The Impact of Distinct Chemical Structures for the Development of a Methamphetamine Vaccine.

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Supporting Information

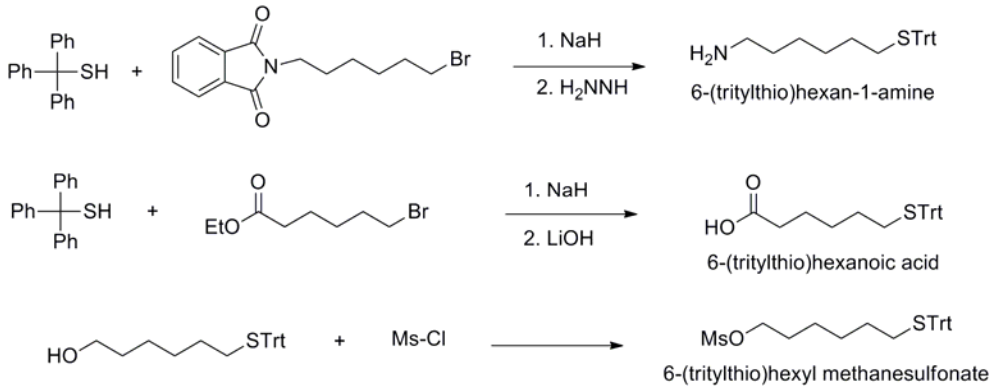
Synthetic Procedures.

General notes. All reactions were performed under an inert atmosphere with dry reagents, solvents, and flame-dried glassware. Unless otherwise noted, all starting materials were purchased from Aldrich, Sigma, Fisher, or Pierce and used as received. All flash column chromatography was performed using silica gel 60 (230-400 mesh). Analytical and preparative thin-layer chromatography (TLC) was performed using Merck Kieselgel 60 F254 silica gel plates (0.25, 0.5, or 1 mm). ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker DRX-600 (600 MHz), DRX-500 (500 MHz), or INOVA-399 (400 MHz) spectrometer and chemical shifts are reported in parts per million (ppm) from an internal standard. Preparative HPLC was done using a C18 column; eluents A (aqueous phase) = 0.1%TFA H_2O, B (organic phase) = 0.1% TFA Acetonitrile; λ = 220nm and 254nm.

Common abbreviations used:
1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide – EDC
Hydroxybenzotriazole – HOBT
Diaza(1,3)bicyclo[5.4.0]undecane - DBU
4-Dimethylaminopyridine – DMAP
Trifluoroacetic acid – TFA
Triisopropylsilane - TIS

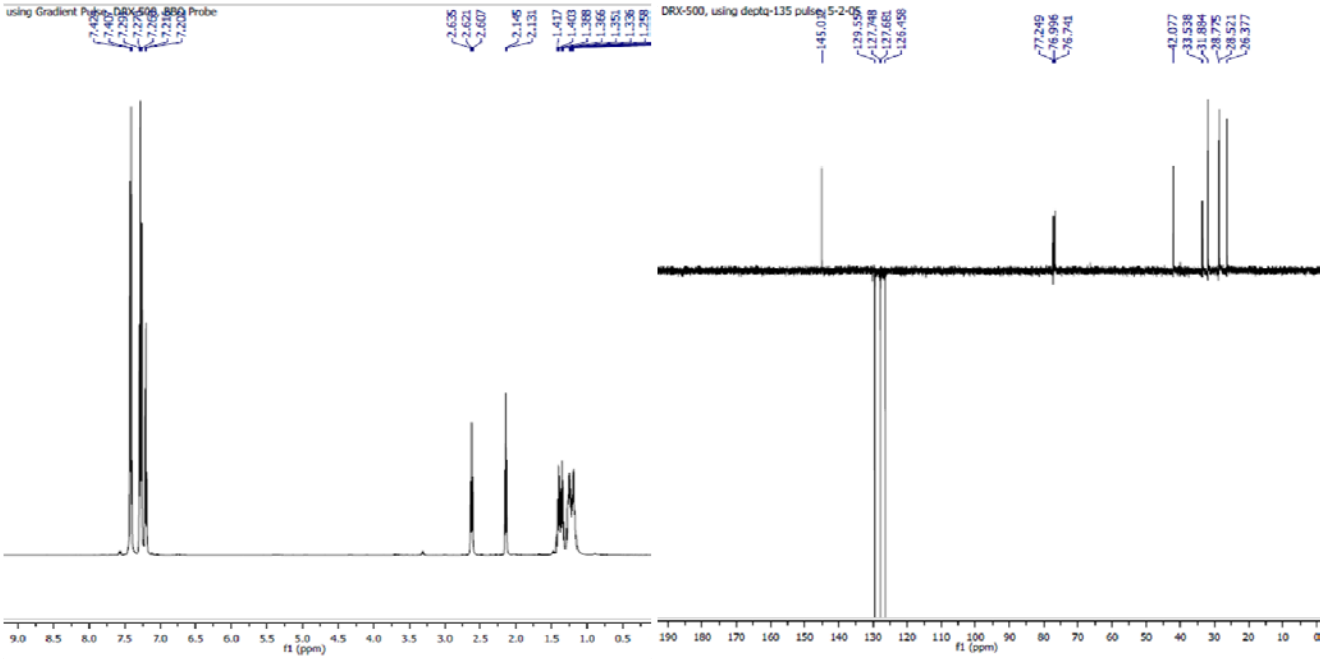
The synthesis of all haptens proceeded by coupling of the appropriate "core" structures and several linkers. Briefly we first present the synthesis of the three linker structures required.

Scheme 1. Synthesis of linker structures.

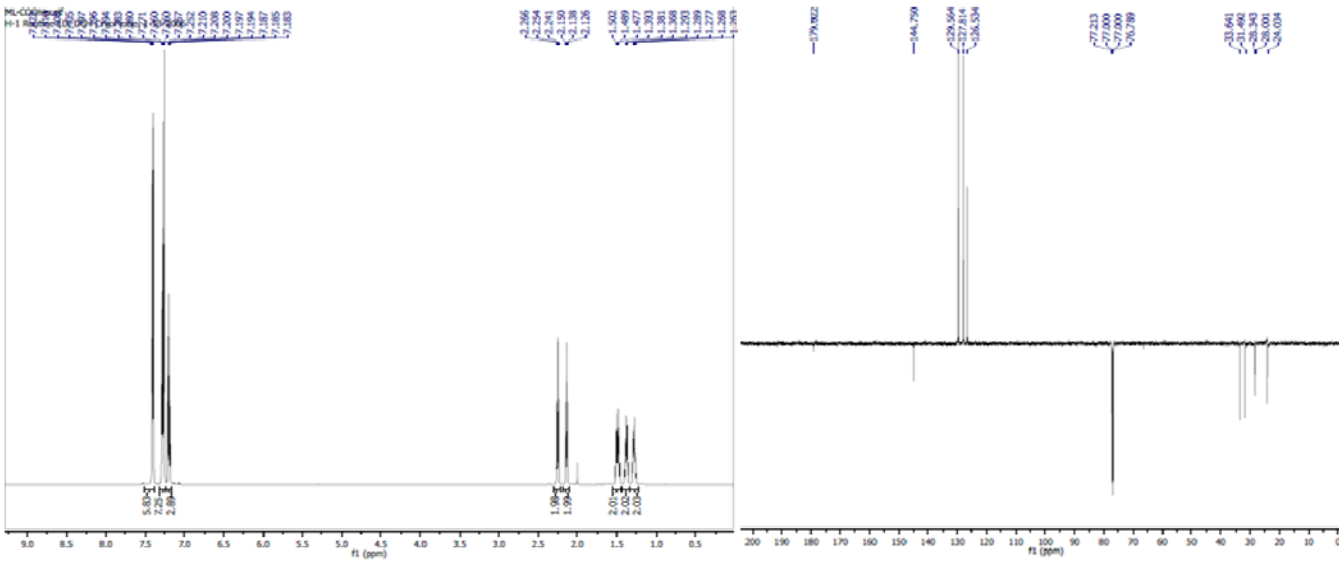


6-(tritylthio)hexan-1-amine. Commercially available triphenylmethanethiol (0.773mmol) was added to a cooled stirred solution of NaH (0.838mmol) in dry THF (2.1 mL). After 20min, a solution of N-(6-bromohexyl)phthalimide (0.644mmol) in 0.54 mL dry THF was added dropwise at 0°C. The reaction was allowed to stir at room temperature for 10 hours. Addition of a 4:1 petroleum ether/ether solution led to

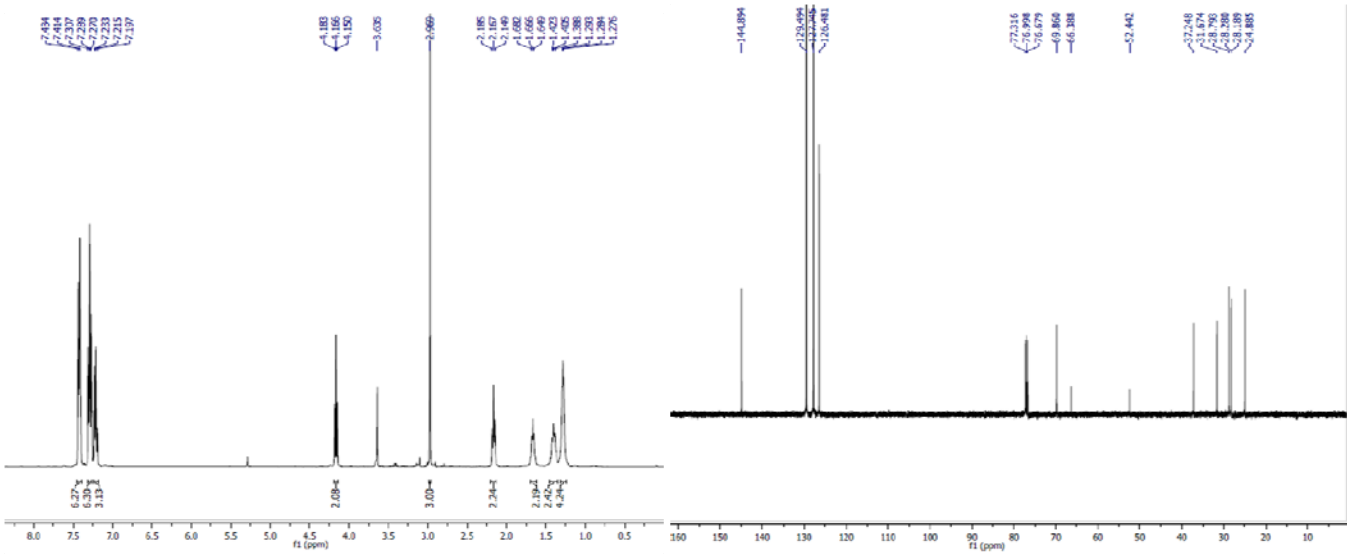
formation of a precipitate which was removed by filtration. Organic layers were combined, dried over Na_2SO_4 and rotovaped to a white solid (64% yield). The final product was obtained by removal of the phthalimide group using hydrazine. Briefly, the starting material (0.413mmol) was dissolved on 2.4mL ethanol, put under argon and 1.23mmol of hydrazine were added dropwise. The reaction mixture was allowed to stir overnight. White precipitate was removed by filtration and washed with ethanol. Filtrates were concentrated under reduced pressure to a film and triturated with chloroform. Additional precipitate was filtered off, and the filtrate was further washed 3X with water, dried over Na_2SO_4 and rotovaped. (88.5% yield ^{1}H NMR (500 MHz , $CDCl_3$) δ 7.42 (d, *J* = 8.0 Hz, 6H), 7.28 (t, *J* = 7.8 Hz, 6H), 7.21 (d, *J* = 7.2 Hz, 3H), 2.62 (t, *J* = 7.0 Hz, 2H), 2.15 (t, *J* = 7.3 Hz, 2H), 1.35 (m, 4H), 1.21 (m, 6H). ^{13}C NMR (500 MHz, $CDCl_3$) δ = 145.01, 129.56, 127.75, 127.68, 126.46, 42.08, 33.54, 31.88, 28.77, 28.52, 26.38. LRMS $(M + H)^+$: calcd for $C_{25}H_{29}NS$ = 376.20 ; found 376.4



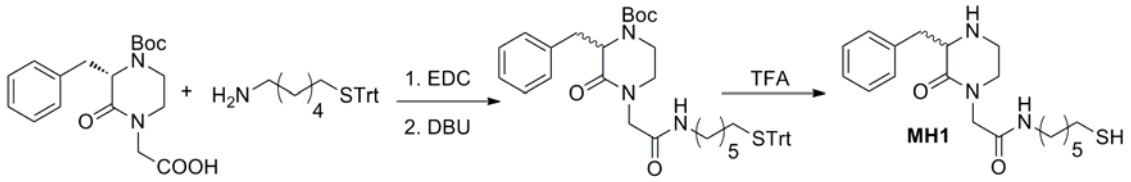
6-(tritylthio)hexanoic acid. Commercially available triphenylmethanethiol (1.1mmol) was added to a cooled stirred solution of NaH (1.16mmol) in dry THF (3 mL). After 20min, ethyl 6-bromohexanoate (0.89mmol) was added neat dropwise at 0°C. The reaction was allowed to stir at room temperature for 10 hours. Addition of a 4:1 petroleum ether/ether solution led to formation of a precipitate which was filtered off. The filtrate was dried over Na_2SO_4 and rotovaped to an oil which solidified upon standing at 4°C (92% crude yield). The crude material (0.821mmol) was dissolved in 9.2 mL of methanol and 4M LiOH (2.46mmol) was added. The solution was stirred at 40°C for 6 hrs. Methanol was rotovaped off, and the residue was re-crystallized from 3-methyl 1-butanol to yield a white solid (74% yield). : ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.51 – 7.38 (m, 6H), 7.31 – 7.24 (m, 6H), 7.20 (ddd, *J* = 6.6, 4.0, 1.2 Hz, 3H), 2.25 (t, *J* = 7.5 Hz, 2H), 2.14 (t, *J* = 7.3 Hz, 2H), 1.55 – 1.44 (m, 2H), 1.44 – 1.34 (m, 2H), 1.34 – 1.22 (m, 2H). ^{13}C NMR (600 MHz, $CDCl_3$) δ = 179.02, 144.75, 129.56, 127.81, 126.53, 33.64, 31.49, 28.34, 28.00, 24.03. LC-MS $(M - H)^-$: calcd for $C_{25}H_{26}O_2S$ = 389.17 ; found 389.2



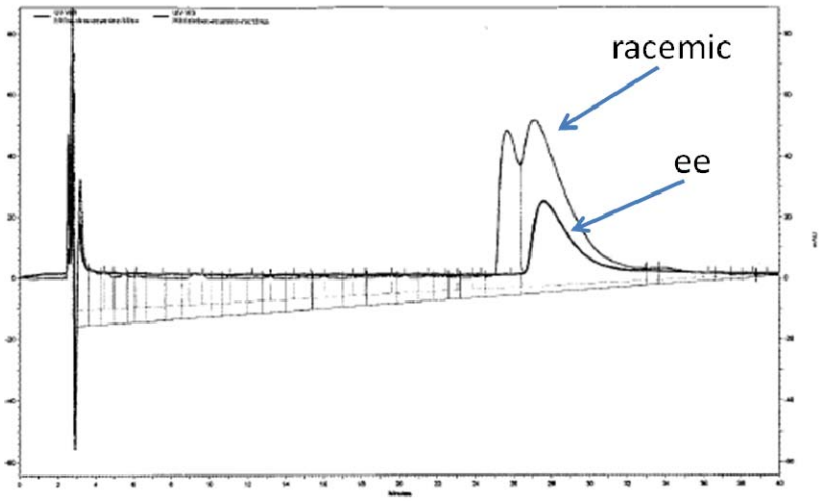
6-(tritylthio)hexyl methanesulfonate. A solution of commercially available 6-(tritylthio)hexan-1-ol (0.5mmol) and triethylamine (0.7mmol) in 1.3 mL dry DCM was cooled to -10°C. Methanesulfonyl chloride (0.6mmol) was added dropwise. After 2 hrs, the reaction was quenched by addition of water. The organic layer was washed 1X with water, dried and rotovaped. (94% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (d, J = 8.1 Hz, 6H), 7.29 (t, J 7.6 Hz, 6H), 7.22 (t, J = 7.2 Hz, 3H), 4.17 (t, J = 6.6 Hz, 2H), 2.97 (s,3H), 2.17 (t, J = 7.2 Hz, 2H), 1.67 (p, J = 6.6 Hz, 2H), 1.45 - 1.36 (m,2H), 1.32 - 1.22 (m, 4H). ^{13}C NMR (400 MHz, $CDCl_3$) δ = 144.89, 129.49, 127.74, 126.48, 69.86, 66.39, 52.44, 37.25, 31.67, 28.79, 28.28, 28.19, 24.89.



Scheme 2. Synthesis of MH1

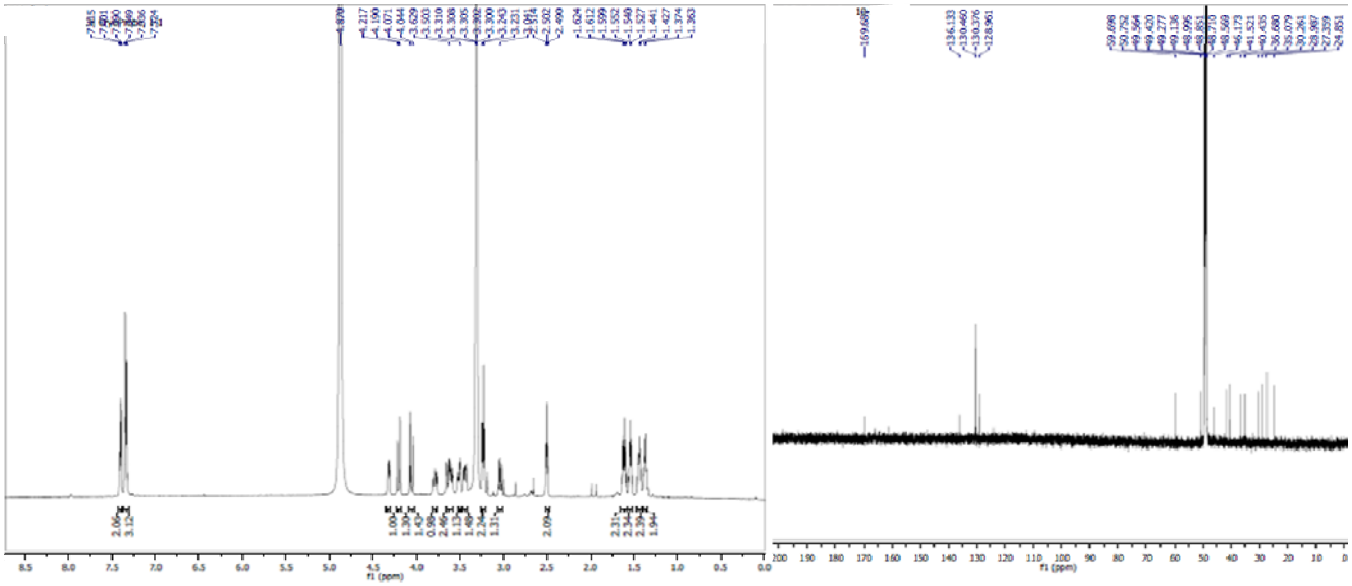


(±)-MH1 was synthesized by racemization of the pure (S)-enantiomer under treatment with base. (3S)-4-Boc-1-carboxymethyl-3-benzyl-piperazin-2-one was purchased from NeoMPS and used without further purification. Racemization conditions were first studied in a model system where (3S)-4-Boc-1-carboxymethyl-3-benzyl-piperazin-2-one was coupled to enantiomerically pure (S)-(-)-α-methylbenzylamine in order to introduce a second stereocenter. Treatment with 0.15 molar equivalents of DBU base in DMF at 80°C under argon for 19 hours allowed for racemization at the C3 position as evidenced by HPLC. Using an isocratic gradient at 15%B (acetonitrile) we were able to clearly observe both diastereomers.

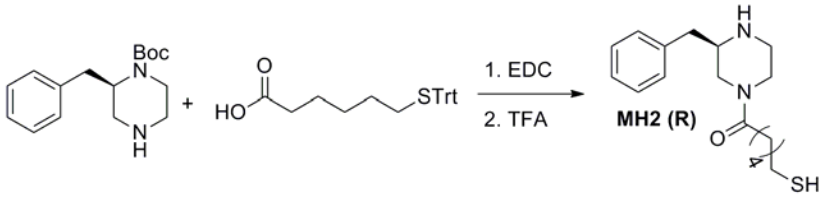


Synthesis of **MH1** proceeded as follows, (3S)-4-Boc-1-carboxymethyl-3-benzyl-piperazin-2-one (0.287mmol) was mixed with EDC (0.373mmol) and HOBT (0.373mmol) in 1.3mL DCM at 0°C. The mixture was stirred for 30min before a solution of 6-(tritylthio)hexan-1-amine (0.344mmol) in 0.1mL DCM was added. 4-methylmorpholine (0.574mmol) was subsequently added dropwise and the solution was stirred for 10hrs. The solution was then dropped in 6mL ethyl acetate and the organic layer washed 3X with saturated sodium bicarbonate, 3X with 10% citric acid and 1X with water. The organic layer was dried and rotovaped. The resulting oil was passed through a short plug of silica to obtain a chromatographically homogeneous material (Rf=0.35 on 80% EtOAc/hexane) which was carried forward without further purification. Racemization occurred by addition of 0.15 molar equivalents of DBU in DMF and heating to 80°C under argon for 19hours. DMF was removed under vacuum and the residue was dissolved in ethyl acetate and passed through a short plug of silica topped with acidic alumina. The resulting material was once again chromatographically homogeneous, as determined by TLC. Finally, global deprotection was achieved by addition of trifluoroacetic acid in a 1:1 dilution with DCM. Two drops of triisopropylsilane were added to scavenge the trityl groups. After 2 hrs, the mixture was rotovaped and purified by preparative HPLC. Method = 0-5min 25%B, to 28%B over 2 min, to 33%B over 23min, to 95%B over 5min, hold at 95% for 5min, re-equilibrate. Product retention time = 20min. Experimental Yield over 3 steps = 43%. ^{1}H NMR (600 MHz, MeOD) δ 7.43 – 7.38 (m, 2H), 7.37 – 7.31 (m, 3H), 4.35 – 4.31 (m, 1H), 4.20 (d, *J* = 16.2 Hz, 1H), 4.06 (d, *J* = 16.2 Hz, 1H), 3.79 (d, *J* = 1.8 Hz, 1H), 3.66 – 3.58 (m, 2H), 3.51 (d, *J* = 3.2 Hz, 1H), 3.46 – 3.42 (m, 1H),

3.24 (d, *J* = 7.0 Hz, 2H), 3.04 (dd, *J* = 15.0, 10.0 Hz, 1H), 2.50 (t, *J* = 7.1 Hz, 2H), 1.65 – 1.58 (m, 2H), 1.57 – 1.51 (m, 2H), 1.43 (d, *J* = 8.3 Hz, 2H), 1.37 (d, *J* = 7.0 Hz, 2H). ^{13}C NMR (600 MHz, MeOD) δ = 169.68, 136.13, 130.46, 130.38, 128.96, 59.70, 50.75, 49.56, 46.17, 41.52, 40.44, 36.68, 35.08, 30.26, 28.99, 27.36, 24.85. LRMS $(M + H)^+$: calcd for $C_{19}H_{29}N_3O_2S$ = 364.20 found = 364.2

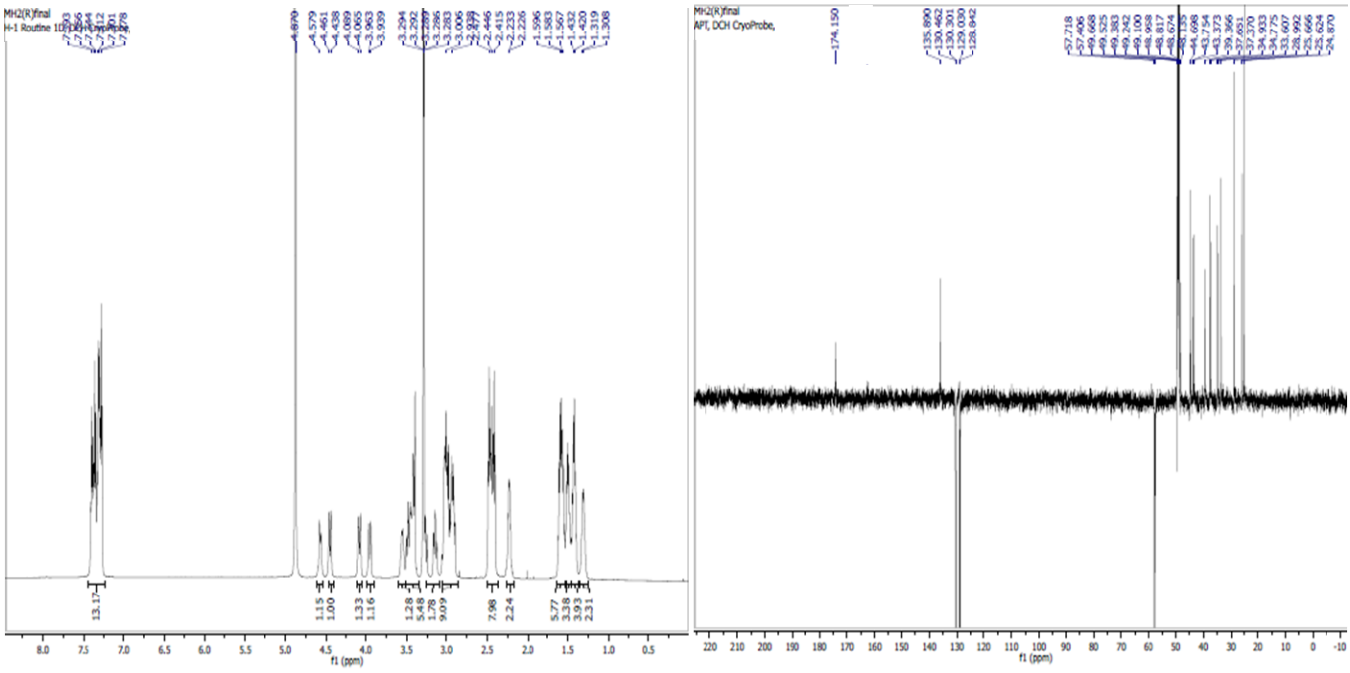


Scheme 3: Synthesis of MH2(R)

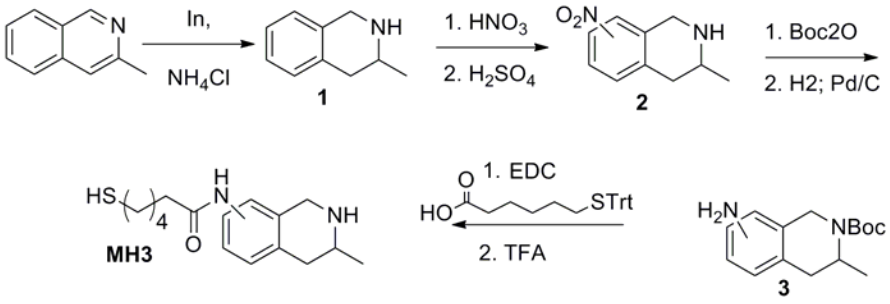


(R)-1-Boc-2-Benzylpiperazine was purchased from Synthonix and used without further purification. 0.153mmol of 6-(tritylthio)hexanoic acid were mixed with 0.2mmol EDC and 0.046mmol DMAP in 0.7mL DCM. 0.184mmol of (R)-1-Boc-2-Benzylpiperazine and 0.3mmol 4-methylmorpholine were added and the reaction mixture was allowed to stir under argon at room temperature for 3 hours. The mixture was then diluted with ethyl acetate and the organic solution was washed 3X with saturated sodium bicarbonate, 3X with 10% citric acid and 1X with water. The organic layer was then dried over sodium sulfate and rotovaped. The residue was then passed through a short plug of silica using 80% ethyl acetate/hexane as eluent. The crude product was used without further purification. Global deprotection was achieved by addition of trifluoroacetic acid in a 1:1 dilution with DCM. Two drops of triisopropylsilane were added to scavenge the trityl groups. After 2 hrs, the mixture was rotovaped and purified by preparative HPLC. Method = 0-5min 30%B, to 33%B over 2 min, to 40% B over 27min, to 95%B over 5 min, hold for 10min, requilibrate.

Product retention time = 13min. Experimental Yield over 2 steps = 56%. Observe rotamers on NMR. ^{1}H NMR (600 MHz, MeOD) δ 7.44 – 7.24 (m, 13H), 4.58 (s, 1H), 4.44 (s, 1H), 4.08 (d, *J* = 14.4 Hz, 1H), 3.95 (d, *J* = 14.5 Hz, 1H), 3.54 (dd, *J* = 15.6, 12.2 Hz, 1H), 3.50 – 3.34 (m, 5H), 3.16 (ddd, *J* = 29.6, 24.5, 15.1 Hz, 2H), 3.05 – 2.85 (m, 9H), 2.51 – 2.36 (m, 8H), 2.23 (d, *J* = 4.2 Hz, 2H), 1.64 – 1.54 (m, 6H), 1.53 – 1.46 (m, 3H), 1.43 (d, *J* = 7.1 Hz, 4H), 1.31 (d, *J* = 6.9 Hz, 2H).^{13}C NMR (600 MHz, MeOD) δ = 174.15, 135.89, 130.46, 130.30, 129.03, 128.84, 57.72, 57.41, 49.67, 48.14, 44.70, 43.75, 43.37, 39.37, 37.65, 37.37, 34.93, 34.78, 33.61, 28.99, 25.67, 25.62, 24.87. LRMS $(M + H)^{+}$: calcd for $C_{17}H_{26}N_2OS$ = 307.18, found 307.1

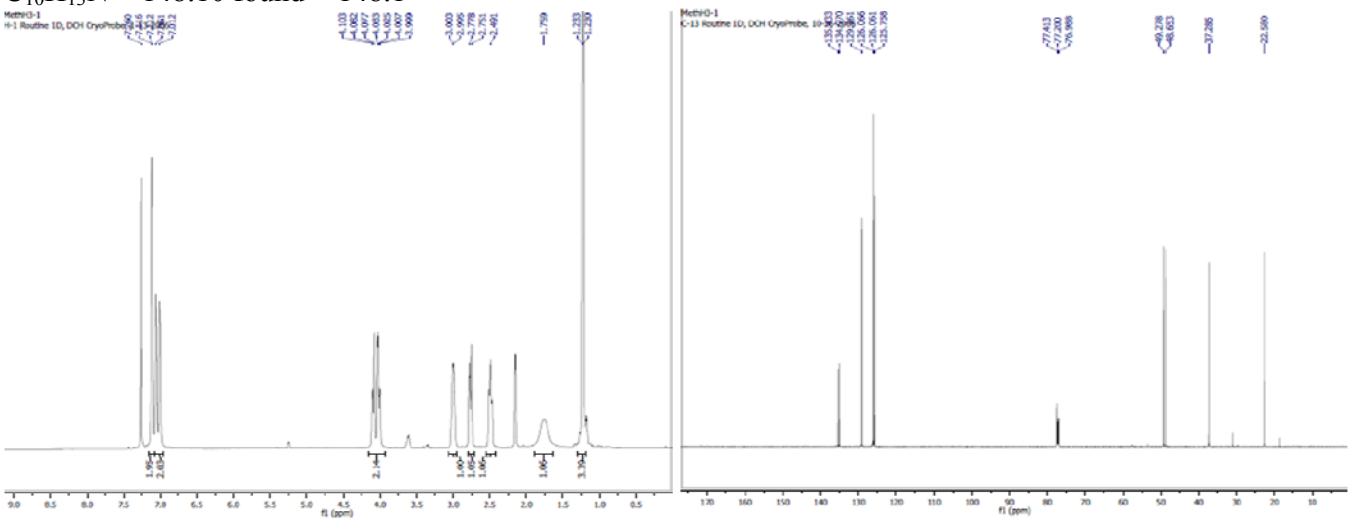


Scheme 4: Synthesis of MH3

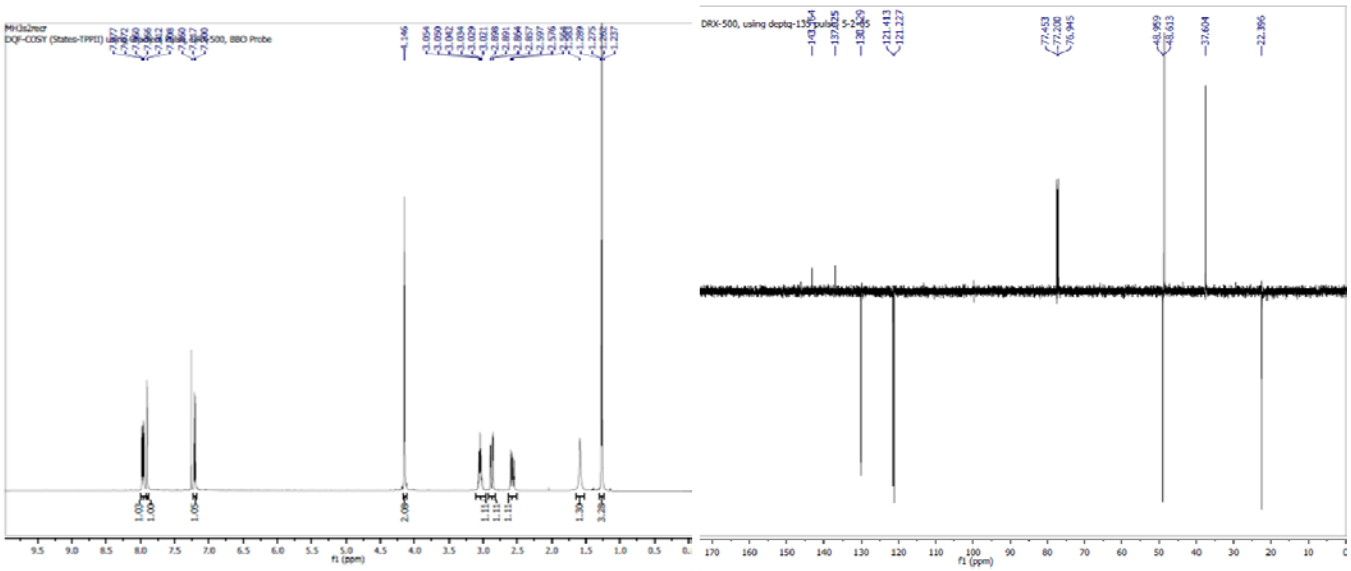


Synthesis of 3-methyl-1,2,3,4-tetrahydroisoquinoline (**1**). Commercially available 3-methylisoquinoline (3.49mmol) was dissolved in 18 mL ethanol. 5.5 mL of aqueous saturated ammonium chloride and 3.67g of indium powder were added. The mixture was stirred under reflux and monitored by TLC for 5 days. After 5 days, the reaction was cooled to room temperature and 75mL water were added. The pH of the reaction was adjusted to 9 and the mixture was filtered through a celite plug. The aqueous layer was extracted 3X with DCM and 3X with chloroform. Organics were dried over sodium sulfate and rotovaped to an oil. Experimental Yield = 74.2%. TLC conditions 5% MeOH/$CHCl_3$ Rf = 0.21 ^{1}H NMR (600 MHz, CDCl3) δ

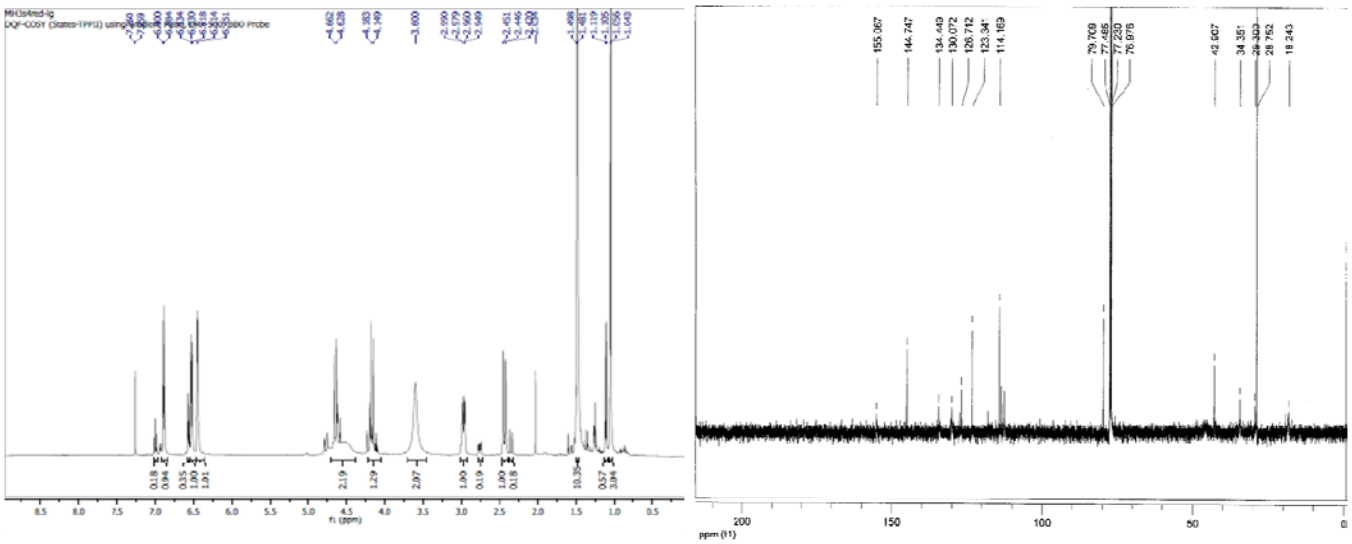
7.11 (d, J = 2.3 Hz, 2H), 7.04 (d, J = 29.1 Hz, 2H), 4.16 – 3.94 (m, 2H), 3.00 (d, J = 4.4 Hz, 1H), 2.76 (d, J = 16.2 Hz, 1H), 2.49 (m, 1H), 1.76 (broad s, 1H), 1.23 (d, J = 1.9 Hz, 3H). ^{13}C NMR (600 MHz, CDCl3) δ = 135.43, 134.97, 129.16, 126.07, 126.06, 125.76, 49.28, 48.65, 37.29, 22.58. LRMS $(M + H)^+$: calcd for $C_{10}H_{13}N$ = 148.10 found = 148.1



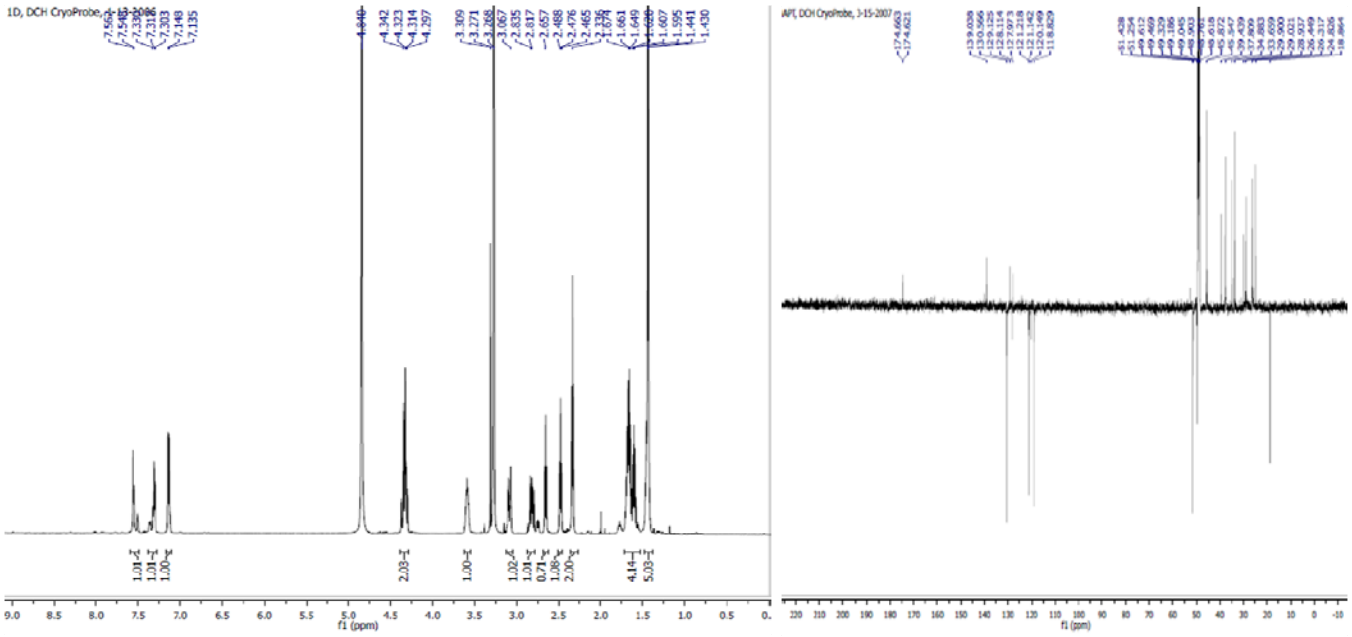
Synthesis of 3-methyl-6-nitro-1,2,3,4-tetrahydroisoquinoline and 3-methyl-7-nitro-1,2,3,4-tetrahydroisoquinoline (**2**). 0.646mmol of 1 were dissolved in 0.86mL of 1:1 t-butyl methyl ether:THF and cooled to 0°C. Concentrated nitric acid (15.8M solution, 0.646mmol) was slowly added and the mixture stirred for 1.5 hrs. The solid was filtered and rinsed with t-butyl methyl ether and put on a desiccant overnight. The solid was then added in portions to a solution of 0.823 mL DCM and 4.15mmol conc. H_2SO_4 at 0°C and stirred for 1.5 hrs. The solution was then slowly added to ~2mL of cold water and basified with NaOH to pH 10. The aqueous layer was extracted 3X with DCM. Organic layers were pooled together, dried over sodium sulfate and rotovaped to a yellow solid. The product gave a single spot on the TLC and was carried forward without further purification. NMR characterization of the crude showed creation of regioisomers at the 6 and 7 positions with a ratio of 5:1. Experimental Yield = 68.7%, TLC conditions 5% MeOH/$CHCl_3$ Rf = 0.37. Recrystallization of the major isomer with ethyl acetate gave the clean spectra detailed below. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (dd, J = 8.4, 2.3 Hz, 1H), 7.91 (d, J = 2.0 Hz, 1H), 7.21 (d, J = 8.4 Hz, 1H), 4.15 (s, 2H), 3.04 (dqd, J = 10.2, 6.3, 3.9 Hz, 1H), 2.88 (dd, J = 17.1, 3.7 Hz, 1H), 2.57 (dd, J = 16.5, 10.5 Hz, 1H), 1.58 (broad s, 1H), 1.30 – 1.23 (d, 3H). ^{13}C NMR (500 MHz, $CDCl_3$) δ = 143.16, 137.025, 130.13, 121.41, 121.23, 48.96, 48.61, 37.60, 22.40. LRMS $(M + H)^+$: calcd for $C_{10}H_{12}N_2O_2$ = 193.09 found = 193.0



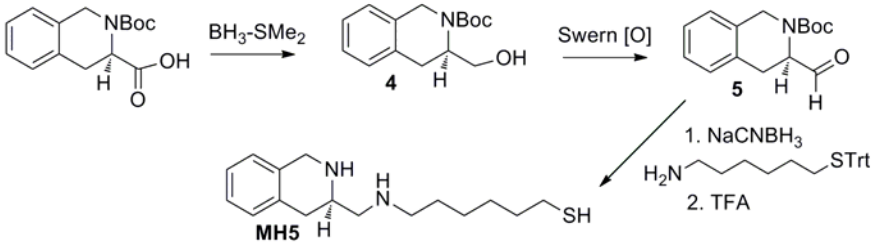
Synthesis of tert-butyl 6-amino-3-methyl-3,4-dihydroisoquinoline-2(1H)-carboxylate and tert-butyl 7-amino-3-methyl-3,4-dihydroisoquinoline-2(1H)-carboxylate (**3**). 1.41mmol of crude **2** was dissolved in dry DCM and 2.11mmol of triethylamine were added. The solution was cooled to 0^{o}C prior to addition of 1.69mmol Boc anhydride in portions. The reaction was monitored by TLC and left to stir for 10hrs. Water was then added to the reaction mixture and the solution cooled to 0^{o}C, the aqueous layer was acidified using 10% citric acid and extracted 3X with DCM. Organic layers were pooled together, dried over sodium sulfate and rotovaped. The residue was dissolved in 6.6mL MeOH, 63mg of 10% activated palladium on carbon were added along with a hydrogen balloon and the reaction monitored by TLC. After 4 days no more starting material was observed. Product was purified by column chromatography with 15% Ethyl acetate/hexane as the eluent. Experimental Yield= 80.6%. TLC conditions 20% EtOAc/hex Rf = 0.2 NMR characterization of showed creation of regioisomers at the 6 and 7 positions with a ratio of 5:1. ^{1}H NMR (500MHz, $CDCl_3$) δ 6.89 (d, *J* = 8.0 Hz, 1H), 6.52 (dd, *J* = 8.0, 2.2 Hz, 1H), 6.45 (s, 1H), 4.64 (d, *J* = 16.8 Hz, 2H), 4.17 (d, *J* = 16.8 Hz, 1H), 3.60 (broad s, 2H), 2.97 (dd, *J* = 15.3, 5.6 Hz, 1H), 2.43 (dd, *J* = 15.4, 2.3 Hz, 1H), 1.49 (d, *J* = 8.6 Hz, 9H), 1.05 (d, *J* = 6.7 Hz, 3H). 13C NMR (DRX-500, $CDCl_3$) δ = 155.07, 144.75, 134.45, 130.07, 126.71, 123.34, 114.17, 79.71, 42.91, 34.35, 29.30, 28.75, 18.24. LRMS $(M + H)^{+}$: calcd for $C_{15}H_{22}N_2O_2$ = 263.17 found = 263.0



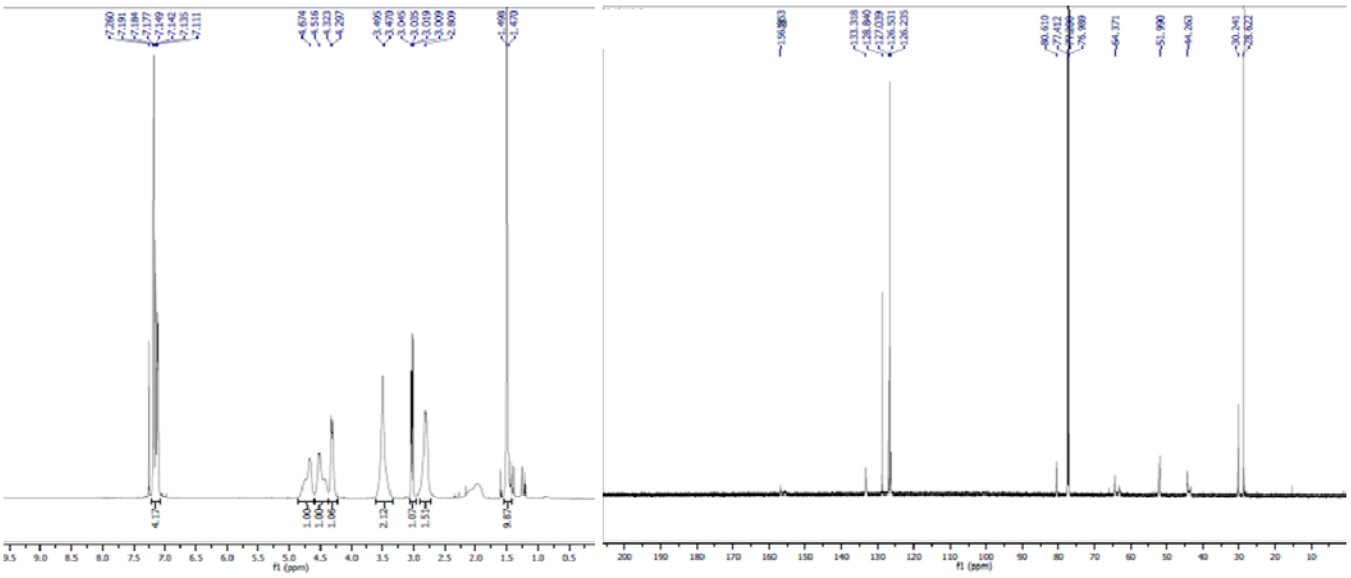
Synthesis of **MH3.** 0.256mmol of 6-(tritylthio)hexanoic acid, 0.332mmol EDC and 0.332mmol HOBT were dissolved in 1.1 mL DCM at 0°C. The mixture was stirred for 30 min before a solution of 0.307mmol of **3** in 0.15 mL DCM and 0.512mmol of 4-methyl morpholine were added. The mixture was then stirred for 10 hours before being dropped in 5mL ethyl acetate. Organic layer was washed 3X with saturated sodium bicarbonate, 2X with 10% citric acid and 1X with water. The organic layer was then dried over sodium sulfate and rotovaped. The material was passed through a short plug of silica and concentrated to yield a pale yellow oil. (TLC conditions 30% EtOAc/hex Rf = 0.45). The crude product was used without further purification. Global deprotection was achieved by addition of trifluoroacetic acid in a 1:1 dilution with DCM. Two drops of triisopropylsilane were added to scavenge the trityl groups. After 2 hrs, the mixture was rotovaped and purified by preparative HPLC. Method = 0-5min 27%B, to 30%B over 2 min, to 40% B over 27min, to 95%B over 5 min, hold for 10min, requilibrate. Product retention time = 14min. Experimental Yield over 2 steps = 35%. ^{1}H NMR (600 MHz, MeOD) δ 7.56 (d, *J* = 8.4 Hz, 1H), 7.32 (t, *J* = 8.2 Hz, 1H), 7.14 (d, *J* = 8.2 Hz, 1H), 4.32 (dd, *J* = 16.1, 10.9 Hz, 2H), 3.62 – 3.55 (m, 1H), 3.08 (dt, *J* = 16.9, 4.1 Hz, 1H), 2.83 (d, *J* = 10.9 Hz, 1H), 2.66 (t, 1H), 2.48 (t, *J* = 7.1 Hz, 1H), 2.34 (t, 2H), 1.72 – 1.55 (m, 4H), 1.44 (d, *J* = 6.5 Hz, 5H). ^{13}C NMR (600 MHz, MeOD) δ = 174.66, 174.62, 139.04, 130.57, 129.13, 128.11, 127.97, 121.22, 121.14, 120.15, 118.83, 51.43, 51.25, 49.61, 45.87, 45.55, 39.44, 37.81, 34.88, 33.66, 29.90, 29.02, 28.94, 26.45, 26.32, 24.83, 18.86. LRMS $(M + H)^+$: calcd for $C_{16}H_{24}N_2OS$= 293.16, found 293.6



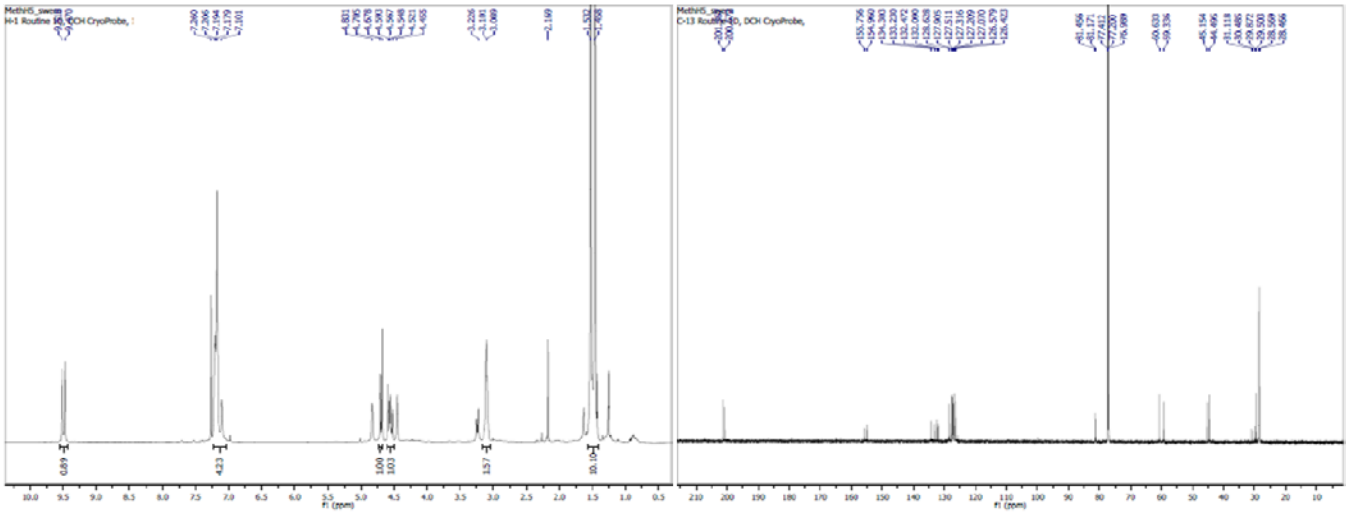
Scheme 5: Synthesis of MH5



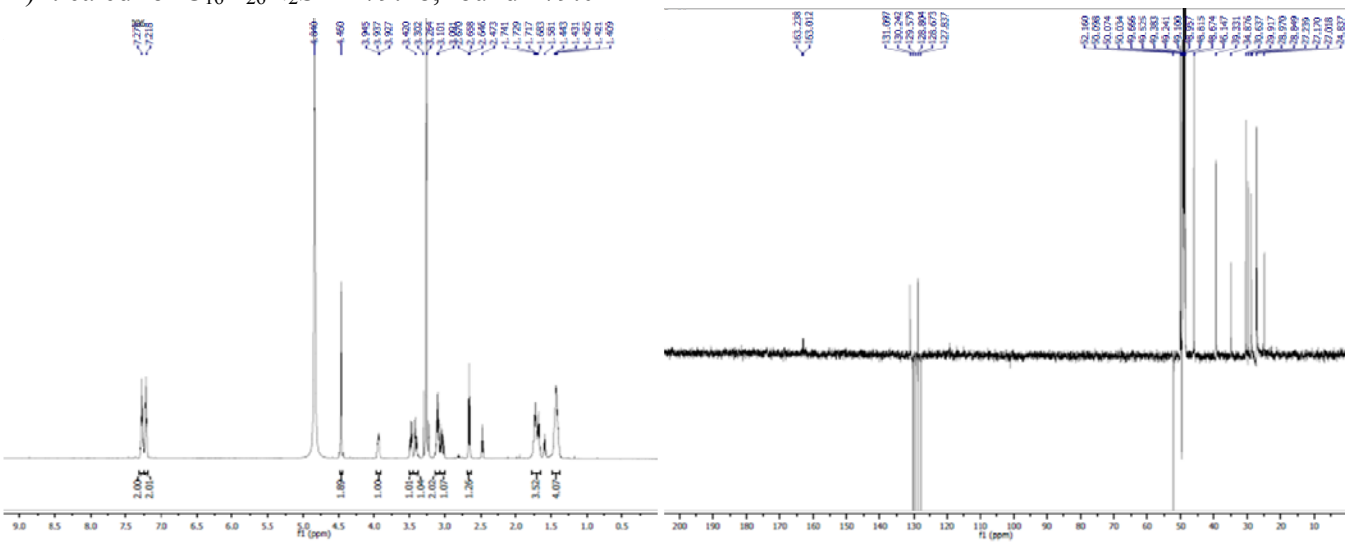
Synthesis of (R)-tert-butyl 3-(hydroxymethyl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (**4**). 0.36mmol of commercially available (R)-N-BOC-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Fluka) was dissolved in 1.38mL dry THF and cooled to 0°C. 0.43mmol of dimethylsulfide borane (10M solution in DMSO) was added dropwise and the reaction was stirred at room temperature for 10 hrs. The reaction was cooled to 0°C and quenched by the addition of saturated sodium bicarbonate. The aqueous layer was extracted 3X with DCM. Organics were combined, dried over sodium sulfate and rotovaped. The residue was passed through a short plug of silica topped with basic alumina using ethyl ether to give a single spot on TLC. Experimental Yield= 91%. TLC conditions 20% EtOAc/hex Rf = 0.16. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.21 – 7.07 (m, 4H), 4.67 (broad d, 1H), 4.52 (broad d, 1H), 4.31 (d, J = 15.4 Hz, 1H), 3.48 (broad s, 2H), 3.03 (dd, J = 15.9, 6.0 Hz, 1H), 2.81 (broad s, 1H), 1.48 (d, J = 16.9 Hz, 9H). ^{13}C NMR (600 MHz, $CDCl_3$) δ = 156.85, 133.32, 128.84, 127.04, 126.53, 80.61, 64.37, 51.99, 44.26, 30.24, 28.62. LRMS $(M + Na)^+$: calcd for $C_{15}H_{21}NO_3$= 286.14, found 286.4



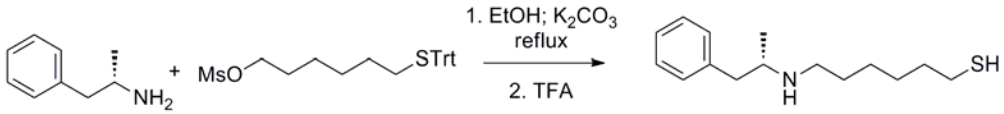
Synthesis of (R)-tert-butyl 3-formyl-3,4-dihydroisoquinoline-2(1H)-carboxylate (**5**). 0.626mmol of extra dry DMSO were dissolved in 1.6mL dry DCM and cooled to -78^{o}C. 0.459mmol of oxalyl chloride were added drop wise and mixture was stirred for 30min. 0.208mmol of **4** were dissolved in 0.834mL dry DCM and added drop wise. Mixture was again stirred for 30min. 0.898mmol of triethylamine was then added drop wise and the reaction was allowed to gradually warm up to room temperature. Reaction was monitored by TLC and deemed complete after 8 hours. Once reaction was complete, it was immediately quenched by addition of ethyl ether. Organic layer was washed 1X with saturated ammonium chloride, 1X saturated sodium bicarbonate, 1X brine and 1X water. The organic layer was dried over sodium sulfate and rotovaped to an oil. Experimental Yield= 80%. TLC conditions 20% EtOAc/hex Rf = 0.45. ^{1}H NMR (600 MHz, CDCl3) δ 9.49 (d, J = 28.9 Hz, 1H), 7.17 (dd, J = 36.0, 27.3 Hz, 4H), 4.69 (d, J = 16.2 Hz, 1H), 4.56 (dd, J = 27.6, 16.1 Hz, 1H), 3.10 (d, J = 6.9 Hz, 2H), 1.50 (d, J = 44.4 Hz, 9H). ^{13}C NMR (600 MHz, CDCl3) δ = 201.37, 200.67, 155.76, 154.96, 134.30, 133.23, 132.47, 132.09, 128.63, 127.90, 127.51, 127.32, 127.21, 127.07, 126.58, 126.42, 81.46, 81.17, 60.63, 59.34, 45.15, 44.50, 31.12, 30.49, 29.87, 29.50, 28.57, 28.47, 1.19.



Synthesis of **MH5**. 0.398mmol of 6-(tritylthio)hexan-1-amine were dissolved in 1mL methanol. 0.398mmol of sodium cyanoborohydride were added along with a drop of bromocresol green pH indicator. 0.018mL of glacial acidic acid were added followed by 0.306mmol of **5** in 1mL of methanol. The mixture was stirred and 0.011mL of glacial acidic acid were further added to maintain acidic pH of solution. After 30min, the methanol was evaporated under vacuum and water was added. The aqueous layer was extracted 3X with ethyl ether. Aqueous layer was basified with potassium hydroxide, saturated with brine and extracted 5X with ether which was subsequently dried over sodium sulfate and rotovaped to an oil. The residue was dissolved in a 1:1 mixture of TFA and DCM to achieve global deprotection. Two drops of TIS were added as a scavenger and the reaction was stirred for 2 hrs. After 2 hrs, the mixture was rotovaped and purified by preparative HPLC. Method = 0-5min 15%B, to 20%B over 2 min, to 30% B over 27min, to 95%B over 5 min, hold for 10min, re-equilibrate. Product retention time = 20min. Experimental Yield over 2 steps = 39%. ^{1}H NMR (600 MHz, MeOD) δ 7.27 (m, 2H), 7.22 (m, 2H), 4.46 (s, 2H), 3.97 – 3.91 (m, 1H), 3.47 (m, 1H), 3.42 (m, 1H), 3.10 (m, 2H), 3.03 (dd, J = 17.3, 11.2 Hz, 1H), 2.66 (t, J = 7.2 Hz, 1H), 1.72 (m, 4H), 1.48 – 1.37 (m, 4H). ^{13}C NMR (600MHz, MeOD) δ = 131.10, 130.24, 129.58, 128.80, 128.67, 127.84, 52.16, 50.10, 50.07, 50.03, 49.67, 46.15, 39.33, 34.88, 30.64, 29.92, 28.97, 28.85, 27.24, 27.17, 27.02, 24.84 LRMS (M + H)$^{+}$: calcd for $C_{16}H_{26}N_2S$ = 279.18, found 279.0

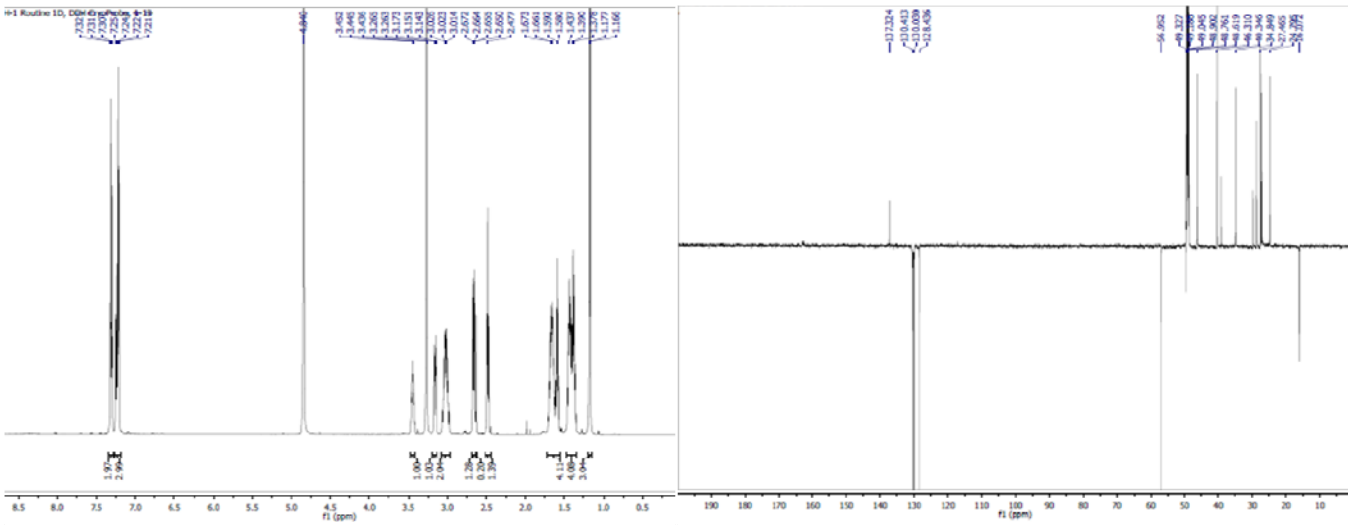


Scheme 6: Synthesis of MH6

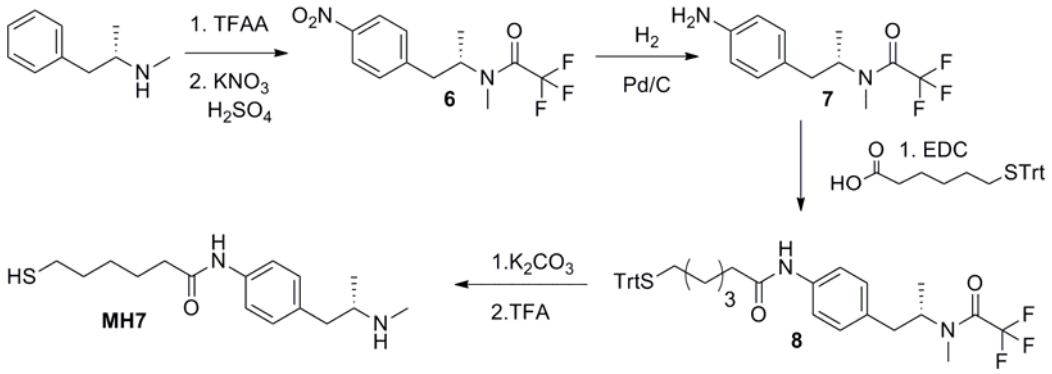


Synthesis of **MH6**. 0.126mmol of d-amphetamine sulfate was dissolved in 0.9mL of ethanol. 0.164mmol of 6-(tritylthio)hexyl methanesulfonate and 0.38mmol of potassium carbonate were added and the solution was heated to reflux overnight. After 14 hrs, ethanol was removed under vacuum and the residue dissolved in ethyl acetate. The organic layer was washed 2X with water, dried over sodium sulfate and rotovaped. The residue was then dissolved in 5mL of 10% TFA/DCM, and two drops of TIS were added as a trityl scavenger. The solution was stirred for 1 hr and then rotovaped, and the residue was purified by prep HPLC. Method = 0-5min 35%B, to 40%B over 2 min, to 44% B over 27min, to 95%B over 5 min, hold for 10min, requilibrate. Product retention time = 17min. Experimental Yield over 2 steps = 50%. ^{1}H NMR (600 MHz,

MeOD) δ 7.31 (m, *J* = 7.5 Hz, 2H), 7.23 (m, 3H), 3.48 – 3.41 (m, 1H), 3.16 (dd, *J* = 13.2, 4.4 Hz, 1H), 3.07 – 2.97 (m, 2H), 2.66 (dd, *J* = 9.3, 3.9 Hz, 1H), 2.48 (t, *J* = 7.6 Hz, 1H), 1.73 – 1.55 (m, 4H), 1.47 – 1.35 (m, 4H), 1.17 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (600MHz, MeOD) δ = 137.32, 130.41, 130.03, 128.44, 56.95, 49.61, 46.31, 40.35, 39.22, 34.85, 29.88, 28.92, 28.82, 27.47, 27.26, 27.11, 24.79, 16.07. LRMS $(M + H)^+$: calcd for $C_{15}H_{25}NS$ = 252.17, found 252.2

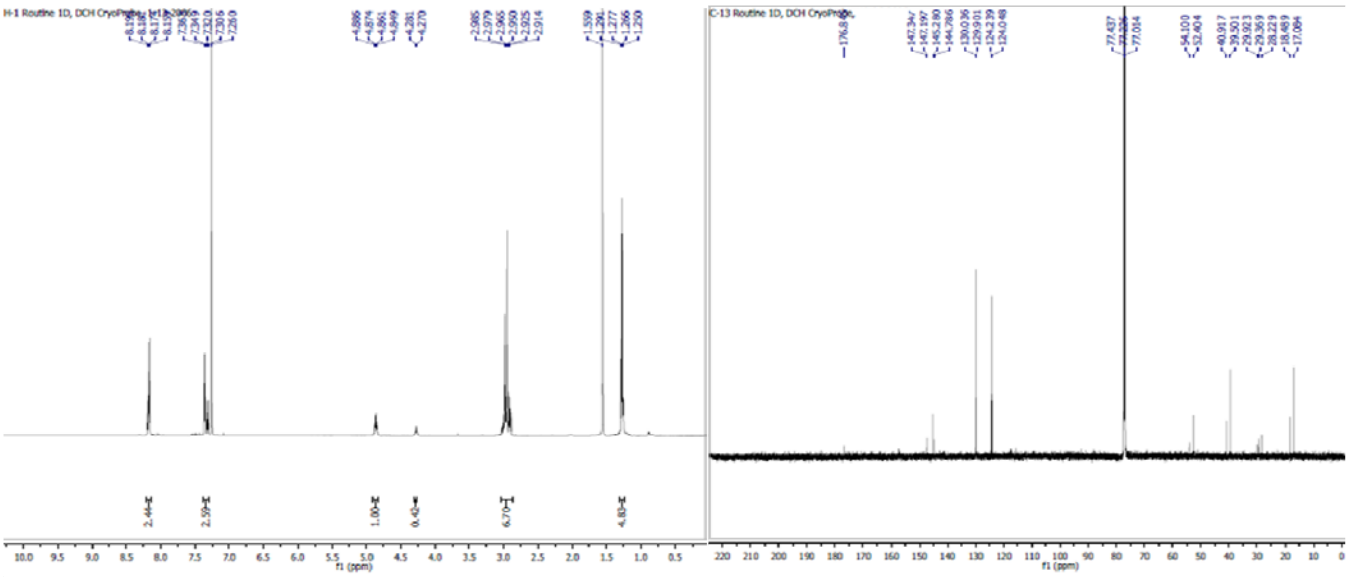


Scheme 7: Synthesis of MH7

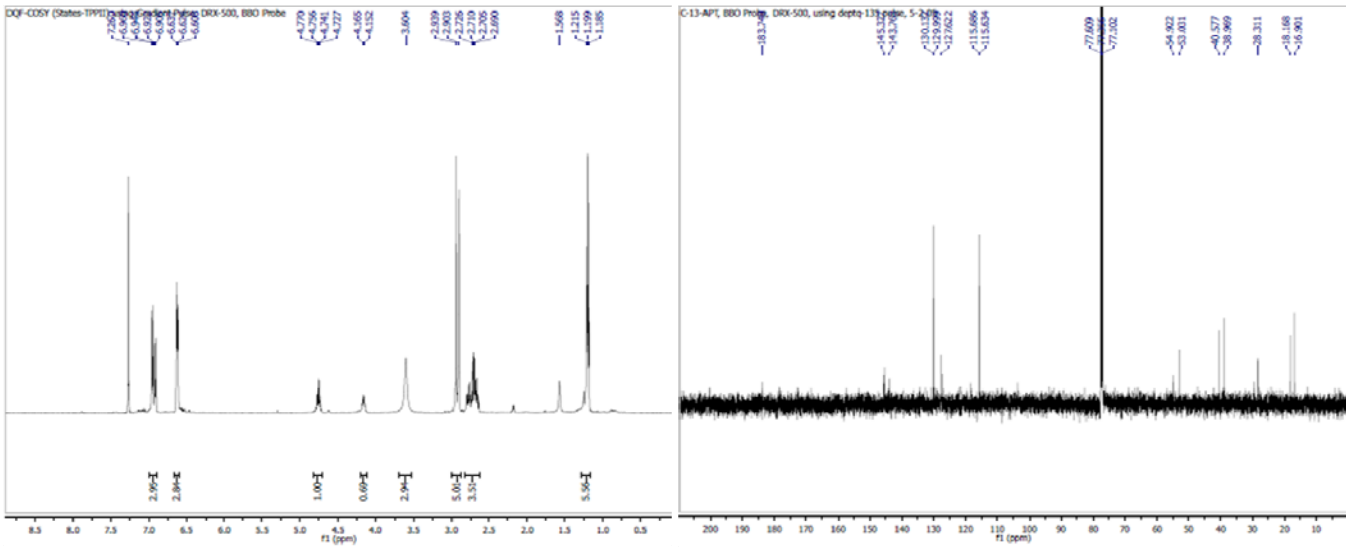


Synthesis of (S)-2,2,2-trifluoro-N-methyl-N-(1-(4-nitrophenyl)propan-2-yl)acetamide (**6**). 0.269mmol of d-methamphetamine hydrochloride were dissolved in 1.8mL DCM. 0.538mmol of triethylamine were added and mixture was cooled to 0°C. 0.323mmol of trifluoroacetic anhydride were added dropwise and mixture was stirred at room temperature for 2.5 hrs. The solvent was removed under vacuum and residue dissolved in ethyl ether which incurred in the formation of a precipitate. The solution was passed through a short plug of silica topped with basic alumina. The filtrates were combined and rotovaped to yield a single spot by TLC which was carried forward without further purification. The residue was dissolved in 0.51mL DCM and added dropwise to a chilled solution consisting of 3.26mmol of potassium nitrate and 3.09mmol of concentrated sulfuric acid previously dissolved in 1.63mL of DCM. The slurry was stirred overnight at room temperature. Aqueous solution of sodium sulfate was added slowly to quench the reaction. The two layers were separated and the organic layer was washed twice more with aqueous sodium sulfate. Organics were

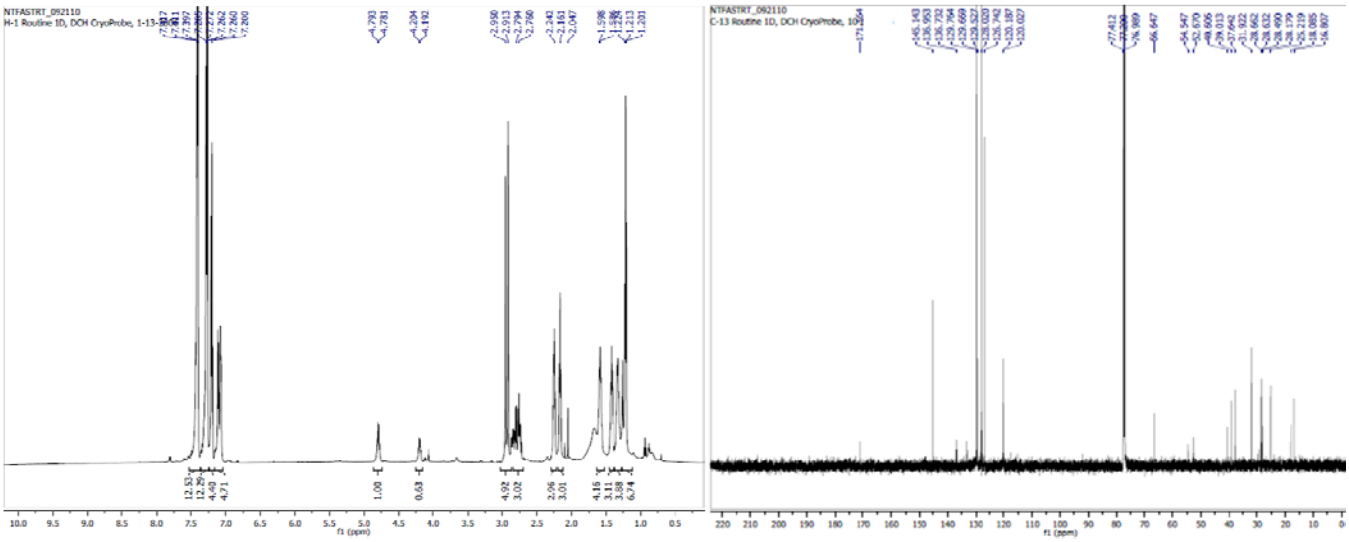
combined, dried with anhydrous sodium sulfate and rotovaped. The product was purified using silica chromatography with 10% Ethyl acetate/hexane as the eluent. The major product of reaction was para-substituted as evidenced by NMR. Experimental Yield= 54% (para-isomer). TLC conditions 15% EtOAc/hex Rf = 0.16. Observe rotamers 1:0.5 by NMR, shifts and integration given for main rotamer. ^{1}HNMR (600 MHz, $CDCl_3$) δ 8.17 (d, *J* = 8.7 Hz, 2H), 7.36 (d, *J* = 8.6 Hz, 2H), 4.87 (dq, *J* = 13.8, 6.9 Hz, 1H), 3.00 – 2.90 (m, 5H), 1.29 – 1.25 (m, 3H). ^{13}C NMR (600 MHz, CDCl3) δ = 176.84, 147.35, 147.20, 145.28, 144.79, 130.04, 129.90, 124.24, 124.05, 54.10, 52.40, 40.92, 39.50, 29.92, 29.37, 28.23, 18.49, 17.08. LRMS $(M + H)^+$: calcd for $C_{12}H_{13}F_3N_2O_3$= 291.09, found 291.4



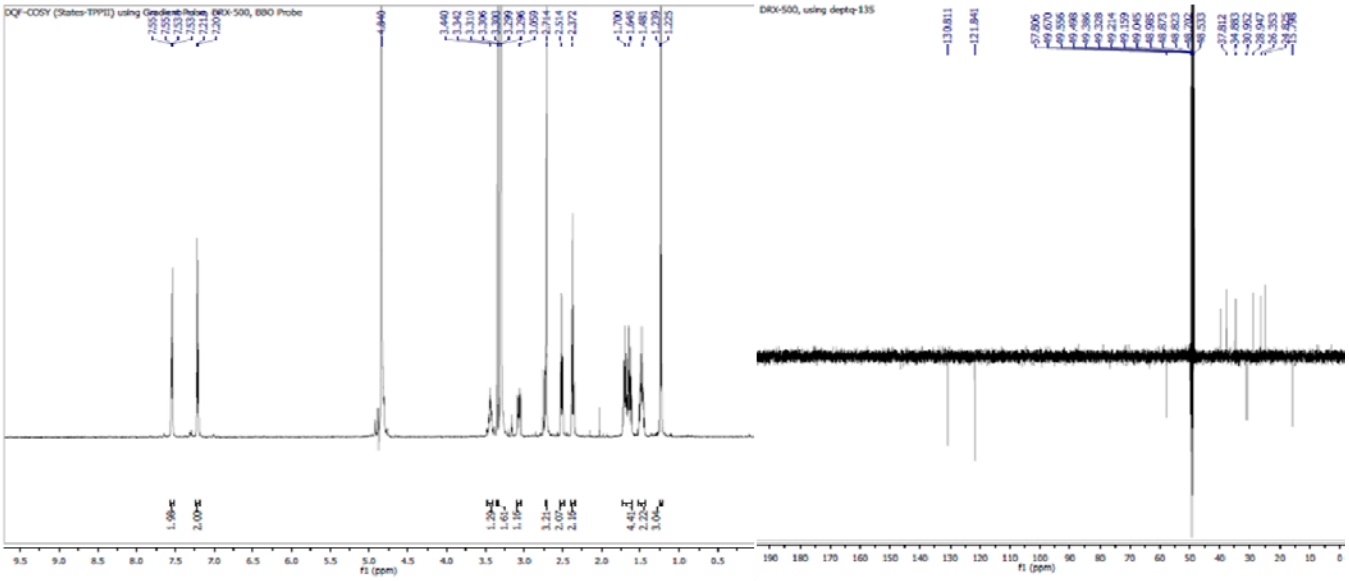
Synthesis of (S)-N-(1-(4-aminophenyl)propan-2-yl)-2,2,2-trifluoro-N-methylacetamide (**7**). 0.174mmol of **6** were dissolved in MeOH and 12mg of 10% activated palladium on carbon were added along with a hydrogen balloon. The reaction was stirred for 2 hrs before being filtered on a celite plug. The plug was washed with methanol, filtrates were combined and solvent was removed under vacuum. The residue was purified using silica chromatography using 20% Ethyl acetate/hexane as eluent. Experimental Yield= 97%. TLC conditions 30% EtOAc/hex Rf = 0.3. Observe rotamers by NMR 1: 0.7, shifts and integration given for main rotamer. ^{1}HNMR (500 MHz, $CDCl_3$)δ 6.95 (d, *J* = 8.2 Hz, 2H), 6.66 – 6.59 (m, 2H), 4.82 – 4.70 (m, 1H), 3.60 (broad s, 2H), 2.92 (s, 3H), 2.83 – 2.62 (m, 2H), 1.29 – 1.17 (m, 3H). ^{13}CNMR (500 MHz, $CDCl_3$) δ = 183.74, 145.33, 143.76, 130.13, 130.00, 127.62, 115.69, 115.63, 54.92, 53.03, 40.58, 38.97, 28.31, 18.17, 16.90. LRMS $(M + H)^+$: calcd for $C_{12}H_{15}F_3N_2O$= 261.11, found 261.1



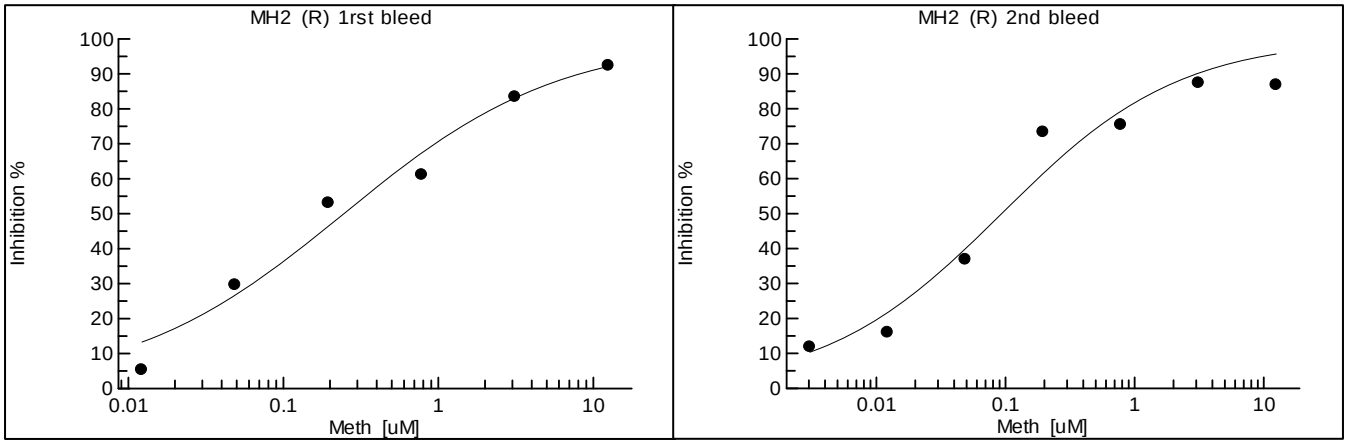
Synthesis of (S)-N-(4-(2-(2,2,2-trifluoro-N-methylacetamido)propyl)phenyl)-6-(tritylthio)hexanamide (8). 0.172mmol of 7 were dissolved in 0.368mL DCM. 0.157 mmol of 6-(tritylthio)hexanoic acid, 0.204mmol of EDC, and 0.047mmol of DMAP were added and the mixture stirred. 0.314mmol of 4-methyl morpholine were added and reaction was stirred for 4hrs. 3mL of ethyl acetate were added and washed 2X with saturated sodium bicarbonate, 4X with 10% citric acid and 1X with water. The organic layer was dried over sodium sulfate and rotovaped to a yellow oil which corresponded to single spot on TLC. Experimental Yield= 93%. TLC conditions 20% EtOAc/hex Rf = 0.13. Observe rotamers 1: 0.63 ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41 (dd, J = 12.7, 8.7 Hz, 13H), 7.27 (dd, J = 10.5, 4.3 Hz, 12H), 7.20 (t, J = 7.6 Hz, 4H), 7.16 - 7.04 (m, 5H), 4.85 - 4.73 (m, 1H), 2.91 (s, J = 25.0 Hz, 5H), 2.87 - 2.70 (m, 3H), 2.25 (dd, J = 12.1, 7.2 Hz, 3H), 2.16 (t, J = 6.9 Hz, 3H), 1.64 - 1.53 (m, 4H), 1.45 - 1.38 (m, 3H), 1.37 - 1.27 (m, 4H), 1.21 (t, J = 7.0 Hz, 7H). ^{13}C NMR (600 MHz, $CDCl_3$) δ = 171.16, 145.14, 136.95, 136.73, 129.76, 129.67, 129.53, 128.02, 126.74, 120.19, 120.03, 66.65, 54.55, 52.67, 40.61, 39.01, 37.64, 31.92, 28.66, 28.63, 28.49, 28.18, 25.22, 18.08, 16.81. LRMS $(M + Na)^+$: calcd for $C_{37}H_{39}F_3N_2O_2S$= 655.25, found 655.3

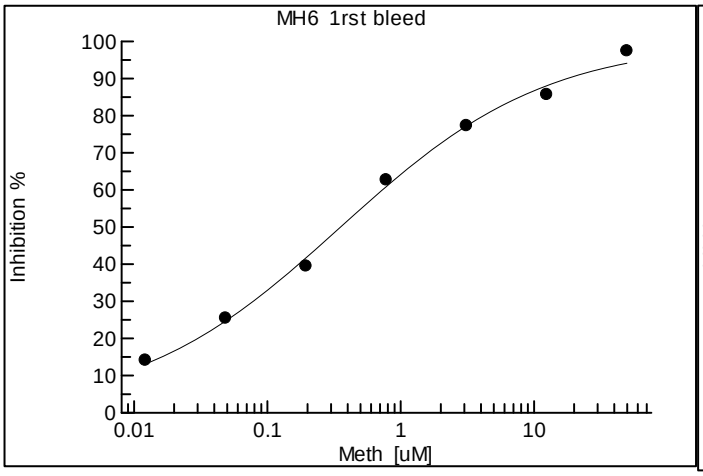


Synthesis of **MH7**. 0.145mmol of **8** were dissolved in 1mL of methanol and drops of water. 0.436mmol of potassium carbonate were added and the mixture stirred at room temperature for 50hrs. Methanol was removed under vacuum and the residue was dropped in water. The aqueous layer was basified and extracted 3X with DCM. The organic layers were combined, dried over sodium sulfate and rotovaped. The residue was dissolved in 6mL of 10% TFA/DCM and drops of TIS were added to scavenge the trityl groups. The solution was stirred for 1 hr and then rotovaped and purified by prep HPLC. Method = 0-5min 25%B, to 30%B over 2 min, to 40% B over 27min, to 95%B over 5 min, hold for 10min, requilibrate. Product retention time = 19min. Experimental Yield over 2 steps = 40%. ^{1}H NMR (500 MHz, MeOD) δ 7.54 (dd, *J* = 8.6, 2.4 Hz, 2H), 7.21 (d, *J* = 8.5 Hz, 2H), 3.48 – 3.40 (m, 1H), 3.34 (s, 1H), 3.07 (dd, *J* = 13.7, 5.3 Hz, 1H), 2.71 (s, 3H), 2.51 (t, *J* = 7.6 Hz, 2H), 2.37 (t, *J* = 7.5 Hz, 2H), 1.74 – 1.61 (m, 4H), 1.52 – 1.45 (m, 2H), 1.23 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (500 MHz, MeOD) δ = 130.81, 121.84, 57.81, 39.71, 37.81, 34.88, 30.95, 28.95, 26.35, 24.83, 15.80**.** LRMS $(M + H)^{+}$: calcd for $C_{16}H_{26}N_2OS$ = 295.18, found 295.2



Immunologic assays.

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60
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80
90
100
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1
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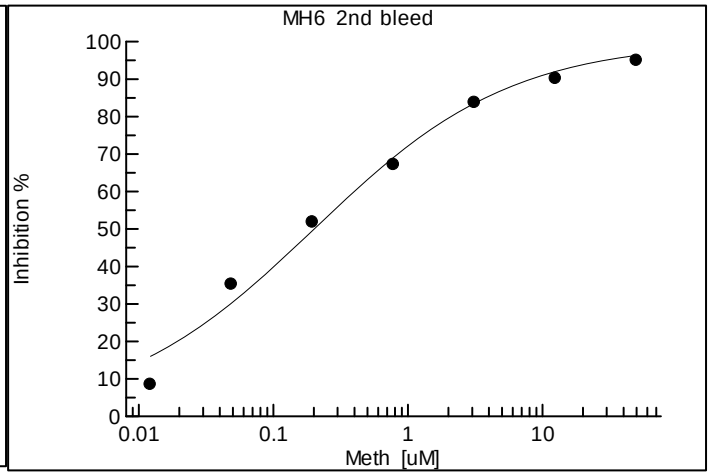
MH6 2nd bleed
Inhibition %
Meth [uM]
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80
90
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1
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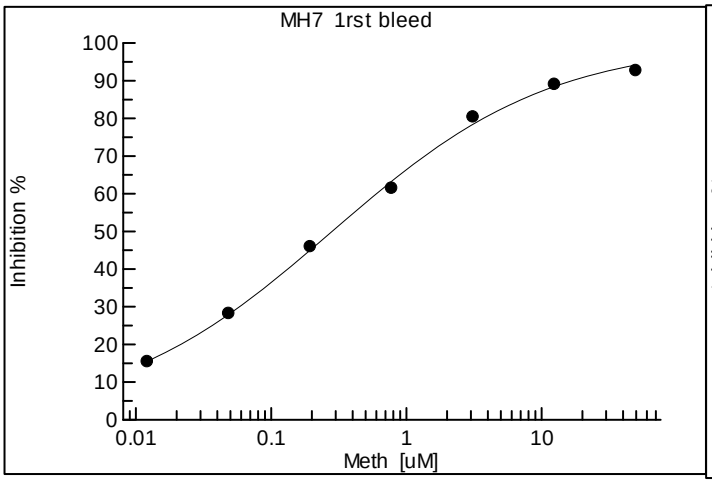
MH7 1rst bleed
Inhibition %
Meth [uM]
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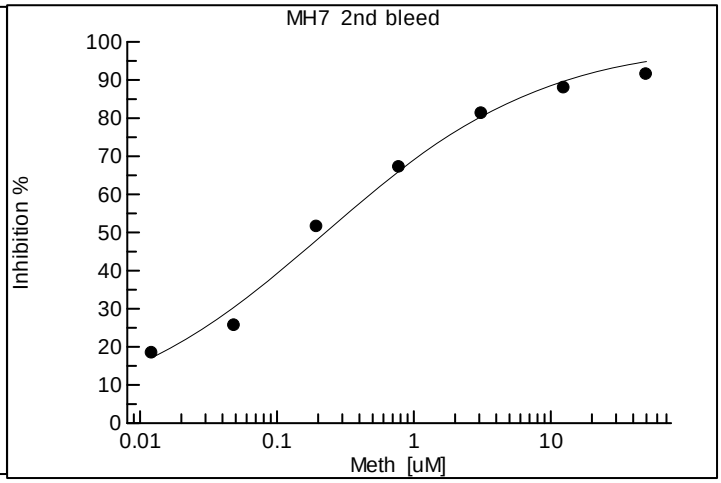
MH7 2nd bleed
Inhibition %
Meth [uM]
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0.01
0.1
1
10