Supporting information

Broadly neutralizing synthetic cannabinoid vaccines

Mingliang Lin, Jinny Claire Lee, Steven Blake, Beverly Ellis, Lisa M. Eubanks, and Kim D. Janda*

Department of Chemistry, Department of Immunology and Microbial Science, The Skaggs Institute for Chemical Biology, and The Worm Institute for Research and Medicine (WIRM), The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037 (USA)

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Chemistry method

Synthesis procedure

General procedure 1 (NaH mediated alkylation): To an Ar protected stirring solution of sodium hydride (60% in mineral oil, 5eq) in 8mL dry DMF, was treated with indole or indazole dissolved in DMF dropwise at 0°C. After stirred for 30min, bromo-reactant (1.2eq) was treated dropwise. Then the mixture was allowed to react at room temperature for 1h. After quenched with water and extracted into EtOAc by adding saturated sodium bicarbonate or 1M HCl, the combined organic phase was washed with water followed by brine wash and dried over $MgSO_4$. If not otherwise mentioned, flash chromatography was performed to afford the alkylated product.

General procedure 2 (Me_2AlCl mediated acylation): To an Ar protected stirring solution of indole derivatives in 8mL dry DCM, was treated with dimethyl aluminum chloride (1M in hexanes, 1.2eq) dropwise at 0°C. After stirred for 30min, acyl chloride (1.2eq) was treated dropwise. Then the mixture was allowed to react at room temperature for 1h. After quenched with water and extracted into EtOAc by adding saturated sodium bicarbonate or 1M HCl, the combined organic phase was washed with water followed by brine wash and dried over $MgSO_4$. Solvent was directly removed via rotary evaporation to afford the acylated product.

General procedure 3 (TFAA mediated acylation): To an Ar protected stirring solution of indole derivatives in 4mL TFA, was treated with carboxylic acid (1eq), TFAA (2eq). Mixture was allowed to react at room temperature for 4h. After quenched with saturated sodium bicarbonate and extracted into EtOAc by adding saturated sodium bicarbonate or 1M HCl, the combined organic phase was washed with water followed by brine wash and dried over $MgSO_4$. Solvent was directly removed via rotary evaporation to afford the acylated product.

General procedure 4 (EDC/HOBT mediated amidation): To an Ar protected stirring solution of carboxylic acid dissolved in 8mL dry DMF, was treated with amine (1.2eq), EDC (1.2eq), HOBT (1.2eq), DIPEA (5eq). Mixture was allowed to react at room temperature overnight. After quenched with water and extracted into EtOAc by adding saturated sodium bicarbonate or 1M HCl, the combined organic phase was washed with water followed by brine wash and dried over $MgSO_4$. If not otherwise mentioned, reversed phase chromatography was performed, after removal of CH3CN in vacuo, the residual water phase was lyophilized or extracted into EtOAc to afford the amidation product.

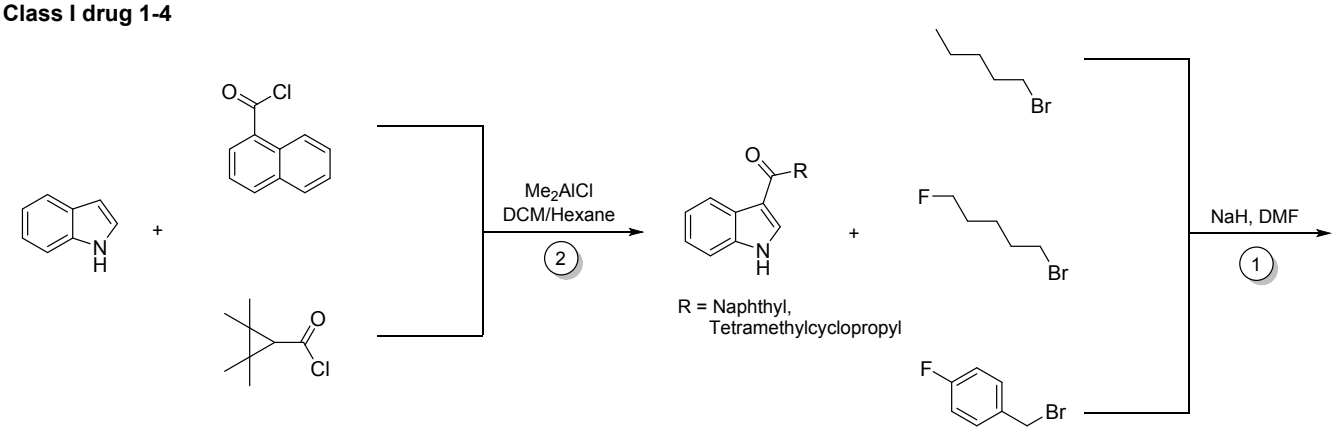
General procedure 5 (Pd/C catalyzed hydrogenation): To an Ar protected two-neck flask containing a stirring solution of either olefin or benzyl ester in 12mL MeOH, was treated with 10%Pd/C. Evacuated the air until the solution was bubbling, refilled the system with H_2 and repeated twice. Mixture was allowed to react at room temperature for 1h. After filtered through a pad of Celite, the filtrate was directly removed via rotary evaporation to afford the hydrogenated or deprotected product.

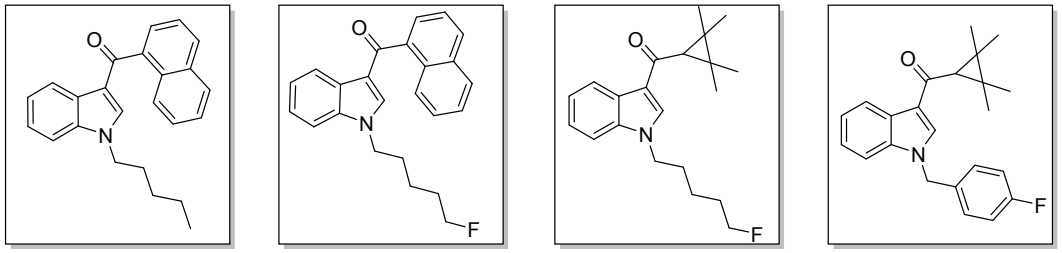
General procedure 6 (EDC/DMAP mediated esterification): To an Ar protected stirring solution of carboxylic acid derivatives in 8mL dry DCM, was treated with alcohol phenol or 8-hydroxyquinoline (2eq), EDC (1.2eq), DMAP (0.3eq). Mixture was allowed to react at room temperature for 4h. After quenched with water and extracted into DCM by adding saturated sodium bicarbonate or 1M HCl, the combined organic phase was washed with water followed by brine wash and dried over $MgSO_4$. If not otherwise mentioned, flash chromatography was performed to afford the ester product.

General procedure 7 (Cesium carbonate mediated substitution): To an Ar protected stirring solution of phenol or hydroxyindole in 6mL dry DMSO was treated with cesium carbonate (3eq) and bromo-reactant (1.2eq) dropwise. Mixture was allowed to react at room temperature overnight. After quenched with water and extracted into EtOAc by adding saturated sodium bicarbonate or 1M HCl, the combined organic phase was washed with water followed by brine wash and dried over $MgSO_4$. Solvent was directly removed via rotary evaporation to afford the ether product.

General procedure was labeled as ①, ②, ③, ④, ⑤, ⑥, ⑦ in the synthesis schemes.

Class I drug 1-4





JWH-018 AM-2201 XLR-11 FUB-144

Given that fluoroalkane and dimethyl aluminum chloride led to side reaction, sequence was chosen as general procedure 2 first using indole (117mg, 1mmol) and 1-naphthoyl chloride (228mg, 1.2mmol) or 2,2,3,3-tetramethylcyclopropane-1-carbonyl chloride (192mg, 1.2mmol) as acylation source, followed by general procedure 1 without purification using 1-bromo-pentyl (181mg, 1.2mmol), 1-bromo-5-fluoropentane (203mg, 1.2mmol) or 4-fluorobenzyl bromide (227mg, 1.2mmol) as alkylation source to afford the final product JWH-018 and AM-2201 as yellow solid, XLR-11 and FUB-144 as off white solid, total yield 70%-80%.

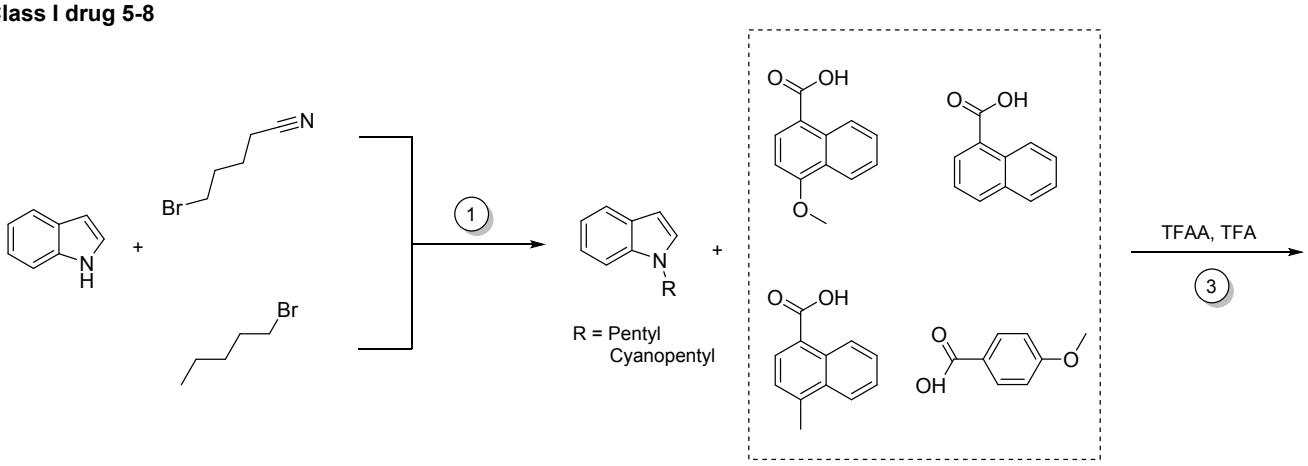
JWH-018 ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.54 – 8.46 (m, 1H), 8.20 (dd, J = 8.3, 1.3 Hz, 1H), 7.97 (dt, J = 8.4, 1.1 Hz, 1H), 7.94 – 7.88 (m, 1H), 7.67 (dd, J = 6.9, 1.3 Hz, 1H), 7.57 – 7.45 (m, 3H), 7.41 – 7.35 (m, 4H), 4.06 (t, J = 7.3 Hz, 2H), 1.81 (p, J = 7.3 Hz, 2H), 1.36 – 1.20 (m, 4H), 0.86 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 192.16, 139.25, 138.07, 137.17, 133.87, 130.93, 130.08, 128.29, 127.13, 126.87, 126.41, 126.13, 125.96, 124.68, 123.72, 123.06, 122.98, 117.67, 110.11, 47.29, 29.60, 29.02, 22.29, 13.99. HRMS (ESI) m/z: $[M+H]^+$ calculated=342.1852; found=342.1859.

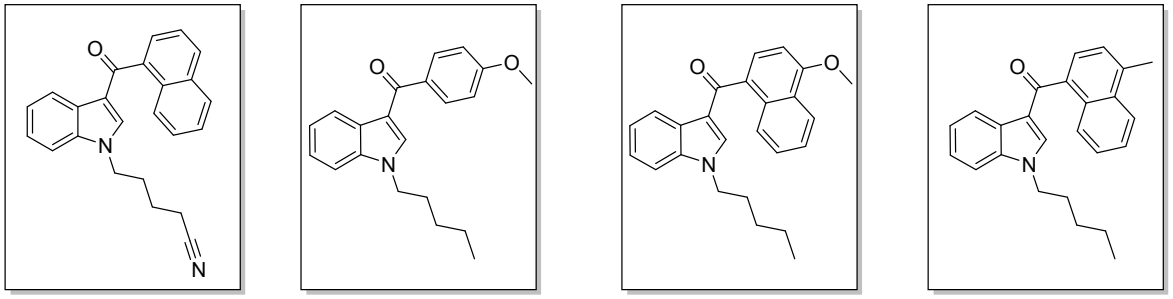
AM-2201 ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.53 – 8.46 (m, 1H), 8.19 (d, J = 8.8 Hz, 1H), 7.97 (d, J = 8.2 Hz, 1H), 7.92 (dd, J = 8.1, 1.4 Hz, 1H), 7.66 (dd, J = 6.9, 1.3 Hz, 1H), 7.57 – 7.44 (m, 3H), 7.43 – 7.34 (m, 4H), 4.43 (t, J = 5.9 Hz, 1H), 4.34 (t, J = 5.9 Hz, 1H), 4.10 (t, J = 7.2 Hz, 2H), 1.87 (p, J = 7.3 Hz, 2H), 1.73 – 1.61 (m, 2H), 1.46 – 1.36 (m, 2H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 192.16, 139.18, 137.95, 137.11, 133.88, 130.92, 130.14, 128.31, 127.14, 126.90, 126.44, 126.11, 125.98, 124.70, 123.83, 123.14, 123.06, 117.83, 110.02, 84.34, 83.03, 47.15, 30.05, 29.89, 29.60, 22.93, 22.89. HRMS (ESI) m/z: $[M+H]^+$ calculated=360.1758; found=360.1766.

XLR-11 ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.43 – 8.38 (m, 1H), 7.67 (s, 1H), 7.37 – 7.26 (m, 3H), 4.49 (t, J = 5.9 Hz, 1H), 4.39 (t, J = 5.9 Hz, 1H), 4.18 (t, J = 7.2 Hz, 2H), 1.94 (q, J = 7.2 Hz, 3H), 1.79 – 1.69 (m, 2H), 1.53 – 1.46 (m, 2H), 1.35 (s, 6H), 1.31 (s, 6H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 194.81, 136.70, 133.54, 126.55, 123.11, 122.90, 122.28, 119.89, 109.69, 84.46, 83.14, 47.01, 41.83, 31.87, 30.16, 30.00, 29.78, 24.21, 23.05, 23.01, 17.16. HRMS (ESI) m/z: $[M+H]^+$ calculated=330.2228; found=330.2236.

FUB-144 ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.42 (dt, J = 7.7, 1.2 Hz, 1H), 7.68 (s, 1H), 7.27 – 7.23 (m, 3H), 7.16 – 7.10 (m, 2H), 7.06 – 6.99 (m, 2H), 5.33 (s, 2H), 1.93 (s, 1H), 1.35 (s, 6H), 1.29 (s, 6H).^{13}C NMR (126 MHz, Chloroform-*d*) δ 194.84, 163.58, 161.61, 136.90, 133.67, 132.01, 131.99, 128.78, 128.72, 126.65, 123.42, 122.93, 122.54, 120.48, 116.19, 116.02, 110.02, 50.13, 41.91, 32.03, 24.18, 17.15. HRMS (ESI) m/z: $[M+H]^+$ calculated=350.1915; found=350.1919.

Class I drug 5-8





AM-2232 RCS-4 JWH-081 JWH-122

General procedure 1 was applied as first step using indole (117mg, 1mmol) and 1-bromo-pentyl (181mg, 1.2mmol) or 5-bromopentanenitrile (194mg, 1.2mmol) as alkylation source, followed by general procedure 3 without purification using 4-methoxy-1-naphthoic acid (242mg, 1.2mmol) or 4-methyl-1-naphthoic acid (223mg, 1.2mmol) or 1-naphthoyl chloride (206mg, 1.2mmol) or 4-methoxybenzoyl chloride (182mg, 1.2mmol) as acylation source to afford the final product JWH-081 and RCS-4 as white solid, AM-2232 and JWH-122 as yellow solid, total yield 70%-80%.

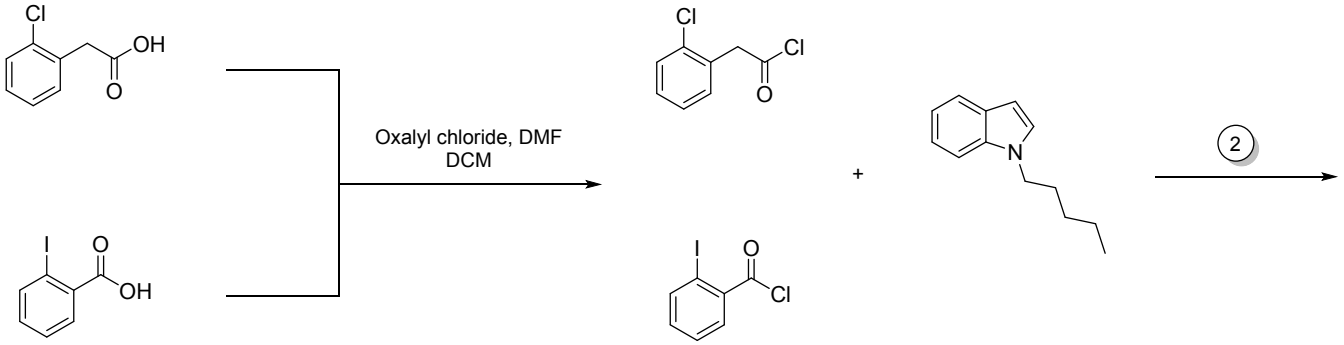
AM-2232 ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.50 (ddt, J = 6.7, 3.2, 1.8 Hz, 1H), 8.18 (d, J = 8.9 Hz, 1H), 7.97 (d, J = 8.2 Hz, 1H), 7.91 (d, J = 8.1 Hz, 1H), 7.65 (dd, J = 7.0, 1.2 Hz, 1H), 7.57 – 7.43 (m, 3H), 7.38 (d, J = 3.3 Hz, 3H), 7.33 (s, 1H), 4.14 (t, J = 6.9 Hz, 2H), 2.30 (t, J = 7.0 Hz, 2H), 2.03 – 1.93 (m, 2H), 1.64 – 1.55 (m, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 192.15, 139.01, 137.62, 136.98, 133.89, 130.87, 130.25, 128.36, 127.12, 126.95, 126.49, 126.02, 125.99, 124.71, 124.07, 123.25, 123.23, 118.94, 118.13, 109.84, 46.36, 29.00, 22.86, 17.02. HRMS (ESI) m/z: $[M+H]^+$ calculated=353.1648; found=353.1655.

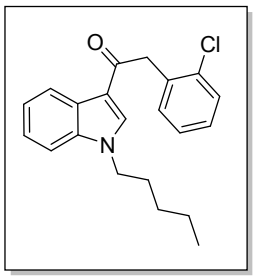
RCS-4 ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.41 – 8.34 (m, 1H), 7.88 – 7.82 (m, 2H), 7.58 (s, 1H), 7.41 – 7.37 (m, 1H), 7.35 – 7.29 (m, 2H), 7.02 – 6.96 (m, 2H), 4.16 (t, J = 7.2 Hz, 2H), 3.89 (s, 3H), 1.88 (p, J = 7.4 Hz, 2H), 1.33 (tdd, J = 12.0, 6.7, 2.2 Hz, 4H), 0.89 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 189.94, 162.33, 136.91, 136.32, 133.64, 131.09, 127.62, 123.48, 122.89, 122.52, 115.75, 113.66, 109.93, 55.57, 47.26, 29.73, 29.13, 22.37, 14.04. HRMS (ESI) m/z: $[M+H]^+$ calculated=322.1801; found=322.1805.

JWH-081 ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.46 (dt, J = 7.1, 3.5 Hz, 1H), 8.39 – 8.27 (m, 2H), 7.67 (d, J = 7.9 Hz, 1H), 7.55 – 7.47 (m, 2H), 7.46 – 7.31 (m, 4H), 6.84 (d, J = 7.9 Hz, 1H), 4.13 – 4.05 (m, 5H), 1.82 (p, J = 7.3 Hz, 2H), 1.37 – 1.22 (m, 4H), 0.86 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 191.89, 157.19, 137.56, 137.13, 132.32, 131.64, 127.94, 127.49, 127.35, 126.00, 125.86, 125.79, 123.56, 123.03, 122.76, 122.18, 117.90, 110.04, 102.31, 55.82, 47.25, 29.66, 29.07, 22.33, 14.01. HRMS (ESI) m/z: $[M+H]^+$ calculated=372.1958; found=372.1968.

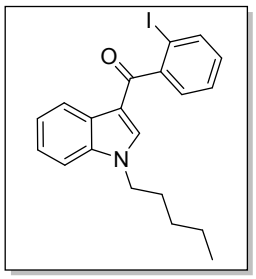
JWH-122 ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.52 – 8.45 (m, 1H), 8.25 (dd, J = 8.4, 1.3 Hz, 1H), 8.08 (dd, J = 8.4, 1.2 Hz, 1H), 7.59 – 7.52 (m, 2H), 7.48 (ddd, J = 8.3, 6.8, 1.3 Hz, 1H), 7.42 – 7.32 (m, 5H), 4.06 (t, J = 7.3 Hz, 2H), 2.78 (s, 3H), 1.81 (p, J = 7.3 Hz, 2H), 1.33 – 1.22 (m, 4H), 0.86 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 192.37, 137.97, 137.71, 137.16, 136.79, 132.96, 131.04, 127.19, 126.77, 126.52, 126.27, 125.97, 125.41, 124.34, 123.65, 123.08, 122.90, 117.83, 110.07, 47.28, 29.63, 29.05, 22.31, 19.96, 14.00. HRMS (ESI) m/z: $[M+H]^+$ calculated=356.2009; found=356.2016.

Class I drug 9-10





JWH-203



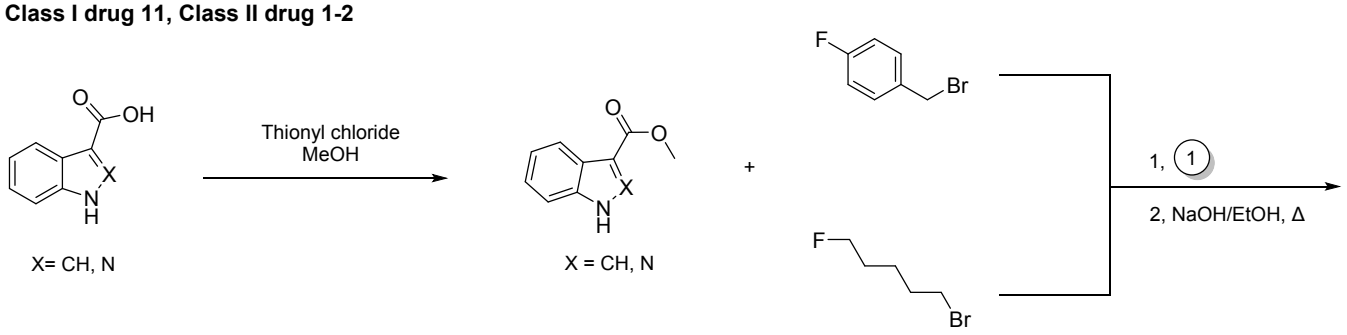
AM-694

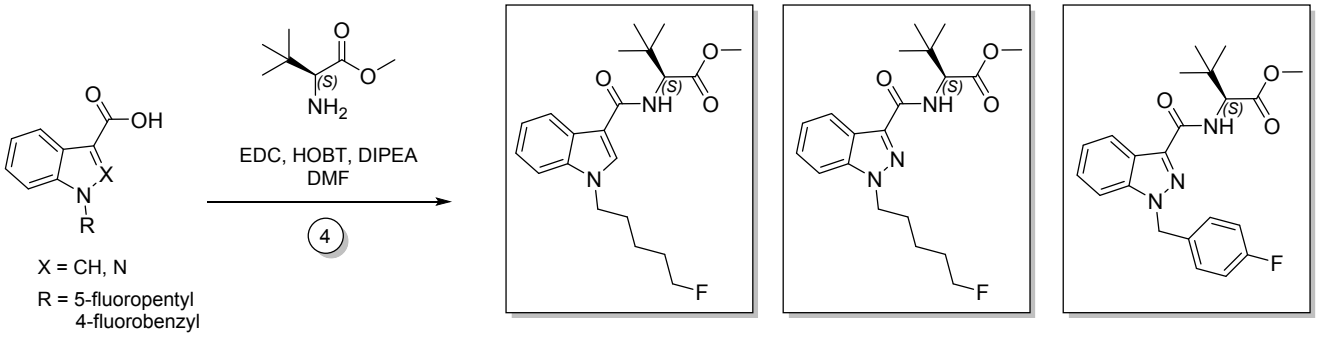
To an Ar protected stirring solution of 2-(2-chlorophenyl)acetic acid (171mg, 1mmol) or 2-iodobenzoic acid (248mg, 1mmol) and catalyst amount of DMF in 8mL DCM was treated with oxalyl chloride (254mg, 2mmol, 2eq). Mixture was allowed to react at room temperature for 1h. Solvent was directly removed via rotary evaporation three times using DCM to get rid of excessive thionyl chloride to produce the acyl chloride product as yellow liquid. Followed by general procedure 2 without purification using 1-pentyl-1*H*-indole (156mg, 0.83mmol) as acylation acceptor to afford the final product JWH-203 as tan solid, AM-694 as white solid, total yield 62%, 66%.

JWH-203 ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.44 – 8.37 (m, 1H), 7.88 (s, 1H), 7.42 – 7.27 (m, 5H), 7.25 – 7.17 (m, 2H), 4.32 (s, 2H), 4.16 (t, J = 7.2 Hz, 2H), 1.89 (p, J = 7.3 Hz, 2H), 1.42 – 1.27 (m, 4H), 0.90 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 191.50, 136.93, 134.97, 134.30, 134.14, 131.81, 129.56, 128.36, 127.05, 126.84, 123.48, 122.94, 122.79, 116.19, 109.97, 47.32, 44.23, 29.64, 29.12, 22.39, 14.05. HRMS (ESI) m/z: $[M+H]^+$ calculated=340.1463; found=340.1471.

AM-694 ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.35 – 8.30 (m, 1H), 7.93 (d, J = 8.0 Hz, 1H), 7.47 – 7.29 (m, 6H), 7.16 (tt, J = 7.7, 1.8 Hz, 1H), 4.14 – 4.07 (m, 2H), 1.85 (p, J = 7.2 Hz, 2H), 1.32 (ddt, J = 13.7, 10.0, 6.2 Hz, 4H), 0.88 (td, J = 7.0, 1.5 Hz, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 191.48, 146.58, 139.79, 138.22, 137.28, 130.61, 128.22, 127.87, 126.89, 123.82, 123.10, 122.96, 115.52, 110.17, 92.78, 47.42, 29.62, 29.06, 22.33, 14.04. HRMS (ESI) m/z: $[M+H]^+$ calculated=418.0662; found=418.0666.

Class I drug 11, Class II drug 1-2





5F-MDMB-PICA 5F-MDMB-PINACA MDMB-FUBINACA

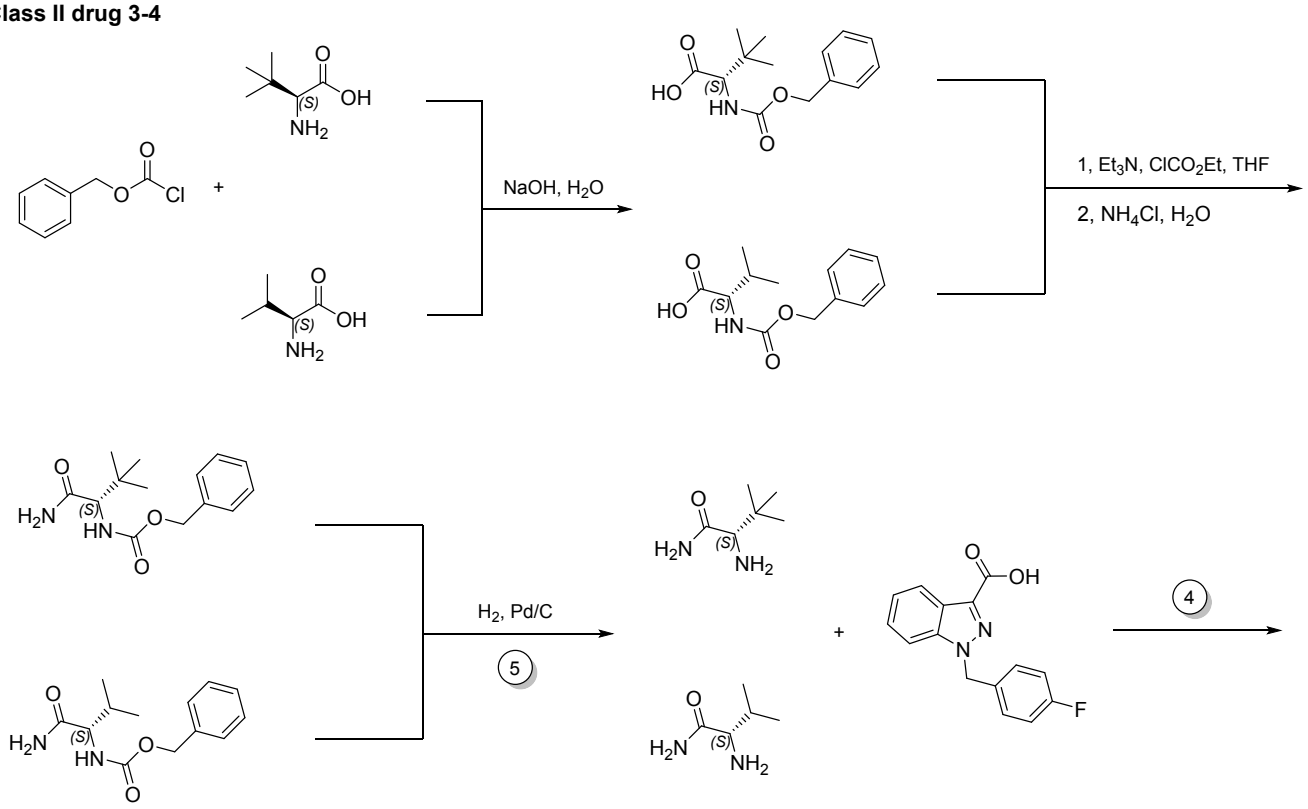
To an Ar protected stirring solution of 1*H*-indole-3-carboxylic acid (161mg, 1mmol) or indazole-3-carboxylic acid (162mg, 1mmol) in 10mL dry MeOH, was treated with thionyl chloride (238mg, 2mmol, 2eq) dropwise. Mixture was allowed to react at room temperature overnight. Solvent was directly removed via rotary evaporation and residual traces of acid was removed by repeated azeotropic entrainment with Et_2O to afford the hydrochloride salt. To a flask containing hydrochloride salt in 6mL DCM, was treated with saturated sodium bicarbonate solution and stirred vigorously for 15min. The basicity of the aqueous layer was tested on pH test paper. The aqueous layer was extracted into DCM and dry over $MgSO_4$. Solvent was removed via rotary evaporation to afford the methylated product as white or red solid, yield 85%. General procedure 1 was applied using 1-bromo-5-fluoropentane (192mg, 1.02mmol) or 4-fluorobenzyl bromide (171mg, 1.02mmol) as alkylation source followed by sodium hydroxide mediated hydrolysis in ethanol under heating condition to afford the N-alkylated indole or indazole 3-carboxylic acid. General procedure 4 was applied as final step using L-tert-Leucine methyl ester (148mg, 1.02mmol) as coupling source to afford the final product 5F-MDMB-PICA, 5F-MDMB-PINACA and MDMB-FUBINACA as white solid, yield 55%. (Intermediate characterization was in agreement with literature[1])

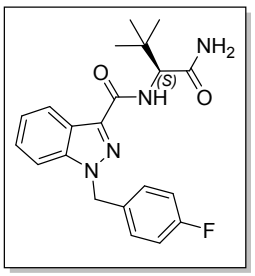
5F-MDMB-PICA ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.01 – 7.94 (m, 1H), 7.79 (s, 1H), 7.42 – 7.37 (m, 1H), 7.32 – 7.27 (m, 2H), 6.56 (d, J = 9.4 Hz, 1H), 4.78 (d, J = 9.3 Hz, 1H), 4.47 (t, J = 5.9 Hz, 1H), 4.37 (t, J = 5.9 Hz, 1H), 4.17 (t, J = 7.1 Hz, 2H), 3.77 (s, 3H), 1.93 (dt, J = 15.1, 7.3 Hz, 2H), 1.79 – 1.65 (m, 2H), 1.51 – 1.41 (m, 2H), 1.09 (s, 9H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 172.80, 164.97, 136.75, 132.26, 125.38, 122.73, 121.90, 120.08, 110.58, 110.50, 84.44, 83.13, 59.95, 52.00, 46.90, 35.23, 30.16, 30.00, 29.77, 26.90, 22.99, 22.95. HRMS (ESI) m/z: $[M+H]^+$ calculated=377.2235; found=377.2238.

5F-MDMB-PINACA ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.35 (dt, J = 8.2, 1.0 Hz, 1H), 7.55 (d, J = 9.7 Hz, 1H), 7.44 – 7.37 (m, 2H), 7.26 (ddd, J = 8.0, 4.7, 3.0 Hz, 1H), 4.73 (d, J = 9.7 Hz, 1H), 4.52 – 4.35 (m, 4H), 3.76 (s, 3H), 2.02 (p, J = 7.3 Hz, 2H), 1.82 – 1.68 (m, 2H), 1.53 – 1.43 (m, 2H), 1.09 (s, 9H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 172.32, 162.45, 140.97, 136.90, 126.88, 123.06, 122.98, 122.77, 109.27, 84.51, 83.20, 59.63, 51.94, 49.32, 35.18, 30.11, 29.95, 29.45, 26.82, 22.83, 22.79. HRMS (ESI) m/z: $[M+H]^+$ calculated=378.2187; found=378.2191.

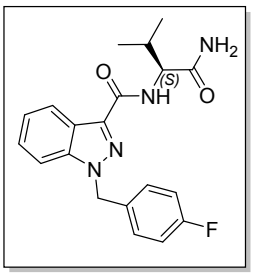
MDMB-FUBINACA ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.36 (dt, J = 8.1, 1.0 Hz, 1H), 7.56 (d, J = 9.7 Hz, 1H), 7.36 (ddd, J = 8.3, 6.9, 1.2 Hz, 1H), 7.34 – 7.16 (m, 2H), 7.22 – 7.17 (m, 2H), 7.06 – 6.96 (m, 2H), 5.59 (s, 2H), 4.74 (d, J = 9.6 Hz, 1H), 3.76 (s, 3H), 1.10 (s, 9H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 172.29, 163.58, 162.31, 161.62, 140.92, 137.47, 131.86, 131.84, 129.13, 129.06 127.21, 123.47, 123.08, 123.01, 116.02, 115.85, 109.58, 59.68, 53.08, 51.96, 35.20, 26.83. HRMS (ESI) m/z: $[M+H]^+$ calculated=398.1874; found=398.1883.

Class II drug 3-4





ADB-FUBINACA



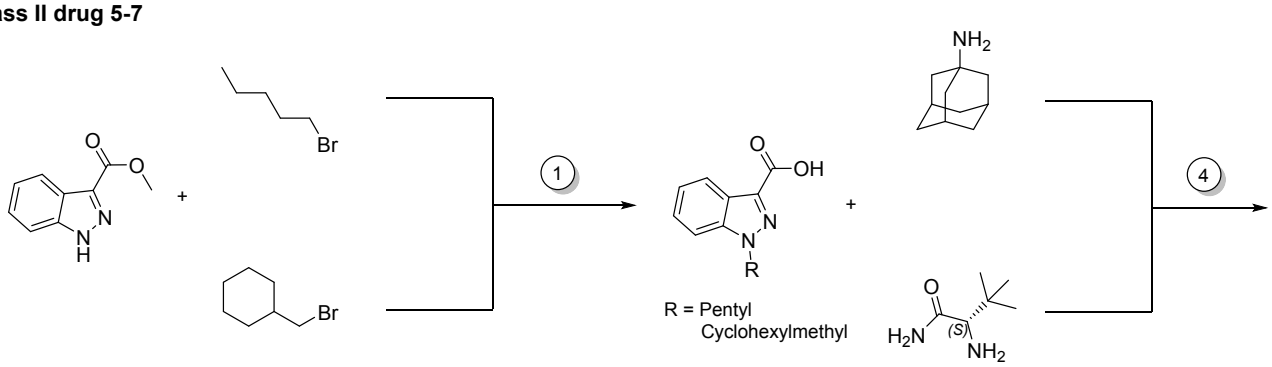
AB-FUBINACA

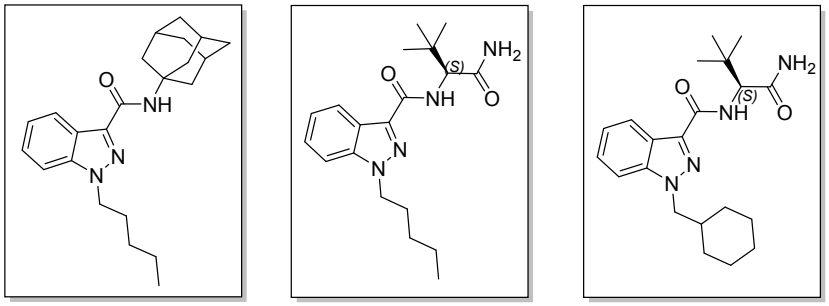
To a stirring solution of L-tert-Leucine (131mg, 1mmol) or L-Valine (117mg, 1mmol) in 8mL water, was treated with sodium hydroxide (88mg, 2.2mmol, 2.2eq), followed by benzyl chloro-formate (205mg, 1.2mmol, 1.2eq) dropwise; phase separation was observed. Mixture was allowed to stir vigorously at room temperature for 2h. After washed with Et_2O once, adjusted the pH to 2-3 by 1M HCl; precipitation was observed. The aqueous solution was extracted into Et_2O followed by brine wash and dried over $MgSO_4$. Solvent was removed via rotary evaporation to afford the Cbz protected product as colorless liquid, yield 88%. Primary amidation can be achieved according to literature[2], to a stirring solution of Cbz-L-tert-Leucine (233mg, 0.88mmol) or Cbz-L-valine (221mg, 0.88mmol) in 10mL THF, was treated with triethylamine (267mg, 2.64mmol, 3eq), ethyl chloroformate (116mg, 1.06mmol, 1.2eq) dropwise at 0°C; white precipitation was observed. After stirred for 30min, ammonium chloride (56mg, 1.06mmol, 1.2eq) dissolved in water was treated. Mixture was allowed to react at 0°C for another 30min. After extracted into EtOAc, the combined organic phase was washed with saturated sodium bicarbonate followed by brine wash and dried over $MgSO_4$. Solvent was removed via rotary evaporation and recrystallized from EtOAc to afford the primary amide product as white solid, yield 88%. General procedure 5 was applied to deprotect the Cbz group followed by general procedure 4 using 1-(4-fluorobenzyl)-1*H*-indazole-3-carboxylic acid (173mg, 0.64mmol) as coupling source to afford final product ADB-FUBINCACA and AB-FUBINACA as white solid, yield 65%. (Intermediate characterization was in agreement with literature[3])

ADB-FUBINACA ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.30 (dd, J = 8.2, 1.1 Hz, 1H), 7.72 (d, J = 9.4 Hz, 1H), 7.40 – 7.16 (m, 5H), 7.04 – 6.95 (m, 2H), 6.39 (s, 1H), 5.68 (s, 1H), 5.58 (s, 2H), 4.65 (d, J = 9.5 Hz, 1H), 1.16 (s, 9H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 172.90, 163.60, 162.64, 161.63, 140.90, 137.33, 131.80, 131.78, 129.18, 129.12, 127.18, 123.42, 123.04, 122.78, 116.03, 115.86, 109.71, 59.81, 53.12, 34.83, 26.91. HRMS (ESI) m/z: $[M+H]^+$ calculated=383.1878; found=383.1882.

AB-FUBINACA ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.32 (dt, J = 8.2, 1.0 Hz, 1H), 7.55 (d, J = 8.9 Hz, 1H), 7.39 – 7.26 (m, 3H), 7.23 – 7.15 (m, 2H), 7.05 – 6.91 (m, 2H), 6.42 (s, 1H), 5.71 (s, 1H), 5.57 (s, 2H), 4.58 (dd, J = 8.9, 6.8 Hz, 1H), 2.36 (hept, J = 6.8 Hz, 1H), 1.09 (dd, J = 6.8, 5.2 Hz, 6H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 173.66, 163.60, 162.89, 161.64, 140.93, 137.25, 131.75, 131.73, 129.17, 129.11, 127.27, 123.38, 123.12, 122.82, 116.04, 115.87, 109.68, 57.97, 53.09, 30.82, 19.61, 18.39. HRMS (ESI) m/z: $[M+H]^+$ calculated=369.1721; found=369.1723.

Class II drug 5-7





APINACA ADB-PINACA MAB-CHMINACA

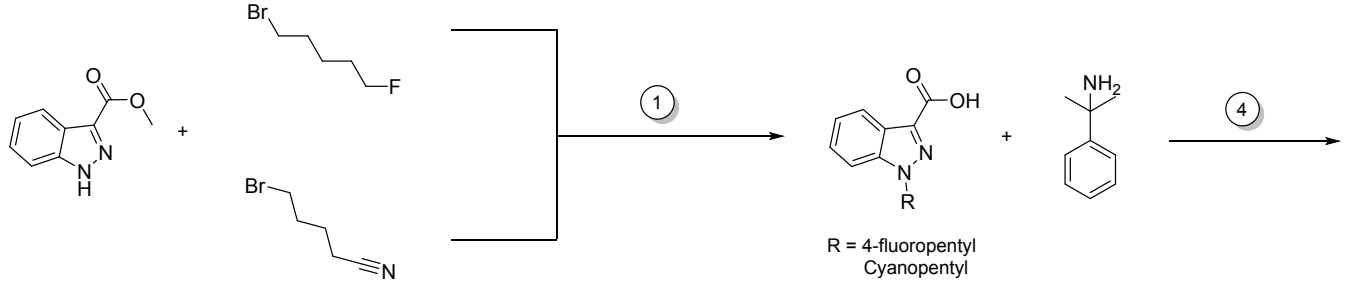
General procedure 1 was applied as first step using methyl 1*H*-indazole-3-carboxylate (176mg, 1mmol) and 1-bromopentyl (181mg, 1.2mmol) or (bromomethyl)cyclohexane (212mg, 1.2mmol) as alkylation source except when (bromomethyl)cyclohexane using as coupling source, longer reaction time usually overnight was required. The methyl ester will spontaneously undergo hydrolysis during the reaction to afford the N-alkylated indazole 3-carboxylic acid. Followed by general procedure 4 without purification using 1-adamantylamine (181mg, 1.2mmol) or L-tert-leucinamide (156mg, 1.2mmol) as coupling source to afford the final product APINACA, ADB-PINACA and MAB-CHMINACA as white solid, total yield 68%.

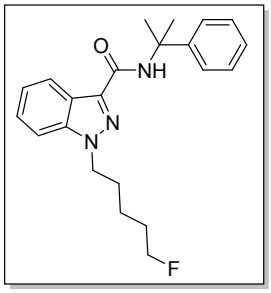
APINACA ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.39 (d, J = 8.2 Hz, 1H), 7.38 (d, J = 3.8 Hz, 2H), 7.23 (dd, J = 6.7, 2.9 Hz, 1H), 6.78 (s, 1H), 4.35 (t, J = 7.2 Hz, 2H), 2.22 (s, 6H), 2.15 (s, 3H), 1.94 (p, J = 7.2 Hz, 2H), 1.76 (q, J = 12.6 Hz, 6H), 1.35 (dp, J = 22.8, 7.4 Hz, 4H), 0.93 – 0.88 (m, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 162.19, 140.99, 138.10, 126.65, 123.26, 122.94, 122.43, 109.23, 51.98, 49.46, 42.03, 36.61, 29.70, 29.62, 29.06, 22.39, 14.06. HRMS (ESI) m/z: $[M+H]^+$ calculated=366.2540; found=366.2541.

ADB-PINACA ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.27 (d, J = 8.2 Hz, 1H), 7.72 (d, J = 9.5 Hz, 1H), 7.45 – 7.36 (m, 2H), 7.25 (td, J = 7.1, 6.2, 1.5 Hz, 1H), 6.79 (s, 1H), 5.93 (s, 1H), 4.70 (d, J = 9.5 Hz, 1H), 4.39 (t, J = 7.2 Hz, 2H), 1.95 (p, J = 7.3 Hz, 2H), 1.41 – 1.28 (m, 4H), 1.16 (s, 9H), 0.89 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 173.42, 162.91, 140.98, 136.46, 126.76, 122.97, 122.79, 122.53, 109.55, 59.70, 49.67, 34.82, 29.56, 29.06, 26.88, 22.36, 14.06. HRMS (ESI) m/z: $[M+H]^+$ calculated=345.2285; found=345.2285.

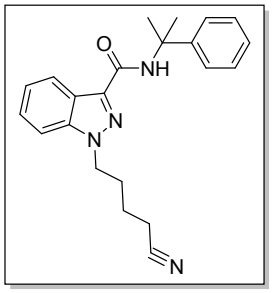
MAB-CHMINACA ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.27 (d, J = 8.1 Hz, 1H), 7.71 (d, J = 9.4 Hz, 1H), 7.44 – 7.35 (m, 2H), 7.26 – 7.21 (m, 1H), 6.60 (s, 1H), 5.74 (s, 1H), 4.68 (d, J = 9.5 Hz, 1H), 4.21 (dd, J = 7.2, 1.5 Hz, 2H), 2.02 (dqt, J = 10.9, 7.1, 3.5 Hz, 2H), 1.75 – 1.54 (m, 5H), 1.26 – 1.19 (m, 2H), 1.16 (s, 9H), 1.04 (td, J = 12.1, 3.5 Hz, 2H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 173.16, 162.90, 141.54, 136.57, 126.68, 122.82, 122.67, 122.50, 109.77, 59.71, 55.88, 38.84, 34.82, 31.05, 31.03, 26.90, 26.35, 25.77, 25.73. HRMS (ESI) m/z: $[M+H]^+$ calculated=371.2441; found=371.2444.

Class II drug 8-9





SGT-25



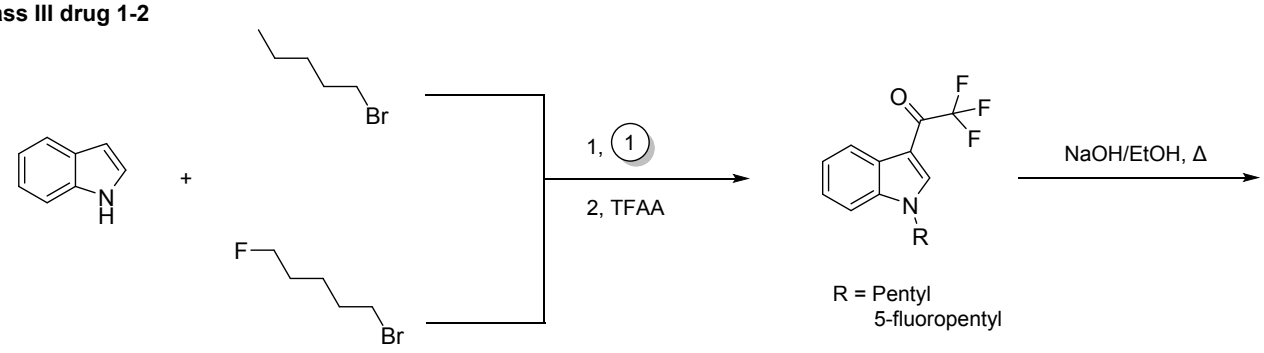
SGT-78

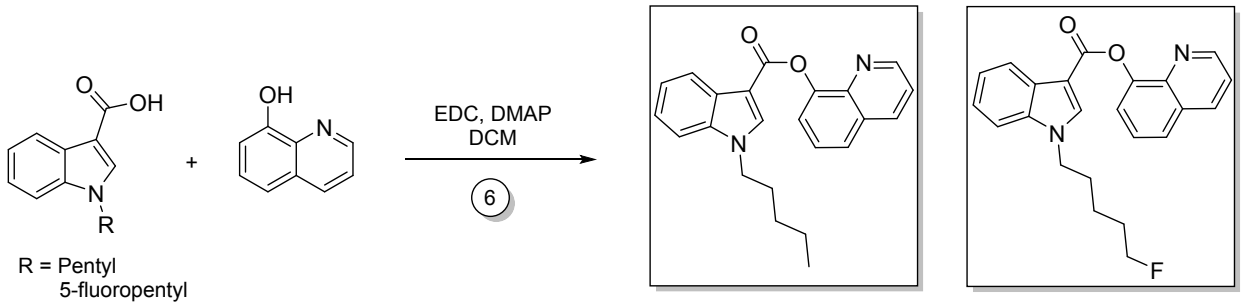
General procedure 1 was applied as first step using methyl 1*H*-indazole-3-carboxylate (176mg, 1mmol) and 1-bromo-pentyl (203mg, 1.2mmol) or 5-bromopentanenitrile (194mg, 1.2mmol) as alkylation source. The methyl ester will spontaneously undergo hydrolysis during the reaction to afford the N-alkylated indazole 3-carboxylic acid. Followed by general procedure 4 without purification using 2-phenylpropan-2-amine (162mg, 1.2mmol) as coupling source to afford the final product SGT-25 and SGT-78 as white solid, total yield 68%.

SGT-25 ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.34 (dd, J = 8.3, 1.1 Hz, 1H), 7.55 – 7.49 (m, 2H), 7.43 – 7.31 (m, 5H), 7.26 – 7.18 (m, 2H), 4.49 (t, J = 5.9 Hz, 1H), 4.44 – 4.37 (m, 3H), 2.02 (p, J = 7.3 Hz, 2H), 1.87 (s, 6H), 1.82 – 1.70 (m, 2H), 1.54 – 1.44 (m, 2H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 161.96, 147.35, 141.00, 138.00, 128.58, 126.87, 126.73, 124.99, 123.38, 122.96, 122.58, 109.12, 84.52, 83.21, 55.94, 49.24, 30.13, 29.98, 29.75, 29.51, 22.87, 22.83. HRMS (ESI) m/z: $[M+H]^+$ calculated=368.2133; found=368.2139.

SGT-78 ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.35 (dt, J = 8.2, 1.0 Hz, 1H), 7.55 – 7.49 (m, 2H), 7.43 – 7.33 (m, 5H), 7.24 (tdd, J = 6.1, 4.3, 2.1 Hz, 2H), 4.45 (t, J = 6.7 Hz, 2H), 2.38 (t, J = 7.0 Hz, 2H), 2.20 – 2.11 (m, 2H), 1.88 (s, 6H), 1.69 (dt, J = 14.8, 7.2 Hz, 2H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 161.77, 147.27, 141.02, 138.40, 128.60, 127.16, 126.76, 124.97, 123.54, 123.01, 122.78, 119.22, 108.88, 55.99, 48.30, 29.71, 28.70, 22.86, 17.02. HRMS (ESI) m/z: $[M+H]^+$ calculated=361.2023; found=361.2029.

Class III drug 1-2





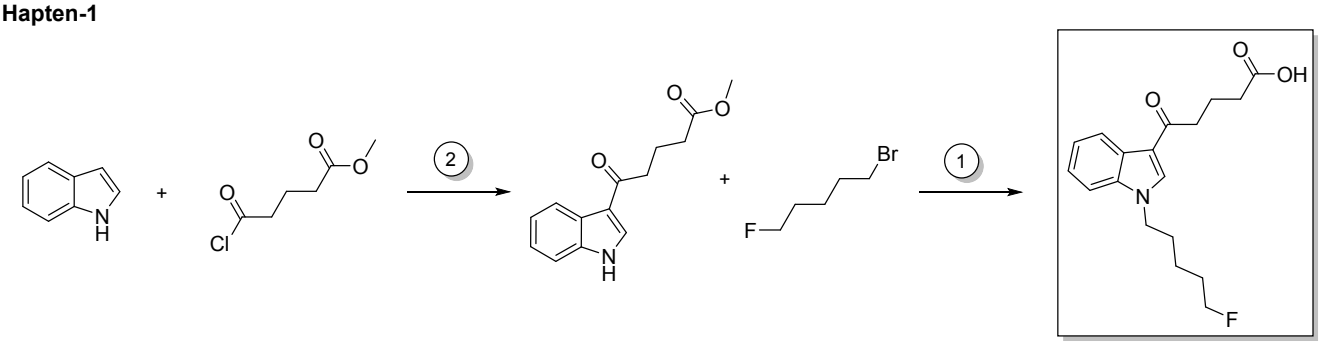
PB-22 5F-PB-22

General procedure 1 was applied as first step using indole (117mg, 1mmol) and 1-bromopentane (181mg, 1.2mmol) or 1-bromo-5-fluoropentane (203mg, 1.2mmol) as alkylation source. Trifluoroacetic anhydride(420mg, 2mmol, 2eq) was then treated and the mixture was allowed to react at room temperature for another 1h. After quenched with water and extracted into EtOAc, the combined organic phase was washed with saturated sodium bicarbonate followed by brine wash and dried over $MgSO_4$. Solvent was directly removed via rotary evaporation to afford the trifluoroacetylated product as yellow liquid. Followed by sodium hydroxide mediated hydrolysis in ethanol under heating condition overnight. The solvent was directly removed via rotary evaporation to afford the crude carboxylic acid product as tan solid. General procedure 6 was applied as final step using 8-hydroxylquinoline (290mg, 2mmol) as coupling source to afford the final product PB-22 as white solid, 5F-PB-22 as yellow solid, total yield 50%. (Intermediate characterization was in agreement with literature[4])

PB-22 ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.93 (dd, J = 4.2, 1.7 Hz, 1H), 8.40 – 8.35 (m, 1H), 8.17 (q, J = 2.9, 1.8 Hz, 2H), 7.74 (dd, J = 8.1, 1.6 Hz, 1H), 7.64 (dd, J = 7.5, 1.5 Hz, 1H), 7.58 (t, J = 7.8 Hz, 1H), 7.46 – 7.37 (m, 2H), 7.37 – 7.30 (m, 2H), 4.16 (t, J = 7.2 Hz, 2H), 1.92 (p, J = 7.2 Hz, 2H), 1.39 (td, J = 8.4, 7.8, 4.8 Hz, 4H), 0.97 – 0.90 (m, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 163.33, 150.59, 147.77, 142.07, 136.79, 135.97, 135.51, 129.61, 127.37, 126.30, 125.62, 122.88, 122.11, 122.08, 122.06, 121.59, 110.12, 105.93, 47.16, 29.65, 29.03, 22.31, 13.96. HRMS (ESI) m/z: $[M+H]^+$ calculated=359.1754; found=359.1760.

5F-PB-22 ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.91 (dd, J = 4.2, 1.7 Hz, 1H), 8.37 – 8.27 (m, 1H), 8.23 – 8.14 (m, 2H), 7.76 (dd, J = 7.5, 2.1 Hz, 1H), 7.65 – 7.55 (m, 2H), 7.47 – 7.36 (m, 2H), 7.32 (tt, J = 7.1, 5.5 Hz, 3H), 4.51 (t, J = 5.9 Hz, 1H), 4.40 (t, J = 5.9 Hz, 1H), 4.23 (t, J = 7.2 Hz, 2H), 2.00 (dq, J = 15.1, 7.1 Hz, 2H), 1.85 – 1.65 (m, 2H), 1.59 – 1.47 (m, 2H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 150.72, 147.81, 136.84, 136.10, 135.50, 129.73, 127.47, 126.43, 125.74, 123.10, 122.30, 122.27, 122.17, 121.71, 110.09, 106.25, 84.44, 83.13, 47.17, 30.18, 30.03, 29.78, 23.04, 23.00. HRMS (ESI) m/z: $[M+H]^+$ calculated=377.1660; found=377.1670.

Hapten-1

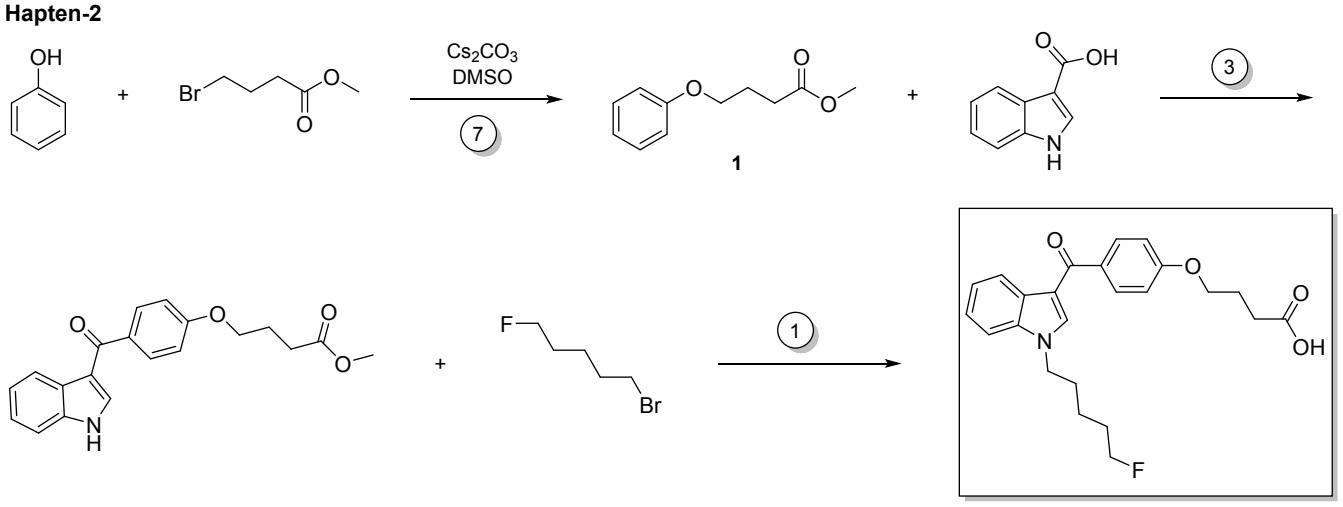


Hapten-1

General procedure 2 was applied as first step using indole (56mg, 0.5mmol) and methyl 5-chloro-5-oxopentanoate (99mg, 0.6mmol) as acylation source, followed by general procedure 1 without purification using 1-bromo-5-fluoropentane (101mg, 0.6mmol) as alkylation source to afford the final product Hapten-1 as orange solid, total yield 65%.

Hapten-1 ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.41 – 8.34 (m, 1H), 7.80 (s, 1H), 7.39 – 7.26 (m, 3H), 4.48 (t, J = 5.8 Hz, 1H), 4.38 (t, J = 5.9 Hz, 1H), 4.17 (t, J = 7.1 Hz, 2H), 2.96 (t, J = 7.2 Hz, 2H), 2.52 (t, J = 7.0 Hz, 2H), 2.17 – 2.08 (m, 2H), 1.94 (p, J = 7.3 Hz, 2H), 1.80 – 1.65 (m, 2H), 1.52 – 1.44 (m, 2H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 195.01, 178.89, 136.88, 134.69, 126.56, 123.49, 122.84, 122.79, 116.56, 109.93, 84.44, 83.13, 47.13, 38.62, 33.38, 30.10, 29.94, 29.64, 23.01, 22.97, 20.19. HRMS (ESI) m/z: $[M+H]^+$ calculated=320.1656; found=320.1664.

Hapten-2



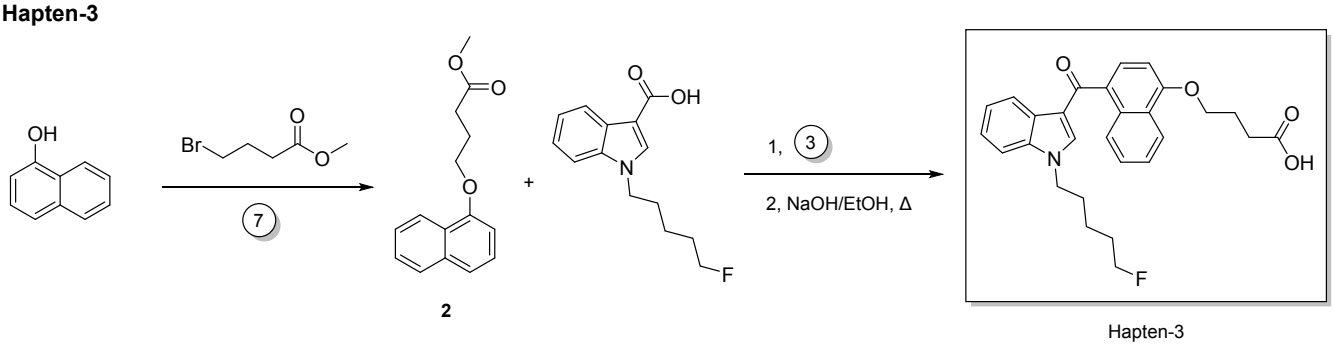
Hapten-2

General procedure 7 was applied as first step using phenol (94mg, 1mmol) and methyl 4-bromobutanoate (217mg, 1.2mmol) as substitution source, given the β-elimination side reaction of methyl 3-bromopropanoate under basic condition, to afford the methyl 4-phenoxybutanoate as colorless liquid, yield 90%. Followed by general procedure 3 using 1*H*-indole-3-carboxylic acid (174mg, 1.08mmol) as acylation source to afford the crude product as tan solid. Without purification, general procedure 1 was applied as final step using 1-bromo-5-fluoropentane (183mg, 1.08mmol) as alkylation source. The methyl ester will spontaneously undergo hydrolysis during the reaction to afford the final product Hapten-2 as orange solid, total yield 48%.

Methyl 4-phenoxybutanoate (1) ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.31 – 7.25 (m, 2H), 6.94 (tt, *J* = 7.4, 1.0 Hz, 1H), 6.91 – 6.86 (m, 2H), 4.00 (t, *J* = 6.1 Hz, 2H), 3.69 (d, *J* = 1.7 Hz, 3H), 2.53 (t, *J* = 7.3 Hz, 2H), 2.11 (tt, *J* = 7.4, 6.1 Hz, 2H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 173.78, 158.88, 129.54, 120.82, 114.55, 66.62, 51.70, 30.66, 24.75.

Hapten-2 ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.39 – 8.32 (m, 1H), 7.86 – 7.79 (m, 2H), 7.58 (s, 1H), 7.44 – 7.27 (m, 3H), 7.04 – 6.94 (m, 2H), 4.47 (t, J = 5.8 Hz, 1H), 4.37 (t, J = 5.8 Hz, 1H), 4.18 (t, J = 7.1 Hz, 2H), 4.12 (t, J = 6.1 Hz, 2H), 2.62 (t, J = 7.2 Hz, 2H), 2.22 – 2.13 (m, 2H), 1.93 (p, J = 7.3 Hz, 2H), 1.78 – 1.64 (m, 2H), 1.56 – 1.41 (m, 2H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 190.05, 178.31, 161.58, 136.85, 136.35, 133.59, 131.14, 127.61, 123.60, 122.93, 122.63, 115.86, 114.17, 109.87, 84.42, 83.11, 66.83, 47.11, 30.49, 30.11, 29.95, 29.68, 24.45, 23.01, 22.97. HRMS (ESI) m/z: $[M+H]^+$ calculated=412.1919; found=412.1927.

Hapten-3



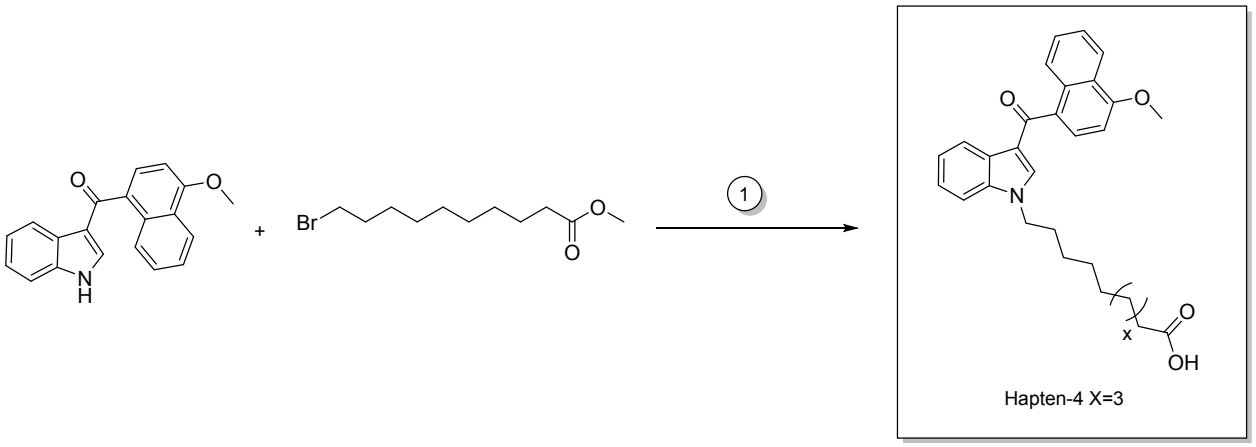
2 Hapten-3

General procedure 7 was applied as first step using naphthol (72mg, 0.5mmol) and methyl 4-bromobutanoate (109mg, 0.6mmol) as substitution source to afford the methyl 4-(naphthalen-1-yloxy)butanoate as colorless liquid, yield 89%. General procedure 3 was then applied using 1-(5-fluoropentyl)-1*H*-indole-3-carboxylic acid (132mg, 0.53mmol) as acylation source followed by sodium hydroxide mediated hydrolysis in ethanol to afford the final product Hapten-3, total yield 75%.

Methyl 4-(naphthalen-1-yloxy)butanoate (2) ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.30 – 8.22 (m, 1H), 7.83 – 7.76 (m, 1H), 7.48 (tt, *J* = 6.8, 5.1 Hz, 2H), 7.44 – 7.33 (m, 2H), 6.83 – 6.77 (m, 1H), 4.19 (t, *J* = 6.0 Hz, 2H), 3.70 (s, 3H), 2.65 (t, *J* = 7.3 Hz, 2H), 2.27 (ddd, *J* = 13.3, 7.3, 5.9 Hz, 2H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 173.82, 154.62, 134.60, 127.57, 126.50, 125.96, 125.73, 125.27, 122.08, 120.38, 104.67, 66.99, 51.78, 30.96, 24.83.

Hapten-3 ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.25 (s, 1H), 8.33 – 8.25 (m, 2H), 8.16 – 8.09 (m, 1H), 7.83 (s, 1H), 7.70 – 7.61 (m, 2H), 7.60 – 7.51 (m, 2H), 7.36 – 7.26 (m, 2H), 7.05 (d, *J* = 8.0 Hz, 1H), 4.42 (t, *J* = 6.0 Hz, 1H), 4.32 (t, *J* = 6.0 Hz, 1H), 4.27 (t, *J* = 6.2 Hz, 2H), 4.23 (t, *J* = 7.1 Hz, 2H), 2.54 (t, *J* = 7.2 Hz, 2H), 2.15 (p, *J* = 6.7 Hz, 2H), 1.77 (p, *J* = 7.3 Hz, 2H), 1.67 – 1.54 (m, 2H), 1.33 – 1.25 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 190.51, 174.16, 155.36, 138.91, 136.75, 131.49, 130.72, 127.89, 127.21, 126.63, 125.68, 125.32, 125.07, 123.15, 122.24, 121.86, 121.76, 116.28, 110.93, 103.70, 84.25, 82.96, 67.27, 45.95, 30.43, 29.32, 29.17, 29.03, 24.25, 21.94, 21.90. HRMS (ESI) m/z: $[M+H]^+$ calculated=462.2075; found=462.2076.

Hapten-4

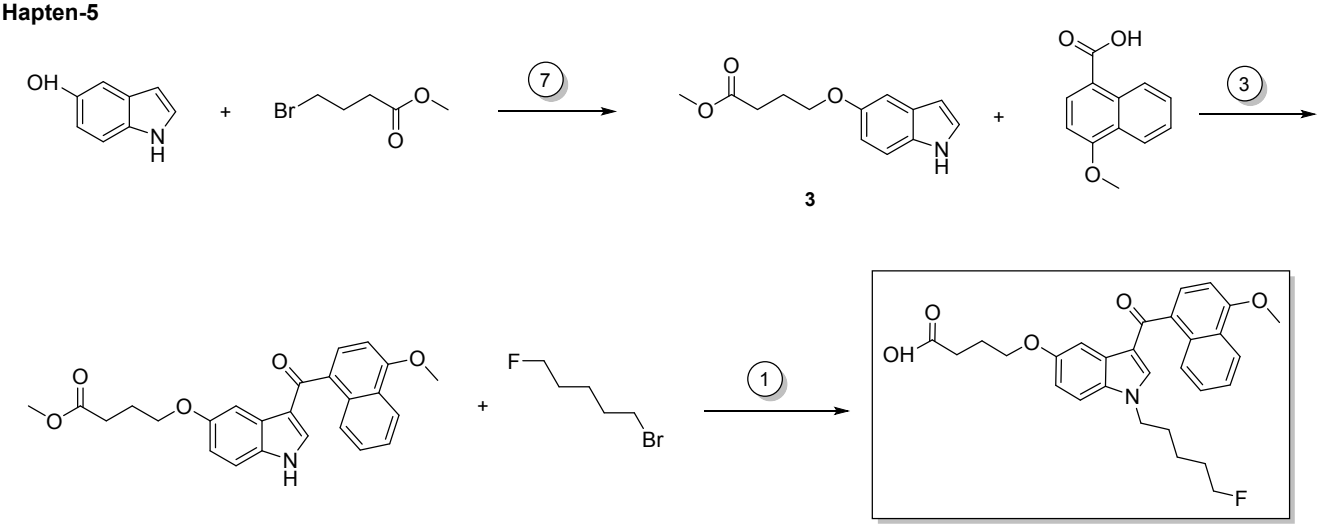


Hapten-4 X=3

General procedure 1 was applied using synthesized (1*H*-indol-3-yl)(4-methoxynaphthalen-1-yl)methanone (75mg, 0.25mmol) and methyl 10-bromo-decanoate (80mg, 0.3mmol) produced by thionyl chloride mediated methylation as alkylation source to afford the final product Hapten-4 as yellow solid, yield 88%.

Hapten-4 ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.48 – 8.40 (m, 1H), 8.38 – 8.26 (m, 2H), 7.66 (d, *J* = 7.9 Hz, 1H), 7.54 – 7.46 (m, 2H), 7.44 – 7.30 (m, 4H), 6.83 (d, *J* = 7.9 Hz, 1H), 4.12 – 4.05 (m, 5H), 2.31 (t, *J* = 7.5 Hz, 2H), 1.81 (p, *J* = 8.0, 7.5 Hz, 2H), 1.59 (p, *J* = 7.4 Hz, 2H), 1.31 – 1.21 (m, 10H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 191.98, 179.24, 157.20, 137.60, 137.12, 132.31, 131.60, 127.95, 127.49, 127.33, 125.98, 125.85, 125.79, 123.56, 123.01, 122.77, 122.18, 117.89, 110.04, 102.31, 55.82, 47.23, 33.99, 29.90, 29.26, 29.16, 29.10, 29.02, 26.88, 24.71. HRMS (ESI) m/z: $[M+H]^+$ calculated=472.2482; found=472.2485.

Hapten-5



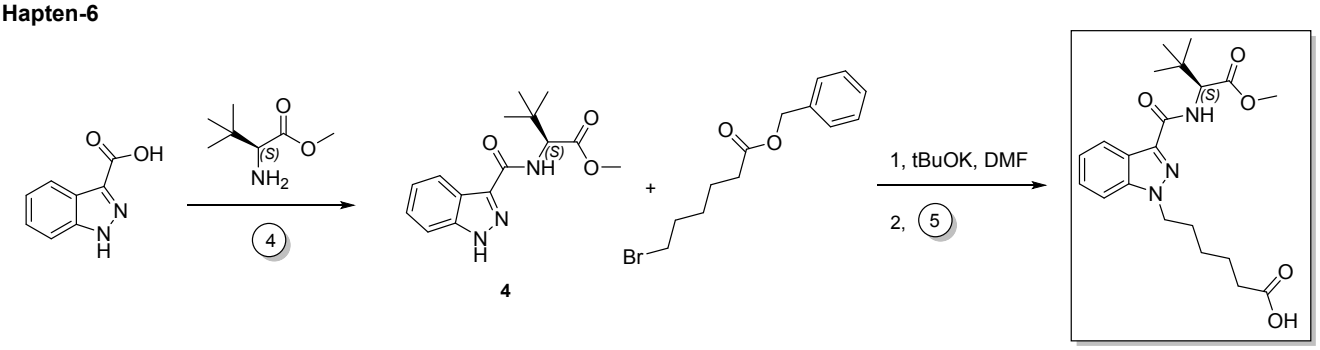
Hapten-5

General procedure 7 was applied as first step using 5-hydroxyindole (133mg, 1mmol) and methyl 4-bromobutanoate (217mg, 1.2mmol) as substitution source to afford the methyl 4-((1*H*-indol-5-yl)oxy)butanoate as colorless oil, yield 91%. Followed by general procedure 3 using 4-methoxy-1-naphthoic acid (222mg, 1.1mmol) as acylation source to afford the crude product as tan solid. Without purification general procedure 1 was applied as final step using methyl 6-bromohexanoate (186mg, 1.1mmol) as alkylation source, after spontaneous hydrolysis to afford the final product Hapten-5 as yellow solid, total yield 62%.

Methyl 4-((1*H*-indol-5-yl)oxy)butanoate (3) ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.88 (s, 1H), 7.28 – 7.23 (m, 2H), 7.01 (d, *J* = 2.4 Hz, 1H), 6.70 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.31 (t, *J* = 2.6 Hz, 1H), 3.94 (t, *J* = 6.3 Hz, 2H), 3.60 (s, 3H), 2.48 (t, *J* = 7.3 Hz, 2H), 1.96 (p, *J* = 6.7 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.13, 152.30, 131.10, 127.98, 125.74, 111.91, 111.56, 102.82, 100.78, 66.90, 51.30, 30.09, 24.46. LRMS (ESI) m/z: $[M+H]^+$ calculated= 234.1; found=234.1.

Hapten-5 ^{1}H NMR (500 MHz, Chloroform-d) δ 8.37 – 8.24 (m, 2H), 7.99 (d, J = 2.5 Hz, 1H), 7.64 (d, J = 7.9 Hz, 1H), 7.54 – 7.46 (m, 2H), 7.33 (s, 1H), 7.26 (d, J = 5.4 Hz, 1H), 6.97 (dd, J = 8.9, 2.5 Hz, 1H), 6.84 (d, J = 7.9 Hz, 1H), 4.43 (t, J = 5.9 Hz, 1H), 4.33 (t, J = 5.9 Hz, 1H), 4.15 (t, J = 6.1 Hz, 2H), 4.06 (d, J = 5.9 Hz, 5H), 2.62 (t, J = 7.3 Hz, 2H), 2.22 – 2.13 (m, 2H), 1.85 (p, J = 7.3 Hz, 2H), 1.74 – 1.59 (m, 2H), 1.39 (tt, J = 10.3, 6.4 Hz, 2H). ^{13}C NMR (126 MHz, Chloroform-d) δ 191.95, 178.29, 157.17, 155.83, 137.62, 132.29, 132.05, 131.58, 128.15, 127.84, 127.47, 125.98, 125.85, 125.80, 122.19, 117.60, 114.56, 110.82, 105.19, 102.34, 84.37, 83.06, 67.37, 55.84, 47.29, 30.78, 30.06, 29.90, 29.64, 24.70, 22.92, 22.89. HRMS (ESI) m/z: $[M+H]^+$ calculated=492.2181; found=492.2180.

Hapten-6



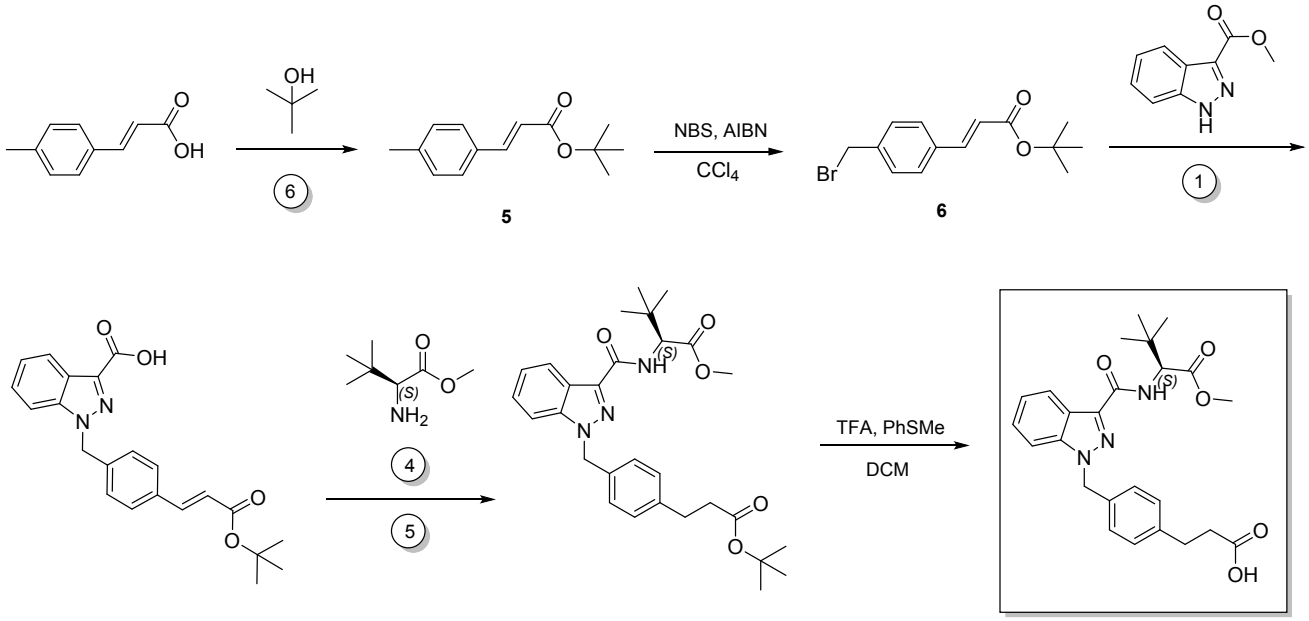
Hapten-6

General procedure 4 was applied as first step using indazole-3-carboxylic acid (162mg, 1mmol) and L-tert-leucine methyl ester (174mg, 1.2mmol) as coupling source to afford the methyl (S)-2-(1*H*-indazole-3-carboxamido)-3,3-dimethylbutanoate as white solid, yield 50%. Followed by altered version of general procedure 1 using t-BuOK as base to prevent spontaneous hydrolysis of methyl ester and benzyl 6-bromohexanoate (171mg, 0.6mmol) as alkylation source. General procedure 5 was applied as final step to deprotect the benzyl ester and afford the final product Hapten-6 as white solid, total yield 25%.

Methyl (S)-2-(1*H*-indazole-3-carboxamido)-3,3-dimethylbutanoate (4) ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.69 (s, 1H), 8.13 (d, *J* = 8.1 Hz, 1H), 7.72 (d, *J* = 9.2 Hz, 1H), 7.65 (d, *J* = 8.4 Hz, 1H), 7.44 (ddd, *J* = 8.3, 6.9, 1.2 Hz, 1H), 7.30 – 7.23 (m, 1H), 4.49 (d, *J* = 9.2 Hz, 1H), 3.70 (s, 3H), 1.02 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.33, 161.87, 141.25, 137.29, 126.75, 122.43, 121.25, 121.23, 110.93, 59.45, 51.80, 34.19, 26.42. LRMS (ESI) m/z: $[M+H]^+$ calculated= 290.1; found=290.1.

Hapten-6 ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.37 – 8.31 (m, 1H), 7.64 (d, J = 9.8 Hz, 1H), 7.40 (dd, J = 3.4, 1.1 Hz, 2H), 7.27 – 7.25 (m, 1H), 4.76 (d, J = 9.8 Hz, 1H), 4.42 (t, J = 6.9 Hz, 2H), 3.77 (s, 3H), 2.43 – 2.28 (m, 2H), 2.03 – 1.94 (m, 2H), 1.73 (dddt, J = 44.5, 13.5, 9.8, 6.5 Hz, 2H), 1.39 (ddt, J = 17.2, 10.0, 7.4 Hz, 2H), 1.10 (s, 9H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 177.81, 172.89, 162.58, 140.89, 136.78, 126.85, 123.12, 122.96, 122.78, 109.28, 59.66, 52.10, 48.91, 35.14, 33.72, 29.25, 26.87, 25.99, 24.16. HRMS (ESI) m/z: $[M+H]^+$ calculated=404.2180; found=404.2185.

Hapten-7



Hapten-7

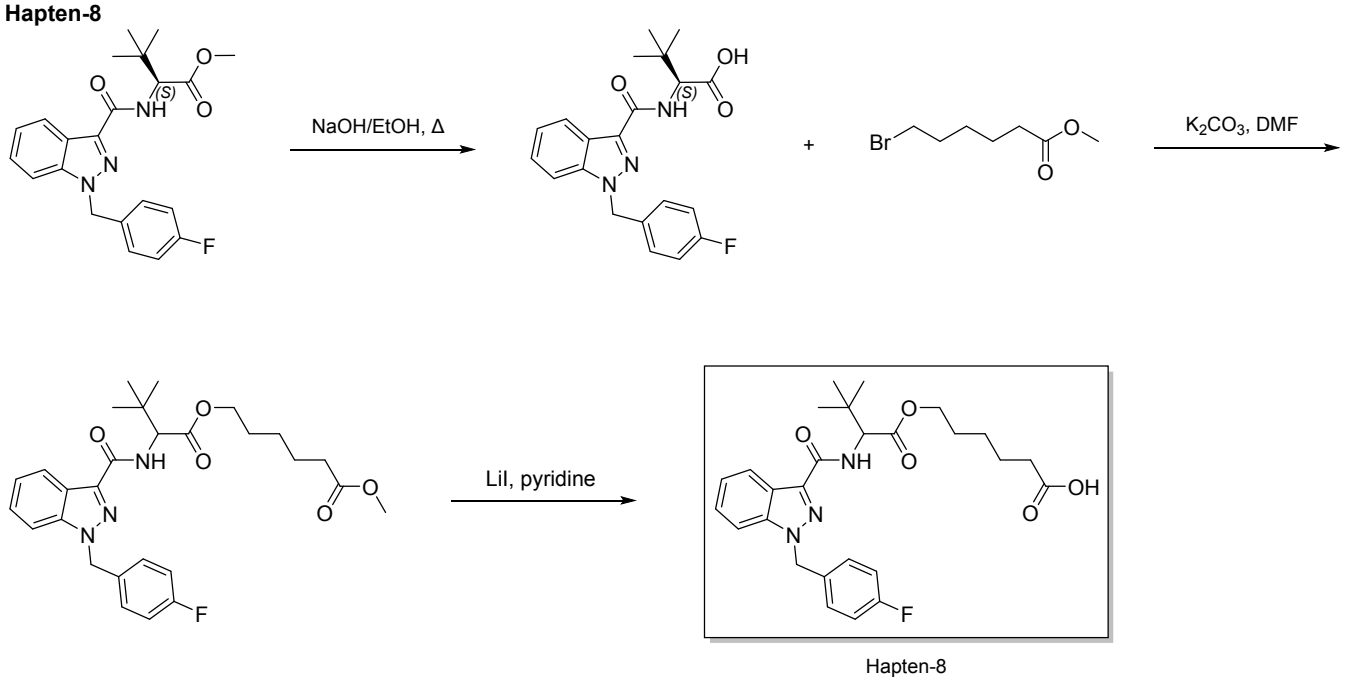
General procedure 6 was applied as first step using 4-methylcinnamic acid (324mg, 2mmol) and t-butanol (1.48g, 20mmol, 10eq) as coupling source to afford Tert-butyl 3-(p-tolyl)acrylate as colorless liquid, yield 25%. Ar protected stirring solution of t-Butyl 4-methylcinnamte (109mg, 0.5mmol), NBS (107mg, 0.6mmol, 1.2eq), AIBN (20mg, 0.12mmol, 0.24eq) was allowed to refluxed overnight in 8mL tetra fluorocarbon. After quenched with water and extracted into EtOAc, the combined organic phase was washed with saturated sodium bicarbonate followed by brine wash and dried over $MgSO_4$. Flash chromatography was performed to afford benzyl bromination product as colorless liquid, yield 70%. Followed by general procedure 1 using methyl 1*H*-indazole-3-carboxylate (51mg, 0.29mmol) as alkylation acceptor, after spontaneous hydrolysis to afford the crude carboxylic acid product as white solid. General procedure 4 was then applied using L-tert-leucine methyl ester (51mg, 0.35mmol) as coupling source followed by general procedure 5 to reduce the olefin. Finally, without purification, to a stirring solution of amide dissolved in 5mL DCM was treated with 1mL TFA and thioanisole (36mg, 0.29mmol, 1eq). Mixture was allowed to react at room temperature for 3h. After quenched with water and extracted into EtOAc, the combined organic phase was washed with saturated sodium bicarbonate followed by brine wash and dried over $MgSO_4$. Flash chromatography was performed to afford the final product Hapten-7 as white solid, yield 45%.

Tert-butyl 3-(p-tolyl)acrylate (5) ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.56 (d, *J* = 15.9 Hz, 1H), 7.40 (d, *J* = 7.8 Hz, 2H), 7.17 (d, *J* = 7.7 Hz, 2H), 6.32 (d, *J* = 16.0 Hz, 1H), 2.36 (s, 3H), 1.53 (s, 9H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 166.68, 143.68, 140.43, 132.07, 129.68, 128.08, 119.24, 80.49, 28.36, 21.57.

Tert-butyl (E)-3-(4-(bromomethyl)phenyl)acrylate (6) ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.56 (d, *J* = 16.0 Hz, 1H), 7.51 – 7.45 (m, 2H), 7.42 – 7.35 (m, 2H), 6.37 (d, *J* = 16.0 Hz, 1H), 4.49 (s, 2H), 1.53 (s, 9H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 166.29, 142.75, 139.62, 134.96, 129.67, 128.47, 121.03, 80.82, 32.96, 28.33.

Hapten-7 ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.35 (dt, J = 8.2, 1.0 Hz, 1H), 7.58 (d, J = 9.7 Hz, 1H), 7.38 – 7.29 (m, 2H), 7.25 (ddd, J = 8.0, 6.5, 1.3 Hz, 1H), 7.18 – 7.11 (m, 4H), 5.60 (s, 2H), 4.74 (d, J = 9.7 Hz, 1H), 3.76 (s, 3H), 2.92 (t, J = 7.7 Hz, 2H), 2.64 (dd, J = 8.2, 7.2 Hz, 2H), 1.09 (s, 9H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 177.64, 172.29, 162.45, 141.01, 140.24, 137.26, 134.18, 128.89, 127.57, 127.10, 123.43, 122.98, 122.94, 109.76, 59.71, 53.51, 51.97, 35.34, 35.19, 30.33, 26.84. HRMS (ESI) m/z: $[M+H]^+$ calculated=452.2180; found=452.2183.

Hapten-8

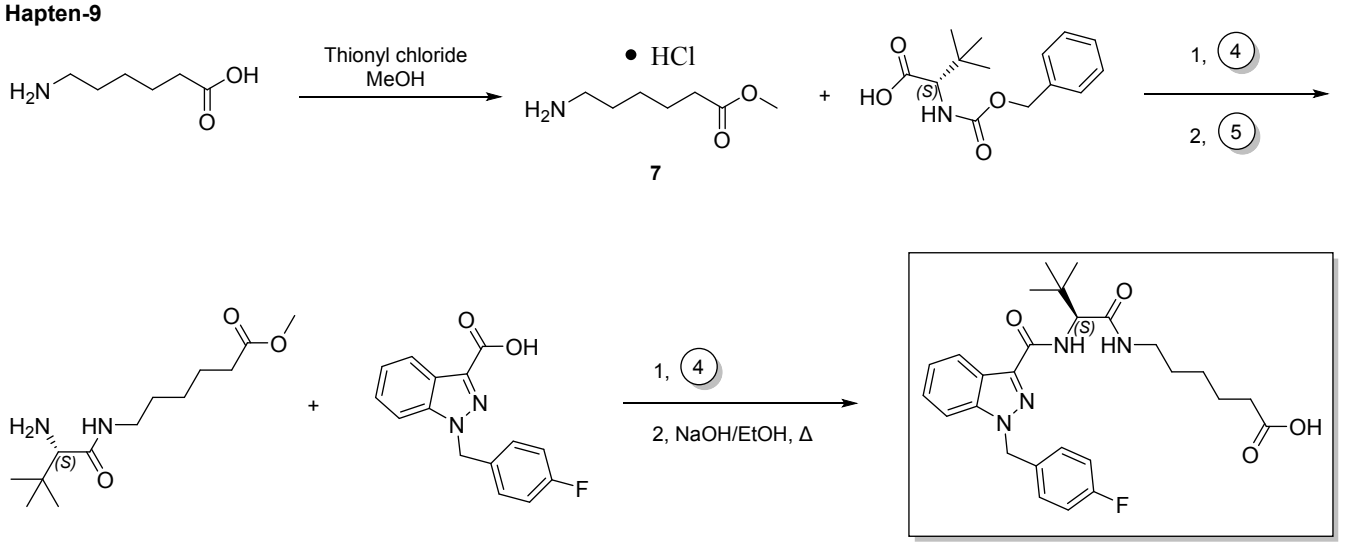


Hapten-8

Starting from the drug MDMB-FUBINACA (198mg, 0.5mmol), sodium hydroxide mediated hydrolysis was applied to afford its carboxylic acid. (characterization see indazole based metabolite 4) Stirring solution of (S)-2-(1-(4-fluorobenzyl)-1*H*-indazole-3-carboxamido)-3,3-dimethylbutanoic acid (186mg, 0.49mmol), methyl 6-bromohexanoate (155mg, 0.74mmol, 1.5eq), K_2CO_3 (260mg, 2.45mmol, 5eq) in DMF was allowed to react overnight to afford the ester, yield 90%. Crude product was added with lithium iodide (590mg, 4.4mmol, 10eq) and refluxing in 3mL pyridine overnight for selectively methyl ester hydrolysis to produce the final product Hapten-8 as white solid after reversed phase chromatography, yield 50%. Partial middle ester hydrolysis was observed.

Hapten-8 ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.36 (dt, *J* = 8.2, 1.1 Hz, 1H), 7.59 (d, *J* = 9.7 Hz, 1H), 7.36 (ddd, *J* = 8.5, 6.8, 1.2 Hz, 1H), 7.30 (dt, *J* = 8.5, 1.0 Hz, 1H), 7.28 – 7.25 (m, 1H), 7.23 – 7.15 (m, 2H), 7.04 – 6.96 (m, 2H), 5.59 (s, 2H), 4.72 (d, *J* = 9.7 Hz, 1H), 4.16 (t, *J* = 6.5 Hz, 2H), 2.34 (t, *J* = 7.4 Hz, 2H), 1.76 – 1.62 (m, 4H), 1.51 – 1.39 (m, 2H), 1.09 (s, 9H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 178.56, 171.80, 163.58, 162.38, 161.62, 140.92, 137.46, 131.85, 131.83, 129.13, 129.06, 127.24, 123.45, 123.07, 123.04, 116.02, 115.85, 109.59, 64.85, 59.77, 53.07, 35.24, 33.80, 28.28, 26.88, 25.49, 24.30. HRMS (ESI) m/z: $[M+H]^+$ calculated=498.2399; found=498.2402.

Hapten-9



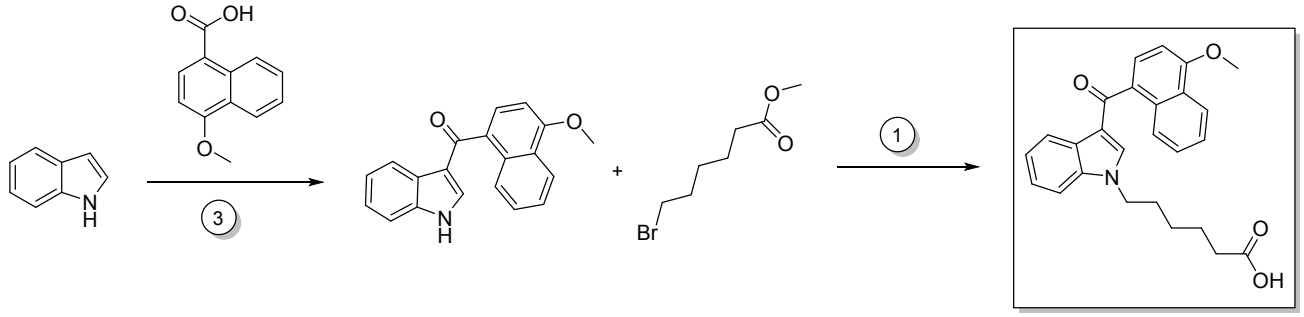
Hapten-9

Thionyl chloride mediated methylation was utilized to afford methyl 6-aminohexanoate hydrochloride (91mg, 0.5mmol) as white solid, yield quantitative. General procedure 4 was then applied using Cbz-L-tert-Leucine (111mg, 0.42mmol) as coupling source followed by general procedure 5 to deprotect the Cbz group to afford the methyl (S)-6-(2-amino-3,3-dimethylbutanamido)hexanoate as colorless liquid, yield 83%. General procedure 4 was then applied using 1-(4-fluorobenzyl)-1*H*-indazole-3-carboxylic acid (135mg, 0.5mmol) as coupling source followed by sodium hydroxide mediated hydrolysis in ethanol to afford the final product Hapten-9 as white solid, yield 42%.

Methyl (S)-6-(2-amino-3,3-dimethylbutanamido)hexanoate (7) ^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.74 (s, 1H), 3.64 (s, 3H), 3.32 – 3.13 (m, 2H), 3.05 (s, 1H), 2.29 (t, *J* = 7.4 Hz, 2H), 1.68 – 1.56 (m, 2H), 1.56 – 1.44 (m, 2H), 1.40 – 1.28 (m, 4H), 0.96 (s, 9H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 174.14, 173.60, 64.59, 51.60, 38.79, 34.13, 33.98, 29.45, 26.82, 26.57, 24.61. LRMS (ESI) m/z: $[M+H]^+$ calculated= 259.2; found=259.2.

Hapten-9 ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.30 (dt, J = 8.2, 1.0 Hz, 1H), 7.83 (d, J = 10.0 Hz, 1H), 7.36 (ddd, J = 8.5, 6.7, 1.1 Hz, 1H), 7.31 (dt, J = 8.6, 1.0 Hz, 1H), 7.29 – 7.22 (m, 1H), 7.23 – 7.18 (m, 2H), 7.05 – 6.95 (m, 2H), 6.75 (t, J = 5.6 Hz, 1H), 5.58 (s, 2H), 4.68 (d, J = 10.0 Hz, 1H), 3.33 – 3.16 (m, 2H), 2.30 (t, J = 7.4 Hz, 2H), 1.67 – 1.55 (m, 2H), 1.55 – 1.44 (m, 2H), 1.35 (dtd, J = 13.5, 6.5, 3.0 Hz, 2H), 1.12 (s, 9H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 177.21, 170.65, 163.61, 162.86, 161.65, 140.89, 137.10, 131.74, 131.72, 129.26, 129.19, 127.22, 123.44, 123.09, 122.80, 116.03, 115.86, 109.75, 60.24, 53.18, 39.21, 35.04, 33.74, 29.02, 26.84, 26.33, 24.46. HRMS (ESI) m/z: $[M+H]^+$ calculated=497.2558; found=497.2568.

Hapten-10

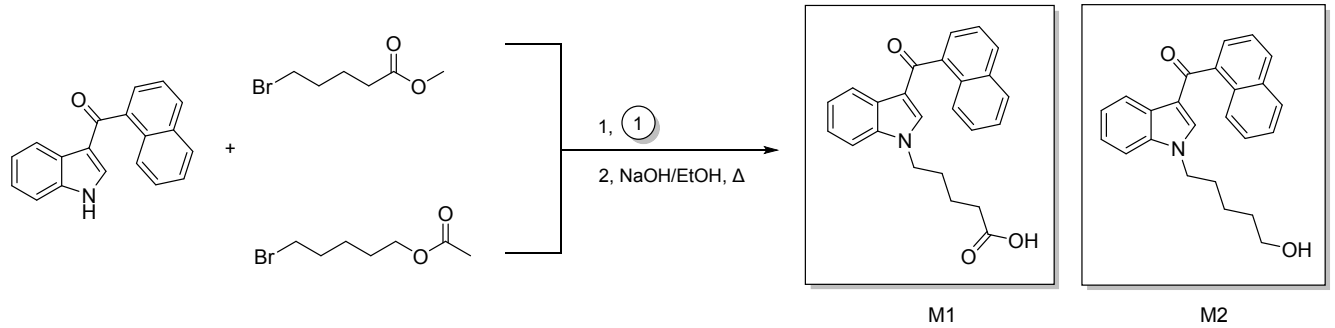


Hapten-10

General procedure 3 was applied as first step using indole (59mg, 0.5mmol) and 4-methoxy-1-naphthoic acid (121mg, 0.6mmol) as acylation source, followed by general procedure 1 without purification using methyl 6-bromohexanoate (125mg, 0.6mmol) as alkylation source, after spontaneous hydrolysis to afford the final product Hapten-10 as yellow solid, total yield 70%.

Hapten-10 ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.43 (ddd, *J* = 7.0, 3.4, 2.0 Hz, 1H), 8.37 – 8.25 (m, 2H), 7.65 (d, *J* = 7.9 Hz, 1H), 7.50 (ddd, *J* = 6.8, 4.7, 3.2 Hz, 2H), 7.41 (s, 1H), 7.39 – 7.31 (m, 3H), 6.83 (d, *J* = 7.9 Hz, 1H), 4.09 (t, *J* = 7.1 Hz, 2H), 4.06 (s, 3H), 2.30 (t, *J* = 7.3 Hz, 2H), 1.84 (p, *J* = 7.3 Hz, 2H), 1.62 (p, *J* = 7.4 Hz, 2H), 1.38 – 1.28 (m, 2H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 191.98, 178.29, 157.25, 137.52, 137.05, 132.30, 131.51, 128.04, 127.52, 127.34, 125.96, 125.86, 125.81, 123.65, 123.07, 122.84, 122.20, 118.01, 109.97, 102.34, 55.83, 46.95, 33.61, 29.61, 26.33, 24.19. HRMS (ESI) m/z: $[M+H]^+$ calculated=416.1856; found=416.1859.

Indole based metabolite 1-3

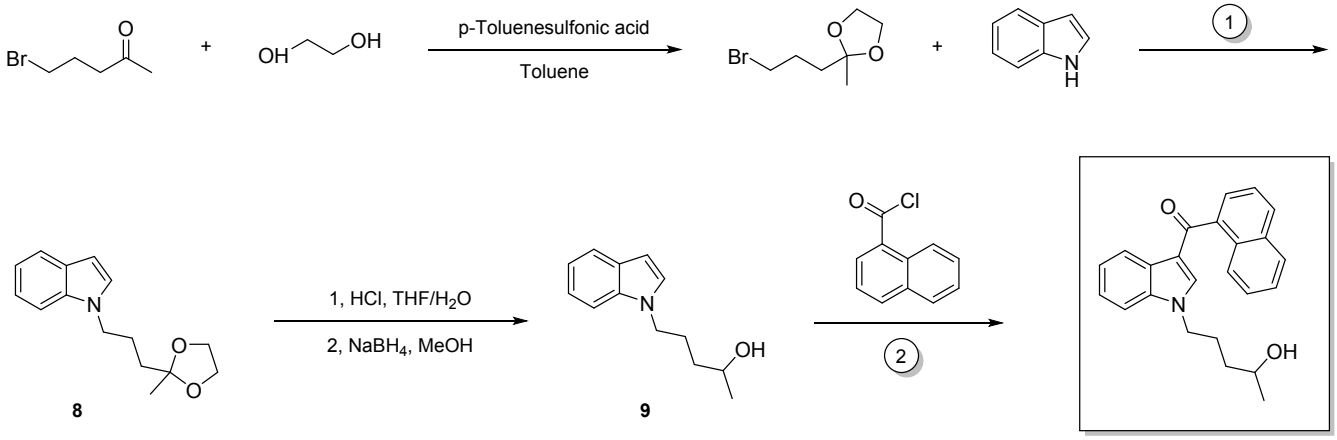


M1 M2

Starting from (1*H*-indol-3-yl)(naphthalen-1-yl)methanone (68mg, 0.25mmol), general procedure 1 was applied as first step using 5-bromopentyl acetate (63mg, 0.3mmol) or methyl 5-bromopentanoate (59mg, 0.3mmol) as alkylation source directly followed by sodium hydroxide mediated hydrolysis in ethanol under heating condition to afford the terminal carboxylic acid M1 as white solid and terminal hydroxyl M2 as colorless liquid, yield 75%.

M1 ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.48 (ddd, J = 6.7, 3.1, 2.1 Hz, 1H), 8.20 – 8.15 (m, 1H), 7.96 (d, J = 8.2 Hz, 1H), 7.90 (dd, J = 8.0, 1.5 Hz, 1H), 7.65 (dd, J = 6.9, 1.2 Hz, 1H), 7.55 – 7.42 (m, 3H), 7.42 – 7.34 (m, 4H), 4.09 (t, J = 7.1 Hz, 2H), 2.32 (t, J = 7.2 Hz, 2H), 1.92 – 1.81 (m, 2H), 1.65 – 1.55 (m, 2H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 192.27, 178.03, 139.08, 137.92, 137.09, 133.87, 130.90, 130.18, 128.32, 127.12, 126.92, 126.44, 126.07, 126.02, 124.70, 123.89, 123.15, 123.11, 117.89, 109.99, 46.90, 33.22, 29.25, 21.97. HRMS (ESI) m/z: $[M+H]^+$ calculated=372.1594; found=372.1603.

M2 ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.52 – 8.44 (m, 1H), 8.21 – 8.16 (m, 1H), 7.97 (d, J = 8.2 Hz, 1H), 7.94 – 7.88 (m, 1H), 7.66 (dd, J = 7.0, 1.3 Hz, 1H), 7.56 – 7.43 (m, 3H), 7.42 – 7.33 (m, 4H), 4.08 (t, J = 7.2 Hz, 2H), 3.57 (t, J = 6.4 Hz, 2H), 1.84 (p, J = 7.4 Hz, 2H), 1.56 – 1.50 (m, 2H), 1.35 (qd, J = 10.1, 9.2, 6.5 Hz, 2H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 192.19, 139.20, 138.04, 137.14, 133.87, 130.92, 130.12, 128.31, 127.13, 126.88, 126.43, 126.11, 125.96, 124.70, 123.79, 123.10, 123.04, 117.76, 110.07, 62.51, 47.22, 32.12, 29.73, 23.25. HRMS (ESI) m/z: $[M+H]^+$ calculated=358.1802; found=358.1804.



M3

To a solution of ethylene glycol(248mg, 4mmol, 4eq) and p-toluenesulfonic acid monohydrate (48mg, 0.25mmol, 0.25eq) in toluene (25 mL) was treated with 5-Bromo-2-pentanone (165mg, 1mmol) and heated at reflux under dean stark conditions for 16 h. The reaction mixture was concentrated and extracted with EtOAc, followed by brine wash and dried over $MgSO_4$. Solvent was directly removed via rotary evaporation to afford the crude acetyl as brown liquid. General procedure 1 was then applied using indole (97mg, 0.83mmol) as alkylation acceptor to afford the 1-(3-(2-methyl-1,3-dioxolan-2-yl)propyl)-1*H*-indole as colorless liquid, yield 89%. To a solution of this intermediate in THF 12mL/H_2O 3mL(4:1) was treated with 2,4mL 1N HCl and heated under 60°C for 3h. After simple workup like extraction and concentration, it was diluted with 8mL MeOH, $NaBH_4$ (84mg, 2.22mmol, 3eq) was added and stirred at room temperature for 1h to afford the 5-(1*H*-indol-1-yl)pentan-2-ol as colorless liquid after flash chromatography, yield 39%. General procedure 2 was applied as final step using 1-naphthoyl chloride (67mg, 0.35mmol) as acylation source to afford the final 4-OH product M3 as colorless liquid, yield 75%.

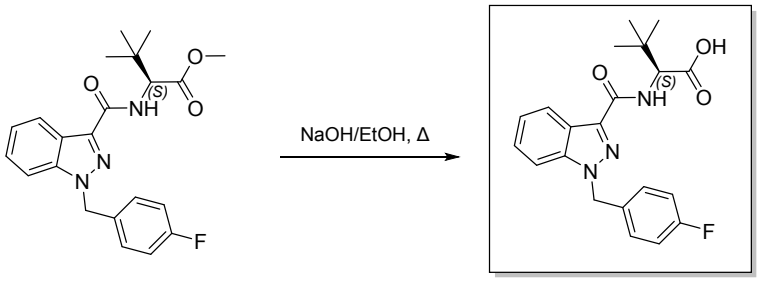
1-(3-(2-methyl-1,3-dioxolan-2-yl)propyl)-1*H*-indole (8) ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.63 (dt, *J* = 7.9, 1.0 Hz, 1H), 7.36 (d, *J* = 8.2 Hz, 1H), 7.21 (ddd, *J* = 8.3, 7.1, 1.3 Hz, 1H), 7.13 – 7.07 (m, 2H), 6.49 (d, *J* = 3.1 Hz, 1H), 4.15 (t, *J* = 7.2 Hz, 2H), 3.99 – 3.84 (m, 4H), 2.03 – 1.92 (m, 2H), 1.72 – 1.66 (m, 2H), 1.30 (s, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 136.11, 128.69, 127.82, 121.49, 121.05, 119.32, 109.80, 109.51, 101.15, 64.83, 46.45, 36.42, 24.96, 24.10. LRMS (ESI) m/z: $[M+H]^+$ calculated= 246.1; found=246.1.

5-(1*H*-indol-1-yl)pentan-2-ol (9) ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.64 (dt, *J* = 7.9, 1.0 Hz, 1H), 7.36 (d, *J* = 7.9 Hz, 1H), 7.21 (ddd, *J* = 8.3, 7.1, 1.3 Hz, 1H), 7.14 – 7.06 (m, 2H), 6.50 (d, *J* = 2.2 Hz, 1H), 4.16 (td, *J* = 7.1, 1.9 Hz, 2H), 3.79 (h, *J* = 6.2 Hz, 1H), 2.06 – 1.83 (m, 2H), 1.48 – 1.44 (m, 2H), 1.17 (d, *J* = 6.1 Hz, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 136.07, 128.72, 127.86, 121.52, 121.10, 119.36, 109.48, 101.16, 67.84, 46.44, 36.48, 26.70, 23.84. LRMS (ESI) m/z: $[M+H]^+$ calculated= 204.1; found=204.1.

M3 ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.51 – 8.46 (m, 1H), 8.18 (dd, *J* = 8.5, 1.2 Hz, 1H), 7.96 (dt, *J* = 8.2, 1.1 Hz, 1H), 7.93 – 7.88 (m, 1H), 7.65 (dd, *J* = 7.0, 1.3 Hz, 1H), 7.56 – 7.44 (m, 3H), 7.42 – 7.31 (m, 4H), 4.10 (t, *J* = 7.1 Hz, 2H), 3.75 (h, *J* = 6.2 Hz, 1H), 2.01 – 1.79 (m, 2H), 1.42 – 1.35 (m, 2H), 1.14 (dd, *J* = 6.2, 1.4 Hz, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 192.20, 139.19, 138.04, 137.15,

133.87, 130.92, 130.12, 128.32, 127.12, 126.89, 126.42, 126.11, 125.96, 124.70, 123.81, 123.09, 123.05, 117.77, 110.11, 67.59, 47.22, 36.03, 26.32, 23.95. ^{13}C NMR (126 MHz, Chloroform-*d*) δ 173.47, 162.79, 140.97, 136.65 (d, *J* = 2.2 Hz), 126.83, 122.92, 122.80, 122.51, 109.50, 67.58 (d, *J* = 7.4 Hz), 59.74, 49.42 (d, *J* = 8.5 Hz), 36.13 (d, *J* = 4.6 Hz), 34.81, 26.86, 26.15 (d, *J* = 5.4 Hz), 23.78 (d, *J* = 2.6 Hz). HRMS (ESI) m/z: $[M+H]^+$ calculated=358.1802; found=358.1807.

Indazole based metabolite 4

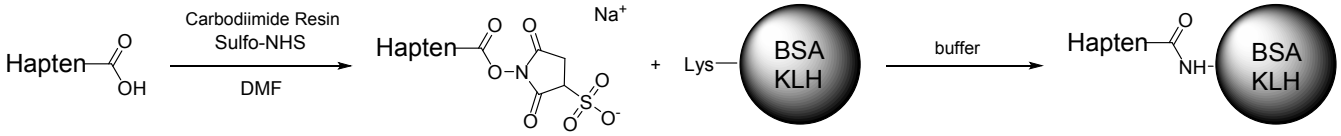


M4

MDMB-FUBIANCA (79mg, 0.2mmol) was directly hydrolyzed by sodium hydroxide mediated hydrolysis in ethanol under heating condition to afford the terminal t-leucine acid M4 as white solid, yield 97%.

M4 ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.35 (dd, *J* = 8.2, 1.9 Hz, 1H), 7.57 (d, *J* = 9.4 Hz, 1H), 7.38 – 7.33 (m, 1H), 7.32 – 7.26 (m, 2H), 7.19 (ddd, *J* = 8.2, 5.4, 2.0 Hz, 2H), 7.00 (td, *J* = 8.7, 2.2 Hz, 2H), 5.59 (s, 2H), 4.76 (dd, *J* = 9.5, 2.1 Hz, 1H), 1.14 (s, 9H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 176.06, 163.59, 162.54, 161.63, 140.94, 137.30, 131.80, 131.77, 129.12, 129.06, 127.29, 123.45, 123.14, 123.04, 116.04, 115.87, 109.62, 59.84, 53.10, 35.04, 26.87. HRMS (ESI) m/z: $[M+H]^+$ calculated=384.1718; found=384.1725.

Hapten conjugation



To test the proper buffer condition, a 4mL glass vial containing 50µl BSA (10mg/mL) was diluted by buffer as listed in Table S1, from PBS to HEPES from low to high concentration of sucrose, to 1mL and was treated with 30µl activated Hapten from stock. Conjugation was allowed to proceed at 4°C overnight.

100µl small scale dialysis was performed on BSA conjugated solution against ddwater and was tested on MALDI-TOF for copy number. Copy number increases concomitant with hapten concentration thus the optimal concentration was concluded to produce the copy number within 15-21. Same conjugate reaction was applied on KLH using the determined hapten concentration. Conjugated solution was transferred into a cuvette and was tested on OD_{600} (Table S1). OD<0.1 was considered as relative transparent. Dialyzed all solution against PBS with or without 10% sucrose depending on the precipitation phenomenon using Slide-A-Lyzer™ 10K MWCO Dialysis Cassettes (Thermo Scientific). It was unlikely to avoid precipitation when OD>0.1. Even with OD<0.1, precipitation could happen overnight.

Although it is generally accepted that aggregation is more effective in inducing immune response,[5] premature precipitation will halt the conjugating progress thus leading to insufficient epitope density. In this regard, we applied HEPES with 40% sucrose as buffer condition for Hapten **2**, **3**, **4**, **5**, **10** while HEPES with 20% sucrose for others.

Table S1. Screening of conjugate buffer conditions.

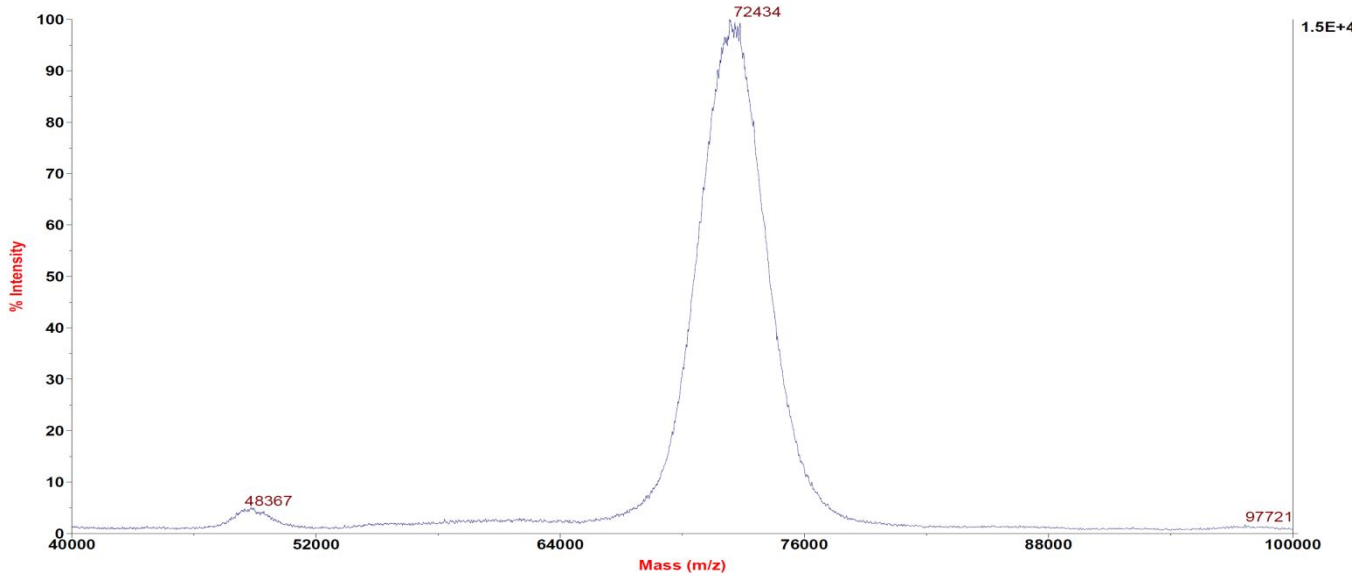
Hapten	PBS	HEPES		
	20% sucrose	10% sucrose	20%	40%
1	0.186	0.074		
2	0.562	0.241	0.128	0.068
4				0.143
5				0.100
9	0.468	0.177	0.072	

Table S2. Copy number of Haptens 1-10.

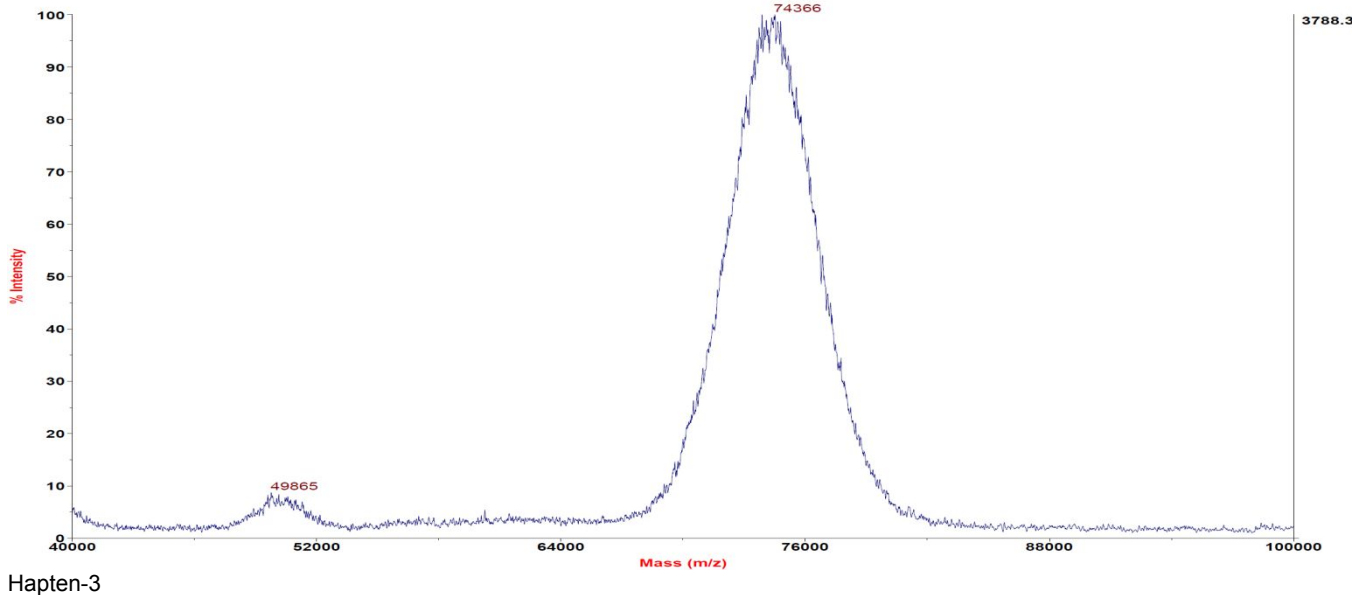
Hapten	MALDI MS	Copy number	Hapten	MALDI MS	Copy number
1	72434	20	6	71861	15
2	74366	20	7	73808	17
3	72952	15	8	75738	19
4	73261	15	9	76566	21
5	73898	15	10	72481	15

MALDI-TOF spectrum of BSA conjugate

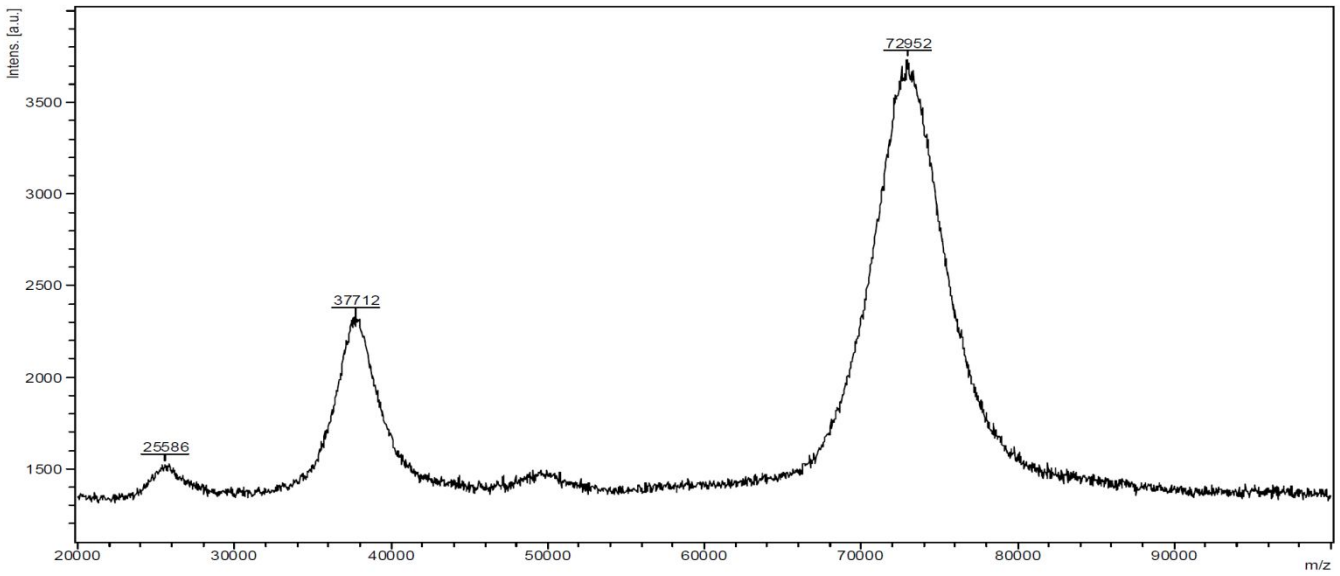
Hapten-1



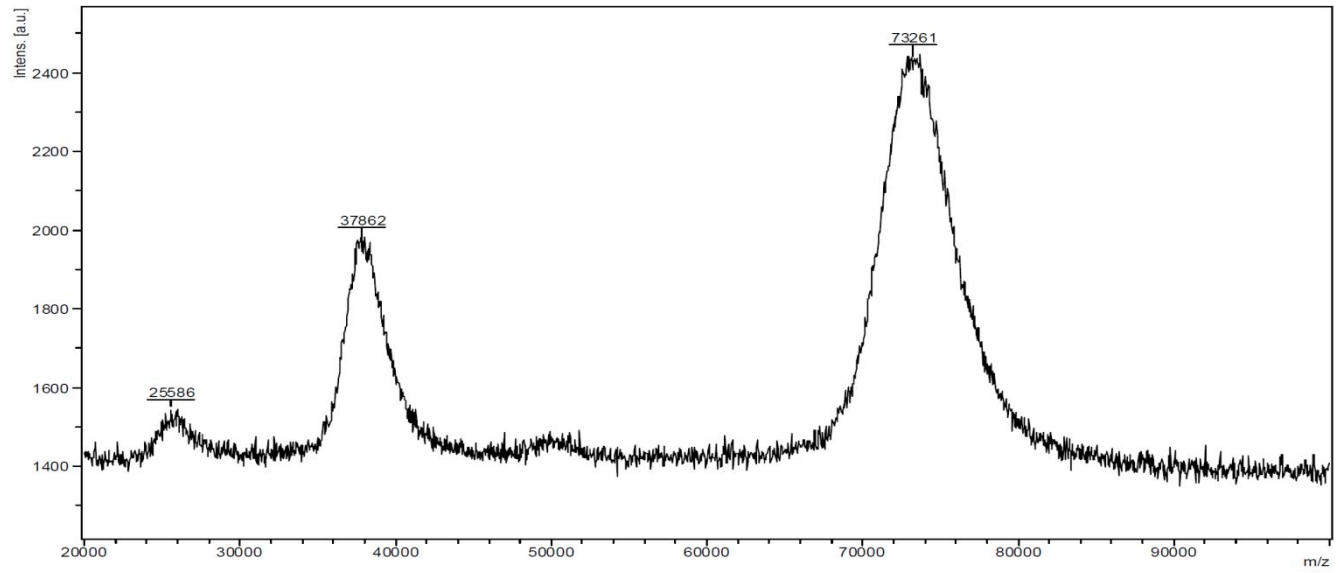
Hapten-2



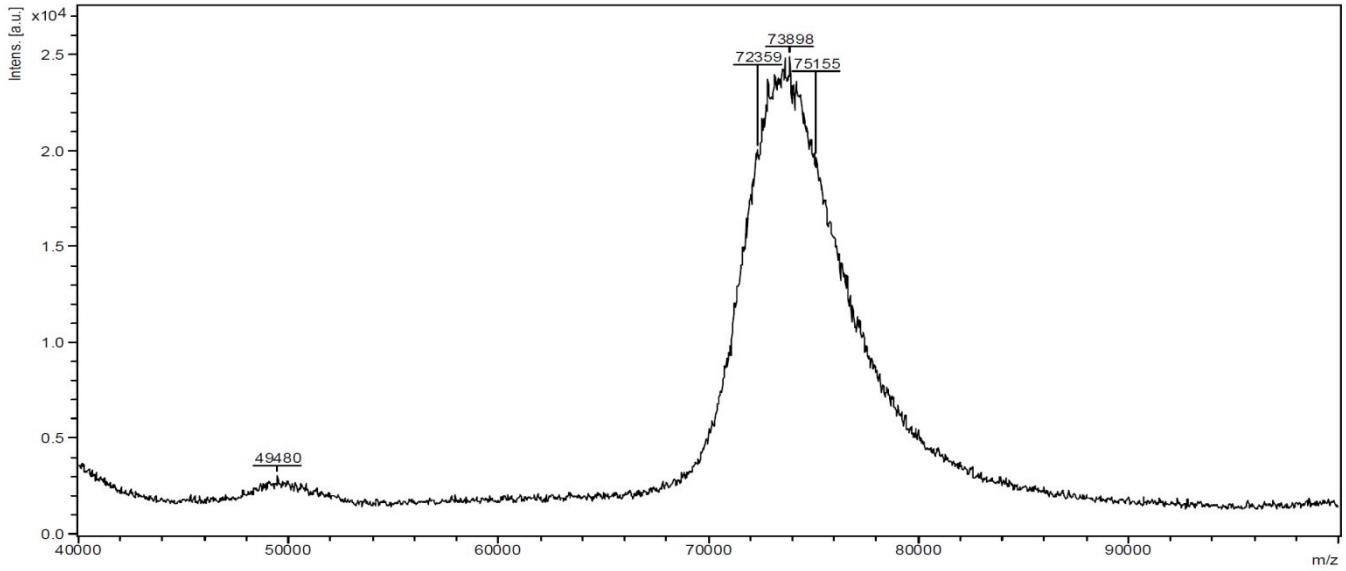
Hapten-3



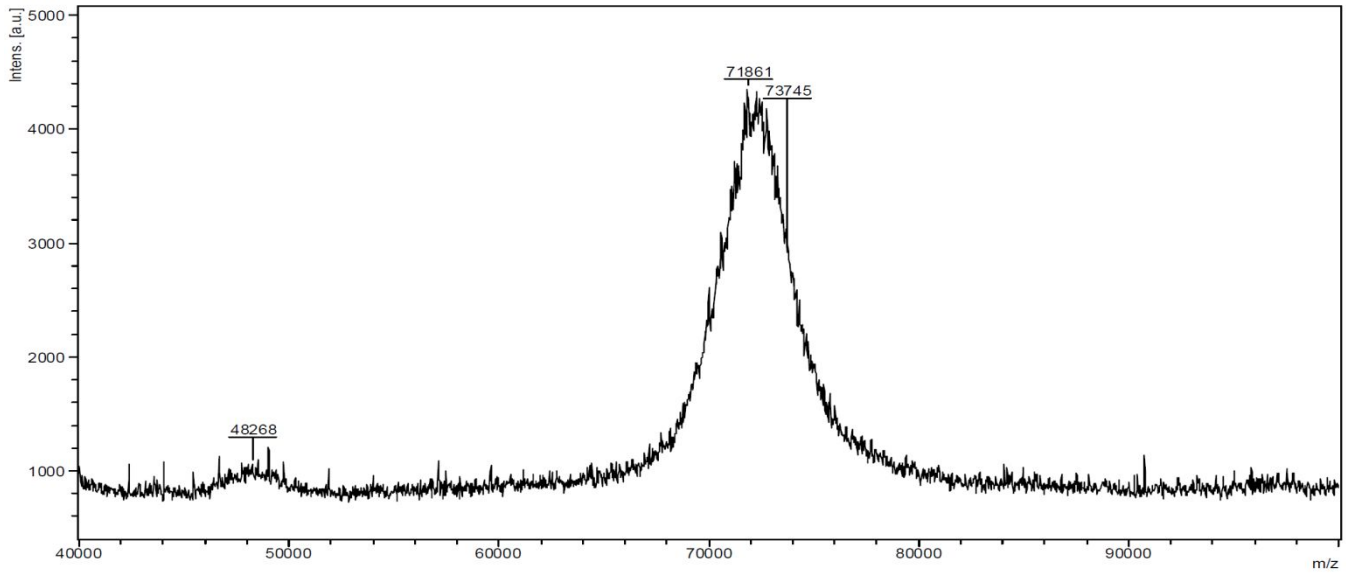
Hapten-4



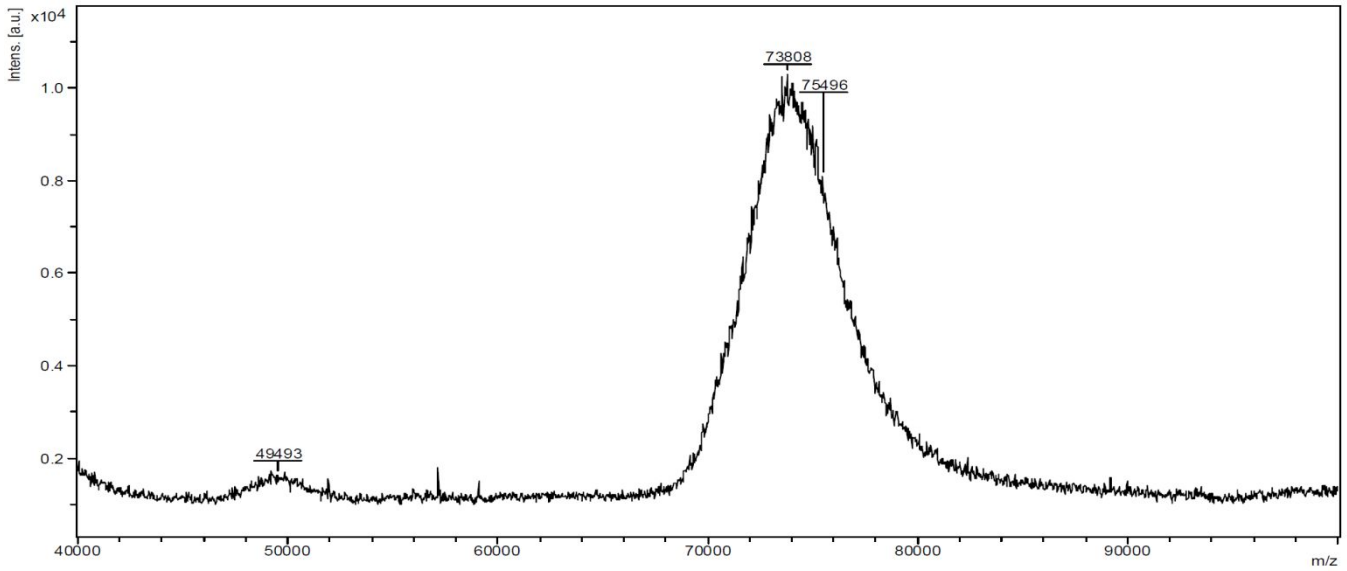
Hapten-5



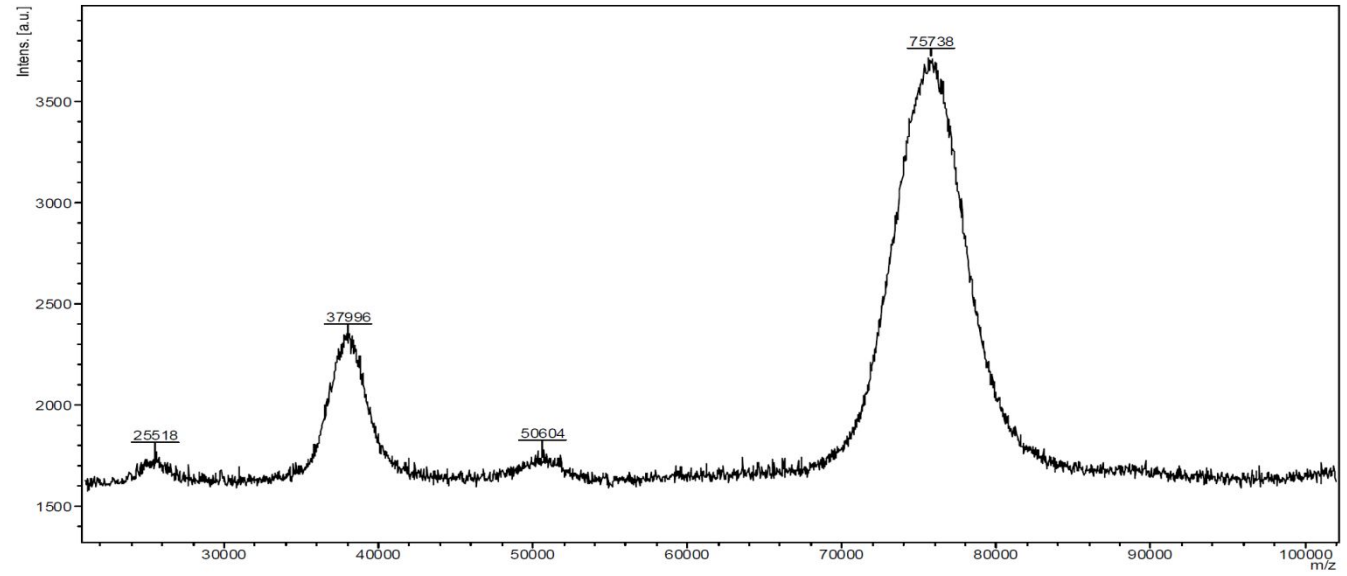
Hapten-6



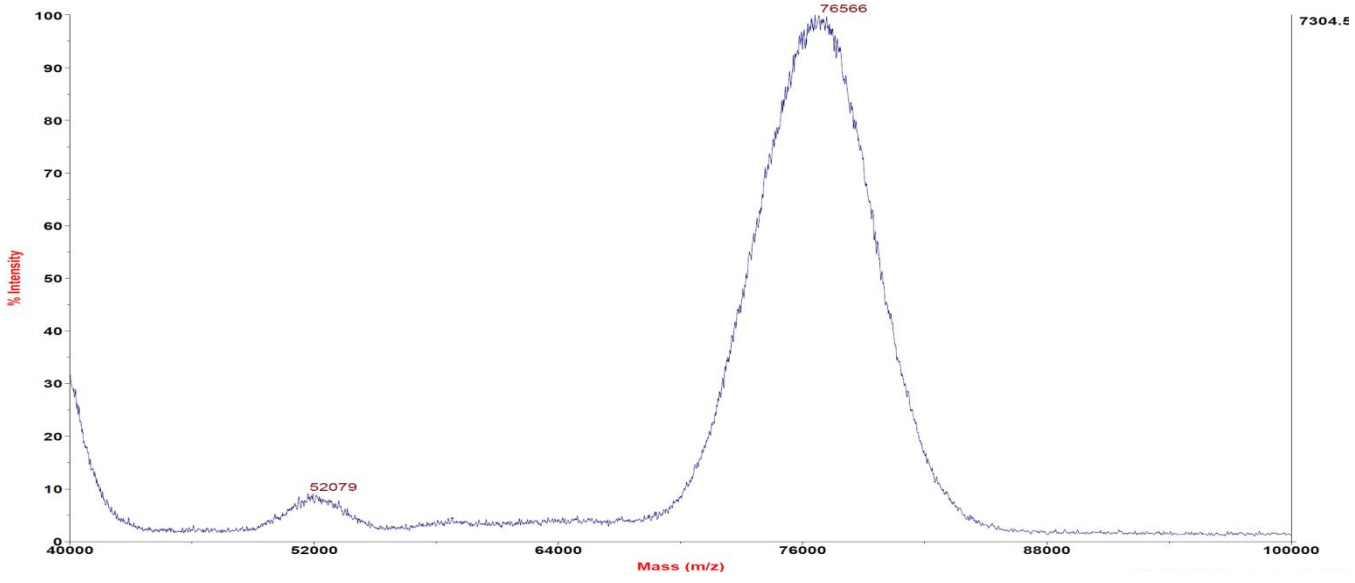
Hapten-7



Hapten-8



Hapten-9



Hapten-10

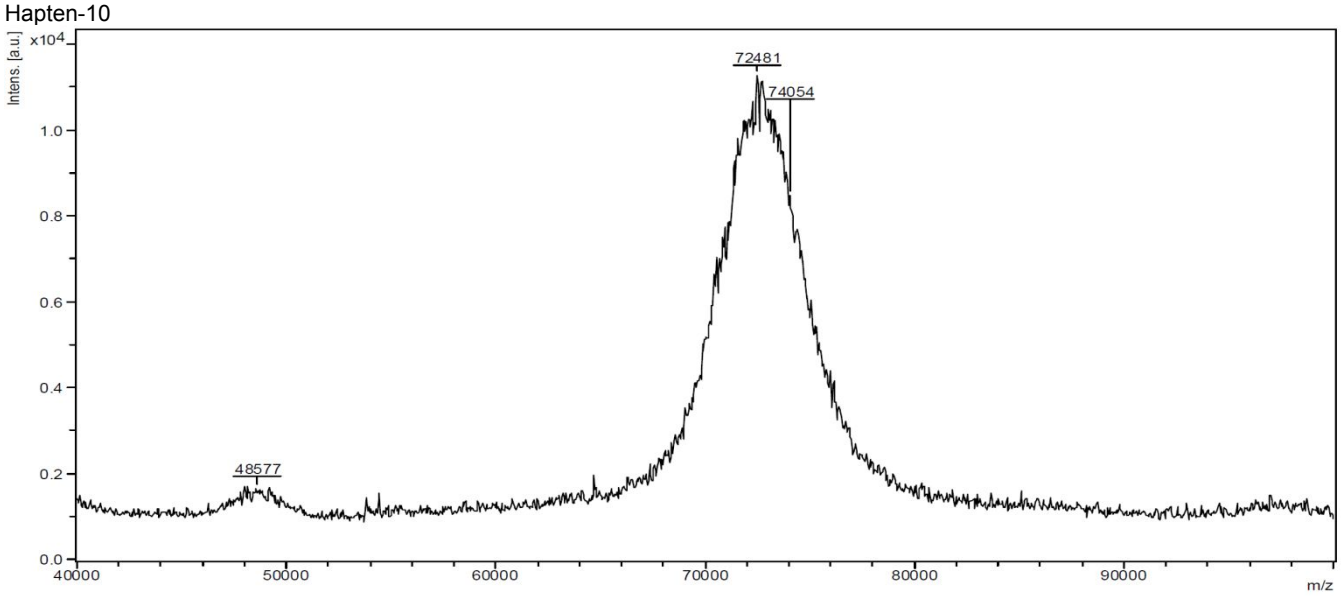
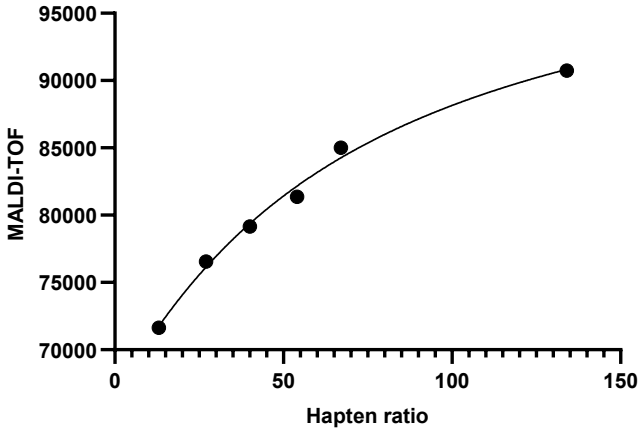


Figure S1. MALDI mass versus Hapten to BSA molar ratio.



Hapten 9 was using as an example. Non-linear regression fit were obtained in GraphPad Prism.

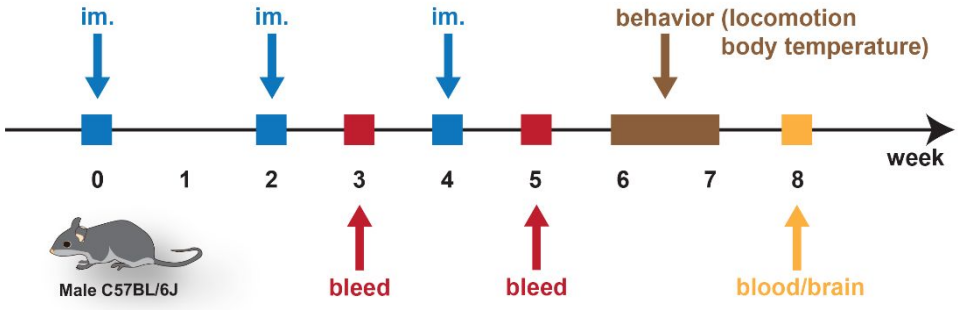
Behavior experiment

Statistical analyses

All statistical analyses in behavior and ELISA were conducted with GraphPad Prism 8.

Vaccine schedule

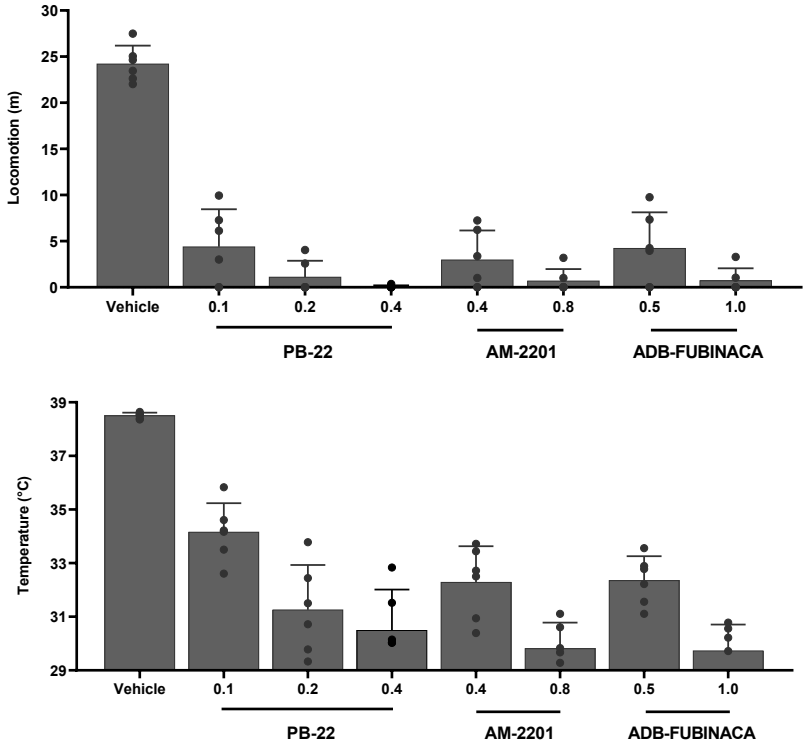
Figure S2. Vaccine schedule.



Vaccination schedule on male C57BL/6J mice, including three immunization, two bleeding periods, behavior tests and blood-brain biodistribution assay.

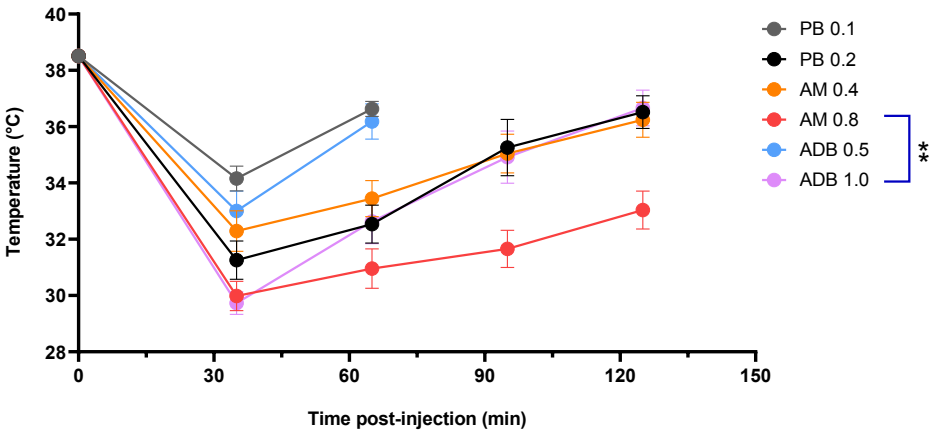
Locomotion and temperature

Figure S3. Behavior results of locomotion and body temperature in male mice.



All experimental groups are significant versus control (n=8, P<0.001) determined by one-way ANOVA, with individual data points shown.

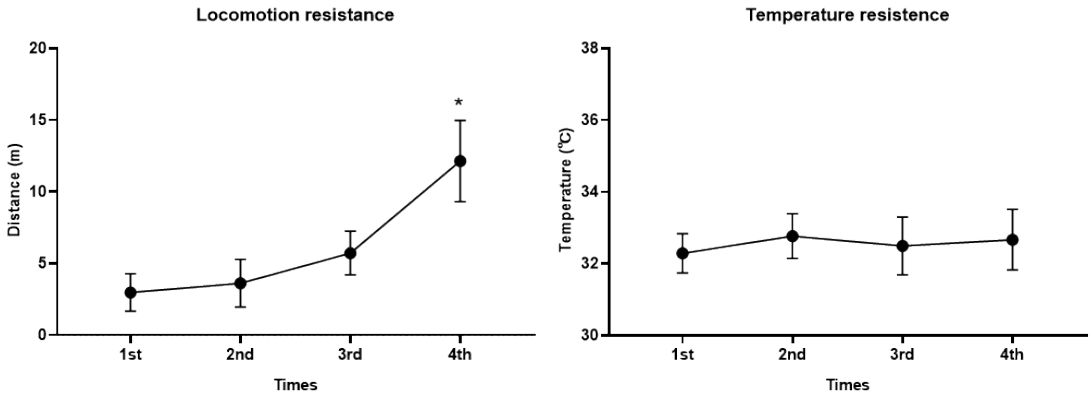
Figure S4. Drug duration effect.



Temperature was measured 35, 65, 95, 125 min after drug administration. Significance in slope of linear regression starting from 35min between 1mg/kg ADB-FUBINACA and 0.8mg/kg AM-2201 is denoted by asterisk (n=8, **P<0.01). All symbols are shown as mean ± SEM.

Tardiness in recover rate especially for Class I owing to their higher hydrophobicity were observed.

Figure S5. Resistance development after repeated administration.



Repeated drug administration was performed once a week. Significance is denoted by an asterisk determined by one-way ANOVA (n=6, *P<0.05). All symbols are shown as mean ± SEM.

Despite tolerance assay usually performed on consecutive daily basis, we were interested in testing once a week which was how long the drug believed to be roughly cleaned from the body and we saw the tolerance started to develop in locomotion but not the temperature. One possible explanation associated with this phenomenon could be the different sensitivity of brain structure in control.[6]

Conditioned place preference

As a standard method for addiction, conditioned place preference has been applied in SCs through a modified paradigm where pre-injection was included to prevent aversion of first time drug encounter.[7]

Mice were tested in a dedicated two chamber CPP apparatus (267 × 483 × 203 mm) distinguished by color (black vs white walls) and texture (smooth vs textured floor). The time spent in each chamber was recorded by ANY-Maze video tracking software (Stoelting Co., Wood Dale, IL). Test was conducted in 4 phases, including pre-test, pre-injection, conditioning and test.

Mice were allowed to freely explore the apparatus for a 20min session on the pre-test day (day 1). Time spent in each chamber was taken as non-treated preference control. The group was assigned into two groups using unbiased design so that the average preference is close to 50% and similar with each other (Table below).

Before conditioning phase, an additional pre-injection phase was engaged to offset any odd feeling when drug was administrated for first few times. One group of mice received four daily (days 2-5) i.p. injection of 0.1 mg/kg drug JWH-081 before the conditioning sessions in their home cage, while the other group just received saline.

Followed by 8 days conditioning phase (days 6-13) when a wall was placed in between the two chambers. All mice received 0.1 mg/kg drug and were put into their paired chamber for 1h on day 6, 8, 10, 12. On alternate day 7, 9, 11, 13, all mice were given vehicle only and put into the unpaired chamber for 1h.

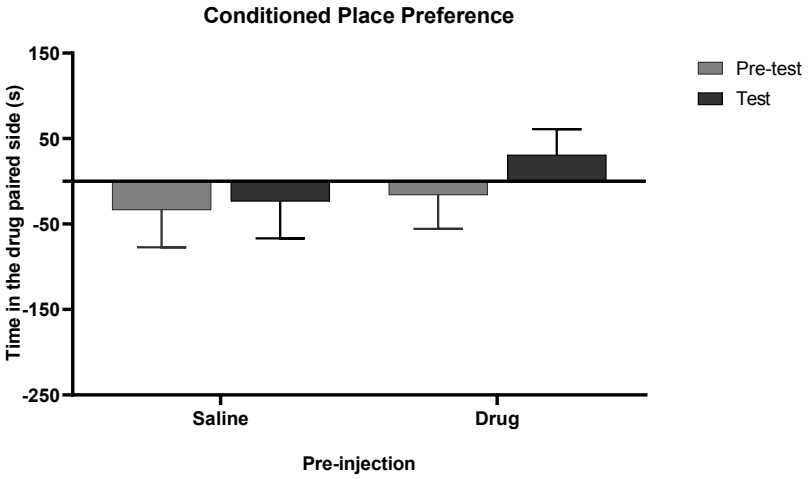
The dividing wall was removed on test day and mice were allowed to freely explore the full apparatus again for 20 min. Time spent in each chamber was recorded and the preference score was calculated by subtracting the time spent in the paired chamber to 600 s, which was then compared with the non-treated preference control.

Table S3. Mice assignment in an unbiased conditioned place preference schedule.

Cage	Mouse	Time on side A	Drug paired side	Pre-injection
3	3	445 s	A	Drug
4	2	455 s	B	Drug
1	2	476 s	A	Drug
4	4	490 s	B	Drug
1	3	503 s	A	Drug
3	2	534 s	B	Drug
2	4	590 s	A	Drug
2	1	663 s	B	Drug
3	1	330 s	A	Saline
1	1	487 s	B	Saline
4	1	495 s	A	Saline
1	4	509 s	B	Saline
2	3	540 s	A	Saline
3	4	547 s	B	Saline
2	2	562 s	A	Saline
4	3	652 s	B	Saline

Group	Time on drug paired side	SEM
Saline	566.50s	43.62s
Drug	584.00s	39.71s

Figure S6. Conditioned place preference.



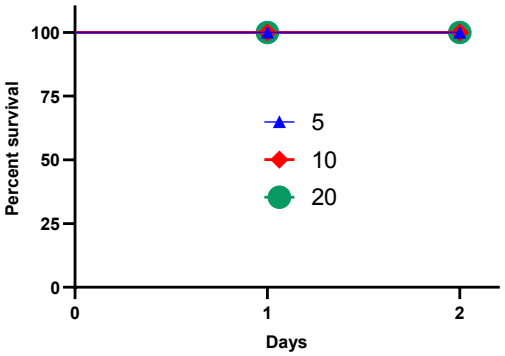
Time spent in the drug paired sites before and after conditioned phase measured in mice (n=8) with or without drug pre-injection. Repeated-measures two-way ANOVA ($P_{interaction}$=0.70, $P_{conditioned}$=0.55). All bars are shown as mean ± SEM.

No preference was induced regardless of pre-injection in this mice strain suggesting that it is not a qualified vaccine model.

Lethality dose

Male C57BL/6J mice (n=6 / group) were injected intraperitoneally with 5 mg/kg, 10 mg/kg and 20 mg/kg AM-2201 in saline. The mice were visually monitored over a period of 48 hours for lethal events.

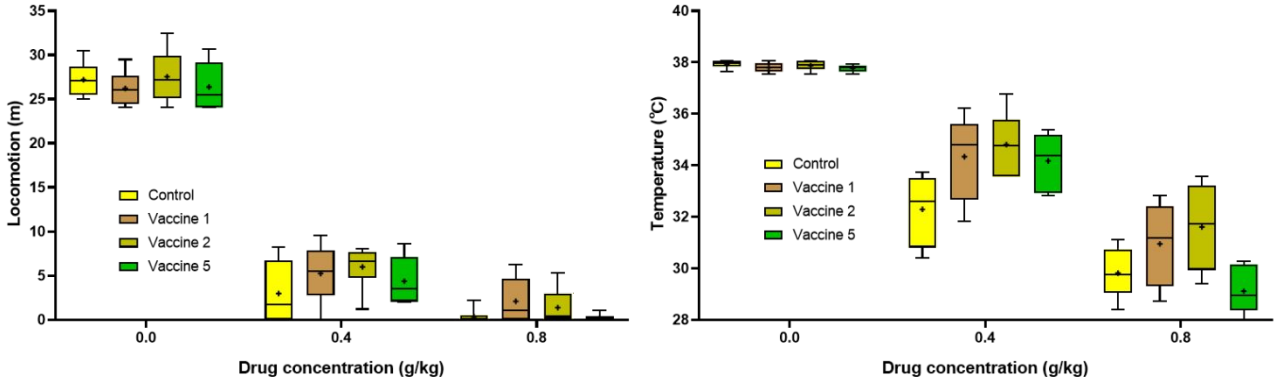
Figure S7. Survival curve.



To our surprise, after several hours of chilling and jerking no death appeared in two days up to dose of 20 mg/kg.

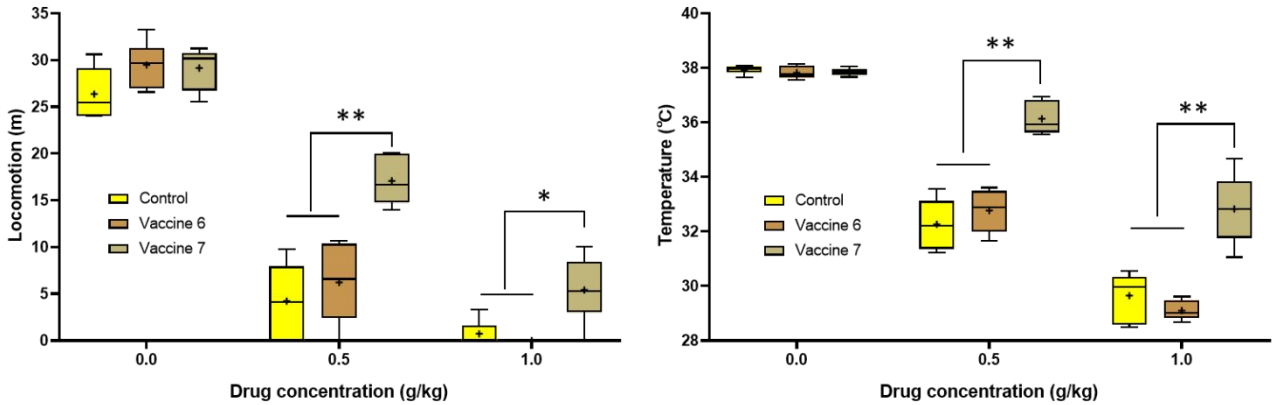
Behavior results of other vaccines

Figure S8. Behavior results of other Class I vaccines.



Behavior results in mice locomotion and body temperature of Vaccine **1**, **2**, and **5** compared to the KLH vaccinated control group. Mice accepted i.p. injection of AM-2201 twice with different dose at one week interval. Data are shown as median with quartiles ± 10–90% CI (n=6); +, mean.

Figure S9. Behavior results of other Class II vaccines.

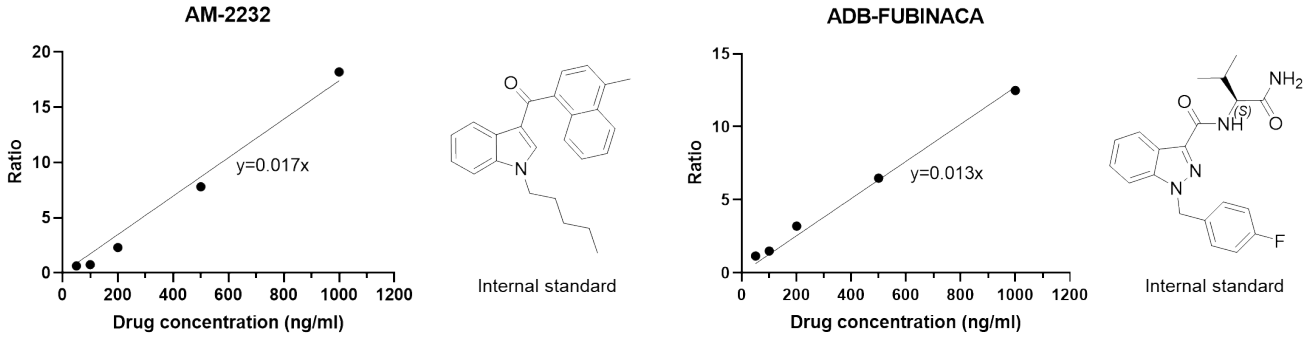


Behavior results in mice locomotion and body temperature of vaccine **6** and **7** compared to the KLH vaccinated control group. Mice accepted i.p. injection of ADB-FUBINACA twice with different dose at one week interval. Data are shown as median with quartiles ± 10–90% CI (n=6); +, mean.

Although not as effective as vaccine **8** and **9**, vaccine **7** did show some protection capability towards ADB-FUBINACA.

Blood/brain biodistribution

Figure S10. Standard curves and internal standard.



Vaping experiment

Figure S11. Vaping apparatus.

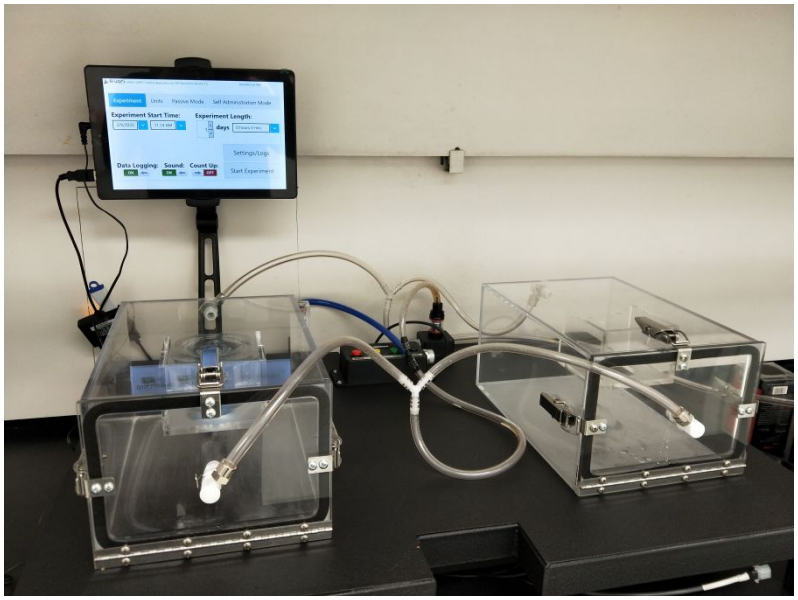
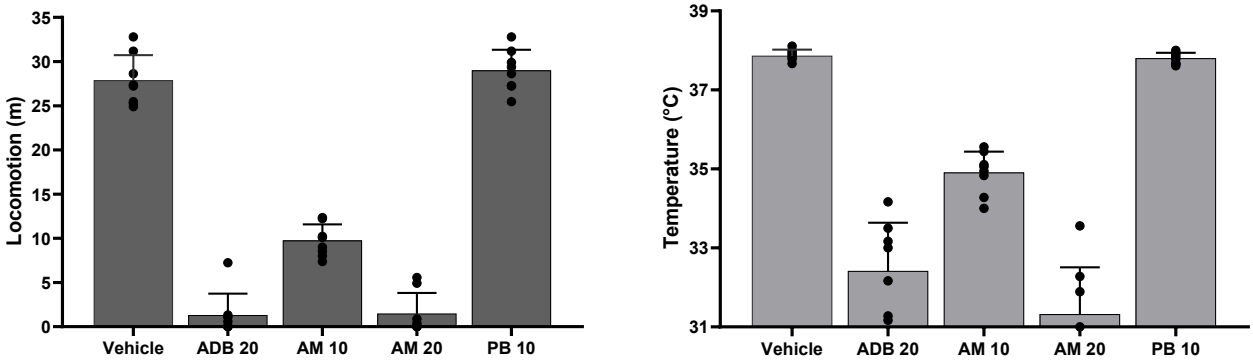
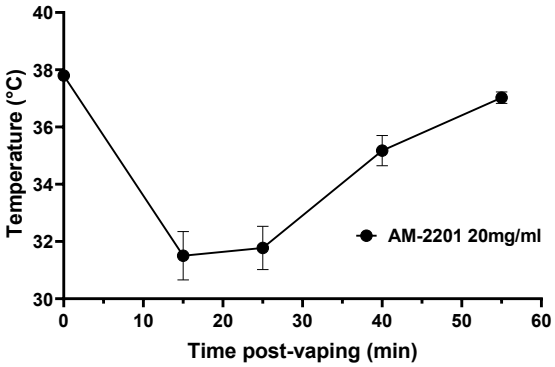


Figure S12. Vaping behavior results.



ADB, ADB-FUBINACA; AM, AM-2201; PB, PB-22; 10,20, dose unit (mg/mL). All experimental groups except PB 10 are significant versus control (n=6, P<0.0001), with individual data points shown. All bars are shown as mean ± SEM.

Figure S13. Vaping duration effect.



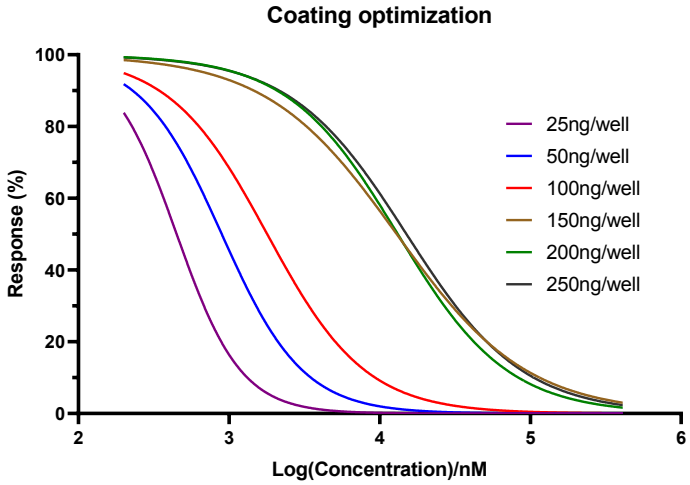
Duration was tested using AM-2201 as surrogate monitored at the time point of 15, 25, 40, 55 min. n=6.

ELISA assay

Antibody titer

There has been some discussions of what concentration of coating antigen to be used for this type of assay. Thus, we examined the titer at a series of coating concentration. While the fitting curve presented a gradual IC50 increase, it was then clear that there was a huge leap between 100 and 150ng/well and it remained relatively steady even the concentration was further increased corresponding to the theory that a stable bivalent binding pattern was achieved.[8]

Figure S14. Optimization of coating concentration.



Using Hapten-9 BSA conjugate as example.

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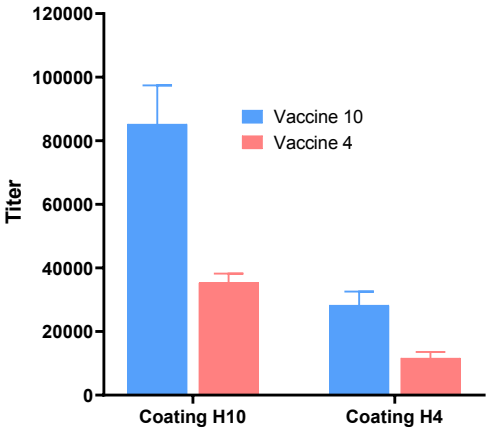
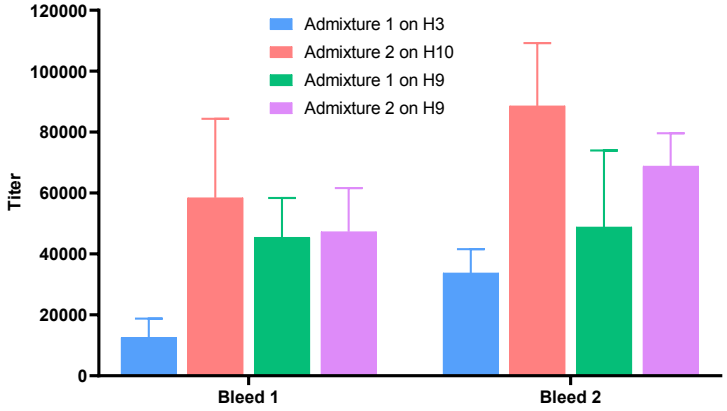
Figure S15. Titer comparison of Hapten 4 and 10.



All bars are shown as mean ± SEM (n=6).

Figure S16. Titer of admixture vaccine.



All bars are shown as mean ± SEM (n=6). No significance concluded when compared to individual vaccine.

Antibody concentration

Interpolated titer ELISA was used to calculated the approximate antibody concentration. 1mg/mL Carfentanyl monoclonal antibodies and its coating hapten BSA conjugate were applied using the same indirect ELISA format to obtain the standard curve (Figure S17). Specific antibody concentrations were back calculated by selecting dilutions of vaccine groups that produce OD 450nm values within the boundary of the standard curve.

Figure S17. Standard curve for antibody concentration.

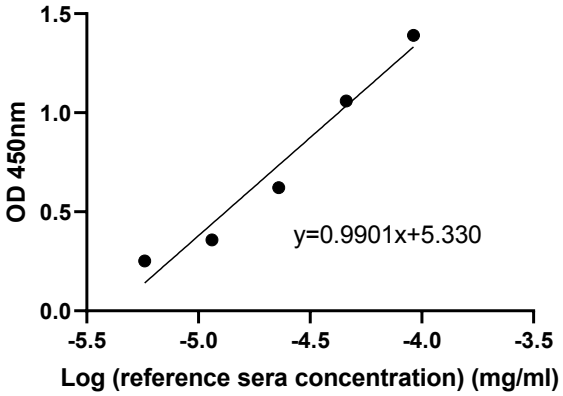
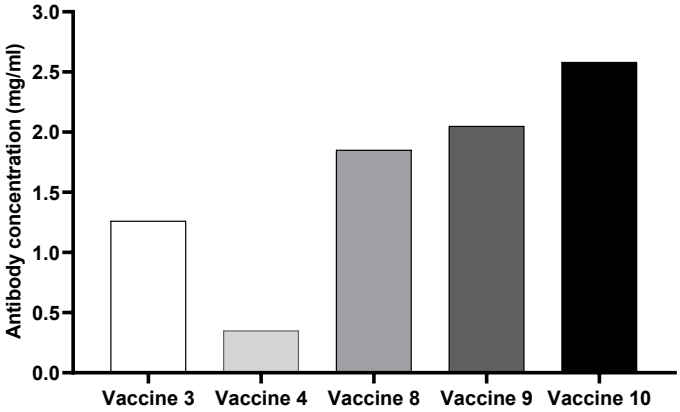


Figure S18. Antibody concentration.



Concentrations were determined using pool anti-sera.

Competitive ELISA - drug affinity

Figure S19. Competitive ELISA curve sets.

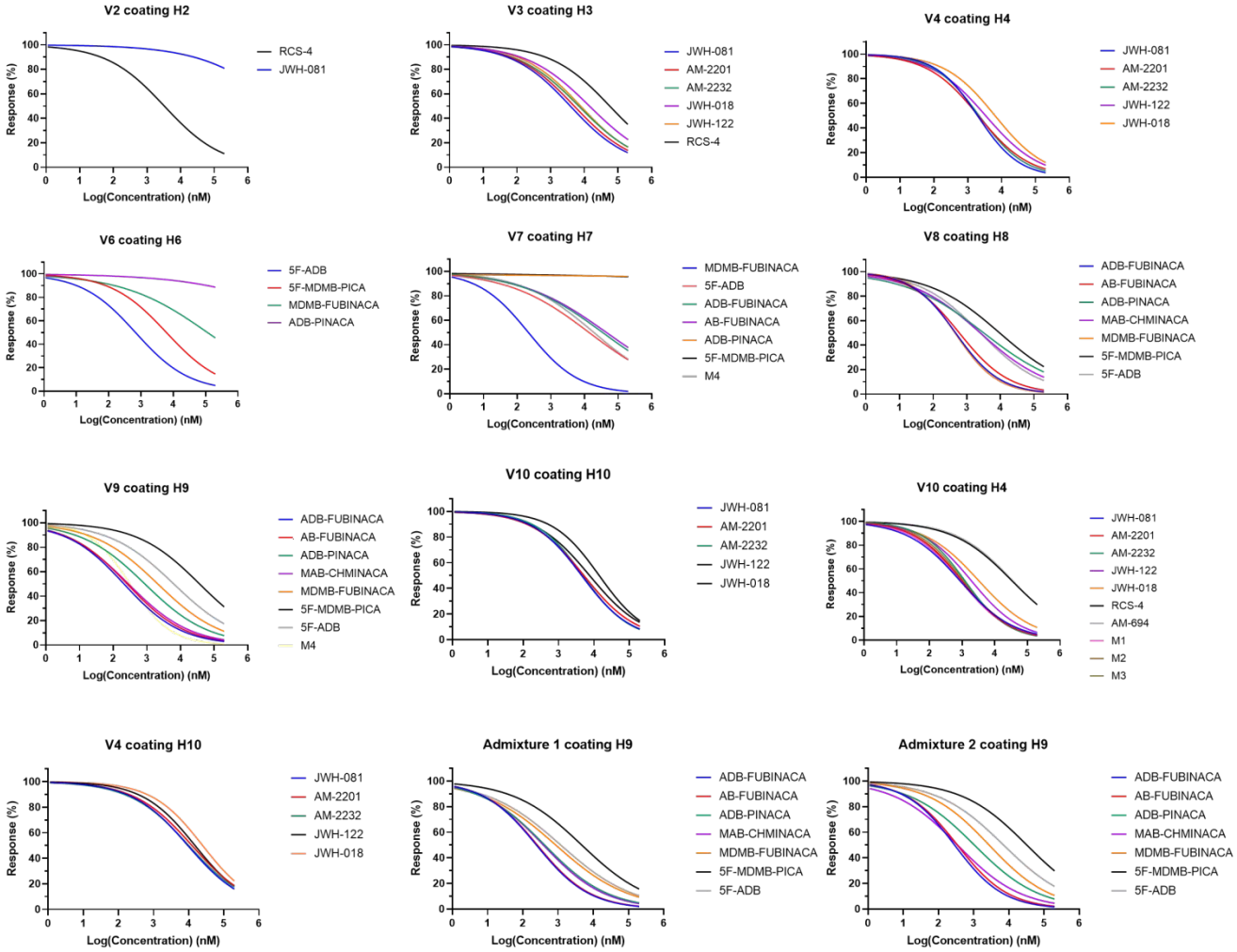


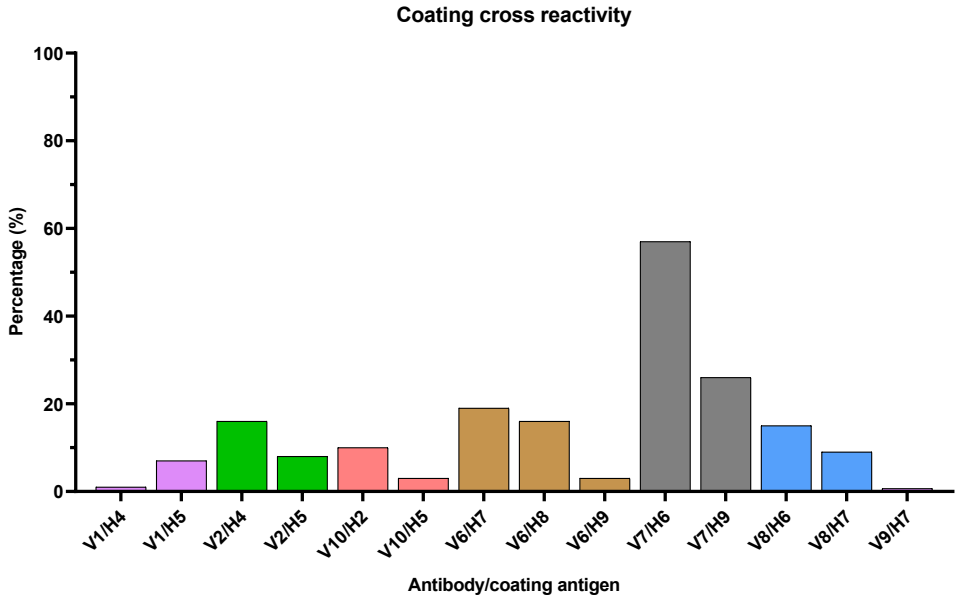
Table S4. Affinity of admixture vaccine 1 and 2 on Class I drugs.

Drug	Admixture 1		Admixture 2	
	Coating Hapten 3		Coating Hapten 4	
	IC_{50} (μM)	CR (%)	IC_{50} (μM)	CR (%)
JWH-081	3.77	100	0.86	100
AM-2201	4.83	78	0.90	96
AM-2232	6.66	57	0.93	93
JWH-122	7.41	51	1.43	60
JWH-018	11.64	32	2.79	31
RCS-4	75.48	5	-	-

Heterogeneous coating

The prerequisite of using heterogenous coating is the presence of decent titer. Of 11 anti-sera/coating antigen pairings, 4 pairs were selected.

Figure S20. Titer cross reactivity using different coating hapten (antibody group/coating antigen).



Using mice serum pooling from all individuals in each vaccine group (n=6). Percentage was calculated by dividing titer to titer values measured in the homologous coating.

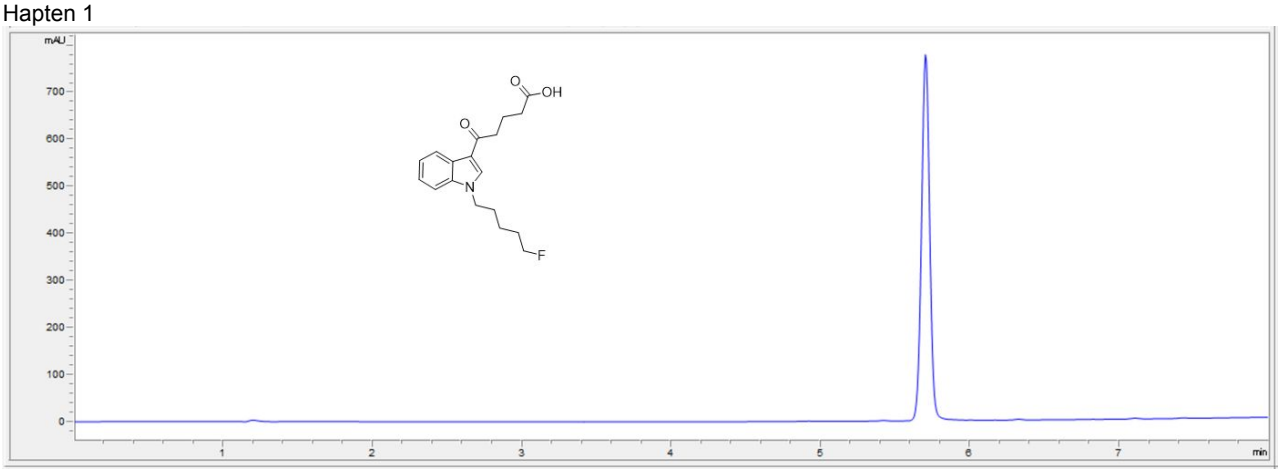
Table S5. Results of Heterogeneous ELISA.[a]

Drug	V10/H2[b]	V1/H5	V2/H4	V2/H5	Drug	V6/H7	V7/H9	V8/H7
	IC_{50} (nM)					IC_{50} (nM)		
JWH-081	24	500	396	228	MDMB-FUBINACA	3708	5	1500
AM-2201	33	601	379	231	5F-ADB	69	644	2125
AM-2232	37	840	643	360	5F-MDMB-PICA	495	-[c]	10415
JWH-122	64	572	385	268	ADB-FUBINACA	-	150	-
JWH-018	71	603	462	274	M4	-	140	-
RCS-4	375	1029	47	36				
M1	28	732	488	285				
M2	31	748	531	312				
M3	34	809	574	339				

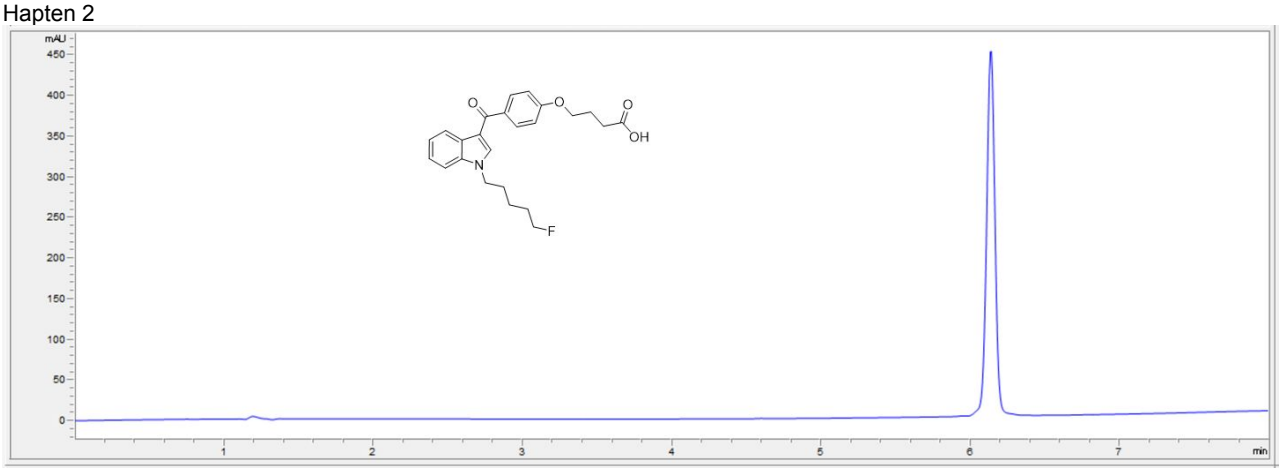
[a] IC50 values are measured by competitive ELISA using mice serum pooling from all individuals in each vaccine group (n=6). [b] paring of antibody groups/coating hapten-BSA conjugates. [c] No affinity detected.

HPLC Trace at 254nm

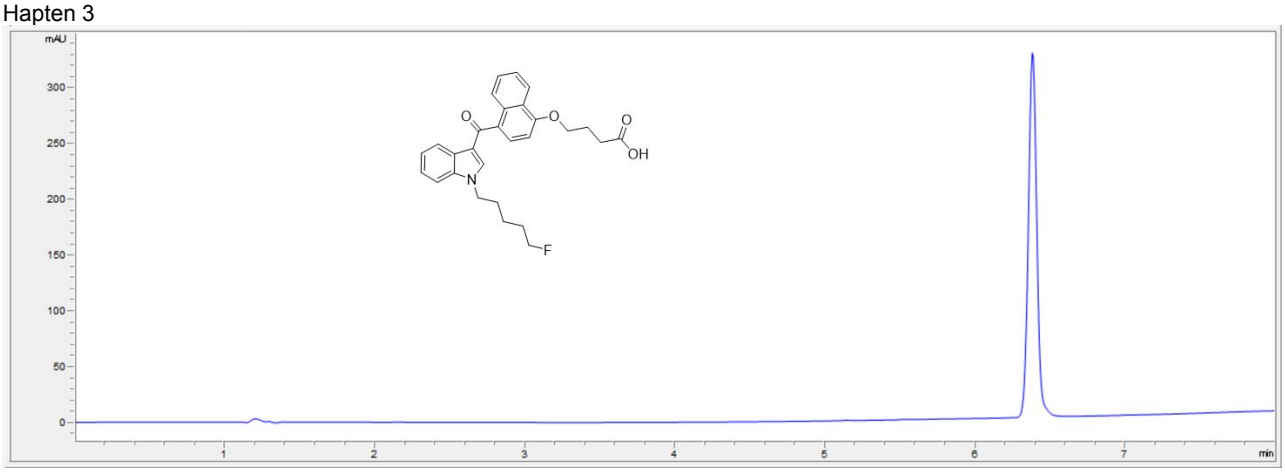
Hapten 1



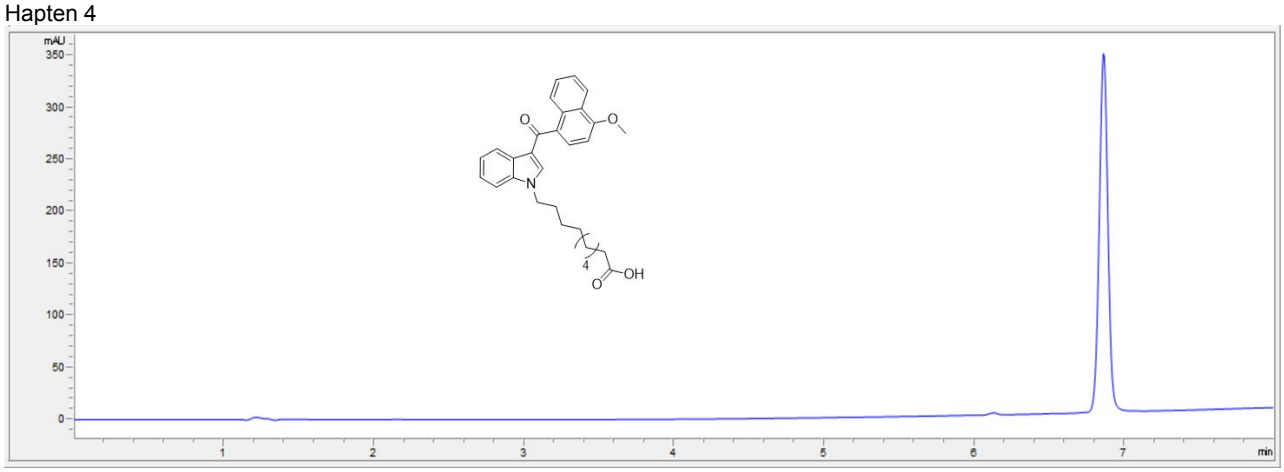
Hapten 2



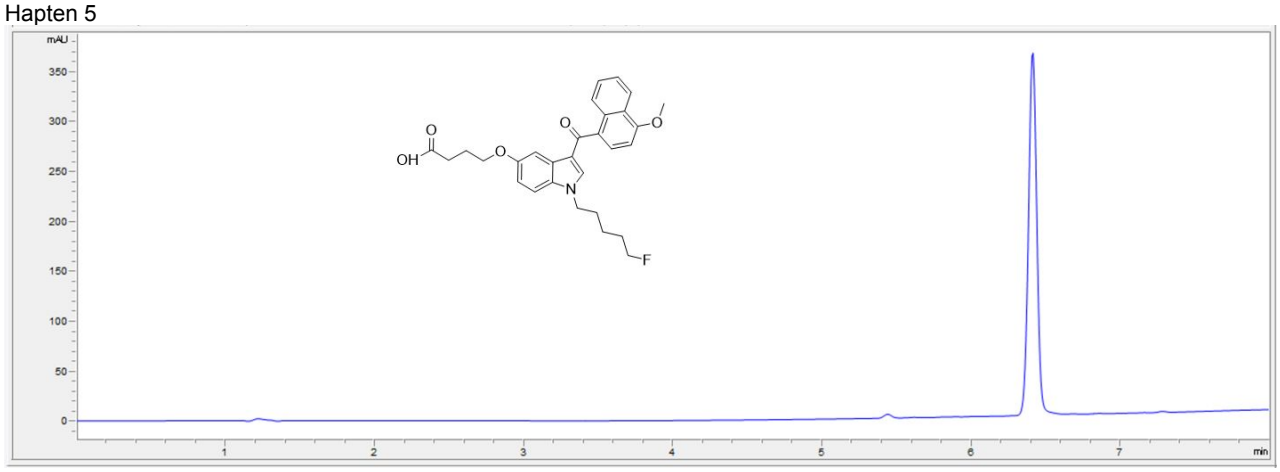
Hapten 3



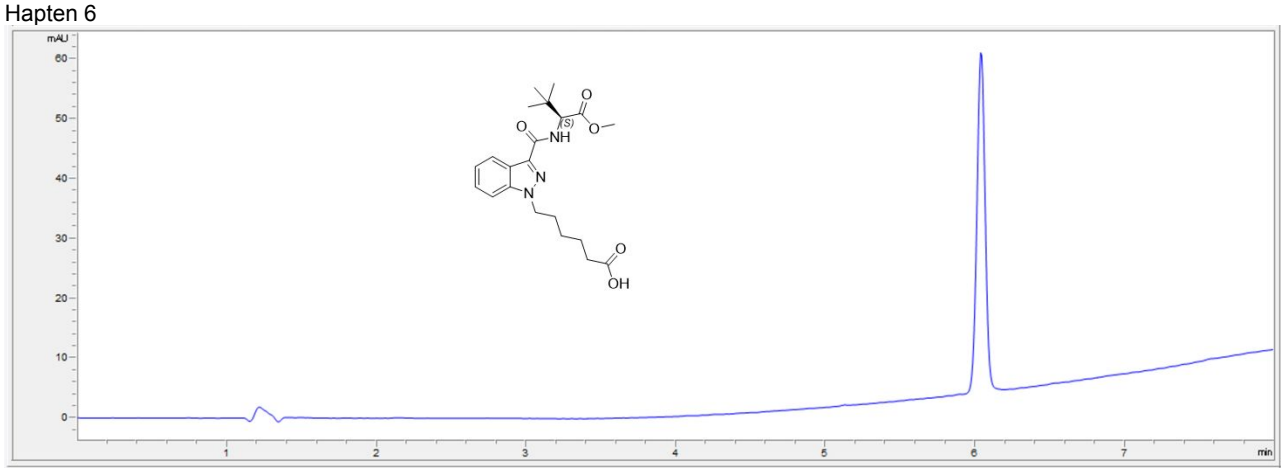
Hapten 4



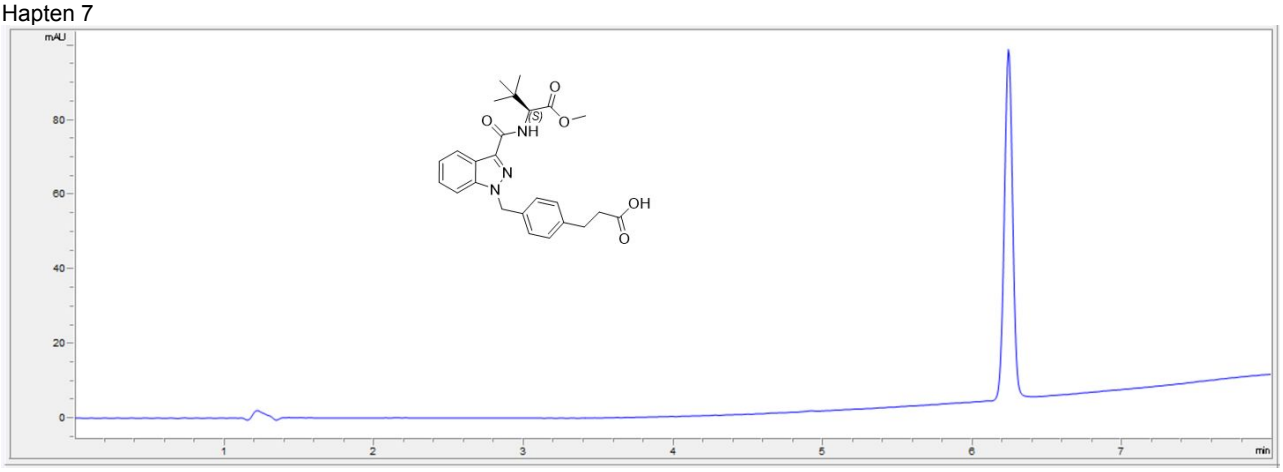
Hapten 5



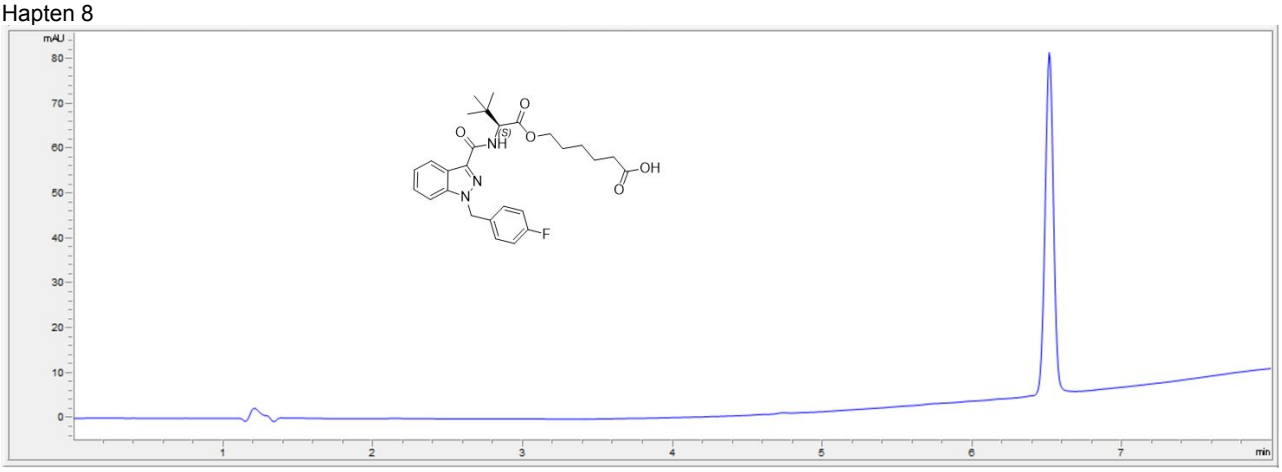
Hapten 6



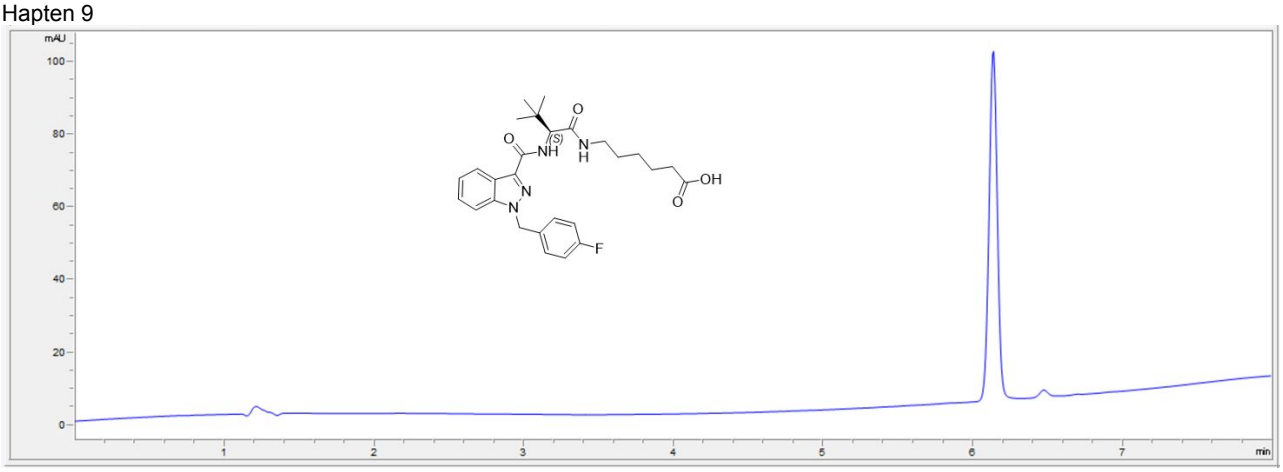
Hapten 7



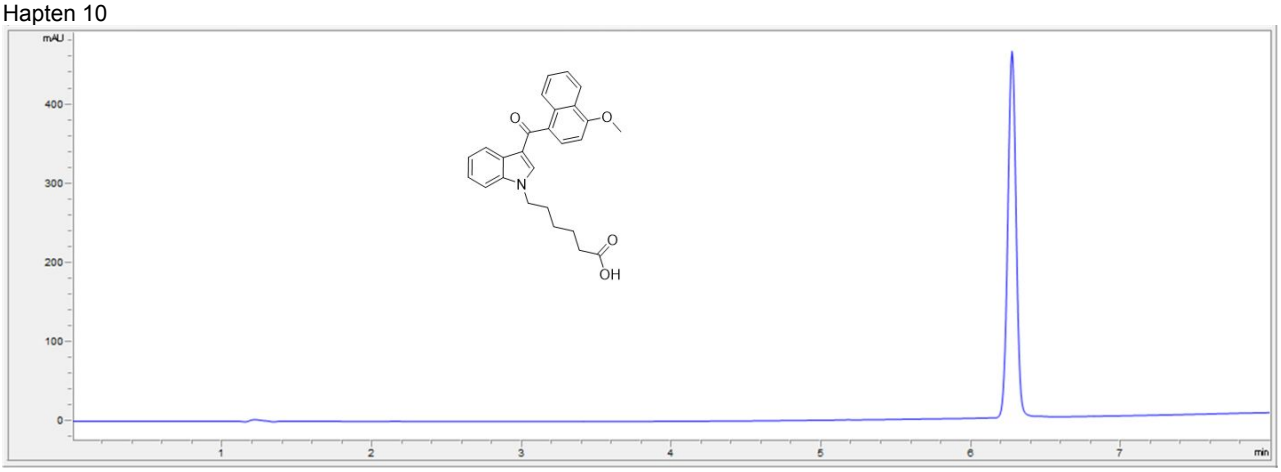
Hapten 8



Hapten 9

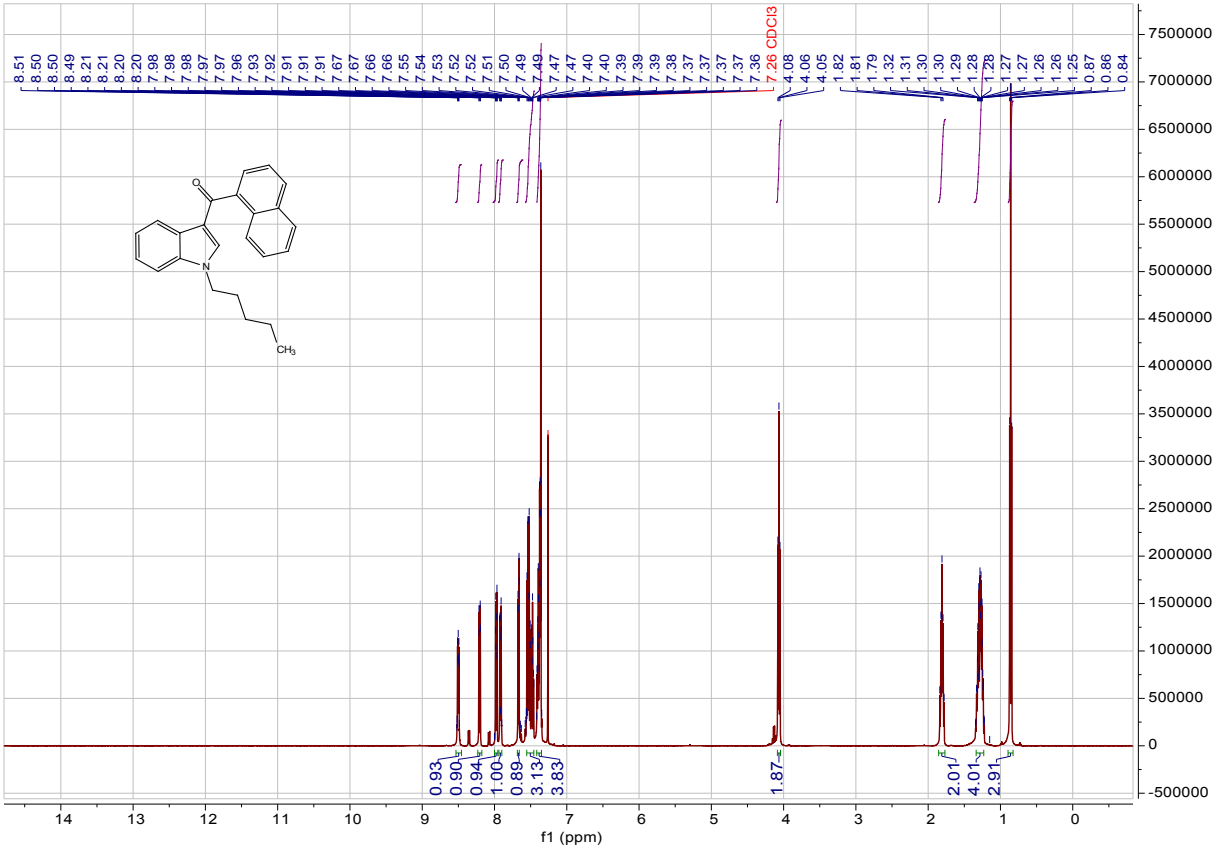


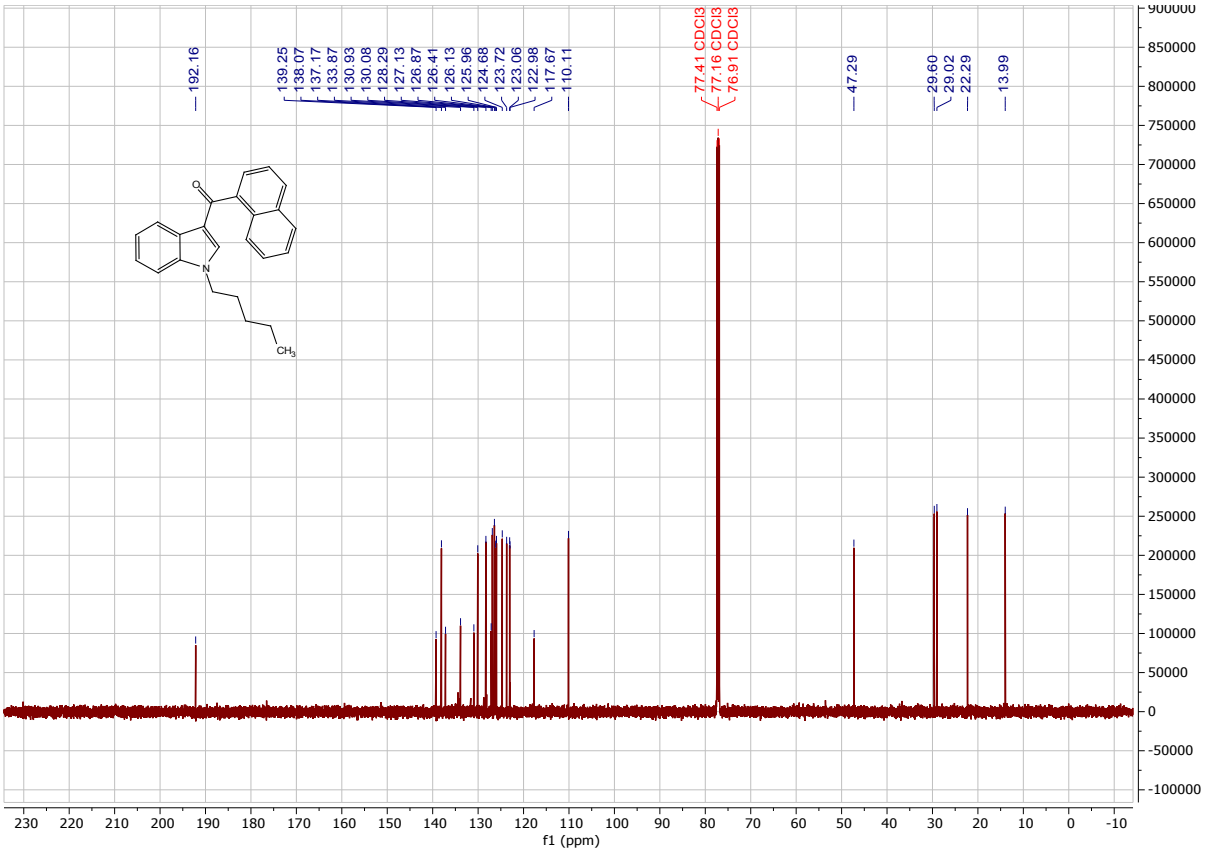
Hapten 10



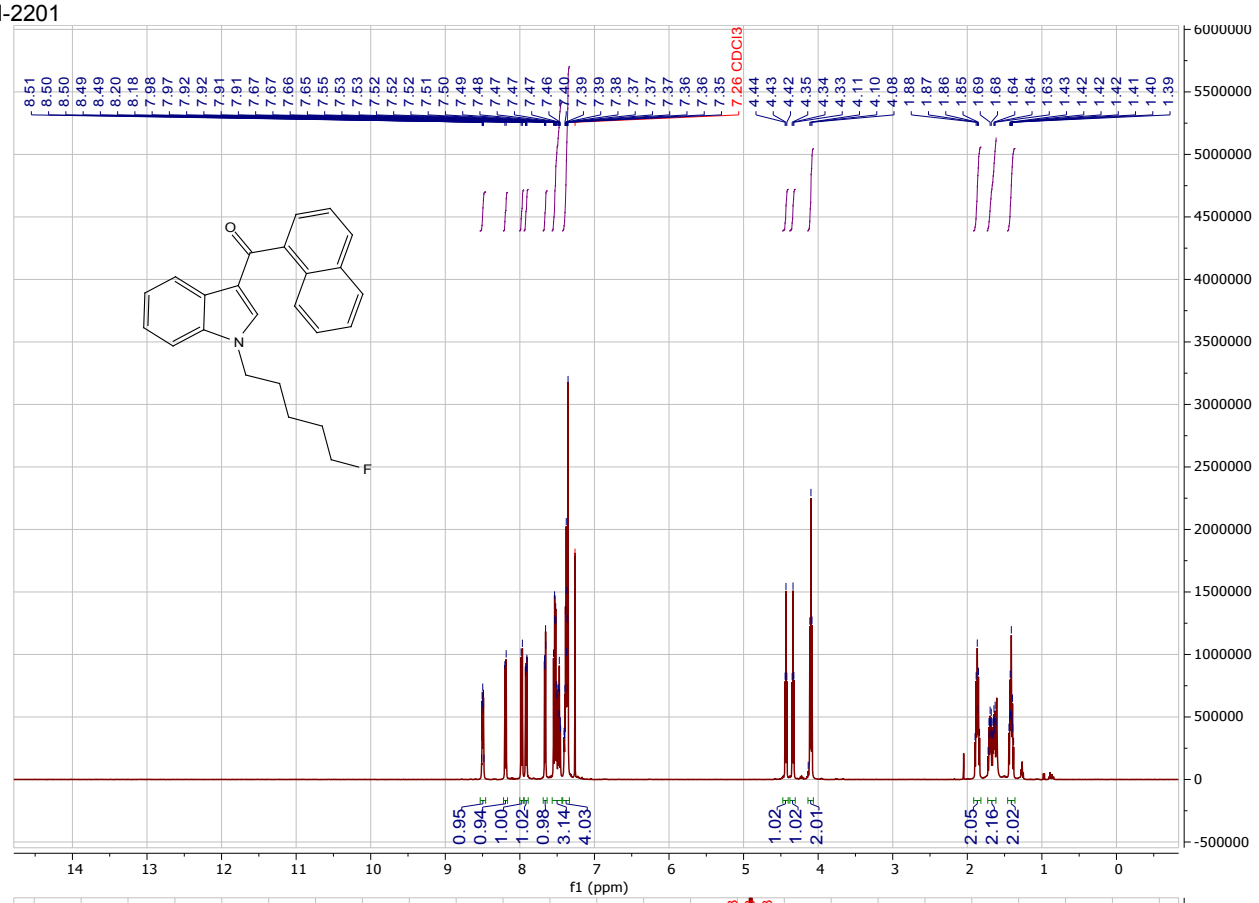
NMR spectrum

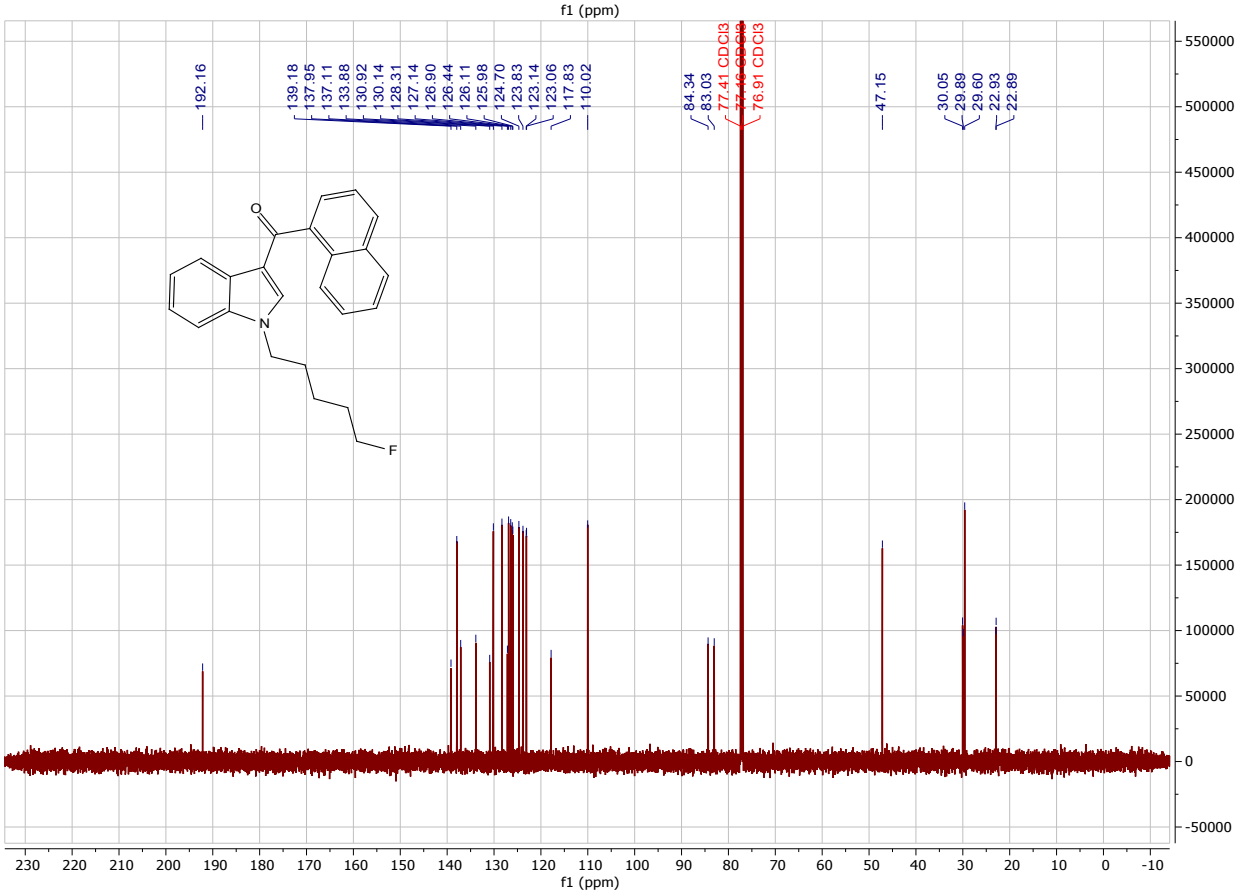
JWH-018



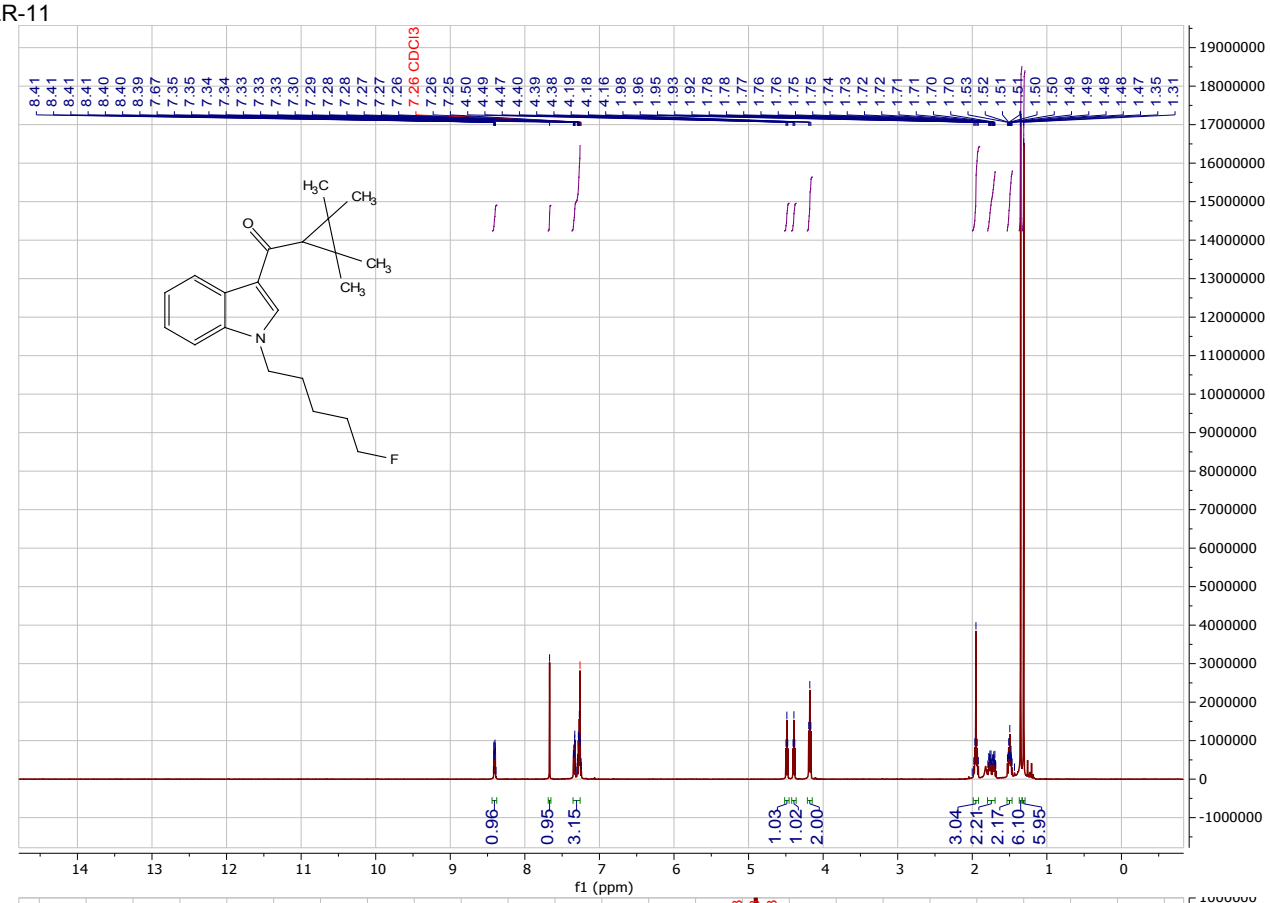


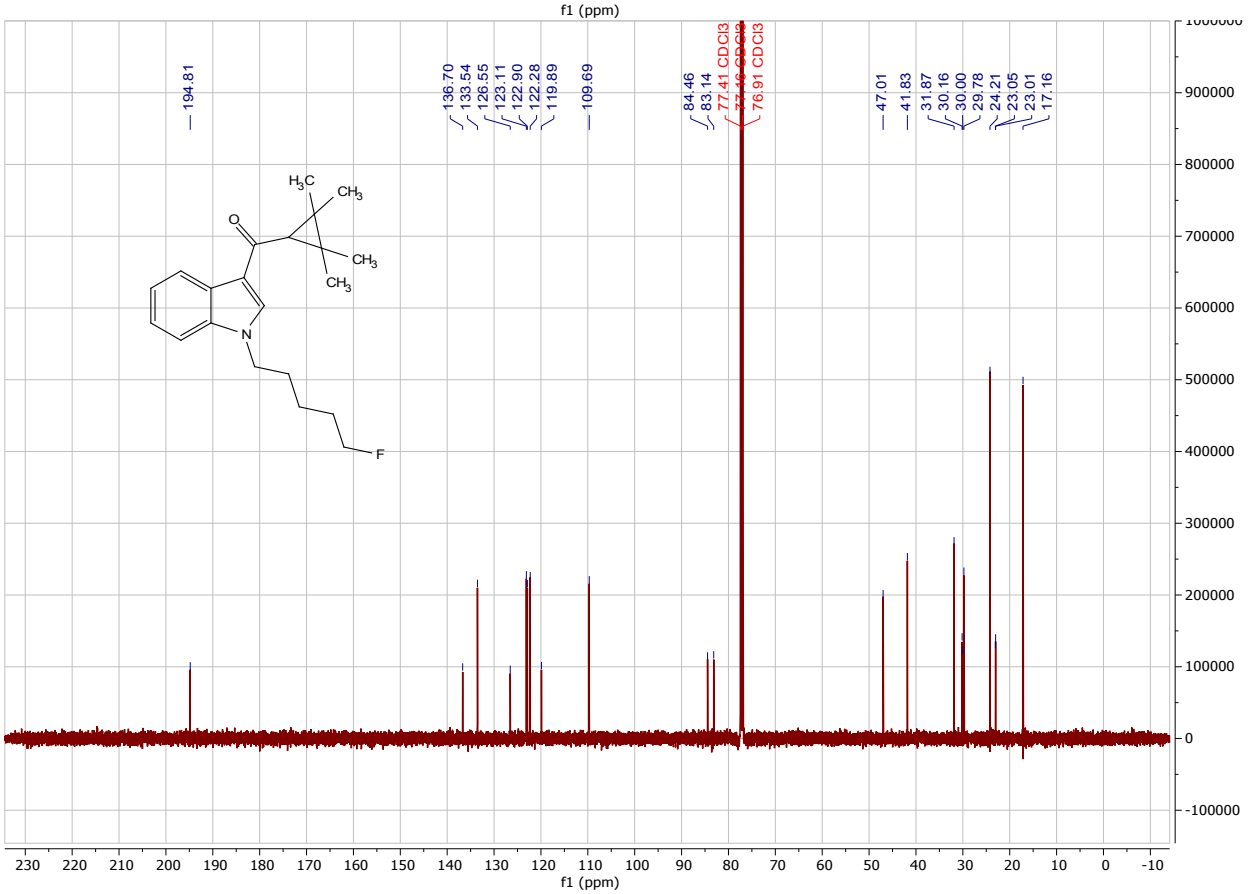
AM-2201


8.51 8.50 8.50 8.49 8.49 8.20 8.18 7.98 7.97 7.92 7.92 7.91 7.91 7.67 7.67 7.66 7.65 7.55 7.53 7.53 7.52 7.52 7.52 7.51 7.50 7.50 7.49 7.48 7.47 7.47 7.47 7.46 7.40 7.39 7.39 7.38 7.37 7.37 7.37 7.36 7.36 7.35 7.26 CDCl3 4.44 4.43 4.42 4.35 4.34 4.33 4.11 4.10 4.08 1.88 1.87 1.86 1.85 1.69 1.68 1.64 1.64 1.63 1.43 1.42 1.42 1.42 1.41 1.40 1.39
O
N
F
0.95 0.94 1.00 1.02 0.98 3.14 4.03 1.02 1.02 2.01 2.05 2.16 2.02
f1 (ppm)

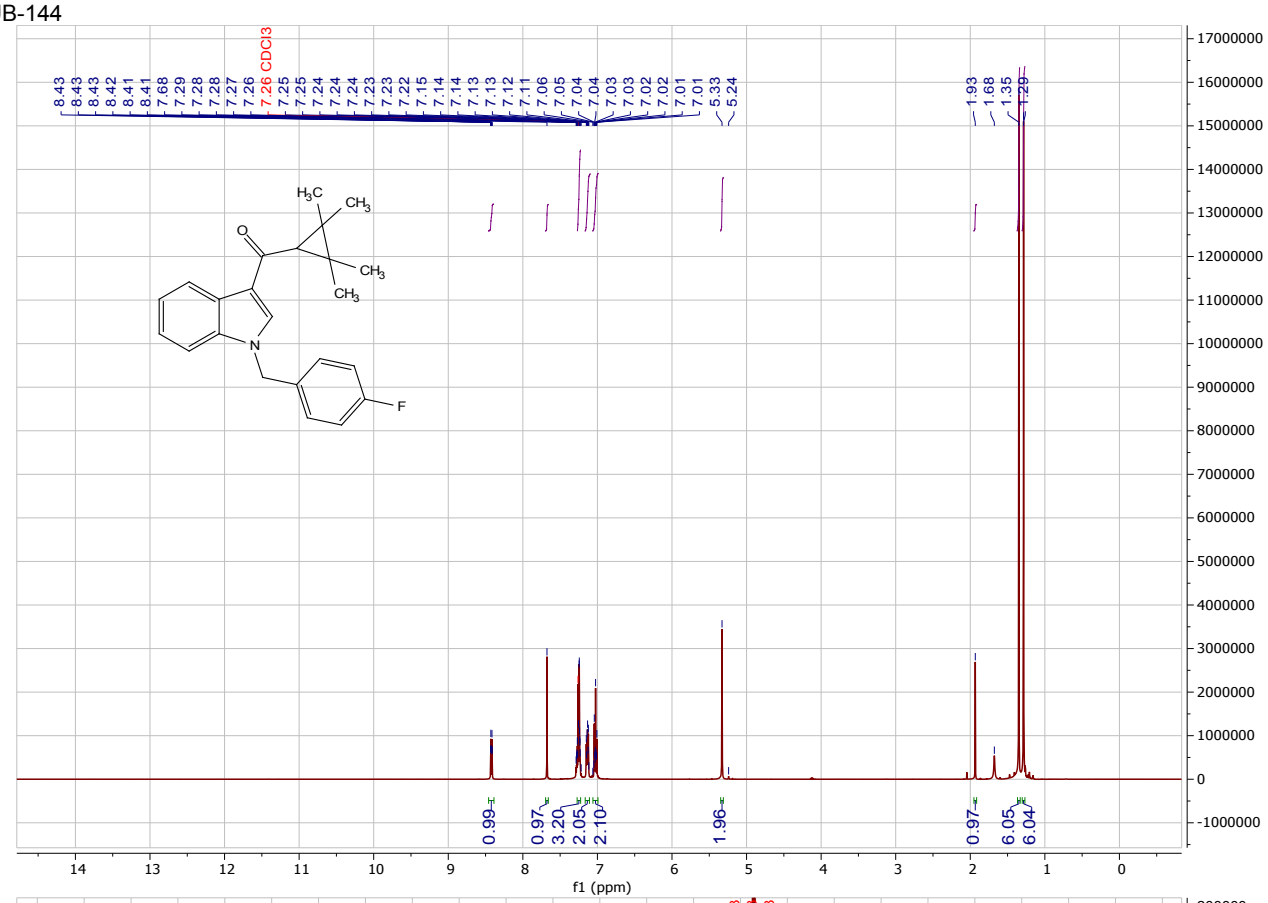

192.16 139.18 137.95 137.11 133.88 130.92 130.14 128.31 127.14 126.90 126.44 126.11 125.98 124.70 123.83 123.14 123.06 117.83 110.02 84.34 83.03 77.41 CDCl3 77.16 CDCl3 76.91 CDCl3 47.15 30.05 29.89 29.60 22.93 22.89
O
N
F
f1 (ppm)

XLR-11


8.41 8.41 8.41 8.41 8.40 8.40 8.39 7.67 7.35 7.35 7.34 7.34 7.33 7.33 7.33 7.30 7.29 7.28 7.28 7.27 7.27 7.26 7.26 CDCl3 7.26 7.25 4.50 4.49 4.47 4.40 4.39 4.38 4.19 4.18 4.16 1.98 1.96 1.95 1.93 1.92 1.78 1.78 1.77 1.76 1.76 1.75 1.75 1.74 1.73 1.72 1.72 1.71 1.71 1.70 1.53 1.52 1.51 1.51 1.50 1.50 1.49 1.49 1.48 1.48 1.47 1.35 1.31
0.96 0.95 3.15 1.03 1.02 2.00 3.04 2.21 2.17 6.10 5.95
f1 (ppm)


194.81
136.70 133.54 126.55 123.11 122.90 122.28 119.89 109.69
84.46 83.14 77.41 CDCl3 77.16 CDCl3 76.91 CDCl3
47.01 41.83 31.87 30.16 30.00 29.78 24.21 23.05 23.01 17.16
f1 (ppm)

FUB-144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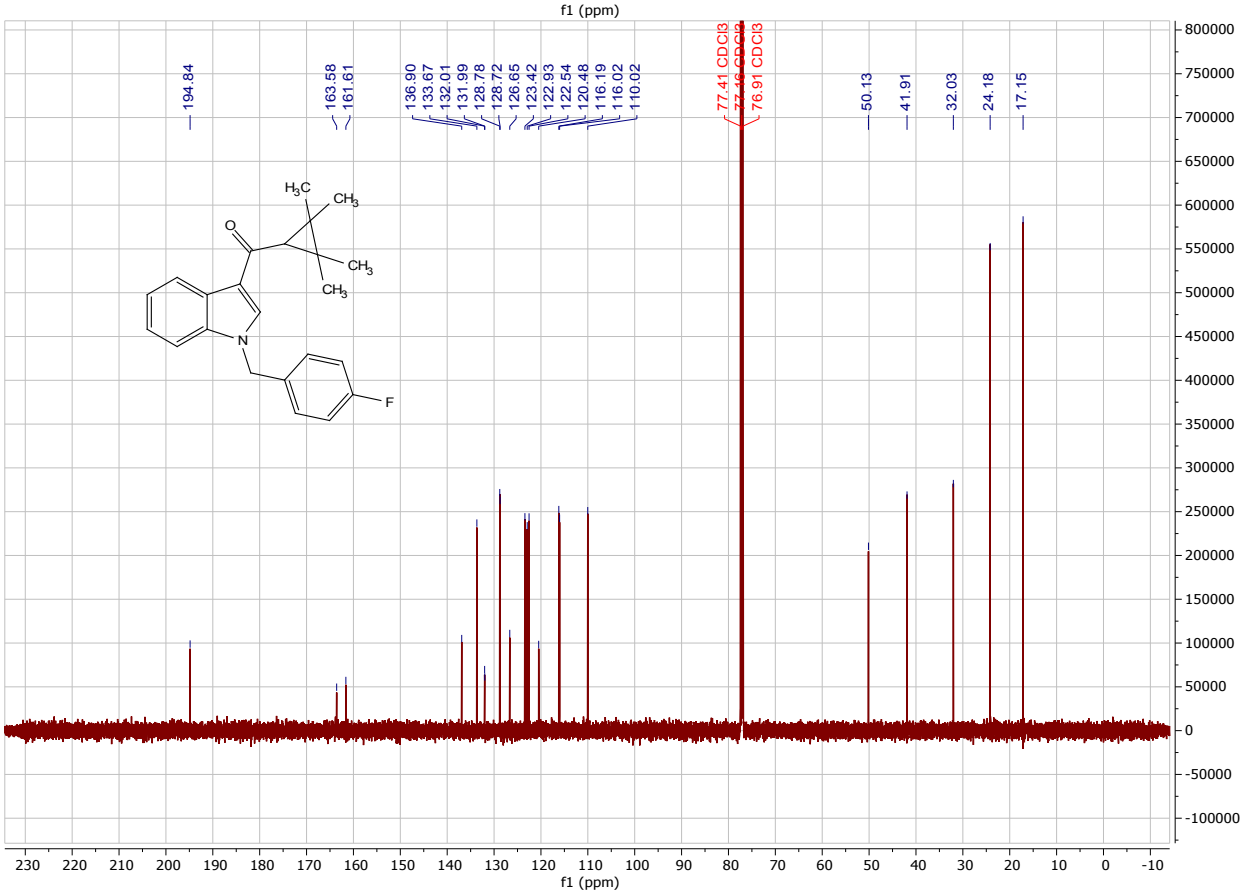
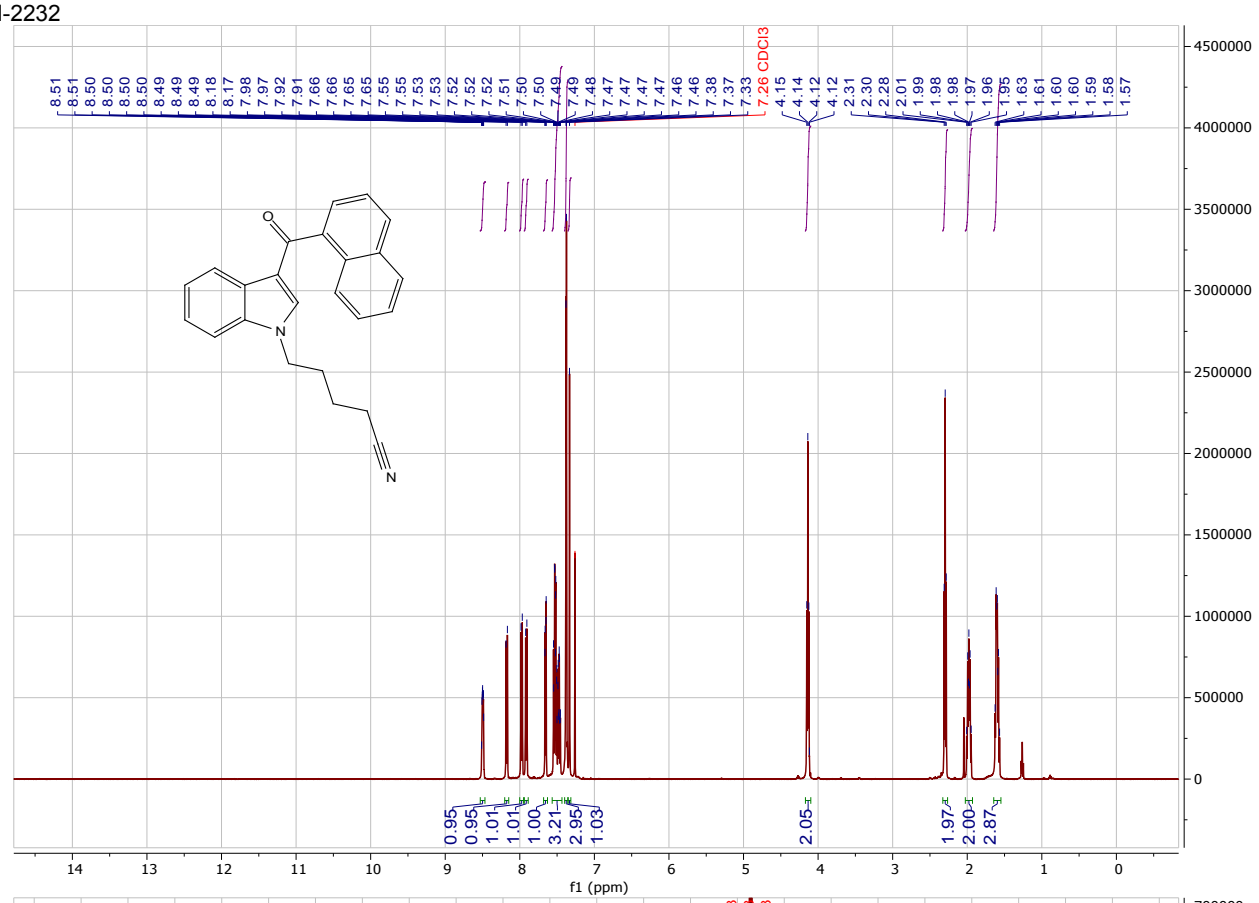
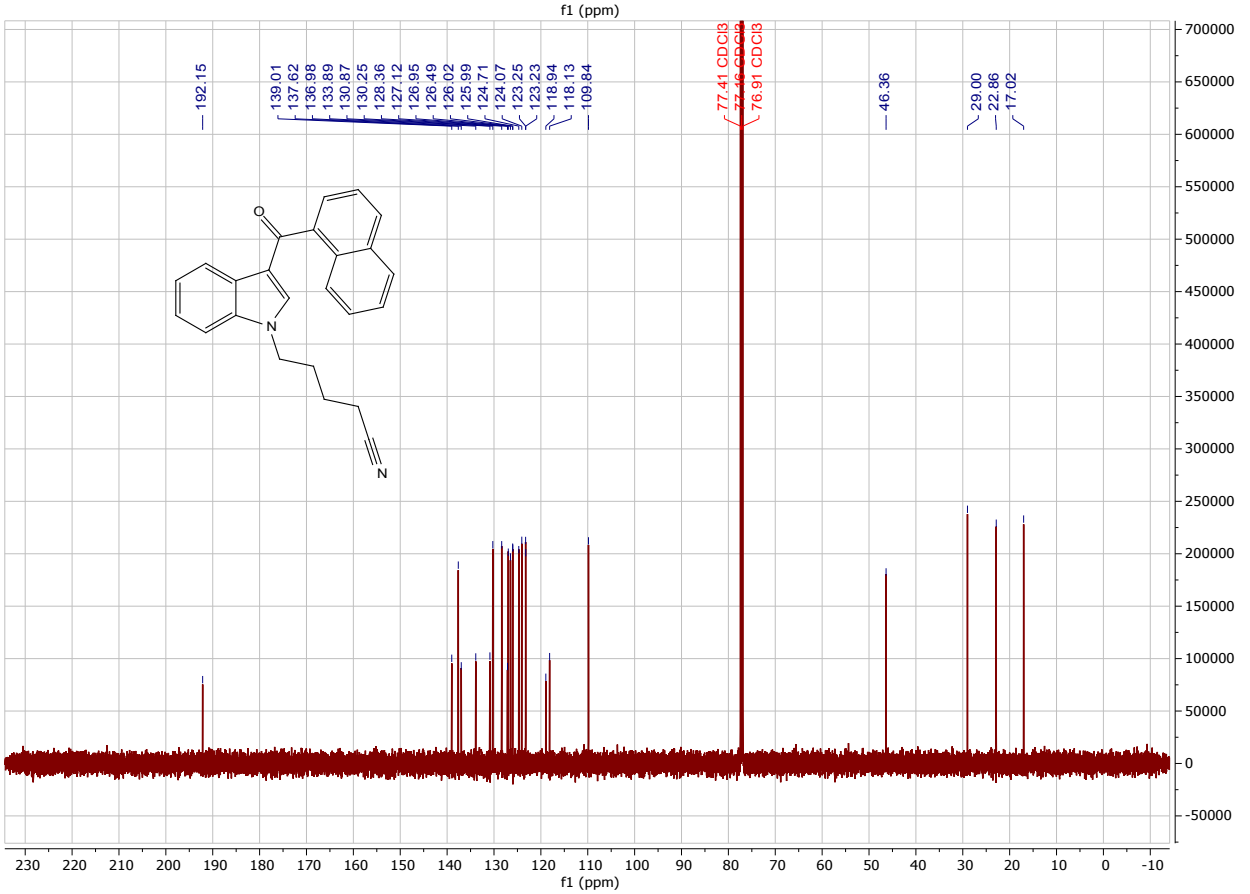
8.43
8.43
8.42
8.41
8.41
7.68
7.29
7.28
7.27
7.26
7.26 CDCl3
7.25
7.25
7.24
7.24
7.24
7.23
7.23
7.22
7.15
7.14
7.14
7.13
7.13
7.12
7.11
7.06
7.05
7.04
7.04
7.03
7.03
7.02
7.02
7.01
7.01
5.33
5.24
1.93
1.68
1.35
1.29
0.99
0.97
3.20
2.05
2.10
1.96
0.97
6.05
6.04
f1 (ppm)

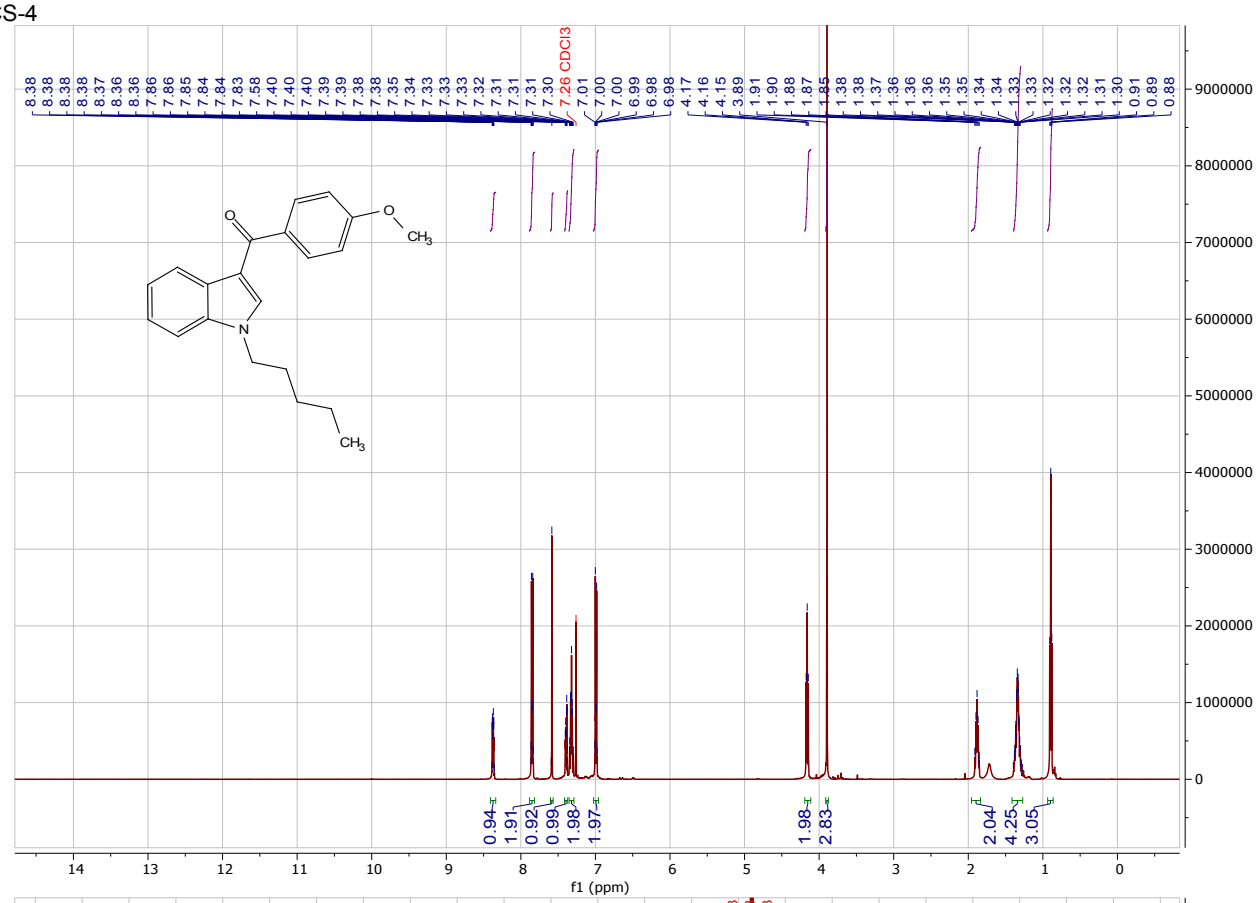

194.84
163.58
161.61
136.90
133.67
132.01
131.99
128.78
128.72
126.65
123.42
122.93
122.54
120.48
116.19
116.02
110.02
77.41 CDCl3
77.16 CDCl3
76.91 CDCl3
50.13
41.91
32.03
24.18
17.15
f1 (ppm)

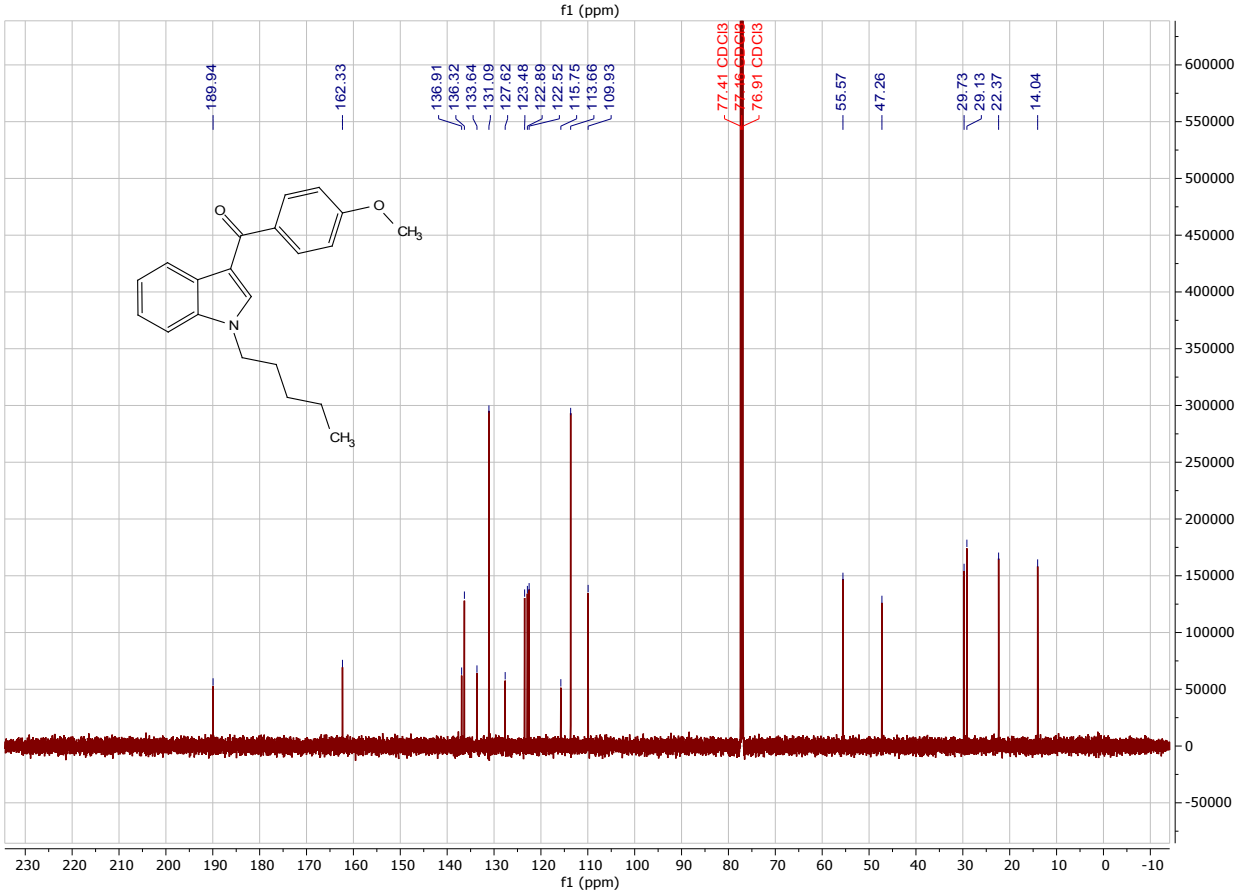
AM-2232


8.51 8.51 8.50 8.50 8.50 8.49 8.49 8.49 8.18 8.17 7.98 7.97 7.92 7.91 7.66 7.66 7.65 7.65 7.55 7.55 7.53 7.53 7.52 7.52 7.52 7.51 7.50 7.50 7.49 7.49 7.48 7.47 7.47 7.47 7.47 7.46 7.46 7.38 7.37 7.33 7.26 CDCl3 4.15 4.14 4.12 4.12 2.31 2.30 2.28 2.01 1.99 1.98 1.98 1.97 1.96 1.95 1.63 1.61 1.60 1.60 1.59 1.58 1.57
O
N
N
0.95 0.95 1.01 1.01 1.00 3.21 2.95 1.03 2.05 1.97 2.00 2.87
f1 (ppm)


192.15 139.01 137.62 136.98 133.89 130.87 130.25 128.36 127.12 126.95 126.49 126.02 125.99 124.71 124.07 123.25 123.23 118.94 118.13 109.84 77.41 CDCl3 77.16 CDCl3 76.91 CDCl3 46.36 29.00 22.86 17.02
O
N
N
f1 (ppm)

RCS-4


8.38 8.38 8.38 8.38 8.37 8.36 8.36 8.36 7.86 7.86 7.85 7.84 7.84 7.83 7.58 7.40 7.40 7.40 7.39 7.39 7.38 7.38 7.35 7.34 7.33 7.33 7.33 7.32 7.31 7.31 7.31 7.30 7.26 CDCl3 7.01 7.00 7.00 6.99 6.98 6.98 4.17 4.16 4.15 3.89 1.91 1.90 1.88 1.87 1.85 1.38 1.38 1.37 1.36 1.36 1.36 1.35 1.35 1.34 1.34 1.33 1.33 1.32 1.32 1.32 1.31 1.30 0.91 0.89 0.88
O
O
CH3
N
CH3
0.94 1.91 0.92 0.99 1.98 1.97 1.98 2.83 2.04 4.25 3.05
f1 (ppm)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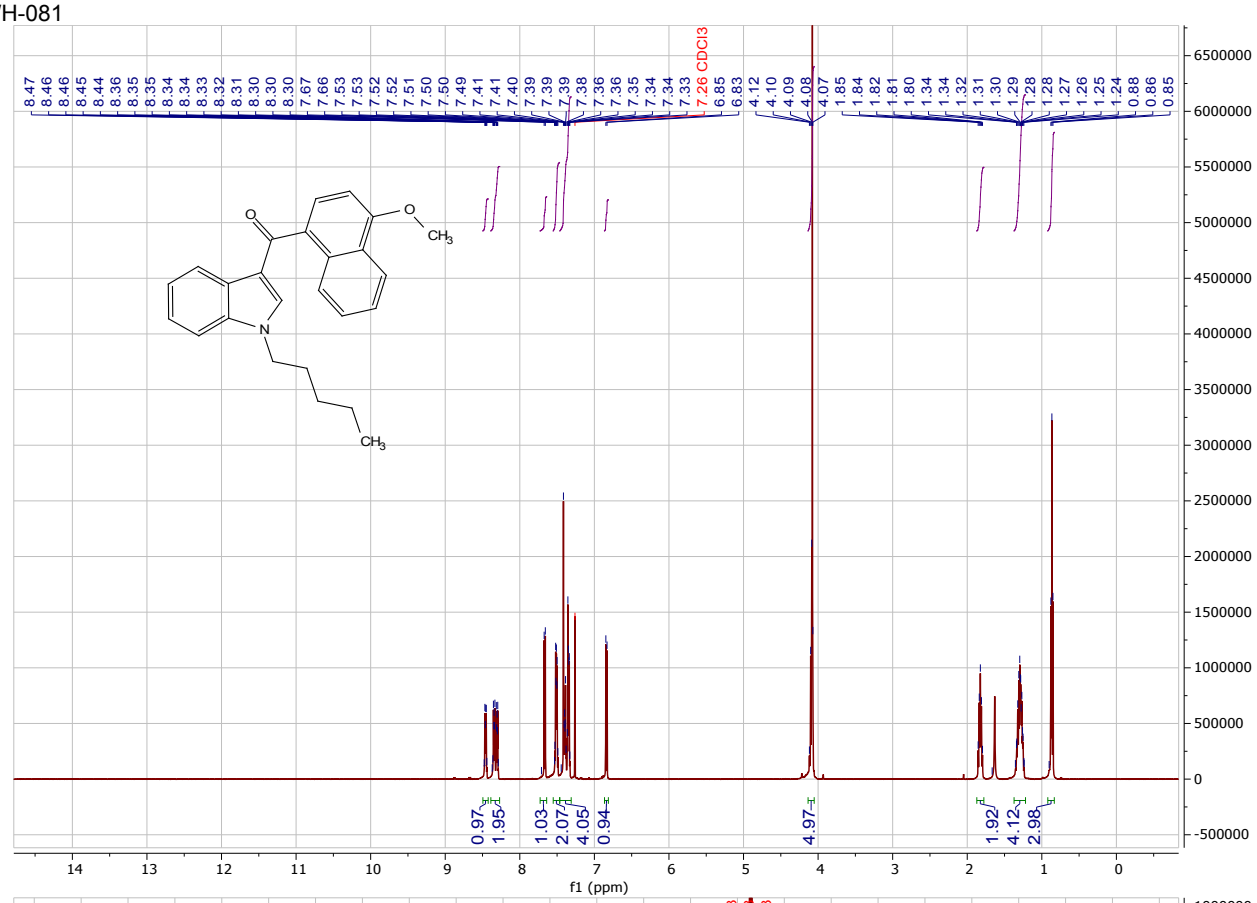
O
O
CH3
N
CH3
f1 (ppm)

JWH-081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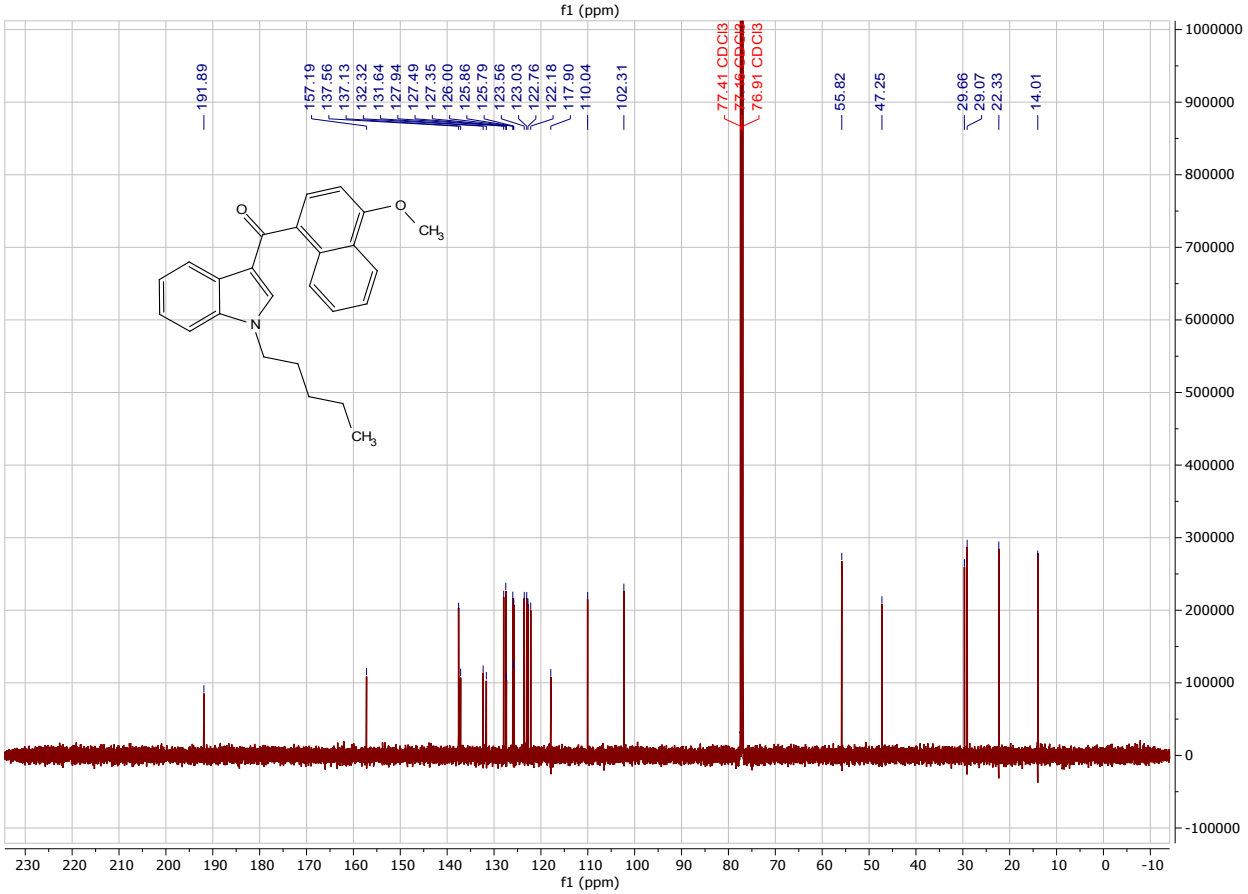
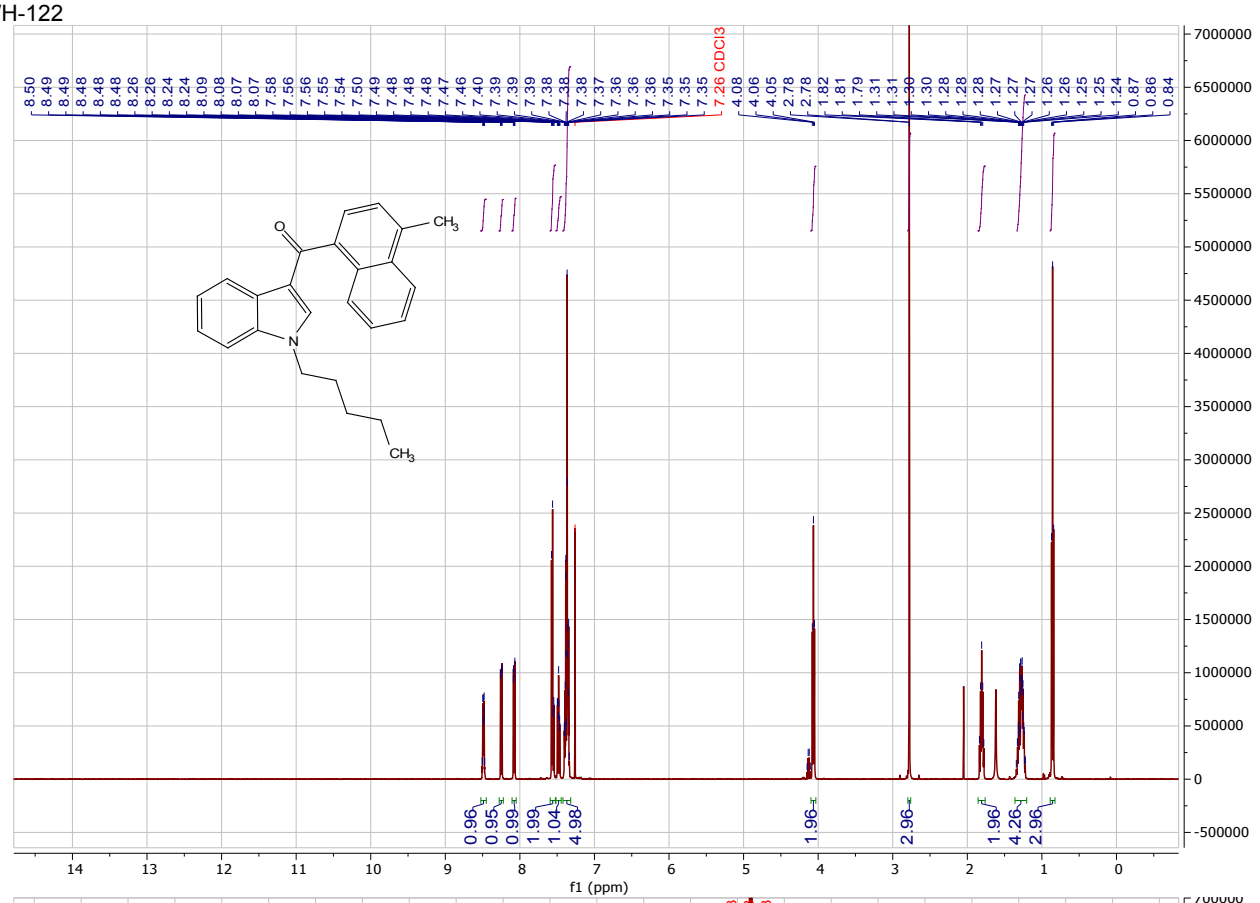
O
O
CH3
N
CH3
0.97 1.95 1.03 2.07 4.05 0.94 4.97 1.92 4.12 2.98
f1 (ppm)


191.89 157.19 137.56 137.13 132.32 131.64 127.94 127.49 127.35 126.00 125.86 125.79 123.56 123.03 122.76 122.18 117.90 110.04 102.31 77.41 CDCl3 77.16 CDCl3 76.91 CDCl3 55.82 47.25 29.66 29.07 22.33 14.01
O
O
CH3
N
CH3
f1 (ppm)

JWH-122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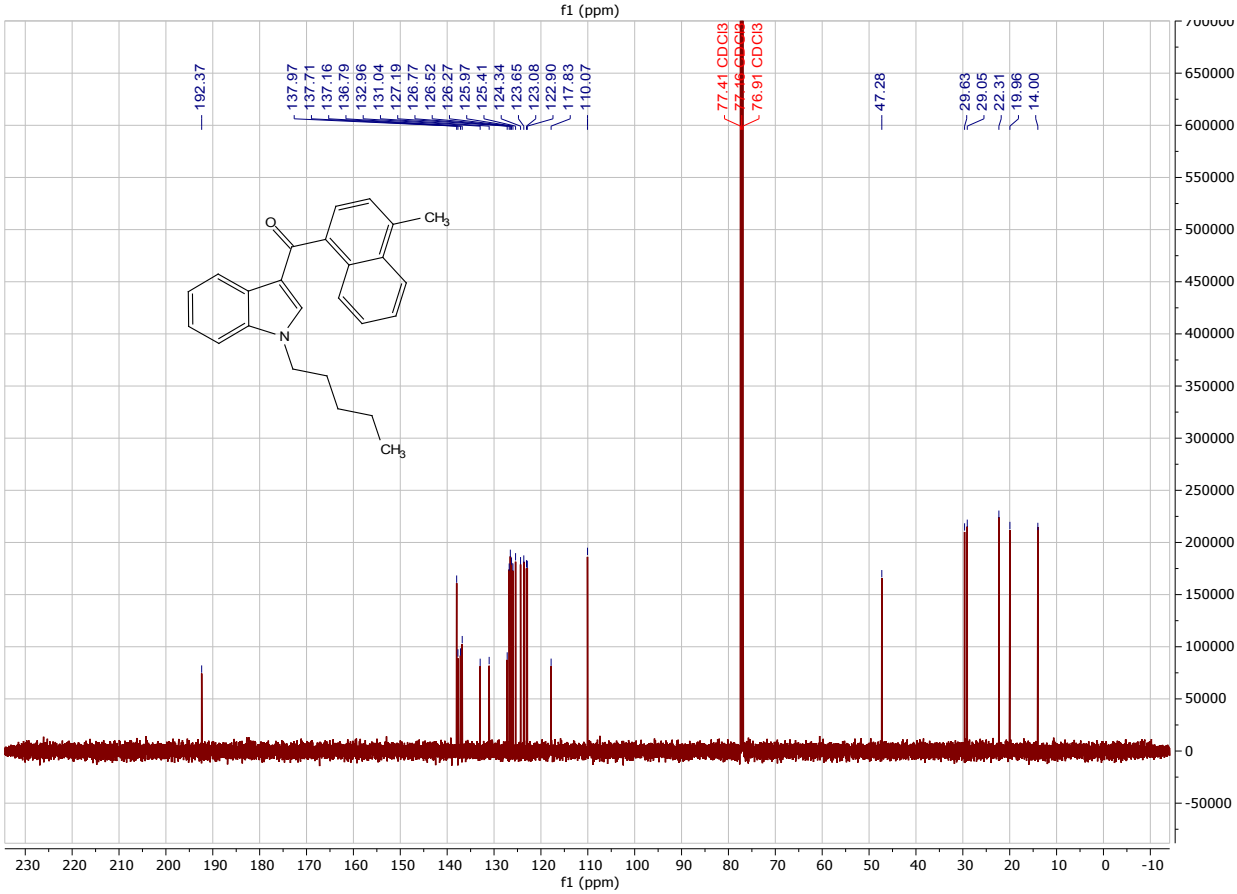
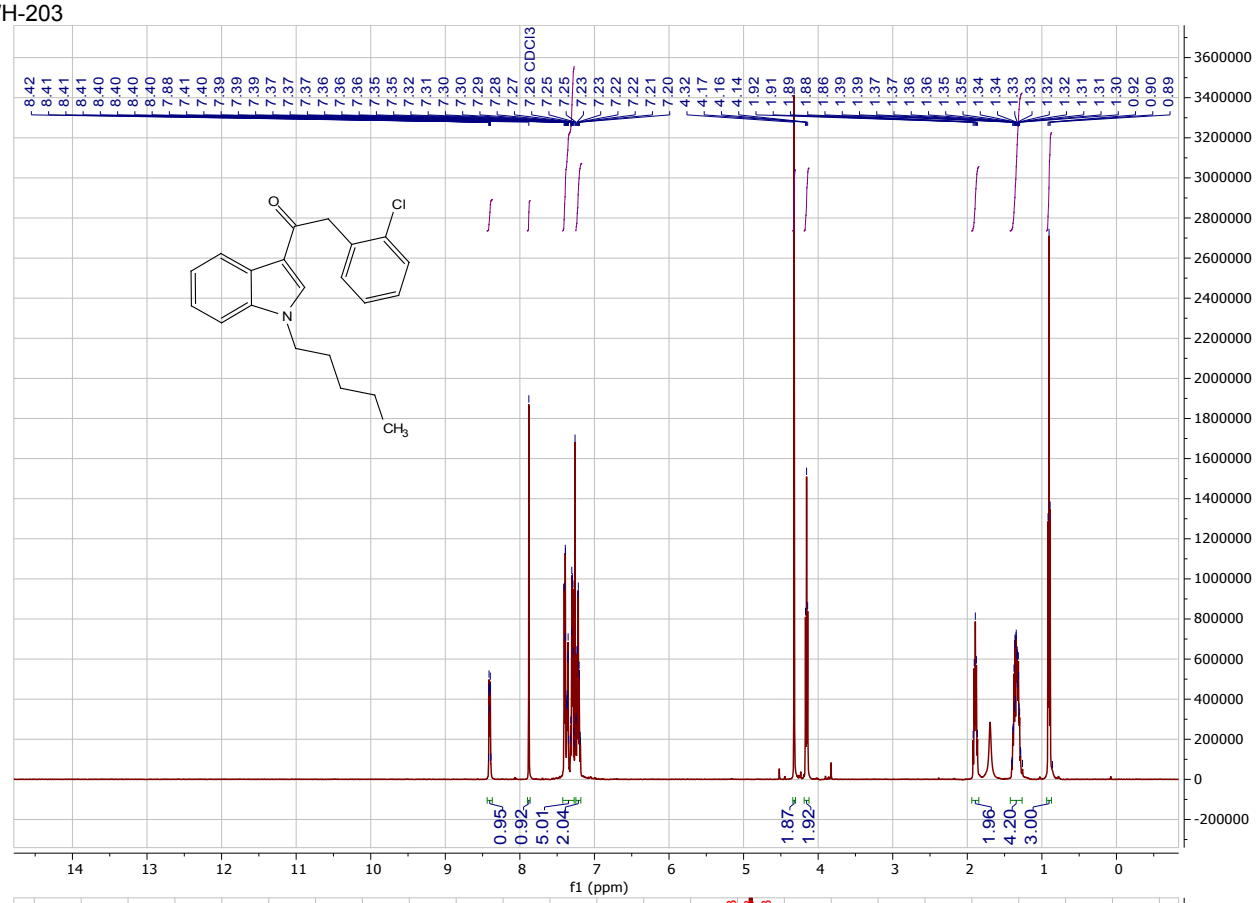
4.08 4.06 4.05 2.78 2.78 1.82 1.81 1.79 1.31 1.31 1.30 1.30 1.28 1.28 1.28 1.27 1.27 1.27 1.26 1.26 1.25 1.25 1.24 0.87 0.86 0.84
O
CH3
N
CH3
0.96 0.95 0.99 1.99 1.04 4.98 1.96 2.96 1.96 4.26 2.96
f1 (ppm)


192.37
137.97 137.71 137.16 136.79 132.96 131.04 127.19 126.77 126.52 126.27 125.97 125.41 124.34 123.65 123.08 122.90 117.83 110.07
77.41 CDCl3 77.16 CDCl3 76.91 CDCl3
47.28
29.63 29.05 22.31 19.96 14.00
O
CH3
N
CH3
f1 (ppm)

JWH-203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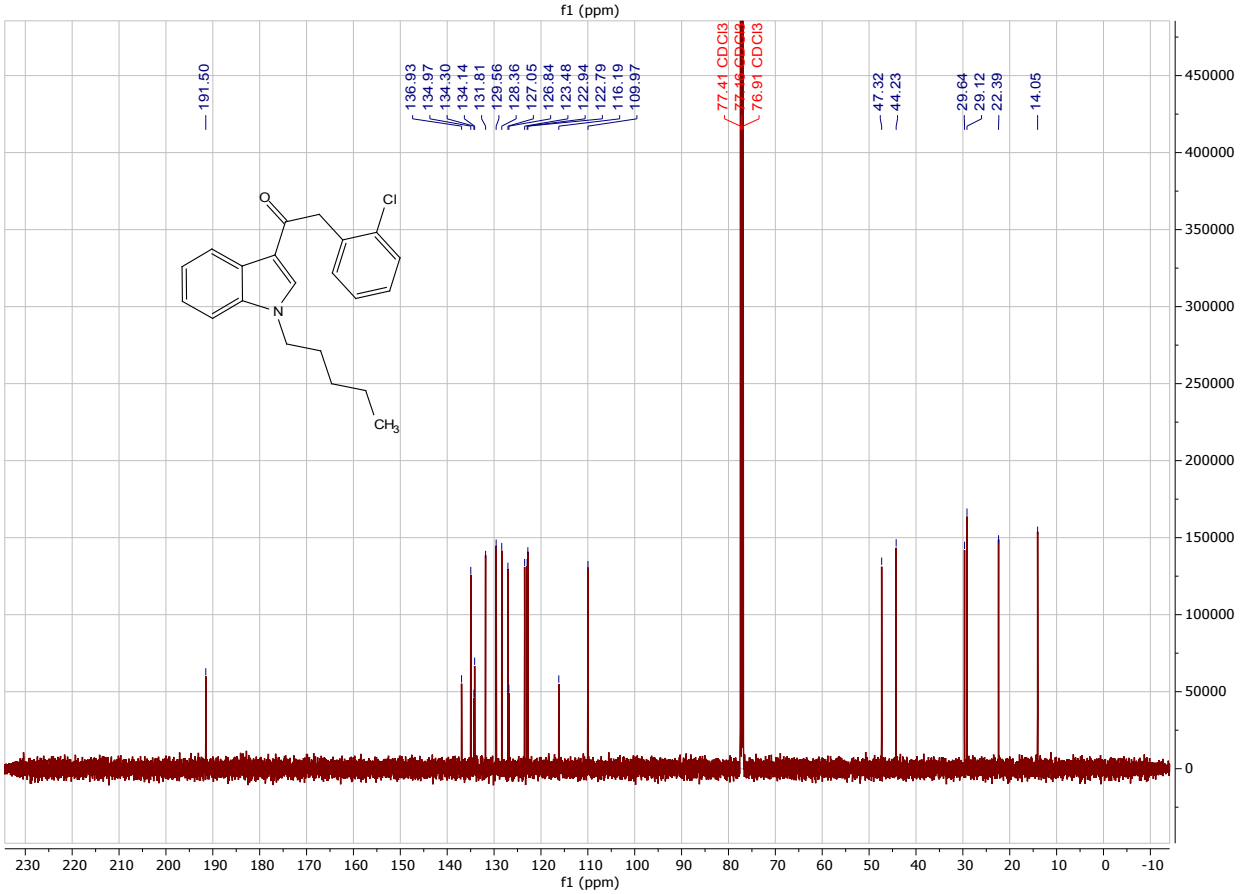
O
Cl
N
CH3
0.95 0.92 5.01 2.04 1.87 1.92 1.96 4.20 3.00
f1 (ppm)


191.50
136.93 134.97 134.30 134.14 131.81 129.56 128.36 127.05 126.84 123.48 122.94 122.79 116.19 109.97
77.41 CDCl3 77.16 CDCl3 76.91 CDCl3
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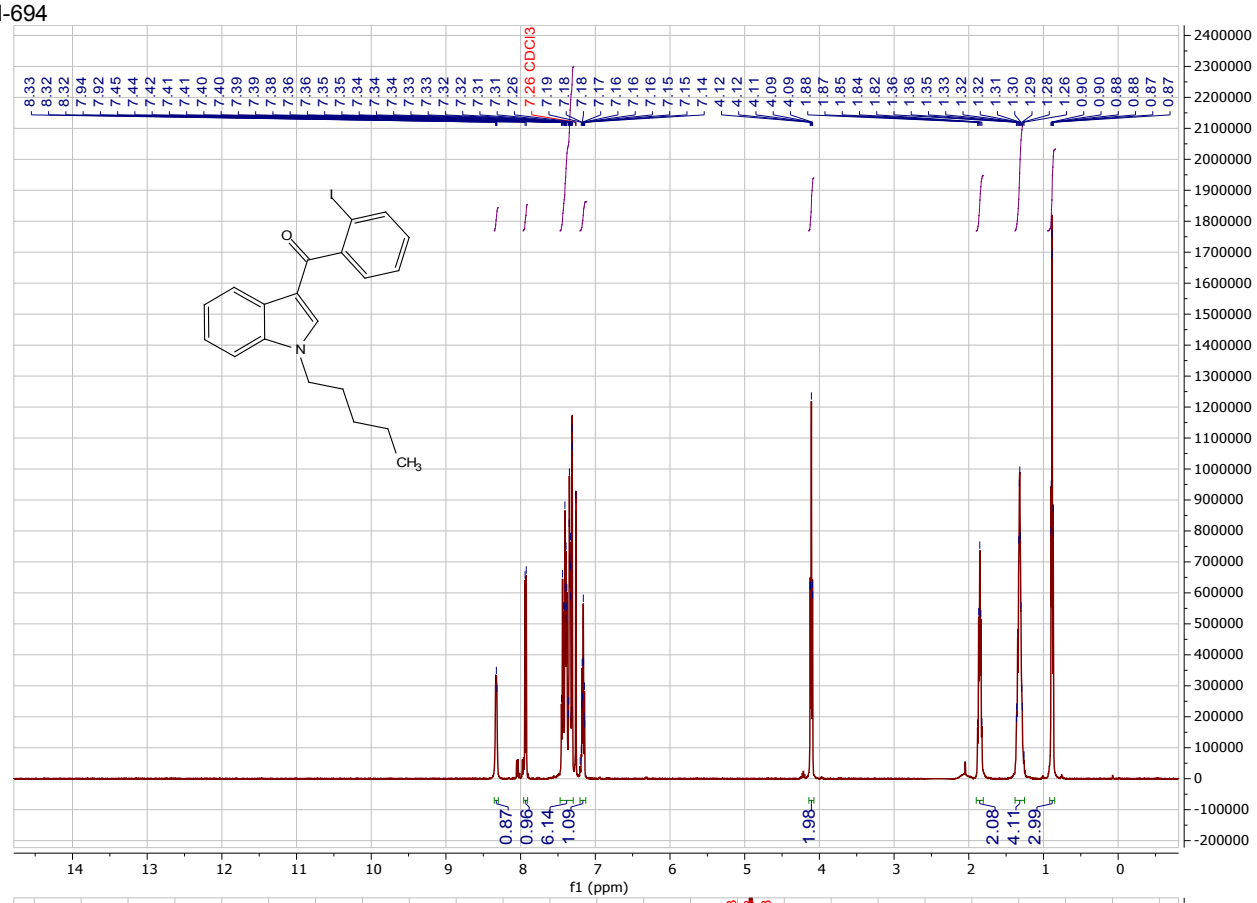
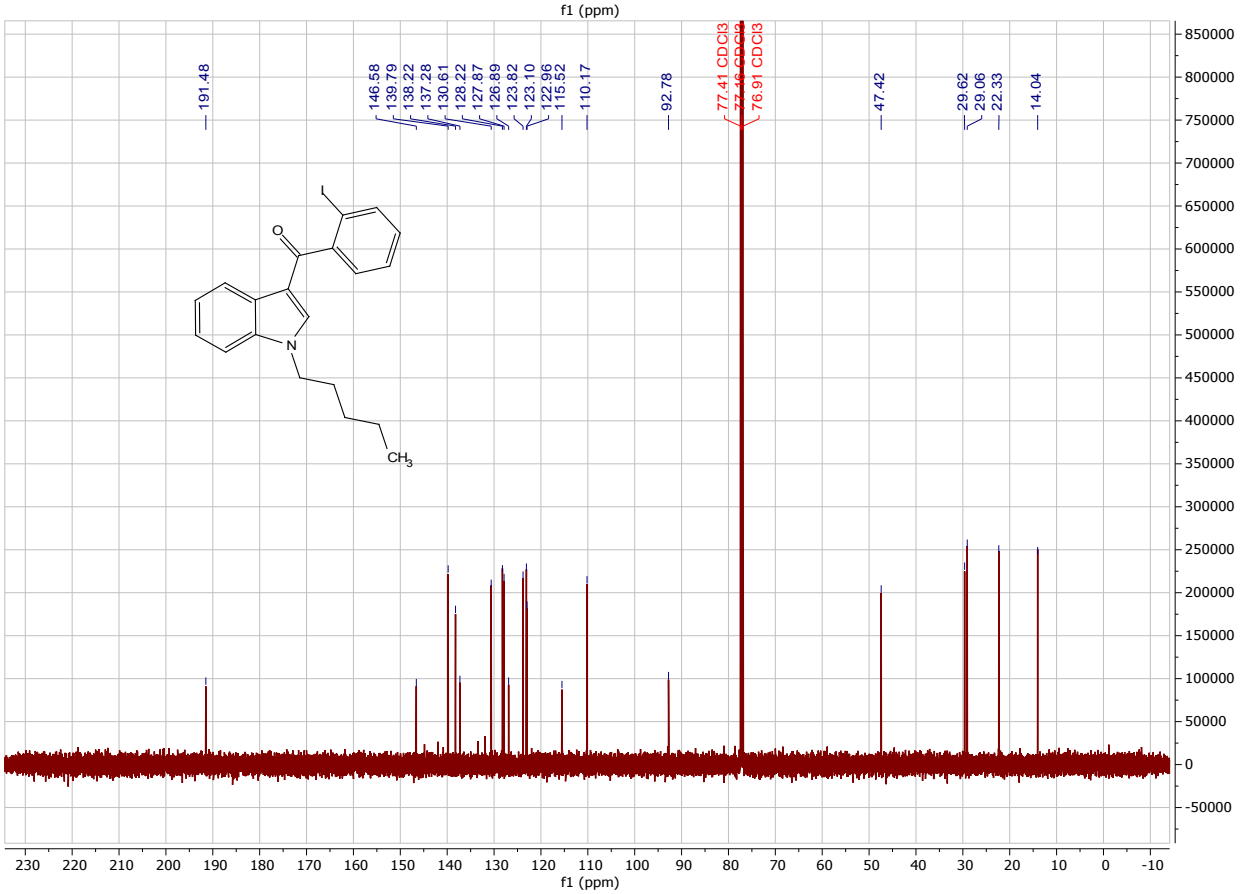
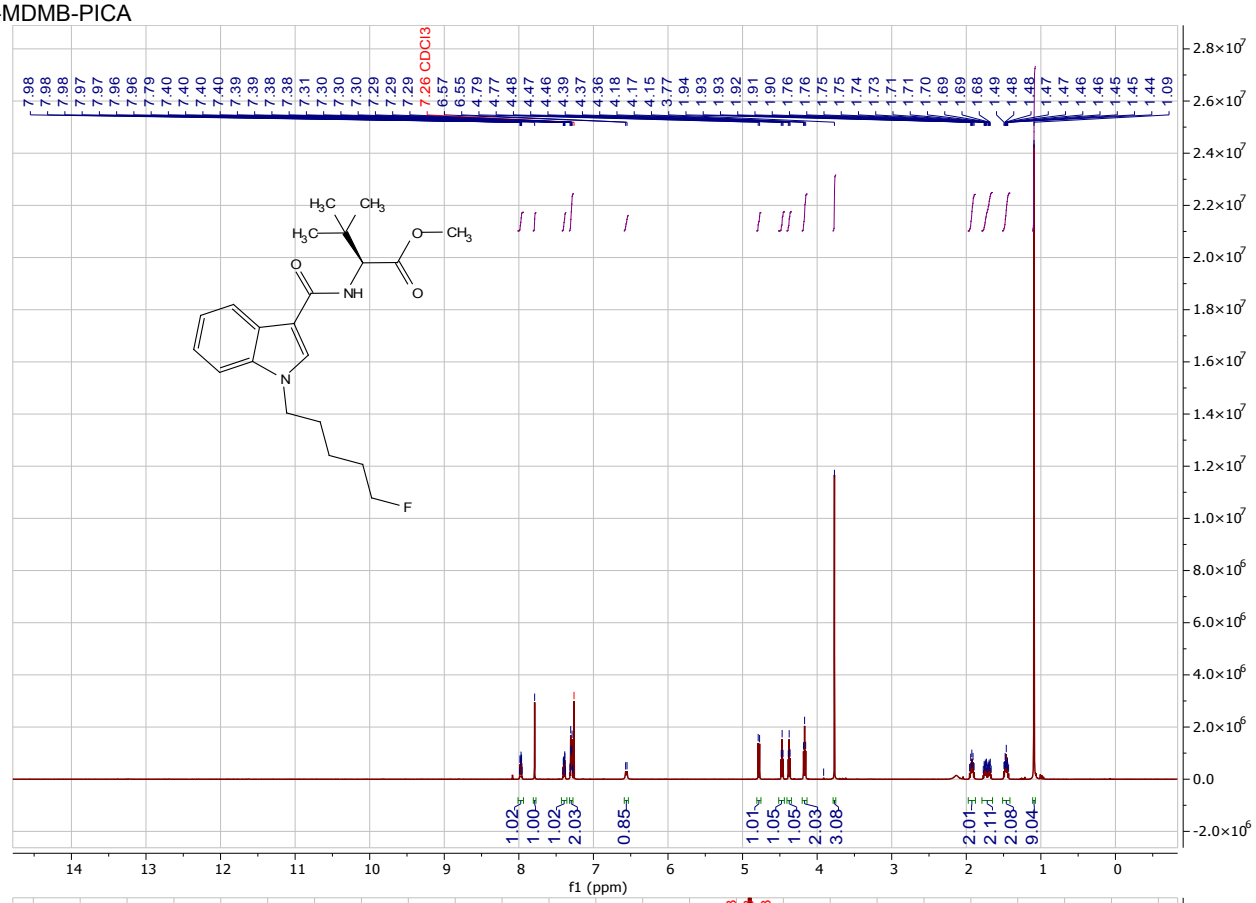
29.64 29.12 22.39
14.05
O
Cl
N
CH3
f1 (ppm)

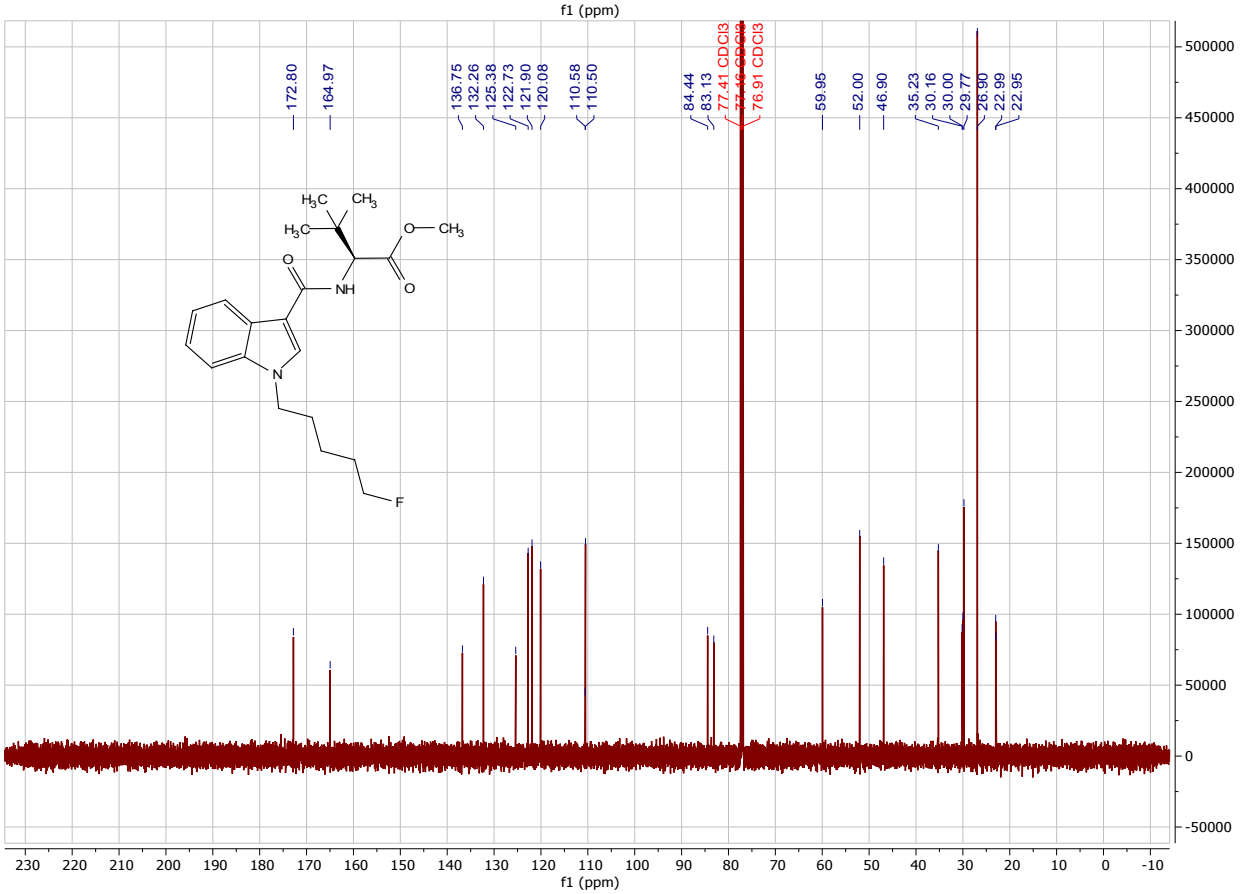
AM-694


8.33 8.32 8.32 7.94 7.92 7.45 7.44 7.42 7.41 7.41 7.40 7.40 7.39 7.39 7.38 7.36 7.36 7.35 7.35 7.34 7.34 7.34 7.33 7.33 7.32 7.32 7.31 7.31 7.26 7.26 CDCl3 7.19 7.18 7.18 7.17 7.16 7.16 7.16 7.15 7.15 7.14 4.12 4.12 4.11 4.09 4.09 1.88 1.87 1.85 1.84 1.82 1.36 1.35 1.33 1.32 1.32 1.31 1.30 1.29 1.28 1.26 0.90 0.90 0.88 0.88 0.87 0.87
0.87 0.96 6.14 1.09 1.98 2.08 4.11 2.99
f1 (ppm)

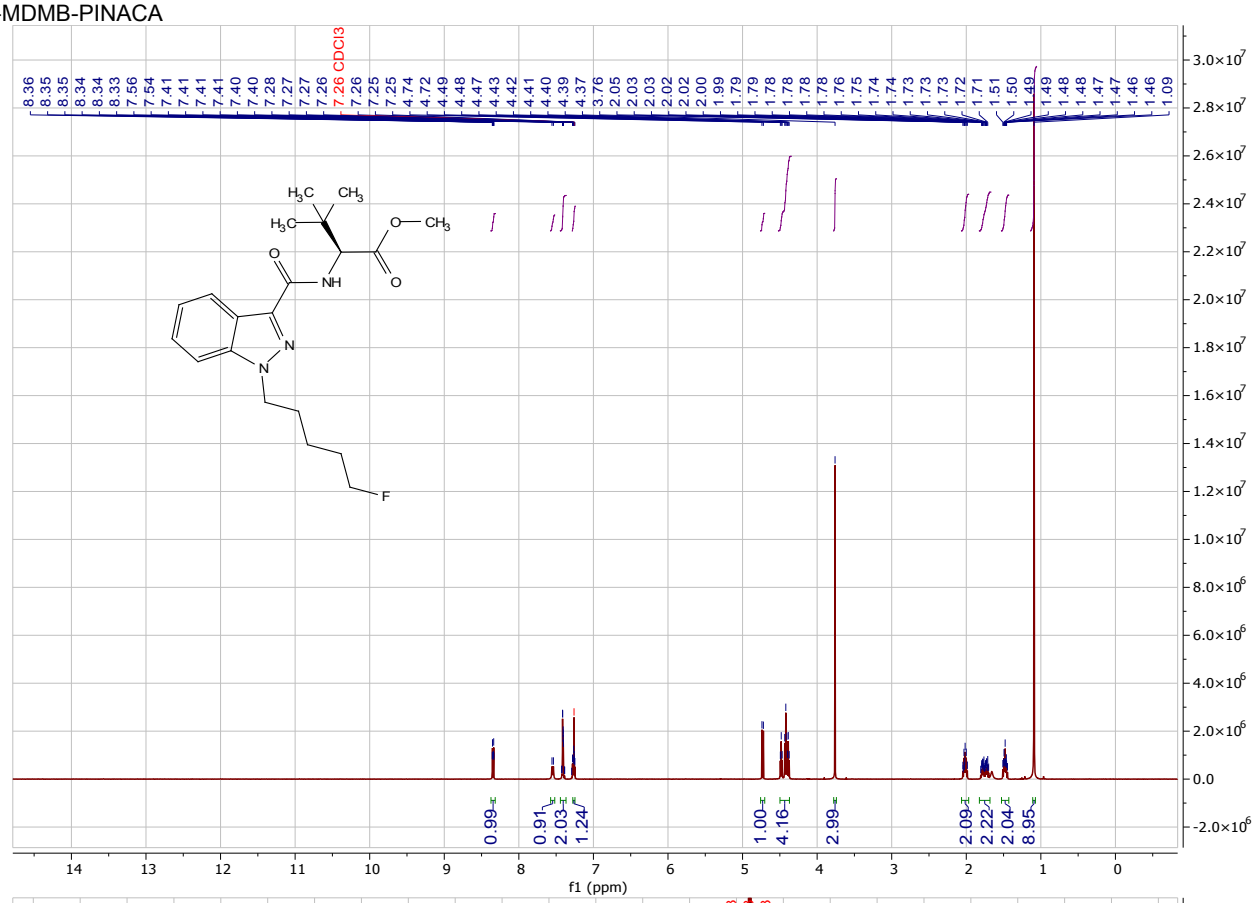

191.48 146.58 139.79 138.22 137.28 130.61 128.22 127.87 126.89 123.82 123.10 122.96 115.52 110.17 92.78 77.41 CDCl3 77.16 CDCl3 76.91 CDCl3 47.42 29.62 29.06 22.33 14.04
f1 (ppm)

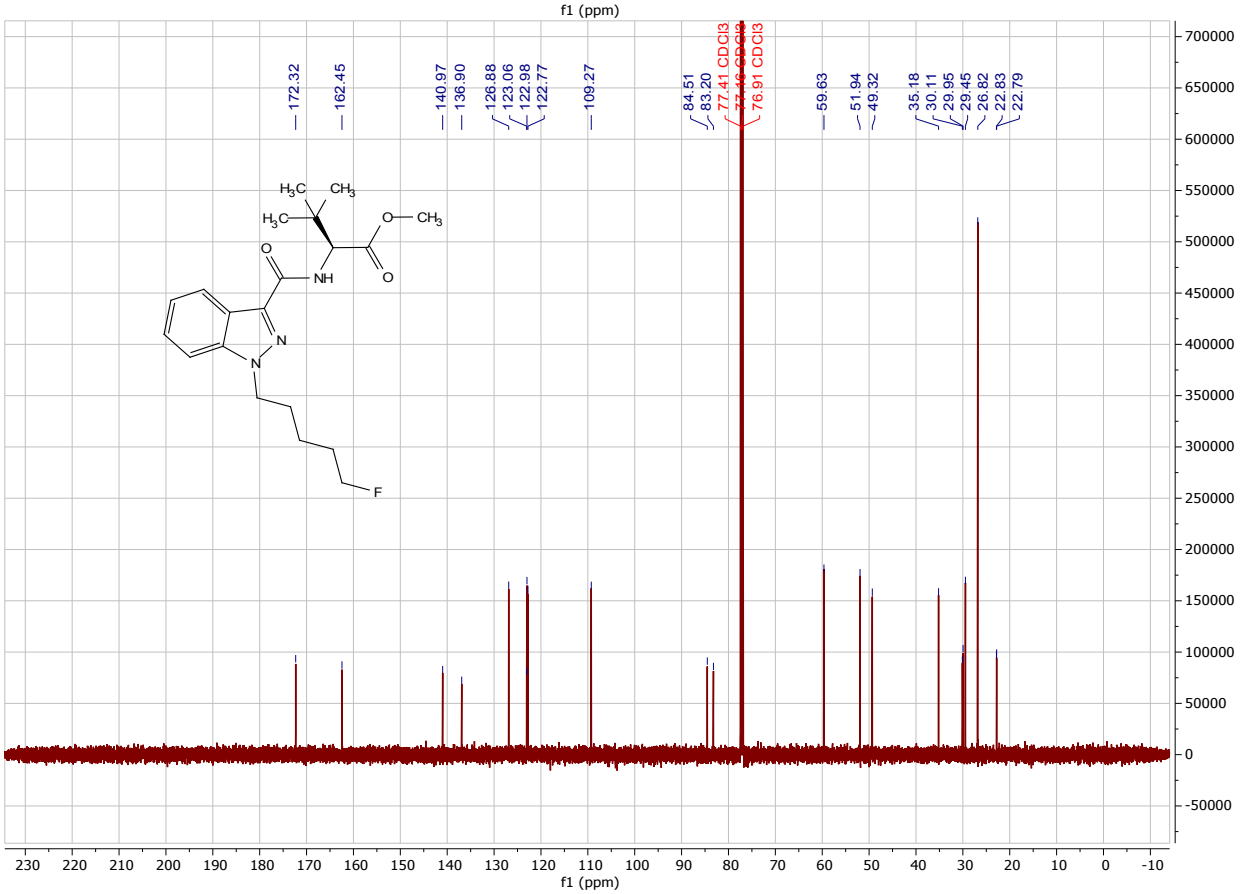
5F-MDMB-PICA


7.98 7.98 7.98 7.97 7.97 7.96 7.96 7.79 7.40 7.40 7.40 7.40 7.39 7.39 7.38 7.38 7.31 7.30 7.30 7.30 7.29 7.29 7.29 7.26 CDCl3 6.57 6.55 4.79 4.77 4.48 4.47 4.46 4.39 4.37 4.36 4.18 4.17 4.15 3.77 1.94 1.93 1.93 1.92 1.91 1.90 1.76 1.76 1.75 1.75 1.74 1.73 1.71 1.71 1.70 1.69 1.69 1.68 1.49 1.48 1.48 1.47 1.47 1.46 1.46 1.45 1.44 1.09
1.02 1.00 1.02 2.03 0.85 1.01 1.05 1.05 2.03 3.08 2.01 2.11 2.08 9.04
f1 (ppm)

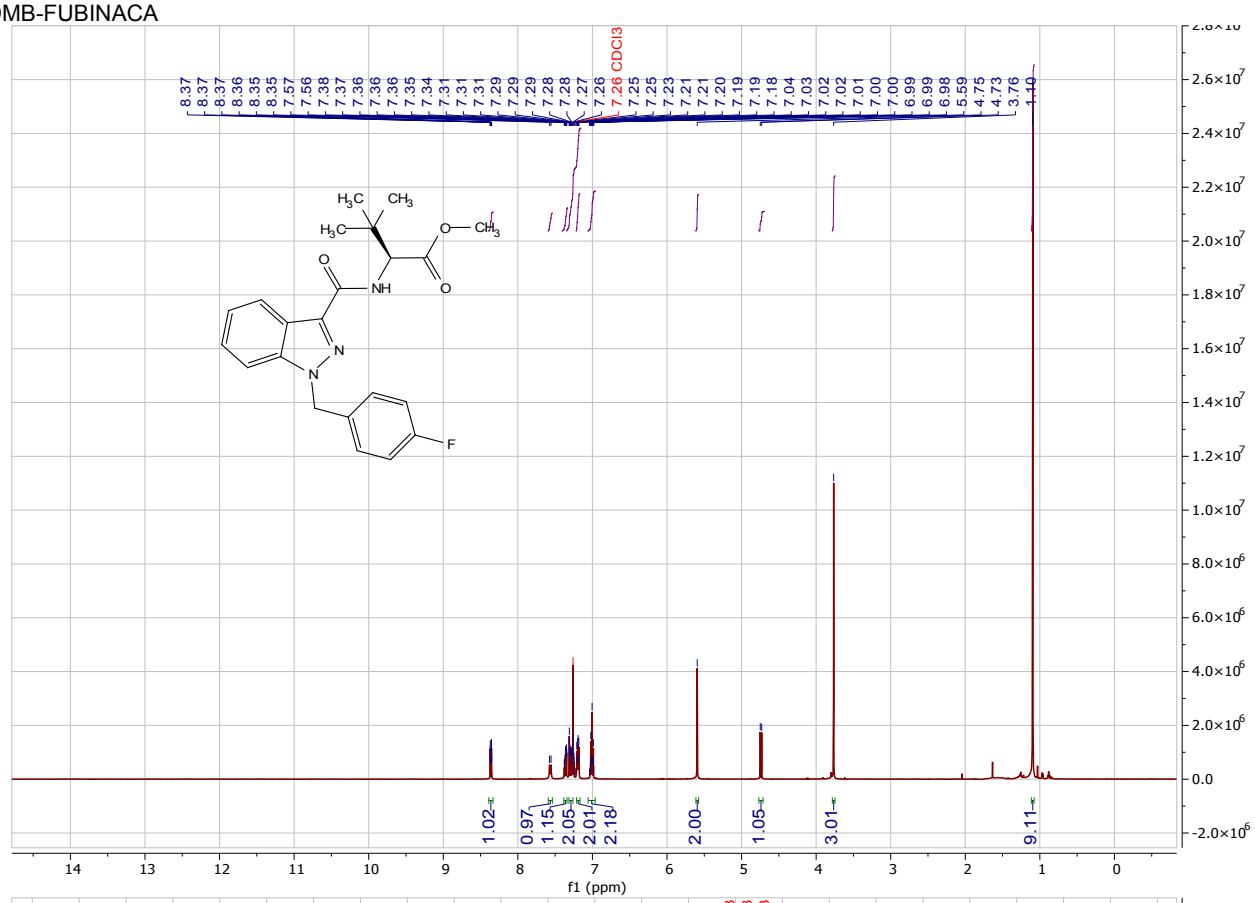

172.80 164.97 136.75 132.26 125.38 122.73 121.90 120.08 110.58 110.50 84.44 83.13 77.41 CDCl3 77.16 CDCl3 76.91 CDCl3 59.96 52.00 46.90 35.23 30.16 30.00 29.77 26.90 22.99 22.95
f1 (ppm)

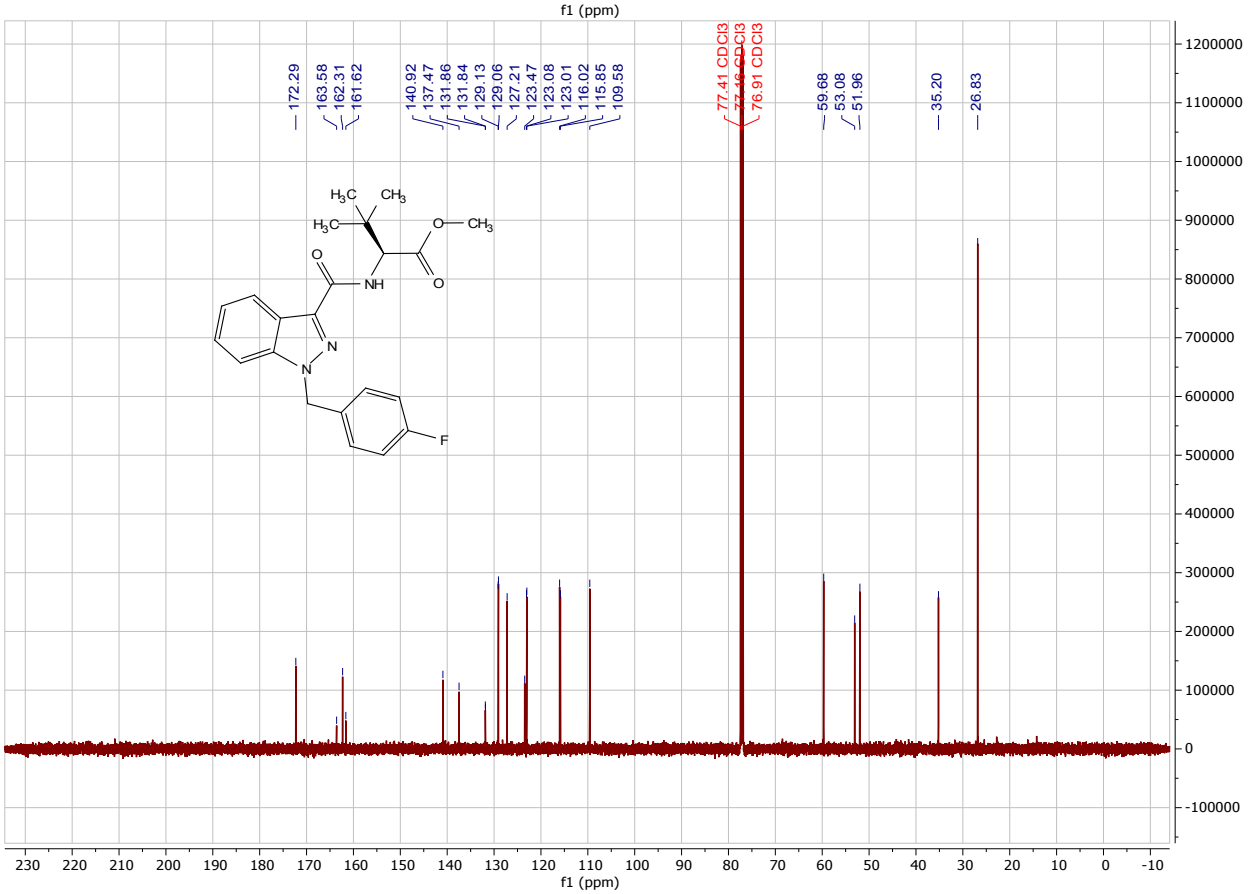
5F-MDMB-PINACA



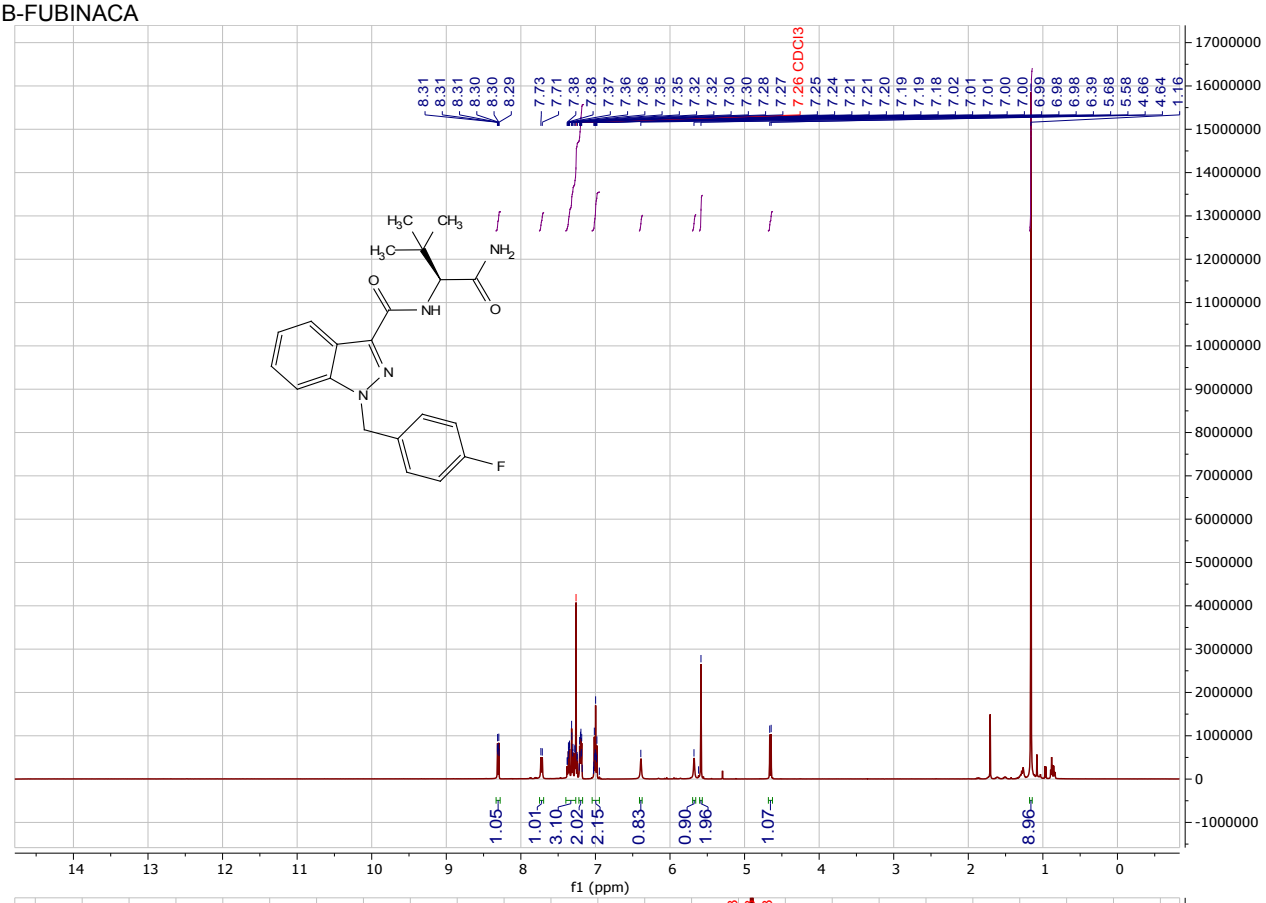


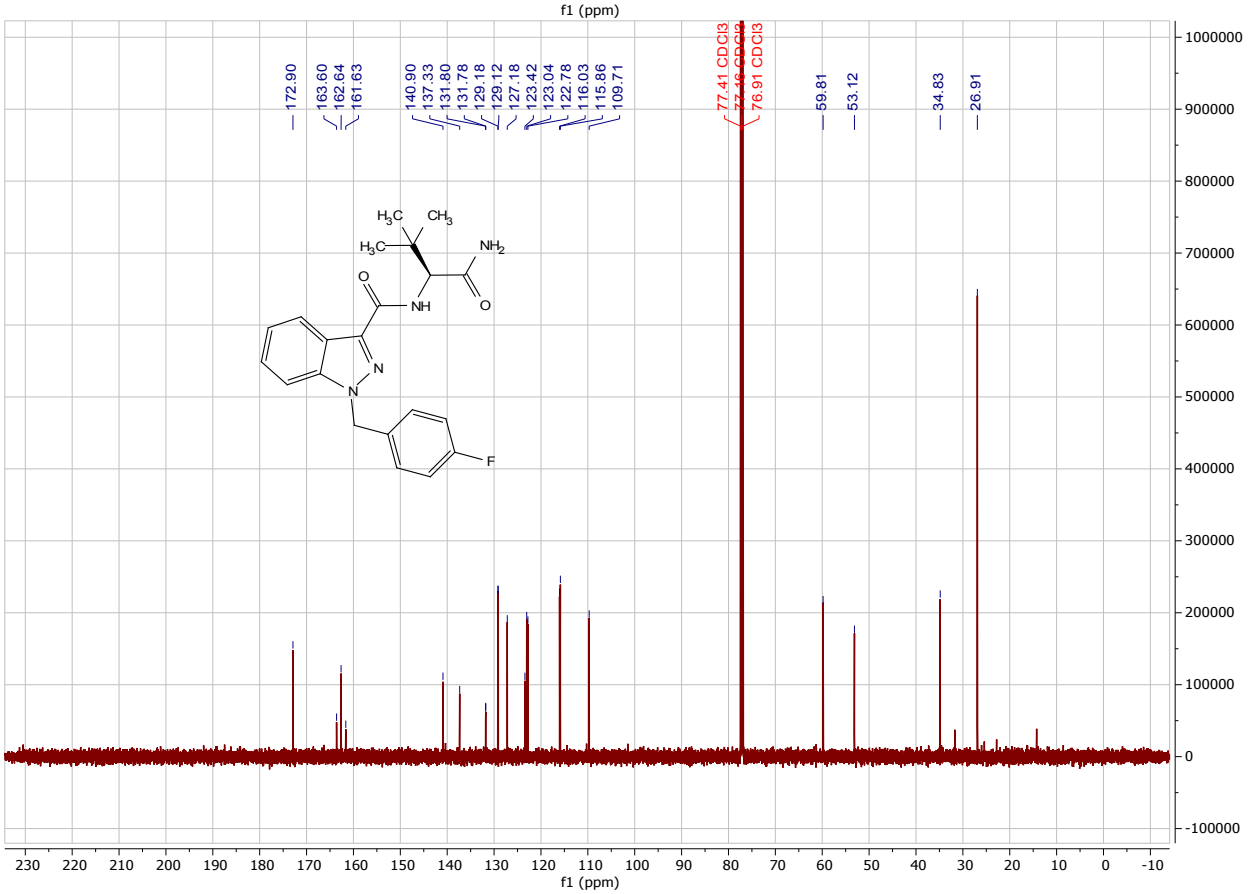
MDMB-FUBINACA



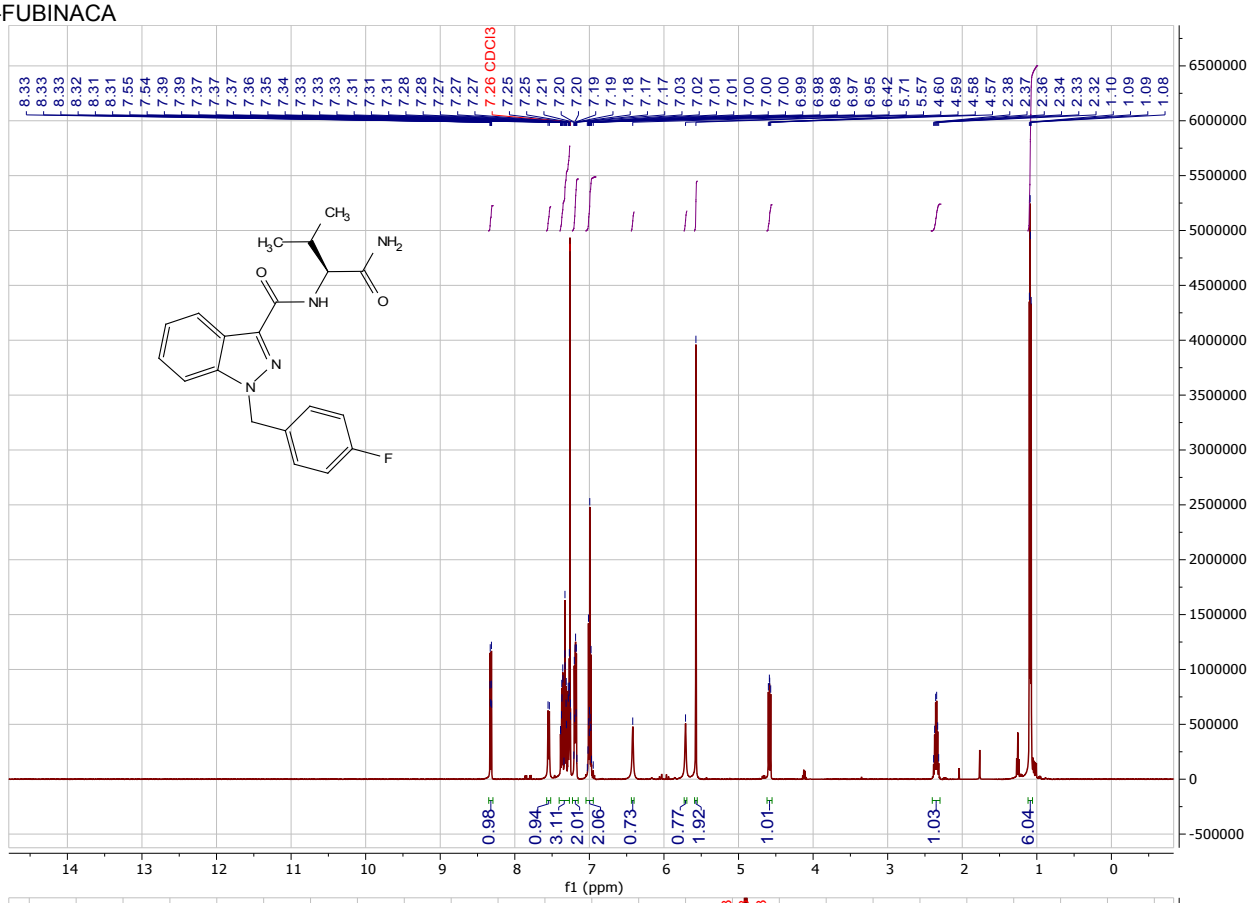


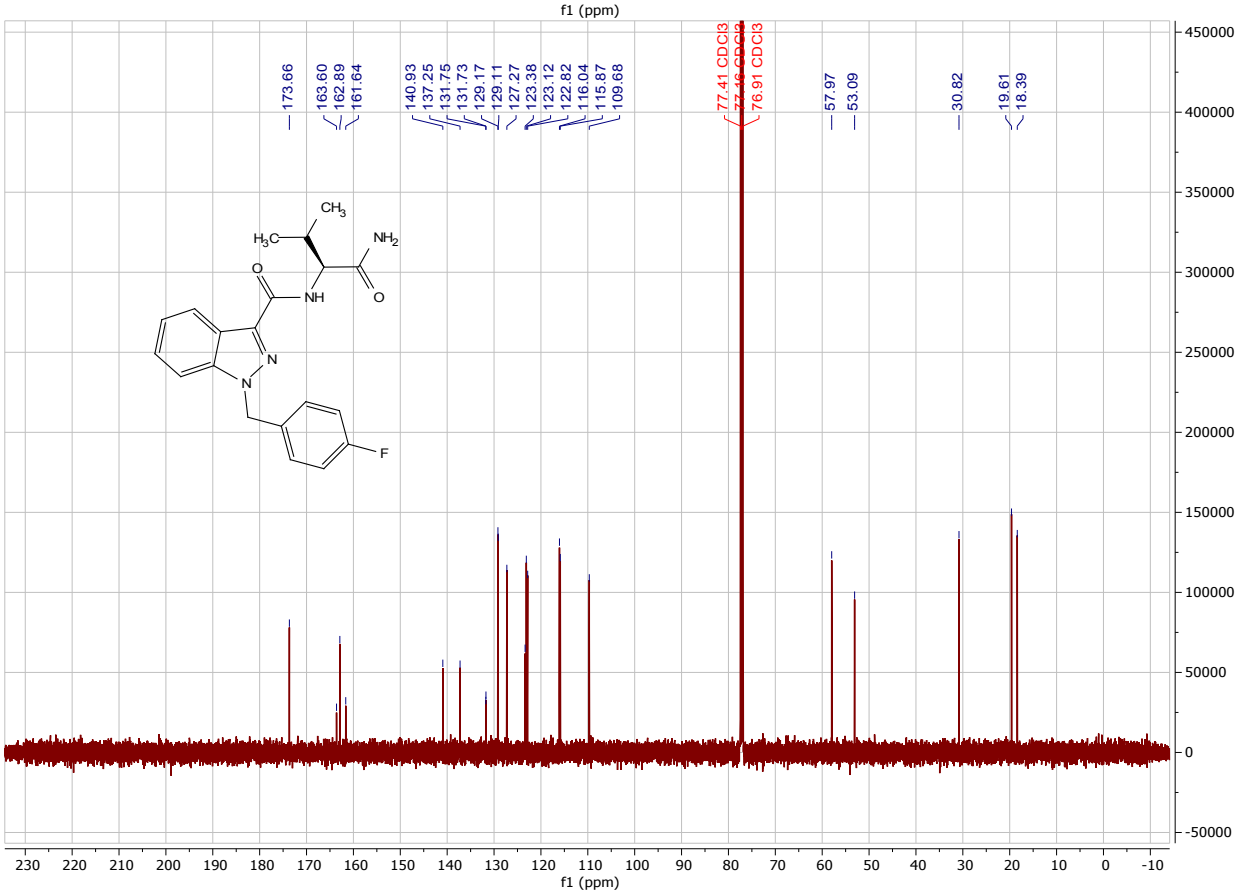
ADB-FUBINACA



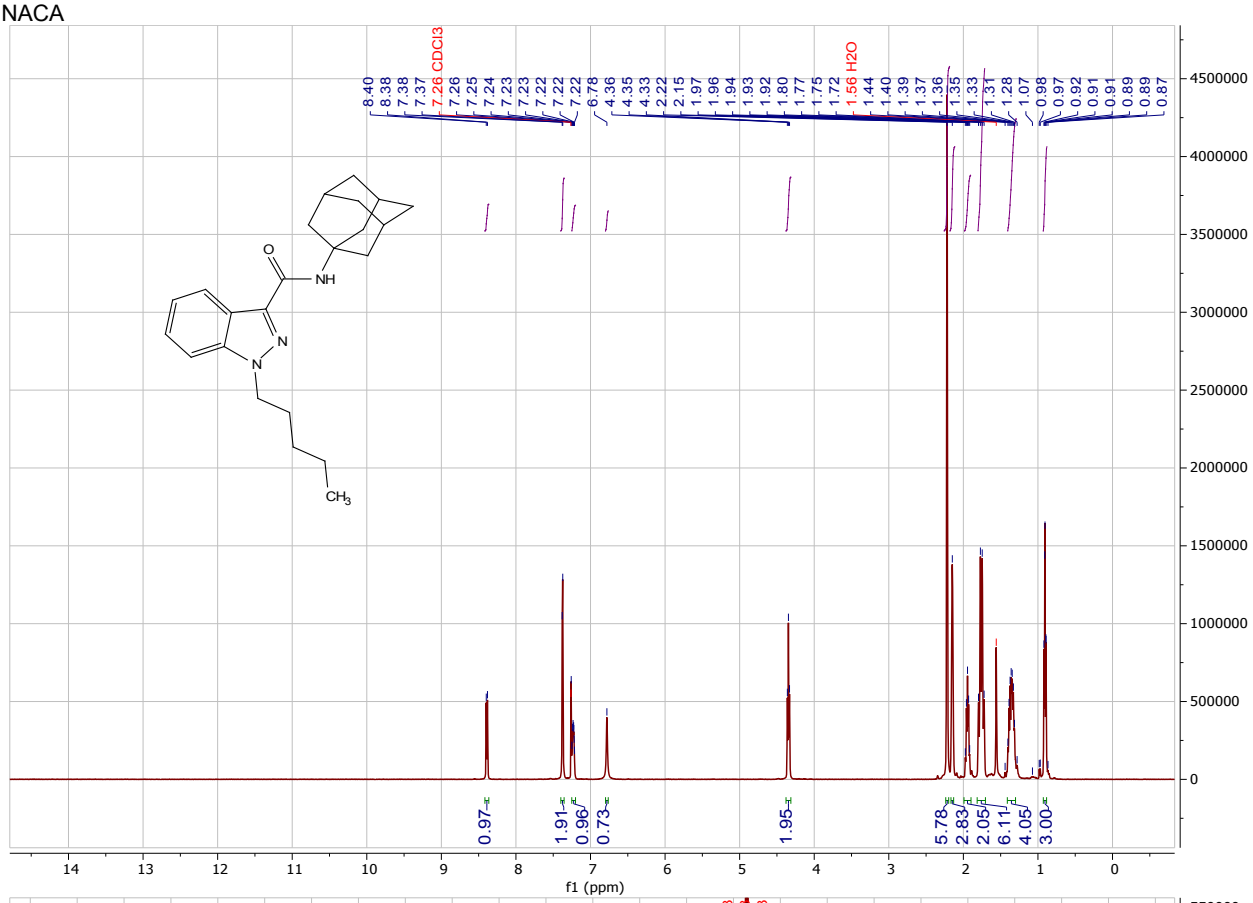


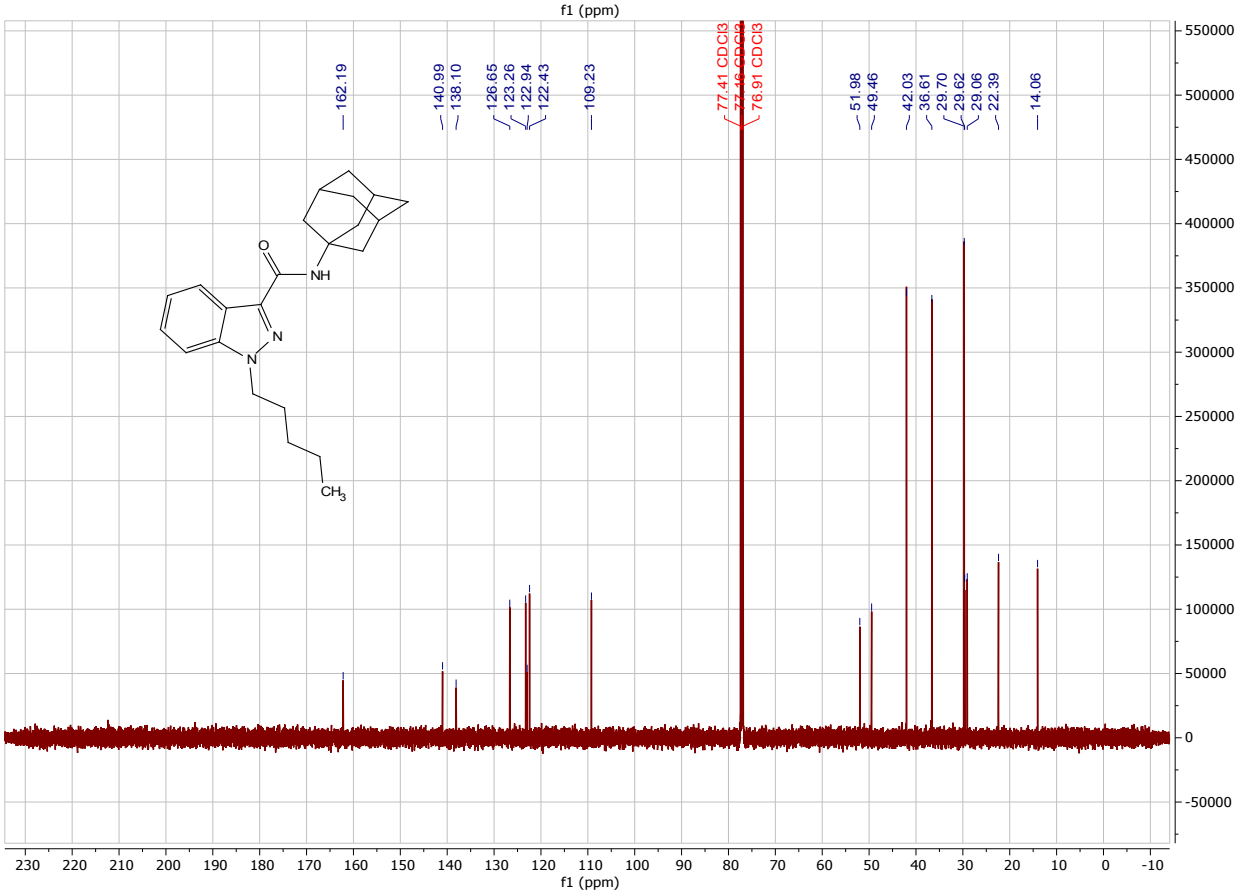
AB-FUBINACA


8.33 8.33 8.33 8.32 8.31 8.31 7.55 7.54 7.39 7.39 7.37 7.37 7.37 7.36 7.35 7.34 7.33 7.33 7.33 7.31 7.31 7.31 7.28 7.28 7.27 7.27 7.27 7.26 CDCl3 7.25 7.25 7.21 7.20 7.20 7.19 7.19 7.18 7.17 7.17 7.17 7.03 7.02 7.01 7.01 7.00 7.00 7.00 6.99 6.98 6.98 6.97 6.95 6.42 5.71 5.57 4.60 4.59 4.58 4.57 2.38 2.37 2.36 2.34 2.33 2.32 1.10 1.09 1.09 1.08
0.98 0.94 3.11 2.01 2.06 0.73 0.77 1.92 1.01 1.03 6.04
f1 (ppm)


173.66 163.60 162.89 161.64 140.93 137.25 131.75 131.73 129.17 129.11 127.27 123.38 123.12 122.82 116.04 115.87 109.68 77.41 CDCl3 77.16 CDCl3 76.91 CDCl3 57.97 53.09 30.82 19.61 18.39
f1 (ppm)

APINACA


8.40
8.38
7.38
7.37
7.26 CDCl3
7.26
7.25
7.24
7.23
7.23
7.22
7.22
7.22
6.78
4.36
4.35
4.33
2.22
2.15
1.97
1.96
1.94
1.93
1.92
1.80
1.77
1.75
1.72
1.56 H2O
1.44
1.40
1.39
1.37
1.36
1.35
1.33
1.31
1.28
1.07
0.98
0.97
0.92
0.91
0.91
0.89
0.89
0.87
O
NH
N
N
CH3
0.97
1.91
0.96
0.73
1.95
5.78
2.83
2.05
6.11
4.05
3.00
f1 (ppm)


162.19
140.99
138.10
126.65
123.26
122.94
122.43
109.23
77.41 CDCl3
77.16 CDCl3
76.91 CDCl3
51.98
49.46
42.03
36.61
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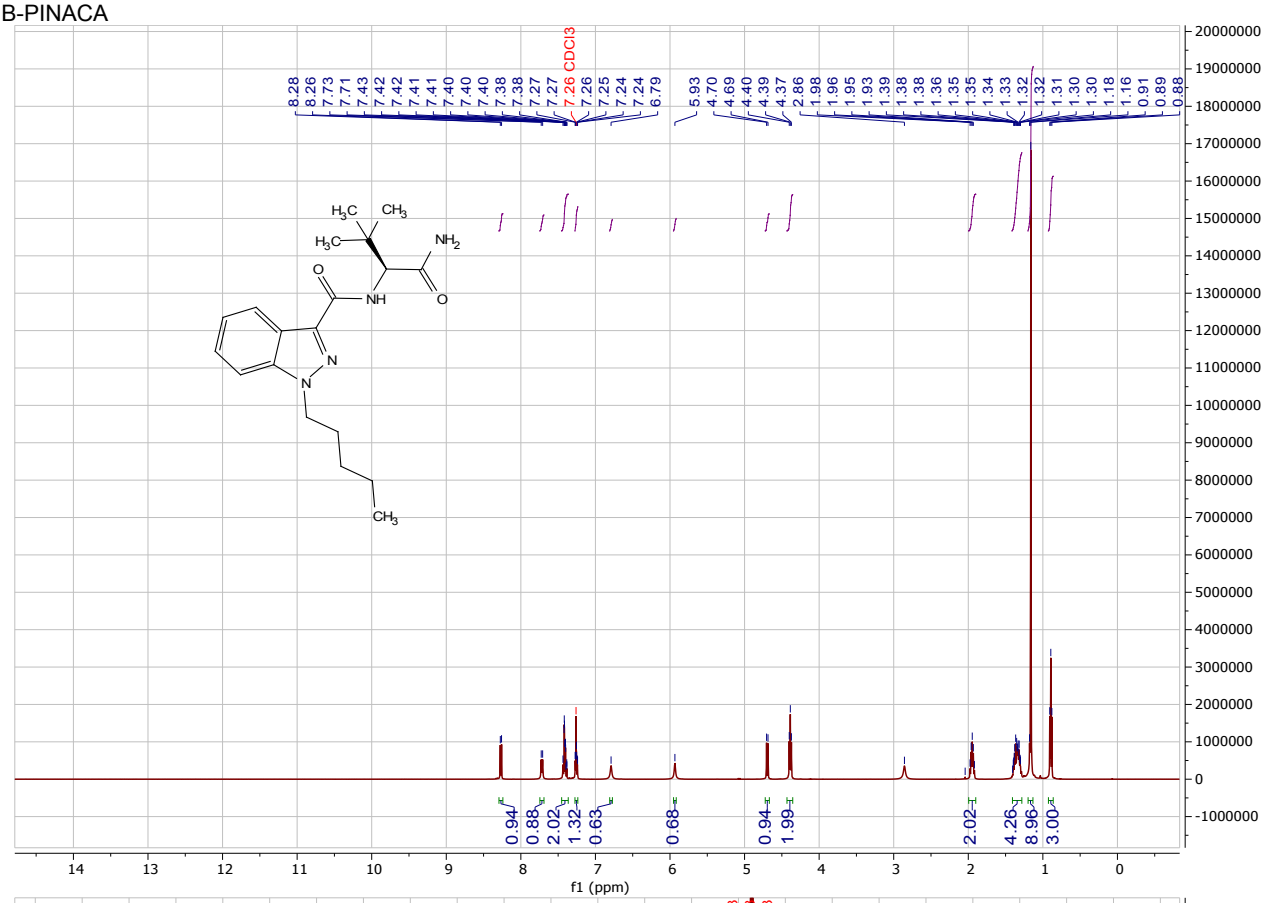
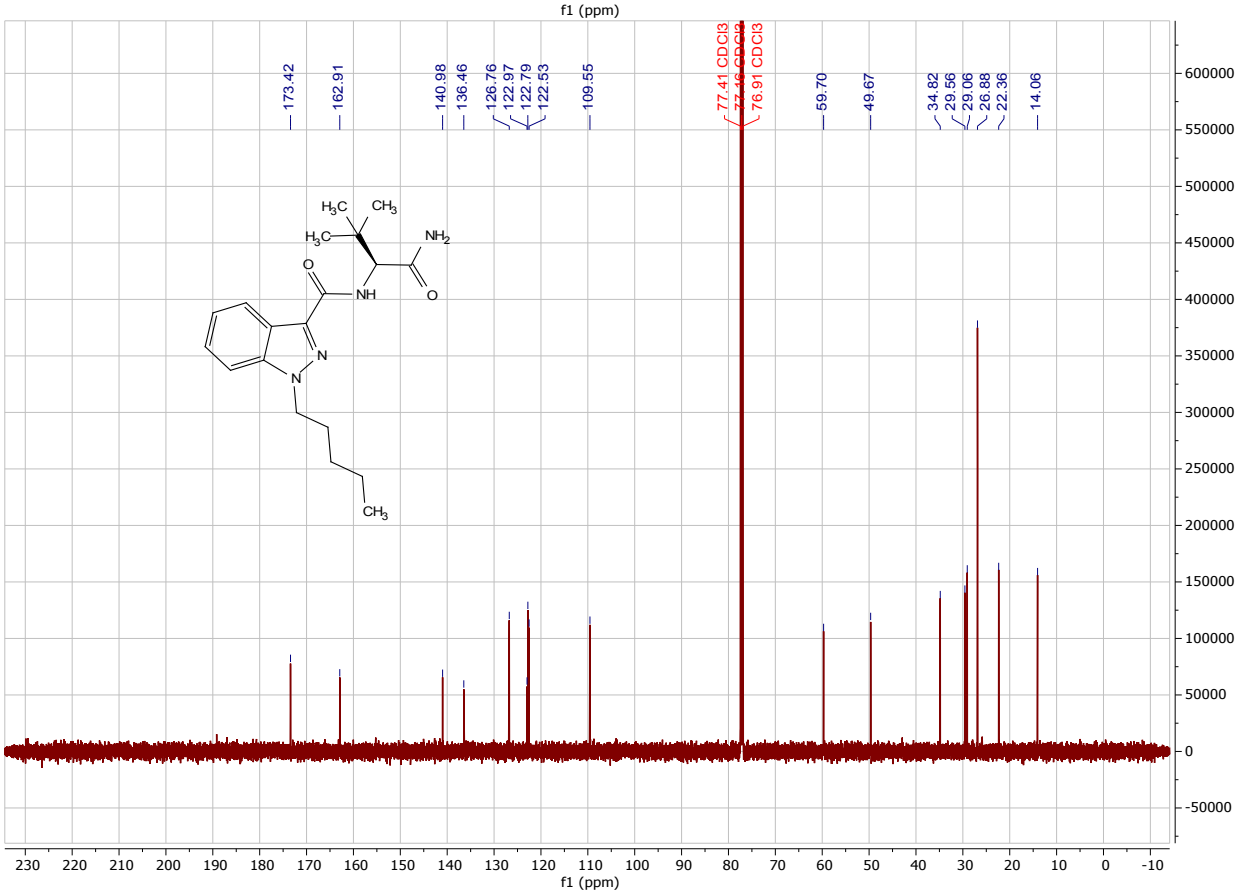
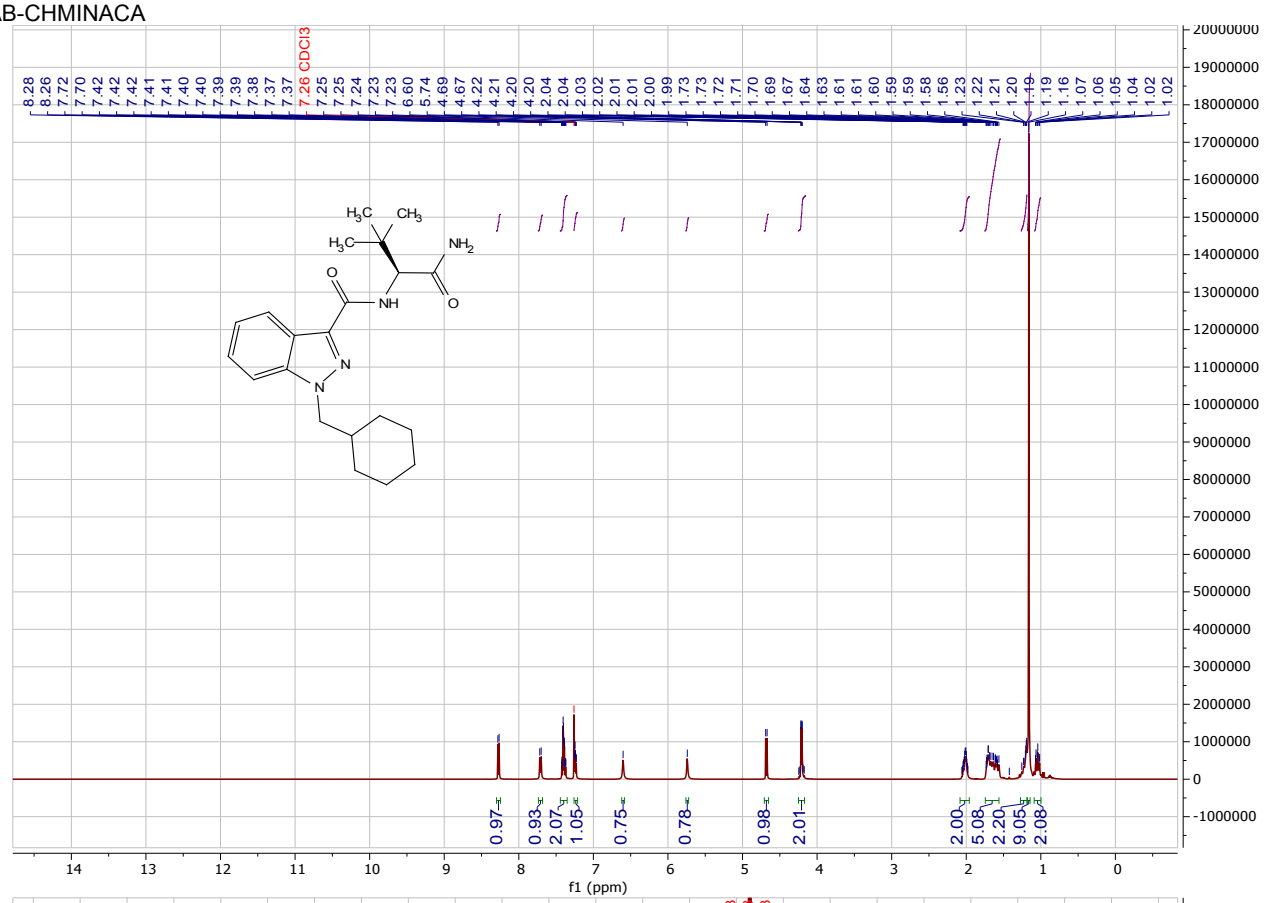
29.62
29.06
22.39
14.06
O
NH
N
N
CH3
f1 (ppm)

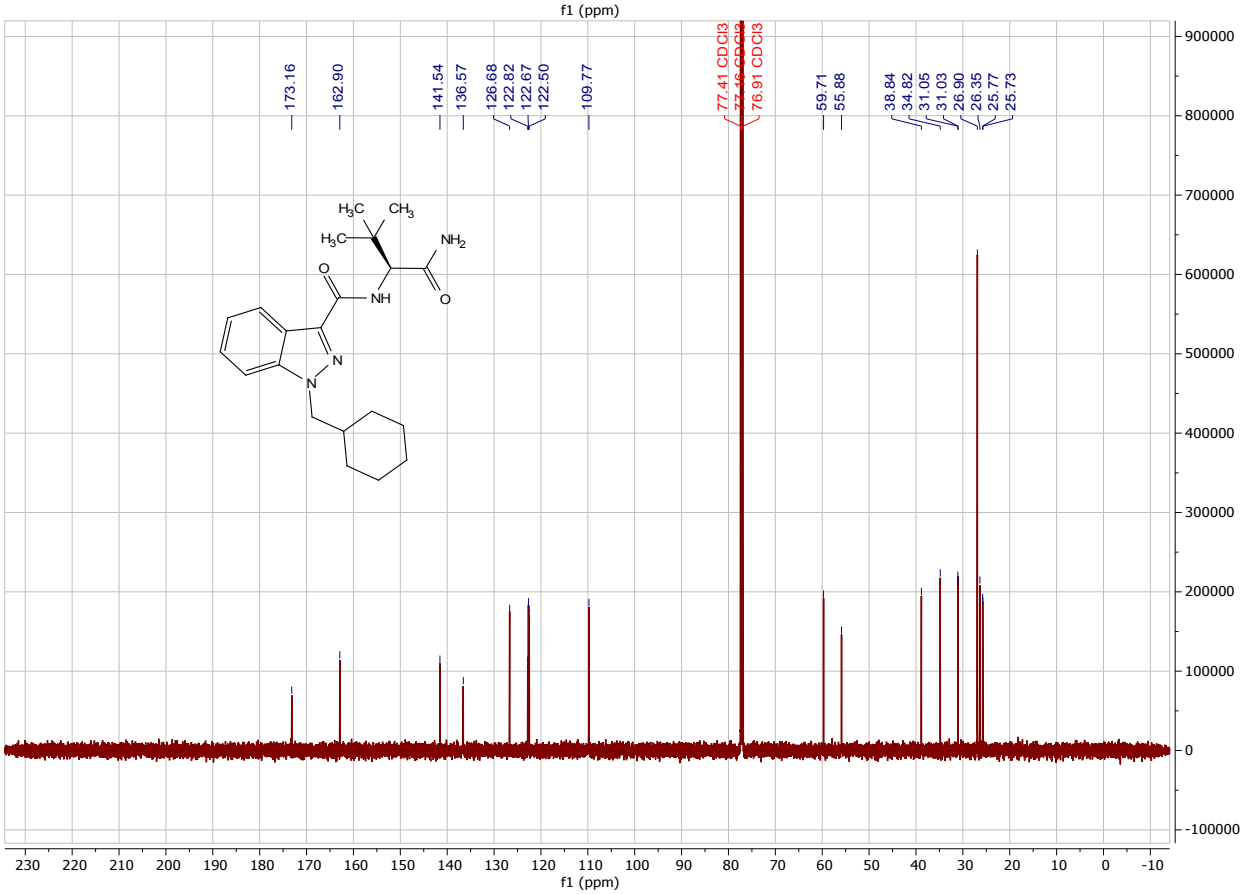
ADB-PINACA


8.28
8.26
7.73
7.71
7.43
7.42
7.42
7.41
7.41
7.40
7.40
7.40
7.38
7.27
7.27
7.26 CDCl3
7.26
7.25
7.24
7.24
6.79
5.93
4.70
4.69
4.40
4.39
4.37
2.86
1.98
1.96
1.95
1.93
1.39
1.38
1.38
1.36
1.35
1.35
1.34
1.33
1.32
1.32
1.31
1.30
1.30
1.18
1.16
0.91
0.89
0.88
0.94
0.88
2.02
1.32
0.63
0.68
0.94
1.99
2.02
4.26
8.96
3.00
f1 (ppm)

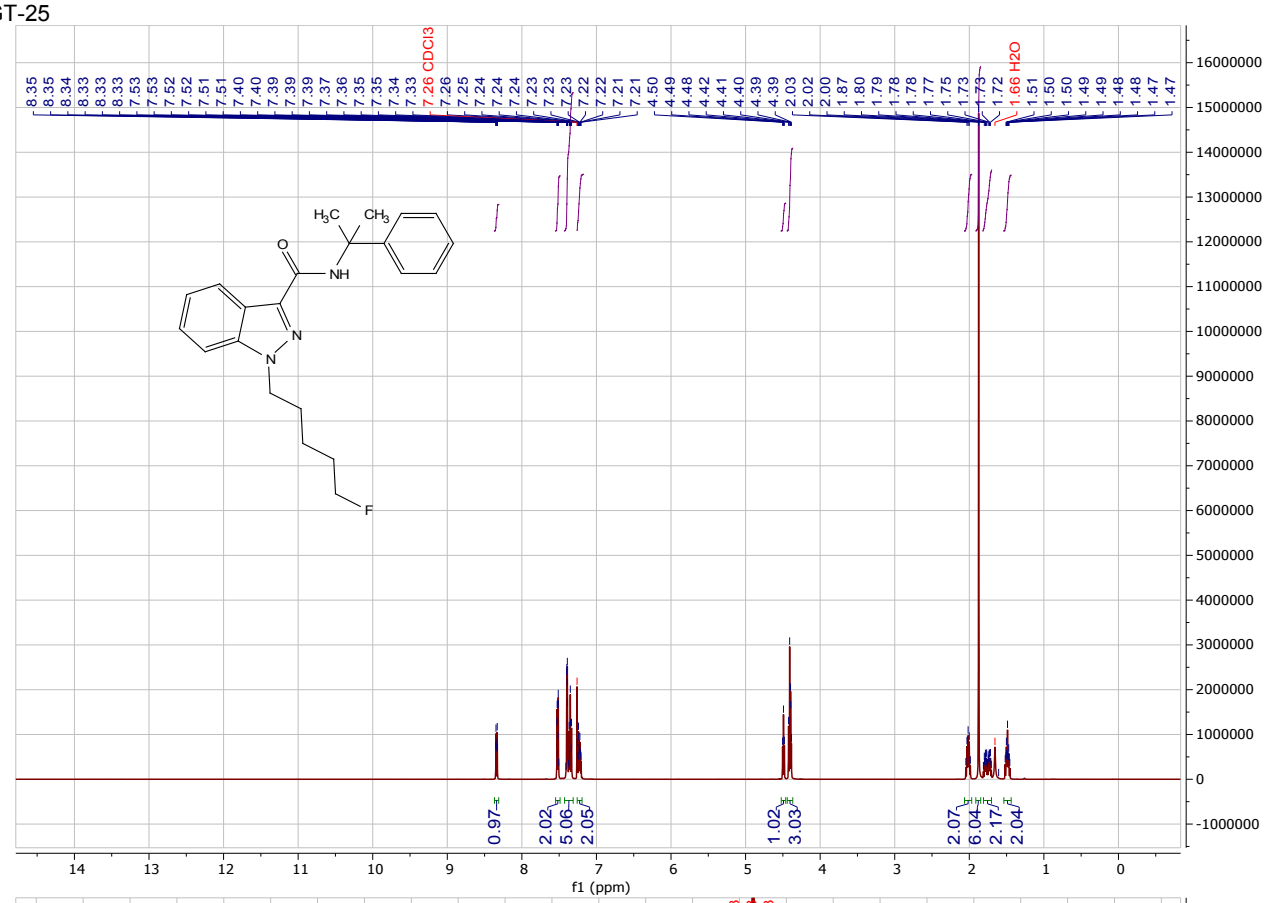

173.42
162.91
140.98
136.46
126.76
122.97
122.79
122.53
109.55
77.41 CDCl3
77.16 CDCl3
76.91 CDCl3
59.70
49.67
34.82
29.56
29.06
26.88
22.36
14.06
f1 (ppm)

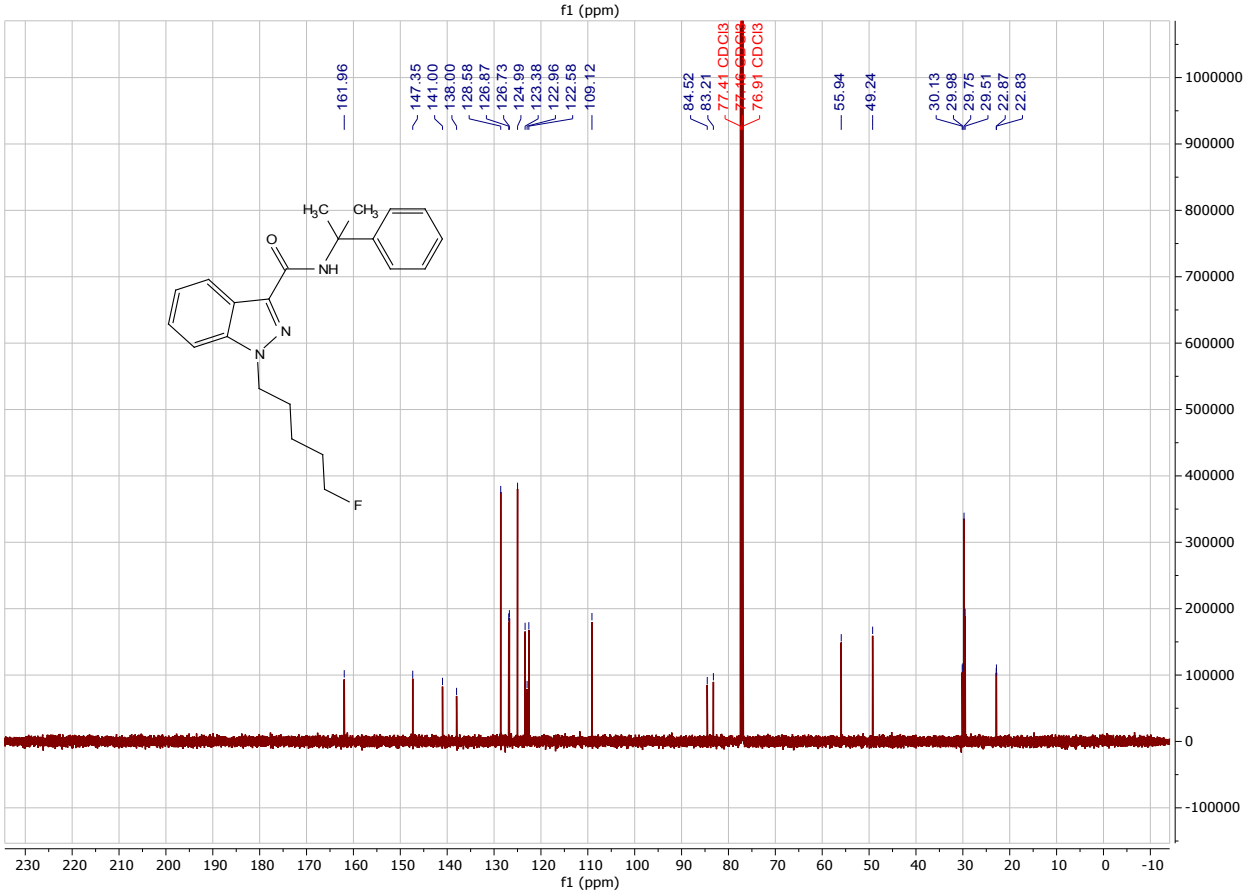
MAB-CHMINACA



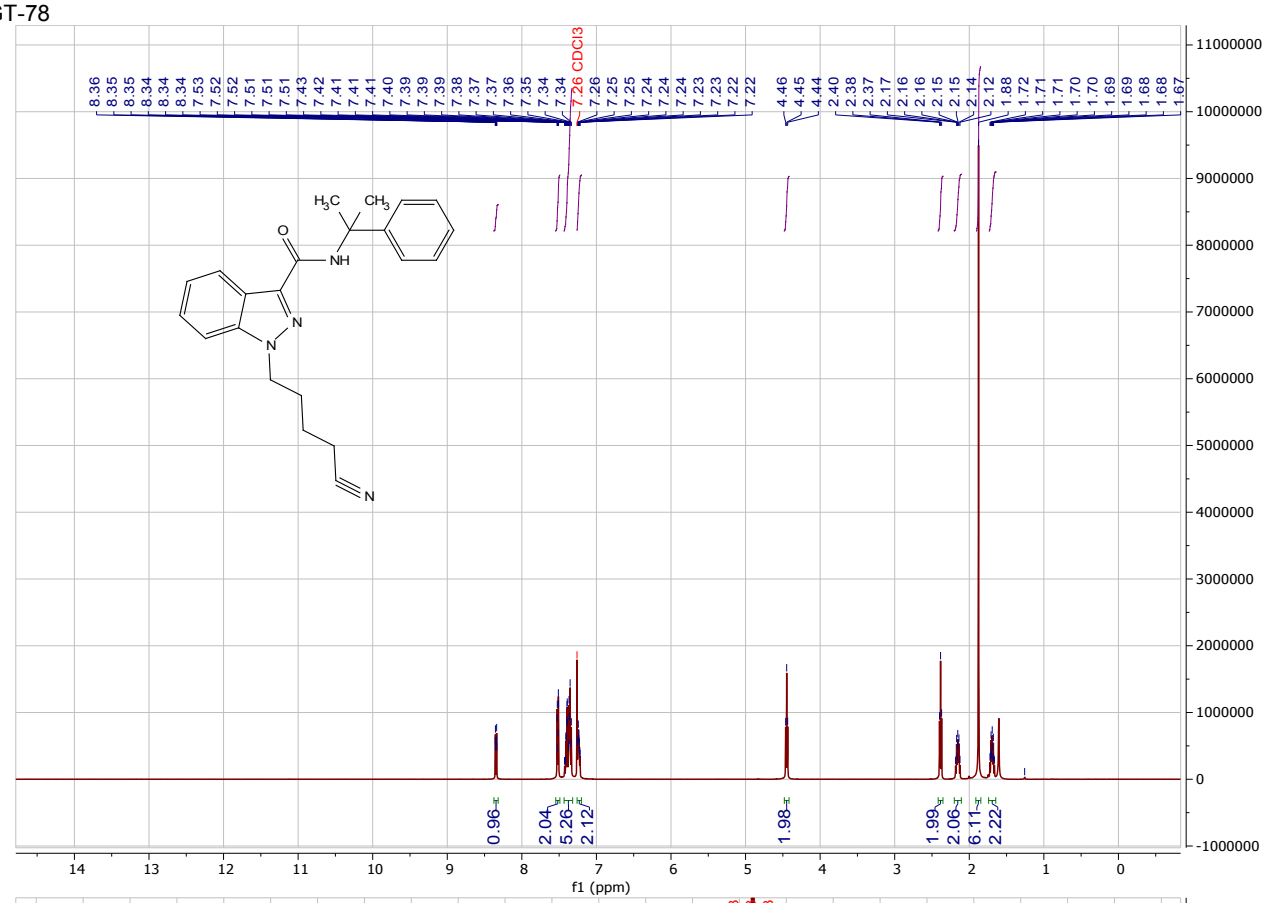


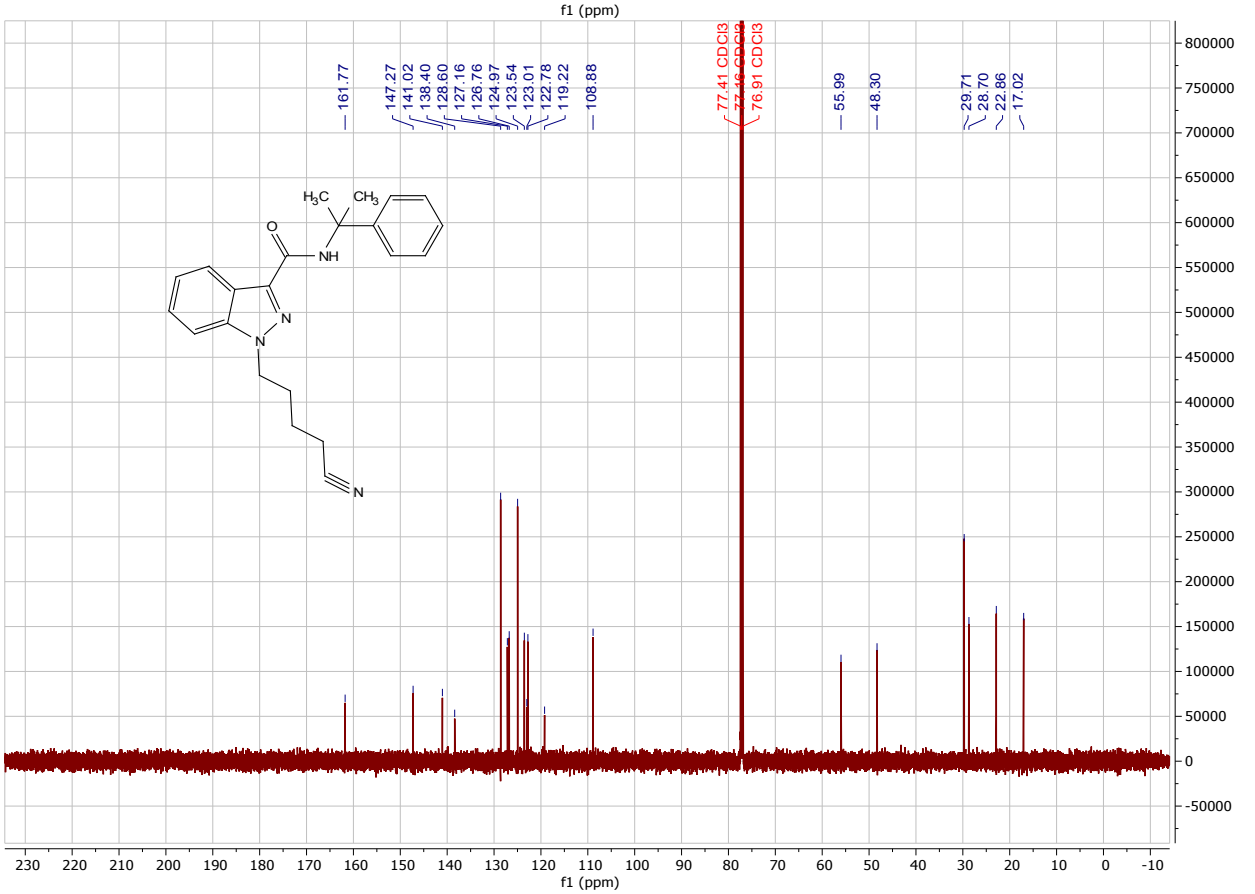
SGT-25


8.35 8.35 8.34 8.33 8.33 8.33 7.53 7.53 7.52 7.52 7.51 7.51 7.40 7.40 7.39 7.39 7.39 7.37 7.36 7.35 7.35 7.34 7.33 7.26 CDCl3 7.26 7.25 7.24 7.24 7.24 7.23 7.23 7.23 7.22 7.22 7.21 7.21 4.50 4.49 4.48 4.42 4.41 4.40 4.39 4.39 2.03 2.02 2.00 1.87 1.80 1.79 1.78 1.77 1.75 1.73 1.73 1.72 1.66 H2O 1.51 1.50 1.50 1.49 1.49 1.48 1.48 1.47 1.47
H3C CH3
O
NH
N
N
F
0.97 2.02 5.06 2.05 1.02 3.03 2.07 6.04 2.17 2.04
f1 (ppm)

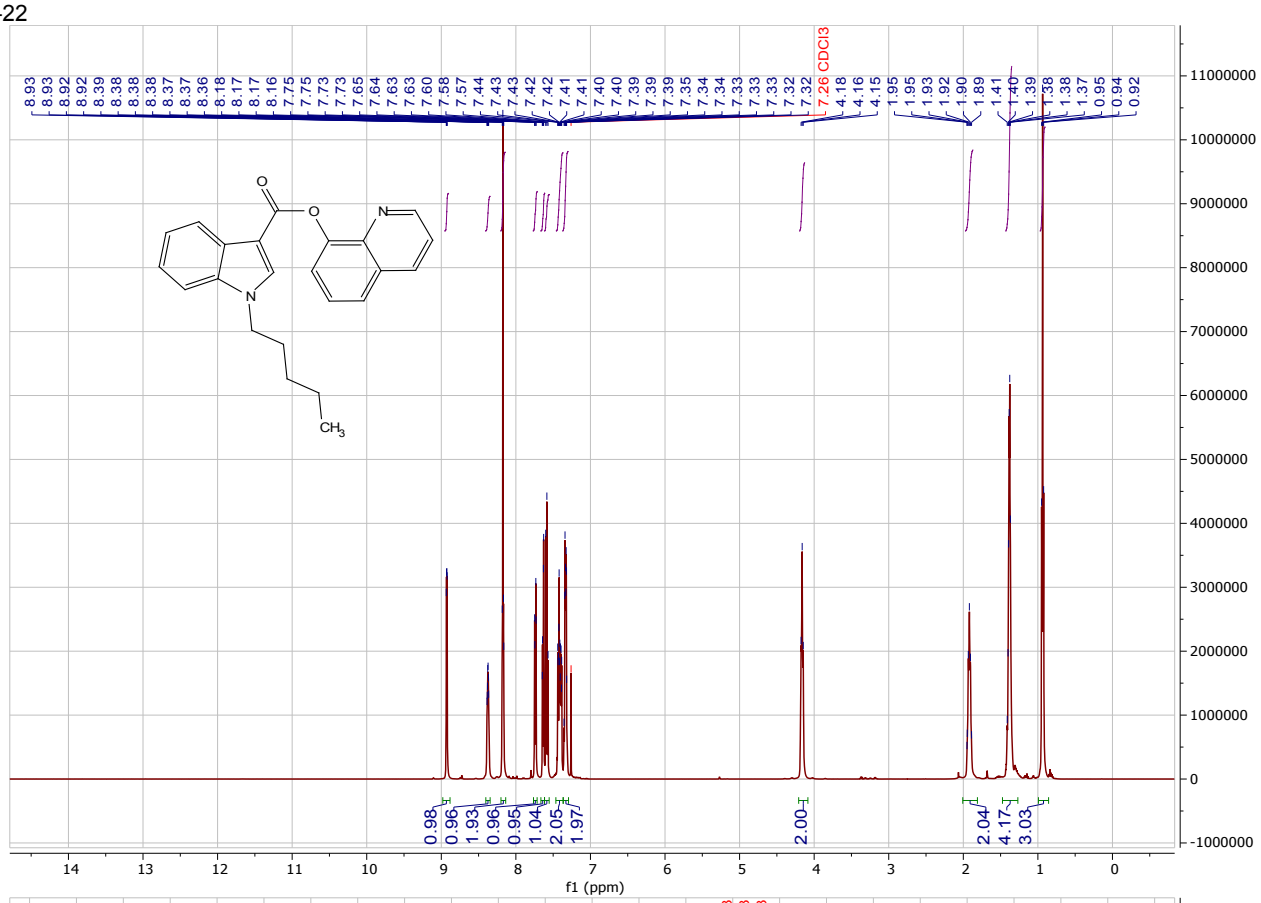

161.96 147.35 141.00 138.00 128.58 126.87 126.73 124.99 123.38 122.96 122.58 109.12 84.52 83.21 77.41 CDCl3 77.16 CDCl3 76.91 CDCl3 55.94 49.24 30.13 29.98 29.75 29.51 22.87 22.83
H3C CH3
O
NH
N
N
F
f1 (ppm)

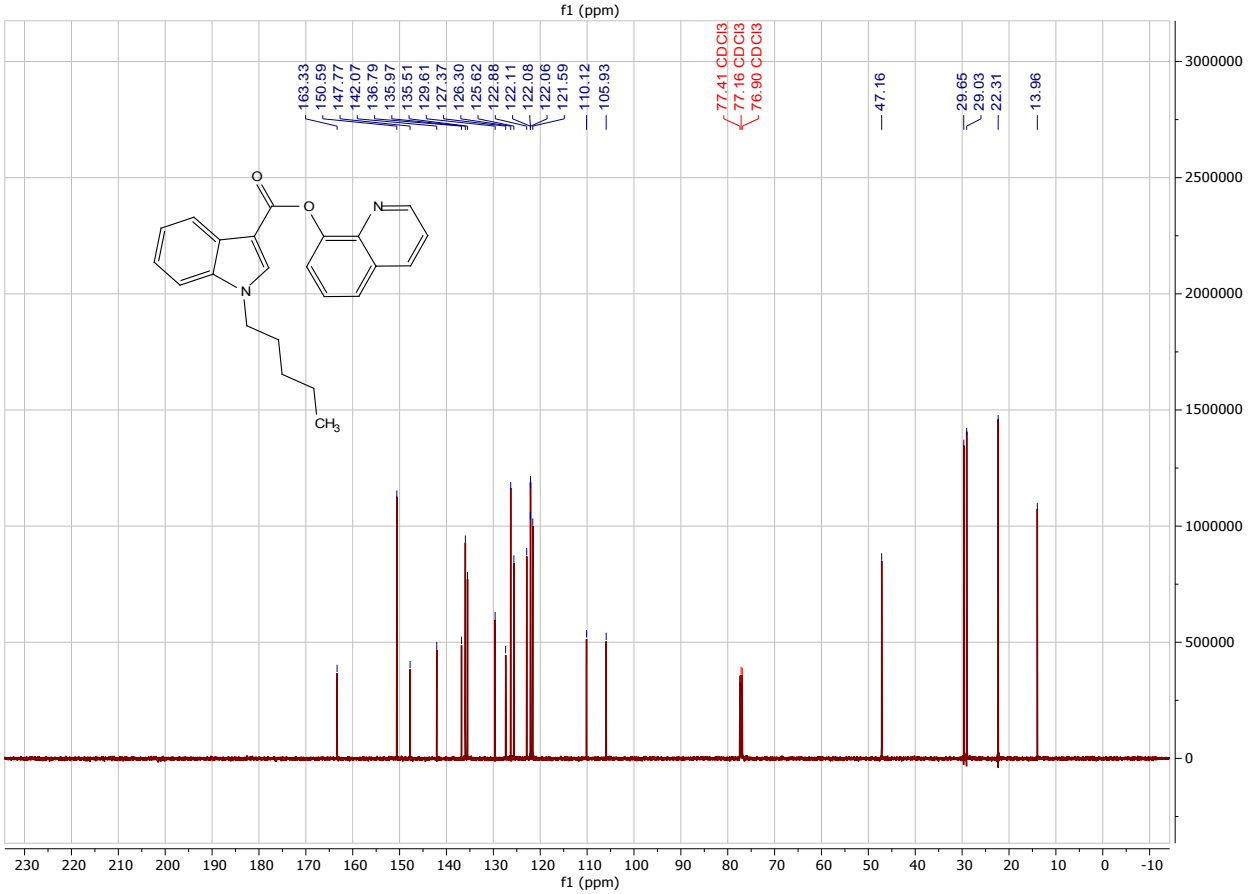
SGT-78


8.36 8.35 8.35 8.34 8.34 8.34 7.53 7.52 7.52 7.51 7.51 7.51 7.43 7.42 7.41 7.41 7.41 7.40 7.39 7.39 7.39 7.38 7.37 7.37 7.36 7.35 7.34 7.34 7.26 CDCl3 7.26 7.25 7.24 7.24 7.24 7.23 7.22 7.22 4.46 4.45 4.44 2.40 2.38 2.37 2.17 2.16 2.16 2.15 2.15 2.14 2.12 1.88 1.72 1.71 1.71 1.70 1.70 1.69 1.69 1.68 1.68 1.67
H3C CH3
O
NH
N
N
N
0.96 2.04 5.26 2.12 1.98 1.99 2.06 6.11 2.22
f1 (ppm)

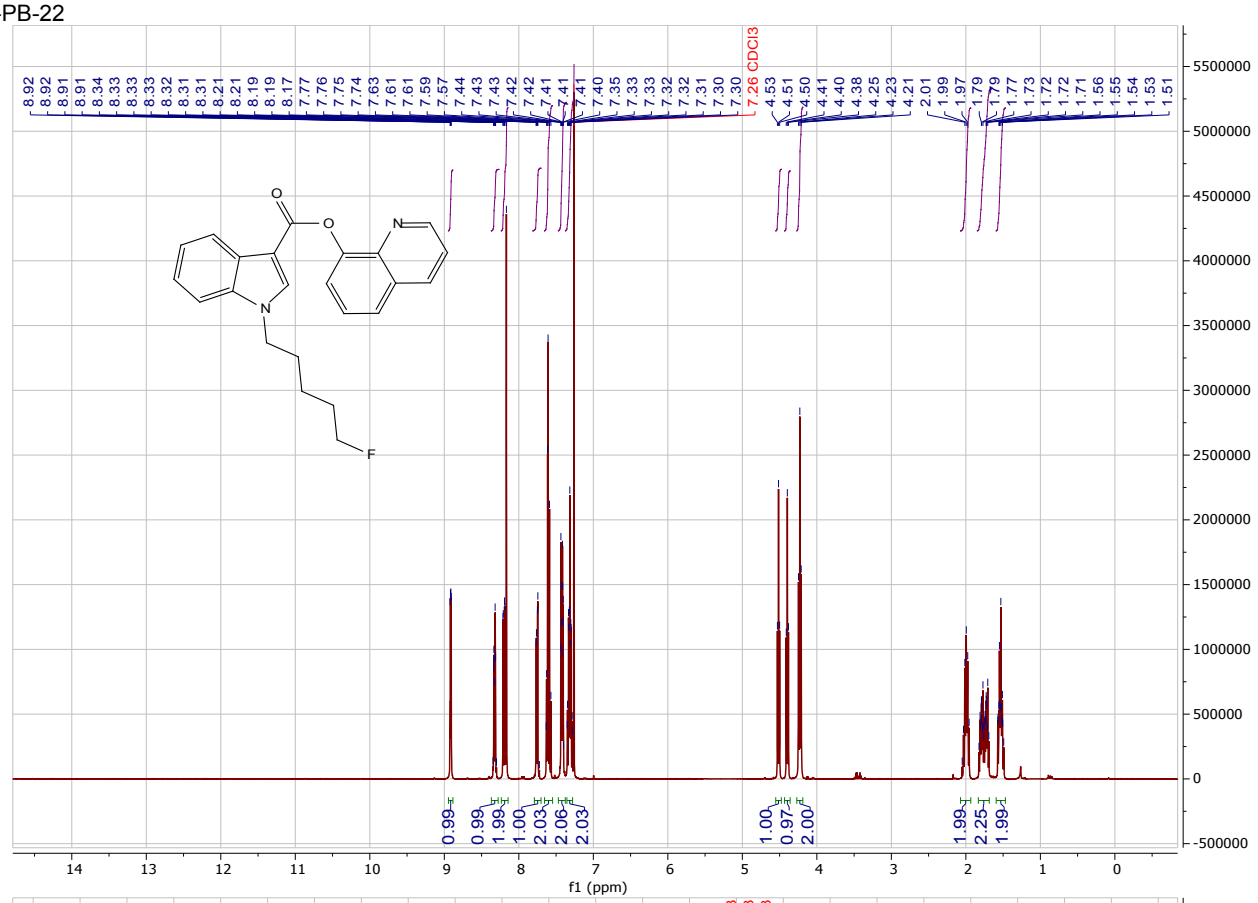

161.77 147.27 141.02 138.40 128.60 127.16 126.76 124.97 123.54 123.01 122.78 119.22 108.88 77.41 CDCl3 77.16 CDCl3 76.91 CDCl3 55.99 48.30 29.71 28.70 22.86 17.02
H3C CH3
O
NH
N
N
N
f1 (ppm)

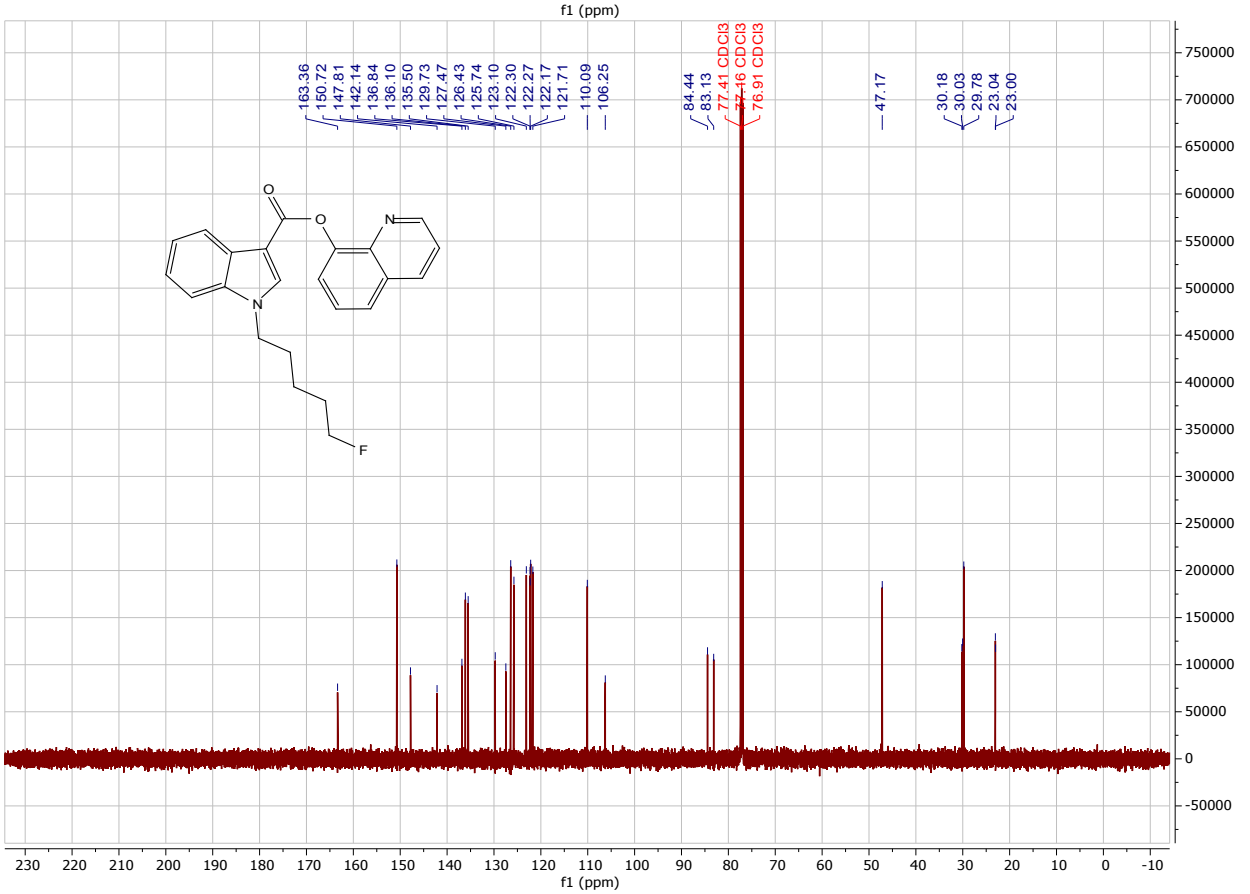
PB-22


8.93 8.93 8.92 8.92 8.39 8.38 8.38 8.37 8.37 8.36 8.18 8.17 8.17 8.16 7.75 7.75 7.73 7.73 7.65 7.64 7.63 7.63 7.60 7.58 7.57 7.44 7.43 7.43 7.42 7.42 7.41 7.41 7.40 7.40 7.39 7.39 7.39 7.35 7.34 7.34 7.33 7.33 7.33 7.32 7.32 7.26 CDCl3 4.18 4.16 4.15 1.95 1.95 1.93 1.92 1.90 1.89 1.41 1.40 1.39 1.38 1.38 1.37 0.95 0.94 0.92
0.98 0.96 1.93 0.96 0.95 1.04 2.05 1.97 2.00 2.04 4.17 3.03
f1 (ppm)

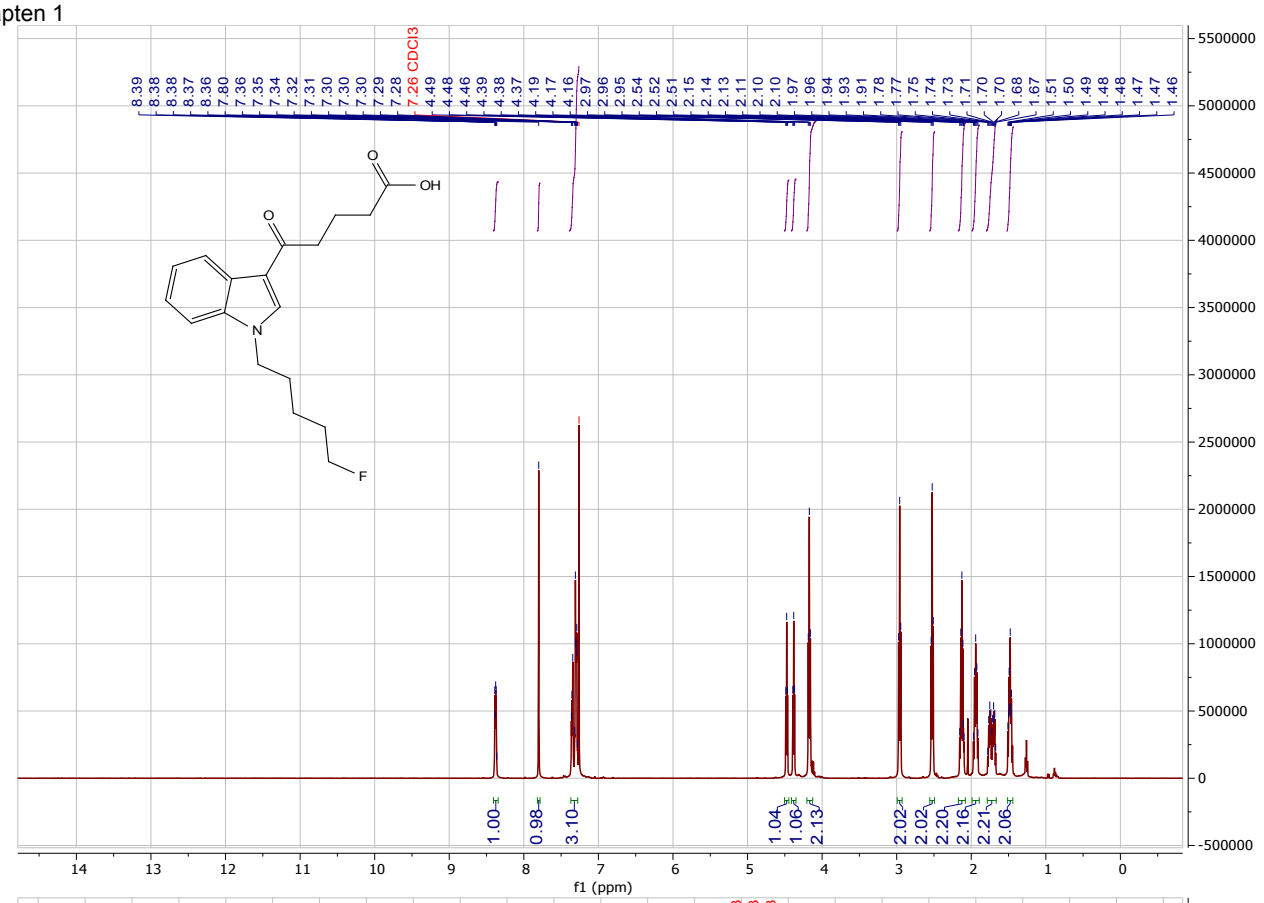

163.33 150.59 147.77 142.07 136.79 135.97 135.51 129.61 127.37 126.30 125.62 122.88 122.11 122.08 122.06 121.59 110.12 105.93
77.41 CDCl3 77.16 CDCl3 76.90 CDCl3 47.16 29.65 29.03 22.31 13.96
f1 (ppm)

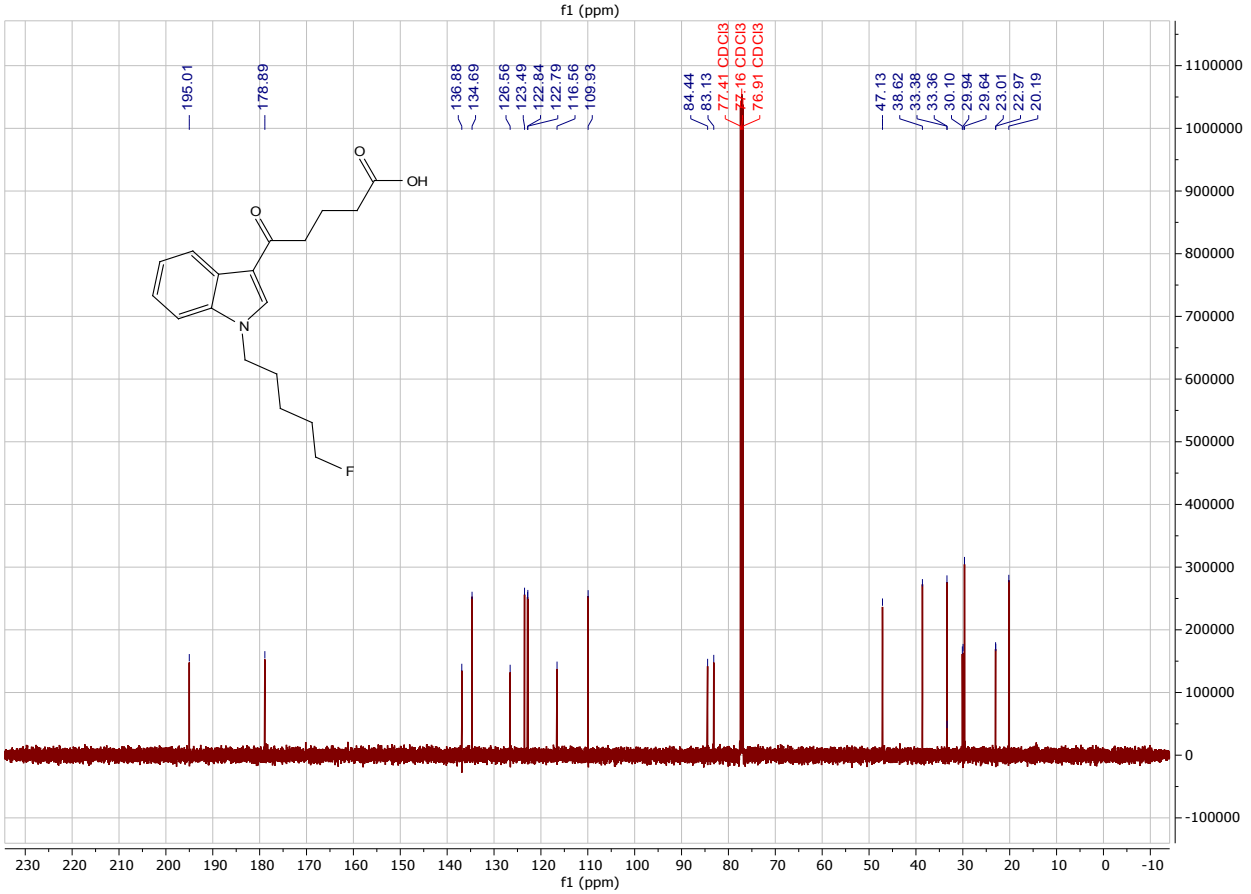
5F-PB-22


8.92 8.92 8.91 8.91 8.34 8.33 8.33 8.32 8.31 8.31 8.21 8.21 8.19 8.19 8.17 7.77 7.76 7.75 7.74 7.63 7.61 7.61 7.59 7.57 7.44 7.43 7.43 7.42 7.42 7.41 7.41 7.41 7.40 7.35 7.33 7.33 7.32 7.31 7.30 7.30 7.26 CDCl3 4.53 4.51 4.50 4.41 4.40 4.38 4.25 4.23 4.21 2.01 1.99 1.97 1.79 1.79 1.77 1.73 1.72 1.72 1.71 1.56 1.55 1.54 1.53 1.51
O
O
N
N
F
0.99 0.99 1.99 1.00 2.03 2.06 2.03 1.00 0.97 2.00 1.99 2.25 1.99
f1 (ppm)

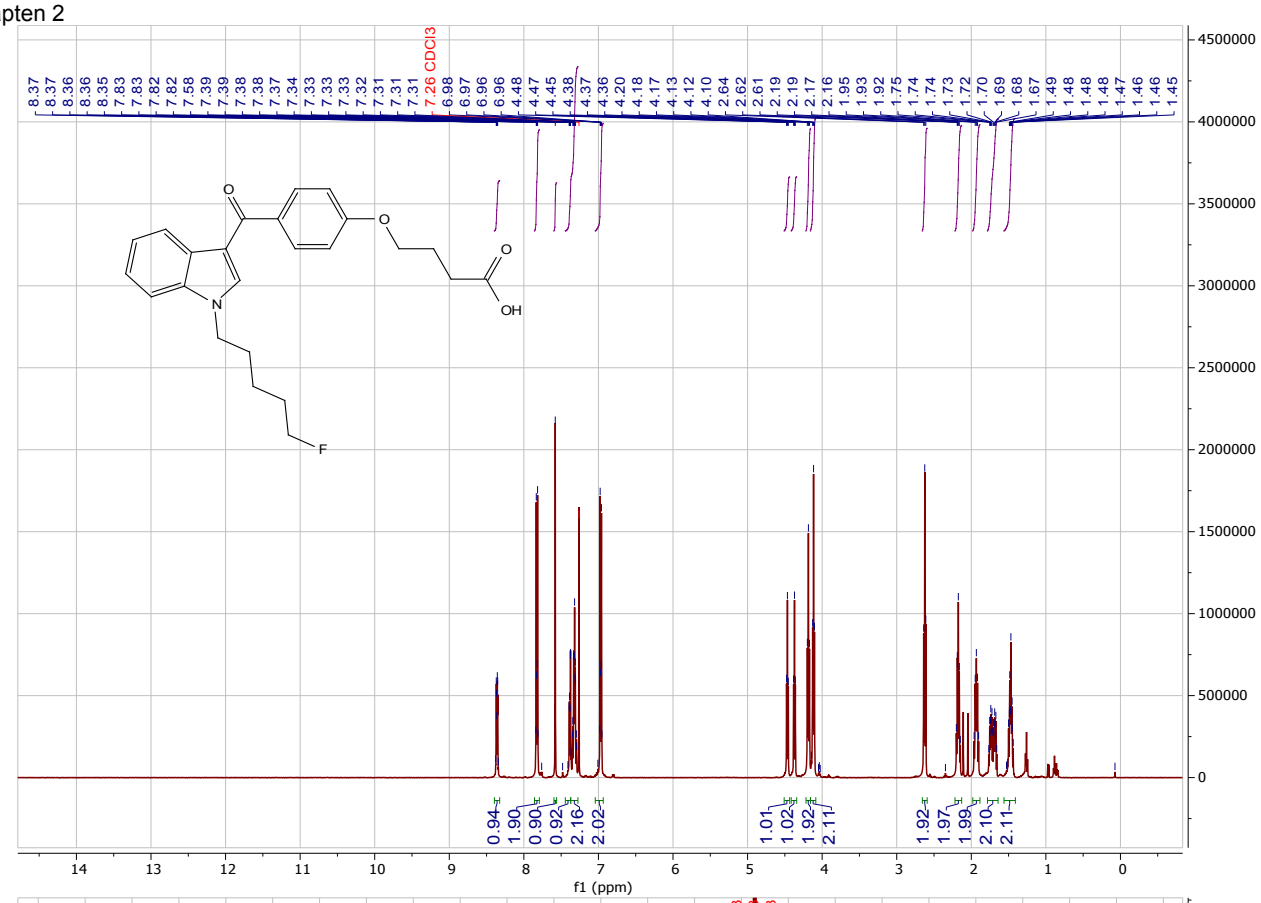

163.36 150.72 147.81 142.14 136.84 136.10 135.50 129.73 127.47 126.43 125.74 123.10 122.30 122.27 122.17 121.71 110.09 106.25 84.44 83.13 77.41 CDCl3 77.16 CDCl3 76.91 CDCl3 47.17 30.18 30.03 29.78 23.04 23.00
O
O
N
N
F
f1 (ppm)

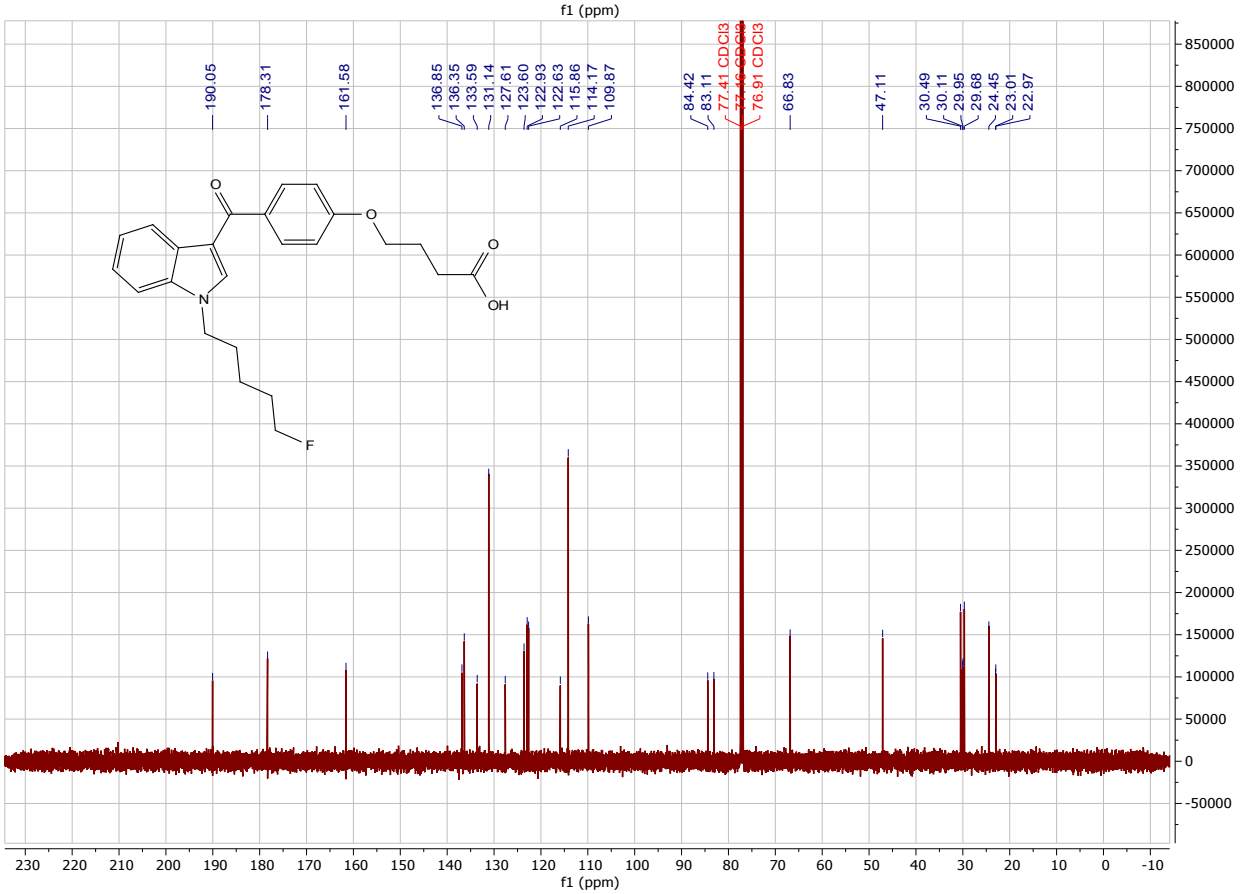
Hapten 1


8.39 8.38 8.38 8.37 8.36 7.80 7.36 7.35 7.34 7.32 7.31 7.30 7.30 7.30 7.29 7.28 7.26 CDCl3 4.49 4.48 4.46 4.39 4.38 4.37 4.19 4.17 4.16 2.97 2.96 2.95 2.54 2.52 2.51 2.15 2.14 2.13 2.11 2.10 2.10 1.97 1.96 1.94 1.93 1.91 1.78 1.77 1.75 1.74 1.73 1.71 1.70 1.70 1.68 1.67 1.51 1.50 1.49 1.48 1.48 1.47 1.47 1.46
O
OH
O
N
F
1.00 0.98 3.10 1.04 1.06 2.13 2.02 2.02 2.20 2.16 2.21 2.06
f1 (ppm)

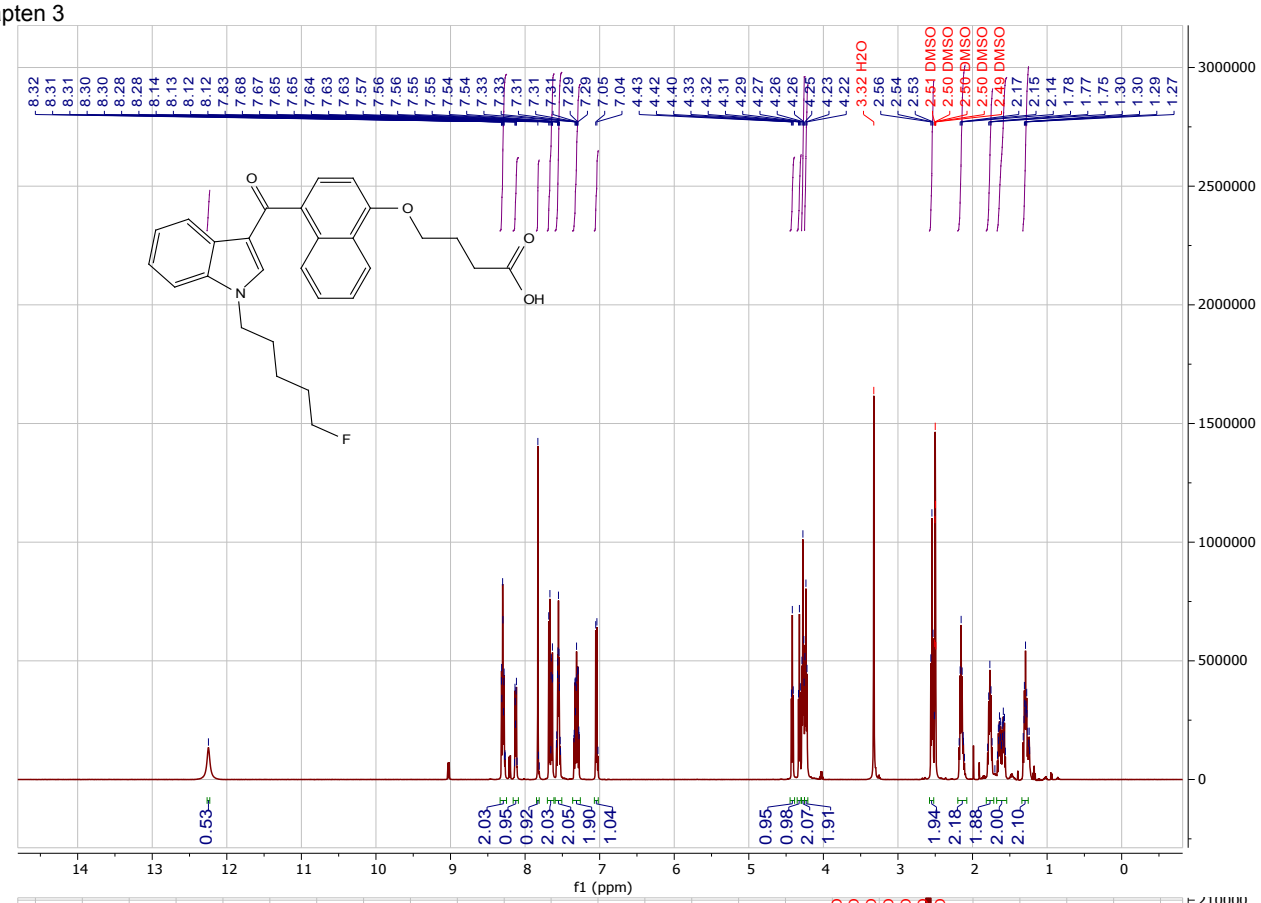

195.01 178.89 136.88 134.69 126.56 123.49 122.84 122.79 116.56 109.93 84.44 83.13 77.41 CDCl3 77.16 CDCl3 76.91 CDCl3 47.13 38.62 33.38 33.36 30.10 29.94 29.64 23.01 22.97 20.19
O
OH
O
N
F
f1 (ppm)

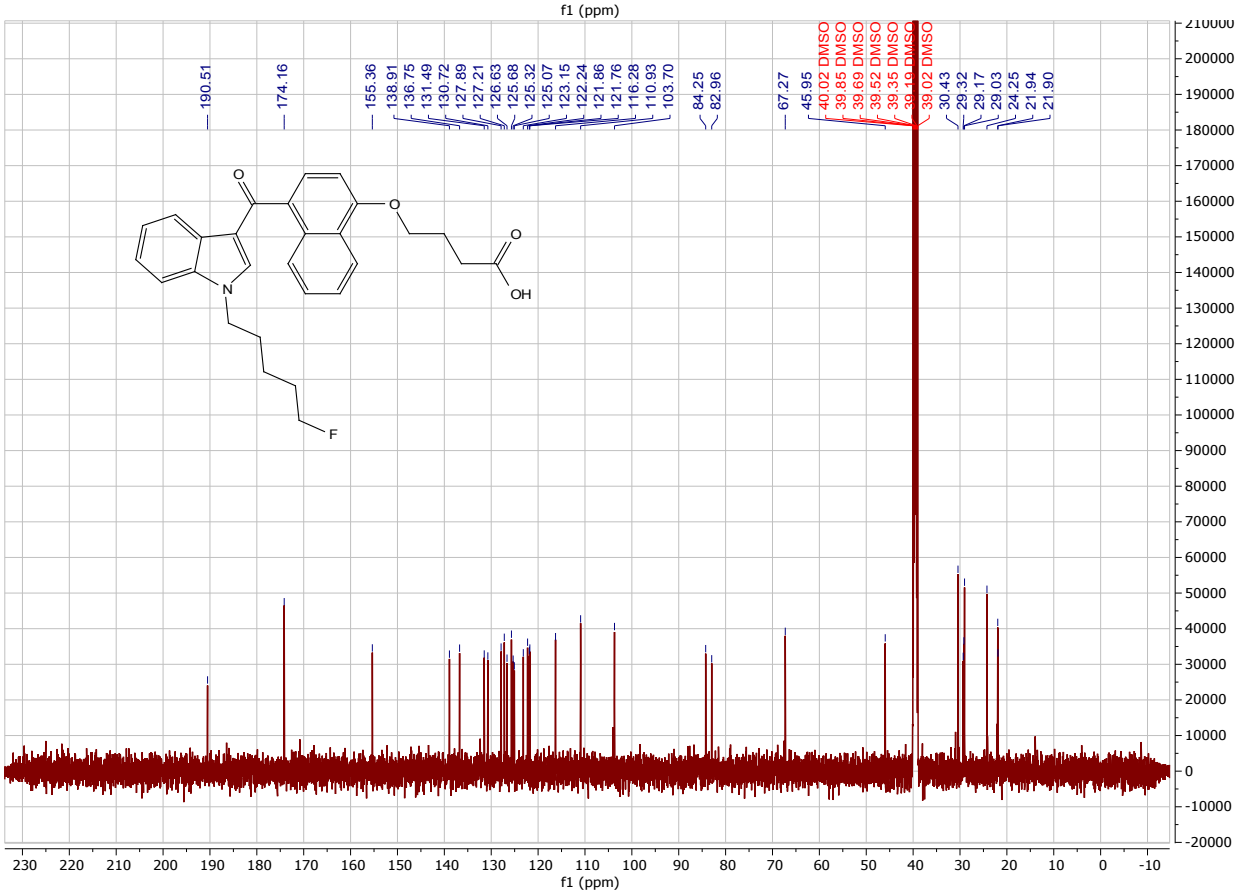
Hapten 2


8.37 8.37 8.36 8.36 8.35 7.83 7.83 7.82 7.82 7.58 7.39 7.39 7.38 7.38 7.37 7.34 7.33 7.33 7.33 7.32 7.31 7.31 7.31 7.26 CDCl3 6.98 6.97 6.96 6.96 4.48 4.47 4.45 4.38 4.37 4.36 4.20 4.18 4.17 4.13 4.12 4.10 2.64 2.62 2.61 2.19 2.19 2.17 2.16 1.95 1.93 1.92 1.75 1.74 1.74 1.73 1.72 1.70 1.69 1.68 1.67 1.49 1.48 1.48 1.48 1.47 1.46 1.46 1.45
O
O
O
OH
N
F
0.94 1.90 0.90 0.92 2.16 2.02 1.01 1.02 1.92 2.11 1.92 1.97 1.99 2.10 2.11
f1 (ppm)

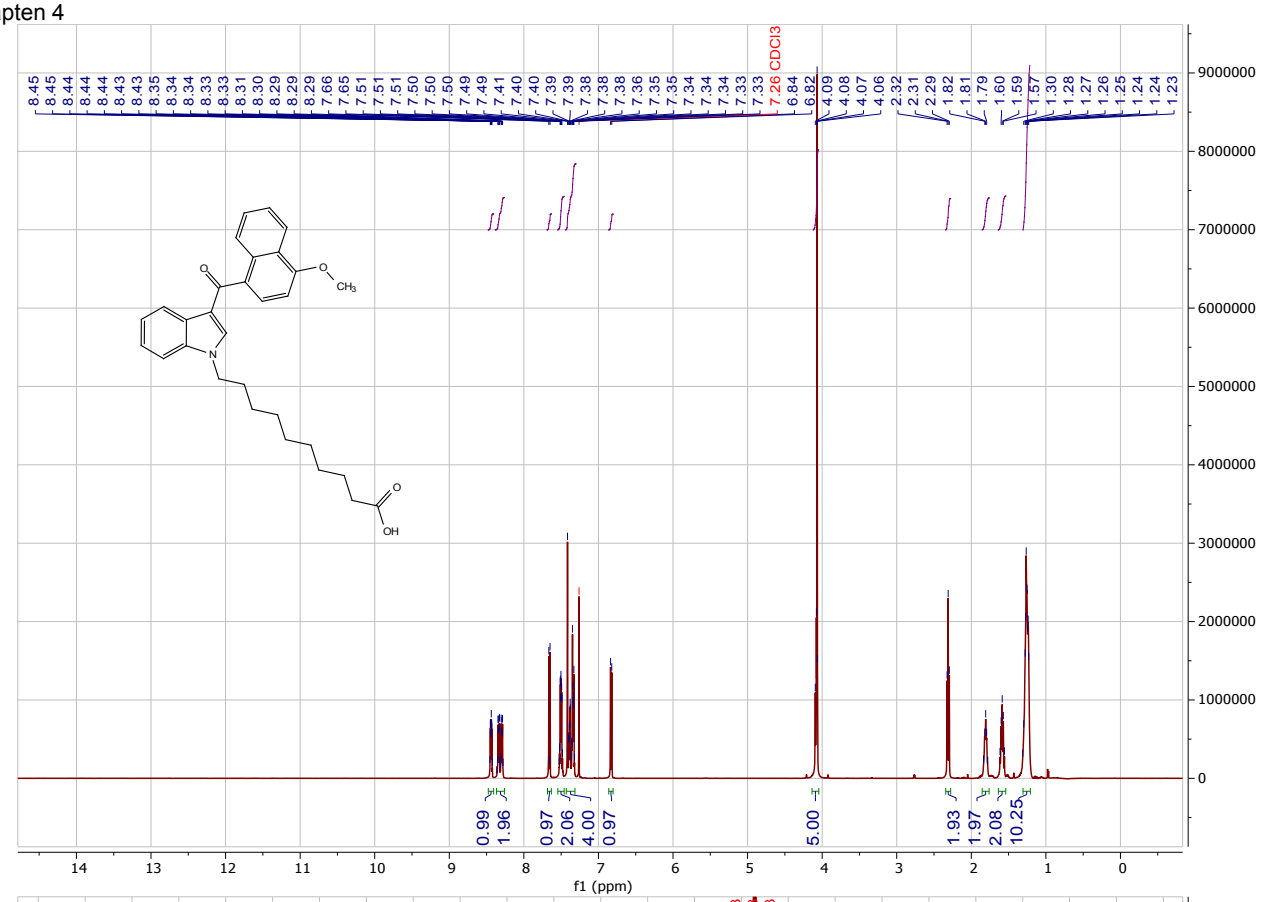

190.05 178.31 161.58 136.85 136.35 133.59 131.14 127.61 123.60 122.93 122.63 115.86 114.17 109.87 84.42 83.11 77.41 CDCl3 77.16 CDCl3 76.91 CDCl3 66.83 47.11 30.49 30.11 29.95 29.68 24.45 23.01 22.97
O
O
O
OH
N
F
f1 (ppm)

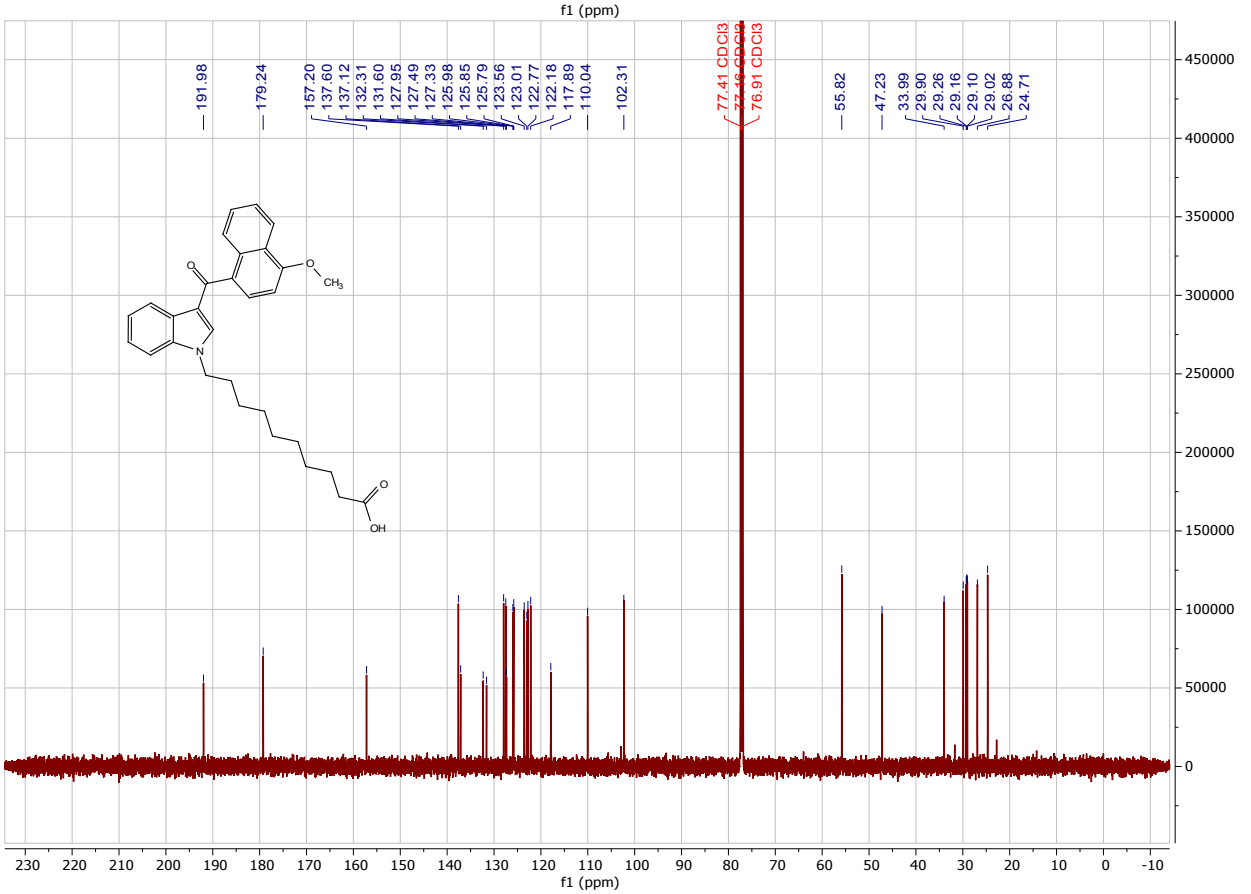
Hapten 3


8.32 8.31 8.31 8.30 8.30 8.28 8.28 8.14 8.13 8.12 8.12 7.83 7.68 7.67 7.65 7.65 7.64 7.63 7.63 7.57 7.56 7.56 7.55 7.55 7.54 7.54 7.33 7.33 7.31 7.31 7.29 7.29 7.05 7.04 4.43 4.42 4.40 4.33 4.32 4.31 4.29 4.27 4.26 4.26 4.25 4.23 4.22 3.32 H2O 2.56 2.54 2.53 2.51 DMSO 2.50 DMSO 2.50 DMSO 2.50 DMSO 2.49 DMSO 2.17 2.15 2.14 1.78 1.77 1.75 1.30 1.29 1.27
O
O
O
OH
N
F
0.53 2.03 0.95 0.92 2.03 2.05 1.90 1.04 0.95 0.98 2.07 1.91 1.94 2.18 1.88 2.00 2.10
f1 (ppm)

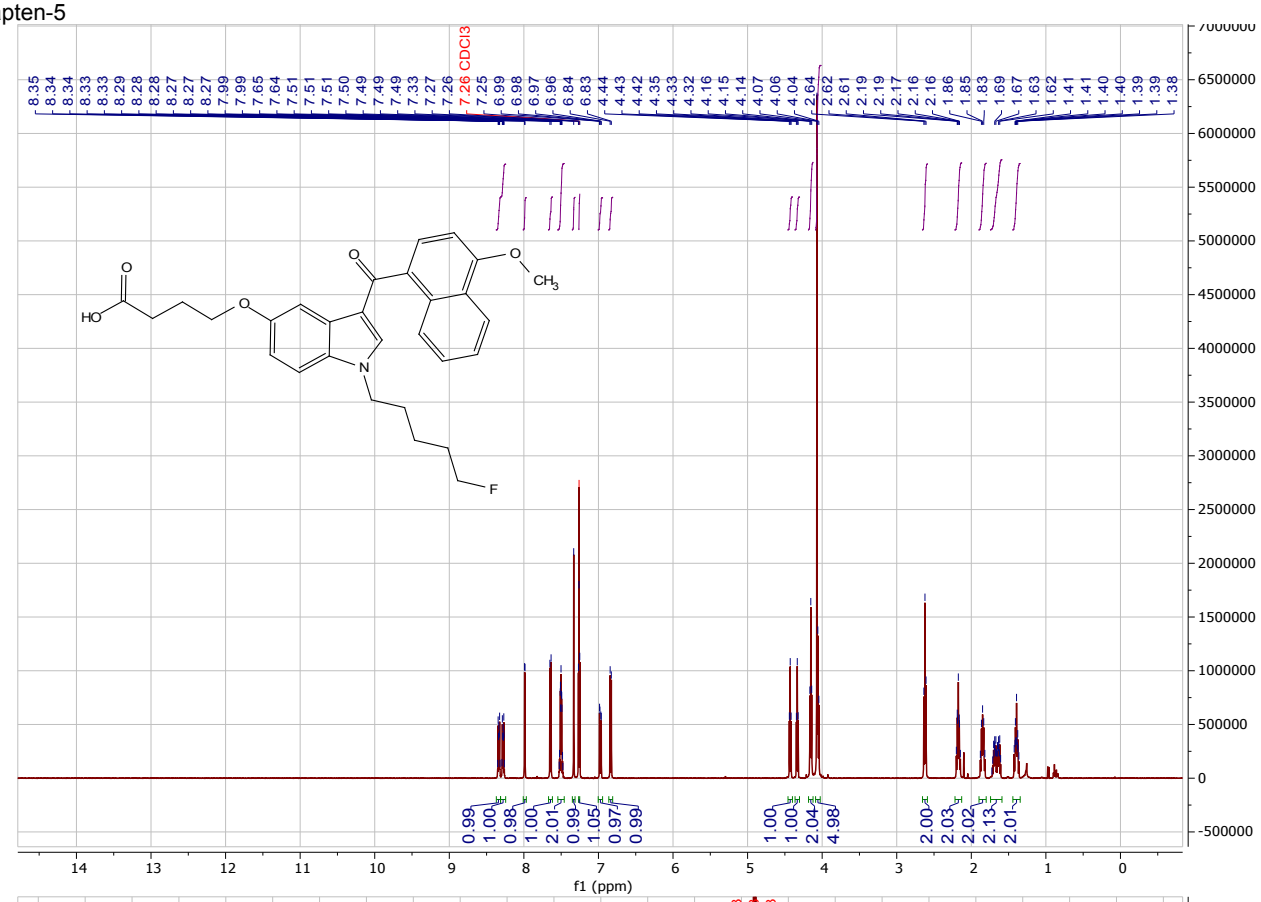

190.51 174.16 155.36 138.91 136.75 131.49 130.72 127.89 127.21 126.63 125.68 125.32 125.07 123.15 122.24 121.86 121.76 116.28 110.93 103.70 84.25 82.96 67.27 45.95 40.02 DMSO 39.85 DMSO 39.69 DMSO 39.52 DMSO 39.35 DMSO 39.19 DMSO 39.02 DMSO 30.43 29.32 29.17 29.03 24.25 21.94 21.90
O
O
O
OH
N
F
f1 (ppm)

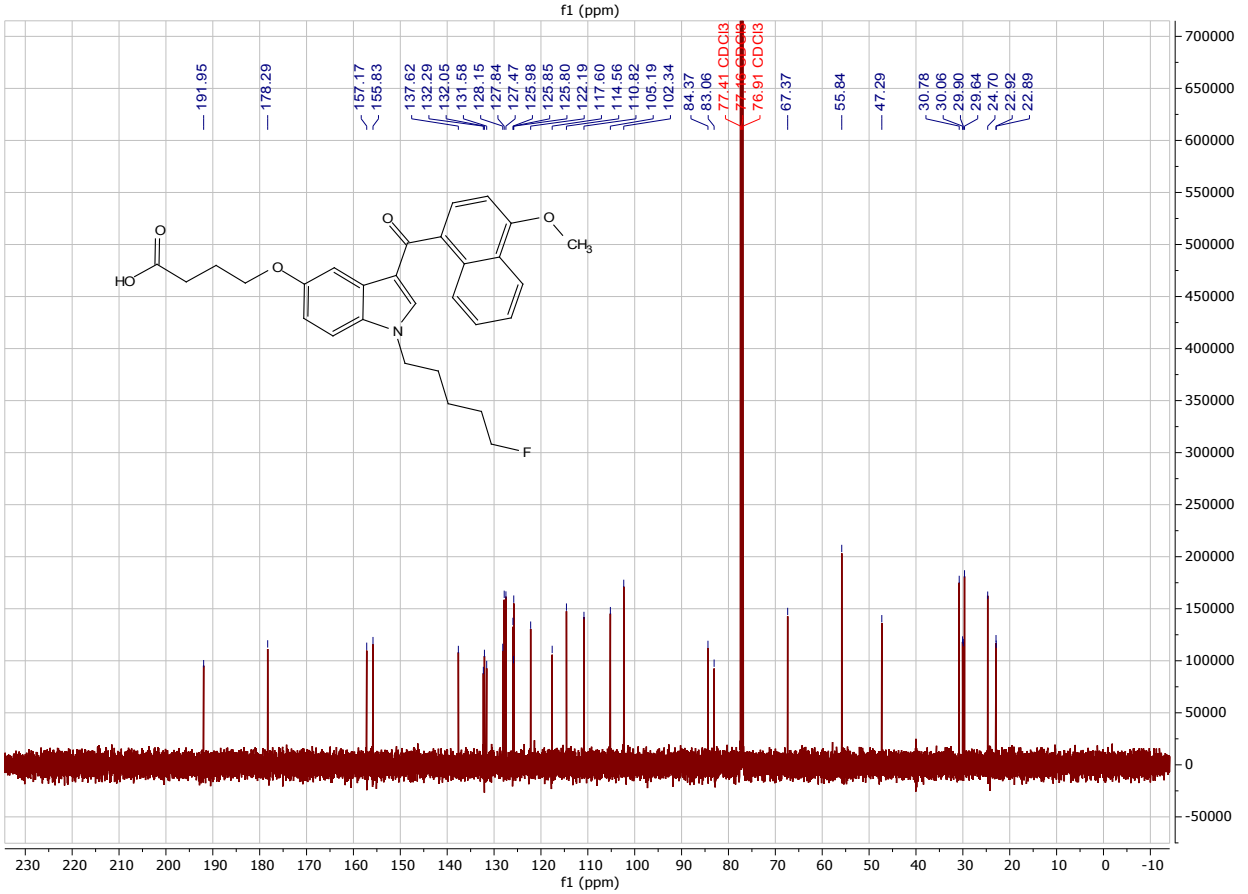
Hapten 4


8.45 8.45 8.44 8.44 8.44 8.43 8.43 8.35 8.34 8.34 8.33 8.33 8.31 8.30 8.29 8.29 8.29 7.66 7.65 7.51 7.51 7.51 7.50 7.50 7.50 7.49 7.49 7.41 7.40 7.40 7.39 7.39 7.38 7.38 7.38 7.36 7.35 7.35 7.34 7.34 7.34 7.33 7.33 7.26 CDCl3 6.84 6.82 4.09 4.08 4.07 4.06 2.32 2.31 2.29 1.82 1.81 1.79 1.60 1.59 1.57 1.30 1.28 1.27 1.26 1.25 1.24 1.24 1.23
0.99 1.96 0.97 2.06 4.00 0.97 5.00 1.93 1.97 2.08 10.25
f1 (ppm)

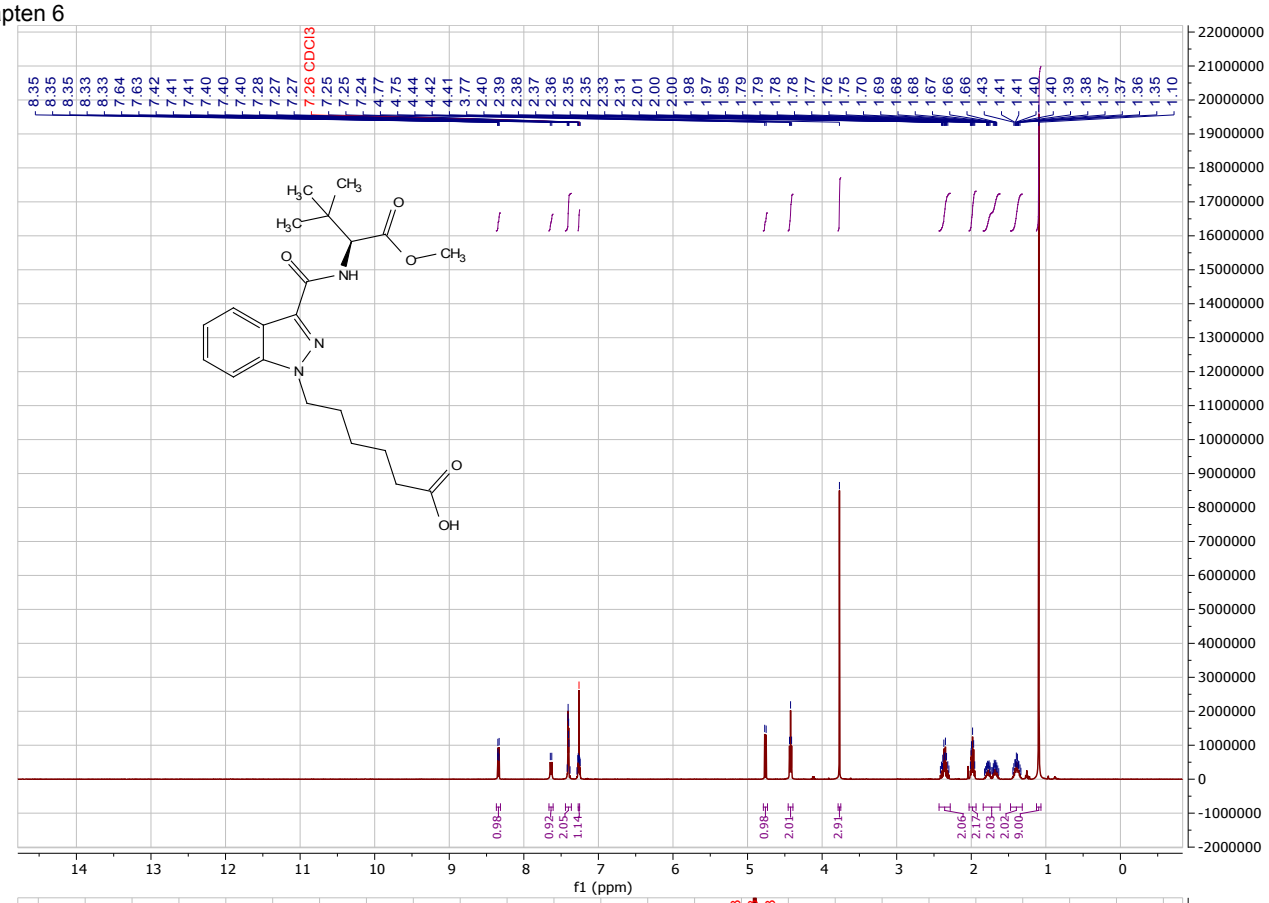

191.98 179.24 157.20 137.60 137.12 132.31 131.60 127.95 127.49 127.33 125.98 125.85 125.79 123.56 123.01 122.77 122.18 117.89 110.04 102.31 77.41 CDCl3 77.16 CDCl3 76.91 CDCl3 55.82 47.23 33.99 29.90 29.26 29.16 29.10 29.02 26.88 24.71
f1 (ppm)

Hapten-5


8.35 8.34 8.34 8.33 8.33 8.29 8.28 8.28 8.27 8.27 8.27 7.99 7.99 7.65 7.64 7.51 7.51 7.51 7.50 7.49 7.49 7.49 7.33 7.27 7.26 7.26 CDCl3 7.25 6.99 6.98 6.97 6.96 6.84 6.83 4.44 4.43 4.42 4.35 4.33 4.32 4.16 4.15 4.14 4.07 4.06 4.04 2.64 2.62 2.61 2.19 2.19 2.17 2.16 2.16 1.86 1.85 1.83 1.69 1.67 1.63 1.62 1.41 1.41 1.40 1.39 1.39 1.38
0.99 1.00 0.98 1.00 2.01 0.99 1.05 0.97 0.99 1.00 1.00 2.04 4.98 2.00 2.03 2.02 2.13 2.01
f1 (ppm)


191.95 178.29 157.17 155.83 137.62 132.29 132.05 131.58 128.15 127.84 127.47 125.98 125.85 125.80 122.19 117.60 114.56 110.82 105.19 102.34 84.37 83.06 77.41 CDCl3 76.91 CDCl3 67.37 55.84 47.29 30.78 30.06 29.90 29.64 24.70 22.92 22.89
f1 (ppm)

Hapten 6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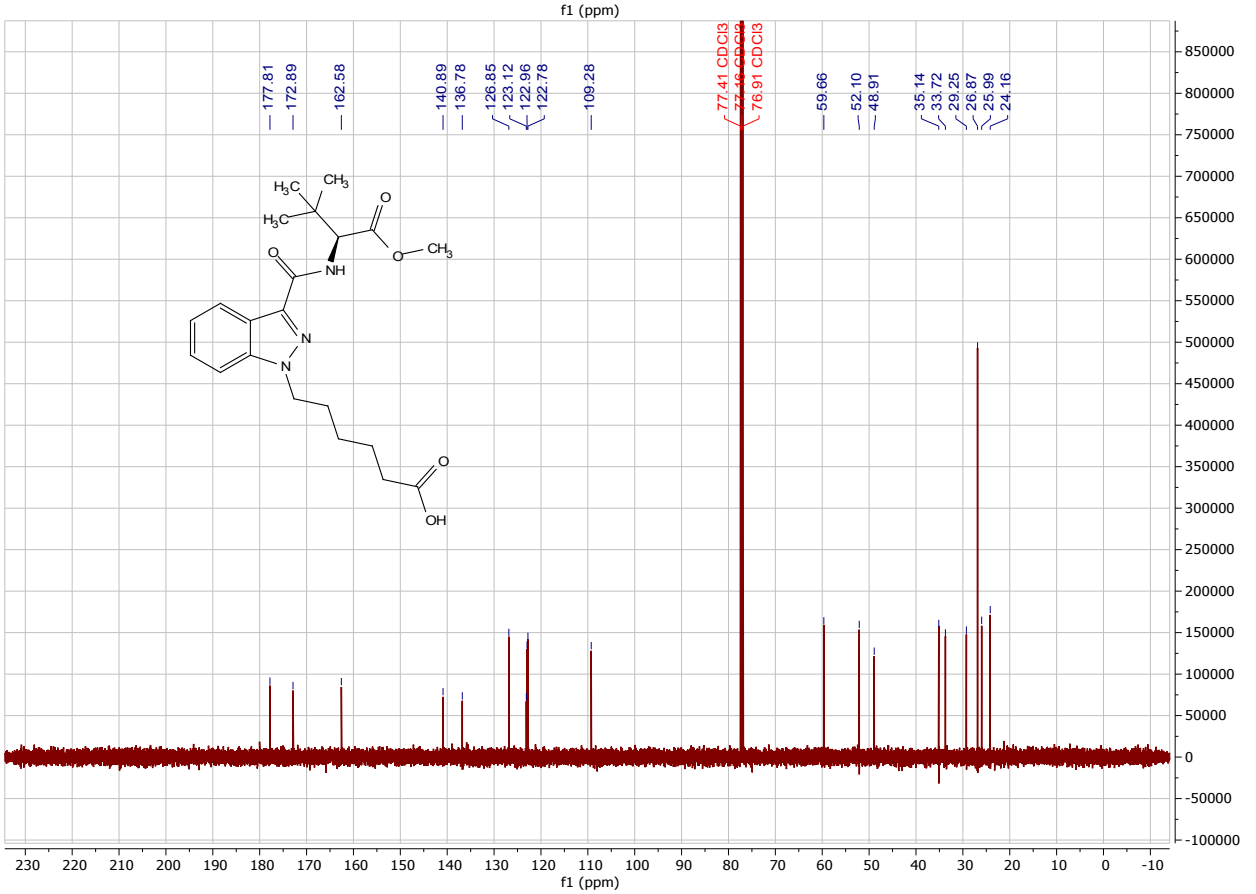
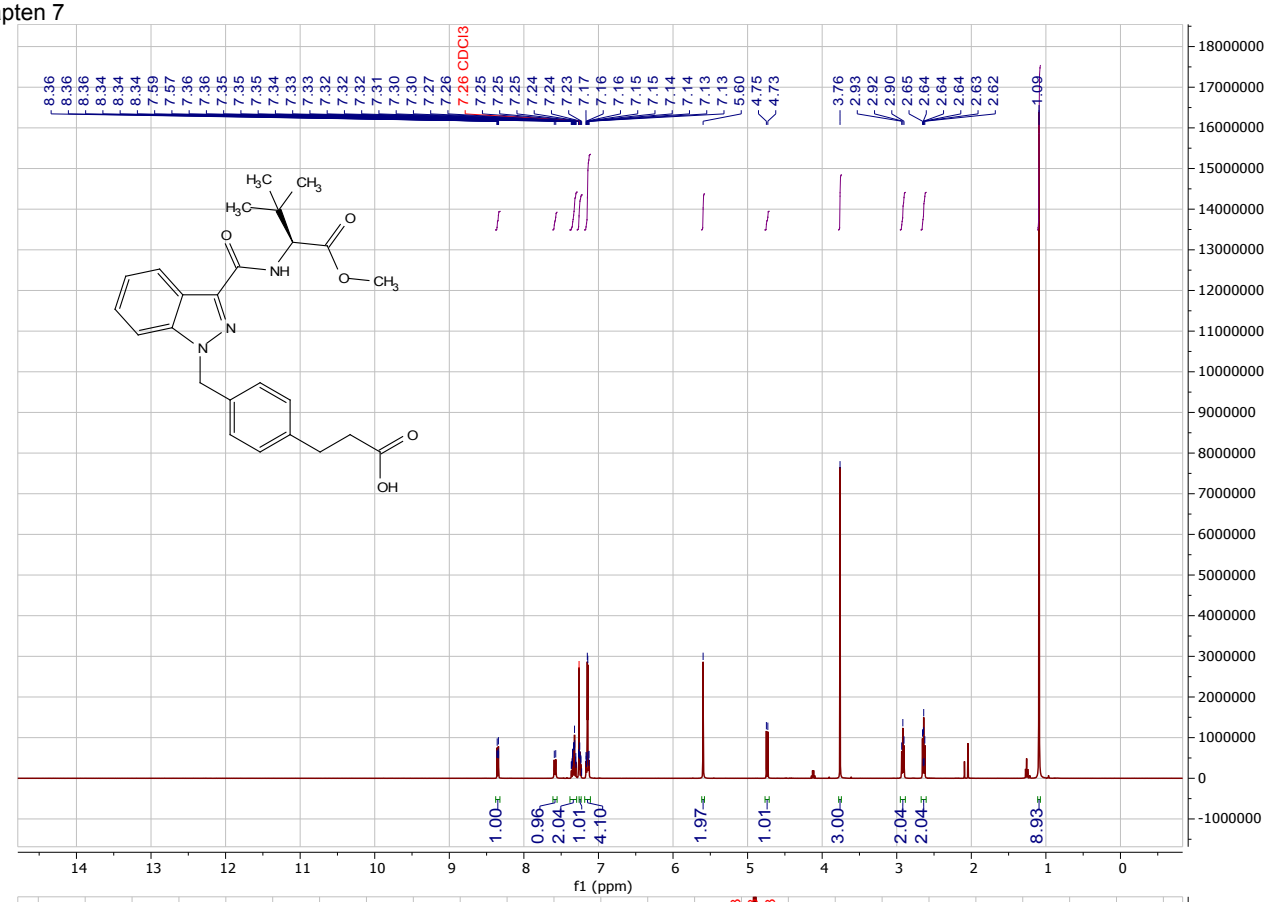
0.98 0.92 2.05 1.14 0.98 2.01 2.91 2.06 2.17 2.03 2.02 9.00
f1 (ppm)


177.81 172.89 162.58 140.89 136.78 126.85 123.12 122.96 122.78 109.28 77.41 CDCl3 77.16 CDCl3 76.91 CDCl3 59.66 52.10 48.91 35.14 33.72 29.25 26.87 25.99 24.16
f1 (ppm)

Hapten 7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8.36
8.34
8.34
8.34
7.59
7.57
7.36
7.36
7.35
7.35
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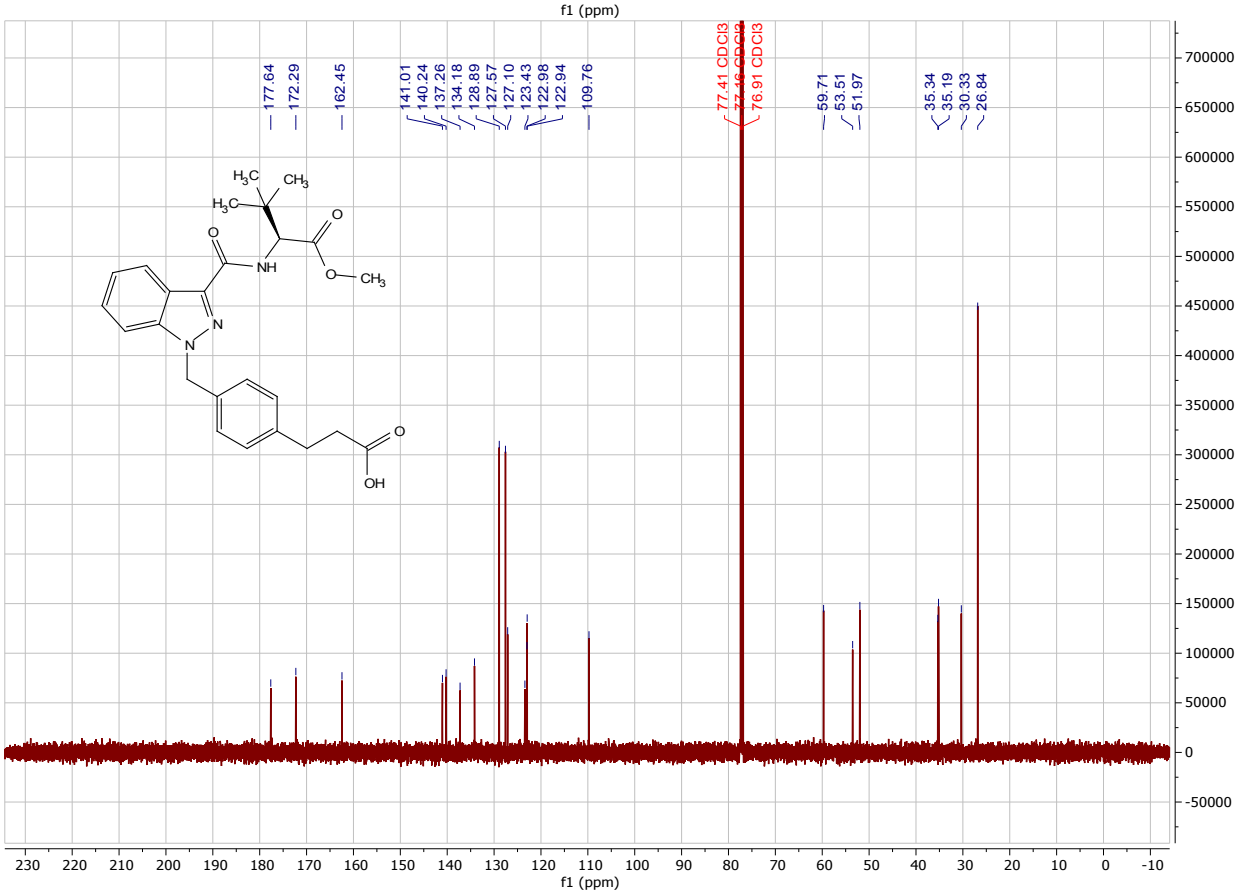
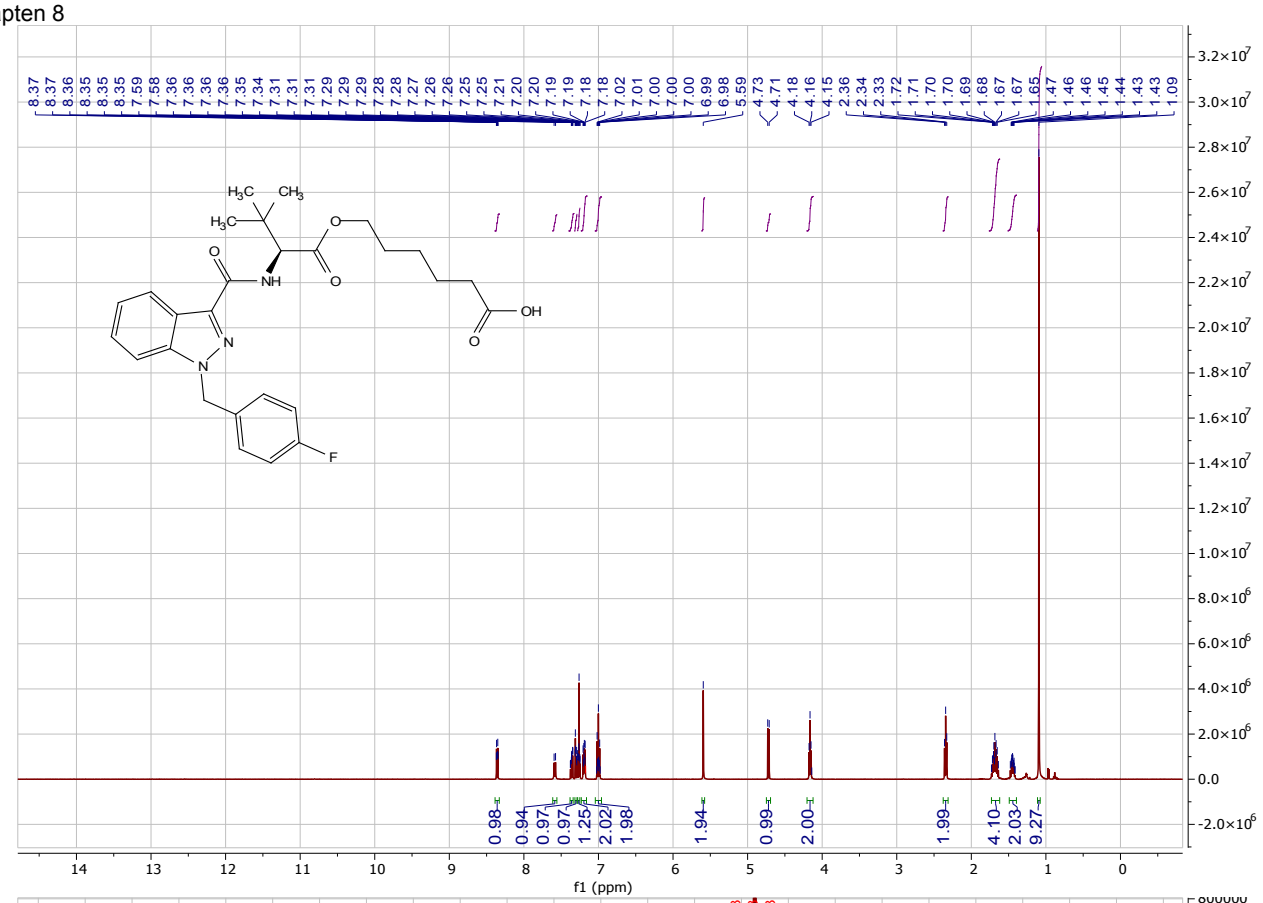
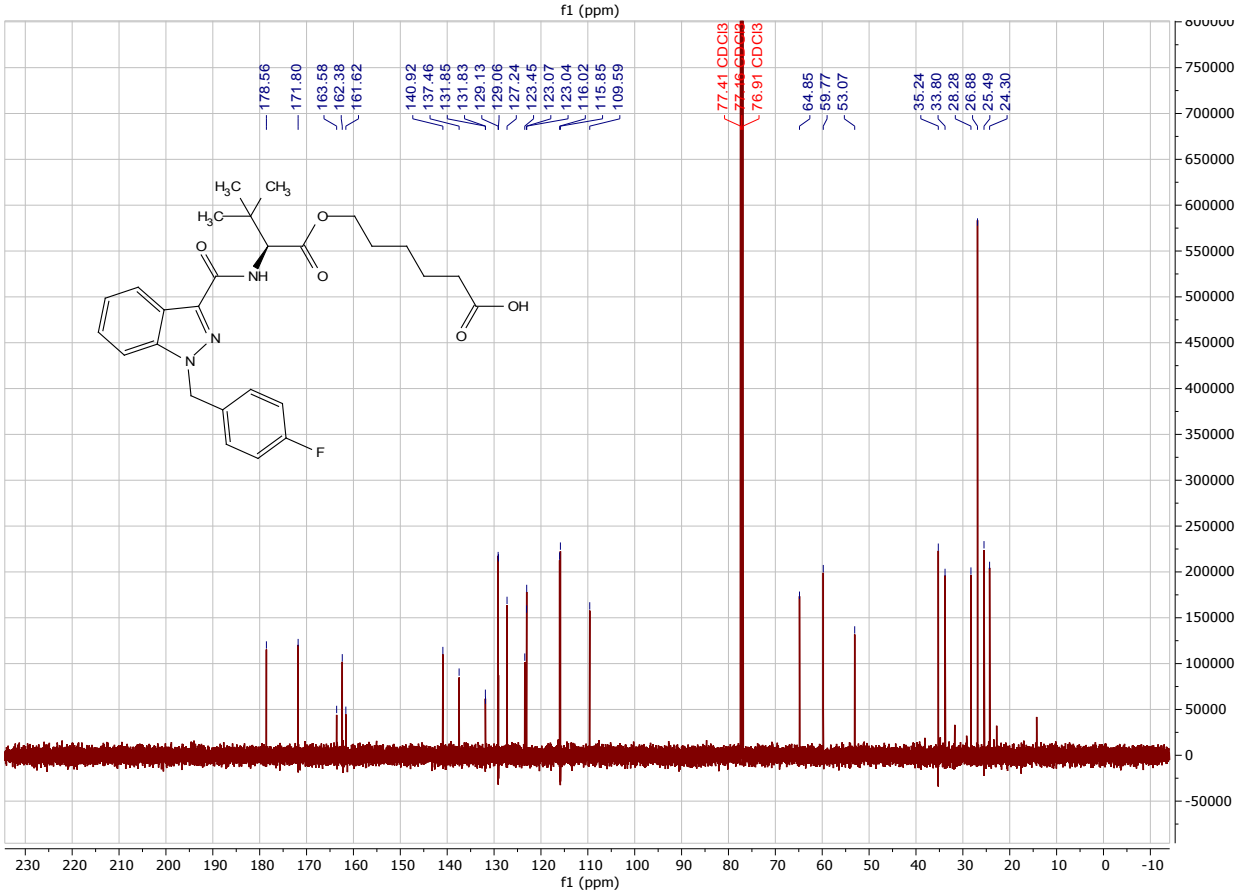
7.34
7.33
7.33
7.32
7.32
7.32
7.31
7.30
7.30
7.27
7.26
7.26 CDCl3
7.25
7.25
7.25
7.24
7.24
7.23
7.17
7.16
7.16
7.15
7.15
7.14
7.14
7.13
7.13
5.60
4.75
4.73
3.76
2.93
2.92
2.90
2.65
2.64
2.64
2.64
2.63
2.62
1.09
1.00
0.96
2.04
1.01
4.10
1.97
1.01
3.00
2.04
2.04
8.93
f1 (ppm)

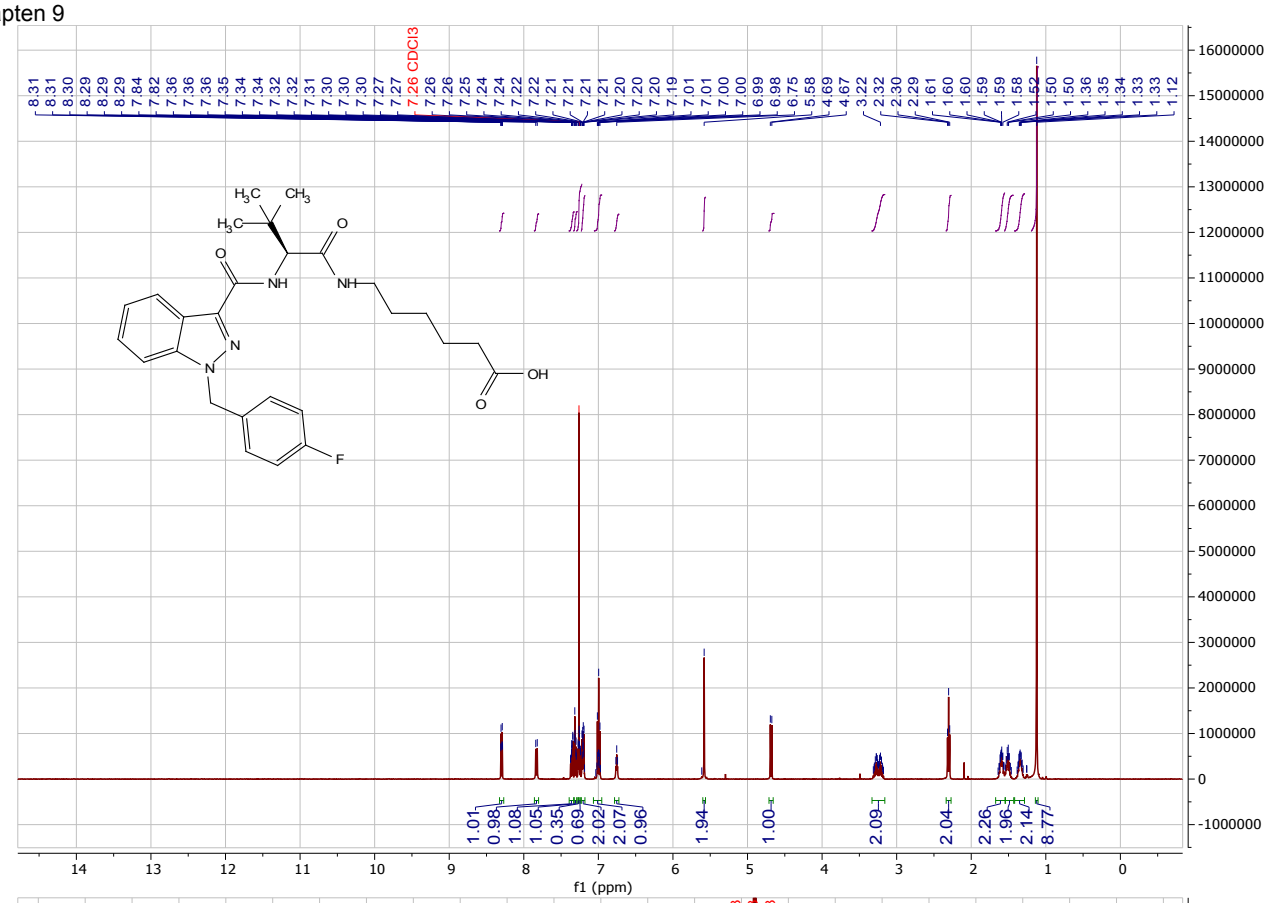

177.64
172.29
162.45
141.01
140.24
137.26
134.18
128.89
127.57
127.10
123.43
122.98
122.94
109.76
77.41 CDCl3
77.16 CDCl3
76.91 CDCl3
59.71
53.51
51.97
35.34
35.19
30.33
26.84
f1 (ppm)

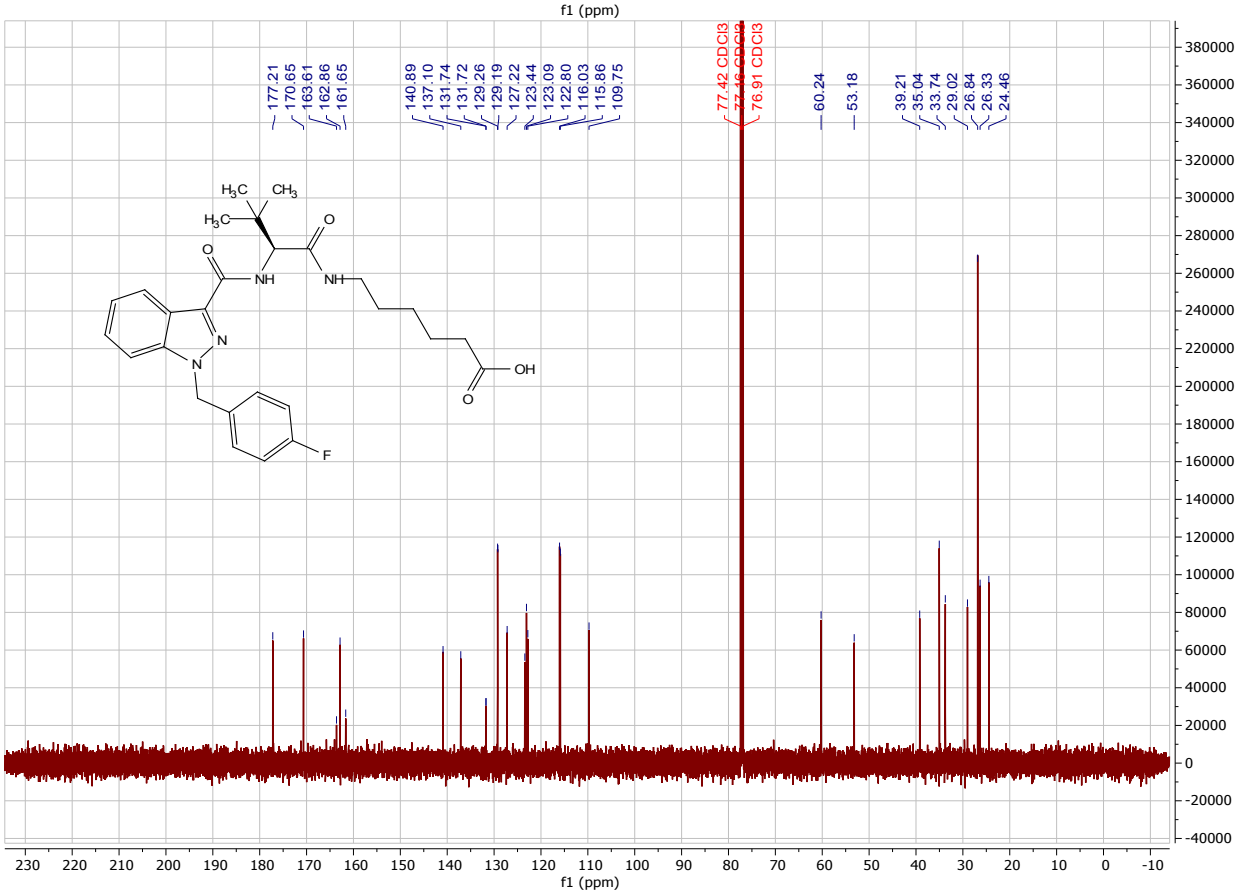
Hapten 8


8.37 8.37 8.36 8.35 8.35 8.35 7.59 7.58 7.36 7.36 7.36 7.35 7.34 7.31 7.31 7.31 7.29 7.29 7.29 7.28 7.28 7.27 7.26 7.26 7.25 7.25 7.21 7.20 7.20 7.19 7.19 7.18 7.18 7.02 7.01 7.00 7.00 7.00 6.99 6.98 5.59 4.73 4.71 4.18 4.16 4.15 2.36 2.34 2.33 1.72 1.71 1.70 1.70 1.69 1.68 1.67 1.67 1.65 1.47 1.46 1.46 1.45 1.44 1.43 1.43 1.09
0.98 0.94 0.97 0.97 1.25 2.02 1.98 1.94 0.99 2.00 1.99 4.10 2.03 9.27
f1 (ppm)

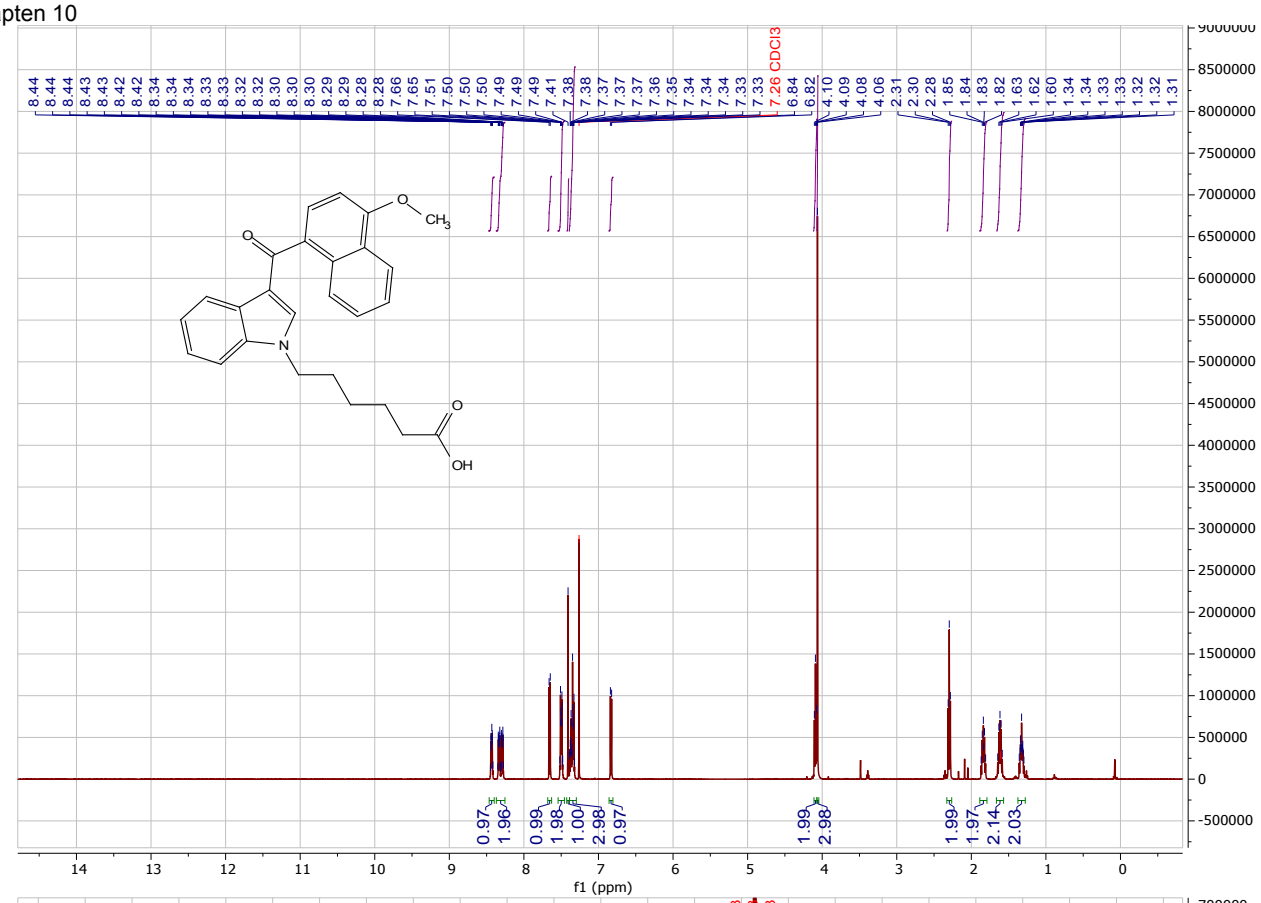

178.56 171.80 163.58 162.38 161.62 140.92 137.46 131.85 131.83 129.13 129.06 127.24 123.45 123.07 123.04 116.02 115.85 109.59 77.41 CDCl3 77.16 CDCl3 76.91 CDCl3 64.85 59.77 53.07 35.24 33.80 28.28 26.88 25.49 24.30
f1 (ppm)

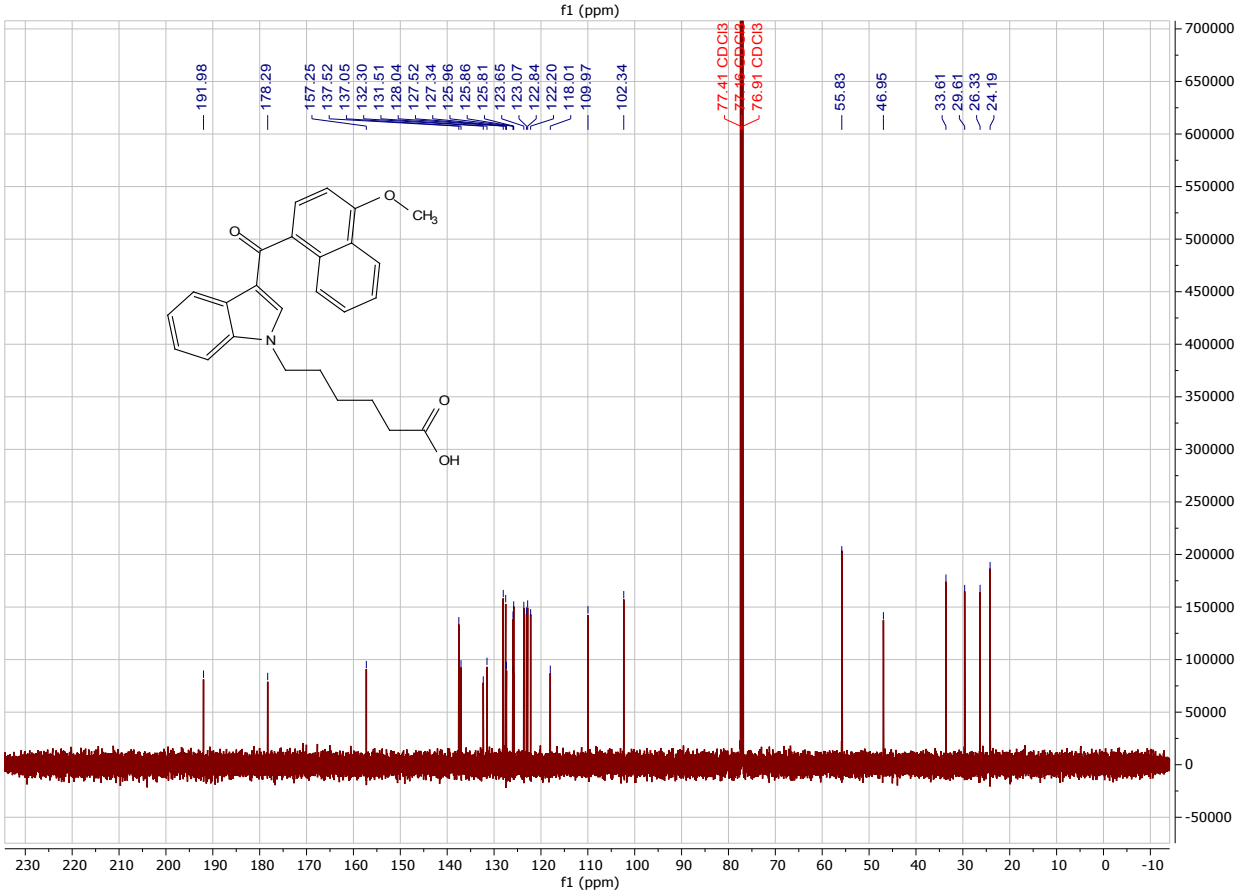
Hapten 9


8.31
8.31
8.30
8.29
8.29
8.29
7.84
7.82
7.36
7.36
7.36
7.35
7.34
7.34
7.32
7.32
7.31
7.30
7.30
7.30
7.27
7.27
7.26 CDCl3
7.26
7.25
7.24
7.24
7.22
7.22
7.21
7.21
7.21
7.21
7.20
7.20
7.19
7.01
7.01
7.00
7.00
6.99
6.98
6.75
5.58
4.69
4.67
3.22
2.32
2.30
2.29
1.61
1.60
1.60
1.59
1.59
1.58
1.52
1.50
1.50
1.36
1.35
1.34
1.33
1.33
1.12
1.01
0.99
1.08
1.05
0.35
0.69
2.02
2.07
0.96
1.94
1.00
2.09
2.04
2.26
1.96
2.14
8.77
f1 (ppm)

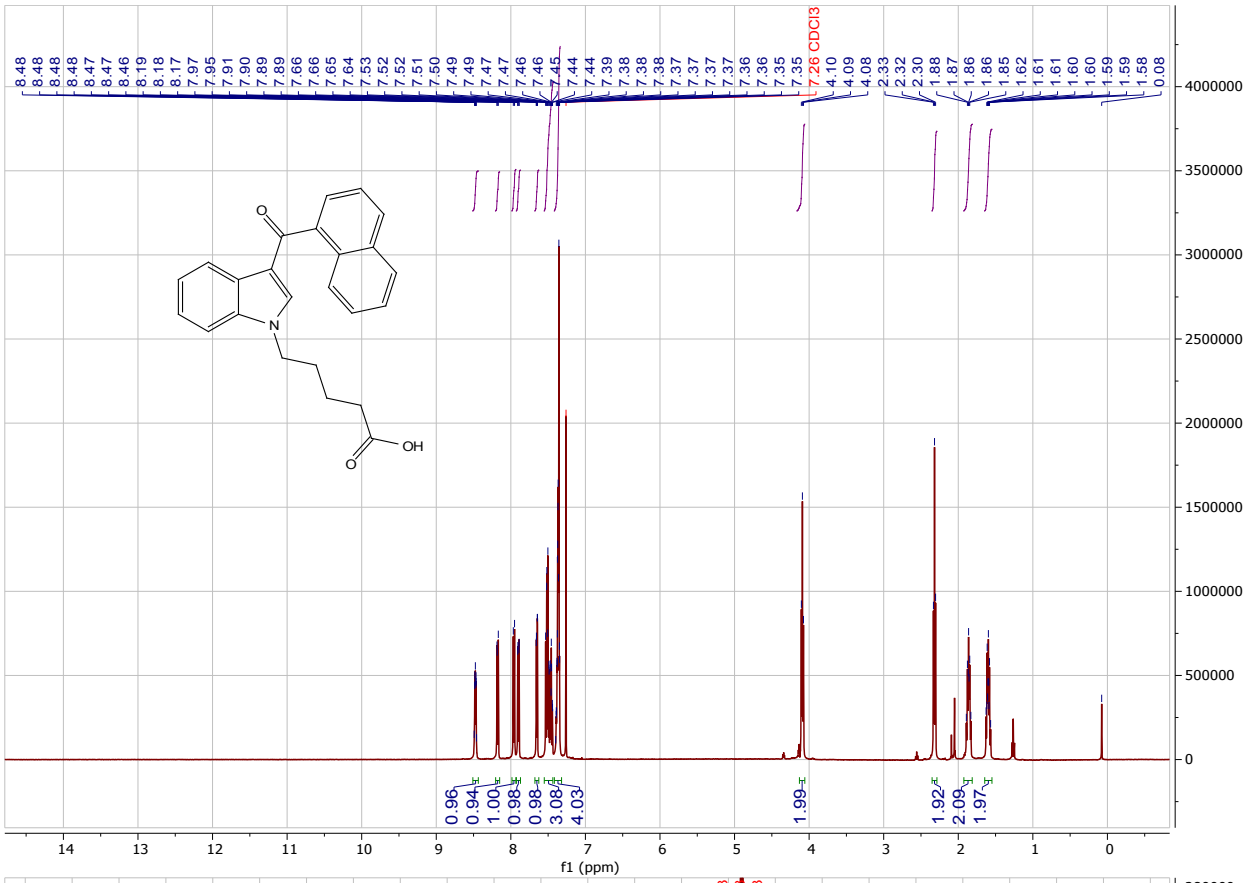

177.21
170.65
163.61
162.86
161.65
140.89
137.10
131.74
131.72
129.26
129.19
127.22
123.44
123.09
122.80
116.03
115.86
109.75
77.42 CDCl3
77.16 CDCl3
76.91 CDCl3
60.24
53.18
39.21
35.04
33.74
29.02
26.84
26.33
24.46
f1 (ppm)

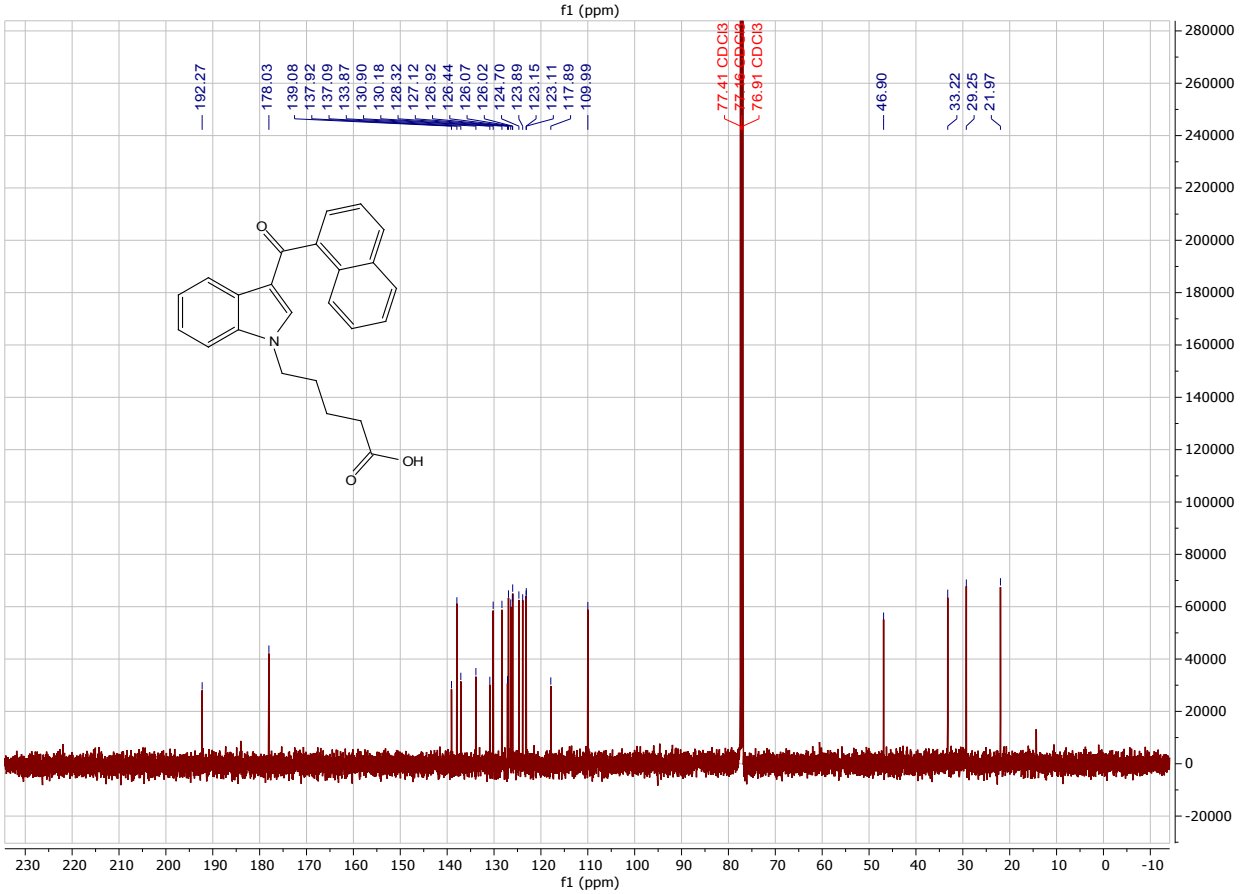
Hapten 10



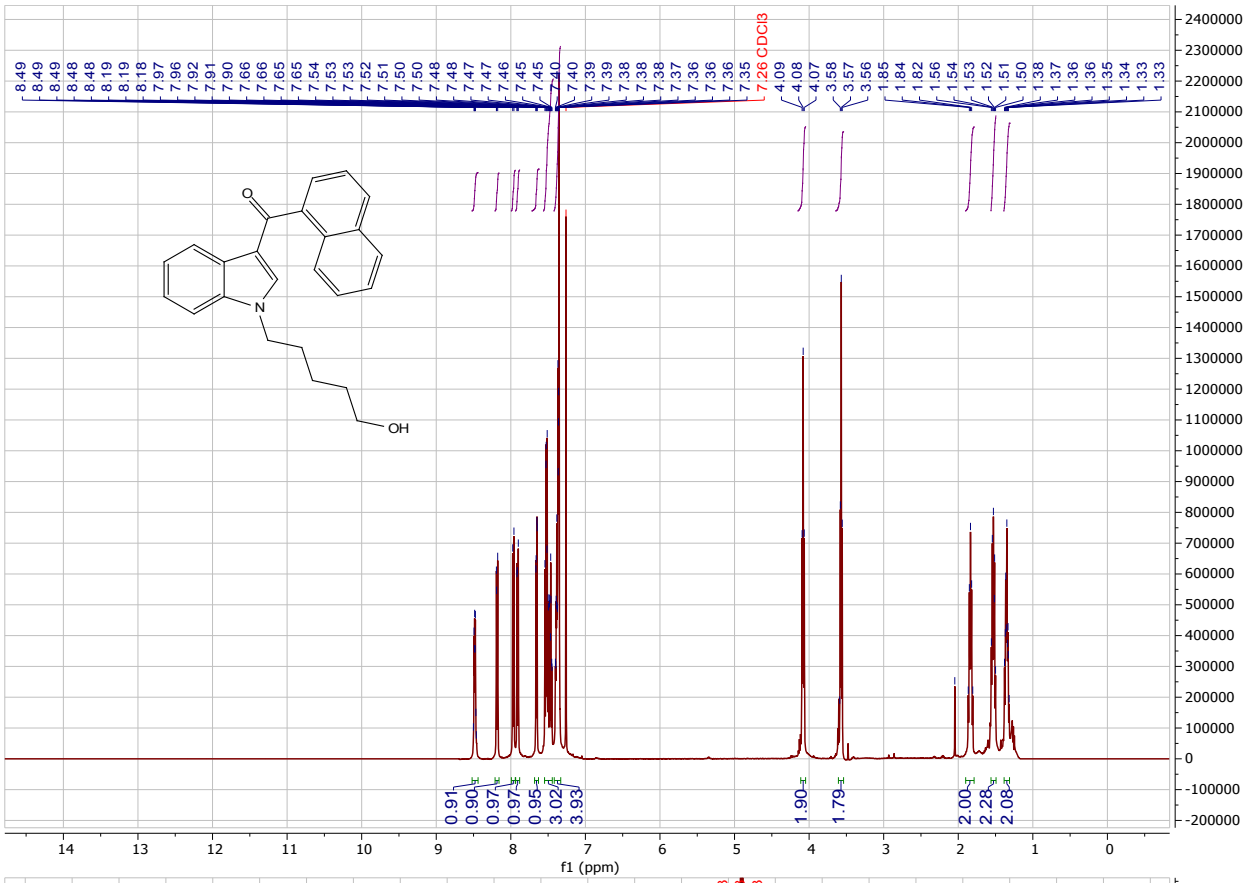


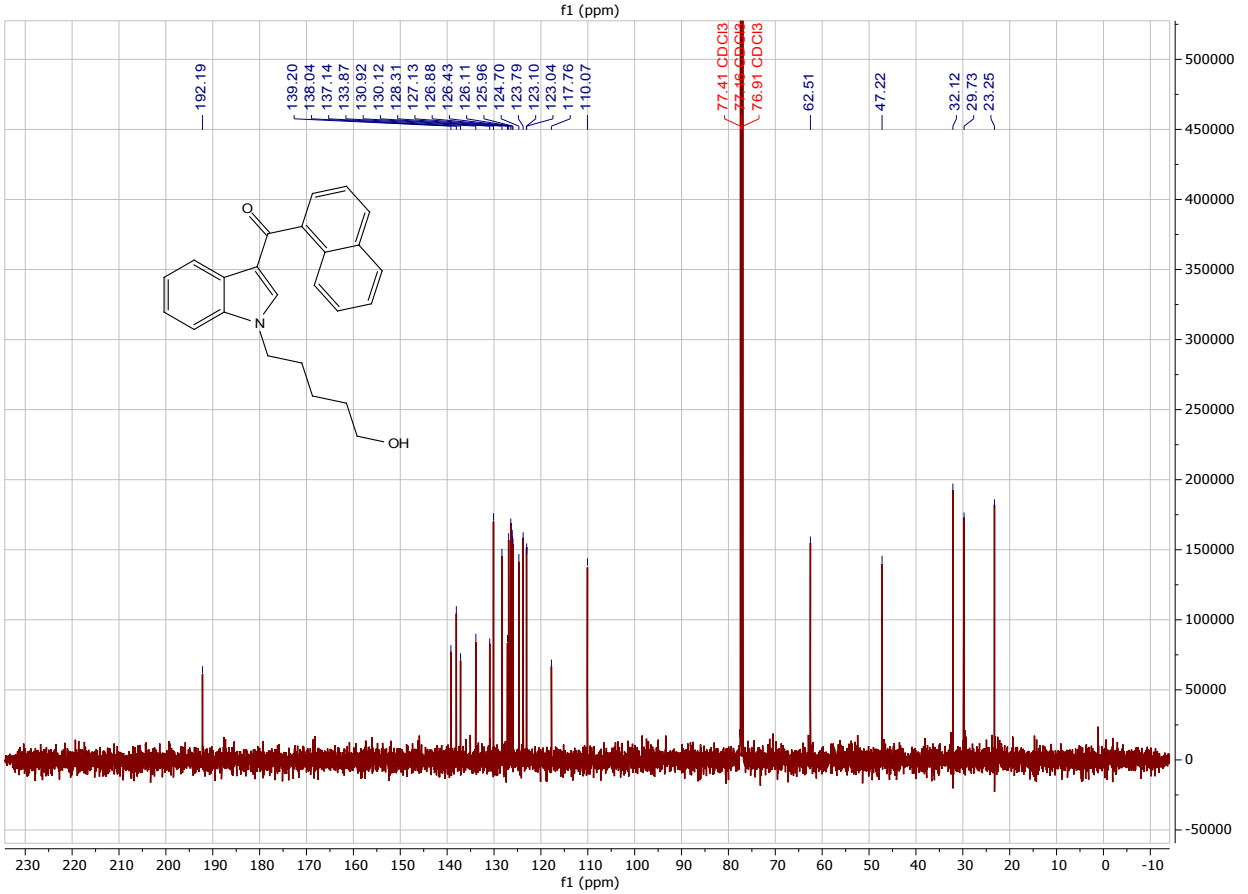
M1


8.48 8.48 8.48 8.48 8.47 8.47 8.46 8.19 8.18 8.17 7.97 7.95 7.91 7.90 7.89 7.89 7.66 7.65 7.64 7.53 7.52 7.52 7.51 7.50 7.49 7.49 7.47 7.47 7.46 7.46 7.45 7.44 7.44 7.39 7.38 7.38 7.37 7.37 7.37 7.37 7.36 7.36 7.35 7.35 7.26 CDCl3 4.10 4.09 4.08 2.33 2.32 2.30 1.88 1.87 1.86 1.86 1.85 1.62 1.61 1.61 1.60 1.60 1.59 1.59 1.58 0.08
O
N
OH
O
0.96 0.94 1.00 0.98 0.98 3.08 4.03 1.99 1.92 2.09 1.97
f1 (ppm)

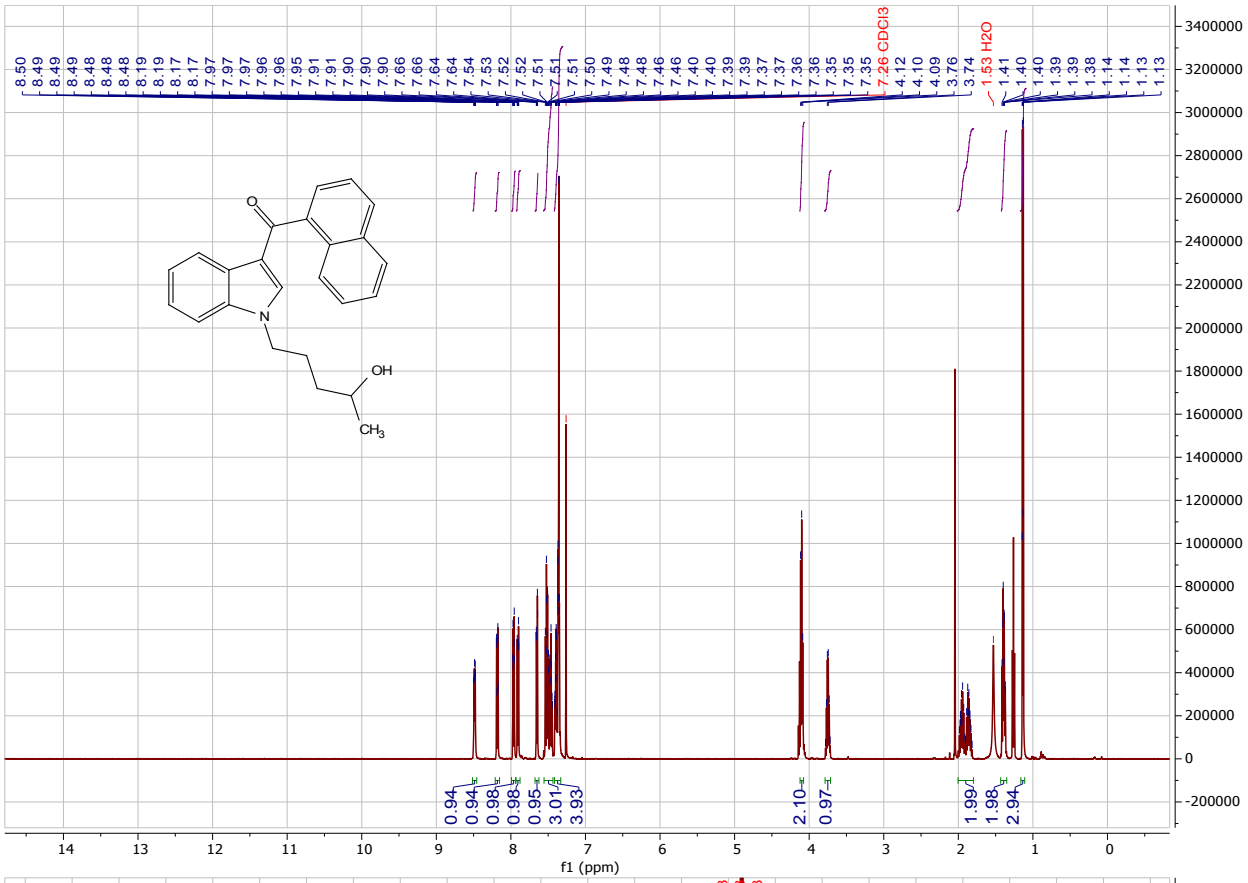

192.27 178.03 139.08 137.92 137.09 133.87 130.90 130.18 128.32 127.12 126.92 126.44 126.07 126.02 124.70 123.89 123.15 123.11 117.89 109.99
77.41 CDCl3 77.16 CDCl3 76.91 CDCl3
46.90 33.22 29.25 21.97
O
N
OH
O
f1 (ppm)

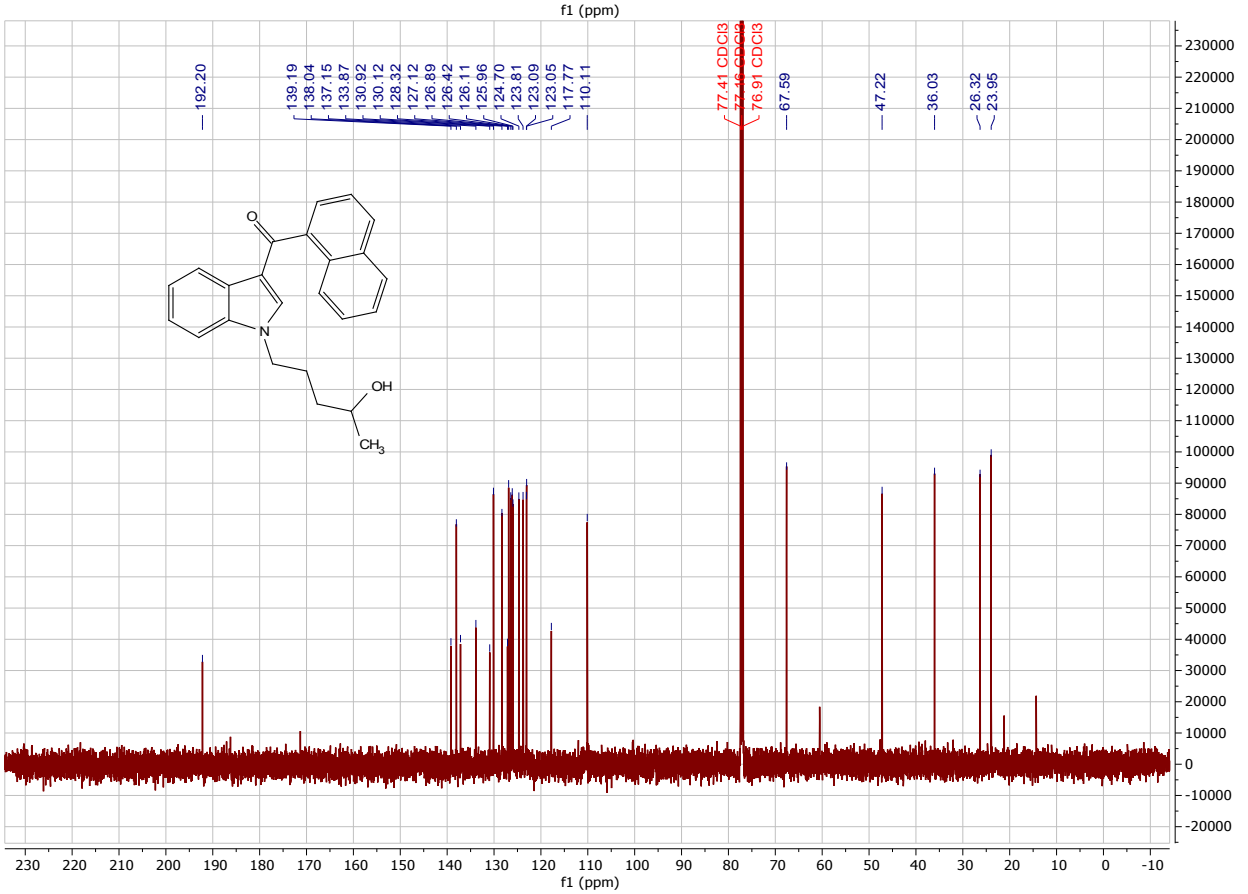
M2


7.26 CDCl3
0.91
0.90
0.97
0.97
0.95
3.02
3.93
1.90
1.79
2.00
2.28
2.08
f1 (ppm)

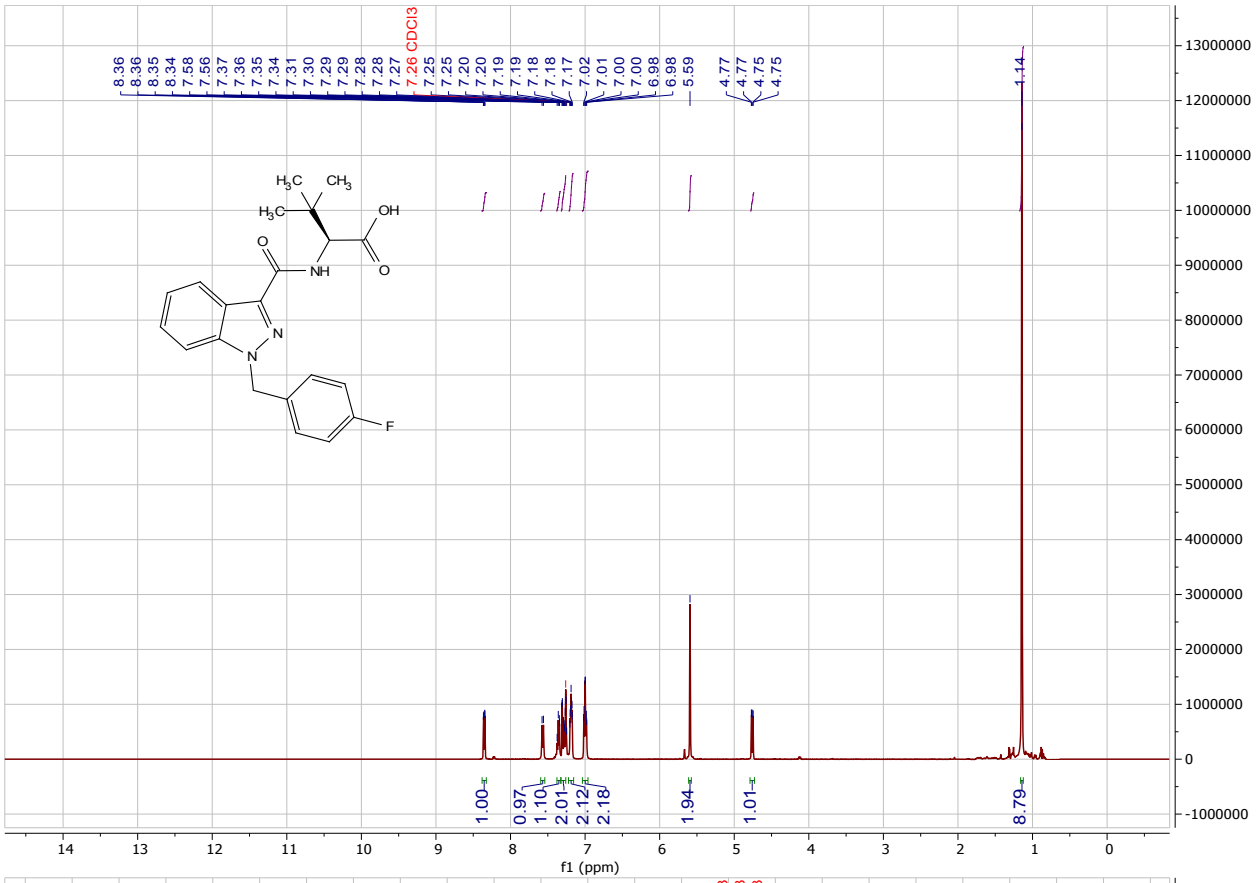

192.19
139.20
138.04
137.14
133.87
130.92
130.12
128.31
127.13
126.88
126.43
126.11
125.96
124.70
123.79
123.10
123.04
117.76
110.07
77.41 CDCl3
77.16 CDCl3
76.91 CDCl3
62.51
47.22
32.12
29.73
23.25
f1 (ppm)

M3


8.50 8.49 8.49 8.49 8.48 8.48 8.48 8.19 8.19 8.17 8.17 7.97 7.97 7.97 7.96 7.96 7.95 7.91 7.91 7.90 7.90 7.90 7.66 7.66 7.64 7.54 7.53 7.52 7.52 7.51 7.51 7.50 7.49 7.48 7.48 7.46 7.46 7.40 7.40 7.39 7.39 7.37 7.37 7.36 7.36 7.35 7.35 7.35 7.26 CDCl3 4.12 4.10 4.09 3.76 3.74 1.53 H2O 1.41 1.40 1.40 1.39 1.39 1.38 1.14 1.13 1.13
O
N
OH
CH3
0.94 0.94 0.98 0.98 0.95 3.01 3.93 2.10 0.97 1.99 1.98 2.94
f1 (ppm)


192.20 139.19 138.04 137.15 133.87 130.92 130.12 128.32 127.12 126.89 126.42 126.11 125.96 124.70 123.81 123.09 123.05 117.77 110.11 77.41 CDCl3 77.16 CDCl3 76.91 CDCl3 67.59 47.22 36.03 26.32 23.95
O
N
OH
CH3
f1 (ppm)

M4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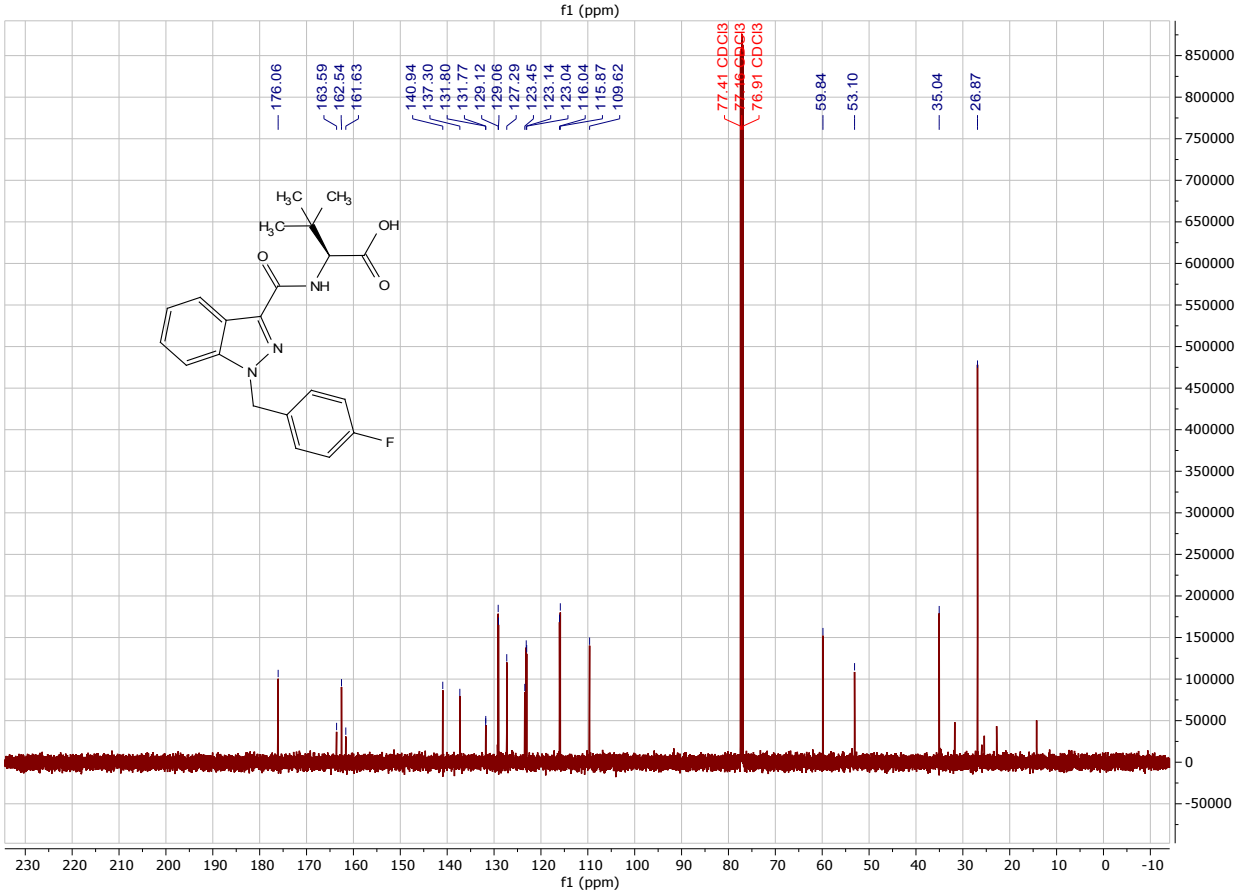
7.56
7.37
7.36
7.35
7.34
7.31
7.30
7.29
7.29
7.28
7.28
7.27
7.26 CDCl3
7.25
7.25
7.20
7.20
7.19
7.19
7.18
7.18
7.17
7.02
7.01
7.00
7.00
6.98
6.98
5.59
4.77
4.77
4.75
4.75
1.14
1.00
0.97
1.10
2.01
2.12
2.18
1.94
1.01
8.79
f1 (ppm)


176.06
163.59
162.54
161.63
140.94
137.30
131.80
131.77
129.12
129.06
127.29
123.45
123.14
123.04
116.04
115.87
109.62
77.41 CDCl3
77.16 CDCl3
76.91 CDCl3
59.84
53.10
35.04
26.87
f1 (ppm)

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(3) Banister, S. D.; Moir, M.; Stuart, J.; Kevin, R. C.; Wood, K. E.; Longworth, M.; Wilkinson, S. M.; Beinat, C.; Buchanan, A. S.; Glass, M.; Connor, M.; McGregor, I. S.; Kassiou, M., Pharmacology of Indole and Indazole Synthetic Cannabinoid Designer Drugs AB-FUBINACA, ADB-FUBINACA, AB-PINACA, ADB-PINACA, 5F-AB-PINACA, 5F-ADB-PINACA, ADBICA, and 5F-ADBICA. *ACS Chem Neurosci* **2015,** *6* (9), 1546-1559.
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