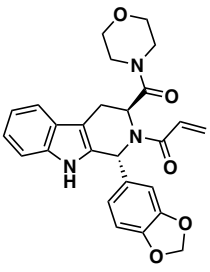


To a solution of **S21** (350 mg, 1.04 mmol) in *N,N*-dimethyl formamide (2 mL) were added HATU (594 mg, 1.56 mmol), morpholine (1.81 g, 20.8 mmol, 1.83 mL), and diisopropylethylamine (269 mg, 2.08 mmol, 363 μL). The mixture was stirred at 25 °C for 1 hour and the reaction was monitored by LC-MS. Upon completion, the reaction mixture was diluted with water (20 mL) and extracted with ethyl acetate (3× 20 mL). The organic layers were combined, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give a residue, which was purified by prep-HPLC (column: Waters Xbridge 150mm×25mm×5μm; mobile phase: [water (10mM NH_4HCO_3)-CH_3CN]; B%: 26%-59%,10min) to give **S22** (300 mg, 70% yield) as a white solid.

LC-MS m/z calculated for $C_{23}H_{24}N_3O_4$ $[M+H]^+$ 406.2. Found 406.2.

^{1}H NMR (400 MHz, CD_3OD): δ 7.50 (d, *J* = 8.0 Hz, 1H), 7.29 (d, *J* = 8.0 Hz, 1H), 7.13-7.06 (m, 1H), 7.06-7.00 (m, 1H), 6.80-6.72 (m, 2H), 6.68-6.62 (m, 1H), 5.94 (s, 2H), 5.27 (s, 1H), 3.97 (dd, *J* = 10.0, 5.0 Hz, 1 H), 3.78-3.47 (m, 8 H), 3.29-3.13 (m, 1 H), 3.05-2.90 (m, 1 H), 2 exchangeable protons not observed.

1-((1*R*,3*S*)-1-(benzo[*d*][1,3]dioxol-5-yl)-3-(morpholine-4-carbonyl)-1,3,4,9-tetrahydro-2*H*-pyrido[3,4-*b*]indol-2-yl)prop-2-en-1-one (WX-02-23) (15)



To a solution of **S22** (70.0 mg, 173 μmol) in dichloromethane (2 mL) were added triethylamine (34.9 mg, 345 μmol, 48.1 μL) and acryloyl chloride (15.6 mg, 173 μmol, 14.1 μL). The mixture was stirred at 0 °C for 10 min and the reaction was monitored by LC-MS. Upon completion, the reaction mixture was concentrated under reduced pressure to give a residue, which was purified by prep-HPLC (column: Waters Xbridge 150mm×25mm×5μm; mobile phase: [water (10mM NH_4HCO_3)-CH_3CN]; B%: 28%-58%,10min) to give **WX-02-23** (23.1 mg, 28% yield) as a white solid.

HRMS ESI-TOF m/z calculated for $C_{26}H_{26}N_3O_5$ $[M+H]^+$ 460.1867. Found 460.1867.

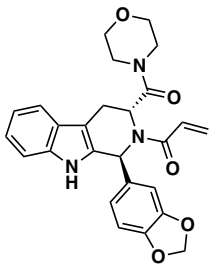
^{1}H NMR (400 MHz, CD_3OD): δ 7.47 (d, *J* = 7.8 Hz, 1H), 7.27 (d, *J* = 7.9 Hz, 1H), 7.08 (ddd, *J* = 8.2, 7.1, 1.3 Hz, 1H), 7.01 (ddd, *J* = 8.1, 7.1, 1.1 Hz, 1H), 6.98-6.72 (m, 4H), 6.42-6.32 (m, 1H), 6.24 (dd,

J = 16.6, 1.8 Hz, 1H), 5.96-5.88 (m, 2H), 5.75 (dd, *J* = 10.6, 1.9 Hz, 1H), 5.14-5.01 (m, 1H), 3.68-3.44 (m, 5H), 3.44-3.12 (m, 5H + solvent residual peak), 1 exchangeable proton not observed.

Synthesis of WX-02-43

Prepared in analogous fashion from *ent*-**S20** (Vinogradova et al., 2020).

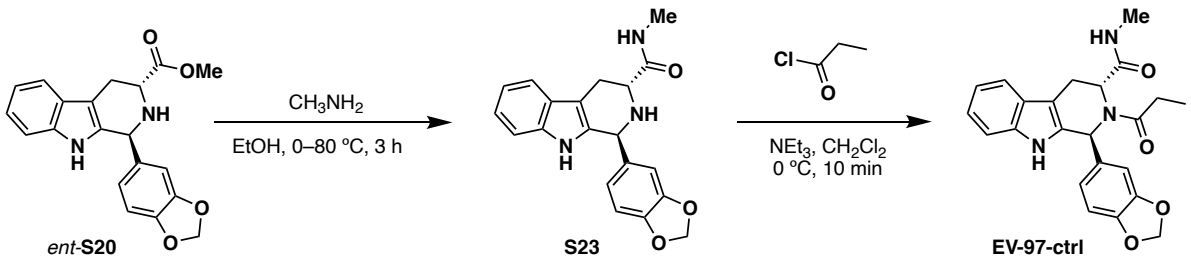
1-((1*S*,3*R*)-1-(benzo[*d*][1,3]dioxol-5-yl)-3-(morpholine-4-carbonyl)-1,3,4,9-tetrahydro-2*H*-pyrido[3,4-*b*]indol-2-yl)prop-2-en-1-one (WX-02-43) (16)



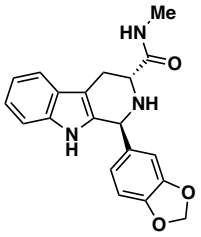
HRMS ESI-TOF m/z calculated for $C_{26}H_{26}N_3O_5$ $[M+H]^+$ 460.1867. Found 460.1870.

^{1}H NMR (400 MHz, CD_3OD): δ 7.47 (d, J = 7.8 Hz, 1H), 7.27 (d, J = 8.0 Hz, 1H), 7.08 (t, J = 7.5 Hz, 1H), 7.01 (t, J = 7.4 Hz, 1H), 6.98-6.66 (m, 4H), 6.42-6.34 (m, 1H), 6.24 (d, J = 16.6 Hz, 1H), 5.93 (s, 2H), 5.76 (dd, J = 10.6, 1.4 Hz, 1H), 5.13-5.02 (m, 1H), 3.72-3.44 (m, 5H), 3.44-3.11 (m, 5H + solvent residual peak), 1 exchangeable proton not observed.

Synthesis of EV-97-ctrl



(1*S*,3*R*)-1-(benzo[*d*][1,3]dioxol-5-yl)-*N*-methyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxamide (S23)

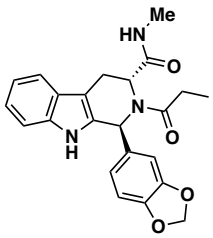


A solution of *ent*-**S20** (1.50 g, 4.28 mmol) (Vinogradova et al., 2020) in ethanol (10 mL) was degassed by purging with nitrogen 3 times, and methylamine (20.3 g, 40% w/w in water) was added. The mixture was stirred at 0 °C for 1 hour, then at 80 °C for 2 hours under nitrogen atmosphere. The reaction was monitored by LC-MS. The reaction mixture was concentrated under reduced pressure to give a residue, which was purified by column chromatography (SiO_2, petroleum ether/EtOAc = 1:0 to 1:1) to give **S23** (1.25 g, 3.28 mmol, 77% yield) as a yellow solid.

LC-MS m/z calculated for $C_{20}H_{20}N_3O_3$ $[M+H]^+$ 350.1. Found 350.1.

^{1}H NMR (400 MHz, CD_3OD): δ 7.47 (d, *J* = 8.0 Hz, 1H), 7.26 (d, *J* = 8.0 Hz, 1H), 7.10-7.04 (m, 1H), 7.03-6.97 (m, 1H), 6.77-6.70 (m, 2H), 6.68-6.60 (m, 1H), 5.89 (s, 2H), 5.24 (s, 1H), 3.65-3.55 (m, 1H), 3.16-3.07 (m, 1H), 2.89-2.78 (m, 1H), 2.75 (s, 3H), 3 exchangeable protons not observed.

(1*S*,3*R*)-1-(benzo[*d*][1,3]dioxol-5-yl)-*N*-methyl-2-propionyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxamide (EV-97-ctrl) (18)



To a solution of **S23** (50.0 mg, 143 μmol) in dichloromethane (2 mL) were added triethylamine (21.7 mg, 215 μmol, 29.9 μL) and propionyl chloride (13.2 mg, 143 μmol, 13.2 μL). The mixture was stirred at 0 °C for 10 min and the reaction was monitored by LC-MS. The reaction mixture was concentrated under reduced pressure to give a residue, which was purified by prep-HPLC (column: Waters Xbridge 150mm×25mm×5μm; mobile phase: [water (10mM NH_4HCO_3)-CH_3CN]; B%: 25%-55%, 10min) to give **EV-97-ctrl** (37.6 mg, 92.8 μmol, 65% yield) as an off-white solid.

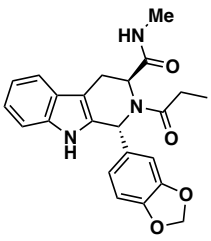
HRMS ESI-TOF m/z calculated for $C_{23}H_{24}N_3O_4$ $[M+H]^+$ 406.1761. Found 406.1760.

^{1}H NMR (400 MHz, CD_3OD): δ 7.42 (d, *J* = 8.0 Hz, 1H), 7.27 (d, *J* = 8.0 Hz, 1H), 7.03 (t, *J* = 7.6 Hz, 1H), 6.97 (t, *J* = 7.3 Hz, 1H), 6.94-6.87 (m, 2H), 6.79-6.67 (m, 1H), 6.22 (s, 1H), 5.88 (s, 2H), 5.18 (m, 1H), 3.53-3.30 (m, 2H + solvent residual peak), 2.61 (s, 3H), 2.58-2.51 (m, 1H), 2.37-2.20 (m, 1H), 1.10-0.94 (m, 3H), 2 exchangeable protons not observed.

Synthesis of EV-96-ctrl

Prepared in analogous fashion from **S20** (Vinogradova et al., 2020).

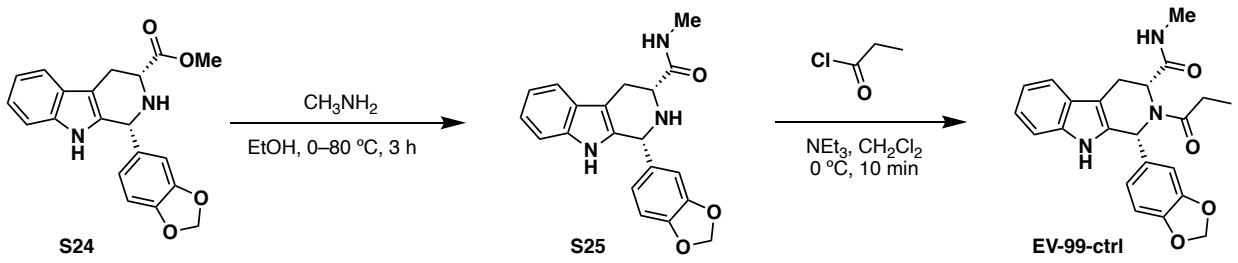
(1*S*,3*R*)-1-(benzo[*d*][1,3]dioxol-5-yl)-*N*-methyl-2-propionyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxamide (EV-96-ctrl) (17)



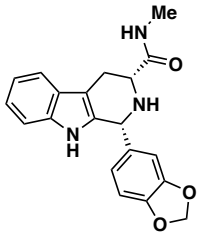
HRMS ESI-TOF m/z calculated for $C_{23}H_{24}N_3O_4$ $[M+H]^+$ 406.1761. Found 406.1761.

^{1}H NMR (400 MHz, CD_3OD): δ 7.42 (d, *J* = 8.0 Hz, 1H), 7.27 (d, *J* = 8.0 Hz, 1H), 7.09-7.02 (m, 1H), 7.01-6.95 (m, 1H), 6.93-6.87 (m, 2H), 6.80-6.68 (m, 1H), 6.23 (s, 1H), 5.91-5.80 (m, 2H), 5.16 (s, 1H), 3.56-3.30 (m, 2H + solvent residual peak), 2.61 (s, 3H), 2.59-2.48 (m, 1H), 2.36-2.25 (m, 1H), 1.03 (m, 3H), 2 exchangeable protons not observed.

Synthesis of EV-99-ctrl



(1*R*,3*R*)-1-(benzo[*d*][1,3]dioxol-5-yl)-*N*-methyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxamide (S25)

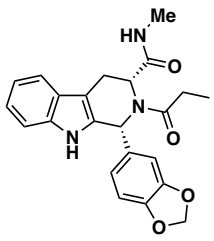


A solution of **S24** (1.50 g, 4.28 mmol) (Vinogradova et al., 2020) in ethanol (10 mL) was degassed by purging with nitrogen 3 times, and methylamine (20.3 g, 40% w/w in water) was added. The mixture was stirred at 0 °C for 1 hour, then 80 °C for 2 hours under nitrogen atmosphere. The reaction was monitored by LC-MS. Upon completion, the reaction mixture was concentrated under reduced pressure to give a residue, which was purified by column chromatography (SiO_2, petroleum ether/EtOAc = 1:0 to 1:1) to give **S25** (1.45 g, 4.03 mmol, 94% yield) as a yellow solid.

LC-MS m/z calculated for $C_{20}H_{20}N_3O_3$ $[M+H]^+$ 350.1. Found 350.1.

^{1}H NMR (400 MHz, CD_3OD): δ 7.44 (d, *J* = 8.0 Hz, 1H), 7.22 (d, *J* = 8.0 Hz, 1H), 7.08-6.95 (m, 2H), 6.90-6.75 (m, 3H), 5.91 (s, 2H), 5.10 (m, 1H), 3.75-3.65 (m, 1H), 3.15-3.05 (m, 1H), 2.90-2.80 (m, 1H), 2.79 (s, 3H), 3 exchangeable protons not observed.

(1*R*,3*R*)-1-(benzo[*d*][1,3]dioxol-5-yl)-*N*-methyl-2-propionyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxamide (EV-99-ctrl) (20)



To a solution of **S25** (50.0 mg, 143 μmol) in dichloromethane (2 mL) were added triethylamine (21.7 mg, 215 μmol, 29.9 μL) and propionyl chloride (13.2 mg, 143 μmol, 13.2 μL). The mixture was stirred at 0 °C for 10 min and the reaction was monitored by LC-MS. Upon completion, the reaction mixture was concentrated under reduced pressure. The resulting residue was purified by prep-HPLC (column: Waters Xbridge 150mm×25mm×5μm; mobile phase: [water (10mM NH_4HCO_3)-CH_3CN]; B%: 29%-59%, 10 min) to give **EV-99-ctrl** (36.9 mg, 90.9 μmol, 64% yield) as a white solid.

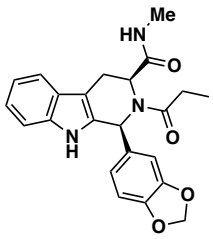
HRMS ESI-TOF m/z calculated for $C_{23}H_{24}N_3O_4$ $[M+H]^+$ 406.1761. Found 406.1756.

^{1}H NMR (400 MHz, CD_3OD): δ 7.53 (d, *J* = 8.0 Hz, 1H), 7.27 (d, *J* = 8.0 Hz, 1H), 7.12-6.95 (m, 3H), 6.86-6.60 (m, 3H), 5.89 (s, 2H), 5.20-5.00 (m, 1H), 3.75-3.55 (m, 1H), 2.97 (dd, *J* = 15.7, 6.8 Hz, 1H), 2.80-2.55 (m, 2H), 2.21 (s, 3H), 1.21 (t, *J* = 8.0 Hz, 3H), 2 exchangeable protons not observed.

Synthesis of EV-98-ctrl

Prepared in analogous fashion from *ent*-**S24** (Vinogradova et al., 2020).

(1*S*,3*S*)-1-(benzo[*d*][1,3]dioxol-5-yl)-*N*-methyl-2-propionyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxamide (EV-98-ctrl) (19)

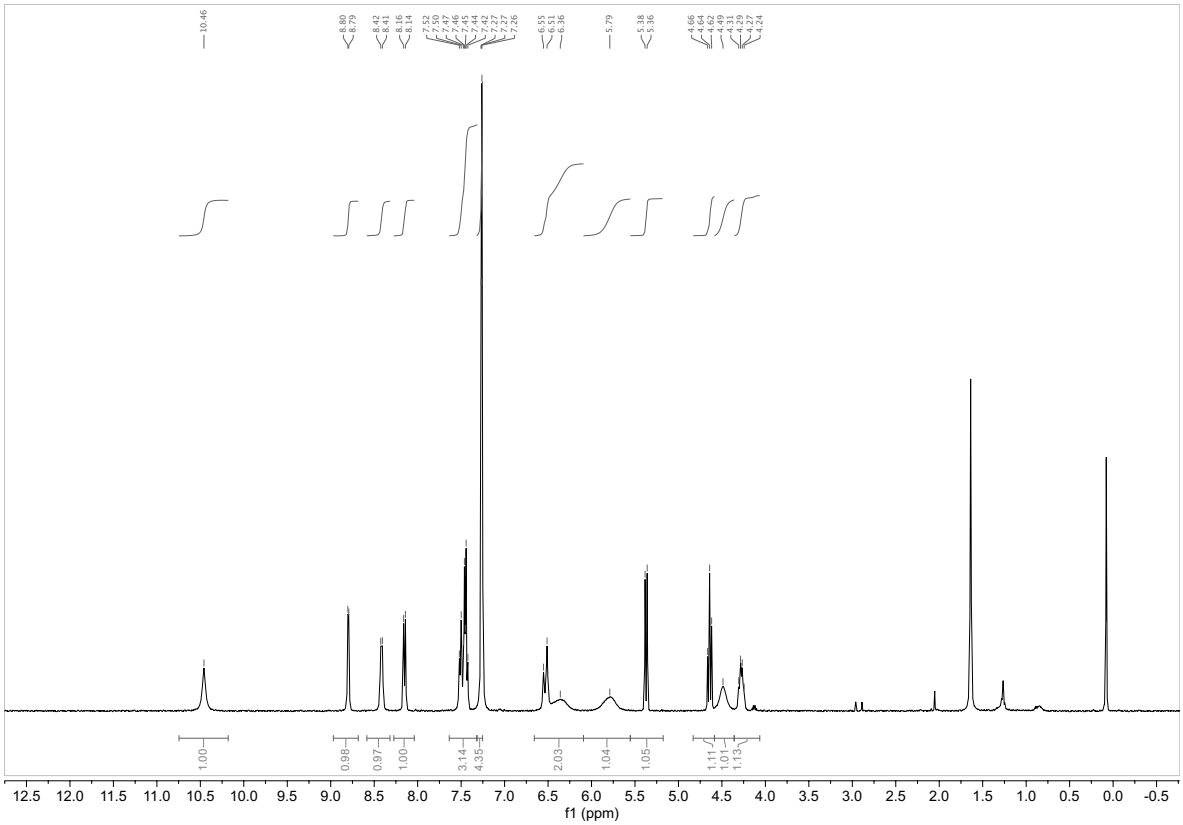


HRMS ESI-TOF m/z calculated for $C_{23}H_{24}N_3O_4$ $[M+H]^+$ 406.1761. Found 406.1762.

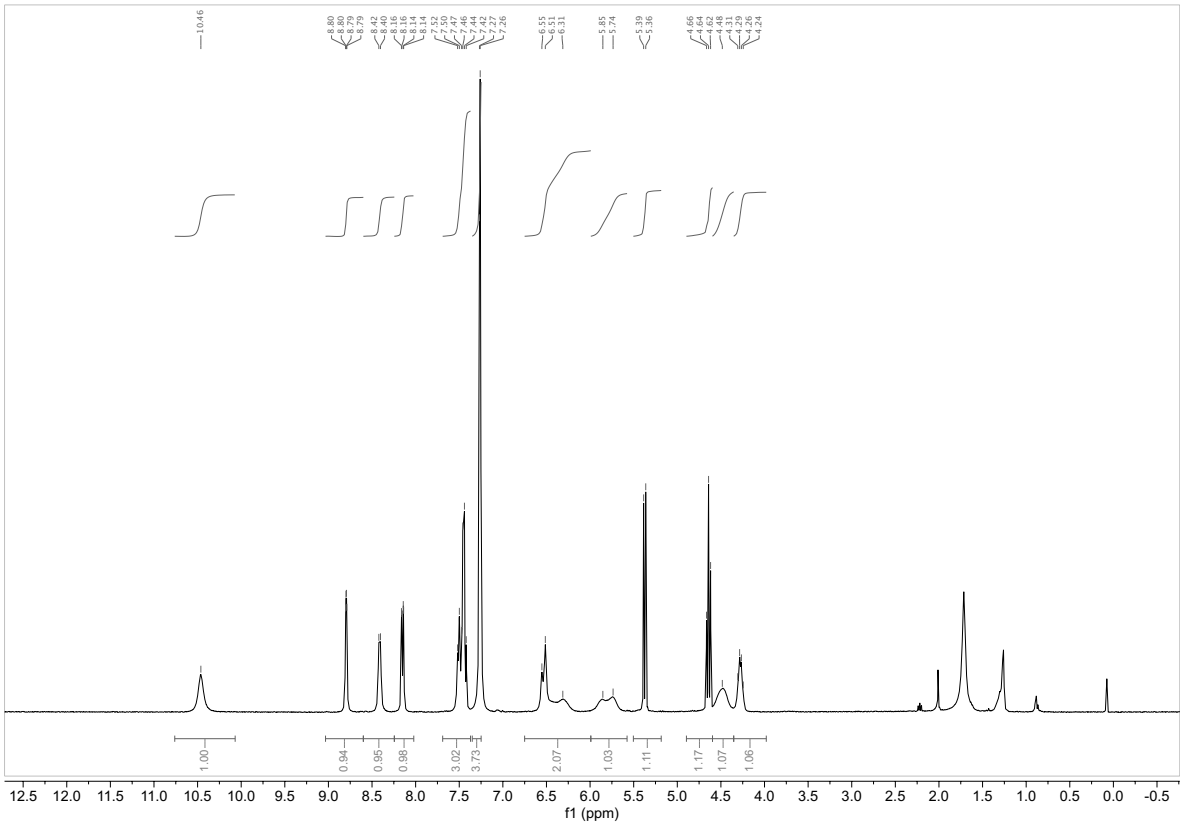
^{1}H NMR (400 MHz, CD_3OD): δ 7.54 (d, *J* = 8.0 Hz, 1H), 7.28 (d, *J* = 8.0 Hz, 1H), 7.14-7.09 (m, 1H), 7.07-6.90 (m, 2H), 6.84 (m, 1H), 6.80-6.62 (m, 2H), 5.90 (s, 2H), 5.25-5.00 (m, 1H), 3.75-3.55 (m, 1H), 2.96 (dd, *J* = 15.9, 6.8 Hz, 1H), 2.75-2.55 (m, 2H), 2.22 (s, 3H), 1.22 (t, *J* = 8.0 Hz, 3H), 2 exchangeable protons not observed.

Spectroscopic and chromatographic data

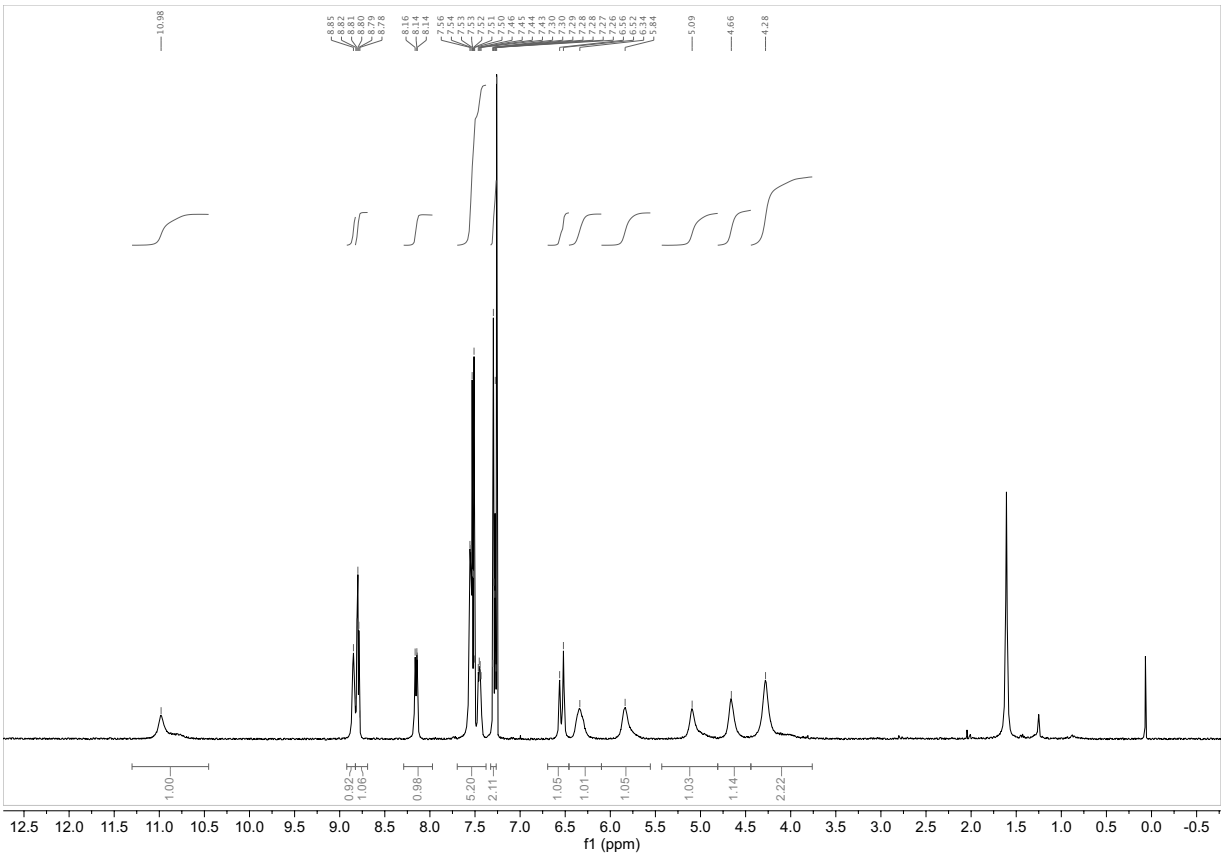
^{1}H NMR of MY-1A (1) in $CDCl_3$



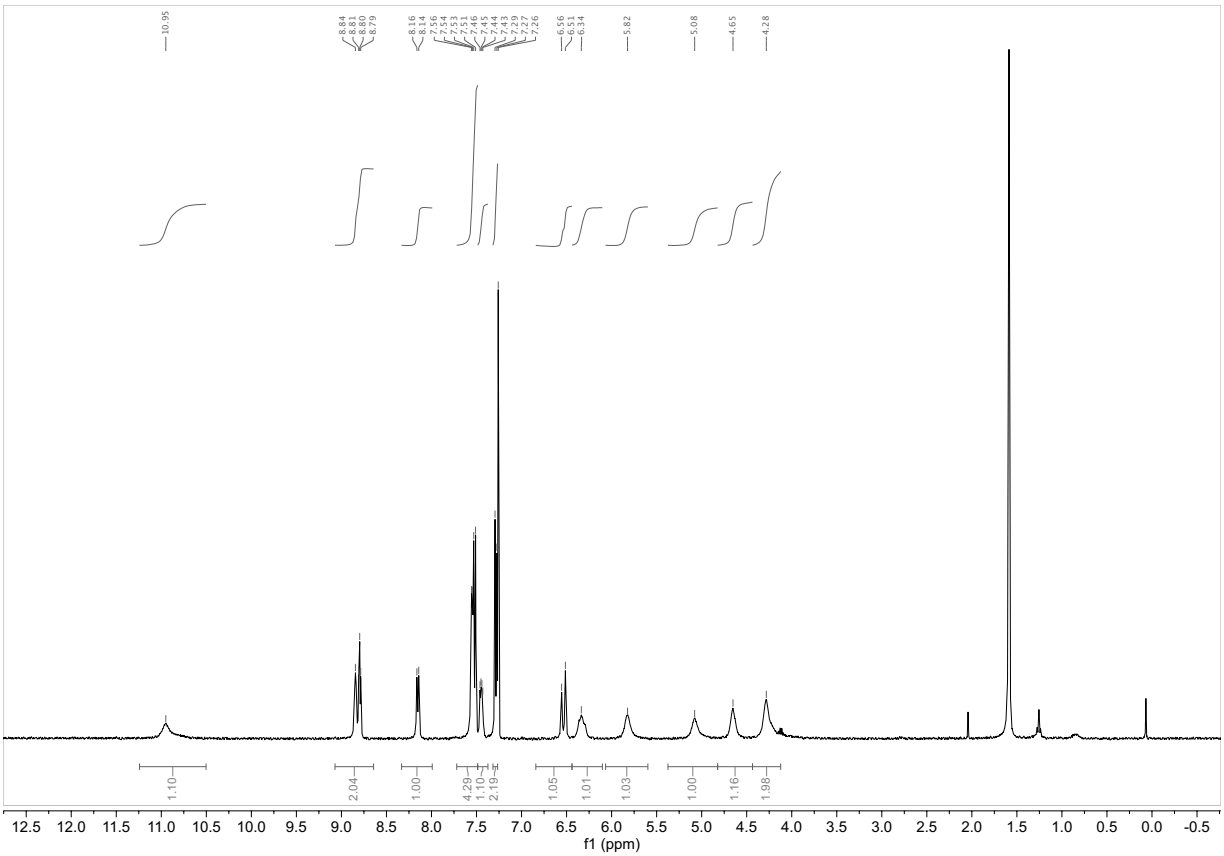
^{1}H NMR of MY-1B (2) in $CDCl_3$



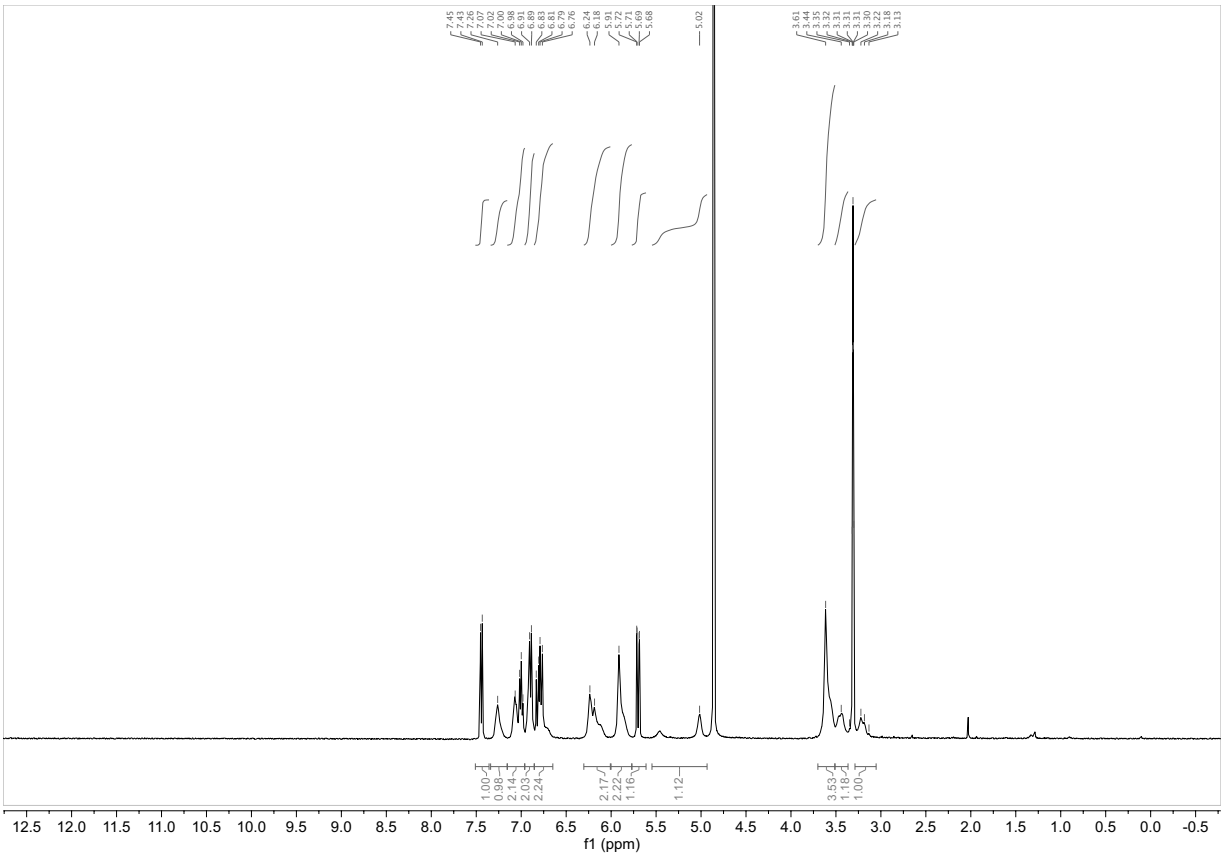
^{1}H NMR of MY-3A (3) in $CDCl_3$



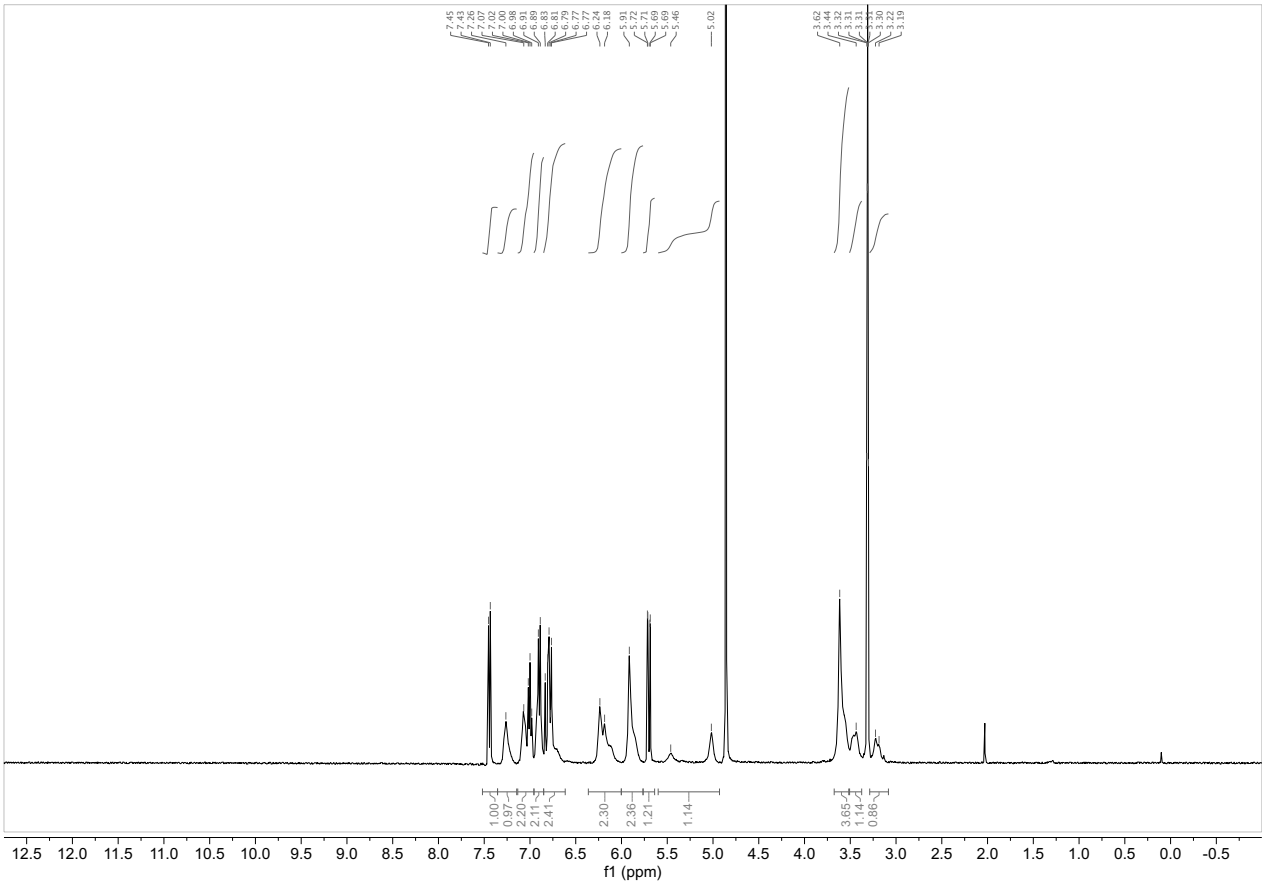
^{1}H NMR of MY-3B (4) in $CDCl_3$



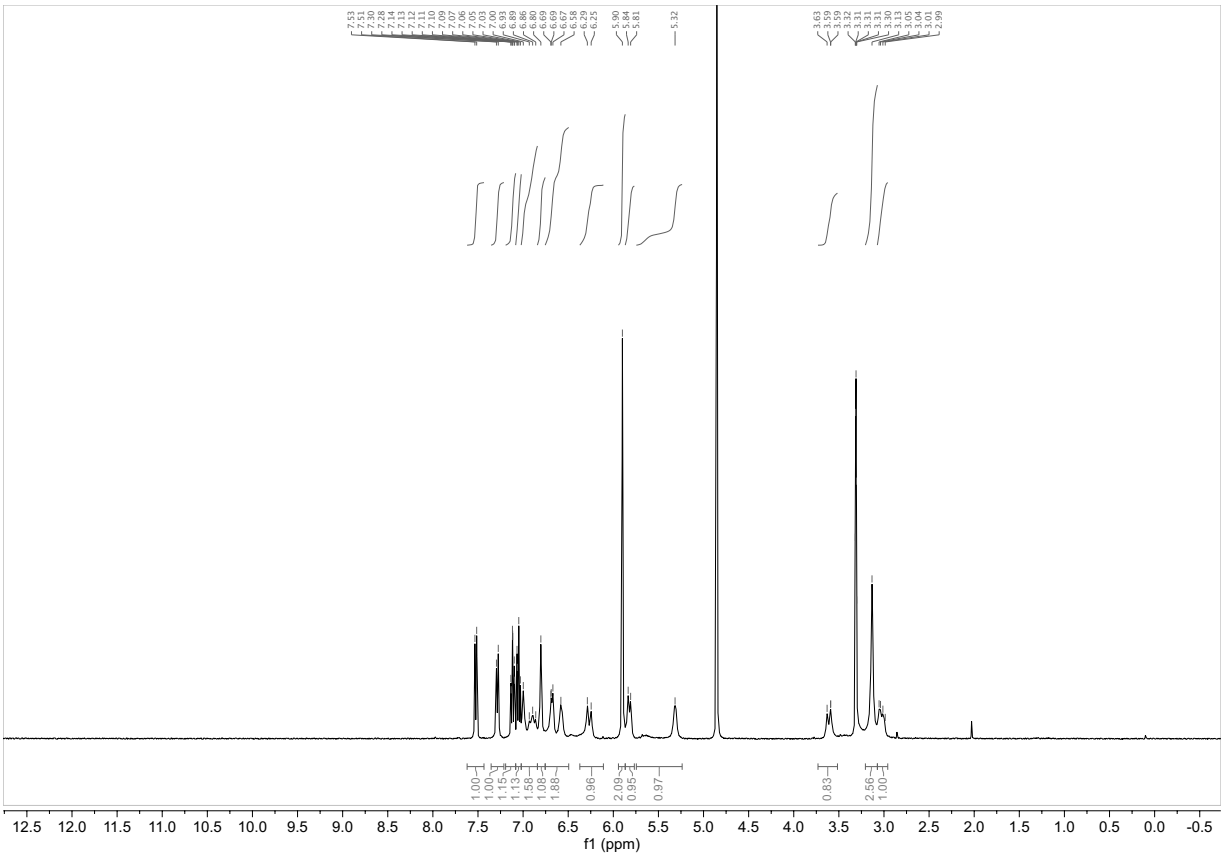
^{1}H NMR of EV-96 (5) in CD_3OD



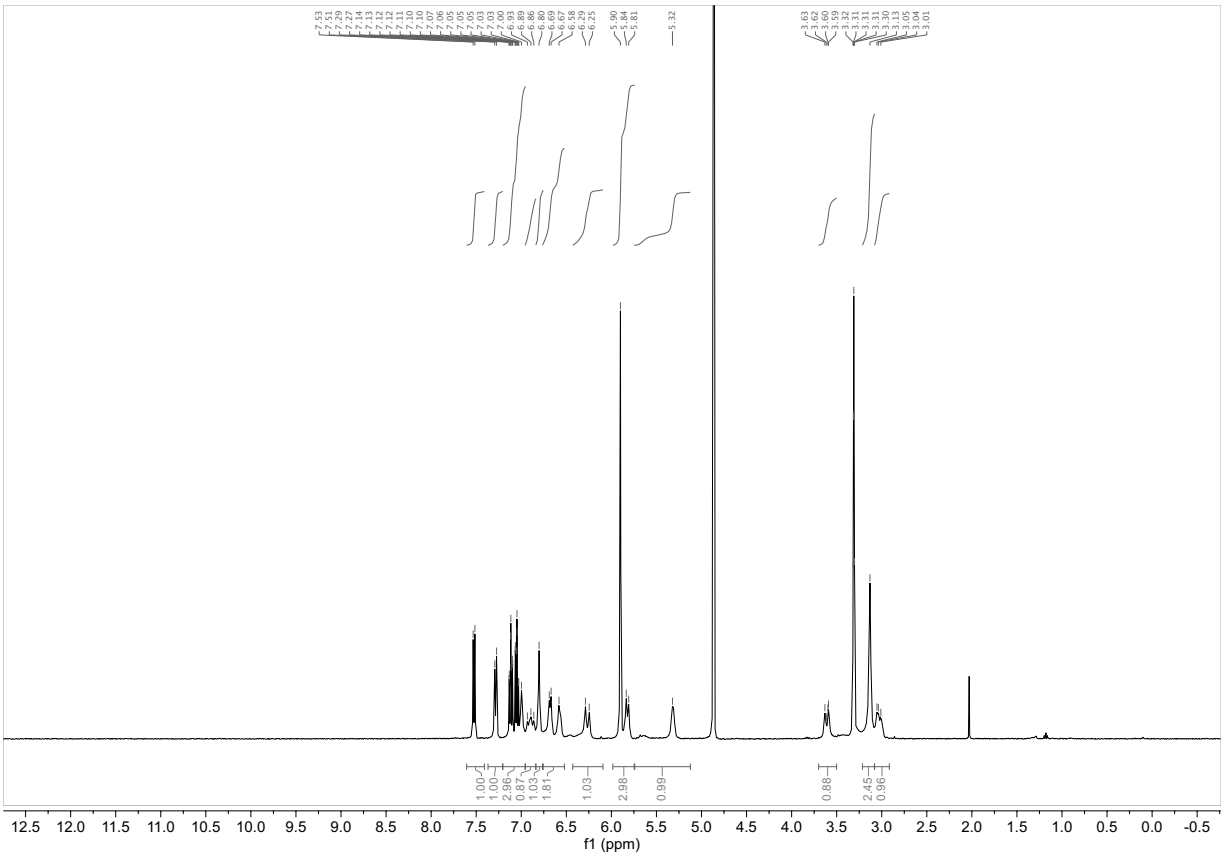
^{1}H NMR of EV-97 (6) in CD_3OD



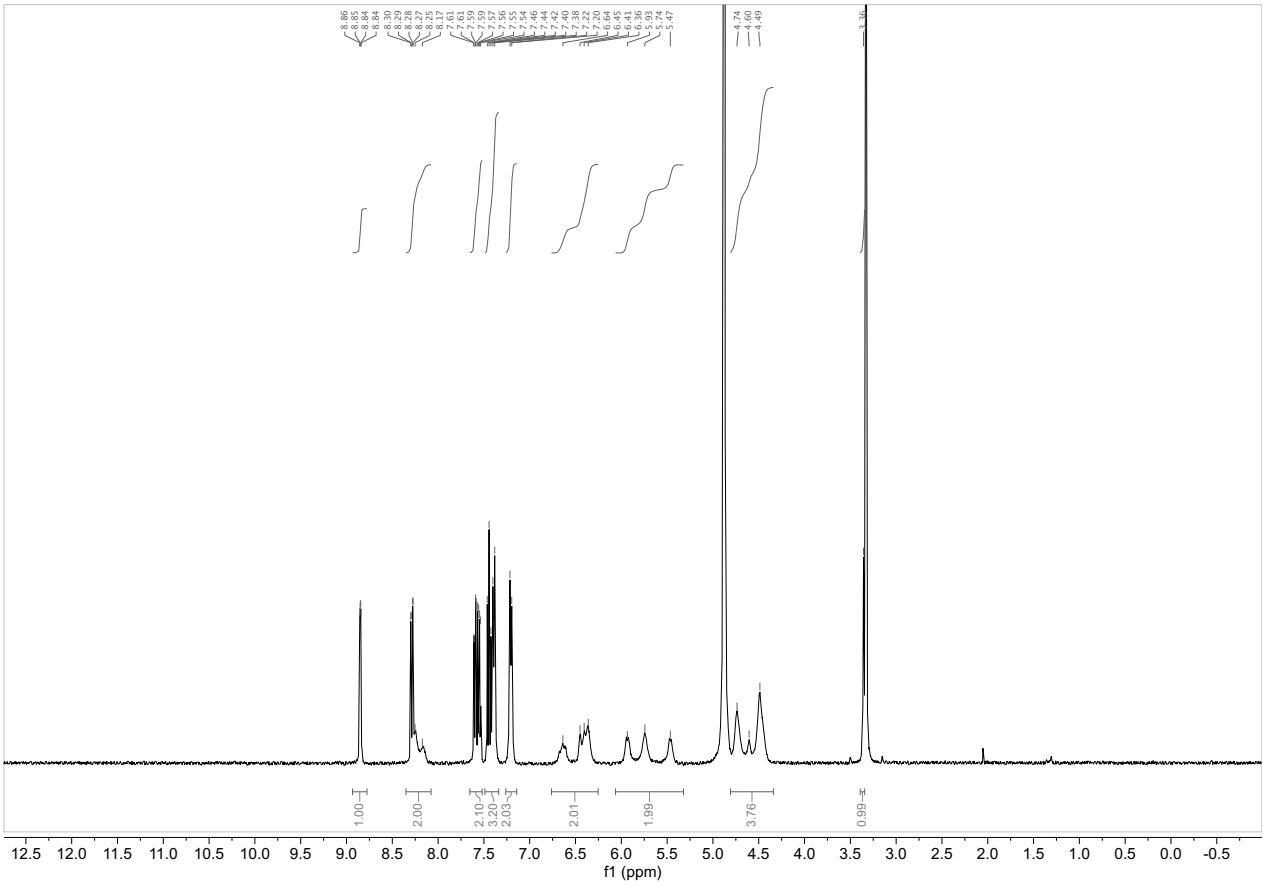
^{1}H NMR of EV-98 (7) in CD_3OD



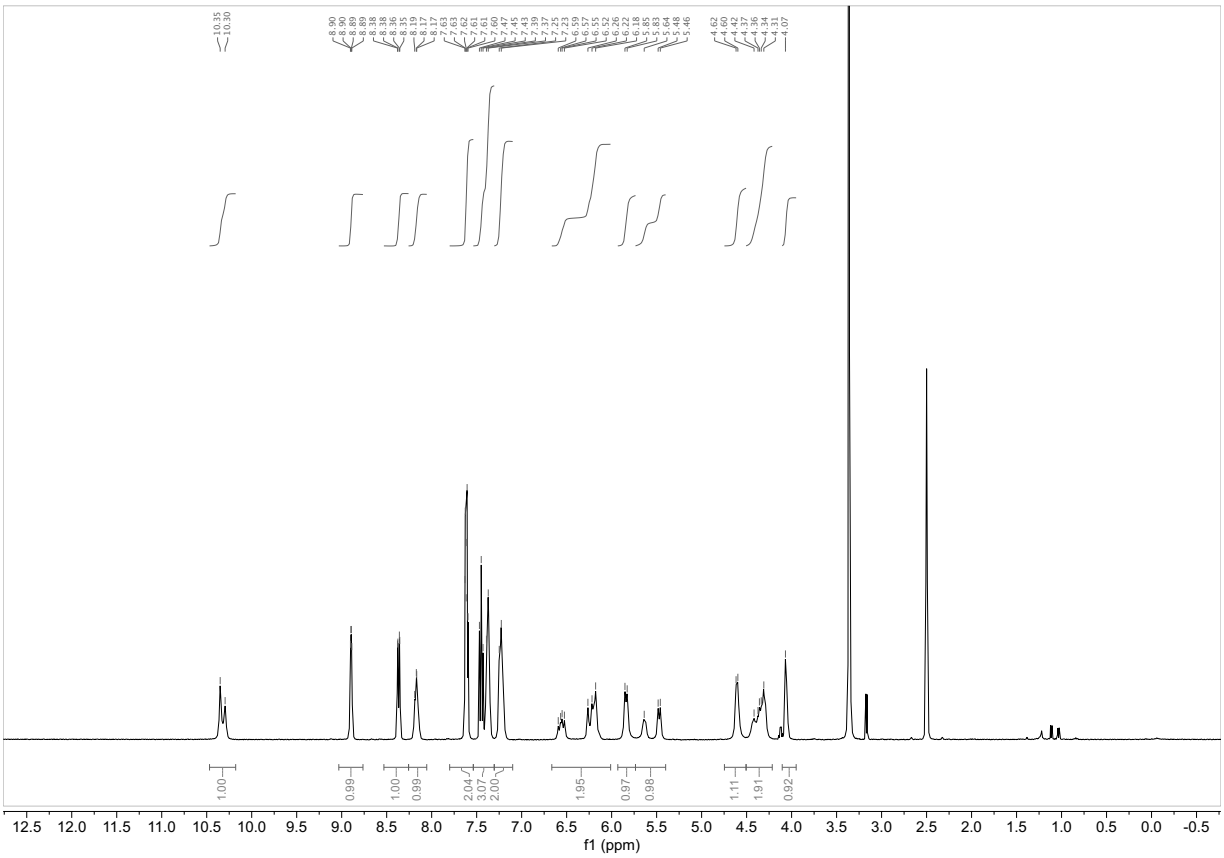
^{1}H NMR of EV-99 (8) in CD_3OD



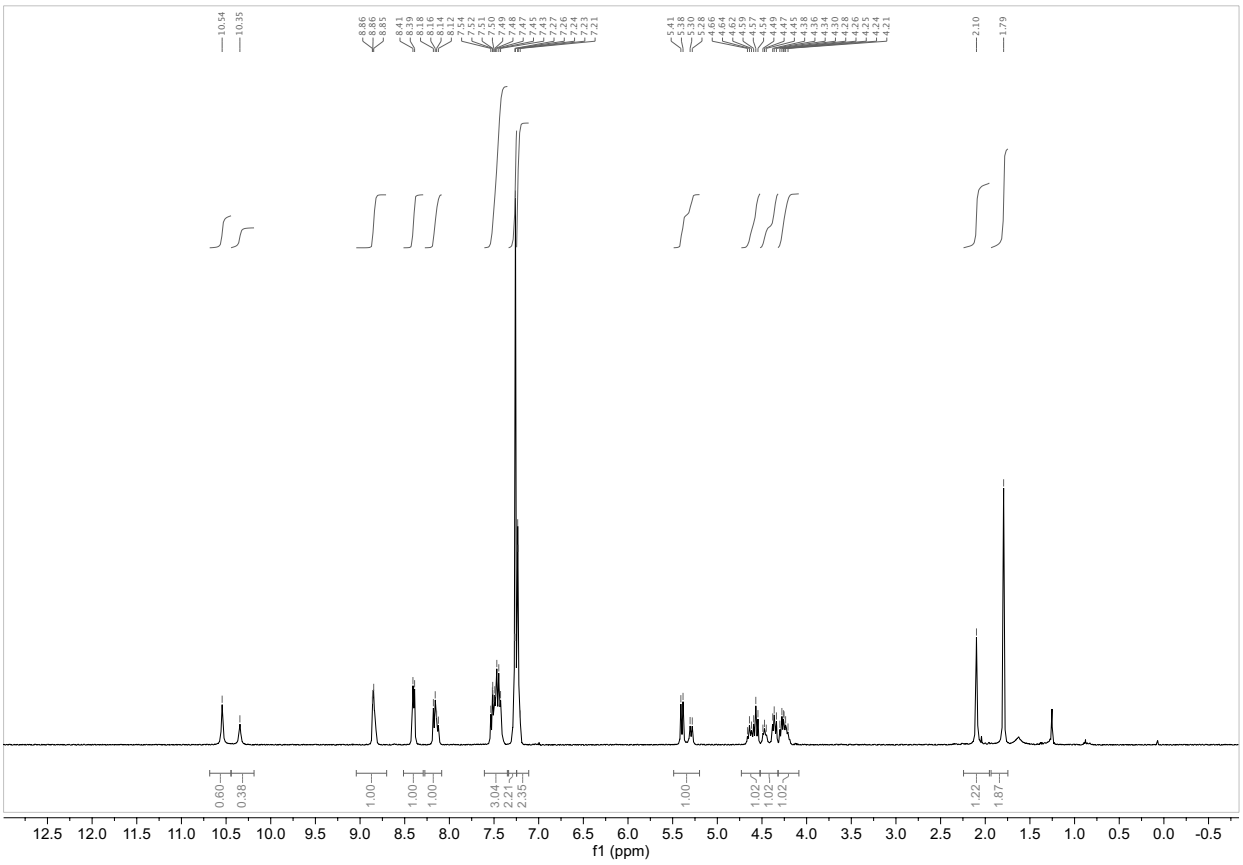
^{1}H NMR of MY-11A (9) in CD_3OD



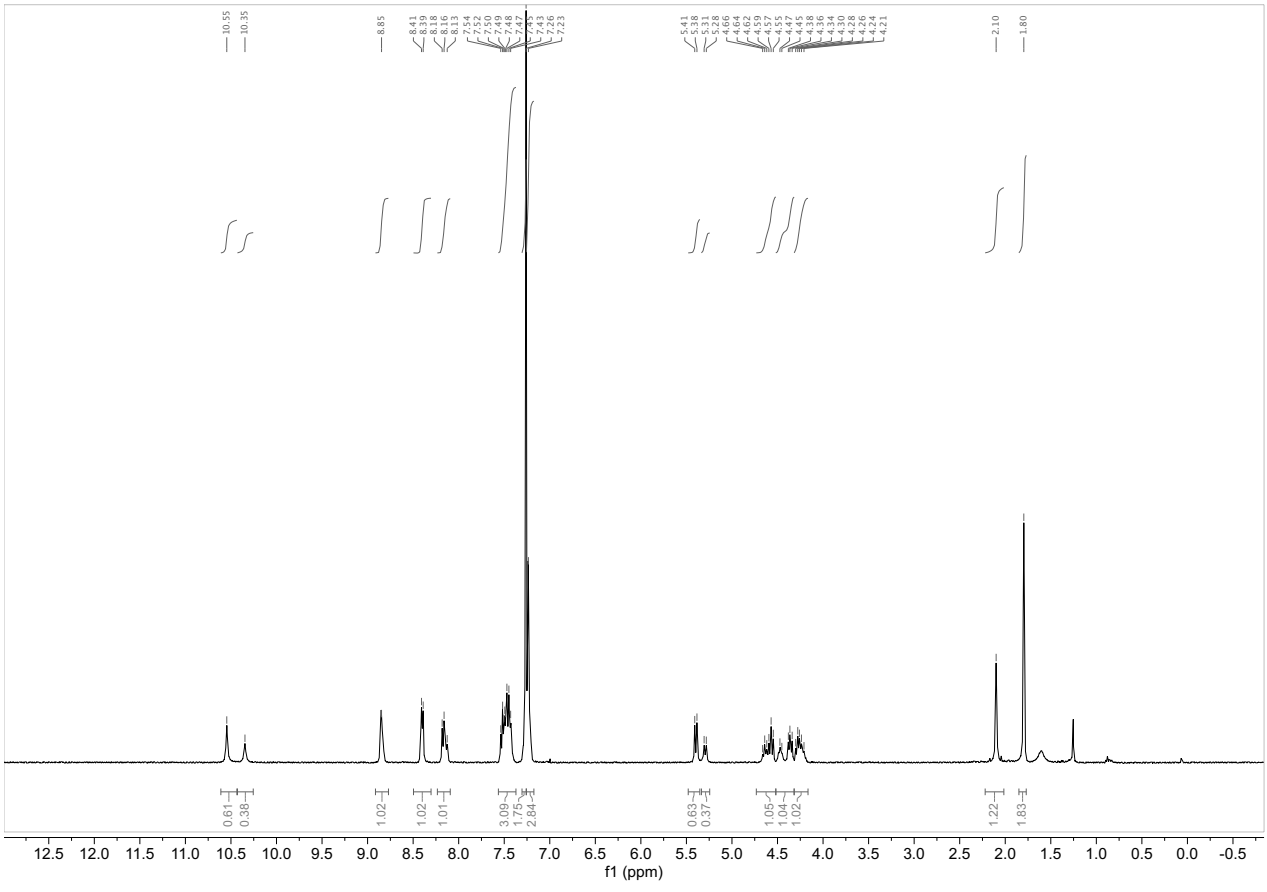
^{1}H NMR of MY-11B (10) in DMSO-*d*$_6$



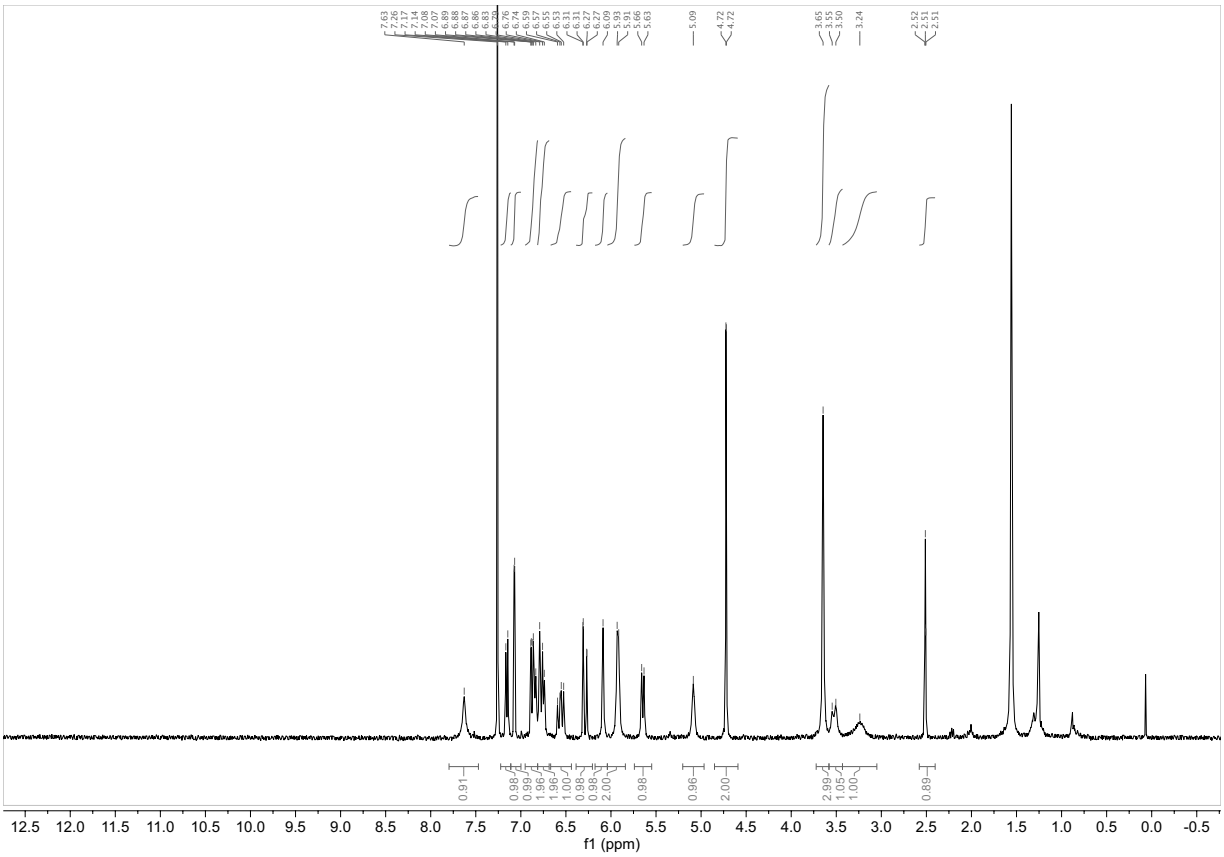
^{1}H NMR of MY-45A (11) in $CDCl_3$



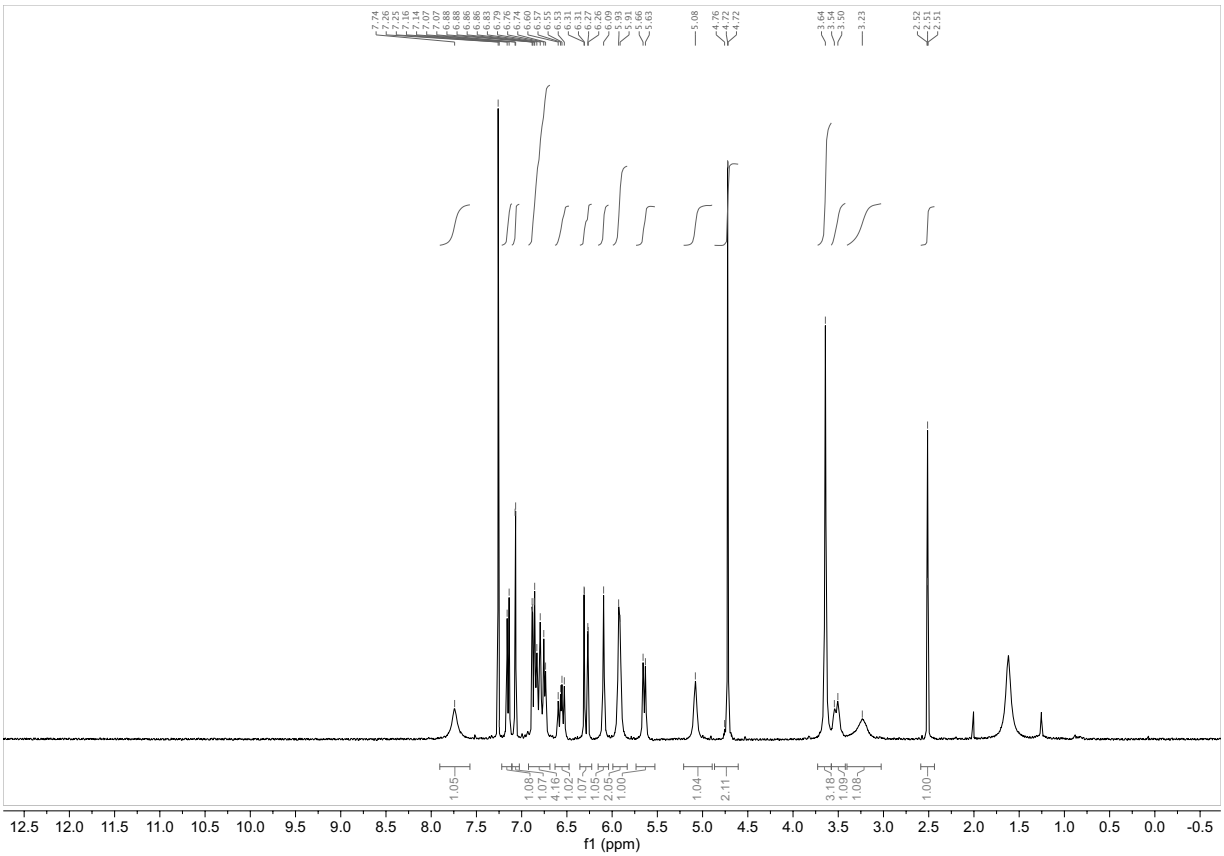
^{1}H NMR of MY-45B (12) in $CDCl_3$



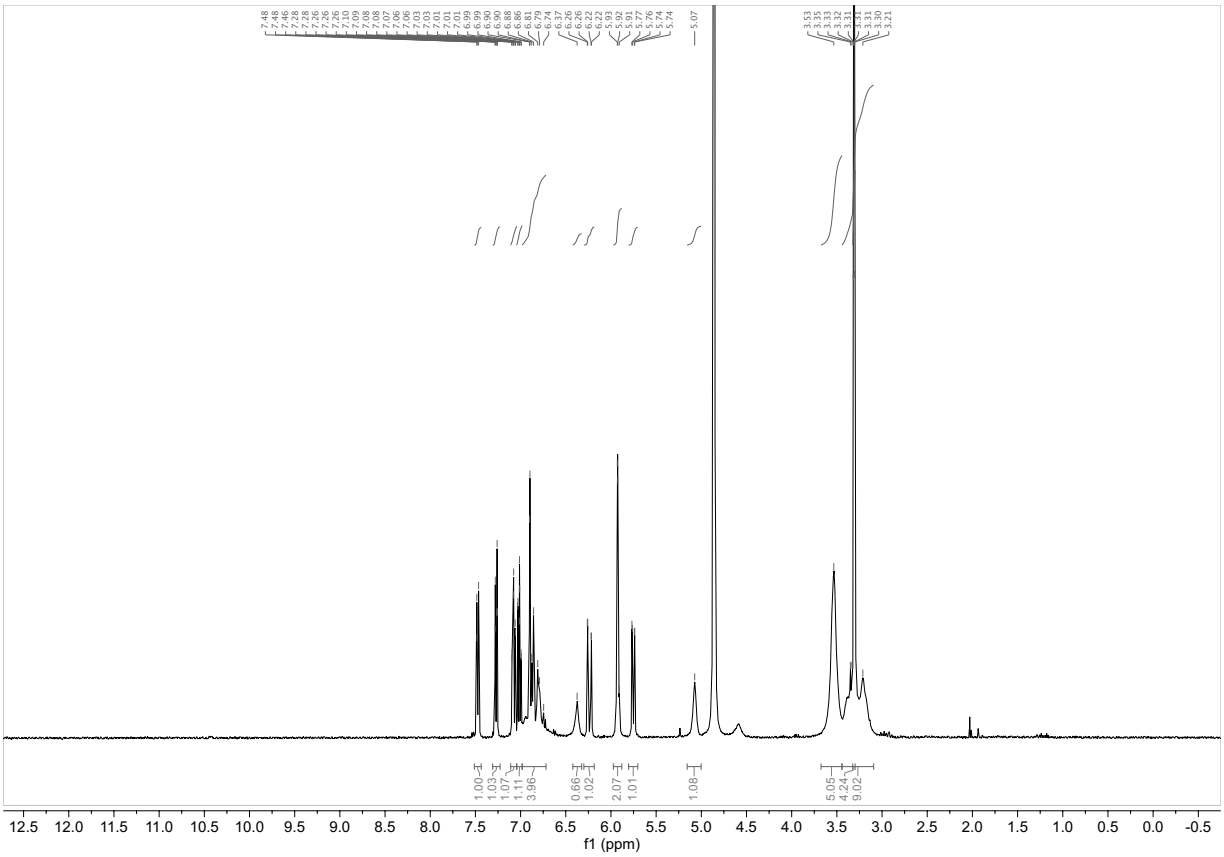
^{1}H NMR of WX-01-10 (13) in $CDCl_3$



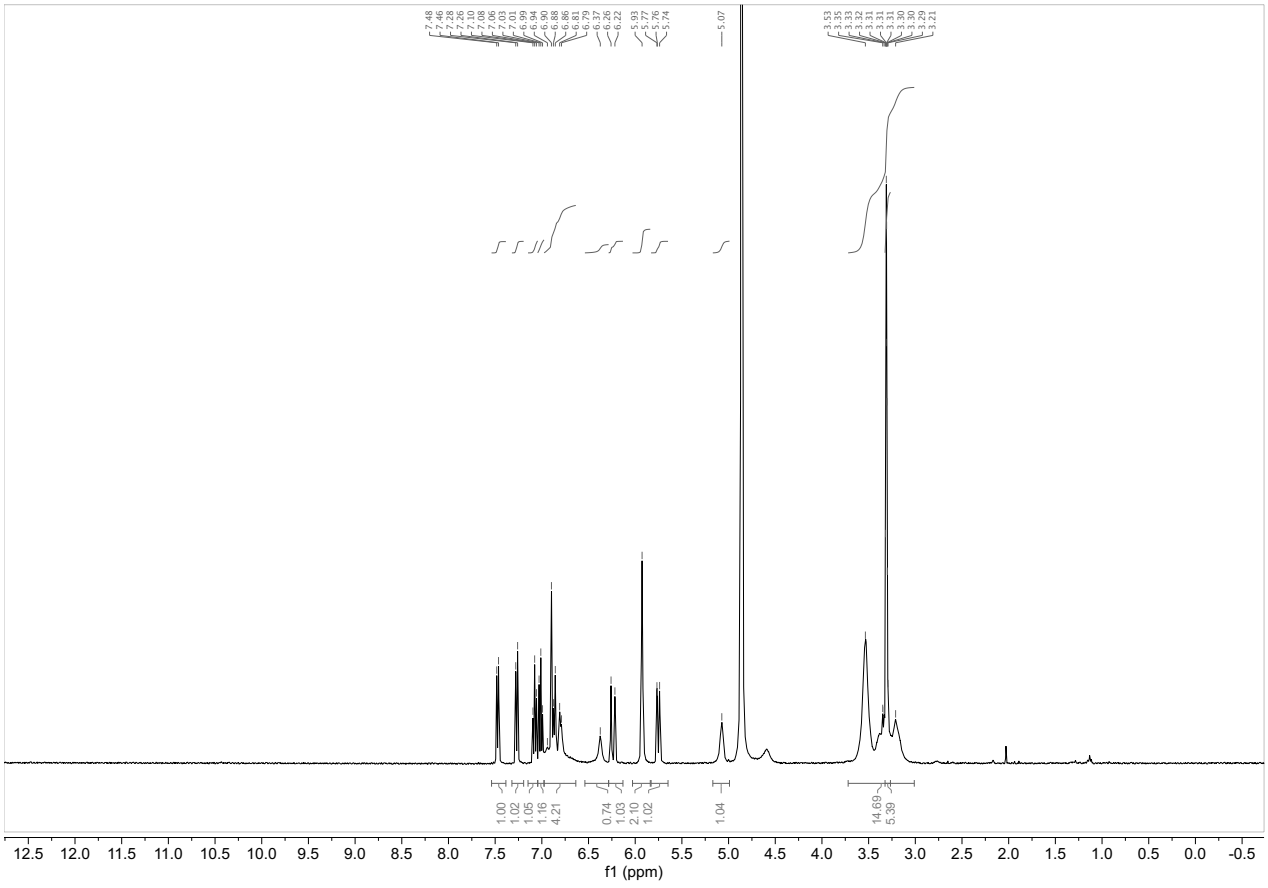
^{1}H NMR of WX-01-12 (14) in $CDCl_3$



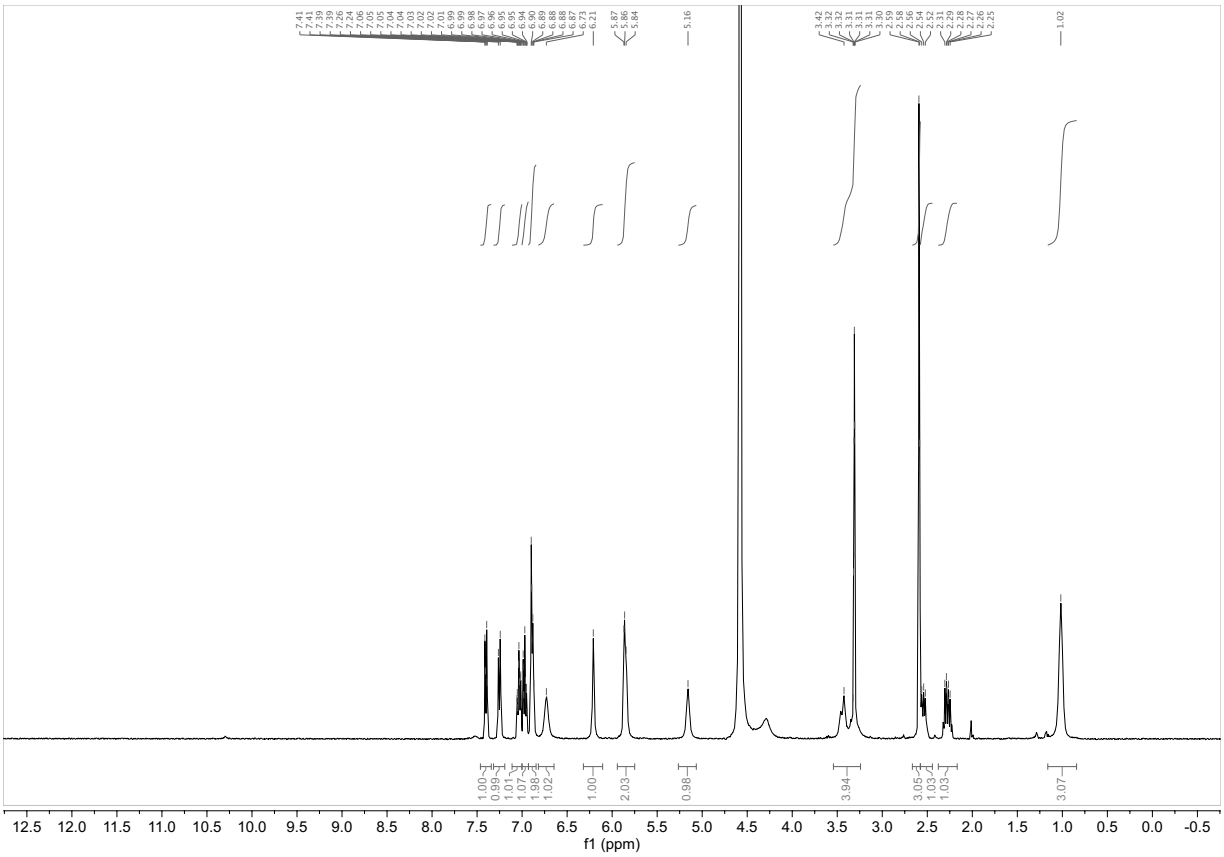
^{1}H NMR of WX-02-23 (15) in CD_3OD



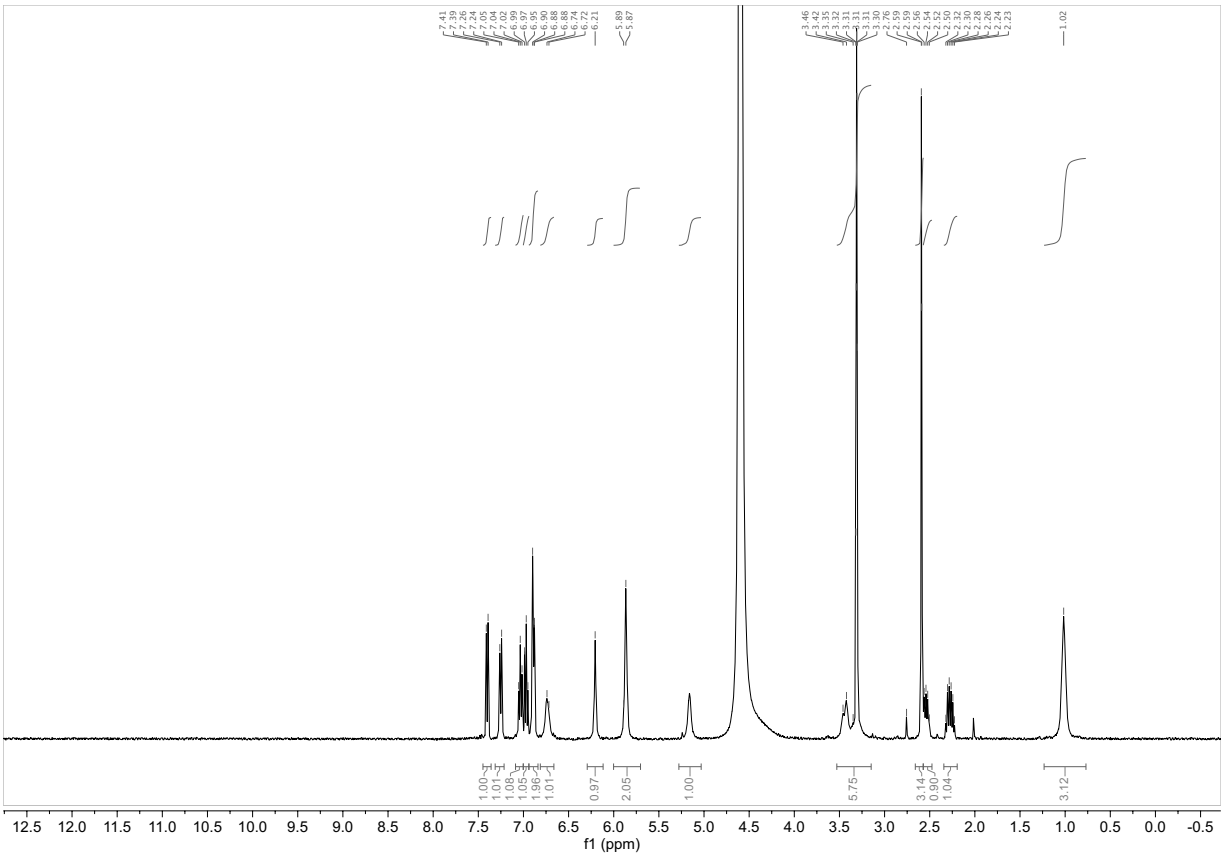
^{1}H NMR of WX-02-43 (16) in CD_3OD



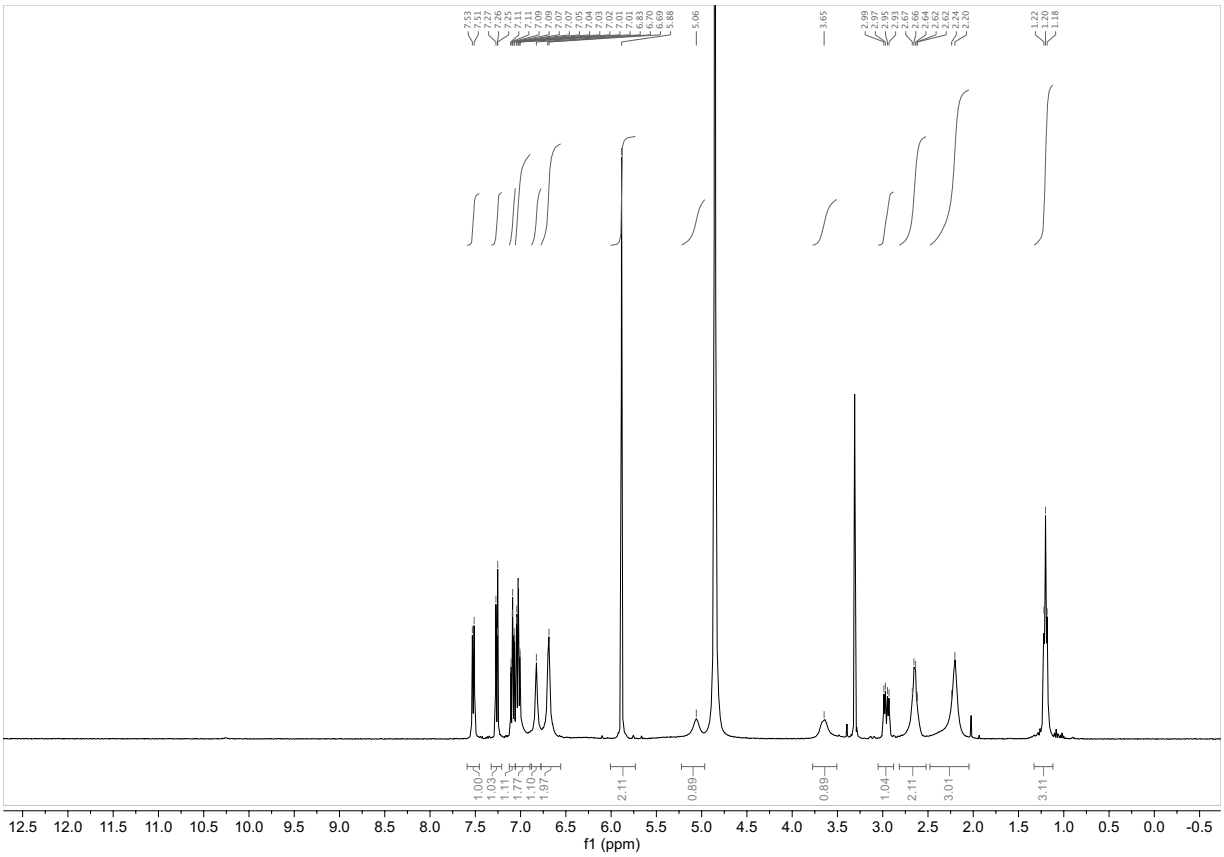
^{1}H NMR of EV-96-ctrl (17) in CD_3OD



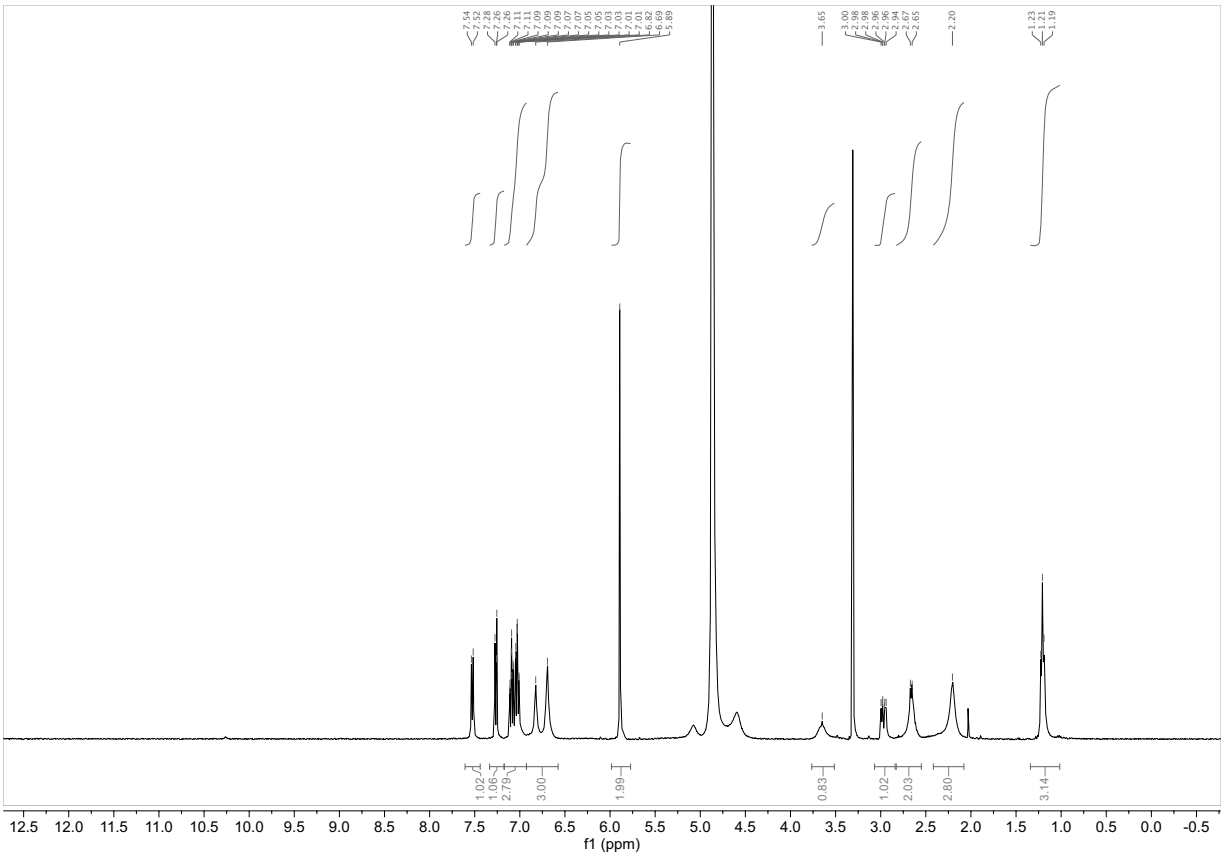
^{1}H NMR of EV-97-ctrl (18) in CD_3OD



^{1}H NMR of EV-98-ctrl (19) in CD_3OD

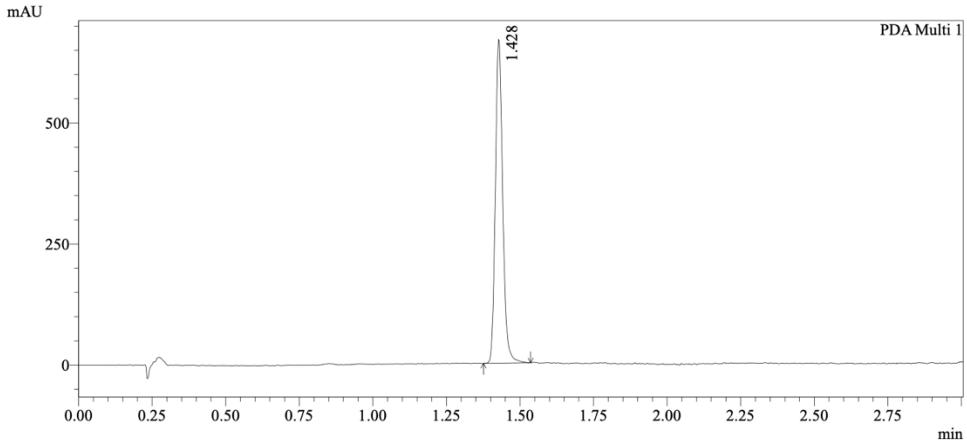


^{1}H NMR of EV-99-ctrl (20) in CD_3OD



Chiral stationary phase SFC:

MY-1A (1)



1 PDA Multi 1 / 220nm,4nm

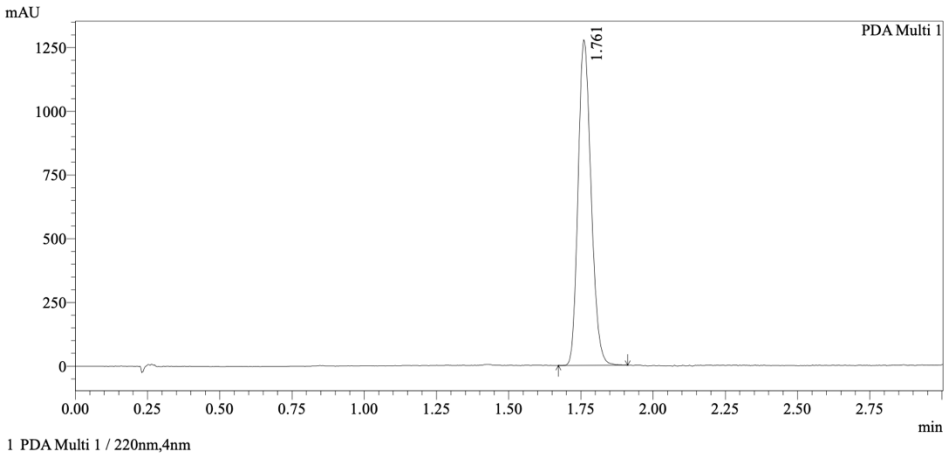
Integration Results

PeakTable

PDA Ch1 220nm

Peak#	Ret. Time	USP Width	Resolution	Height	Area	Area %
1	1.428	0.049	0.000	652902	1161646	100.000
Total				652902	1161646	100.000

MY-1B (2)



1 PDA Multi 1 / 220nm,4nm

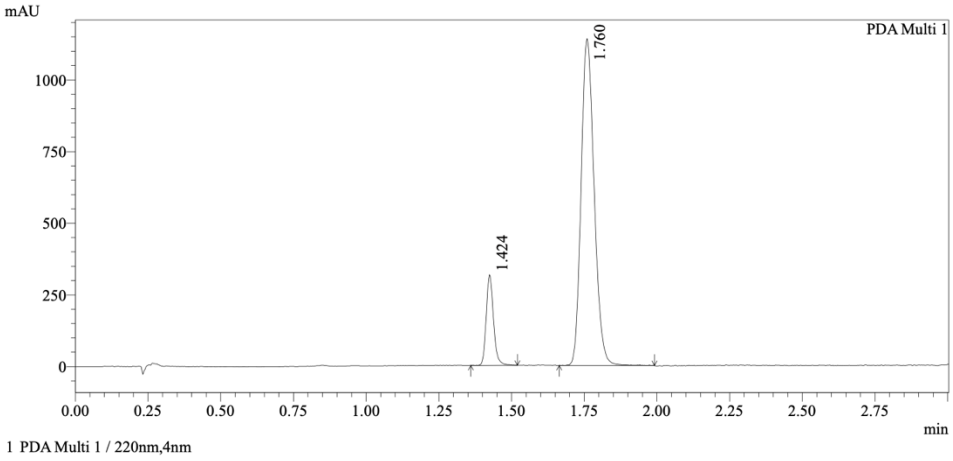
Integration Results

PeakTable

PDA Ch1 220nm

Peak#	Ret. Time	USP Width	Resolution	Height	Area	Area %
1	1.761	0.085	0.000	1254375	4001669	100.000
Total				1254375	4001669	100.000

MY-1A and MY-1B mixture



1 PDA Multi 1 / 220nm,4nm

Integration Results

PeakTable

PDA Ch1 220nm

Peak#	Ret. Time	USP Width	Resolution	Height	Area	Area %
1	1.424	0.049	0.000	296527	545869	13.295
2	1.760	0.085	5.021	1125780	3560048	86.705
Total				1422307	4105918	100.000

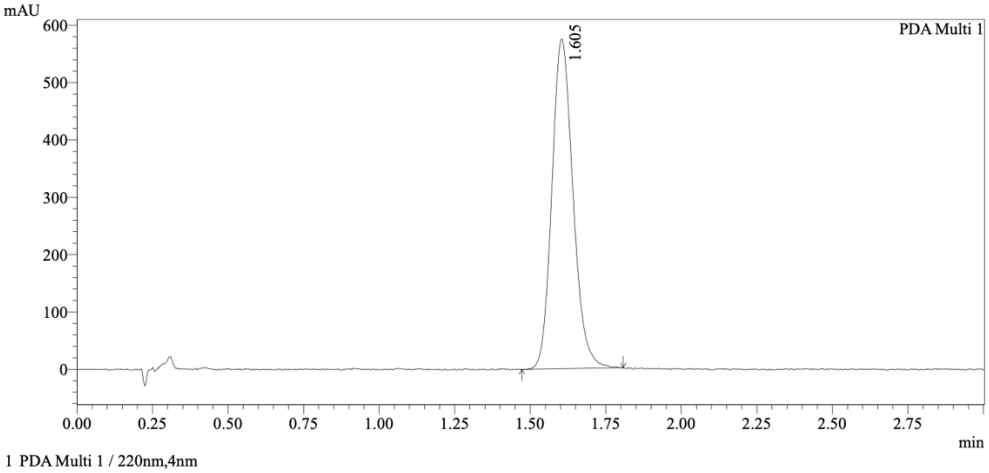
Method

Column: Chiralcel OJ-3 50×4.6mm I.D., 3 μm;

Mobile phase B: MeOH (0.05% DEA);

Gradient elution: 5% to 40% B.

MY-3A (3)



1 PDA Multi 1 / 220nm,4nm

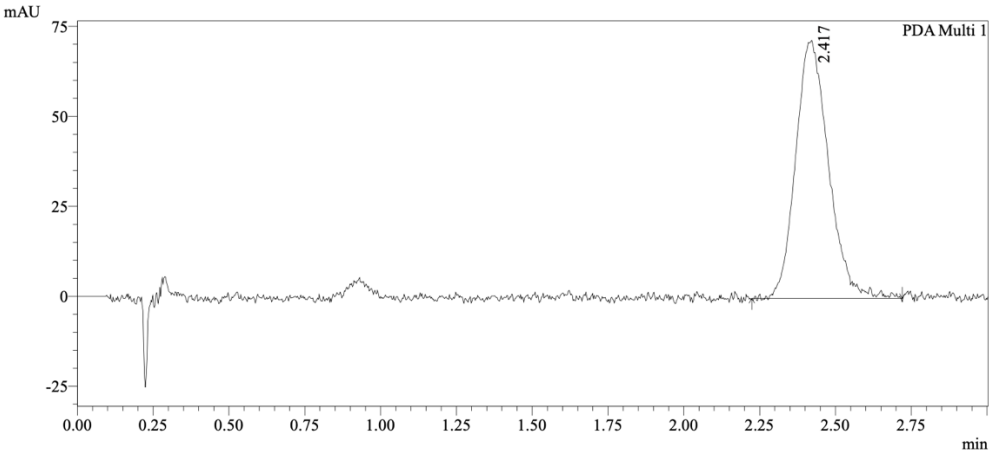
Integration Results

PeakTable

PDA Ch1 220nm

Peak#	Ret. Time	USP Width	Resolution	Height	Area	Area %
1	1.605	0.129	0.000	561301	2805243	100.000
Total				561301	2805243	100.000

MY-3B (4)



1 PDA Multi 1 / 220nm,4nm

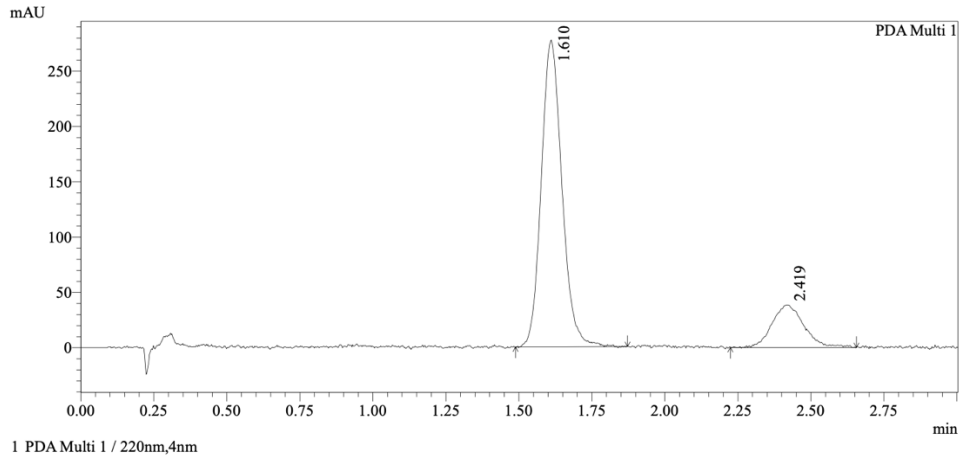
Integration Results

PeakTable

PDA Ch1 220nm

Peak#	Ret. Time	USP Width	Resolution	Height	Area	Area %
1	2.417	0.197	0.000	70006	540538	100.000
Total				70006	540538	100.000

MY-3A and MY-3B mixture



1 PDA Multi 1 / 220nm,4nm

Integration Results

PeakTable

PDA Ch1 220nm

Peak#	Ret. Time	USP Width	Resolution	Height	Area	Area %
1	1.610	0.131	0.000	273503	1365053	82.414
2	2.419	0.197	4.933	37483	291274	17.586
Total				310986	1656326	100.000

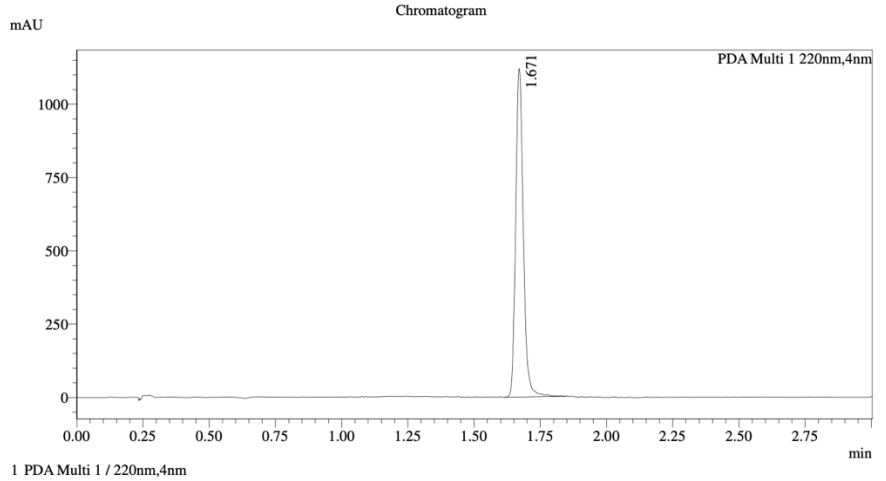
Method

Column: Chiralpak AD-3 50×4.6mm I.D., 3 μm;

Mobile phase: A: CO_2, B: iPrOH (0.05% DEA);

Gradient elution: 40% B (isocratic).

EV-96 (5)



1 PDA Multi 1 / 220nm,4nm

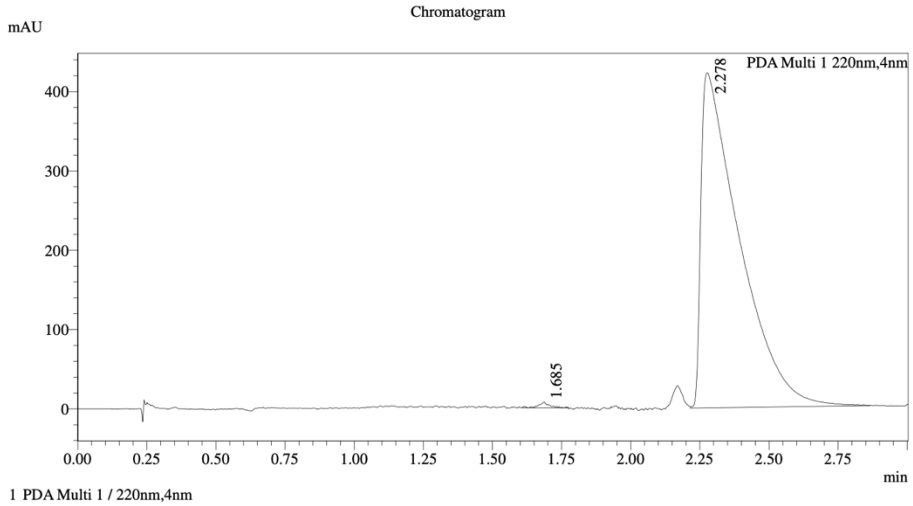
Integration Result

Peak Table

PDA Ch1 220nm

Peak#	Ret. Time	Height	Height%	Resolution(USP)		Area	Area%
1	1.671	1103556	100.000		--	2250054	100.000

EV-97 (6)



1 PDA Multi 1 / 220nm,4nm

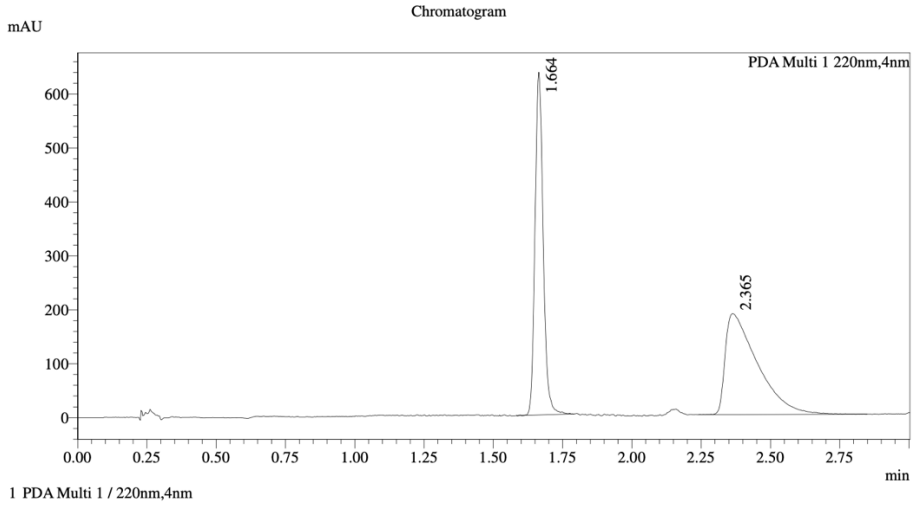
Integration Result

Peak Table

PDA Ch1 220nm

Peak#	Ret. Time	Height	Height%	Resolution(USP)		Area	Area%
1	1.685	6779	1.581		--	18710	0.456
2	2.278	421874	98.419		3.818	4086960	99.544

EV-96 and EV-97 mixture



Integration Result

Peak Table

PDA Ch1 220nm

Peak#	Ret. Time	Height	Height%	Resolution(USP)	Area	Area%
1	1.664	610940	76.585	--	1306910	46.379
2	2.365	186793	23.415	5.162	1511007	53.621

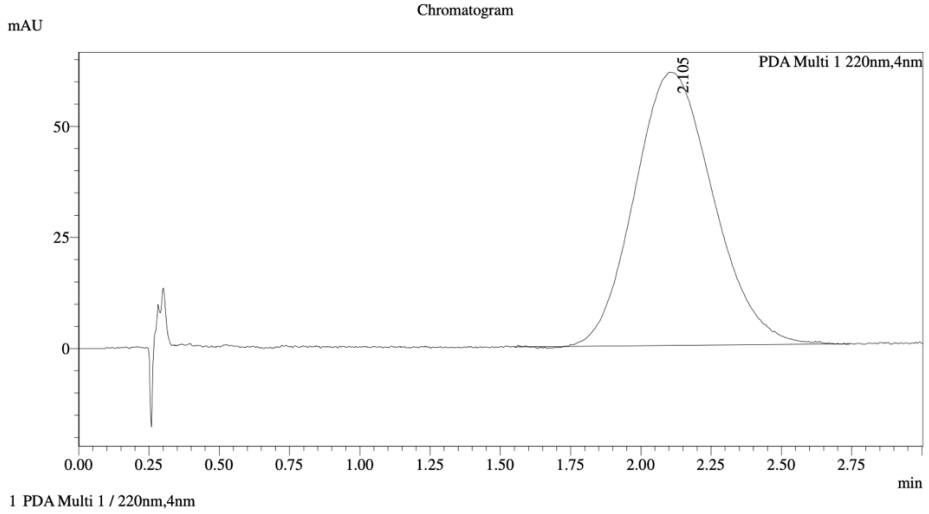
Method

Column: Chiralcel OD-3 50×4.6mm I.D., 3 μm;

Mobile phase B: MeOH (0.05% DEA);

Gradient elution: 5% to 40% B.

EV-98 (7)



1 PDA Multi 1 / 220nm,4nm

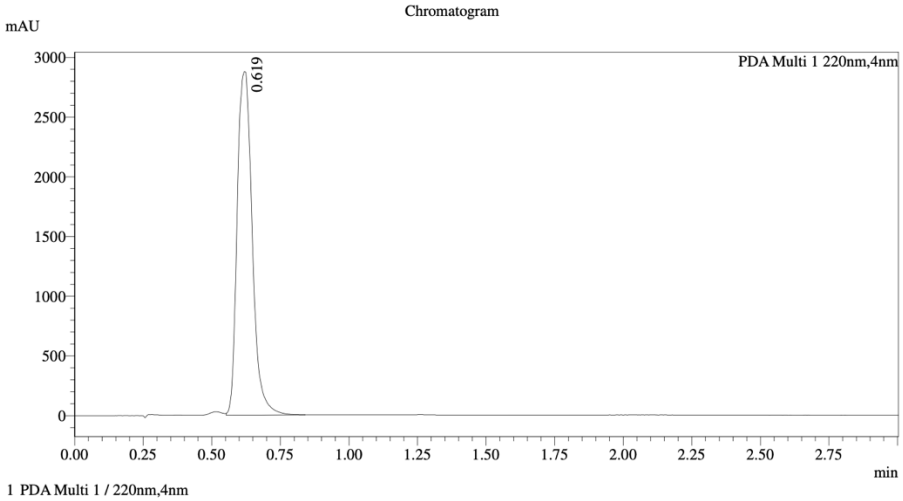
Integration Result

Peak Table

PDA Ch1 220nm

Peak#	Ret. Time	Height	Height%	Resolution(USP)		Area	Area%
1	2.105	61452	100.000		--	1196846	100.000

EV-99 (8)



1 PDA Multi 1 / 220nm,4nm

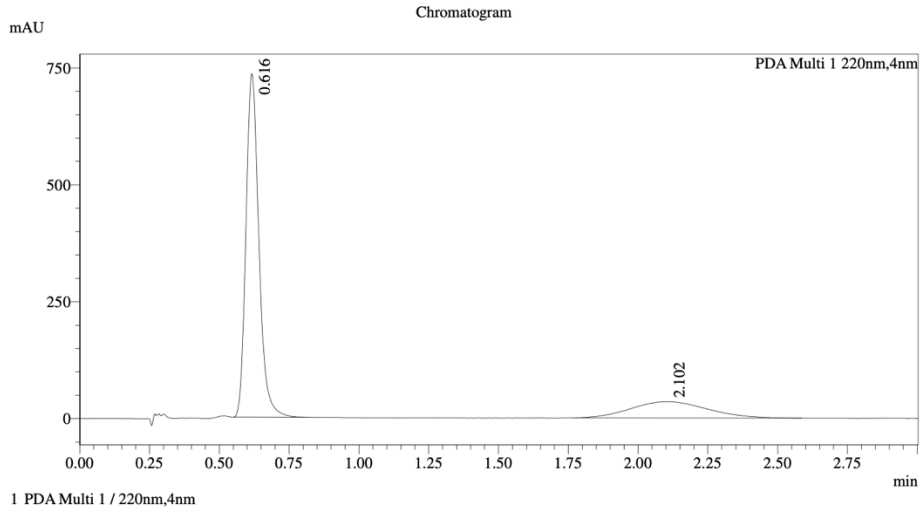
Integration Result

Peak Table

PDA Ch1 220nm

Peak#	Ret. Time	Height	Height%	Resolution(USP)		Area	Area%
1	0.619	2874854	100.000		--	10981838	100.000

EV-98 and EV-99 mixture



Integration Result

Peak Table

PDA Ch1 220nm

Peak#	Ret. Time	Height	Height%	Resolution(USP)	Area	Area%
1	0.616	722077	95.396	--	2366200	78.122
2	2.102	34845	4.604	5.013	662663	21.878

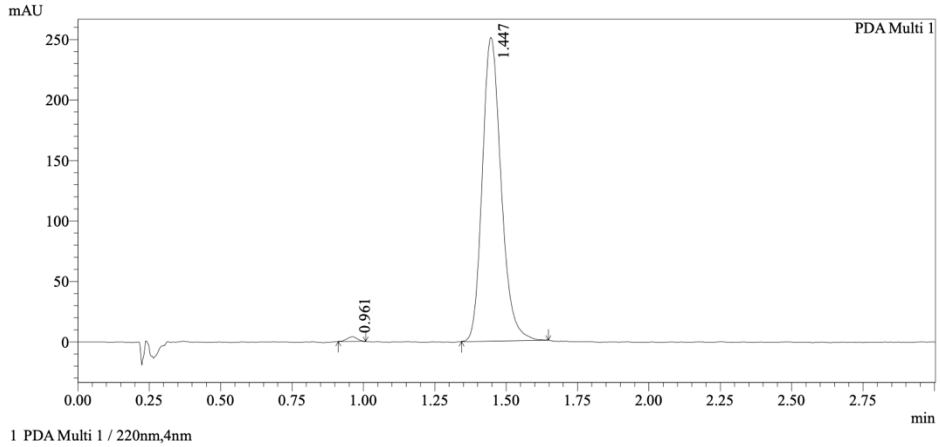
Method

Column: Chiralpak AD-3 50×4.6mm I.D., 3 μm

Mobile phase B: MeOH/CH_3CN (0.05% DEA);

Gradient elution: 40% B (isocratic)

MY-11A (9)



1 PDA Multi 1 / 220nm,4nm

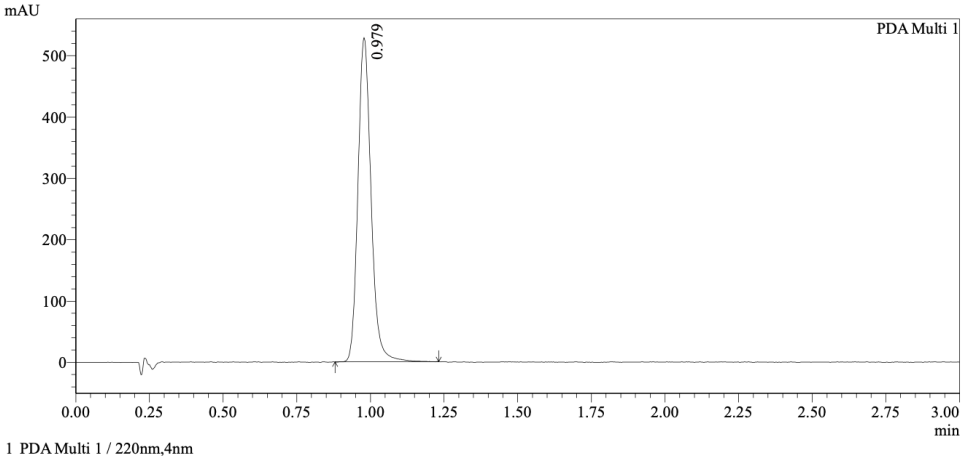
Integration Results

PeakTable

PDA Ch1 220nm

Peak#	Ret. Time	USP Width	Resolution	Height	Area	Area %
1	0.961	0.072	0.000	3299	9386	0.809
2	1.447	0.120	5.055	247033	1151490	99.191
Total				250333	1160876	100.000

MY-11B (10)



1 PDA Multi 1 / 220nm,4nm

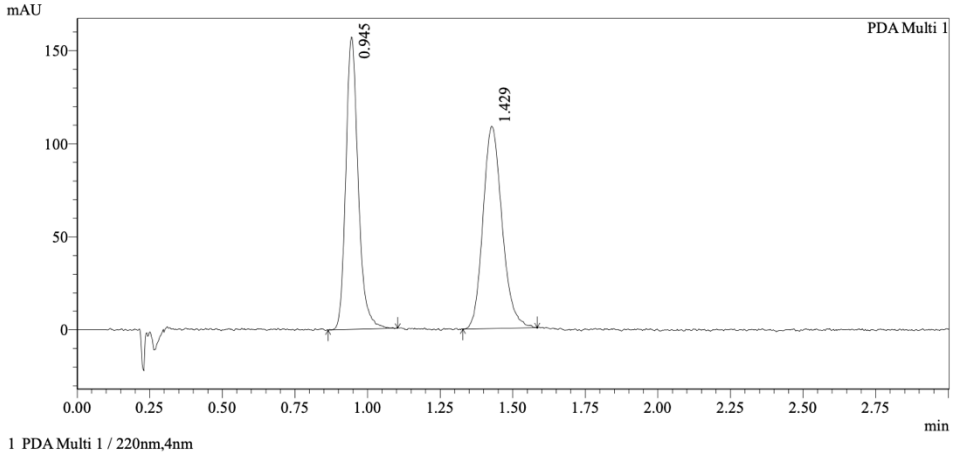
Integration Results

PeakTable

PDA Ch1 220nm

Peak#	Ret. Time	USP Width	Resolution	Height	Area	Area %
1	0.979	0.082	0.000	487407	1637634	100.000
Total				487407	1637634	100.000

MY-11A and MY-11B mixture



1 PDA Multi 1 / 220nm,4nm

Integration Results

PeakTable

PDA Ch1 220nm

Peak#	Ret. Time	USP Width	Resolution	Height	Area	Area %
1	0.945	0.079	0.000	140777	466791	48.372
2	1.429	0.121	4.848	105702	498217	51.628
Total				246479	965007	100.000

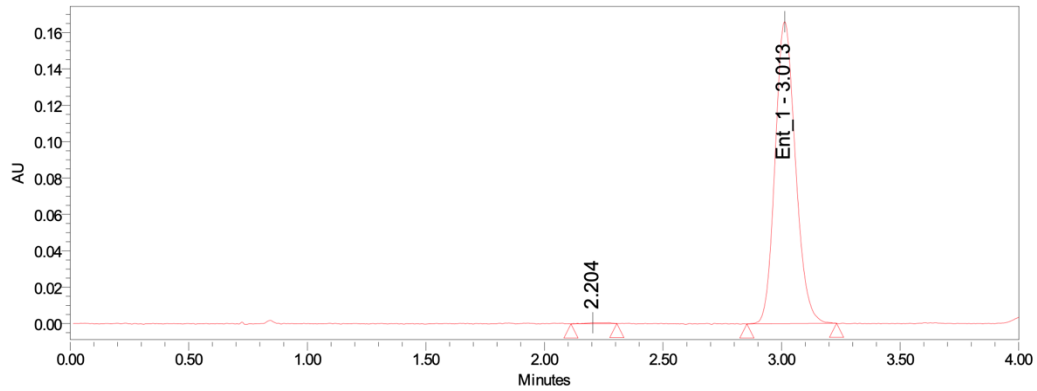
Method

Column: Chiralpak AD-3 50×4.6mm I.D., 3 μm

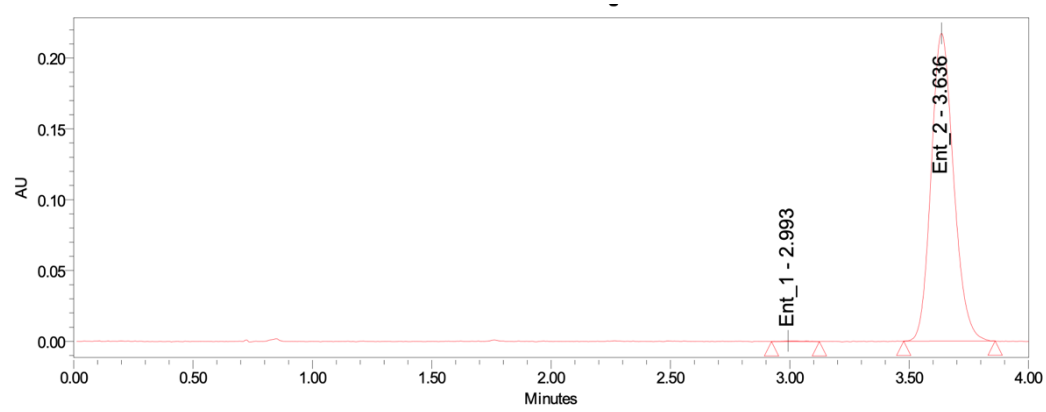
Mobile phase: A: CO_2, B: iPrOH (0.05% DEA);

Gradient elution: 40% B (isocratic).

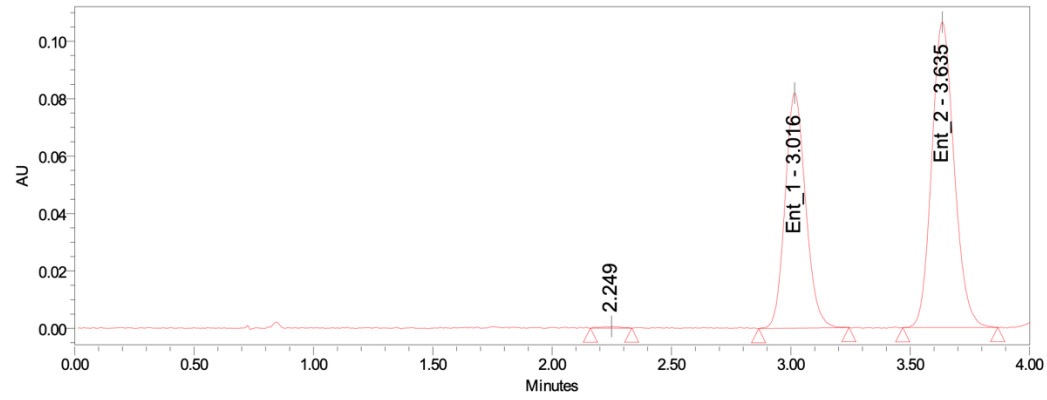
MY-45A (11)



MY-45B (12)



MY-45A and MY-45B mixture



	Peak 1 Area (t_R 3.0 min)		Peak 2 Area (t_R 3.6 min)		
sample	relative (%)	absolute	relative (%)	absolute	ee (%)
MY-45A	100.00	991600	-	-	100.00
MY-45B	0.26	3707	99.74	1423004	-99.48
mixture	41.32	491070	58.68	697307	-17.35

Method

Column: Daicel IBN 250×4.6mm I.D., 3 μm

Column temperature:

Mobile phase: A: CO_2, B: MeOH

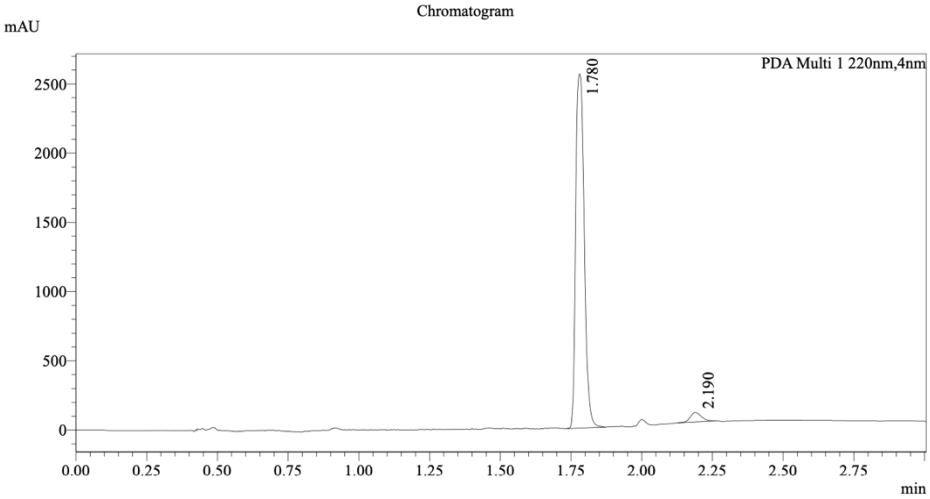
Gradient elution: 40% B (isocratic)

Flow rate: 3.5 mL / min

Back pressure: 110.3 bar

Detection wavelength: 235 nm

WX-01-10 (13)



1 PDA Multi 1 / 220nm,4nm

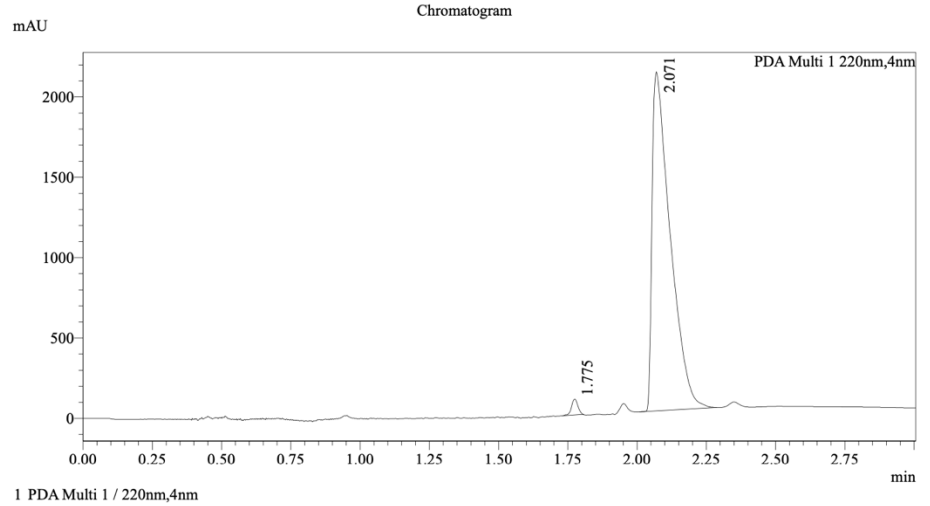
Integration Result

Peak Table

PDA Ch1 220nm

Peak#	Ret. Time	Height	Height%	USP Width	Area	Area%
1	1.780	2547962	97.425	0.055	5360030	96.588
2	2.190	67356	2.575	0.075	189350	3.412

WX-01-12 (14)



1 PDA Multi 1 / 220nm,4nm

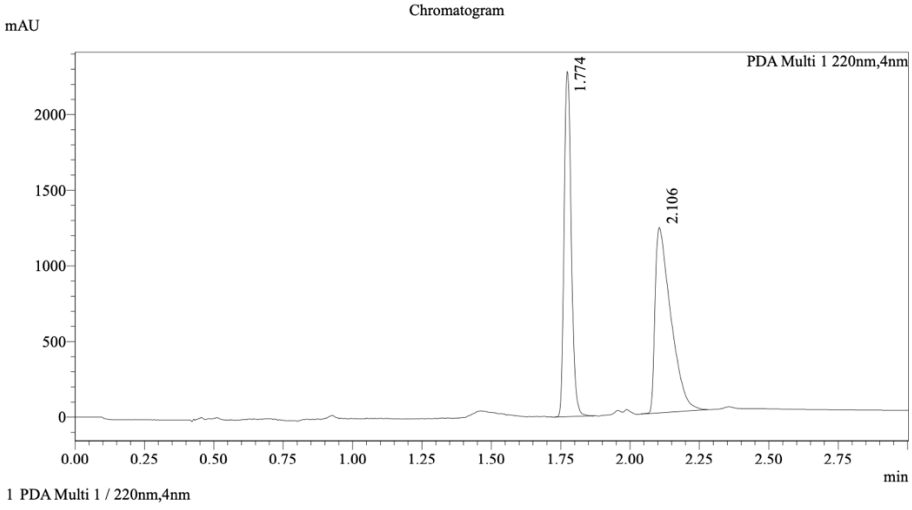
Integration Result

Peak Table

PDA Ch1 220nm

Peak#	Ret. Time	Height	Height%	USP Width	Area	Area%
1	1.775	95306	4.343	0.047	160514	1.646
2	2.071	2099003	95.657	0.122	9589854	98.354

WX-01-10 and WX-01-12 mixture



Integration Result

Peak Table

PDA Ch1 220nm

Peak#	Ret. Time	Height	Height%	USP Width	Area	Area%
1	1.774	2254220	65.173	0.050	4107310	45.914
2	2.106	1204582	34.827	0.108	4838435	54.086

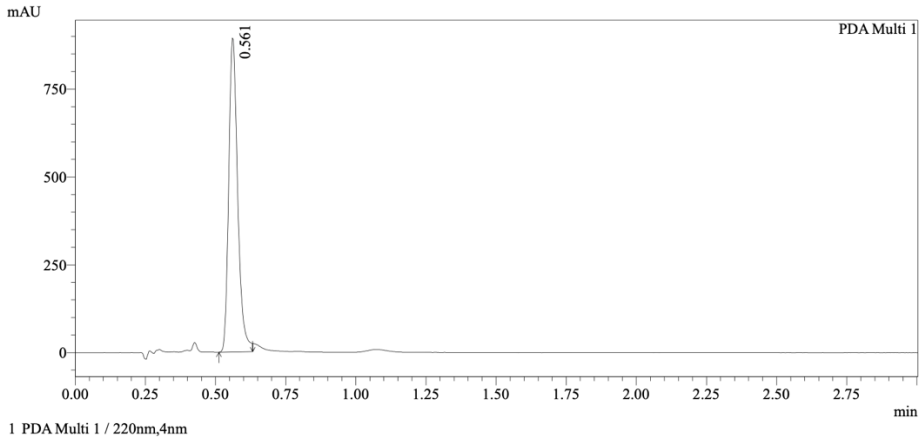
Method

Column: Cellucoat 50×4.6mm I.D., 3 μm

Mobile phase B: EtOH (0.05% DEA);

Gradient elution: 5% to 40% B.

WX-02-23 (15)



1 PDA Multi 1 / 220nm,4nm

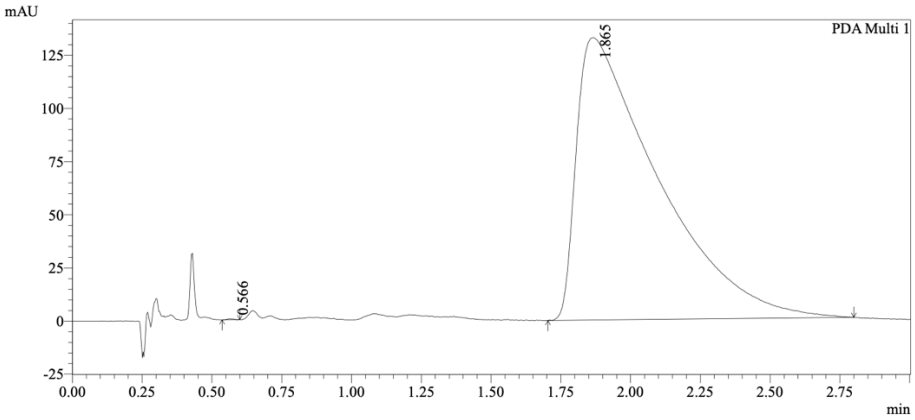
Integration Results

PeakTable

PDA Ch1 220nm

Peak#	Ret. Time	USP Width	Resolution	Height	Area	Area %
1	0.561	0.061	0.000	852432	1989408	100.000
Total				852432	1989408	100.000

WX-02-43 (16)



1 PDA Multi 1 / 220nm,4nm

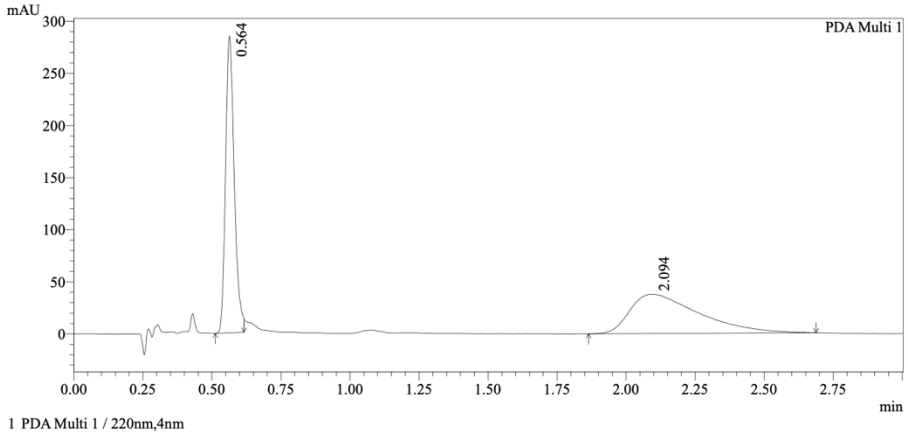
Integration Results

PeakTable

PDA Ch1 220nm

Peak#	Ret. Time	USP Width	Resolution	Height	Area	Area %
1	0.566	0.049	0.000	390	643	0.024
2	1.865	0.553	4.318	132541	2696012	99.976
Total				132931	2696655	100.000

WX-02-23 and WX-02-43 mixture



1 PDA Multi 1 / 220nm,4nm

Integration Results

PeakTable

PDA Ch1 220nm

Peak#	Ret. Time	USP Width	Resolution	Height	Area	Area %
1	0.564	0.061	0.000	278808	626639	48.682
2	2.094	0.453	5.954	37794	660569	51.318
Total				316601	1287208	100.000

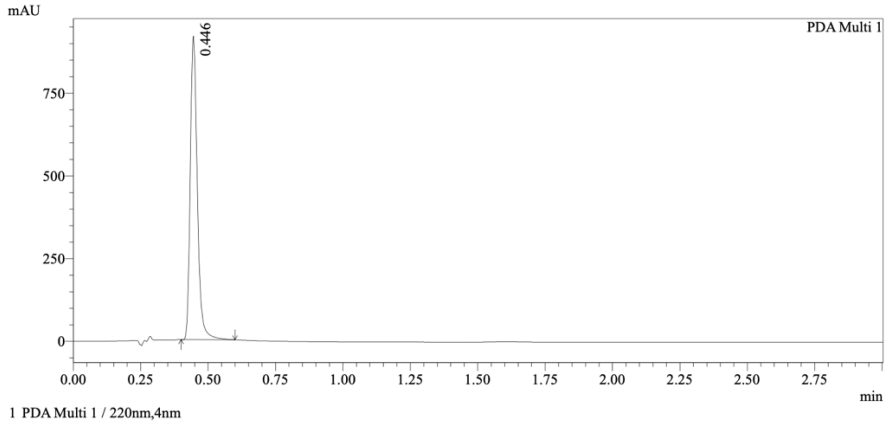
Method

Column: Chiralcel OD-3 50×4.6mm I.D., 3 μm;

Mobile phase B: MeOH (0.05%DEA);

Gradient elution: 40% B (isocratic)

EV-96-ctrl (17)



1 PDA Multi 1 / 220nm,4nm

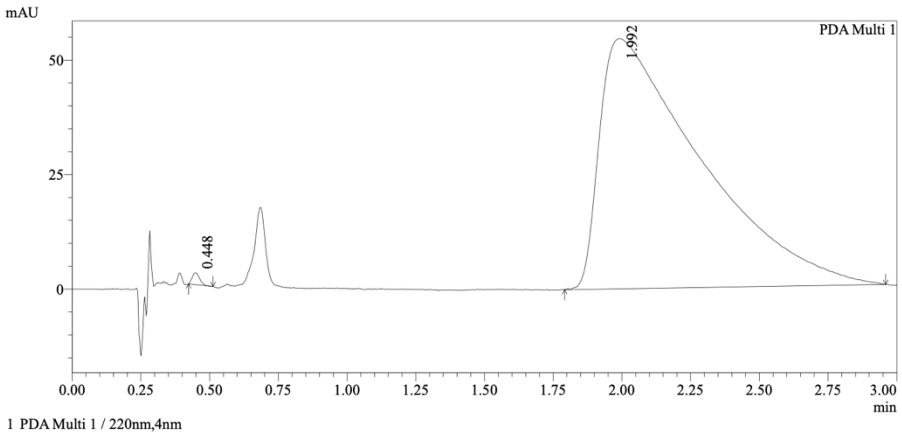
Integration Results

PeakTable

PDA Ch1 220nm

Peak#	Ret. Time	USP Width	Resolution	Height	Area	Area %
1	0.446	0.051	0.000	901243	1679633	100.000
Total				901243	1679633	100.000

EV-97-ctrl (18)



1 PDA Multi 1 / 220nm,4nm

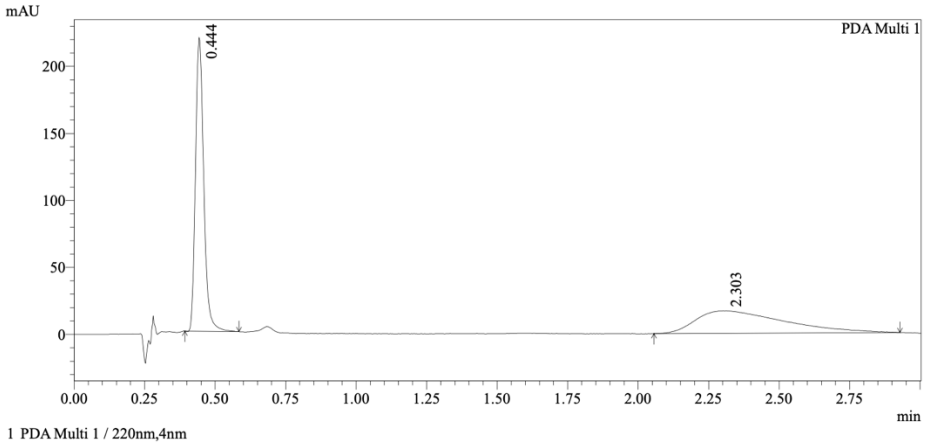
Integration Results

PeakTable

PDA Ch1 220nm

Peak#	Ret. Time	USP Width	Resolution	Height	Area	Area %
1	0.448	0.055	0.000	2504	4904	0.349
2	1.992	0.710	4.040	54596	1398379	99.651
Total				57100	1403283	100.000

Mixture of compounds EV-96 and EV-97



1 PDA Multi 1 / 220nm,4nm

Integration Results

PeakTable

PDA Ch1 220nm

Peak#	Ret. Time	USP Width	Resolution	Height	Area	Area %
1	0.444	0.056	0.000	214301	447926	55.168
2	2.303	0.569	5.944	16753	364004	44.832
Total				231054	811931	100.000

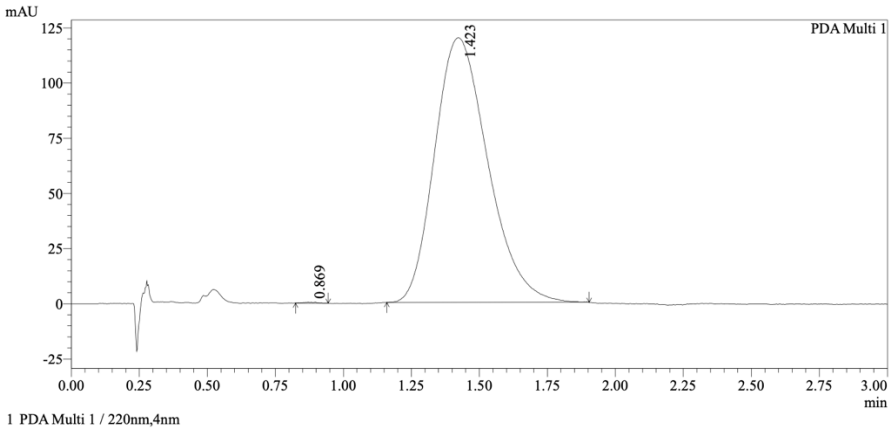
Method

Column: Chiralcel OD-3 50×4.6mm I.D., 3 μm;

Mobile phase B: MeOH (0.05%DEA);

Gradient elution: 40% B (isocratic).

EV-98-ctrl (19)



1 PDA Multi 1 / 220nm,4nm

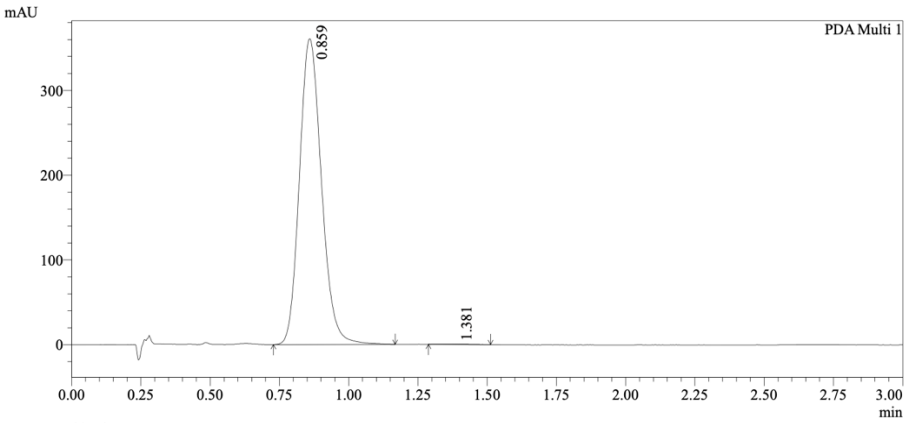
Integration Results

PeakTable

PDA Ch1 220nm

Peak#	Ret. Time	USP Width	Resolution	Height	Area	Area %
1	0.869	0.096	0.000	348	1159	0.071
2	1.423	0.358	2.438	119881	1634250	99.929
Total				120229	1635409	100.000

EV-99-ctrl (20)



1 PDA Multi 1 / 220nm,4nm

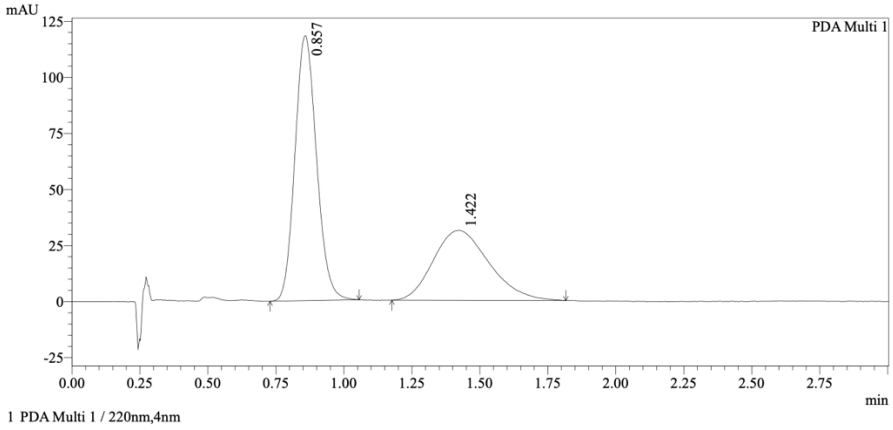
Integration Results

PeakTable

PDA Ch1 220nm

Peak#	Ret. Time	USP Width	Resolution	Height	Area	Area %
1	0.859	0.146	0.000	359647	1997444	99.895
2	1.381	0.160	3.418	316	2095	0.105
Total				359963	1999539	100.000

Mixture of compounds EV-98-ctrl and EV-99-ctrl



1 PDA Multi 1 / 220nm,4nm

Integration Results

PeakTable

PDA Ch1 220nm

Peak#	Ret. Time	USP Width	Resolution	Height	Area	Area %
1	0.857	0.147	0.000	117235	653341	59.496
2	1.422	0.376	2.161	31162	444778	40.504
Total				148397	1098120	100.000

Method

Column: Chiralpak AD-3 50×4.6mm I.D., 3 μm;

Mobile phase B: MeOH (0.05%DEA);

Gradient elution: 40% B (isocratic).

References cited

Maetani, M., Zoller, J., Melillo, B., Verho, O., Kato, N., Pu, J., Comer, E., and Schreiber, S.L. (2017). Synthesis of a Bicyclic Azetidine with In Vivo Antimalarial Activity Enabled by Stereospecific, Directed C(sp(3))-H Arylation. J Am Chem Soc *139*, 11300-11306.

Vinogradova, E.V., Zhang, X., Remillard, D., Lazar, D.C., Suciu, R.M., Wang, Y., Bianco, G., Yamashita, Y., Crowley, V.M., Schafroth, M.A.*, et al.* (2020). An Activity-Guided Map of Electrophile-Cysteine Interactions in Primary Human T Cells. Cell *182*, 1009-1026 e1029.