Electronic Supplementary Material (ESI) for Organic Chemistry Frontiers.

Application of Organocatalysis in Bioorganometallic Chemistry: Asymmetric Synthesis of Multifunctionalized Spirocyclic Pyrazolone-Ferrocene Hybrids as Novel RalA Inhibitors

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Supporting Information

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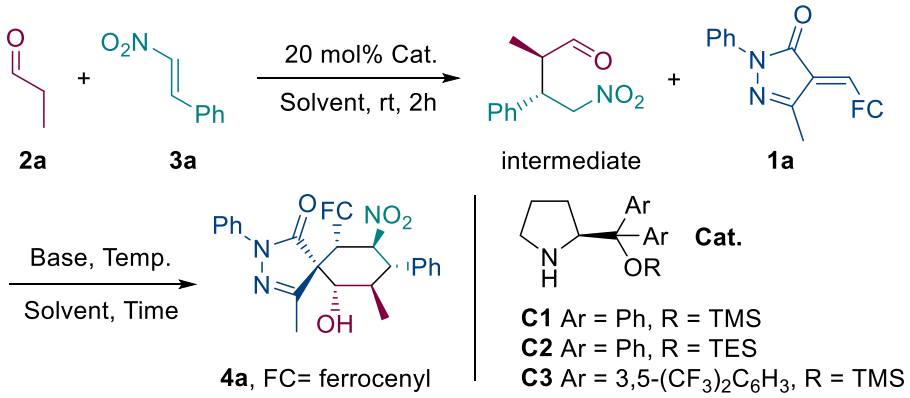
1. Experimental details

1.1 General methods for synthesis

- Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded with Bruker Avance III 400 MHz spectrometers. Proton chemical shifts are reported in parts per million (δ scale), and are referenced using residual protium in the NMR solvent ($CDCl_3$: δ 7.26 ($CHCl_3$)). Data are reported as follows: chemical shift [multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br s = broad singlet), coupling constant(s) (Hz), integration].
- Carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were recorded with Bruker Avance 400 MHz spectrometers. Carbon chemical shifts are reported in parts per million (δ scale), and are referenced using the carbon resonances of the solvent (δ 77.0 ($CHCl_3$)). Data are reported as follows: chemical shift [multiplicity (if not singlet), assignment (C_q = fully substituted carbon)].
- High resolution mass spectra (HRMS) were recorded on a Waters SYNAPT G2 using an electrospray (ESI) ionization source.
- High Performance Liquid Chromatography (HPLC) was analyzed by chiral column in comparison with authentic racemates, using a Daicel Chiralpak AD-H Column (250 x 4.6 mm), Daicel Chiralpak OD-H Column (250 x 4.6 mm), Daicel Chiralpak IC-H Column (250 x 4.6 mm) or Daicel Chiralpak IE-H Column (250 x 4.6 mm). UV detection was performed at 220 nm or 254 nm.
- Optical rotation values were measured with instruments operating at λ = 589 nm, corresponding to the sodium D line at 25 ºC.
- Column chromatography was performed on silica gel (400-500 mesh) eluting with ethyl acetate and petroleum ether. TLC was performed on glass-backed silica plates. UV light and I_2 were used to visualize products.
- Melting points were determined on a Mel-Temp apparatus and are uncorrected.

1.2 Optimization of the model reaction (Table S1)[a]



Entry	Catalyst	Solvent	Base	Temp (°C)	Time[b]	Yield[c] (%)	dr[d]	ee[e] (%)
1	**C1**	Toluene	TEA	rt	12 h	42	90:10	92
2	**C2**	Toluene	TEA	rt	12 h	30	90:10	92
3	**C3**	Toluene	TEA	rt	12 h	28	92:8	94
4	**C1**	CH_2Cl_2	TEA	rt	12 h	37	85:15	90
5	**C1**	THF	TEA	rt	12 h	23	80:20	91
6	**C1**	Acetone	TEA	rt	8 h	55	>20:1	94
7	**C1**	MeCN	TEA	rt	8 h	59	>20:1	93
8	**C1**	MeCN	K_2CO_3	rt	8 h	52	90:10	92
9	**C1**	MeCN	DIPEA	rt	12 h	48	>20:1	92
10	**C1**	**MeCN**	**DBU**	**rt**	**4 h**	**65**	**>20:1**	**94**
11	**C1**	MeCN	DBU	35	4 h	60	92:8	94
12	**C1**	MeCN	DBU	60	2 h	56	88:12	93

[a] Reactions were performed with **2a** (0.80 mmol), **3a** (0.40 mmol), **Cat.** (0.08 mmol) and AcOH (0.08 mmol) in the indicated solvent (4.0 mL) at room temperature for 2 h, after which **1a** (0.3 mmol) and base (0.1 mmol) were added in the indicated solvent. [b] Reaction time in the second step. [c] Yield of isolated **4a**. [d] Calculated based on ^{1}H NMR analysis of the crude reaction mixture. [e] Determined by HPLC using a chiral stationary phase.

We initially investigated the three-component domino reaction using propanal **2a**, nitrostyrene **3a** and pyrazolone substrate **1a** as the standard substrates. The first Michael reaction proceeded in the presence of catalyst **C1** (20 mol%) in toluene at room temperature for two hours. A toluene solution of pyrazolone **1a** and triethylamine was added in a one-pot operation, resulting in successive Michael

and aldol reactions. To our gratification, the tandem reaction proceeded smoothly to afford the desired product **4a** with good diastereoselectivity and excellent enantioselectivity, albeit in moderate yield (entry 1). Subsequently, several chiral secondary amine catalysts were tested. The triethylsilyloxy ether **C2** provided an equally good stereoselectivity, but in relatively low yield (entry 2). High enantiocontrol was also achieved when catalyst **C3** containing electron-withdrawing substituents, but slower reaction rate was observed (entry 3). The same reaction in CH_2Cl_2, THF and acetone also provided **4a** with lower yields or at slower rates for completion (entries 4-6), except for the reactions in acetonitrile that provided 59% yields of the adduct (entry 7). It was found that the base condition in the second Michael step had some influence on the reaction efficiency (entries 8-10), and DBU proved to be the best choice. Conducting the reaction at higher temperature did not improve the reaction rate (entries 11 and 12). The reaction condition in entry 10 was chosen as the optimal one for further investigation on the substrate scope.

1.3 Cell culture and cellular proliferation assay

The human pancreatic cancer cell lines PANC-1 and HPAF-II were obtained from ATCC (American type culture collection) and cultured in our laboratory, the cells were incubated under sterile conditions at 37℃ and were maintained in a humidified atmosphere 5% CO_2 (v/v) with DMEM medium containing 10% fetal bovine serum (GIBCO, Waltham, MA, USA). MTT assay was performed to evaluate the cellular proliferation inhibitory activities of tested compound by a panel of cancer cells. In general, cells were seeded into 96-well plates and treated with a series of concentration of test drugs for 48 hours. The MTT reagent (5 mg/ml) was added per well for 3 h at 37℃. After that, the MTT was removed and 150 μl DMSO was added to dissolve the formazan crystals. Then, optical density (OD) was measured at 570 nm of the solution. The control group consisted of untreated cells. The percentage of cell viability averaged from three individual experiments.

1.4 The HTRF based assay of RalA-substrate interaction

The enzymatic assay of test compounds are using the HTRF based method provided by Cisbio Co. Ltd., in brief, the HTRF assay used a GST-tagged RalA protein, and then the biotinylated substrate peptide of C3 exoenzyme and two HTRF detection reagents are added. The HTRF signal is

proportional to the amount of interaction between GST-tagged RalA and biotinylated substrate C3 exoenzyme peptide, the detailed experimental procedures are according to the manufacturer's protocols.

1.5 Transmission electronic microscopy

The ultrastructure of mitochondrial morphology was observed using a Hitachi transmission electron microscope similar to our previous reports. Briefly, collected cells were fixed with 2% glutaraldehyde for 2 hours, washed with PBS and then post- fixed with 1% OsO_4 for 1.5 hours at 4°C. The samples were then washed and dehydrated with graded alcohol. After dehydration, the samples were infiltrated and embedded in 618 epoxy resin. Ultrathin sections were cut, stained with uranyl acetate and lead citrate and then examined under the transmission electron microscope (Hitachi 7000, Hitachi, Japan).

1.6 Apoptosis assay by Flow Cytometry (FCM) and fluorescent microscopy

PANC-1 cells were seeded in six-well plates for 12 hours, and then treated with compound 5b (2 or 5 μM, respectively) or vehicle (DMSO) for 24 hours. Cells were collected, then fixed with 75% ice-cold ethanol and stored at -20 °C for another one hour. The flow cytometric analysis was used to identify different micelles apoptosis inducing effect. PANC-1 cells treated with compound 5b or blank solvent (control) were gently trypsinized without EDTA and centrifuged at 2000 rpm for 5 minutes. Then, the harvested cells were washed with 1.0 ml ice cold PBS and re-suspended in 500 μl 1×binding buffer, and incubated with 5 μl of Annexin V-FITC and 5 μl of propidium iodide (PI) for 15 min at room temperature. Followed by FCM (BD FACS Calibur, BD, USA) using the FL1 channel for Annexin V-FITC and the FL2 channel of PI. Both early apoptotic (Annexin V+/PI-) and late apoptotic (Annexin V+/PI+) cells are included in the cell assay of apoptosis. In addition, PANC-1 cells were plated in six-well plates, the cells were grew and adhered for 24 hours, then incubated with 2 or 5 μM of 5b for an additional 12 hours followed by Hoechst 33258 addition. The morphology of nuclei was visualized under an Olympus fluorescence microscope.

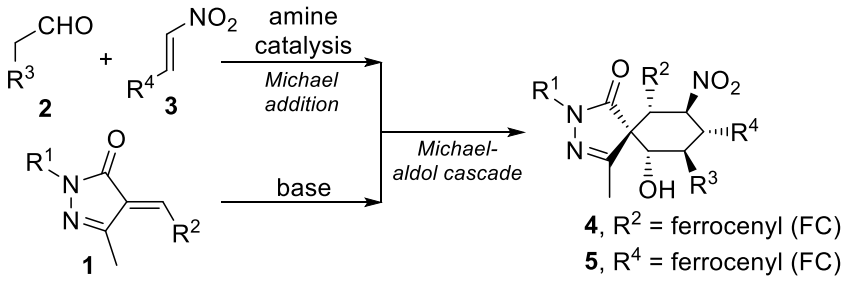
1.7 GST pull down assay

To analyze effects of compound 5b on RalA activation, PANC-1 cells were seeded in six-plate dishes and cultured for 5 hours, after which cells were washed and lysed as described above. Lysates were cleared by centrifugation at 16,000 g for 5 min, and supernatants were incubated with 10μl of RalBP1-glutathione transferase (GST) bound to glutathione-Sepharose (GSH) beads for 1 h at 4°C with rocking as previously described. The beads were washed twice with lysis buffer, resuspended in Laemmli electrophoresis sample buffer, resolved, and immunoblotted for RalA as described above.

1.8 Western blot analysis

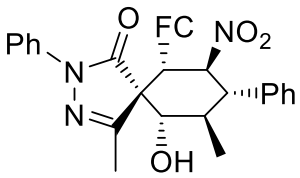
The different concentrations of drug treated cells were harvested and washed with cold 1×PBS. Total cell lysates were prepared in lysis RIPA buffer (Invitrogen, CA, USA) on ice for 30 min, followed by centrifugation at 13000 rpm for 30 min at 4℃. After collecting supernatant, protein concentration was determined by a bicinchoninic acid protein assay kit (Thermo, USA). The protein was resolved on a 10-15% SDS-polyacrylamide gel, electro blotted onto nitrocellulose membranes, and then incubated with proper primary antibodies which were purchased from Cell Signaling Technology or Santa Cruz Biotechnology and secondary antibodies before visualization by chemiluminescence Kit (Millpore, USA).

2. Asymmetric synthesis of spirocyclic pyrazolone-ferrocene derivates



The reaction was carried out with saturated aldehyde **2** (0.8 mmol) and nitroolefin **3** (0.4 mmol) in the presence of catalyst **C1** (0.08 mmol) and acetic acid (0.08 mmol) in acetonitrile (4.0 mL) at room temperature for 2 h to afford the Michael adduct intermediate. When the reaction was complete, the pyrazolone substrate (0.3 mmol) and DBU (0.1 mmol in 1.0 mL MeCN) were added in one-pot. The reaction mixture was stirred at room temperature for a specified reaction time until the reaction completed (monitored by TLC). Then the reaction mixture was concentrated and the residue was purified by flash chromatography on silica gel to give the spirocyclic pyrazolone-ferrocene derivate.

(*5S,6S,7R,8S,9S,10S*)-1,7-dimethyl-3,8-diphenyl-10-ferrocenyl-9-nitro-6-hydroxyl-2,3-diazaspiro[4.5]dec-1-ene-4-one



4a

Prepared according to the general procedure using (*E*)-(2-nitrovinyl) benzene **3a** (59.66 mg, 0.40 mmol, 1.0 equiv), propionaldehyde **2a** (46.46 mg, 0.8 mmol, 2 equiv) and (*Z*)-4-ferrocenylidene-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one **1a** (111.07 mg, 0.30 mmol, 0.75 equiv). Purification of the crude product via column chromatography delivered **4a** as an orange solid with 65% yield (116.8 mg). The diastereomeric ratio was determined to be >20:1 by crude ^{1}H-NMR analysis, and the enantiomeric excess of the major product was determined to be 94% by chiral HPLC analysis on Chiralpak AD-H column (10% 2-propanol/*n*-hexane, 1 mL/min, UV 254 nm), t_{major} = 13.45 min, t_{minor} = 8.08 min; $[\alpha]_D^{20}$ = -45.1 (*c* = 0.21 in CH_2Cl_2); m.p. 118-121℃.

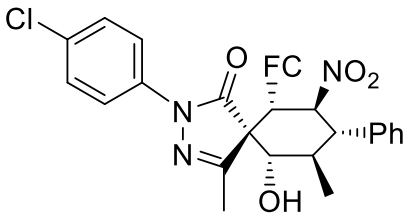
NMR and HRMS data for the product **4a**:

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 7.63 (br s, 1H), 7.51 - 7.43 (m, 3H), 7.35 - 7.28 (m, 4H), 7.17 - 7.12 (m, 1H), 7.05 (br s, 1H), 5.78 (t, *J* = 10.8 Hz, 1H), 4.15 (dd, *J* = 3.6, 1.2 Hz, 1H), 4.02 - 3.99 (m, 7H), 3.86 (dd, *J* = 3.6, 1.2 Hz, 1H), 3.74 (dd, *J* = 9.6, 6.4 Hz, 1H), 3.57 (d, *J* = 11.2 Hz, 1H), 2.96 - 2.93 (m, 2H), 2.32 (s, 3H), 1.67 (d, *J* = 6.4 Hz, 1H), 0.84 (d, *J* = 6.0 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 172.0 , 159.8 , 137.7 ,137.0 , 128.6 , 128.2 , 125.6 , 120.0 , 88.5 , 81.5 , 75.4 , 69.8 , 67.8 , 67.3 , 66.1 , 66.0 , 65.6 , 54.2 , 42.3 , 35.5 , 16.0 , 13.9 .

HRMS (ESI): *m/z* calculated for $C_{32}H_{31}FeN_3O_4Na^+$: 600.1556, found 600.1564.

(*5S,6S,7R,8S,9S,10S*)-1,7-dimethyl-3-(2-chlorophenyl)-8-phenyl-10-ferrocenyl-9-nitro-6-hydroxyl-2,3-diazaspiro[4.5]dec-1-ene-4-one



4b

Prepared according to the general procedure using (*E*)-(2-nitrovinyl) benzene **3a** (59.66 mg, 0.40 mmol, 1.0 equiv), propionaldehyde **2a** (46.46 mg, 0.8 mmol, 2 equiv) and (*Z*)-4-ferrocenylidene-5-methyl-2-(2-chlorophenyl)-2,4-dihydro-3H-pyrazol-3-one **1b** (121.40 mg, 0.30 mmol, 0.75 equiv). Purification of the crude product via column chromatography delivered **4b** as a yellow solid with 58% yield (110.9 mg). The diastereomeric ratio was determined to be >20:1 by crude ^{1}H-NMR analysis, and the enantiomeric excess of the major product was determined to be 93% by chiral HPLC analysis on Chiralpak IC-H column (10% 2-propanol/*n*-hexane, 1 mL/min, UV 254 nm), t_{major} = 22.97 min, t_{minor} = 38.22 min; $[\alpha]_D^{20}$ = -21.7 (*c* = 0.21 in CH_2Cl_2); m.p. >185℃.

NMR and HRMS data for the product **4b**:

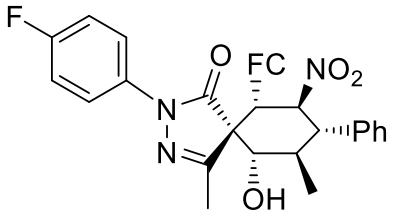
^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 7.58 (br s, 1H), 7.41 (dd, *J* = 8.0, 1.2 Hz, 2H), 7.34 - 7.27 (m, 2H), 7.26 (s, 0H), 7.25 - 7.19 (m, 2H), 7.05 (br s, 1H), 6.83 (dd, *J* = 7.6, 2.0 Hz, 1H), 5.78 (t, *J* = 10.8 Hz, 1H), 4.22 - 4.21 (m, 1H), 4.20 - 4.18 (m, 1H), 4.17 - 4.16 (m, 1H), 4.04 (s, 5H), 3.96 - 3.95(m,

1H), 3.76 (dd, *J* = 9.6, 7.6 Hz, 1H), 3.64 (d, *J* = 11.2 Hz, 1H), 2.95 (t, *J* = 11.6 Hz, 1H), 2.92 - 2.85 (m, 1H), 2.34 (s, 3H), 1.68 (d, *J* = 7.6 Hz, 1H), 0.86 (d, *J* = 6.0 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 172.2 , 160.0 , 137.7 , 131.7 , 133.9 , 130.2 , 129.9 , 128.8 , 128.1 , 127.4 , 88.5 , 82.0 , 75.4 , 70.0 , 67.8 , 67.5 , 66.7 , 65.6 , 65.3 , 54.3 , 42.2 , 35.6 , 16.0 , 14.0 .

HRMS (ESI): *m/z* calculated for $C_{32}H_{30}ClFeN_3O_4Na^+$: 634.1172, found 634.1179.

(*5S,6S,7R,8S,9S,10S*)-1,7-dimethyl-3-(4-fluorophenyl)-8-phenyl-10-ferrocenyl-9-nitro-6-hydroxyl-2,3-diazaspiro[4.5]dec-1-ene-4-one



4c

Prepared according to the general procedure using (*E*)-(2-nitrovinyl) benzene **3a** (59.66 mg, 0.40 mmol, 1.0 equiv), propionaldehyde **2a** (46.46 mg, 0.8 mmol, 2 equiv) and (*Z*)-4-ferrocenylidene-5-methyl-2-(4-fluorophenyl)-2,4-dihydro-3H-pyrazol-3-one **1c** (116.47 mg, 0.30 mmol, 0.75 equiv). Purification of the crude product via column chromatography delivered **4c** as a yellow solid with 67% yield (124.4 mg). The diastereomeric ratio was determined to be >20:1 by crude ^{1}H-NMR analysis, and the enantiomeric excess of the major product was determined to be >99% by chiral HPLC analysis on Chiralpak AD-H column (10% 2-propanol/*n*-hexane, 1 mL/min, UV 254 nm), t_{major} = 11.98 min, t_{minor} = 8.16 min; $[\alpha]_D^{20}$ = -60.2 (*c* = 0.24 in CH_2Cl_2); m.p. 135-137℃.

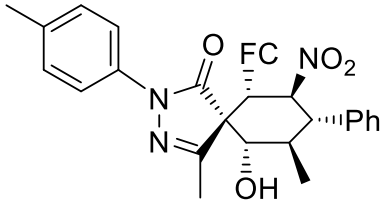
NMR and HRMS data for the product **4c**:

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 7.60 (br s, 1H), 7.48 - 7.38 (m, 3H), 7.37 - 7.28 (m, 2H), 7.10 - 6.94 (m, 3H), 5.75 (t, *J* = 11.2 Hz, 1H), 4.12 (dd, *J* = 3.6, 2.0 Hz, 1H), 4.01 - 3.99 (m, 7H), 3.85 - 3.84 (m, 1H), 3.72 (dd, *J* = 9.6, 6.0 Hz, 1H), 3.56 (d, *J* = 11.2 Hz, 1H), 2.97 - 2.89 (m, 2H), 2.29 (s, 3H), 1.86 (d, *J* = 6.4 Hz, 1H), 0.83 (d, *J* = 5.6 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 170.1 , 158.4 (d, J_{CF}= 243.6 Hz), 158.1 , 135.8 , 131.3 (d, J_{CF}= 2.9 Hz), 126.3 , 120.0 , 120.0 , 113.6 , 113.4 , 86.6 , 79.6 , 73.4 , 68.0 , 65.9 , 65.4 , 64.2 , 64.1 , 63.7 , 52.3 , 40.4 , 33.6 , 14.1 , 12.0 .

HRMS (ESI): *m/z* calculated for $C_{32}H_{30}FFeN_3O_4Na^+$: 618.1462, found 618.1464.

(*5S,6S,7R,8S,9S,10S*)-1,7-dimethyl-3-(p-tolyl)-8-phenyl-10-ferrocenyl-9-nitro-6-hydroxyl-2,3-diazaspiro[4.5]dec-1-ene-4-one



4d

Prepared according to the general procedure using (*E*)-(2-nitrovinyl) benzene **3a** (59.66 mg, 0.40 mmol, 1.0 equiv), propionaldehyde **2a** (46.46 mg, 0.8 mmol, 2 equiv) and (*Z*)-4-ferrocenylidene-5-methyl-2-(p-tolyl)-2,4-dihydro-3H-pyrazol-3-one **1d** (115.28 mg, 0.30 mmol, 0.75 equiv). Purification of the crude product via column chromatography delivered **4d** as an orange solid with 57% yield (101.6 mg). The diastereomeric ratio was determined to be >20:1 by crude ^{1}H-NMR analysis, and the enantiomeric excess of the major product was determined to be 95% by chiral HPLC analysis on Chiralpak AD-H column (10% 2-propanol/*n*-hexane, 1 mL/min, UV 254 nm), t_{major} = 13.40 min, t_{minor} =8.77 min; $[\alpha]_D^{20}$ = -38.1 (*c* = 0.23 in CH_2Cl_2); m.p. 126-128℃.

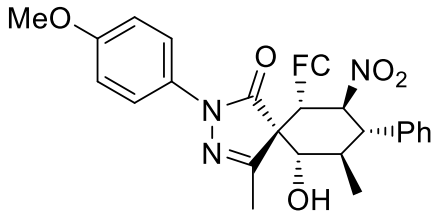
NMR and HRMS data for the product **4d**:

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 7.62 (br s, 1H), 7.46 (br s, 1H), 7.36 - 7.29 (m, 4H), 7.13 - 7.09 (m, 2H), 7.05 (br s, 1H), 5.78 (t, *J* = 10.8 Hz, 1H), 4.15 - 4.14 (m, 1H), 4.04 - 3.99 (m, 7H), 3.86 - 3.85 (m, 1H), 3.73 (dd, *J* = 10.0, 6.8 Hz, 1H), 3.56 (d, *J* = 11.2 Hz, 1H), 2.98 - 2.90 (m, 2H), 2.31 (s, 3H), 2.30 (s, 3H), 1.67 (d, *J* = 6.8 Hz, 1H), 0.84 (d, *J* = 5.6 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 171.8 , 159.6 , 137.7 , 135.4 , 134.6 , 129.2 , 128.1, 120.1 , 88.6 , 81.6 , 75.4 , 69.8 , 67.8 , 67.3, 66.1 , 66.0 , 65.6 , 54.2 , 42.3 , 35.5 , 21.0 , 16.0 , 13.9 .

HRMS (ESI): *m/z* calculated for $C_{33}H_{34}FeN_3O_4^+$: 592.1893, found 592.1895.

(*5S,6S,7R,8S,9S,10S*)-1,7-dimethyl-3-(4-methoxyphenyl)-8-phenyl-10-ferrocenyl-9-nitro-6-hydroxyl-2,3-diazaspiro[4.5]dec-1-ene-4-one



4e

Prepared according to the general procedure using (*E*)-(2-nitrovinyl) benzene **3a** (59.66 mg, 0.40 mmol, 1.0 equiv), propionaldehyde **2a** (46.46 mg, 0.8 mmol, 2 equiv) and (*Z*)-4-ferrocenylidene-5-methyl-2-(4-methoxyphenyl)-2,4-dihydro-3H-pyrazol-3-one **1e** (120.08 mg, 0.30 mmol, 0.75 equiv). Purification of the crude product via column chromatography delivered **4e** as a yellow solid with 61% yield (114.4 mg). The diastereomeric ratio was determined to be >20:1 by crude ^{1}H-NMR analysis, and the enantiomeric excess of the major product was determined to be >99% by chiral HPLC analysis on Chiralpak AD-H column (10% 2-propanol/*n*-hexane, 1 mL/min, UV 254 nm), t_{major} =21.02 min, t_{minor} =12.06 min; $[\alpha]_D^{20}$ = -46.5 (*c* = 0.23 in CH_2Cl_2); m.p. 142-144℃.

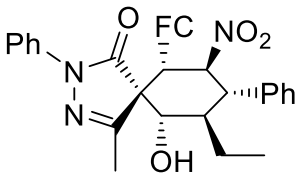
NMR and HRMS data for the product **4e**:

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 7.62 (br s, 1H), 7.45 (br s, 1H), 7.36 - 7.28 (m, 4H), 7.04 (br s, 1H), 6.84 - 6.81 (m, 2H), 5.78 (t, *J* = 11.2 Hz, 1H), 4.16 - 4.15 (m, 1H), 4.05 - 4.02 (m, 2H), 4.01 (s, 5H), 3.87 - 3.86 (m, 1H), 3.78 (s, 3H), 3.73 (dd, *J* = 9.6, 6.4 Hz, 1H), 3.56 (d, *J* = 11.2 Hz, 1H), 2.98 - 2.90 (m, 2H), 2.30 (s, 3H), 1.71 (d, *J* = 6.4 Hz, 1H), 0.84 (d, *J* = 5.6 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 171.8 , 159.6 , 157.5 , 137.7 , 130.1 , 128.1 , 122.2 , 113.8 , 88.5 , 81.6 , 75.4 , 69.9 , 67.8 , 67.3 , 66.1 , 65.9 , 65.6 , 55.5 , 54.2 , 42.3 , 35.5 , 16.0 , 13.9 .

HRMS (ESI): *m/z* calculated for $C_{33}H_{33}FeN_3O_5Na^+$: 630.1662, found 630.1662.

(*5S,6S,7R,8S,9S,10S*)-1-methyl-7-ethyl-3,8-diphenyl-10-ferrocenyl-9-nitro-6-hydroxyl-2,3-diazaspiro[4.5]dec-1-ene-4-one



4f

Prepared according to the general procedure using (*E*)-(2-nitrovinyl) benzene **3a** (59.66 mg, 0.40 mmol, 1.0 equiv), butyraldehyde **2b** (57.69 mg, 0.8 mmol, 2 equiv) and (*Z*)-4-ferrocenylidene-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one **1a** (111.07 mg, 0.30 mmol, 0.75 equiv). Purification of the crude product via column chromatography delivered **4f** as an orange solid with 69% yield (127.7 mg). The diastereomeric ratio was determined to be >20:1 by crude ^{1}H-NMR analysis, and the enantiomeric excess of the major product was determined to be 95% by chiral HPLC analysis on Chiralpak AD-H column (10% 2-propanol/*n*-hexane, 1 mL/min, UV 254 nm), t_{major} =9.11 min, t_{minor} = 6.08 min; $[\alpha]_D^{20}$ = -38.6 (*c* = 0.21 in CH_2Cl_2); m.p. 126-128℃.

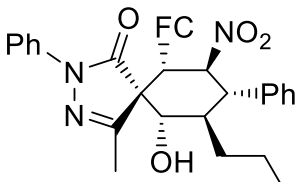
NMR and HRMS data for the product **4f**:

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 7.64 (br s, 1H), 7.50 - 7.43 (m, 3H), 7.35 - 7.27 (m, 4H), 7.17 - 7.12 (m, 1H), 7.07 (br s, 1H), 5.76 (t, *J* = 11.2 Hz, 1H), 4.14 - 4.13 (m, 1H), 4.02 - 3.99 (m, 7H), 3.99 - 3.94 (m, 1H), 3.86 - 3.85 (m, 1H), 3.54 (d, *J* = 11.2 Hz, 1H), 3.15 (dd, *J* = 12.4, 11.2 Hz, 1H), 3.05 - 2.97 (m, 1H), 2.33 (s, 3H), 1.56 (d, *J* = 6.4 Hz, 1H), 1.53 - 1.45 (m, 1H), 1.24 - 1.17 (m, 1H), 0.77 (t, *J* = 7.6 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 172.1 , 159.9 , 137.5 , 137.0 , 128.6 , 128.1 , 125.6 , 120.0 , 89.0 , 81.5 , 71.7 , 69.8 , 67.8 , 67.3 , 66.1 , 66.0 , 65.5 , 50.4 , 42.2 , 39.0, 20.0 , 14.0 , 7.5 .

HRMS (ESI): *m/z* calculated for $C_{33}H_{33}FeN_3O_4Na^+$: 614.1713, found 614.1722.

(*5S,6S,7R,8S,9S,10S*)-1-methyl-7-propyl-3,8-diphenyl-10-ferrocenyl-9-nitro-6-hydroxyl-2,3-diazaspiro[4.5]dec-1-ene-4-one



4g

Prepared according to the general procedure using (*E*)-(2-nitrovinyl) benzene **3a** (59.66 mg, 0.40 mmol, 1.0 equiv), butyraldehyde **2c** (68.91 mg, 0.8 mmol, 2 equiv) and (*Z*)-4-ferrocenylidene-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one **1a** (111.07 mg, 0.30 mmol, 0.75 equiv). Purification of the crude product via column chromatography delivered **4g** as an orange solid with 62% yield (112.0 mg).The diastereomeric ratio was determined to be >20:1 by crude ^{1}H-NMR analysis, and the enantiomeric excess of the major product was determined to be 97% by chiral HPLC analysis on Chiralpak AD-H column (10% 2-propanol/*n*-hexane, 1 mL/min, UV 254 nm), t_{major} = 7.04 min, t_{minor} = 5.81 min; $[\alpha]_D^{20}$ = -34.9 (*c* = 0.21 in CH_2Cl_2); m.p. 110-113℃.

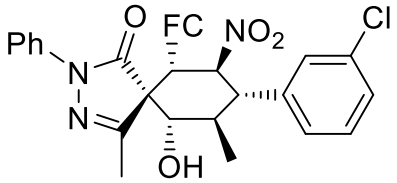
NMR and HRMS data for the product **4g**:

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 7.64 (br s, 1H), 7.52 - 7.43 (m, 3H), 7.36 - 7.28 (m, 4H), 7.14 (tt, *J* = 7.2, 1.2 Hz, 1H), 7.05 (br s, 1H), 5.76 (t, *J* = 11.2 Hz, 1H), 4.14 (dd, *J* = 3.6, 2.0 Hz, 1H), 4.00 (s, 7H), 3.93 (d, *J* = 10.4 Hz, 1H), 3.85 (dd, *J* = 3.6, 1.6 Hz, 1H), 3.53 (d, *J* = 11.2 Hz, 1H), 3.13 (t, *J* = 12.0 Hz, 1H), 2.96 (tt, *J* = 11.2, 3.2 Hz, 1H), 2.30 (s, 3H), 1.75 (br s, 1H), 1.43 - 1.25 (m, 2H), 1.17 - 1.07 (m, 2H), 0.69 (t, *J* = 5.6 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 172.1 , 159.9 , 137.6 , 137.0 , 128.6 , 128.1 , 125.6 , 120.0 , 88.9 , 81.6 , 72.7 , 69.8 , 67.8 , 67.3 , 66.2 , 66.0 , 65.5 , 51.4 , 42.2 , 38.9 , 30.4 , 17.2 , 14.4 , 14.0 .

HRMS (ESI): *m/z* calculated for $C_{34}H_{36}FeN_3O_4^+$: 606.2050, found 606.2047.

(*5S,6S,7R,8S,9S,10S*)-1,7-dimethyl-3-phenyl-8-(3-chlorophenyl)-10-ferrocenyl-9-nitro-6-hydroxyl-2,3-diazaspiro[4.5]dec-1-ene-4-one



4h

Prepared according to the general procedure using (*E*)-1-chloro-3-(2-nitrovinyl) benzene **3h** (73.44 mg, 0.40 mmol, 1.0 equiv), propionaldehyde **2a** (46.46 mg, 0.8 mmol, 2 equiv) and (*Z*)-4-ferrocenylidene-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one **1a** (111.07 mg, 0.30 mmol, 0.75 equiv). Purification of the crude product via column chromatography delivered **4h** as an orange solid

with 63% yield (116.2 mg). The diastereomeric ratio was determined to be >20:1 by crude ^{1}H-NMR analysis, and the enantiomeric excess of the major product was determined to be 93% by chiral HPLC analysis on Chiralpak AD-H column (10% 2-propanol/*n*-hexane, 1 mL/min, UV 254 nm), t_{major} = 12.41 min, t_{minor} = 7.47 min; $[\alpha]_D^{20}$ = -52.2 (*c* = 0.25 in CH_2Cl_2); m.p. 123-125℃.

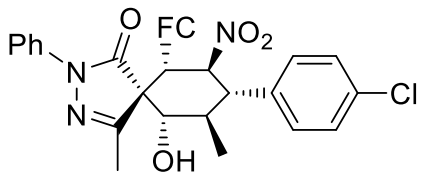
NMR and HRMS data for the product **4h**:

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 7.65 - 7.50 (m, 1H), 7.50 - 7.36 (m, 3H), 7.34 – 7.29 (m, 3H), 7.18 – 7.12 (m, 1H), 7.10 – 6.91 (m, 1H), 5.76 (br s, 1H), 4.15 (s, 1H), 4.04 – 3.99 (m, 7H), 3.86 (dd, *J* = 3.6, 1.6 Hz, 1H), 3.72 (dd, *J* = 10.0, 6.4 Hz, 1H), 3.55 (d, *J* = 11.2 Hz, 1H), 2.93-2.91 (m, 2H), 2.31 (s, 3H), 1.67 (d, *J* = 6.4 Hz, 1H), 0.87 – 0.83 (d, *J* = 6.4 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 171.9 , 159.6 , 139.8 , 137.0 , 128.6 , 125.7 , 120.0 , 88.2 , 81.3 , 75.2 , 69.9 , 67.9 , 67.4 , 66.0 , 65.6 , 53.8 , 42.3 , 30.9 , 16.0 , 13.9 .

HRMS (ESI): *m/z* calculated for $C_{32}H_{31}ClFeN_3O_4^+$: 612.1347, found 612.1332.

(*5S,6S,7R,8S,9S,10S*)-1,7-dimethyl-3-phenyl-8-(4-chlorophenyl)-10-ferrocenyl-9-nitro-6-hydroxyl-2,3-diazaspiro[4.5]dec-1-ene-4-one



4i

Prepared according to the general procedure using (*E*)-1-chloro-4-(2-nitrovinyl) benzene **3i** (73.44 mg, 0.40 mmol, 1.0 equiv), propionaldehyde **2a** (46.46 mg, 0.8 mmol, 2 equiv) and (*Z*)-4-ferrocenylidene-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one **1a** (111.07 mg, 0.30 mmol, 0.75 equiv). Purification of the crude product via column chromatography delivered **4i** as a yellow solid with 62% yield (114.1 mg). The diastereomeric ratio was determined to be >20:1 by crude ^{1}H-NMR analysis, and the enantiomeric excess of the major product was determined to be 93% by chiral HPLC analysis on Chiralpak AD-H column (10% 2-propanol/*n*-hexane, 1 mL/min, UV 254 nm), t_{major} = 10.84 min, t_{minor} = 8.24 min; $[\alpha]_D^{20}$ = -80.97 (*c* = 0.24 in CH_2Cl_2); decomp. >169℃.

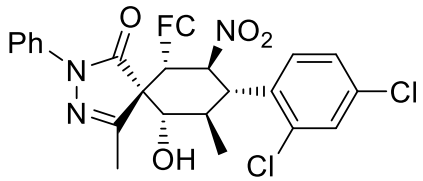
NMR and HRMS data for the product **4i**:

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 7.56 (br s, 1H), 7.50 - 7.39 (m, 3H), 7.34 - 7.27 (m, 3H), 7.15 (tt, *J* = 7.2, 1.2 Hz, 1H), 6.99 (br s, 1H), 5.75 (t, *J* = 10.8 Hz, 1H), 4.13 (dd, *J* = 3.6, 1.6 Hz, 1H), 4.02 (t, *J* = 2.0 Hz, 2H), 4.00 (s, 5H), 3.86 (dd, *J* = 3.6, 1.6 Hz, 1H), 3.72 (dd, *J* = 9.6, 6.4 Hz, 1H), 3.55 (d, *J* = 11.2 Hz, 1H), 2.97 - 2.86 (m, 2H), 2.31 (s, 3H), 1.68 (d, *J* = 6.4 Hz, 1H), 0.84 (d, *J* = 6.0 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 171.9 , 159.6 , 137.0 , 136.3 , 134.1 , 128.6 , 125.7 , 120.0 , 88.4 , 81.3 , 75.2 , 69.9 , 67.9 , 67.4 , 66.0 , 66.0 , 65.6 , 53.5 , 42.3 , 35.5 , 16.0 , 13.9 .

HRMS (ESI): *m/z* calculated for $C_{32}H_{31}ClFeN_3O_4^+$: 612.1347, found 612.1336.

(*5S,6S,7R,8S,9S,10S*)-1,7-dimethyl-3-phenyl-8-(2,4-dichlorophenyl)-10-ferrocenyl-9-nitro-6-hydroxyl-2,3-diazaspiro[4.5]dec-1-ene-4-one



4j

Prepared according to the general procedure using (*E*)-1,3-dichloro-4-(2-nitrovinyl) benzene **3j** (87.21 mg, 0.40 mmol, 1.0 equiv), propionaldehyde **2a** (46.46 mg, 0.8 mmol, 2 equiv) and (*Z*)-4-ferrocenylidene-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one **1a** (111.07 mg, 0.30 mmol, 0.75 equiv). Purification of the crude product via column chromatography delivered **4j** as a yellow solid with 54% yield (99.9 mg). The diastereomeric ratio was determined to be >20:1 by crude ^{1}H-NMR analysis, and the enantiomeric excess of the major product was determined to be 99% by chiral HPLC analysis on Chiralpak AD-H column (10% 2-propanol/*n*-hexane, 1 mL/min, UV 254 nm), t_{major} = 12.70 min, t_{minor} = 7.90 min; $[\alpha]_D^{20}$ = -36.9 (*c* = 0.24 in CH_2Cl_2); m.p. 160-162℃.

NMR and HRMS data for the product **4j**:

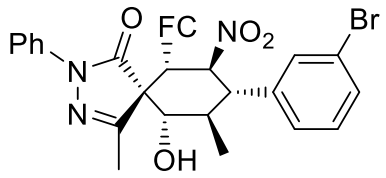
^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 7.65 (d, *J* = 8.4 Hz, 1H), 7.48 - 7.44 (m, 2H), 7.42 (d, *J* = 2.0 Hz, 1H), 7.38 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.34 - 7.28 (m, 2H), 7.15 (tt, *J* = 7.6, 1.2 Hz, 1H), 5.79 (t, *J* = 11.2 Hz, 1H), 4.13 (dd, *J* = 3.6, 1.6 Hz, 1H), 4.04 - 4.02 (m, 2H), 4.01 (s, 5H), 3.89 - 3.77 (m, 3H),

3.61 (d, J = 11.2 Hz, 1H), 2.80 (td, *J* = 11.2, 6.4 Hz, 1H), 2.32 (s, 3H), 1.76 -1.74 (m, 1H), 0.87 (d, *J* = 6.4 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 172.0 , 159.7 , 136.9 , 135.5 , 134.5 , 129.6 , 129.1 , 128.7 , 128.4 , 125.8 , 120.1 , 87.2 , 81.2 , 75.0 , 69.9 , 67.9 , 67.4 , 66.0 , 65.8 , 65.7 , 47.7 , 42.3 , 36.9 , 15.0 , 14.0 .

HRMS (ESI): *m/z* calculated for $C_{32}H_{30}Cl_2FeN_3O_4^+$: 646.0957, found 646.0958.

(*5S,6S,7R,8S,9S,10S*)-1,7-dimethyl-3-phenyl-8-(3-bromophenyl)-10-ferrocenyl-9-nitro-6-hydroxyl-2,3-diazaspiro[4.5]dec-1-ene-4-one



4k

Prepared according to the general procedure using (*E*)-1-bromo-3-(2-nitrovinyl) benzene **3k** (87.21 mg, 0.40 mmol, 1.0 equiv), propionaldehyde **2a** (46.46 mg, 0.8 mmol, 2 equiv) and (*Z*)-4-ferrocenylidene-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one **1a** (111.07 mg, 0.30 mmol, 0.75 equiv). Purification of the crude product via column chromatography delivered **4k** as an orange solid with 60% yield (121.7 mg). The diastereomeric ratio was determined to be >20:1 by crude ^{1}H-NMR analysis, and the enantiomeric excess of the major product was determined to be 92% by chiral HPLC analysis on Chiralpak AD-H column (10% 2-propanol/*n*-hexane, 1 mL/min, UV 254 nm), t_{major} = 12.28 min, t_{minor} = 7.89 min; $[\alpha]_D^{20}$ = -65.14 (*c* = 0.22 in CH_2Cl_2); m.p. 138-140℃.

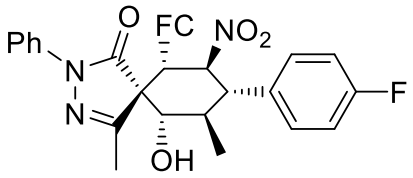
NMR and HRMS data for the product **4k**:

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 7.79 - 7.43 (m, 4H), 7.33 - 7.28 (m, 2H), 7.24 - 7.12 (m, 2H), 6.99 (br s, 1H), 5.76 (br s, 1H), 4.19 - 3.97 (m, 8H), 3.86 (dd, *J* = 3.6, 1.2 Hz, 1H), 3.72 (dd, *J* = 9.6, 6.4 Hz, 1H), 3.55 (d, *J* = 11.2 Hz, 1H), 2.91 (br s, 2H), 2.31 (s, 3H), 1.69 (d, *J* = 6.4 Hz, 1H), 0.85 (d, *J* = 6.0 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 171.9 , 159.6 , 140.1 , 137.0 , 128.6 , 125.7 , 120.0 , 100.0 , 88.2 , 81.3 , 75.2 , 69.9 , 67.9 , 67.4 , 66.0 , 65.6 , 53.8 , 42.3 , 30.9 , 16.0 , 13.9 .

HRMS (ESI): *m/z* calculated for $C_{32}H_{30}BrFeN_3O_4Na^+$: 678.0661, found 678.0670.

(*5S,6S,7R,8S,9S,10S*)-1,7-dimethyl-3-phenyl-8-(4−fluorophenyl)-10-ferrocenyl-9-nitro-6-hydroxyl-2,3-diazaspiro[4.5]dec-1-ene-4-one



4l

Prepared according to the general procedure using (*E*)-1-fluoro-4-(2-nitrovinyl) benzene **3l** (66.86 mg, 0.40 mmol, 1.0 equiv), propionaldehyde **2a** (46.46 mg, 0.8 mmol, 2 equiv) and (*Z*)-4-ferrocenylidene-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one **1a** (111.07 mg, 0.30 mmol, 0.75 equiv). Purification of the crude product via column chromatography delivered **4l** as a yellow solid with 64% yield (119.4 mg). The diastereomeric ratio was determined to be >20:1 by crude ^{1}H-NMR analysis, and the enantiomeric excess of the major product was determined to be 98% by chiral HPLC analysis on Chiralpak AD-H column (10% 2-propanol/*n*-hexane, 1 mL/min, UV 254 nm), t_{major} =7.93 min, t_{minor} = 10.61 min; $[\alpha]_D^{20}$ = -46.5 (*c* = 0.23 in CH_2Cl_2); m.p. 137-138℃.

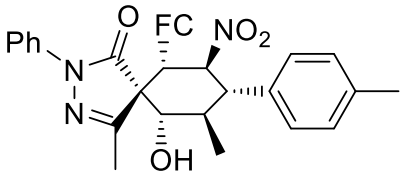
NMR and HRMS data for the product **4l**:

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 7.60 (br s, 1H), 7.50 - 7.44 (m, 2H), 7.34 - 7.28 (m, 2H), 7.15 (tt, *J* = 7.6, 1.2 Hz, 2H), 7.00 (br s, 2H), 5.74 (t, *J* = 10.8 Hz, 1H), 4.13 (dd, *J* = 3.6, 1.6 Hz, 1H), 4.02 (t, *J* = 1.6 Hz, 2H), 4.01 (s, 5H), 3.86 (dd, *J* = 3.6, 1.6 Hz, 1H), 3.73 (dd, *J* = 10.0, 6.0 Hz, 1H), 3.55 (d, *J* = 11.2 Hz, 1H), 2.98 - 2.87 (m, 2H), 2.31 (s, 3H), 1.68 (d, *J* = 6.4 Hz, 1H), 0.84 (d, *J* = 5.6 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 172.0 , 162.5 (d, J_{CF}= 245.5 Hz), 159.8 , 137.0 , 133.5 (d, J_{CF}= 3.0 Hz), 128.6 , 125.7 , 120.0 , 88.6 , 81.4 , 75.2 , 69.9 , 67.8 , 67.4 , 66.0 , 66.0 , 65.6 , 53.4 , 42.3 , 35.6 , 15.9 , 13.9 .

HRMS (ESI): *m/z* calculated for $C_{32}H_{30}FFeN_3O_4Na^+$: 618.1462, found 618.1474.

(*5S,6S,7R,8S,9S,10S*)-1,7-dimethyl-3-phenyl-8-(p-tolyl)-10-ferrocenyl-9-nitro-6-hydroxyl-2,3-diazaspiro[4.5]dec-1-ene-4-one



4m

Prepared according to the general procedure using (*E*)-1-methyl-4-(2-nitrovinyl) benzene **3m** (65.27 mg, 0.40 mmol, 1.0 equiv), propionaldehyde **2a** (46.46 mg, 0.8 mmol, 2 equiv) and (*Z*)-4-ferrocenylidene-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one **1a** (111.07 mg, 0.30 mmol, 0.75 equiv). Purification of the crude product via column chromatography delivered **4m** as an orange solid with 70% yield (124.7 mg). The diastereomeric ratio was determined to be >20:1 by crude ^{1}H-NMR analysis, and the enantiomeric excess of the major product was determined to be 98% by chiral HPLC analysis on Chiralpak AD-H column (10% 2-propanol/*n*-hexane, 1 mL/min, UV 254 nm), t_{major} = 9.61 min, t_{minor} = 7.90 min; $[\alpha]_D^{20}$ = -52.9 (*c* = 0.20 in CH_2Cl_2); decomp. >171℃.

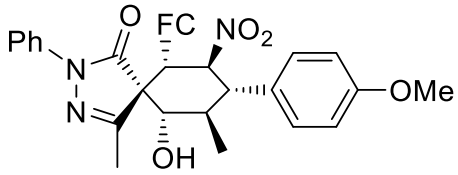
NMR and HRMS data for the product **4m**:

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 7.54 - 7.45 (m, 3H), 7.32 - 7.27 (m, 3H), 7.17 - 7.06 (m, 2H), 6.93 (br s, 1H), 5.75 (t, *J* = 10.8 Hz, 1H), 4.14 (dd, *J* = 3.6, 2.0 Hz, 1H), 4.00 (s, 7H), 3.85 (dd, *J* = 3.6, 1.6 Hz, 1H), 3.72 (d, *J* = 8.4 Hz, 1H), 3.55 (d, *J* = 11.2 Hz, 1H), 2.94 - 2.85 (m, 2H), 2.35 (s, 3H), 2.31 (s, 3H), 1.76 (s, 1H), 0.84 (d, *J* = 5.6 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 172.0 , 159.8, 137.8 , 137.0 , 134.6 , 128.6 , 125.6 , 120.0 , 88.6 , 81.6 , 75.4 , 69.8 , 67.8 , 67.3 , 66.1 , 66.0 , 65.6 , 53.8 , 42.3 , 35.5 , 21.2 , 16.0 , 13.9 .

HRMS (ESI): *m/z* calculated for $C_{33}H_{34}FeN_3O_4^+$: 592.1893, found 592.1895.

(5S,6S,7R,8S,9S,10S)-1,7-dimethyl-3-phenyl-8-(4-methoxyphenyl)-10-ferrocenyl-9-nitro-6-hydroxyl-2,3-diazaspiro[4.5]dec-1-ene-4-one



4n

Prepared according to the general procedure using (*E*)-1-methyl-4-(2-nitrovinyl) benzene **3m** (71.67 mg, 0.40 mmol, 1.0 equiv), propionaldehyde **2a** (46.46 mg, 0.8 mmol, 2 equiv) and (*Z*)-4-ferrocenylidene-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one **1a** (111.07 mg, 0.30 mmol, 0.75 equiv). Purification of the crude product via column chromatography delivered **4n** as an orange solid with 69% yield (125.9 mg). The diastereomeric ratio was determined to be >20:1 by crude ^{1}H-NMR analysis, and the enantiomeric excess of the major product was determined to be 94% by chiral HPLC analysis on Chiralpak AD-H column (10% 2-propanol/*n*-hexane, 1 mL/min, UV 254 nm), t_{major} =15.56 min, t_{minor} =10.59 min; $[\alpha]_D^{20}$ = -67.3 (*c* = 0.23 in CH_2Cl_2); decomp. >176℃.

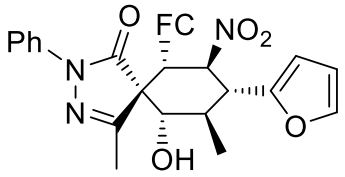
NMR and HRMS data for the product **4n**:

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 7.56 - 7.44 (m, 3H), 7.33 - 7.27 (m, 2H), 7.14 (tt, *J* = 7.2, 1.2 Hz, 1H), 6.99 (br s, 2H), 6.80 (br s, 1H), 5.73 (t, *J* = 11.2 Hz, 1H), 4.14 (dd, *J* = 3.6, 1.6 Hz, 1H), 4.01 (s, 7H), 3.85 (dd, *J* = 3.6, 1.6 Hz, 1H), 3.81 (s, 3H), 3.73 - 3.69 (m, 1H), 3.54 (d, *J* = 11.2 Hz, 1H), 2.91 - 2.84 (m, 2H), 2.30 (s, 3H), 1.80 (d, *J* = 6.4 Hz, 1H), 0.83 (d, *J* = 5.6 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 172.0 , 159.8 , 159.3 , 137.0 , 129.6 , 128.6 , 125.6 , 120.0 , 88.7 , 81.6 , 75.4 , 69.8 , 67.8 , 67.3 , 66.1 , 66.0 , 65.5 , 55.2 , 53.4 , 42.2 , 35.6 , 16.0 , 13.9 .

HRMS (ESI): *m/z* calculated for $C_{33}H_{34}FeN_3O_5^+$: 608.1842, found 608.1839.

(*5S,6S,7R,8S,9S,10S*)-1,7-dimethyl-3-phenyl-8-furyl-10-ferrocenyl-9-nitro-6-hydroxyl-2,3-diazaspiro[4.5]dec-1-ene-4-one



4o

Prepared according to the general procedure using (*E*)-2-(2-nitrovinyl)furan **3o** (55.64 mg, 0.40 mmol,

1.0 equiv), propionaldehyde **2a** (46.46 mg, 0.8 mmol, 2 equiv) and (*Z*)-4-ferrocenylidene-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one **1a** (111.07 mg, 0.30 mmol, 0.75 equiv). Purification of the crude product via column chromatography delivered **4o** as a yellow solid with 55% yield (97.4 mg). The diastereomeric ratio was determined to be >20:1 by crude ^{1}H-NMR analysis, and the enantiomeric excess of the major product was determined to be 92% by chiral HPLC analysis on Chiralpak AD-H column (10% 2-propanol/*n*-hexane, 1 mL/min, UV 254 nm), t_{major} = 14.65 min, t_{minor} =8.28 min; $[\alpha]_D^{20}$ = -61.0 (*c* = 0.24 in CH_2Cl_2); decomp. >150℃.

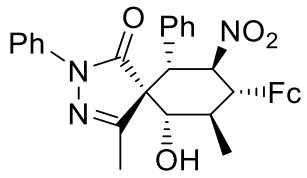
NMR and HRMS data for the product **4o**:

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 7.50 - 7.44 (m, 3H), 7.30 - 7.26 (m, 2H), 7.13 (tt, *J* = 7.2, 1.2 Hz, 1H), 6.33 (dd, *J* = 3.2, 2.0 Hz, 1H), 6.24 (d, *J* = 3.2 Hz, 1H), 5.81 (t, *J* = 11.2 Hz, 1H), 4.14 (dd, *J* = 3.6, 1.6 Hz, 1H), 4.04 - 3.98 (m, 7H), 3.83 (dd, *J* = 3.6, 1.6 Hz, 1H), 3.65 (dd, *J* = 10.8, 6.4 Hz, 1H), 3.50 (d, *J* = 11.2 Hz, 1H), 3.12 (t, *J* = 11.2 Hz, 1H), 3.09 - 2.99 (m, 1H), 2.27 (s, 3H), 1.90 (d, *J* = 6.8 Hz, 1H), 0.90 (d, *J* = 6.4 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 171.7 , 159.6 , 150.2 , 143.0 , 137.0 , 128.6 , 125.5 , 119.8 , 110.2 , 109.1 , 86.5 , 81.4 , 75.2 , 69.9 , 67.8 , 67.3 , 66.1 , 66.0 , 65.5 , 47.8 , 42.0 , 33.8 , 15.8 , 13.8 .

HRMS (ESI): *m/z* calculated for $C_{30}H_{29}FeN_3O_5Na^+$: 590.1349, found 590.1367.

(*5S,6S,7R,8R,9R,10S*)-1,7-dimethyl-3,10-diphenyl-8-ferrocenyl-9-nitro-6-hydroxyl-2,3-diazaspiro[4.5]dec-1-ene-4-one



5a

Prepared according to the general procedure using (*E*)-2-(2-nitrovinyl)ferrocene **3p** (102.83 mg, 0.40 mmol, 1.0 equiv), propionaldehyde **2a** (46.46 mg, 0.8 mmol, 2 equiv) and (*Z*)-4-benzylidene-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one **1f** (78.69 mg, 0.30 mmol). Purification of the crude product via column chromatography delivered **5a** as a yellow solid with 54% yield (93.7 mg). The diastereomeric ratio was determined to be 86:14 by crude ^{1}H-NMR analysis, and the enantiomeric

excess of the major product was determined to be 94% by chiral HPLC analysis on Chiralpak AD-H column (10% 2-propanol/*n*-hexane, 1 mL/min, UV 254 nm), t_{major} =15.44 min, t_{minor} = 20.62 min; $[\alpha]_D^{20}$ = 301.0 (*c* = 0.23 in CH_2Cl_2); m.p. 175-178℃.

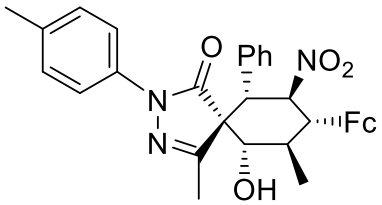
NMR and HRMS data for the product **5a**:

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 7.69 - 7.64 (m, 2H), 7.38 - 7.33 (m, 2H), 7.21 - 7.10 (m, 5H), 5.81 (dd, *J* = 12.0, 10.8 Hz, 1H), 4.24 - 4.20 (m, 2H), 4.12 (s, 5H), 4.12 - 4.08 (m, 2H), 3.77 (dd, *J* = 10.4, 6.4 Hz, 1H), 3.48 (d, *J* = 11.6 Hz, 1H), 2.97 (t, *J* = 11.2 Hz, 1H), 2.70 - 2.60 (m, 1H), 2.12 (s, 3H), 1.79 (d, *J* = 6.4 Hz, 1H), 1.29 (d, *J* = 6.4 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 172.2 , 159.6 , 138.8 , 137.3 , 129.8 , 128.8 , 125.6 , 119.5 , 89.0 , 87.1 , 75.8 , 69.6 , 68.9 , 67.8 , 67.6 , 67.0 , 65.9 , 63.8 , 50.2 , 45.0 , 37.2 , 21.1 , 17.1 , 13.4 .

HRMS (ESI): *m/z* calculated for $C_{32}H_{32}FeN_3O_4^+$: 578.1737, found 578.1724.

(*5S,6S,7R,8R,9R,10S*)-1,7-dimethyl-3-phenyl-10-(p-tolyl)-8-ferrocenyl-9-nitro-6-hydroxyl-2,3-diazaspiro[4.5]dec-1-ene-4-one



5b

Prepared according to the general procedure using (*E*)-2-(2-nitrovinyl)ferrocene **3p** (102.83 mg, 0.40 mmol, 1.0 equiv), propionaldehyde **2a** (46.46 mg, 0.8 mmol, 2 equiv) and (*Z*)-4-benzylidene-5-methyl-2-(p-tolyl)-2,4-dihydro-3H-pyrazol-3-one **1g** (82.84 mg, 0.30 mmol). Purification of the crude product via column chromatography delivered **5b** as a yellow solid with 64% yield (118.7 mg). The diastereomeric ratio was determined to be >20:1 by crude ^{1}H-NMR analysis, and the enantiomeric excess of the major product was determined to be >99% by chiral HPLC analysis on Chiralpak AD-H column (10% 2-propanol/*n*-hexane, 1 mL/min, UV 254 nm), t_{major} = 28.58 min, t_{minor} = 14.52 min; $[\alpha]_D^{20}$ = 343.0 (*c* = 0.21 in CH_2Cl_2); m.p. 138-140℃.

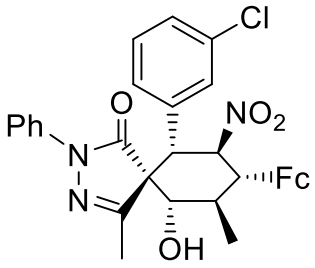
NMR and HRMS data for the product **5b**:

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 7.54 - 7.49 (m, 2H), 7.22 - 7.14 (m, 7H), 5.79 (dd, *J* = 11.2, 10.8 Hz, 1H), 4.22 (t, *J* = 1.6 Hz, 2H), 4.14 - 4.08 (m, 7H), 3.73 (dd, *J* = 10.4, 6.4 Hz, 1H), 3.45 (d, *J* = 11.6 Hz, 1H), 2.94 (t, *J* = 11.2 Hz, 1H), 2.69 - 2.59 (m, 1H), 2.33 (s, 3H), 2.08 (s, 3H), 1.88 (d, *J* = 6.8 Hz, 1H), 1.27 (d, *J* = 6.4 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 171.9 , 159.4 , 135.4 , 134.8 , 132.9 , 129.3 , 129.0 , 128.9 , 119.7 , 88.9 , 87.2 , 75.8 , 69.7 , 67.8 , 67.6 , 67.1 , 66.1 , 63.6 , 50.5 , 45.0 , 37.1 , 21.0 , 17.1 , 13.4 .

HRMS (ESI): *m/z* calculated for $C_{33}H_{33}FeN_3O_4Na^+$:614.1713, found 614.1719.

(*5S,6S,7R,8R,9R,10S*)-1,7-dimethyl-3-phenyl-10-(3-chlorophenyl)-8-ferrocenyl-9-nitro-6-hydroxyl-2,3-diazaspiro[4.5]dec-1-ene-4-one



5c

Prepared according to the general procedure using (*E*)-2-(2-nitrovinyl)ferrocene **3p** (102.83 mg, 0.40 mmol, 1.0 equiv), propionaldehyde **2a** (46.46 mg, 0.8 mmol, 2 equiv) and (*Z*)-4-(3-chlorobenzylidene)-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one **1h** (89.03 mg, 0.30 mmol). Purification of the crude product via column chromatography delivered **5c** as a yellow solid with 60% yield (113.7 mg). The diastereomeric ratio was determined to be 96:4 by crude ^{1}H-NMR analysis, and the enantiomeric excess of the major product was determined to be 99% by chiral HPLC analysis on Chiralpak AD-H column (10% 2-propanol/*n*-hexane, 1 mL/min, UV 254 nm), t_{major} = 24.52 min, t_{minor} = 17.11 min; $[\alpha]_D^{20}$ = 323.9 (*c* = 0.21 in CH_2Cl_2); m.p. 130-132℃.

NMR and HRMS data for the product **5c**:

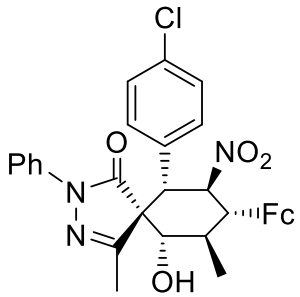
^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 7.68 - 7.63 (m, 2H), 7.39 - 7.34 (m, 2H), 7.24 - 6.96 (m, 5H), 5.77 (dd, *J* = 12.0, 10.8 Hz, 1H), 4.24 - 4.21 (m, 2H), 4.13 - 4.11 (m, 6H), 4.08 - 4.07 (m, 1H), 3.75

(d, *J* = 10.4 Hz, 1H), 3.45 (d, *J* = 11.6 Hz, 1H), 2.95 (t, *J* = 11.6 Hz, 1H), 2.68 - 2.60 (m, 1H), 2.12 (s, 3H), 1.80 (br s, 1H), 1.28 (d, *J* = 6.8 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 171.8 , 159.2, 137.0 , 134.9 , 130.2 , 129.4 , 128.8 , 125.9 , 119.7 , 88.6 , 86.8 , 75.7 , 69.6 , 69.0 , 67.7 , 67.1 , 65.9 , 63.5 , 49.9 , 45.0 , 37.1 , 17.0 , 13.4 .

HRMS (ESI): *m/z* calculated for $C_{32}H_{30}ClFeN_3O_4Na^+$: 634.1166, found 634.1171.

(*5S,6S,7R,8R,9R,10S*)-1,7-dimethyl-3-phenyl-10-(4-chlorophenyl)-8-ferrocenyl-9-nitro-6-hydroxyl-2,3-diazaspiro[4.5]dec-1-ene-4-one



5d

Prepared according to the general procedure using (*E*)-2-(2-nitrovinyl)ferrocene **3p** (102.83 mg, 0.40 mmol, 1.0 equiv), propionaldehyde **2a** (46.46 mg, 0.8 mmol, 2 equiv) and (*Z*)-4-(4-chlorobenzylidene)-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one **1i** (89.03 mg, 0.30 mmol). Purification of the crude product via column chromatography delivered **5d** as a yellow solid with 63% yield (120.3 mg). The diastereomeric ratio was determined to be 86:14 by crude ^{1}H-NMR analysis, and the enantiomeric excess of the major product was determined to be 98% by chiral HPLC analysis on Chiralpak AD-H column (10% 2-propanol/*n*-hexane, 1 mL/min, UV 254 nm), t_{major} = 15.75 min, t_{minor} = 10.96 min; $[\alpha]_D^{20}$ = 318.0 (*c* = 0.23 in CH_2Cl_2); m.p. 124-127℃.

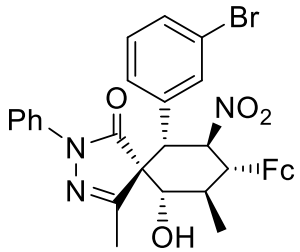
NMR and HRMS data for the product **5d**:

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 7.71 - 7.66 (m, 2H), 7.39 - 7.34 (m, 2H), 7.22 - 7.04 (m, 5H), 5.77 (dd, *J* = 11.8, 10.4 Hz, 1H), 4.24 - 4.21 (m, 2H), 4.13 - 4.10 (s, 6H), 4.08 - 4.06 (m, 1H), 3.76 (dd, *J* = 10.4, 6.8 Hz, 1H), 3.47 (d, *J* = 12.0 Hz, 1H), 2.96 (t, *J* = 10.8 Hz, 1H), 2.68 - 2.60 (m, 1H), 2.12 (s, 3H), 1.73 (d, *J* = 6.4 Hz, 1H), 1.29 (d, *J* = 6.4 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 171.9 , 159.2 , 137.2 , 135.1 , 131.5 , 129.2 , 128.9 , 125.8 , 119.4 , 88.8 , 86.8 , 75.7 , 69.6 , 69.0 , 67.7 , 67.6 , 67.1 , 65.9 , 63.6 , 49.8 , 45.0 , 37.1 , 17.0 , 13.4 .

HRMS (ESI): *m/z* calculated for $C_{32}H_{30}ClFeN_3O_4Na^+$: 634.1166, found 634.1177.

(*5S,6S,7R,8R,9R,10S*)-1,7-dimethyl-3-phenyl-10-(3-bromophenyl)-8-ferrocenyl-9-nitro-6-hydroxyl-2,3-diazaspiro[4.5]dec-1-ene-4-one



5e

Prepared according to the general procedure using (*E*)-2-(2-nitrovinyl)ferrocene **3p** (102.83 mg, 0.40 mmol, 1.0 equiv), propionaldehyde **2a** (46.46 mg, 0.8 mmol, 2 equiv) and (*Z*)-4-(3-bromobenzylidene)-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one **1j** (102.06 mg, 0.30 mmol). Purification of the crude product via column chromatography delivered **5e** as a yellow solid with 58% yield (117.0 mg). The diastereomeric ratio was determined to be 85:15 by crude ^{1}H-NMR analysis, and the enantiomeric excess of the major product was determined to be 99% by chiral HPLC analysis on Chiralpak AD-H column (10% 2-propanol/*n*-hexane, 1 mL/min, UV 254 nm), t_{major} = 26.45 min, t_{minor} = 18.16 min; $[\alpha]_D^{20}$ = 284.1 (*c* = 0.20 in CH_2Cl_2); m.p. 123-126℃.

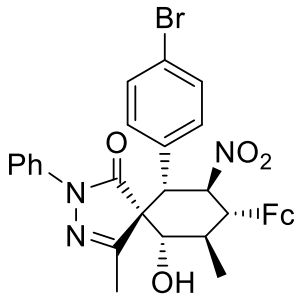
NMR and HRMS data for the product **5e**:

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 7.68 - 7.64 (m, 2H), 7.43 - 7.28 (m, 4H), 7.22 - 7.17 (m, 1H), 7.08 - 6.95 (m, 2H), 5.77 (t, *J* = 11.2 Hz, 1H), 4.24 - 4.21 (m, 2H), 4.13 - 4.11 (m, 6H), 4.09 - 4.07 (m, 1H), 3.76 (dd, *J* = 10.4, 6.4 Hz, 1H), 3.45 (d, *J* = 11.6 Hz, 1H), 2.95 (t, *J* = 10.8 Hz, 1H), 2.68 - 2.61 (m, 1H), 2.13 (s, 3H), 1.74 (d, *J* = 6.4 Hz, 1H), 1.29 (d, *J* = 6.4 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 171.8 , 159.2 , 137.0 , 135.2 , 132.3 , 128.8 , 128.7 , 125.9 , 119.9 , 88.6 , 86.8 , 75.6 , 69.6 , 69.0 , 67.7 , 67.1 , 65.9 , 63.5 , 49.9 , 45.0 , 37.1 , 17.0 , 13.4 .

HRMS (ESI): *m/z* calculated for $C_{32}H_{30}BrFeN_3O_4Na^+$: 678.0661, found 678.0674.

(*5S,6S,7R,8R,9R,10S*)-1,7-dimethyl-3-phenyl-10-(4-bromophenyl)-8-ferrocenyl-9-nitro-6-hydroxyl-2,3-diazaspiro[4.5]dec-1-ene-4-one



5f

Prepared according to the general procedure using (*E*)-2-(2-nitrovinyl)ferrocene **3p** (102.83 mg, 0.40 mmol, 1.0 equiv), propionaldehyde **2a** (46.46 mg, 0.8 mmol, 2 equiv) and (*Z*)-4-(4-bromobenzylidene)-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one **1k** (102.06 mg, 0.30 mmol). Purification of the crude product via column chromatography delivered **5f** as a yellow solid with 59% yield (119.3 mg). The diastereomeric ratio was determined to be 92:8 by crude ^{1}H-NMR analysis, and the enantiomeric excess of the major product was determined to be 98% by chiral HPLC analysis on Chiralpak AD-H column (10% 2-propanol/*n*-hexane, 1 mL/min, UV 254 nm), t_{major} = 16.52 min, t_{minor} = 11.56 min; $[\alpha]_D^{20}$ = 312.7 (*c* = 0.24 in CH_2Cl_2); m.p. 123-125℃.

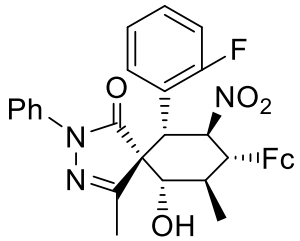
NMR and HRMS data for the product **5f**:

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 7.70 - 7.66 (m, 2H), 7.40 - 7.35 (m, 2H), 7.34 - 7.29 (m, 2H), 7.22 - 7.17 (m, 1H), 7.16 - 6.91 (m, 2H), 5.76 (dd, *J* = 11.6, 10.8 Hz, 1H), 4.24 - 4.21 (m, 2H), 4.13 - 4.10 (m, 6H), 4.08 - 4.06 (m, 1H), 3.74 (dd, *J* = 10.4, 6.4 Hz, 1H), 3.45 (d, *J* = 12.0, H), 2.94 (t, *J* = 11.2 Hz, 1H), 2.66 - 2.58 (m, 1H), 2.10 (s, 3H), 1.81 (d, *J* = 6.4 Hz, 1H), 1.28 (d, *J* = 6.4 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 171.9 , 159.2 , 137.2 , 132.2 , 132.0 , 128.9 , 125.8 , 123.3 , 119.4 , 88.7 , 86.8 , 75.7 , 69.6 , 67.7 , 67.6 , 67.1 , 65.9 , 63.5 , 49.8 , 45.0 , 37.1 , 17.0 , 13.4 .

HRMS (ESI): *m/z* calculated for $C_{32}H_{30}BrN_3O_4Na^+$: 678.0661, found 678.0665.

(*5S,6S,7R,8R,9R,10S*)-1,7-dimethyl-3-phenyl-10-(2-fluorophenyl)-8-ferrocenyl-9-nitro-6-hydroxyl-2,3-diazaspiro[4.5]dec-1-ene-4-one



5g

Prepared according to the general procedure using (*E*)-2-(2-nitrovinyl)ferrocene **3p** (102.83 mg, 0.40 mmol, 1.0 equiv), propionaldehyde **2a** (46.46 mg, 0.8 mmol, 2 equiv) and (*Z*)-4-(2-fluorobenzylidene)-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one **1l** (84.03 mg, 0.30 mmol). Purification of the crude product via column chromatography delivered **5g** as a yellow solid with 55% yield (102.4 mg). The diastereomeric ratio was determined to be 93:7 by crude ^{1}H-NMR analysis, and the enantiomeric excess of the major product was determined to be 99% by chiral HPLC analysis on Chiralpak IC-H column (10% 2-propanol/*n*-hexane, 1 mL/min, UV 254 nm), t_{major} =8.89 min, t_{minor} = 20.22 min; $[\alpha]_D^{20}$ = 313.0 (*c* = 0.21 in CH_2Cl_2); m.p. 125-129℃.

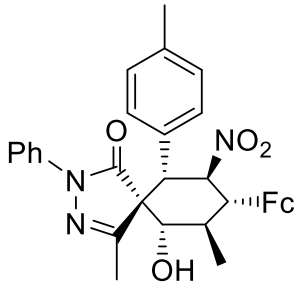
NMR and HRMS data for the product **5g**:

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 7.68 - 7.62 (m, 2H), 7.40 - 7.32 (m, 3H), 7.21 - 7.14 (m, 2H), 7.02 - 6.91 (m, 2H), 5.79 (t, *J* = 11.2 Hz, 1H), 4.24 - 4.20 (m, 2H), 4.17 (d, *J* = 11.6 Hz, 1H), 4.14 - 4.10 (m, 6H), 4.09 - 4.08 (m, 1H), 3.82 (dd, *J* = 10.4, 6.8 Hz, 1H), 3.01 (t, *J* = 11.2 Hz, 1H), 2.70 - 2.59 (m, 1H), 2.17 (s, 3H), 1.80 (d, *J* = 6.4 Hz, 1H), 1.30 (d, *J* = 6.4 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 172.0 , 160.0 (d, J_{CF}= 245.0 Hz), 159.8 , 137.2 , 130.5 (d, J_{CF}= 8.7 Hz), 128.8 , 128.2 (d, J_{CF}= 2.2 Hz), 125.7 , 124.7 (d, J_{CF}= 3.5 Hz), 120.1 (d, J_{CF}= 13.3 Hz), 119.5 , 115.9 (d, J_{CF}= 23.3 Hz), 88.2 , 86.8 , 75.7 , 69.6 , 67.7 , 67.6 , 67.1 , 65.9 , 63.6 , 45.0 , 40.2 (d, J_{CF}= 4.1 Hz), 37.1, 17.0 , 12.8 (d, J_{CF}= 5.6 Hz).

HRMS (ESI): *m/z* calculated for $C_{32}H_{30}FFeN_3O_4Na^+$: 618.1462, found 618.1468.

(*5S,6S,7R,8R,9R,10S*)-1,7-dimethyl-3-phenyl-10-(p-tolyl)-8-ferrocenyl-9-nitro-6-hydroxyl-2,3-diazaspiro[4.5]dec-1-ene-4-one



5h

Prepared according to the general procedure using (*E*)-2-(2-nitrovinyl)ferrocene **3p** (102.83 mg, 0.40 mmol, 1.0 equiv), propionaldehyde **2a** (46.46 mg, 0.8 mmol, 2 equiv) and (*Z*)-4-(4-methylbenzylidene)-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one **1m** (82.90 mg, 0.30 mmol). Purification of the crude product via column chromatography delivered **5h** as a yellow solid with 62% yield (110.3 mg). The diastereomeric ratio was determined to be >20:1 by crude ^{1}H-NMR analysis, and the enantiomeric excess of the major product was determined to be 96% by chiral HPLC analysis on Chiralpak AD-H column (10% 2-propanol/*n*-hexane, 1 mL/min, UV 254 nm), t_{major} = 21.15 min, t_{minor} = 14.92 min; $[\alpha]_D^{20}$ = 343.9 (*c* = 0.21 in CH_2Cl_2); m.p. 119-122℃.

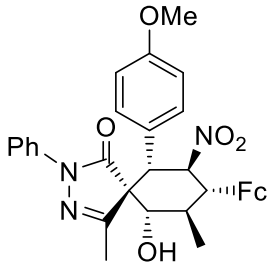
NMR and HRMS data for the product **5h**:

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 7.73 - 7.66 (m, 2H), 7.40 - 7.33 (m, 2H), 7.22 - 6.91 (m, 5H), 5.79 (t, *J* = 11.6, 10.8 Hz, 1H), 4.24 - 4.19 (m, 2H), 4.14 - 4.11 (m, 6H), 4.08 (dd, *J* = 3.6, 1.6 Hz, 1H), 3.76 (dd, *J* = 10.4, 6.8 Hz, 1H), 3.44 (d, *J* = 11.6 Hz, 1H), 2.95 (t, *J* = 10.8 Hz, 1H), 2.68 - 2.58 (m, 1H), 2.21 (s, 3H), 2.11 (s, 3H), 1.71 (d, *J* = 6.4 Hz, 1H), 1.27 (d, *J* = 6.4 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 172.2 , 159.6 , 138.8 , 137.3 , 129.8 , 129.7 , 128.8 , 125.6 , 119.5 , 89.0 , 87.2 , 75.8 , 69.6 , 67.8 , 67.6 , 67.0 , 66.0 , 63.8 , 50.2 , 45.0 , 37.2 , 21.1 , 17.1 , 13.4 .

HRMS (ESI): *m/z* calculated for $C_{33}H_{34}FeN_3O_4^+$: 592.1893, found 592.1840.

(*5S,6S,7R,8R,9R,10S*)-1,7-dimethyl-3-phenyl-10-(4-methoxyphenyl)-8-ferrocenyl-9-nitro-6-hydroxyl-2,3-diazaspiro[4.5]dec-1-ene-4-one



5i

Prepared according to the general procedure using (*E*)-2-(2-nitrovinyl)ferrocene **3p** (102.83 mg, 0.40 mmol, 1.0 equiv), propionaldehyde **2a** (46.46 mg, 0.8 mmol, 2 equiv) and (*Z*)-4-(4-methoxybenzylidene)-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one **1n** (87.70 mg, 0.30 mmol). Purification of the crude product via column chromatography delivered **5i** as a yellow solid with 66% yield (119.6 mg). The diastereomeric ratio was determined to be >20:1 by crude ^{1}H-NMR analysis, and the enantiomeric excess of the major product was determined to be 98% by chiral HPLC analysis on Chiralpak AD-H column (10% 2-propanol/*n*-hexane, 1 mL/min, UV 254 nm), t_{major} = 22.19 min, t_{minor} = 17.54 min; $[\alpha]_D^{20}$ = 342.4 (*c* = 0.22 in CH_2Cl_2); m.p. 130-133℃.

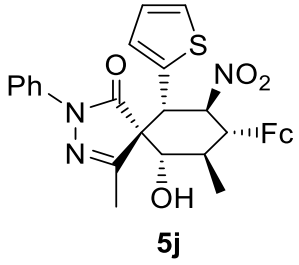
NMR and HRMS data for the product **5i**:

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 7.73 - 7.67 (m, 2H), 7.36 (tt, *J* = 7.6, 2.0 Hz, 2H), 7.21 - 6.94 (m, 3H), 6.72 - 6.67 (m, 2H), 5.76 (t, *J* = 11.2 Hz, 1H), 4.21(dd, *J* = 3.6, 1.6 Hz , 2H), 4.12 - 4.07 (m, 7H), 3.75 (dd, *J* = 10.4, 6.0 Hz, 1H), 3.69 (s, 3H), 3.43 (d, *J* = 11.6 Hz, 1H), 2.95 (t, *J* = 10.8 Hz, 1H), 2.66 - 2.59 (m, 1H), 1.72 (d, *J* = 6.4 Hz, 1H), 1.27 (d, *J* = 6.4 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 172.2 , 159.8 , 159.6 , 137.3 , 128.8 , 125.6 , 124.8 , 119.5 , 89.1 , 87.1 , 75.8 , 69.6 , 67.7 , 67.6 , 67.0 , 65.9 , 63.9 , 55.1 , 49.8 , 45.0 , 37.2 , 17.1 , 13.4 .

HRMS (ESI): *m/z* calculated for $C_{33}H_{34}FeN_3O_5^+$: 608.1842, found 608.1834.

(*5S,6S,7R,8R,9R,10S*)-1,7-dimethyl-3-phenyl-10-thienyl-8-ferrocenyl-9-nitro-6-hydroxyl-2,3-diazaspiro[4.5]dec-1-ene-4-one



5j

Prepared according to the general procedure using (*E*)-2-(2-nitrovinyl)ferrocene **3p** (102.83 mg, 0.40 mmol, 1.0 equiv), propionaldehyde **2a** (46.46 mg, 0.8 mmol, 2 equiv) and (*Z*)-4-thienylidene-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one **1o** (80.50 mg, 0.30 mmol). Purification of the crude product via column chromatography delivered **5j** as a yellow solid with 51% yield (88.7 mg). The diastereomeric ratio was determined to be >20:1 by crude ^{1}H-NMR analysis, and the enantiomeric excess of the major product was determined to be >99% by chiral HPLC analysis on Chiralpak AD-H column (10% 2-propanol/*n*-hexane, 1 mL/min, UV 254 nm), t_{major} =22.80 min, t_{minor} = 25.66 min; $[\alpha]_D^{20}$ = 314.1 (*c* = 0.25 in CH_2Cl_2); m.p. 145-145℃.

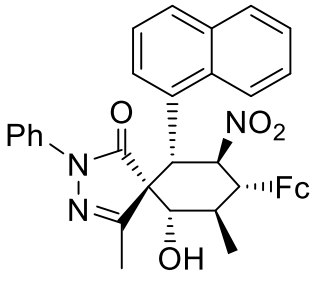
NMR and HRMS data for the product **5j**:

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 7.80 - 7.74 (m, 2H), 7.41 - 7.35 (m, 2H), 7.22 - 7.17 (m, 1H), 7.15 - 7.12 (m, 1H), 6.88 (dd, *J* = 3.6, 1.2 Hz, 1H), 6.82 (dd, *J* = 5.2, 3.6 Hz, 1H), 5.74 (dd, *J* = 11.6 , 10.8, 1H), 4.23-4.21 (m, 2H), 4.14 - 4.11 (m, 6H), 4.06 (dd, *J* = 3.6, 1.6 Hz, 1H), 3.83 (d, *J* = 11.6 Hz, 1H), 3.74 (dd, *J* = 10.4, 6.4 Hz, 1H), 2.94 (t, *J* = 10.8 Hz, 1H), 2.64 - 2.56 (m, 1H), 2.15 (s, 3H), 1.77 (d, *J* = 6.4 Hz, 1H), 1.27 (d, *J* = 6.4 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 171.6 , 159.2 , 137.4 , 134.5 , 128.8 , 127.2 , 127.0 , 126.1 , 125.6 , 119.4 , 90.2 , 86.9 , 75.6 , 69.6 , 67.8 , 67.6 , 67.1 , 66.0 , 64.0 , 45.3 , 44.9 , 37.0 , 17.0 , 13.3 .

HRMS (ESI): *m/z* calculated for $C_{30}H_{30}FeN_3O_4S^+$:584.1301, found 584.1290.

(*5S,6S,7R,8R,9R,10S*)-1,7-dimethyl-3-phenyl-10-naphthyl-8-ferrocenyl-9-nitro-6-hydroxyl-2,3-diazaspiro[4.5]dec-1-ene-4-one



5k

Prepared according to the general procedure using (*E*)-2-(2-nitrovinyl)ferrocene **3p** (102.83 mg, 0.40 mmol, 1.0 equiv), propionaldehyde **2a** (46.46 mg, 0.8 mmol, 2 equiv) and (*Z*)-4-naphthylidene-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one **1p** (93.71 mg, 0.30 mmol). Purification of the crude

product via column chromatography delivered **5k** as a yellow solid with 49% yield (96.5 mg). The diastereomeric ratio was determined to be >20:1 by crude ^{1}H-NMR analysis, and the enantiomeric excess of the major product was determined to be >99% by chiral HPLC analysis on Chiralpak AD-H column (10% 2-propanol/*n*-hexane, 1 mL/min, UV 254 nm), t_{major} = 34.61 min, t_{minor} =18.09 min; $[\alpha]_D^{20}$ = 444.1 (*c* = 0.22 in CH_2Cl_2); decomp. >197℃.

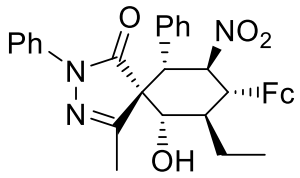
NMR and HRMS data for the product **5k**:

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 8.13 (d, *J* = 8.4 Hz, 1H), 7.80 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.71 - 7.67 (m, 3H), 7.63 (dd, *J* = 7.6, 1.2 Hz, 1H), 7.59 - 7.55 (m, 1H), 7.49 - 7.45 (m, 1H), 7.40 - 7.35 (m, 2H), 7.24 - 7.18 (m, 2H), 5.97 (dd, *J* =11.6 ,10.4 Hz, 1H), 4.64 (d, *J* = 11.6 Hz, 1H), 4.24 - 4.21 (m, 2H), 4.15 - 4.14 (m, 1H), 4.12 - 4.10 (m, 6H), 3.92 (dd, *J* = 10.4, 6.4 Hz, 1H), 3.14 (t, *J* = 10.8 Hz, 1H), 2.80 - 2.70 (m, 1H), 1.79 (d, *J* = 6.4 Hz, 1H), 1.35 (d, *J* = 6.8 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 172.6 , 159.6 , 137.3 , 134.1 , 131.2 , 129.5 , 129.4 , 128.8 , 126.7 , 125.8 , 125.7 , 125.3 , 125.2 , 121.8 , 119.5 , 89.3 , 87.0 , 76.3 , 69.6 , 67.7 , 67.6 , 67.1 , 66.0 , 64.2 , 45.6 , 42.5 , 37.4 , 17.2 , 13.6 .

HRMS (ESI): *m/z* calculated for $C_{36}H_{33}FeN_3O_4Na^+$: 650.1713, found 650.1716.

(*5S,6S,7R,8R,9R,10S*)-1-methyl-7-ethyl-3,10-diphenyl-8-ferrocenyl-9-nitro-6-hydroxyl-2,3-diazaspiro[4.5]dec-1-ene-4-one



5l

Prepared according to the general procedure using (*E*)-2-(2-nitrovinyl)ferrocene **3p** (102.83 mg, 0.40 mmol, 1.0 equiv), propionaldehyde **2b** (57.69 mg, 0.8 mmol, 2 equiv) and (*Z*)-4-benzylidene-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one **1f** (78.69 mg, 0.30 mmol). Purification of the crude product via column chromatography delivered **5l** as a yellow solid with 59% yield (108.1 mg). The diastereomeric ratio was determined to be 89:11 by crude ^{1}H-NMR analysis, and the enantiomeric excess of the major product was determined to be 92% by chiral HPLC analysis on Chiralpak AD-H

column (10% 2-propanol/*n*-hexane, 1 mL/min, UV 254 nm), t_{major} =14.46 min, t_{minor} = 7.85 min; $[\alpha]_D^{20}$ = 386.7 (*c* = 0.21 in CH_2Cl_2); m.p. 128-132℃.

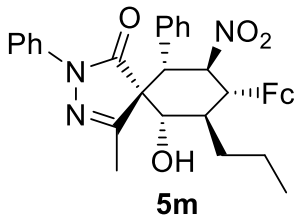
NMR and HRMS data for the product **5l**:

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 7.69 - 7.66 (m, 2H), 7.38 - 7.34 (m, 2H), 7.24 - 7.08 (m, 6H), 5.84 (dd, *J* = 11.6, 10.4 Hz, 1H), 4.24 - 4.22 (m, 2H), 4.16 (dd, *J* = 3.6, 1.6 Hz, 1H), 4.11 (s, 5H), 4.07 - 4.06 (m, 1H), 3.99 (dd, *J* = 10.8, 6.4 Hz, 1H), 3.41 (d, *J* = 11.6 Hz, 1H), 3.15 (t, *J* = 11.2 Hz, 1H), 2.61 (tt, *J* = 11.2, 3.6 Hz, 1H), 2.11 (s, 3H), 1.2 - 1.70 (m, 2H), 1.68 (d, *J* = 6.4 Hz, 1H), 0.96 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 172.2 , 159.6 , 137.3 , 133.0 , 129.0 , 129.0 , 128.8 , 125.6 , 119.6 , 88.8 , 87.4 , 72.2 , 69.6 , 69.5 , 67.6 , 67.3 , 67.0 , 65.8 , 63.6 , 50.6 , 41.2 , 40.8 , 19.5 , 13.5 , 8.0 .

HRMS (ESI): *m/z* calculated for $C_{33}H_{33}FeN_3O_4Na^+$: 614.1713, found 614.1713.

(*5S,6S,7R,8R,9R,10S*)-1-methyl-7-propyl-3,10-diphenyl-8-ferrocenyl-9-nitro-6-hydroxyl-2,3-diazaspiro[4.5]dec-1-ene-4-one



5m

Prepared according to the general procedure using (*E*)-2-(2-nitrovinyl)ferrocene **3p** (102.83 mg, 0.40 mmol, 1.0 equiv), propionaldehyde **2c** (68.91 mg, 0.8 mmol, 2 equiv) and (*Z*)-4-benzylidene-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one **1f** (78.69 mg, 0.30 mmol). Purification of the crude product via column chromatography delivered **5m** as a yellow solid with 58% yield (108.3 mg). The diastereomeric ratio was determined to be 82:18 by crude ^{1}H-NMR analysis, and the enantiomeric excess of the major product was determined to be 92% by chiral HPLC analysis on Chiralpak AD-H column (10% 2-propanol/*n*-hexane, 1 mL/min, UV 254 nm), t_{major} = 10.66 min, t_{minor} = 7.27 min; $[\alpha]_D^{20}$ = 361.8 (*c* = 0.23 in CH_2Cl_2); m.p. 114-116℃.

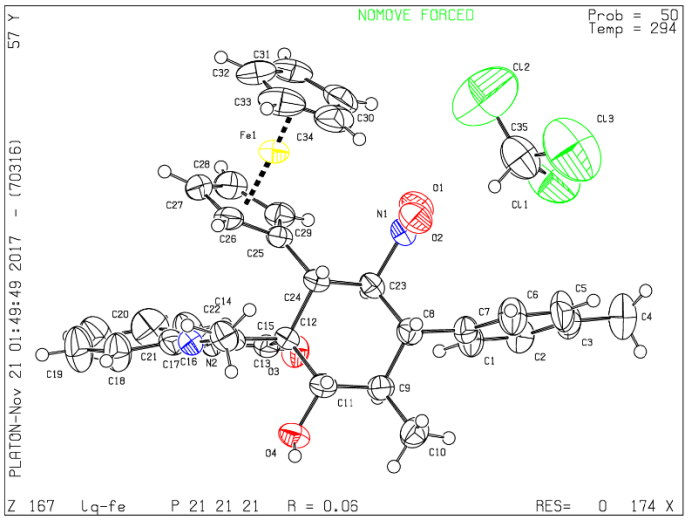
NMR and HRMS data for the product **5m**:

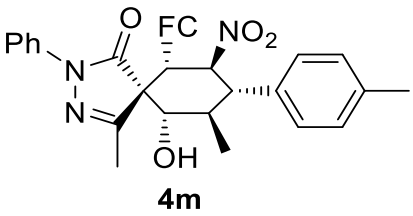
^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 7.71 - 7.66 (m, 2H), 7.39 - 7.34 (m, 2H), 7.25 - 7.07 (m, 6H), 5.91 (dd, *J* = 11.6, 10.8 Hz, 1H), 4.26 - 4.24 (m, 1H), 4.23 - 4.21 (m, 1H), 4.21 - 4.19 (m, 1H), 4.10 (s, 5H), 4.03 - 4.02 (m, 1H), 3.96 (dd, *J* = 10.8, 6.4 Hz, 1H), 3.42 (d, *J* = 11.6 Hz, 1H), 3.14 (t, *J* = 11.2 Hz, 1H), 2.53 (tt, *J* = 11.2, 3.6 Hz, 1H), 2.11 (s, 3H), 1.58 (d, *J* = 6.8 Hz, 1H), 1.51 - 1.25 (m, 4H), 0.90 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 172.2 , 159.6 , 137.3 , 133.0 , 129.1 , 128.8 , 125.6 , 119.6 , 88.2 , 87.4 , 73.0 , 69.6 , 69.6 , 67.7 , 67.6 , 66.8 , 65.6 , 63.7 , 50.8 , 41.8 , 41.7 , 29.8 , 17.3 , 14.4 , 13.4 .

HRMS (ESI): *m/z* calculated for $C_{34}H_{35}FeN_3O_4Na^+$: 628.1869, found 628.1875.

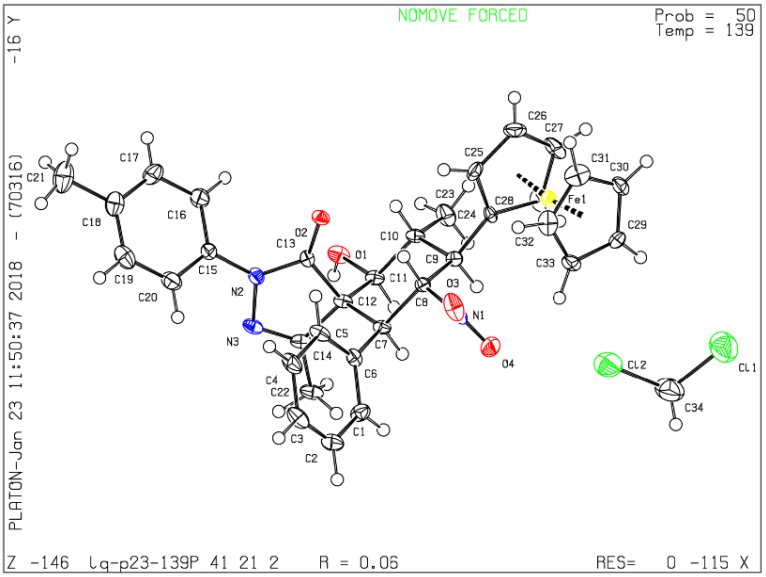
3. Crystal data of 4m and 5b

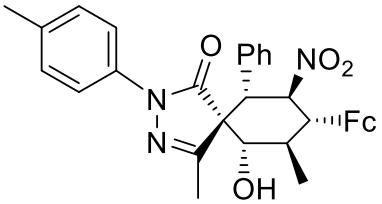




4m

Empirical formula	$C_{35}H_{34}Cl_3FeN_2O_4$
Formula weight	708.84
Temperature/K	294.24(10)
Crystal system	orthorhombic
Space group	$P2_12_12_1$
a/Å	9.54049(16)
b/Å	13.5186(2)
c/Å	26.0238(5)
α/°	90
β/°	90
γ/°	90
Volume/Å^3	3356.40(10)
Z	4
ρ_{calc}g/cm^3	1.403
μ/mm^{-1}	6.132
F(000)	1468.0
Crystal size/mm^3	0.65 × 0.3 × 0.25
Radiation	CuKα (λ = 1.54184)
2Θ range for data collection/°	9.434 to 145.112
Index ranges	-11 ≤ h ≤ 7, -16 ≤ k ≤ 14, -32 ≤ l ≤ 31
Reflections collected	18187
Independent reflections	6542 [R_{int} = 0.0428, R_{sigma} = 0.0457]
Data/restraints/parameters	6542/0/410
Goodness-of-fit on F^2	1.028
Final R indexes [I>=2σ (I)]	R_1 = 0.0626, wR_2 = 0.1645
Final R indexes [all data]	R_1 = 0.0776, wR_2 = 0.1788
Largest diff. peak/hole / e Å^{-3}	0.61/-0.72
Flack parameter	-0.001(4)

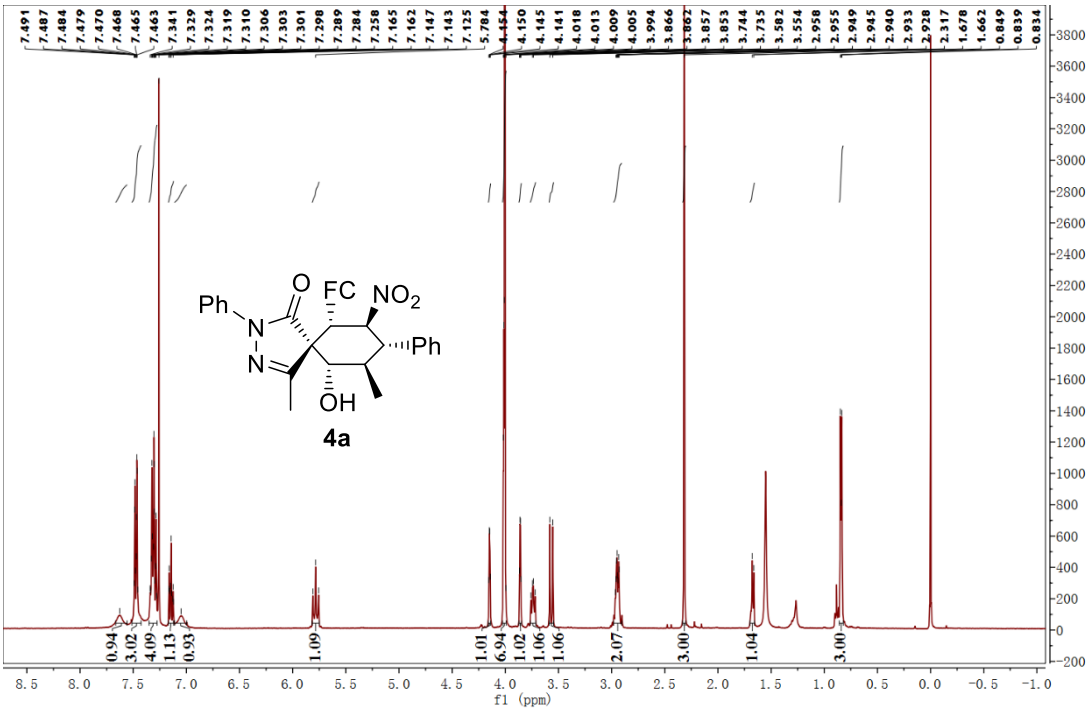


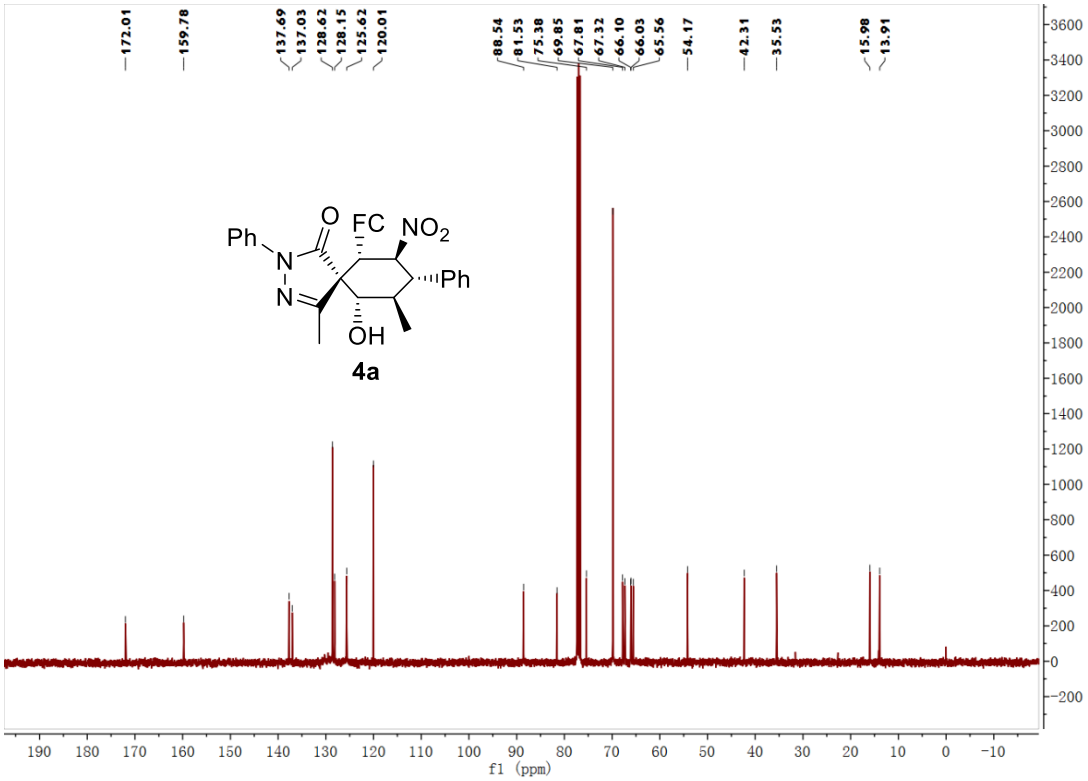


5b

Empirical formula	$C_{34}H_{35}Cl_2FeN_3O_4$
Formula weight	676.40
Temperature/K	139.05(10)
Crystal system	tetragonal
Space group	$P4_12_12$
a/Å	12.0535(3)
b/Å	12.0535(3)
c/Å	43.8649(13)
α/°	90
β/°	90
γ/°	90
Volume/Å^3	6373.0(3)
Z	8
ρ_{calc}g/cm^3	1.410
μ/mm^{-1}	5.686
F(000)	2816.0
Crystal size/mm^3	0.7 × 0.55 × 0.35
Radiation	CuKα (λ = 1.54184)
2Θ range for data collection/°	7.606 to 146.404
Index ranges	$-14 \leq h \leq 11$, $-13 \leq k \leq 14$, $-54 \leq l \leq 54$
Reflections collected	34873
Independent reflections	6315 [R_{int} = 0.0790, R_{sigma} = 0.0521]
Data/restraints/parameters	6315/0/401
Goodness-of-fit on F^2	1.070
Final R indexes [I>=2σ (I)]	R_1 = 0.0630, wR_2 = 0.1467
Final R indexes [all data]	R_1 = 0.0745, wR_2 = 0.1552
Largest diff. peak/hole / e Å^{-3}	0.67/-0.53
Flack parameter	-0.001(3)

4 NMR spectra and HPLC chromatograms





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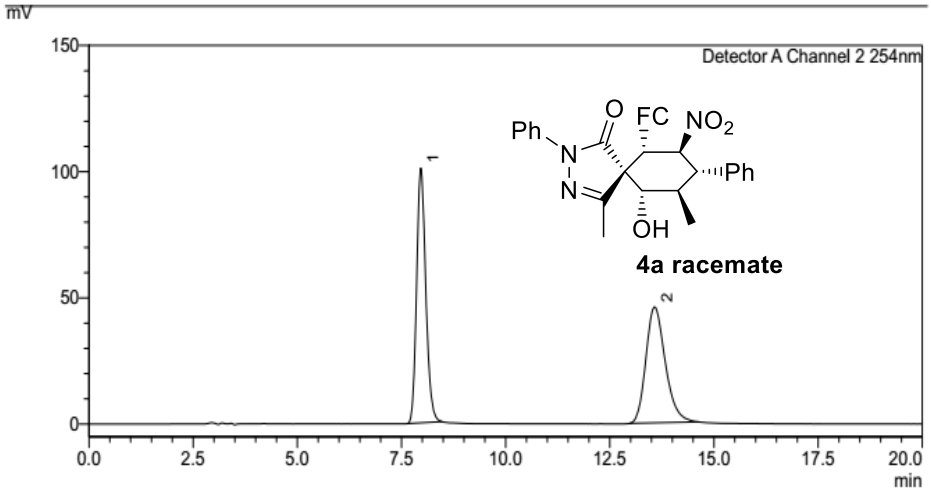

<Peak Table>

Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
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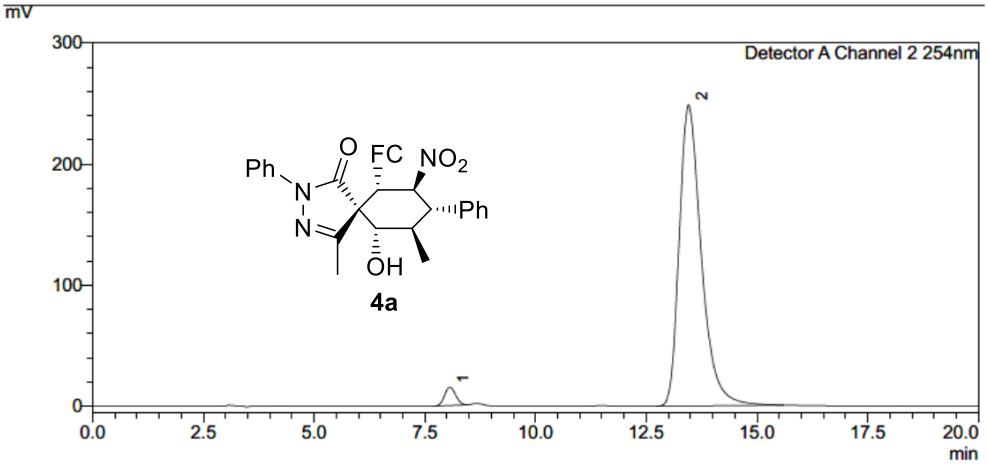
2	13.575	1484402	45867	49.049	49.049
Total		3026367	146772		100.000

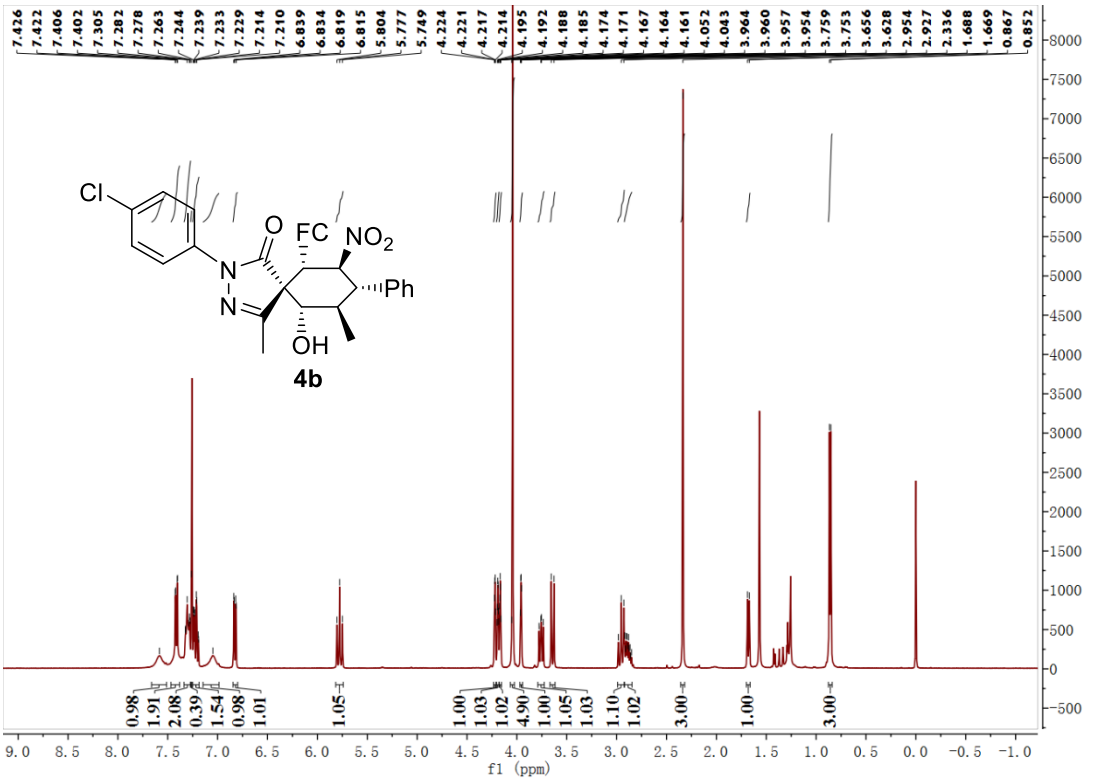
<Chromatogram>

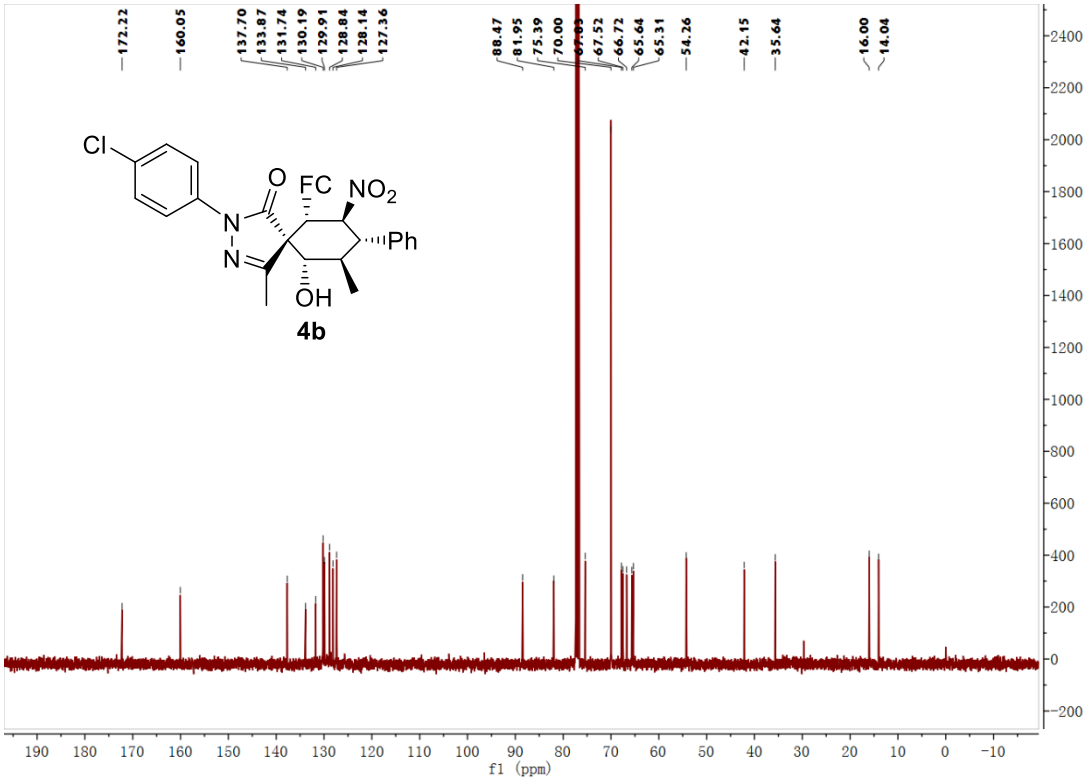


<Peak Table>

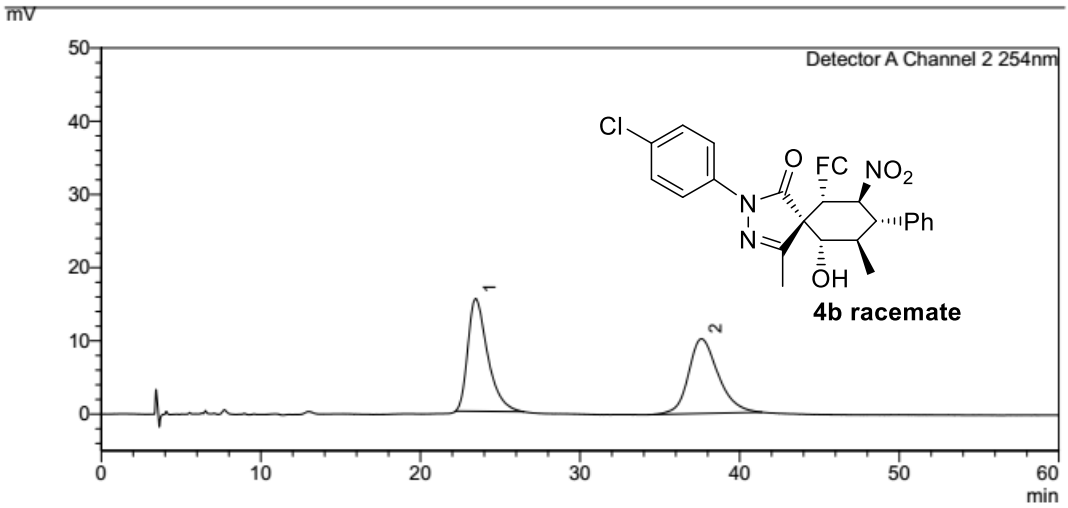
Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	8.083	261182	15161	2.945	2.945
2	13.453	8608073	248482	97.055	97.055
Total		8869254	263643		100.000


Cl
O
FC
NO2
N
N
Ph
OH
4b
f1 (ppm)


Cl
O
FC
NO2
N
N
Ph
OH
4b
f1 (ppm)

<Chromatogram>

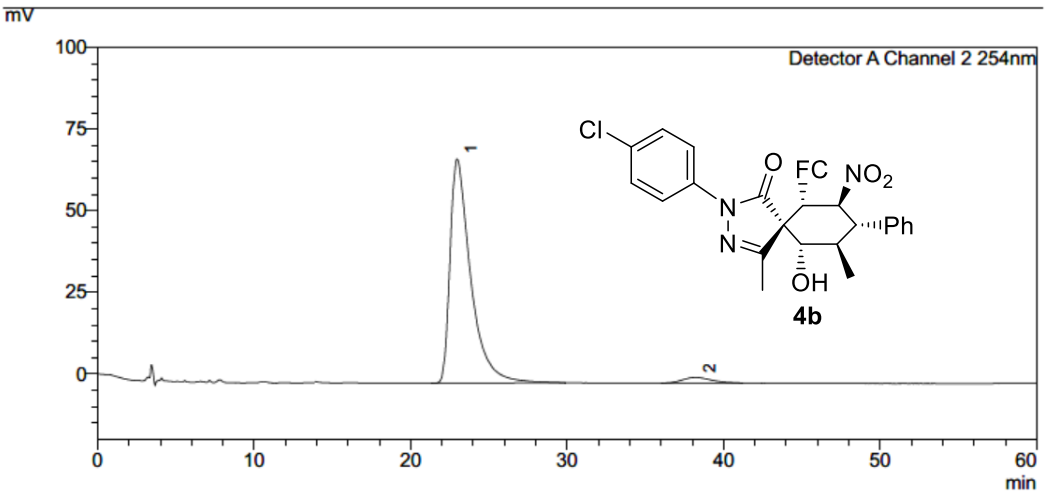


<Peak Table>

Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	23.461	1330575	15372	50.150	50.150
2	37.599	1322606	10203	49.850	49.850
Total		2653181	25575		100.000

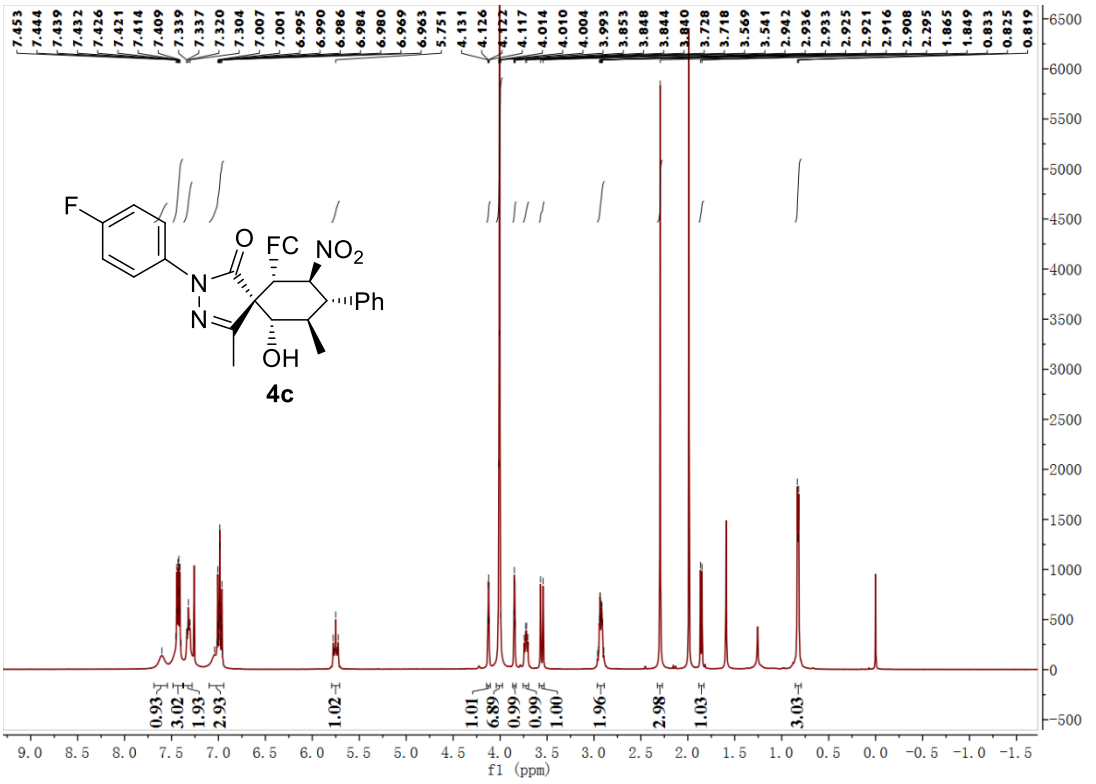
<Chromatogram>

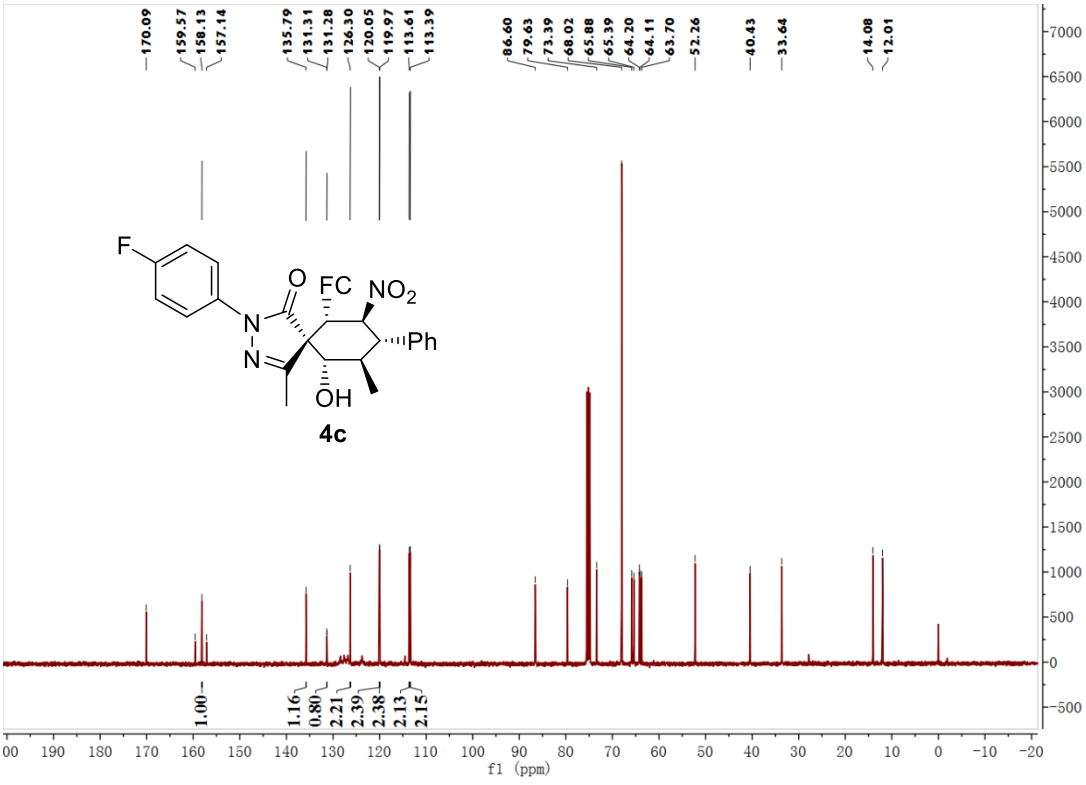


<Peak Table>

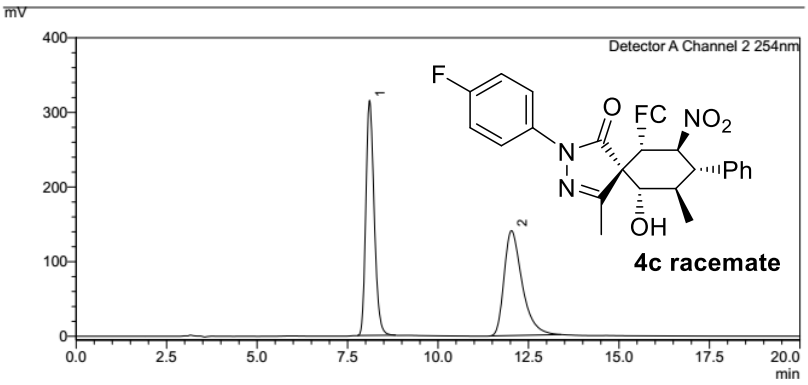
Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	22.968	6290616	68702	96.665	96.665
2	38.217	217008	1709	3.335	3.335
Total		6507625	70411		100.000


F
O
FC
NO2
N
N
Ph
OH
4c
f1 (ppm)


F
O
FC
NO2
N
N
Ph
OH
4c
f1 (ppm)

<Chromatogram>

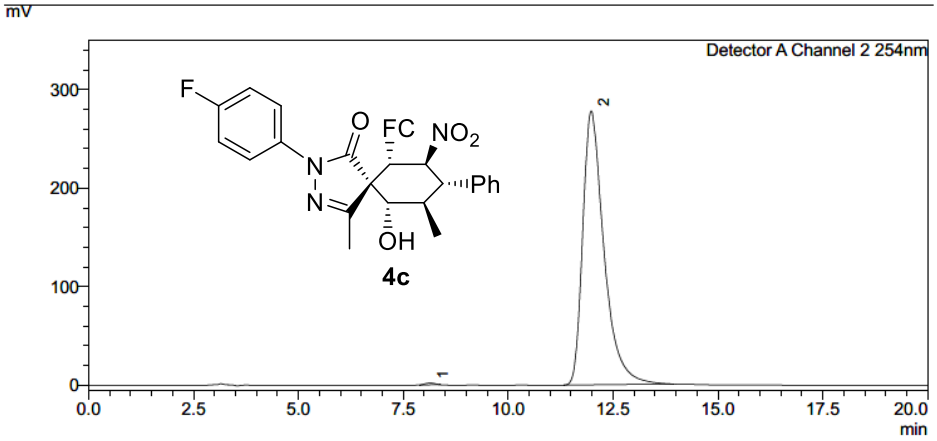


<Peak Table>

Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	8.109	5013190	314957	50.696	50.696
2	12.029	4875524	140589	49.304	49.304
Total		9888713	455546		100.000

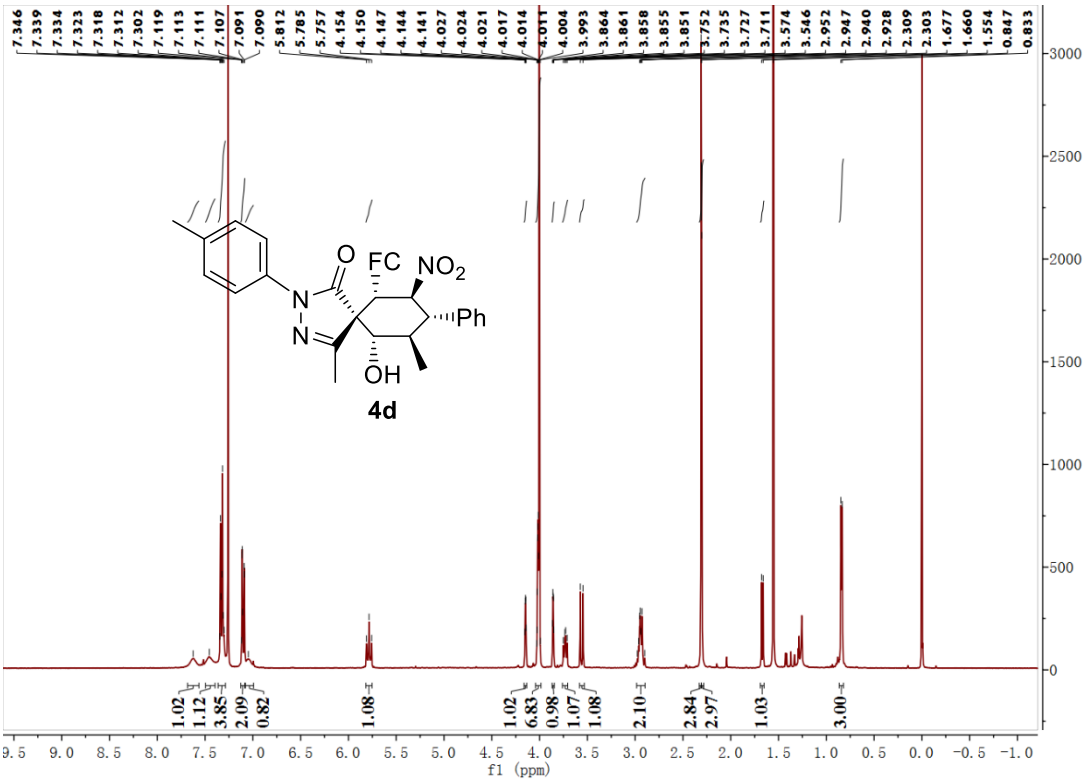
<Chromatogram>

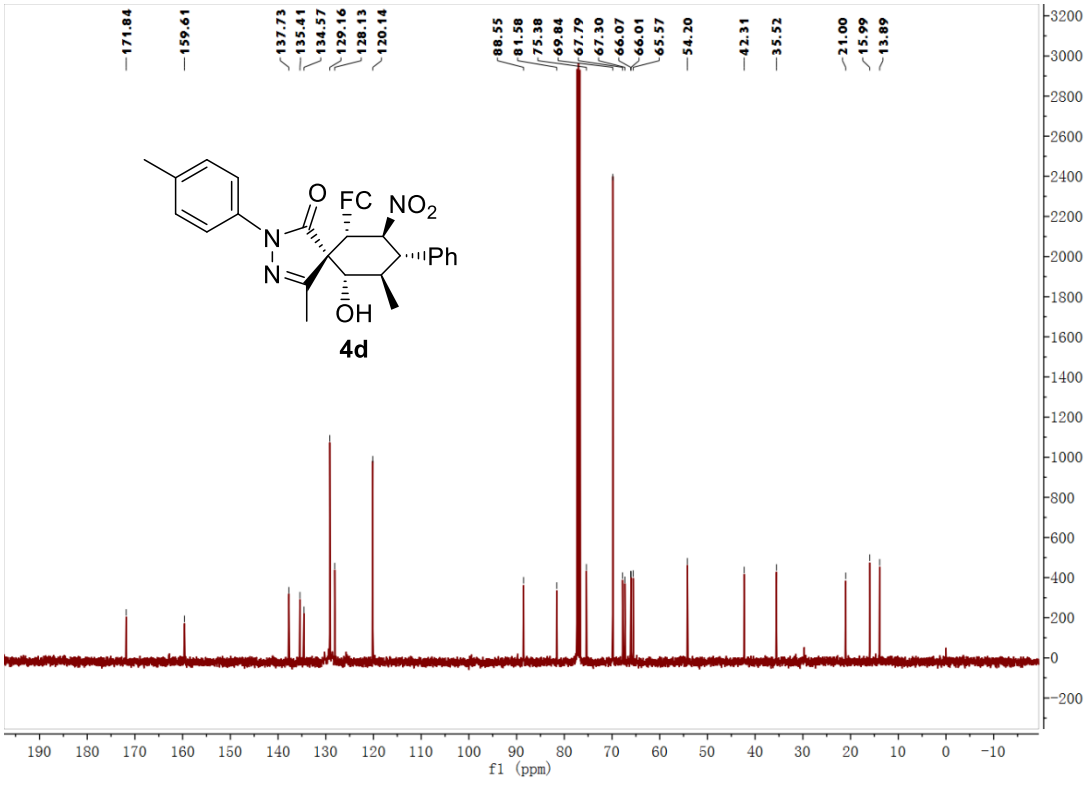


<Peak Table>

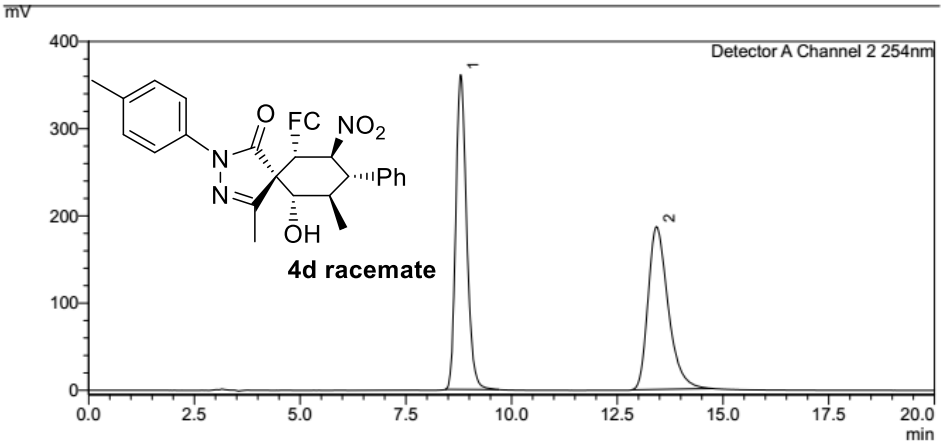
Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	8.155	29402	1907	0.299	0.299
2	11.979	9809138	278101	99.701	99.701
Total		9838539	280008		100.000


4d
f1 (ppm)


4d
f1 (ppm)

<Chromatogram>

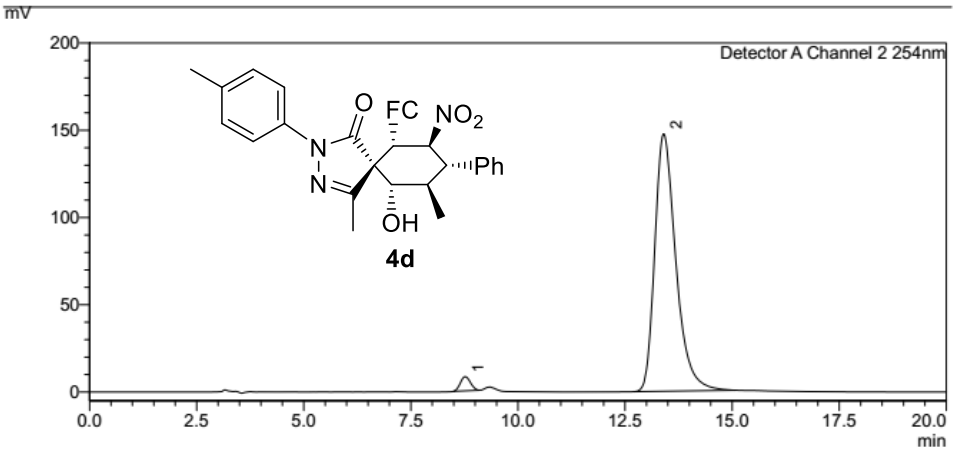


<Peak Table>

Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	8.797	6424165	360845	50.989	50.989
2	13.429	6174928	186589	49.011	49.011
Total		12599093	547435		100.000

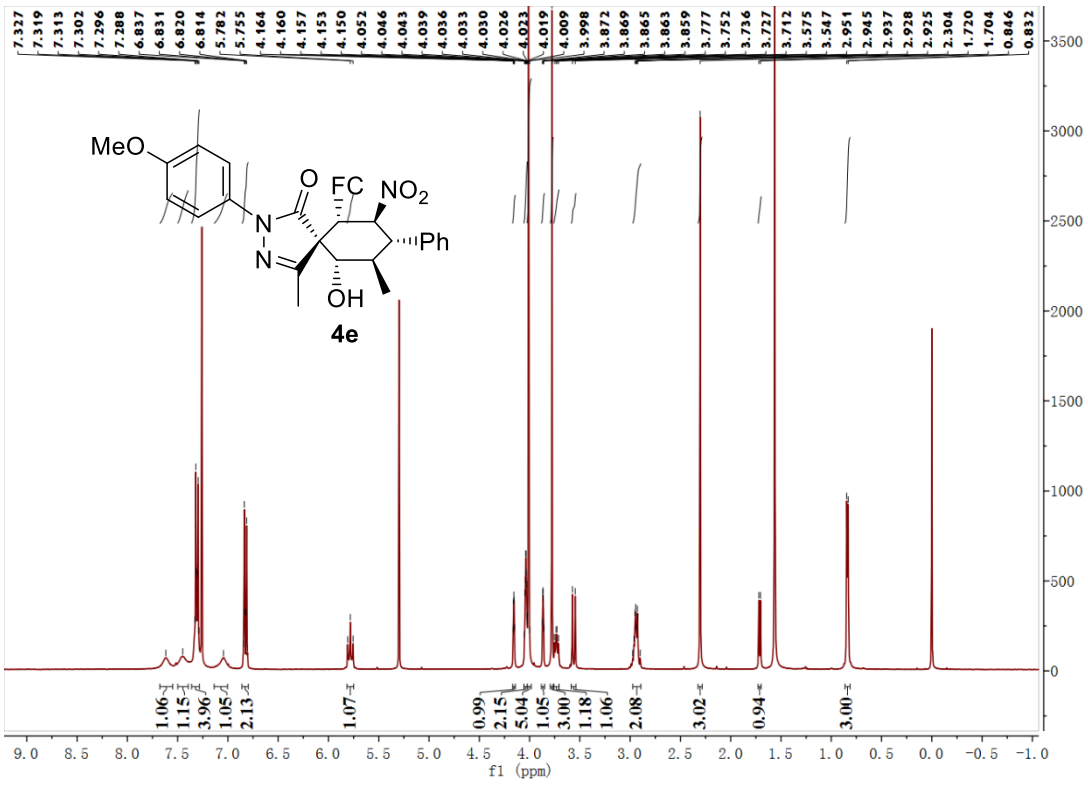
<Chromatogram>

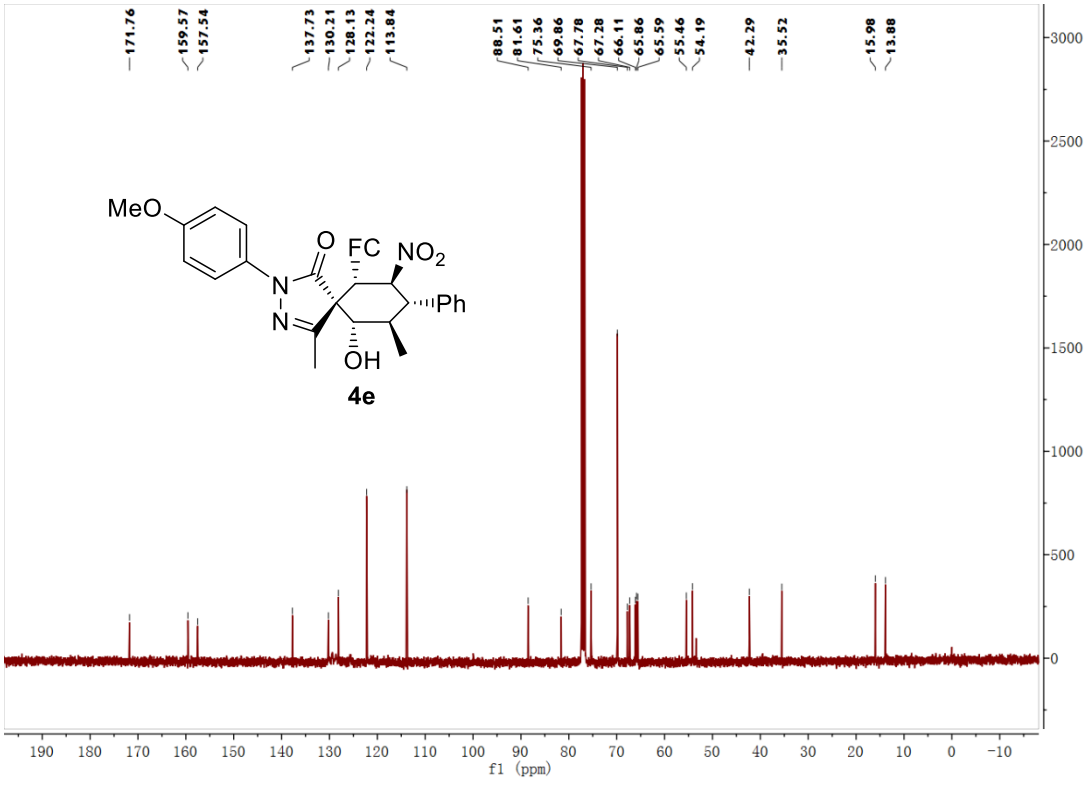


<Peak Table>

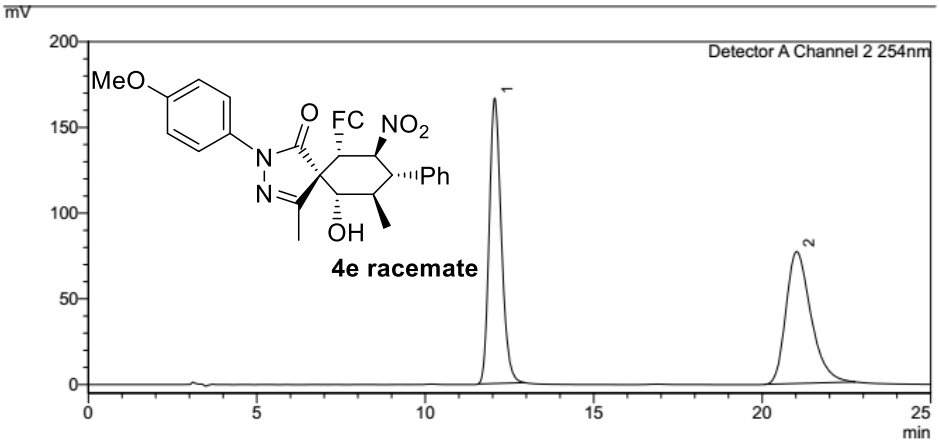
Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	8.768	132256	8137	2.555	2.555
2	13.402	5044208	147511	97.445	97.445
Total		5176464	155648		100.000


MeO
O
FC
NO2
N
N
Ph
OH
4e
f1 (ppm)


MeO
O
FC
NO2
N
N
Ph
OH
4e
f1 (ppm)

<Chromatogram>

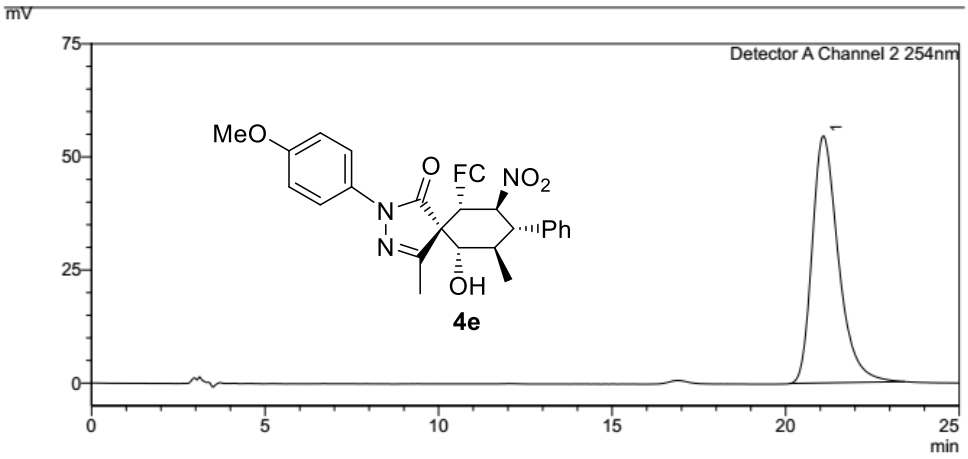


<Peak Table>

Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	12.062	4134118	166518	51.069	51.069
2	21.023	3961028	77018	48.931	48.931
Total		8095146	243536		100.000

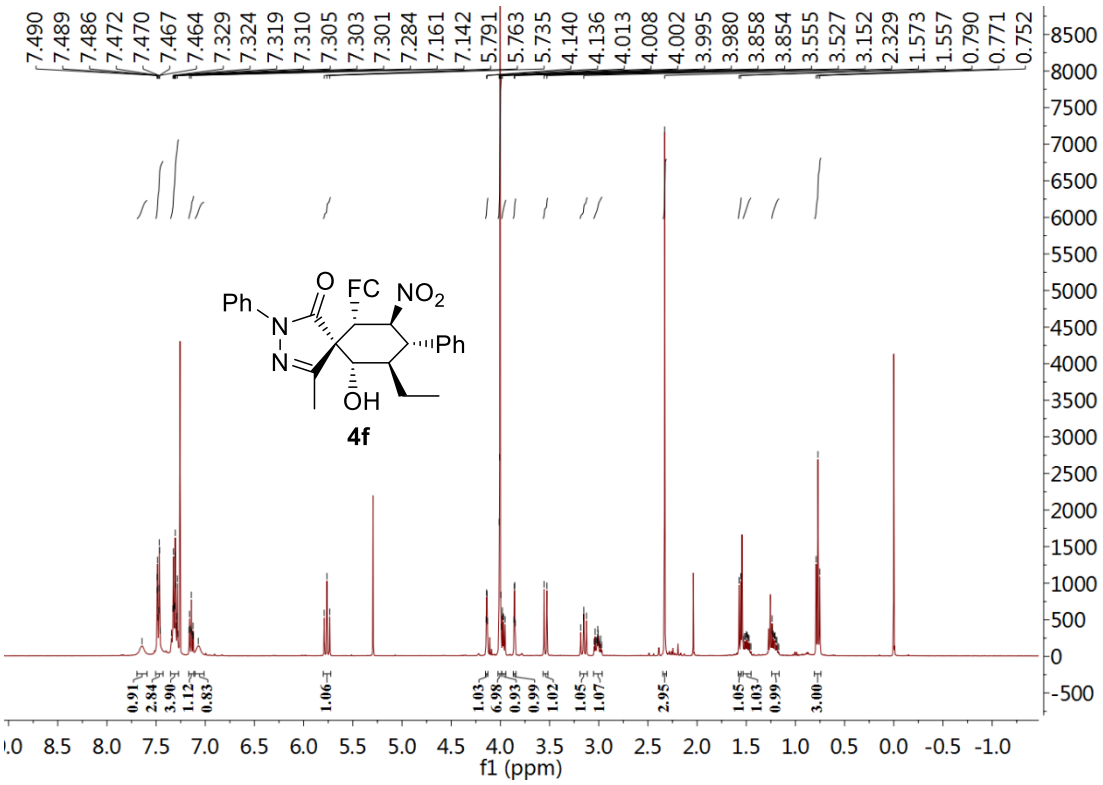
<Chromatogram>

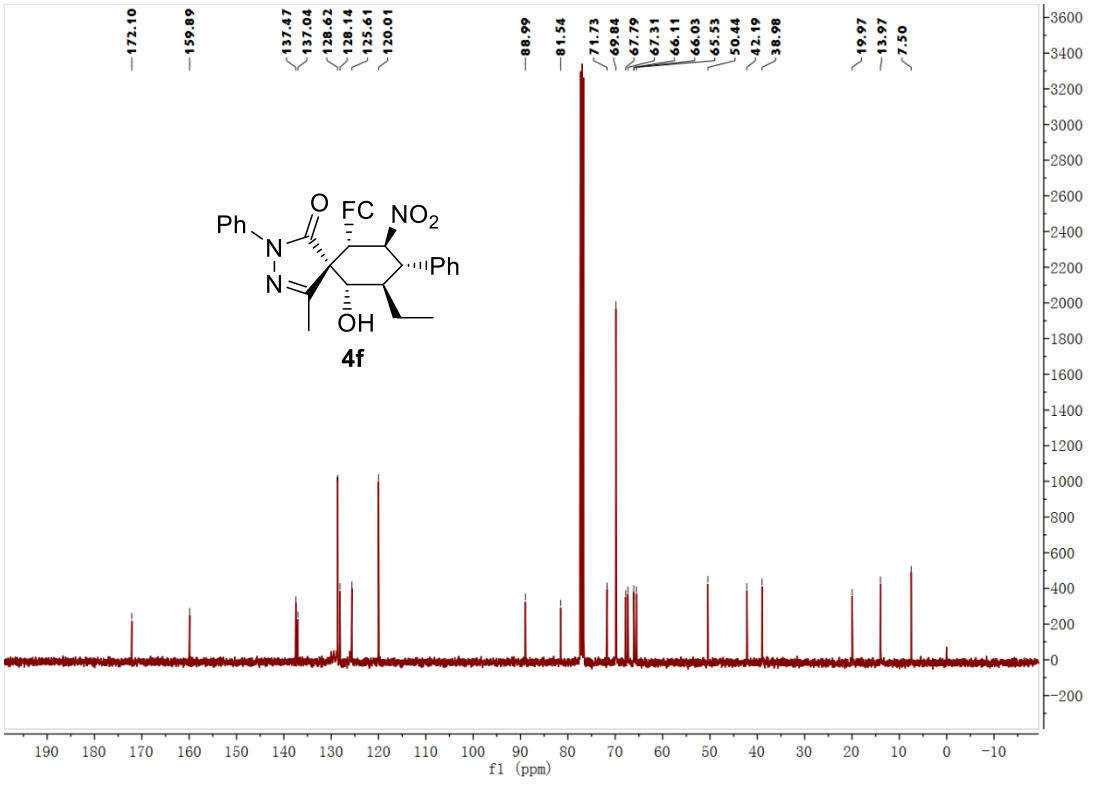


<Peak Table>

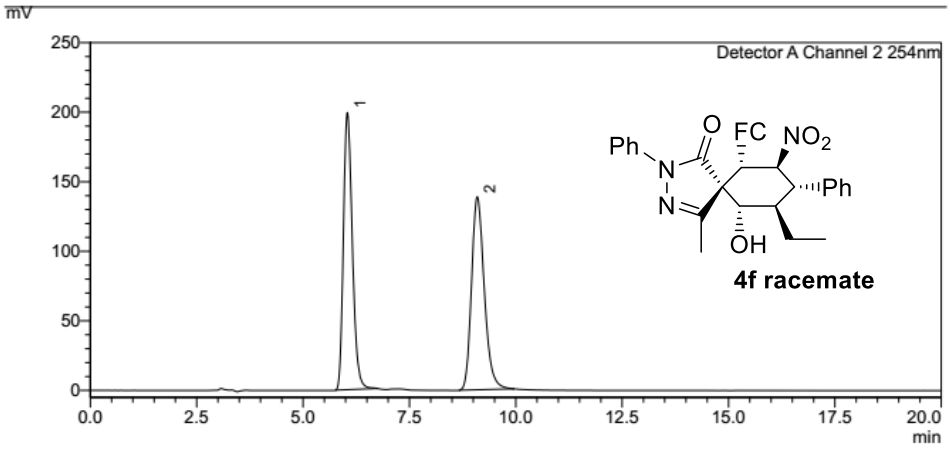
Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	21.088	2865704	54610	100.000	100.000
Total		2865704	54610		100.000


7.490 7.489 7.486 7.472 7.470 7.467 7.464 7.329 7.324 7.319 7.310 7.305 7.303 7.301 7.284 7.161 7.142 5.791 5.763 5.735 4.140 4.136 4.013 4.008 4.002 3.995 3.980 3.858 3.854 3.555 3.527 3.152 2.329 1.573 1.557 0.790 0.771 0.752
0.91 2.84 3.90 1.12 0.83 1.06 1.03 6.98 0.93 0.99 1.02 1.05 1.07 2.95 1.05 1.03 0.99 3.00
f1 (ppm)
Ph
N
N
O
FC
NO2
Ph
OH
4f


172.10 159.89 137.47 137.04 128.62 128.14 125.61 120.01 88.99 81.54 71.73 69.84 67.79 67.31 66.11 66.03 65.53 50.44 42.19 38.98 19.97 13.97 7.50
f1 (ppm)
Ph
N
N
O
FC
NO2
Ph
OH
4f

<Chromatogram>

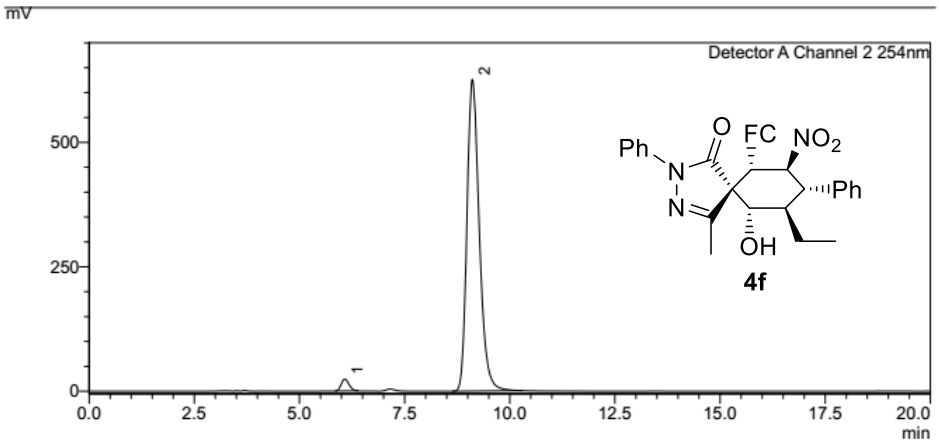


<Peak Table>

Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	6.042	2901768	199280	50.081	50.081
2	9.095	2892325	138731	49.919	49.919
Total		5794093	338011		100.000

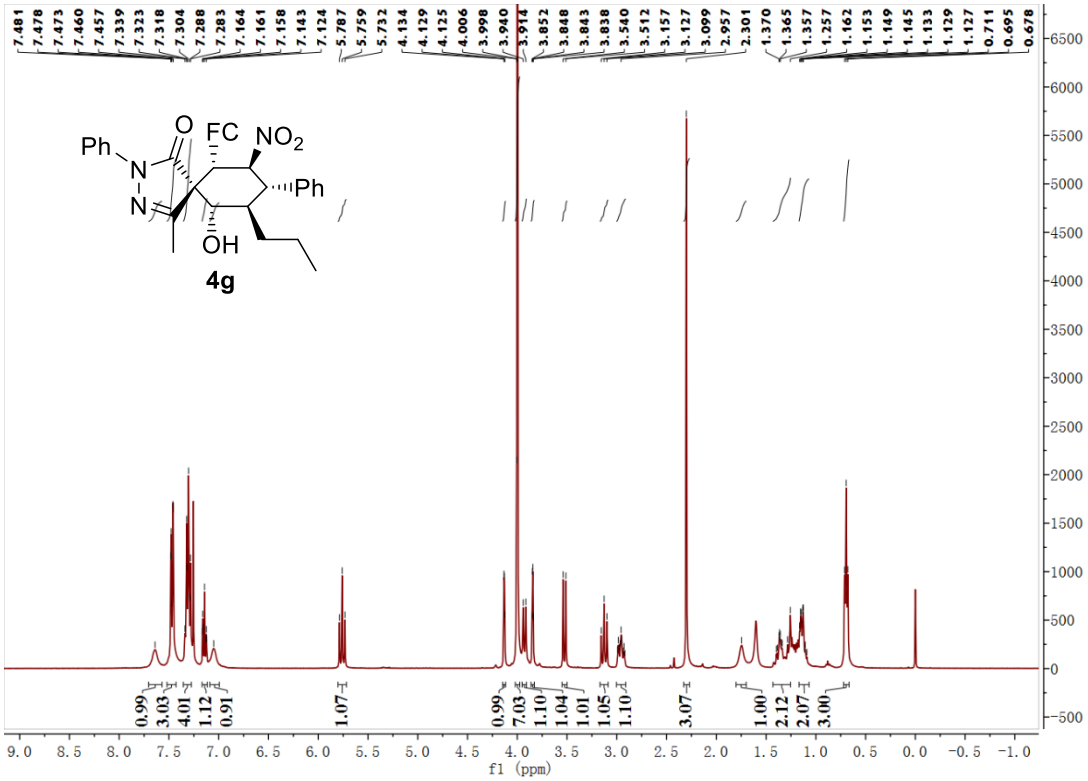
<Chromatogram>

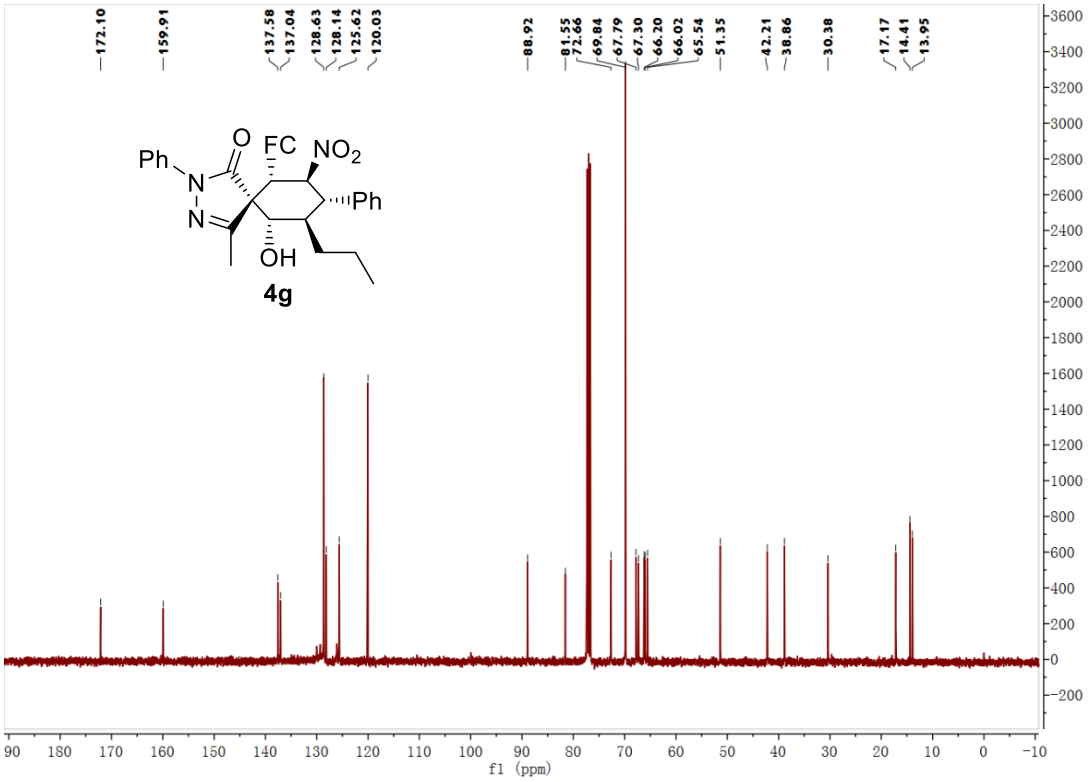


<Peak Table>

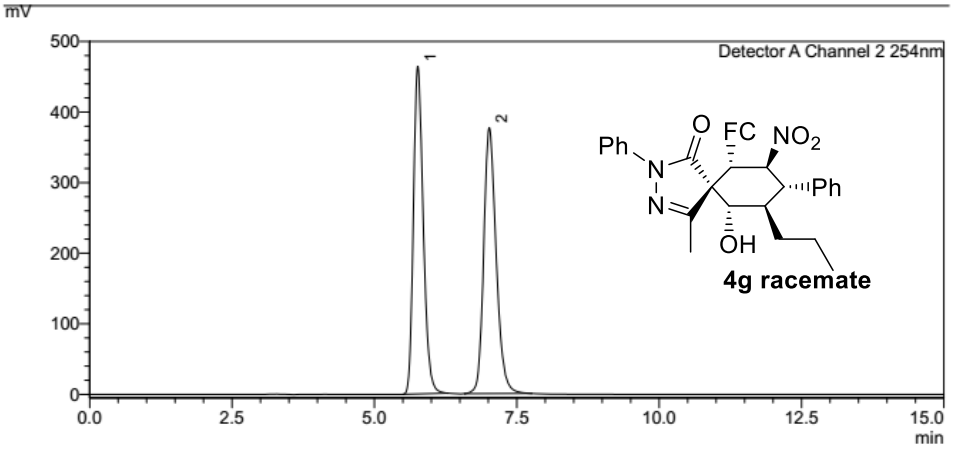
Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	6.079	305504	23709	2.338	2.338
2	9.111	12761424	626248	97.662	97.662
Total		13066927	649957		100.000


4g
f1 (ppm)


4g
f1 (ppm)

<Chromatogram>

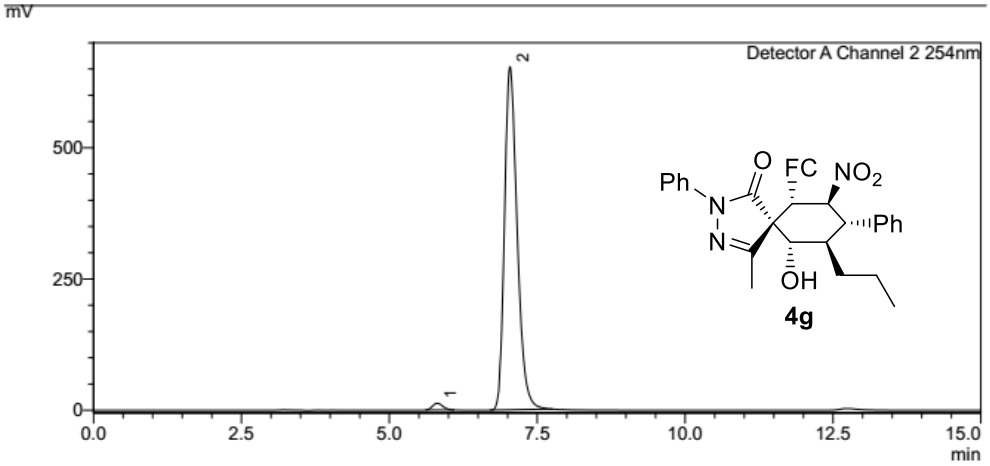


<Peak Table>

Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	5.762	5573419	464272	49.210	49.210
2	7.017	5752436	376933	50.790	50.790
Total		11325855	841206		100.000

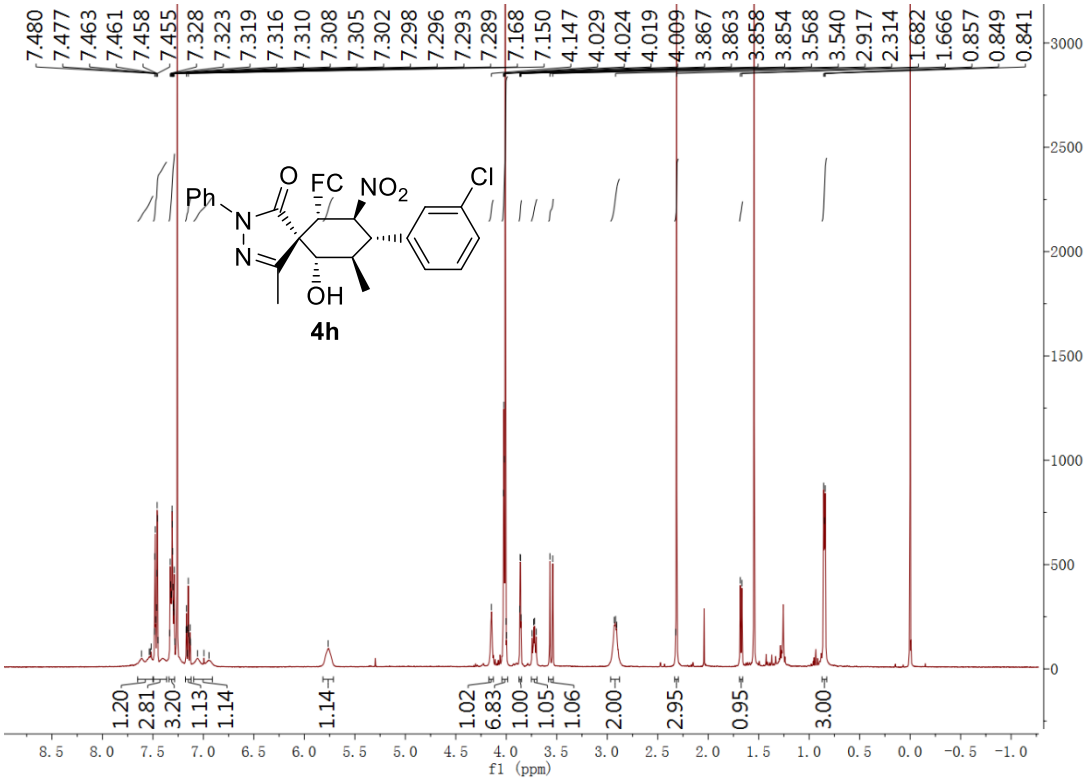
<Chromatogram>

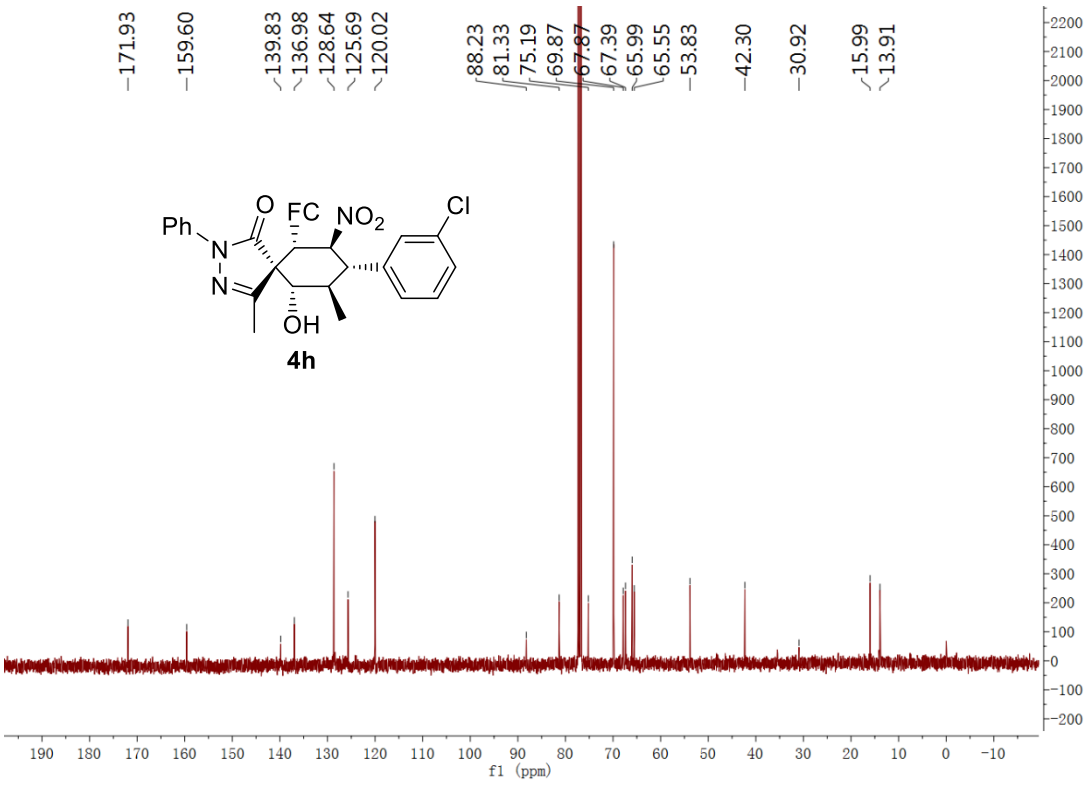


<Peak Table>

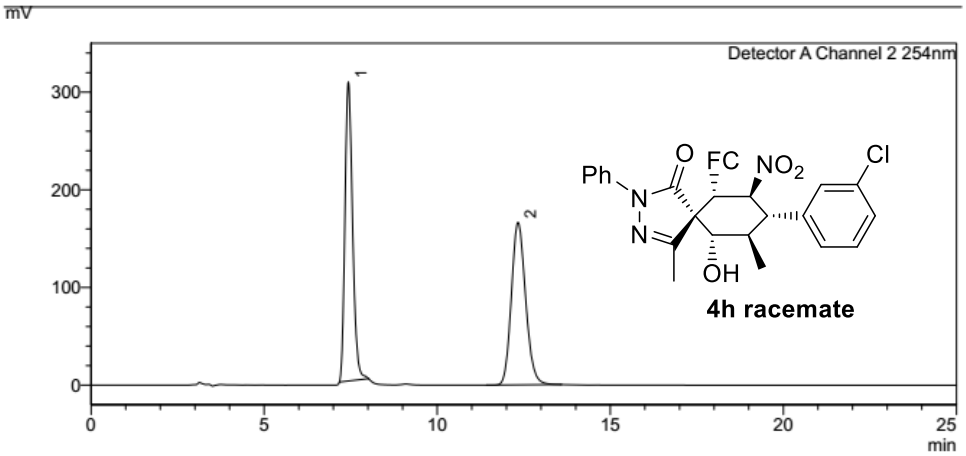
Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	5.811	154043	12790	1.553	1.553
2	7.040	9762495	653750	98.447	98.447
Total		9916538	666540		100.000


7.480 7.477 7.463 7.461 7.458 7.455 7.328 7.323 7.319 7.316 7.310 7.308 7.305 7.302 7.298 7.296 7.293 7.289 7.168 7.150 4.147 4.029 4.024 4.019 4.009 3.867 3.863 3.858 3.854 3.568 3.540 2.917 2.314 1.682 1.666 0.857 0.849 0.841
Ph
N
N
O
FC
NO2
Cl
OH
4h
1.20 2.81 3.20 1.13 1.14 1.14 1.02 6.83 1.00 1.05 1.06 2.00 2.95 0.95 3.00
f1 (ppm)


171.93 159.60 139.83 136.98 128.64 125.69 120.02 88.23 81.33 75.19 69.87 67.87 67.39 65.99 65.55 53.83 42.30 30.92 15.99 13.91
Ph
N
N
O
FC
NO2
Cl
OH
4h
f1 (ppm)

<Chromatogram>

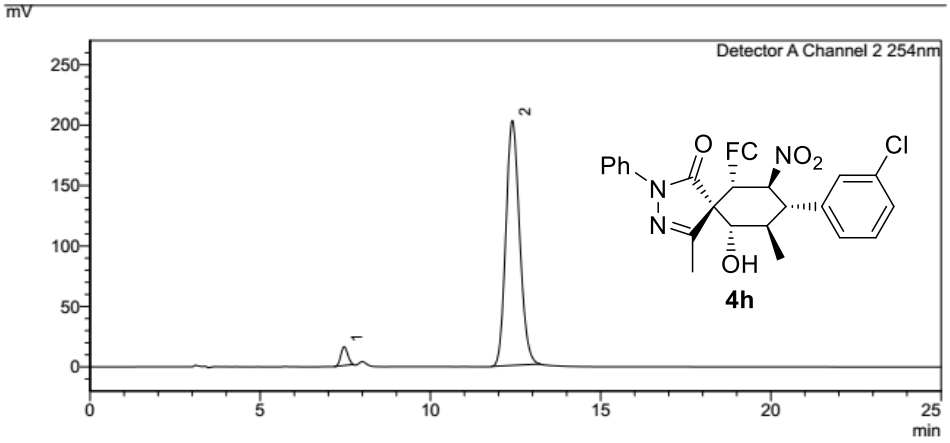


<Peak Table>

Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	7.433	4669901	306107	49.514	49.514
2	12.335	4761626	166457	50.486	50.486
Total		9431527	472565		100.000

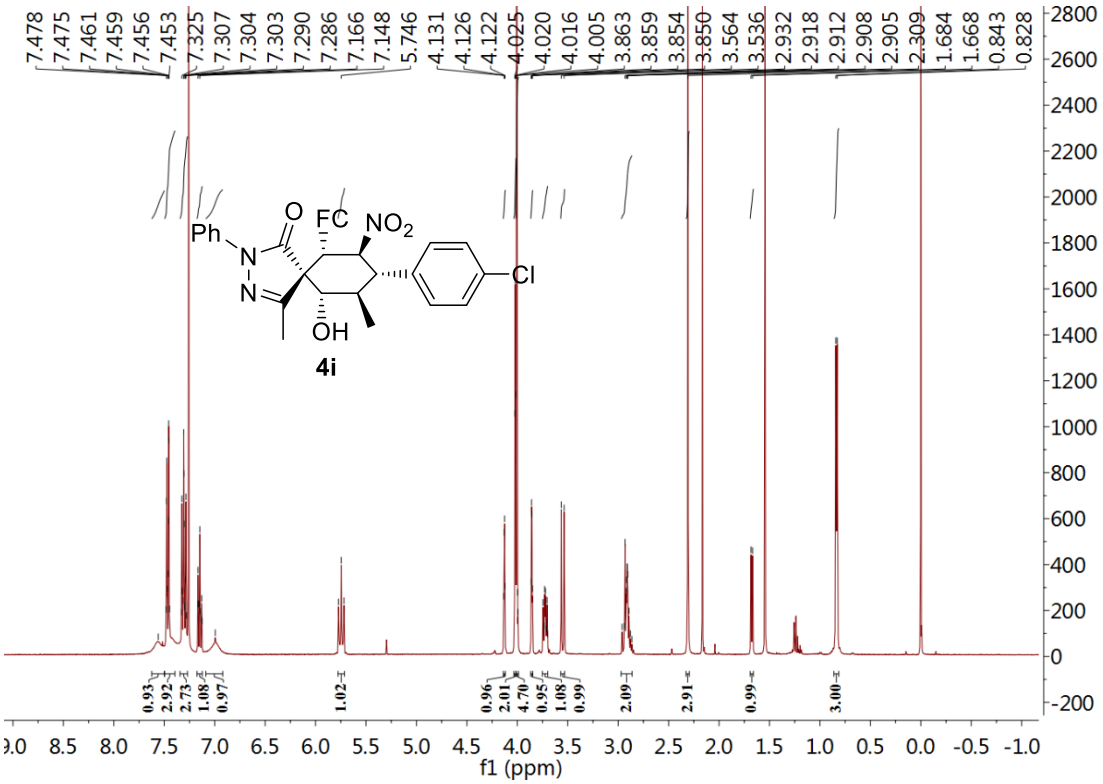
<Chromatogram>

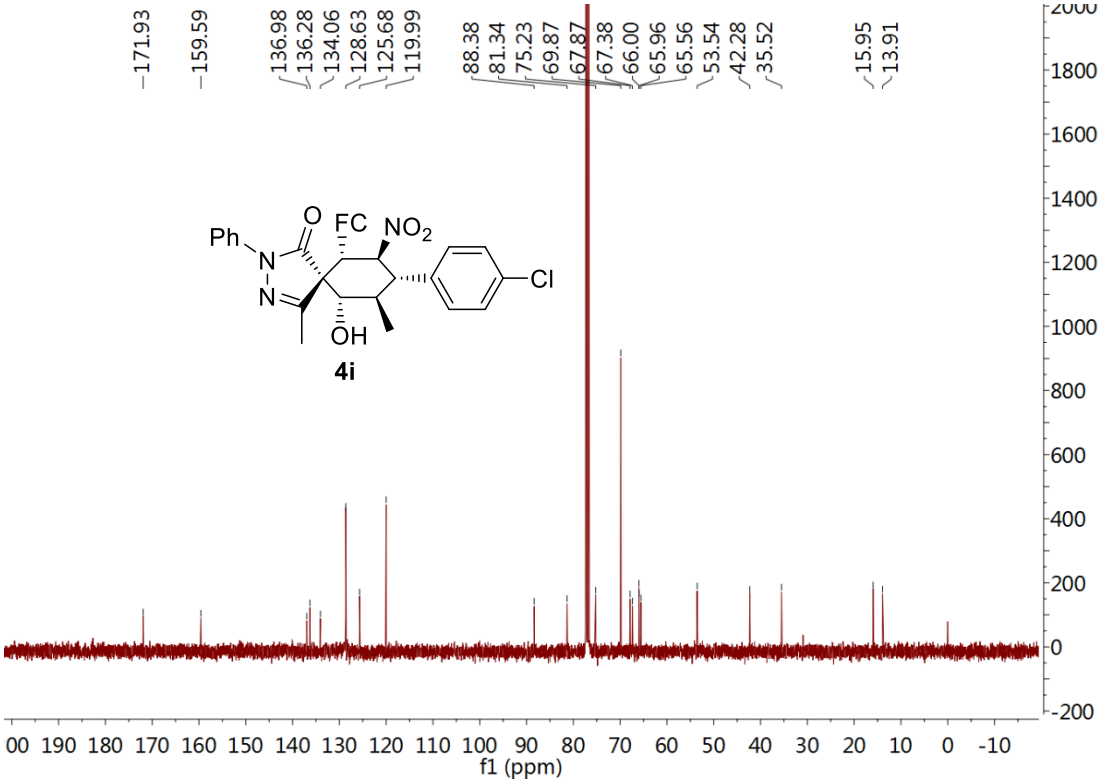


<Peak Table>

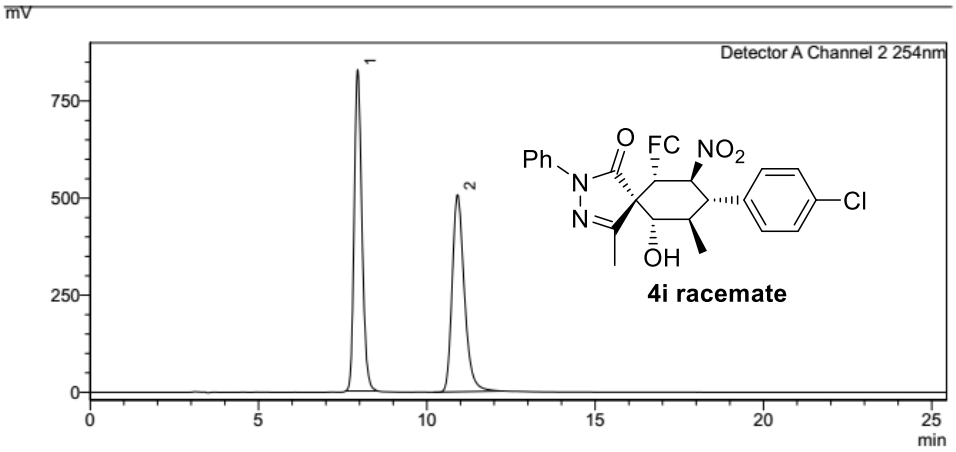
Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	7.466	217318	15468	3.692	3.692
2	12.406	5669085	202753	96.308	96.308
Total		5886403	218221		100.000


7.478
7.475
7.461
7.459
7.456
7.453
7.325
7.307
7.304
7.303
7.290
7.286
7.166
7.148
5.746
4.131
4.126
4.122
4.025
4.020
4.016
4.005
3.863
3.859
3.854
3.850
3.564
3.536
2.932
2.918
2.912
2.908
2.905
2.309
1.684
1.668
0.843
0.828
0.93
2.92
2.73
1.08
0.97
1.02
0.96
2.01
4.70
0.95
1.08
0.99
2.09
2.91
0.99
3.00
f1 (ppm)
Ph
N
N
O
FC
NO2
Cl
OH
4i


171.93
159.59
136.98
136.28
134.06
128.63
125.68
119.99
88.38
81.34
75.23
69.87
67.87
67.38
66.00
65.96
65.56
53.54
42.28
35.52
15.95
13.91
f1 (ppm)
Ph
N
N
O
FC
NO2
Cl
OH
4i

<Chromatogram>

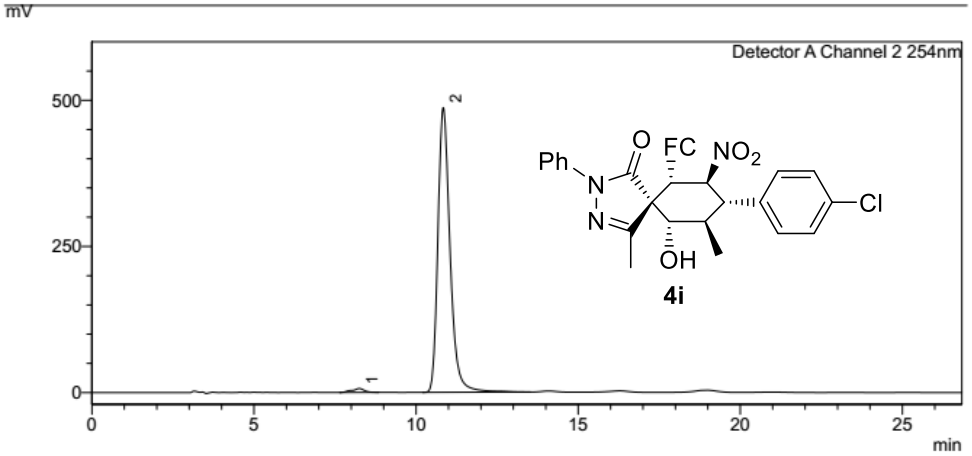


<Peak Table>

Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	7.945	13206475	827492	50.819	50.819
2	10.912	12780796	507796	49.181	49.181
Total		25987271	1335288		100.000

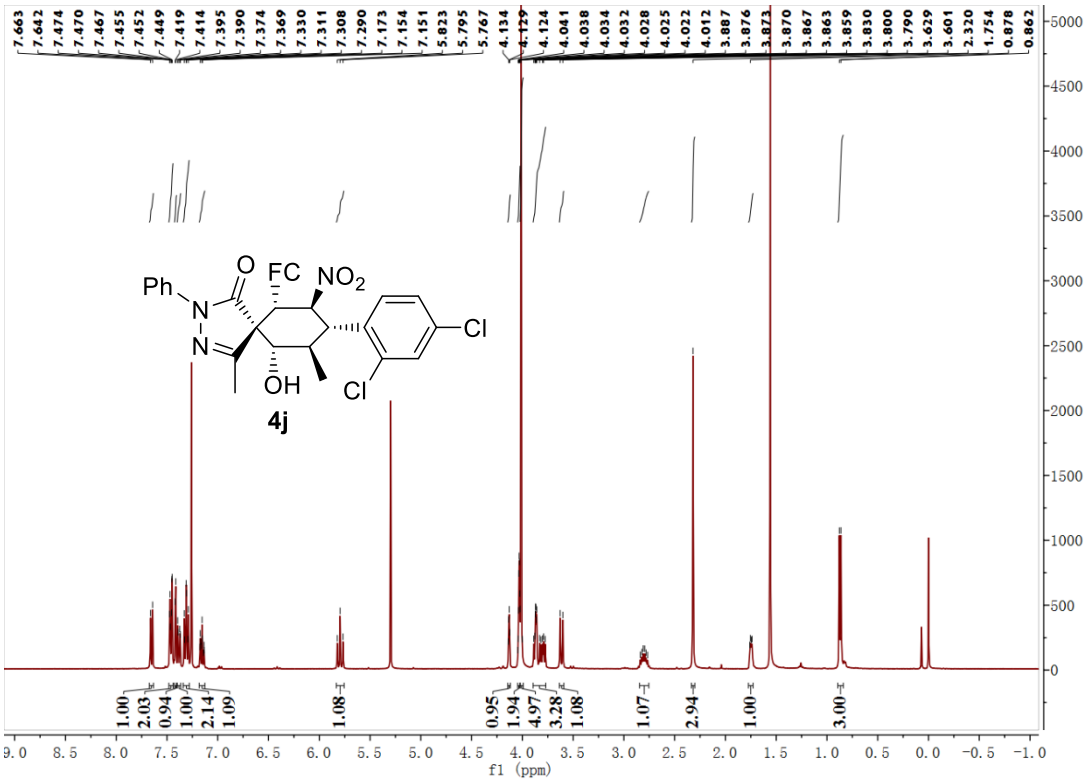
<Chromatogram>

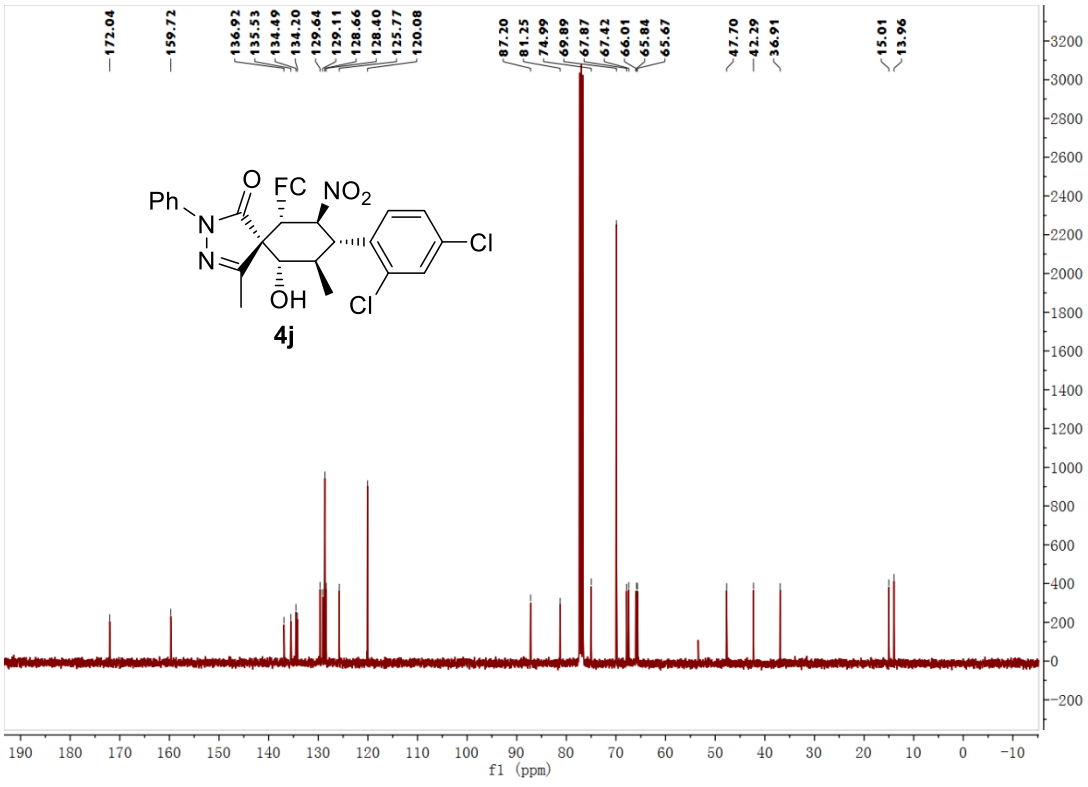


<Peak Table>

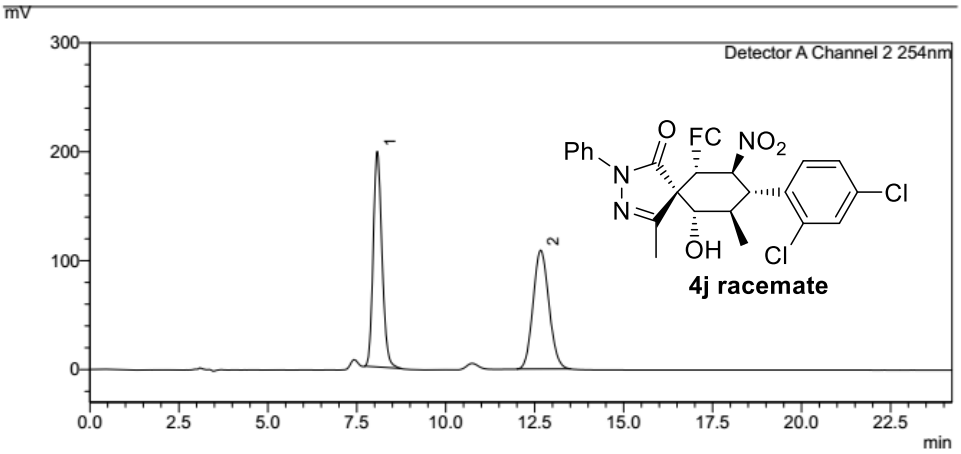
Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	8.244	177042	6692	1.372	1.372
2	10.838	12727112	486946	98.628	98.628
Total		12904154	493638		100.000


Ph
N
N
O
FC
NO2
OH
Cl
Cl
4j
f1 (ppm)


Ph
N
N
O
FC
NO2
OH
Cl
Cl
4j
f1 (ppm)

<Chromatogram>

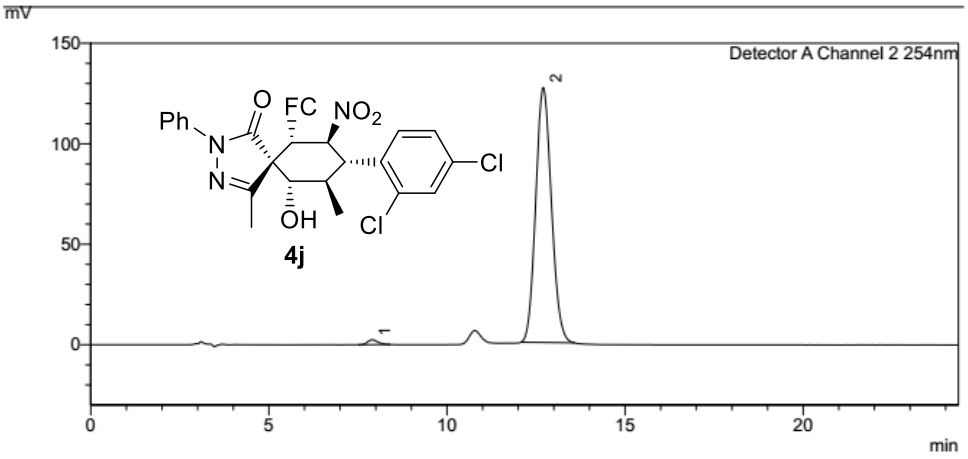


<Peak Table>

Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	8.074	3397603	197824	50.114	50.114
2	12.667	3382177	108951	49.886	49.886
Total		6779780	306775		100.000

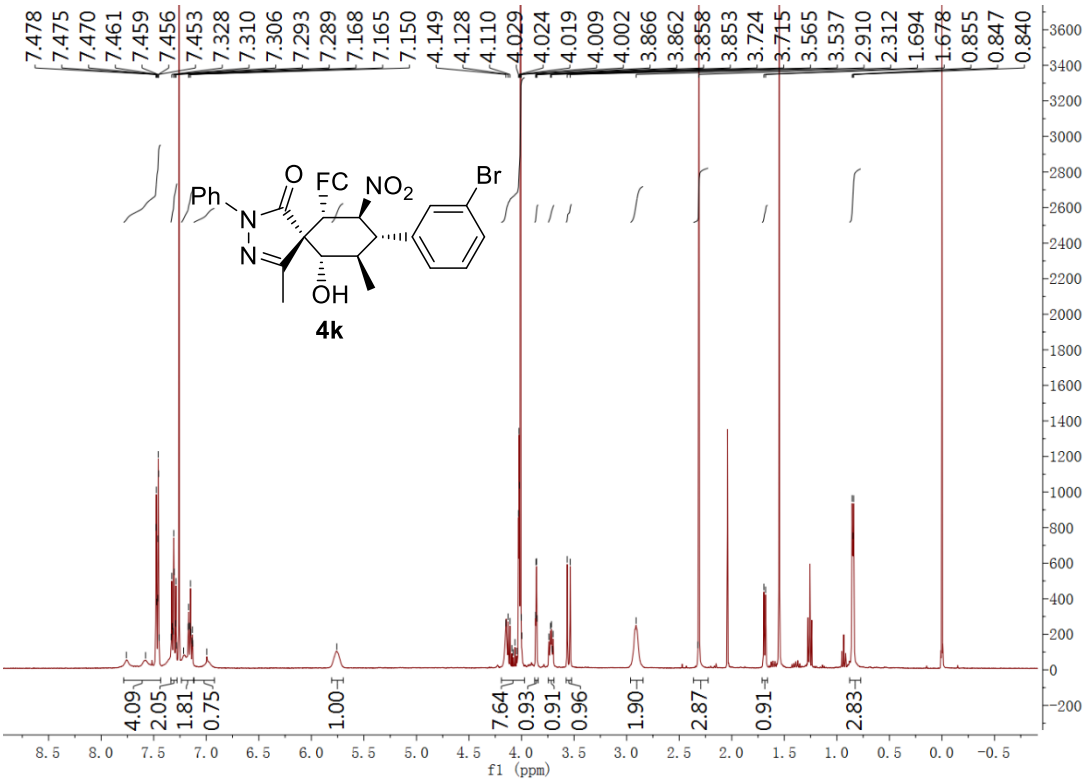
<Chromatogram>

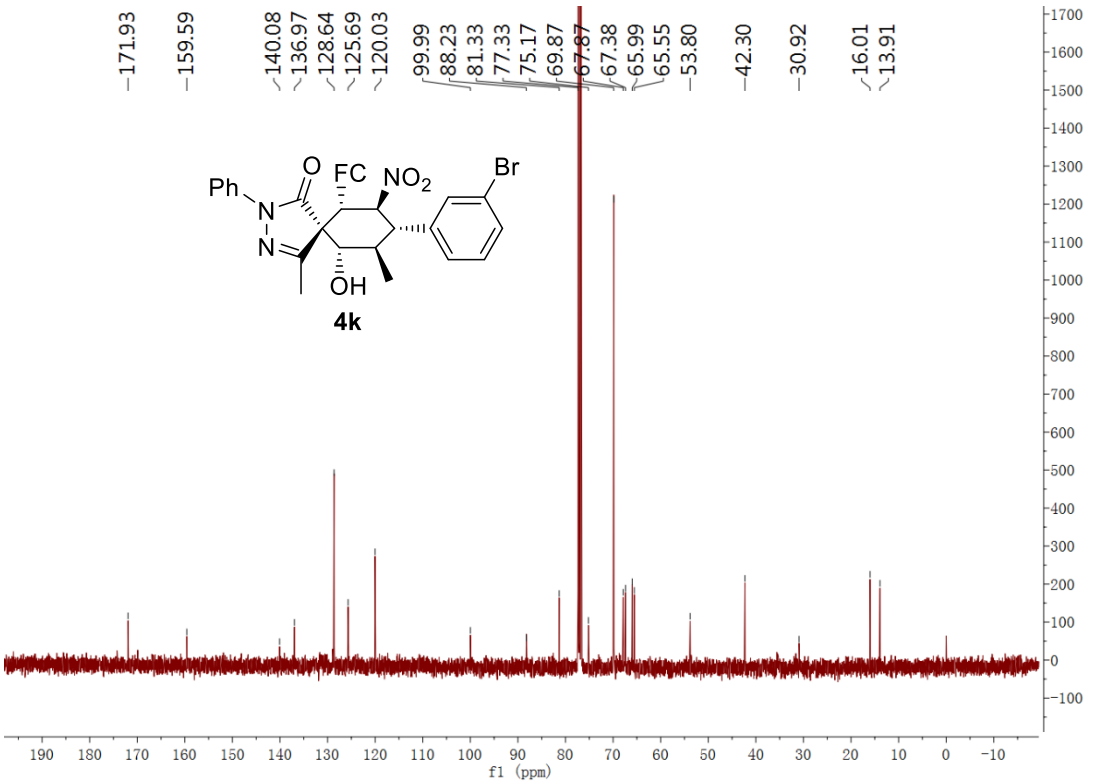


<Peak Table>

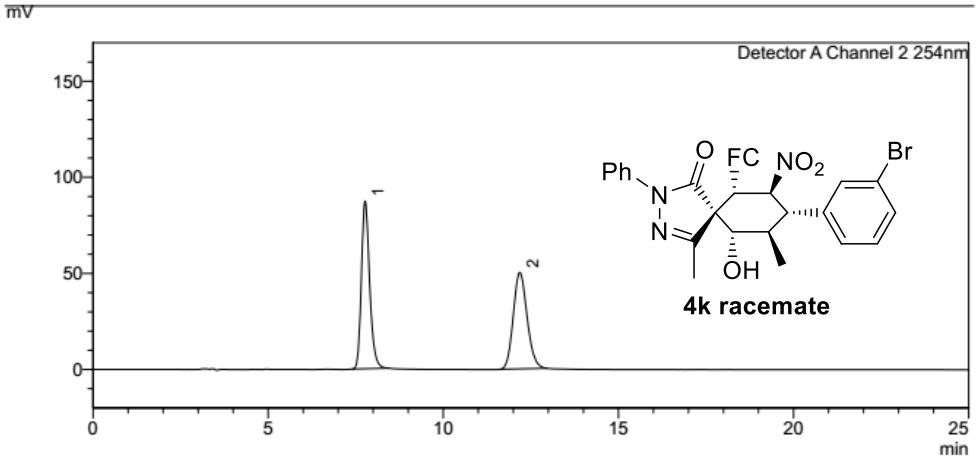
Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	7.902	48976	2436	1.244	1.244
2	12.703	3889490	126878	98.756	98.756
Total		3938466	129314		100.000


7.478
7.475
7.470
7.461
7.459
7.456
7.453
7.328
7.310
7.306
7.293
7.289
7.168
7.165
7.150
4.149
4.128
4.110
4.029
4.024
4.019
4.009
4.002
3.866
3.862
3.858
3.853
3.724
3.715
3.565
3.537
2.910
2.312
1.694
1.678
0.855
0.847
0.840
Ph
N
N
O
FC
NO2
Br
OH
4k
4.09
2.05
1.81
0.75
1.00
7.64
0.93
0.91
0.96
1.90
2.87
0.91
2.83
f1 (ppm)


171.93
159.59
140.08
136.97
128.64
125.69
120.03
99.99
88.23
81.33
77.33
75.17
69.87
67.87
67.38
65.99
65.55
53.80
42.30
30.92
16.01
13.91
Ph
N
N
O
FC
NO2
Br
OH
4k
f1 (ppm)

<Chromatogram>

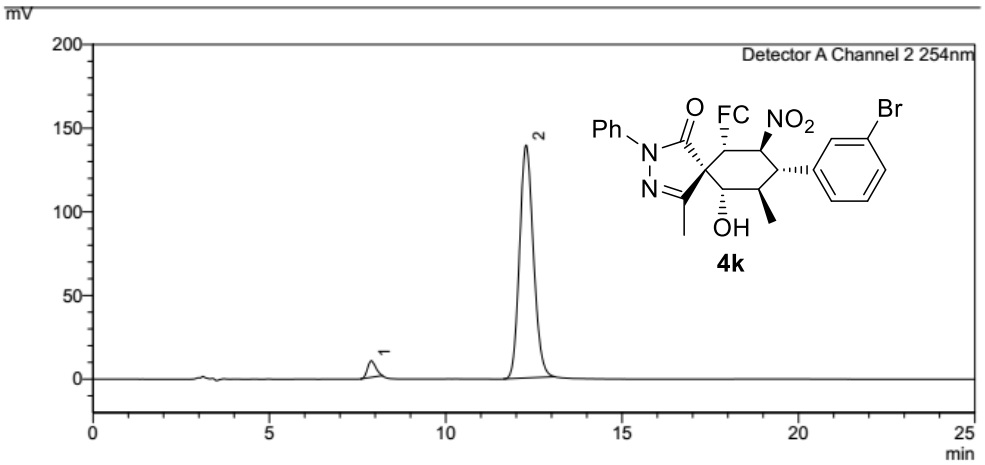


<Peak Table>

Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	7.769	1423222	87286	50.905	50.905
2	12.185	1372615	50190	49.095	49.095
Total		2795837	137477		100.000

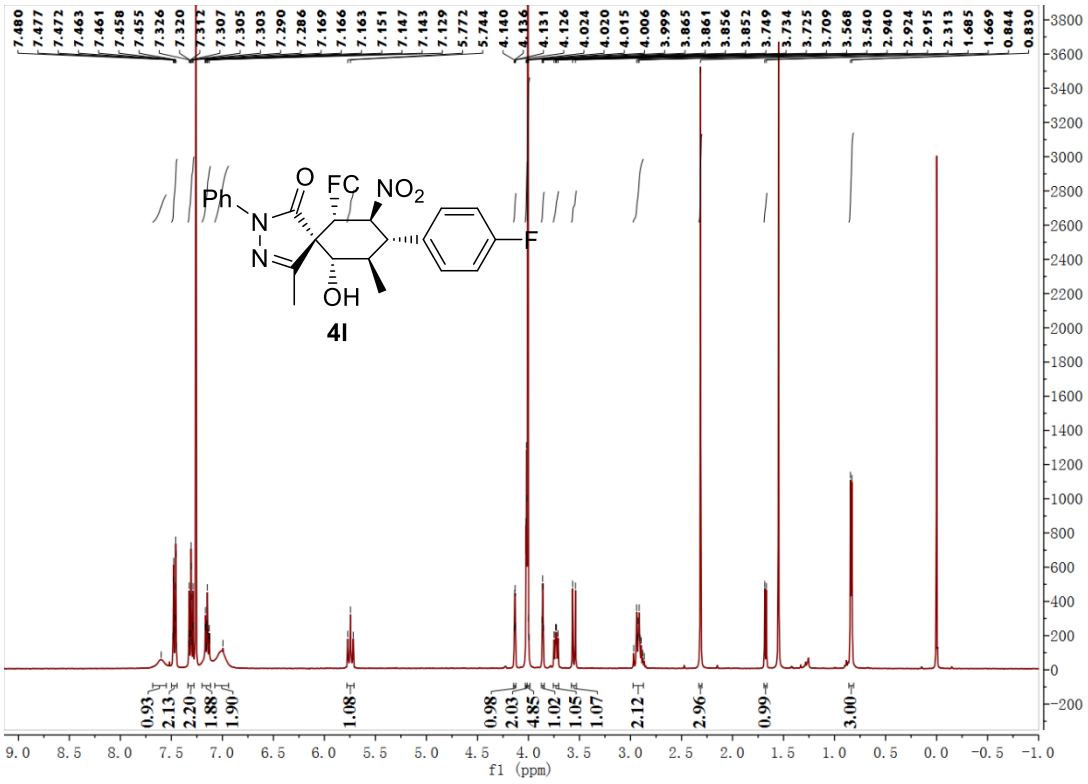
<Chromatogram>

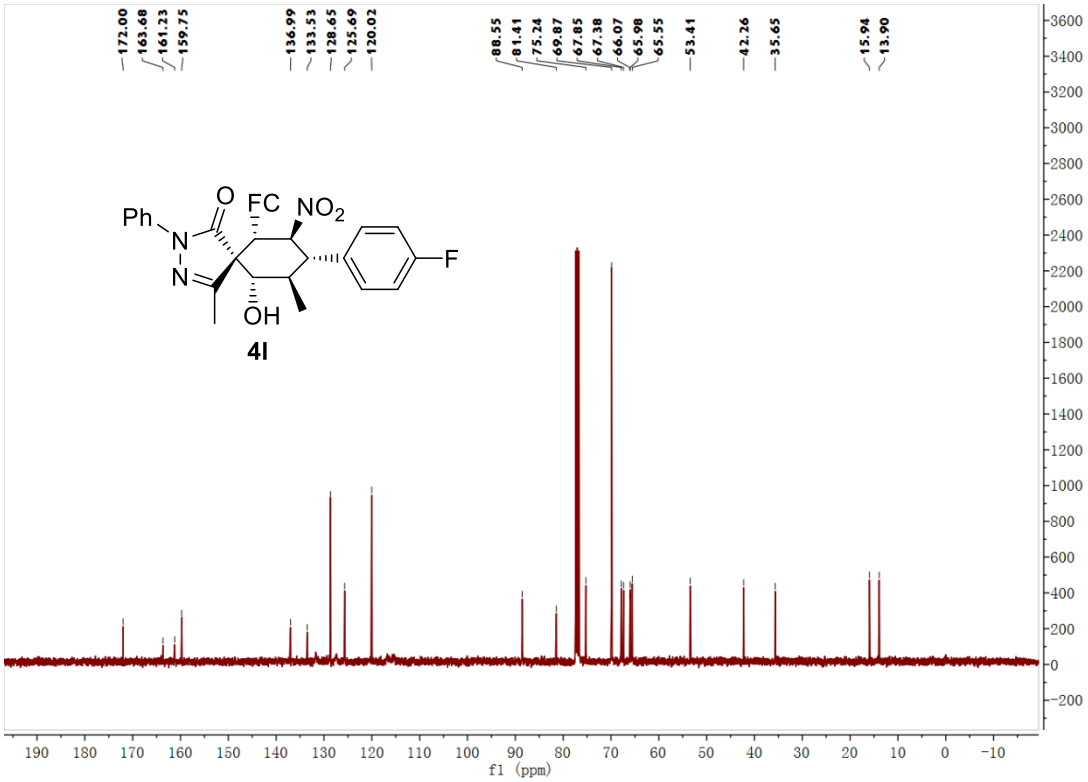


<Peak Table>

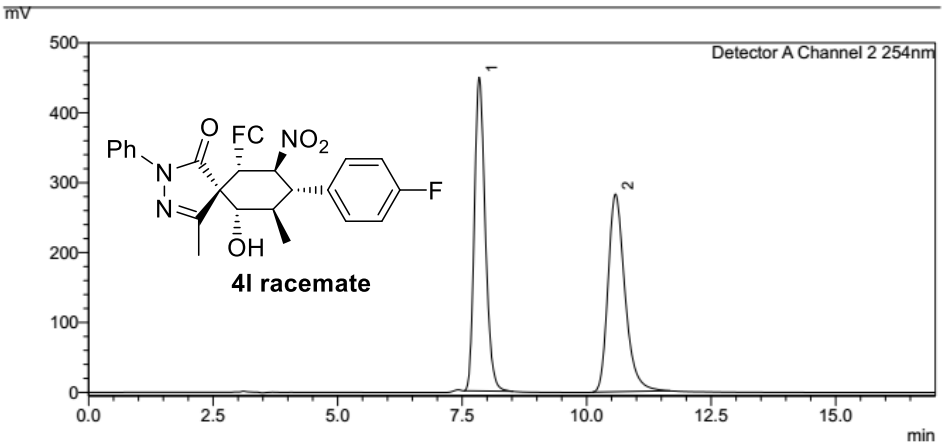
Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	7.891	159587	9850	4.024	4.024
2	12.278	3806703	139222	95.976	95.976
Total		3966290	149072		100.000


7.480 7.477 7.472 7.463 7.461 7.458 7.455 7.326 7.320 7.312 7.307 7.305 7.303 7.290 7.286 7.169 7.166 7.163 7.151 7.147 7.143 7.129 5.772 5.744 4.140 4.136 4.131 4.126 4.024 4.020 4.015 4.006 3.999 3.865 3.861 3.856 3.852 3.749 3.734 3.725 3.709 3.568 3.540 2.940 2.924 2.915 2.313 1.685 1.669 0.844 0.830
0.93 2.13 2.20 1.88 1.90 1.08 0.98 2.03 4.85 1.02 1.05 1.07 2.12 2.96 0.99 3.00
f1 (ppm)
Ph
O
FC
NO2
N
N
OH
F
4l


172.00 163.68 161.23 159.75 136.99 133.53 128.65 125.69 120.02 88.55 81.41 75.24 69.87 67.85 67.38 66.07 65.98 65.55 53.41 42.26 35.65 15.94 13.90
f1 (ppm)
Ph
O
FC
NO2
N
N
OH
F
4l

<Chromatogram>

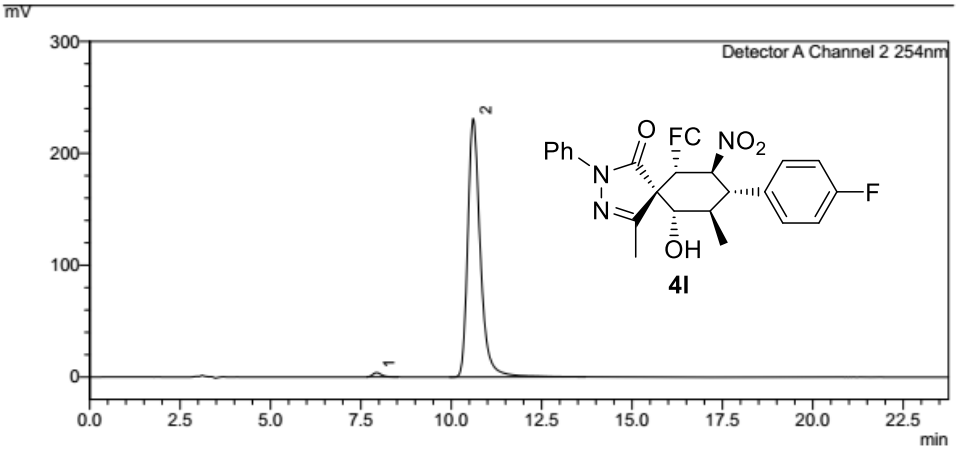


<Peak Table>

Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	7.844	6824867	448607	50.546	50.546
2	10.580	6677426	282715	49.454	49.454
Total		13502294	731322		100.000

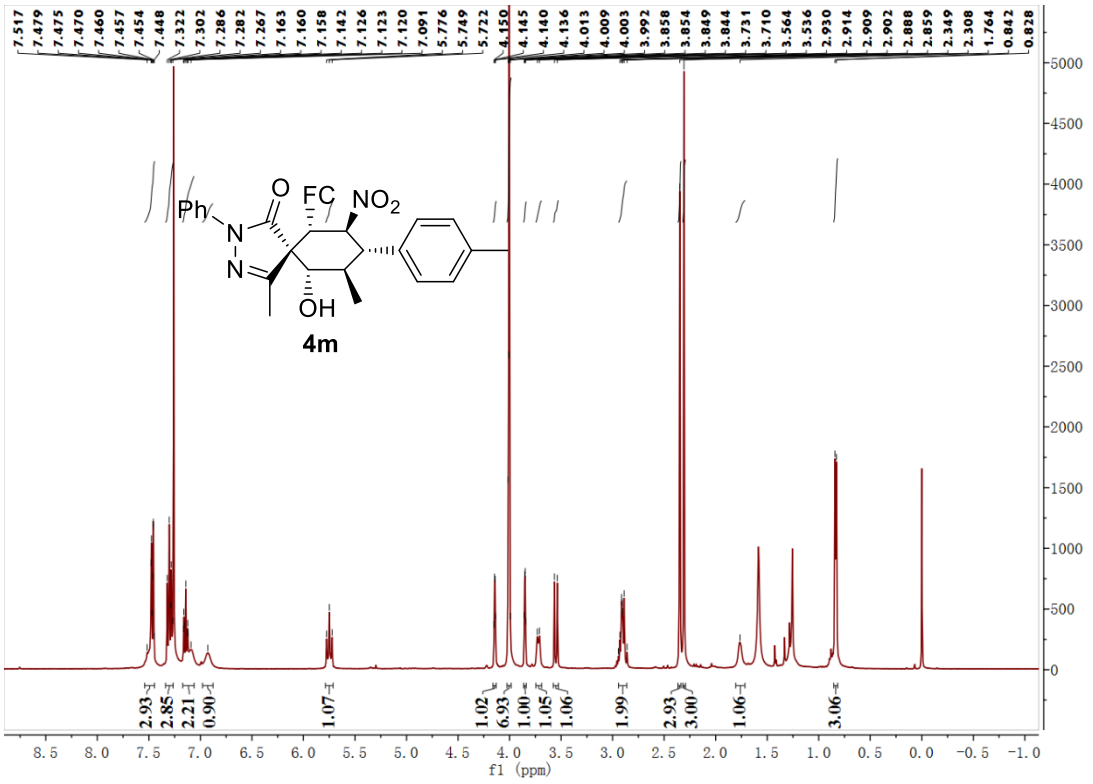
<Chromatogram>

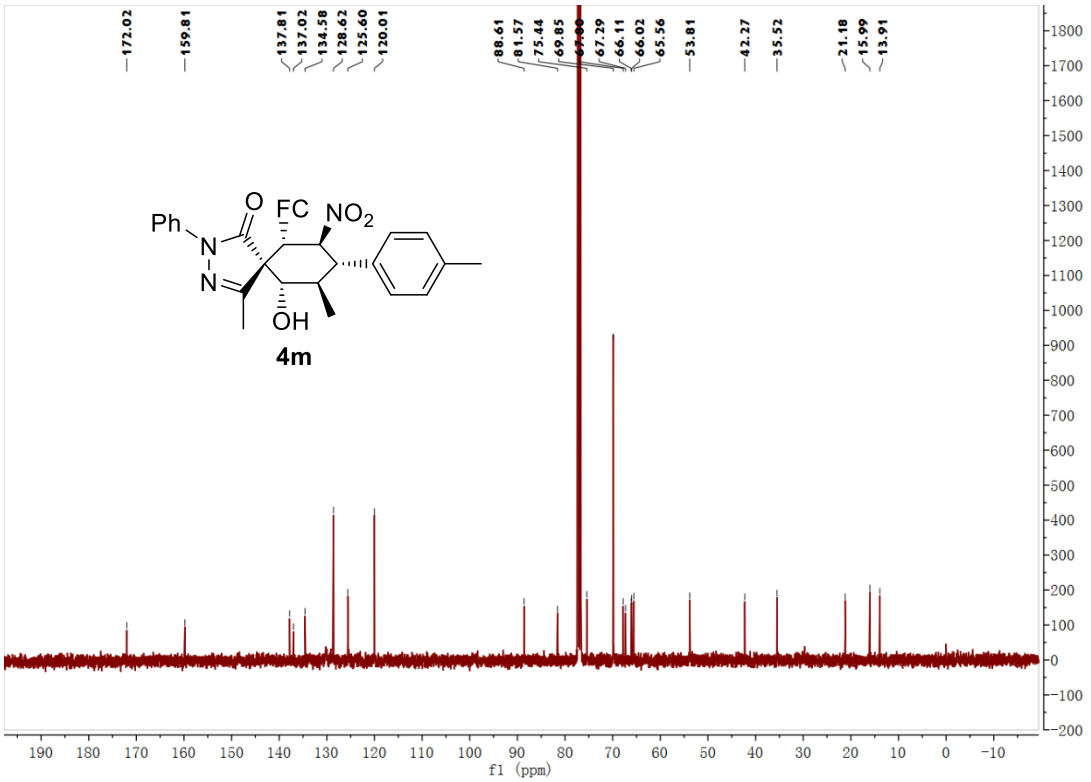


<Peak Table>

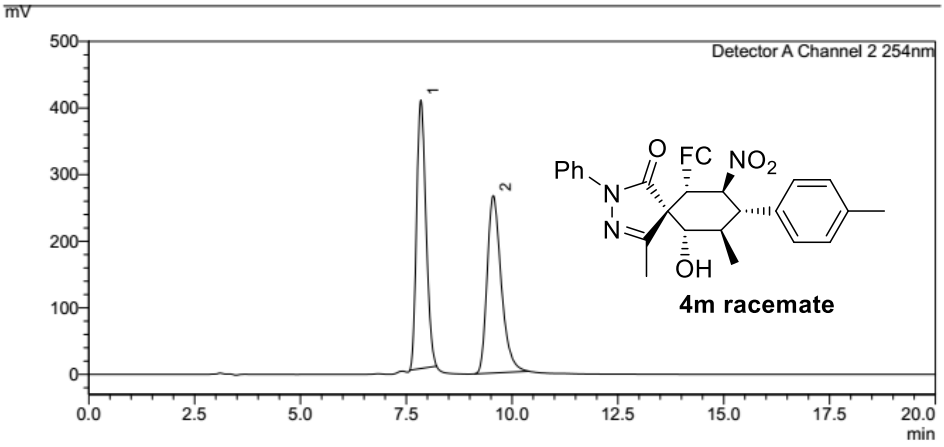
Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	7.934	65317	3878	1.154	1.154
2	10.606	5596065	231075	98.846	98.846
Total		5661383	234953		100.000


7.517 7.479 7.475 7.470 7.460 7.457 7.454 7.448 7.322 7.302 7.286 7.282 7.267 7.163 7.160 7.158 7.142 7.126 7.123 7.120 7.091 5.776 5.749 5.722 4.150 4.145 4.140 4.136 4.013 4.009 4.003 3.992 3.858 3.854 3.849 3.844 3.731 3.710 3.564 3.536 2.930 2.914 2.909 2.902 2.888 2.859 2.349 2.308 1.764 0.842 0.828
Ph
O
FC
NO2
N
N
OH
4m
2.93 2.85 2.21 0.90 1.07 1.02 6.93 1.00 1.05 1.06 1.99 2.93 3.00 1.06 3.06
f1 (ppm)


172.02 159.81 137.81 137.02 134.58 128.62 125.60 120.01 88.61 81.57 75.44 69.85 67.29 66.11 66.02 65.56 53.81 42.27 35.52 21.18 15.99 13.91
Ph
O
FC
NO2
N
N
OH
4m
f1 (ppm)

<Chromatogram>

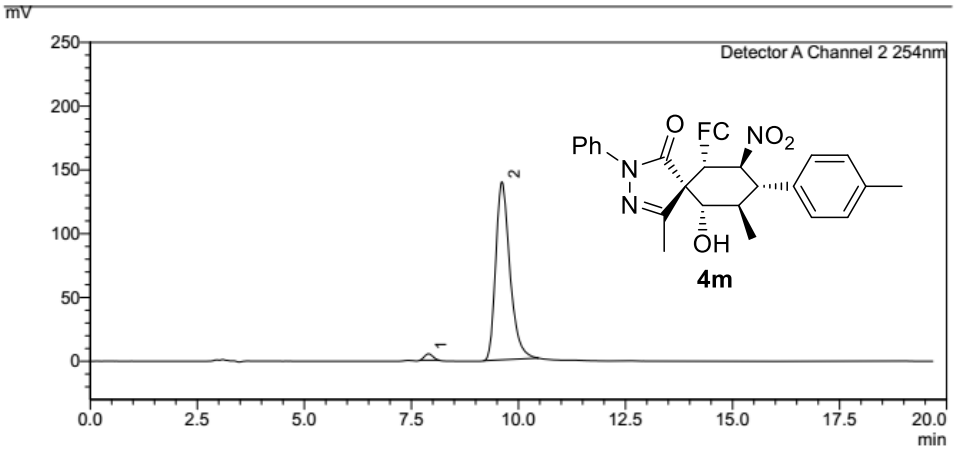


<Peak Table>

Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	7.844	6204052	403026	50.140	50.140
2	9.554	6169322	266391	49.860	49.860
Total		12373375	669417		100.000

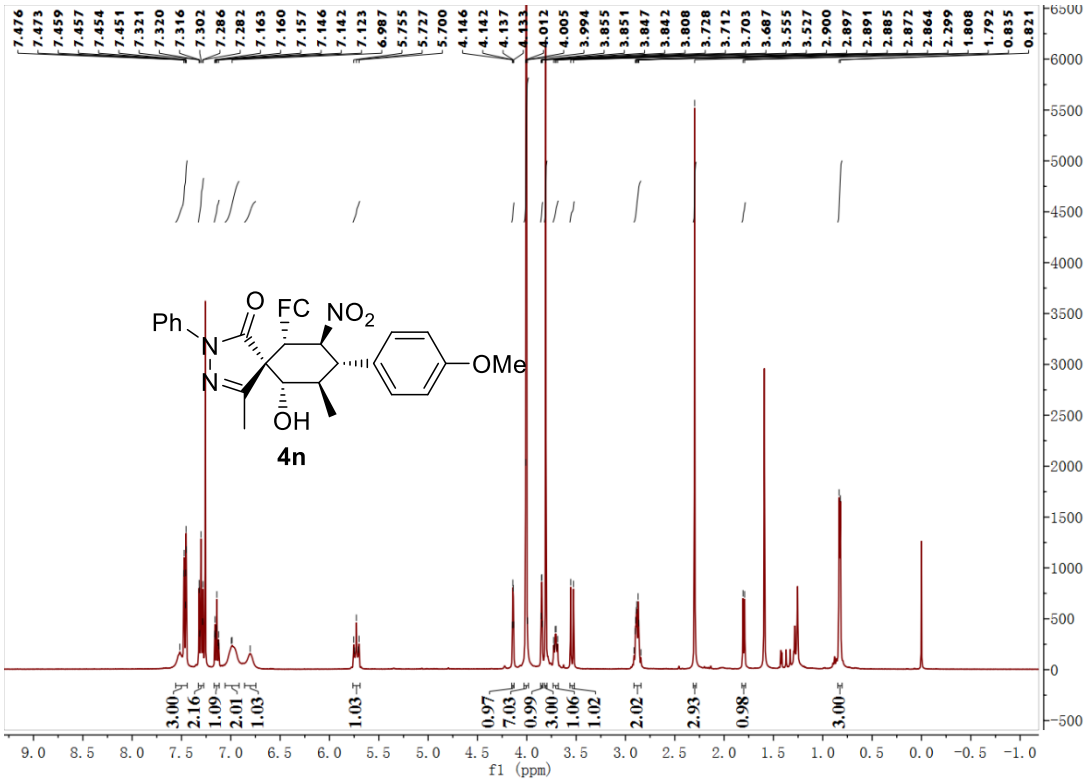
<Chromatogram>

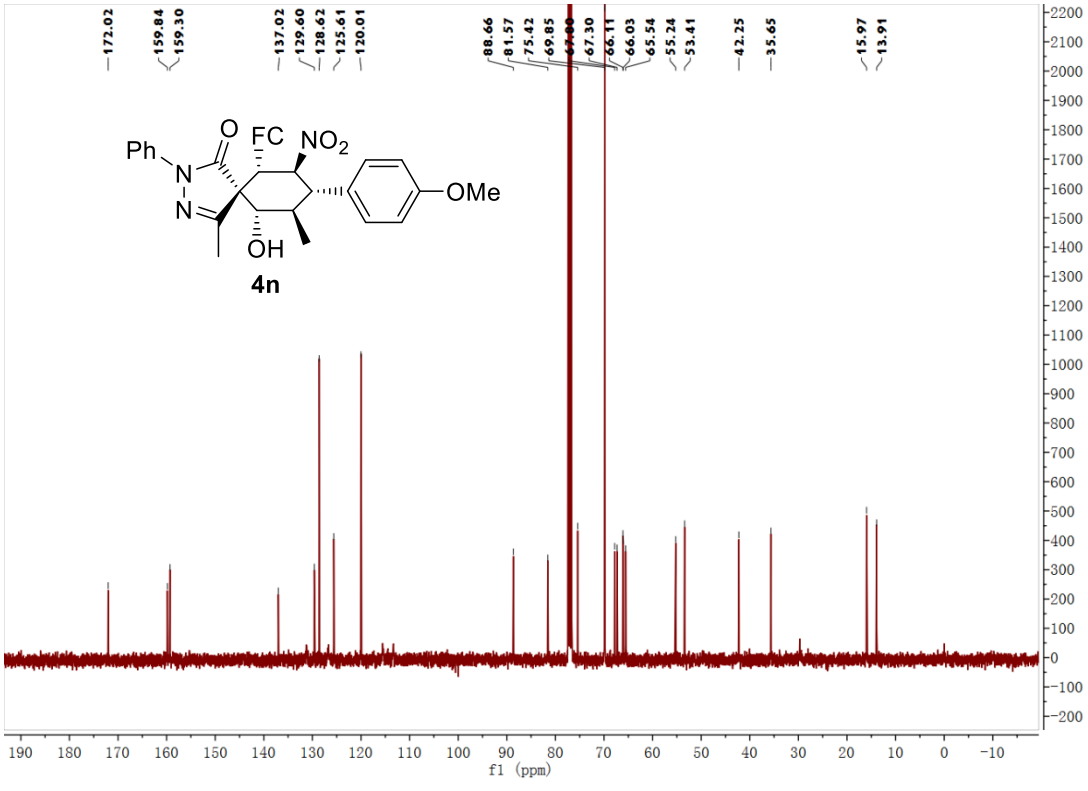


<Peak Table>

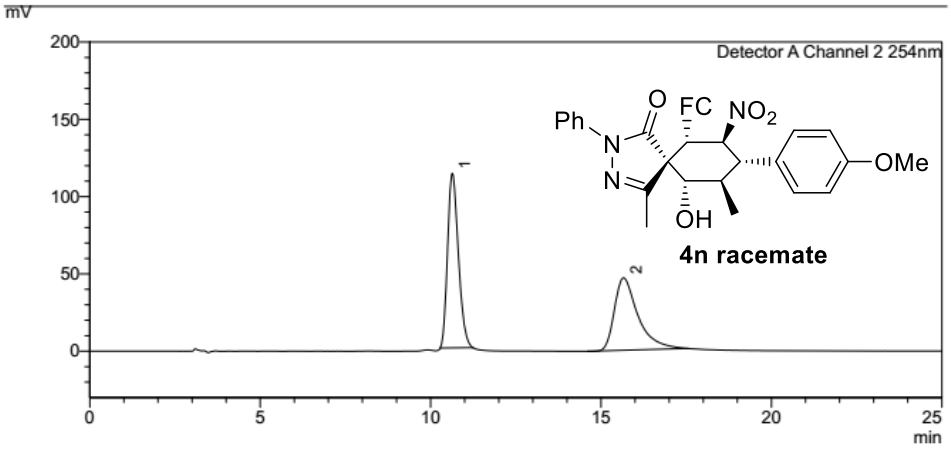
Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	7.899	67049	4989	2.042	2.042
2	9.613	3217253	139587	97.958	97.958
Total		3284303	144577		100.000


Ph
N
N
O
FC
NO2
OMe
OH
4n
f1 (ppm)


Ph
N
N
O
FC
NO2
OMe
OH
4n
f1 (ppm)

<Chromatogram>

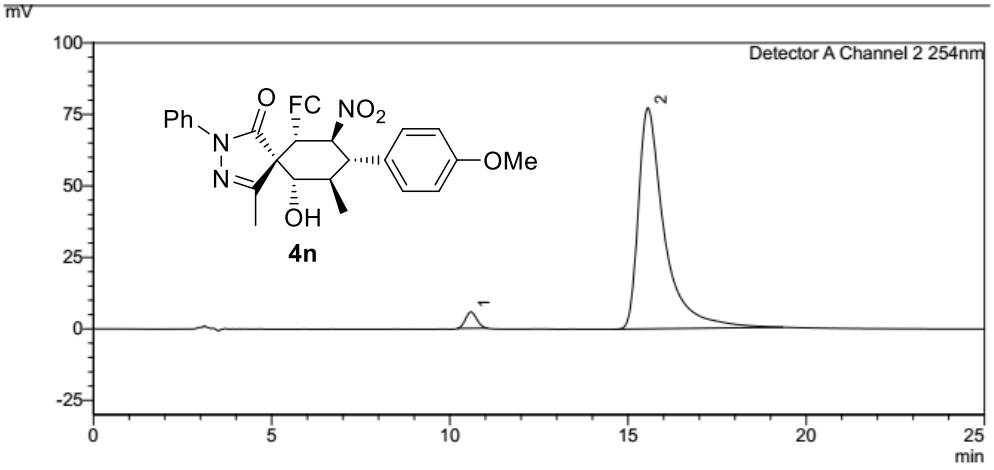


<Peak Table>

Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	10.636	2490418	112976	52.474	52.474
2	15.661	2255545	46833	47.526	47.526
Total		4745963	159808		100.000

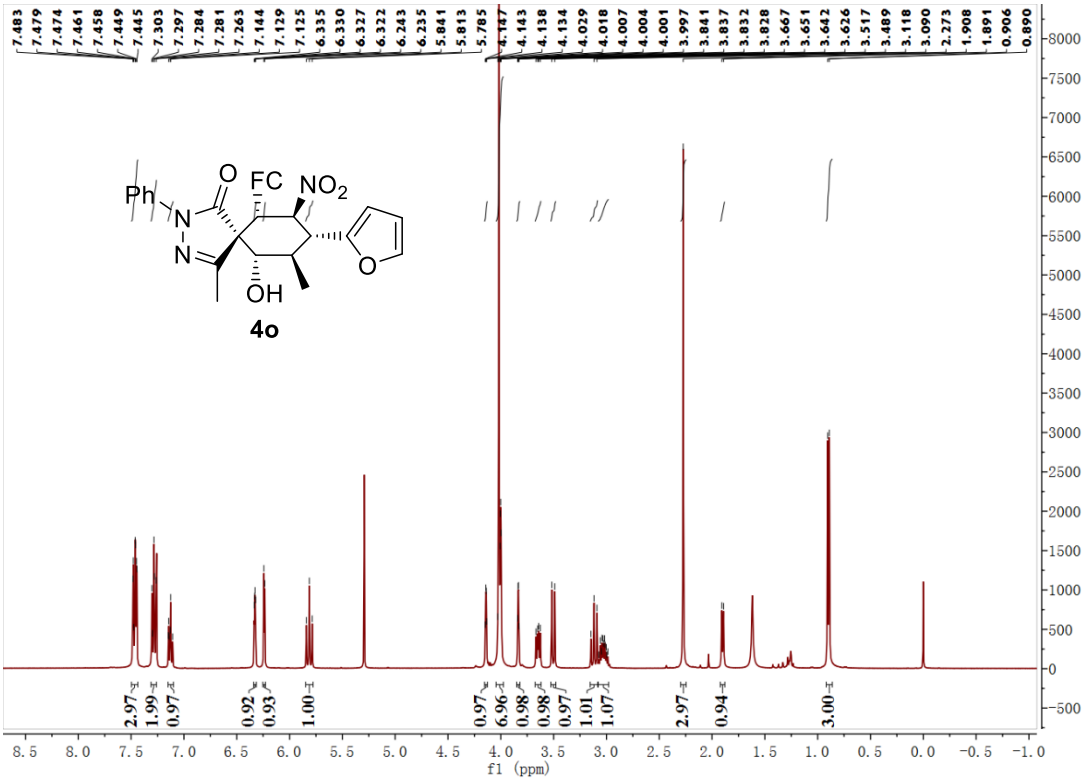
<Chromatogram>

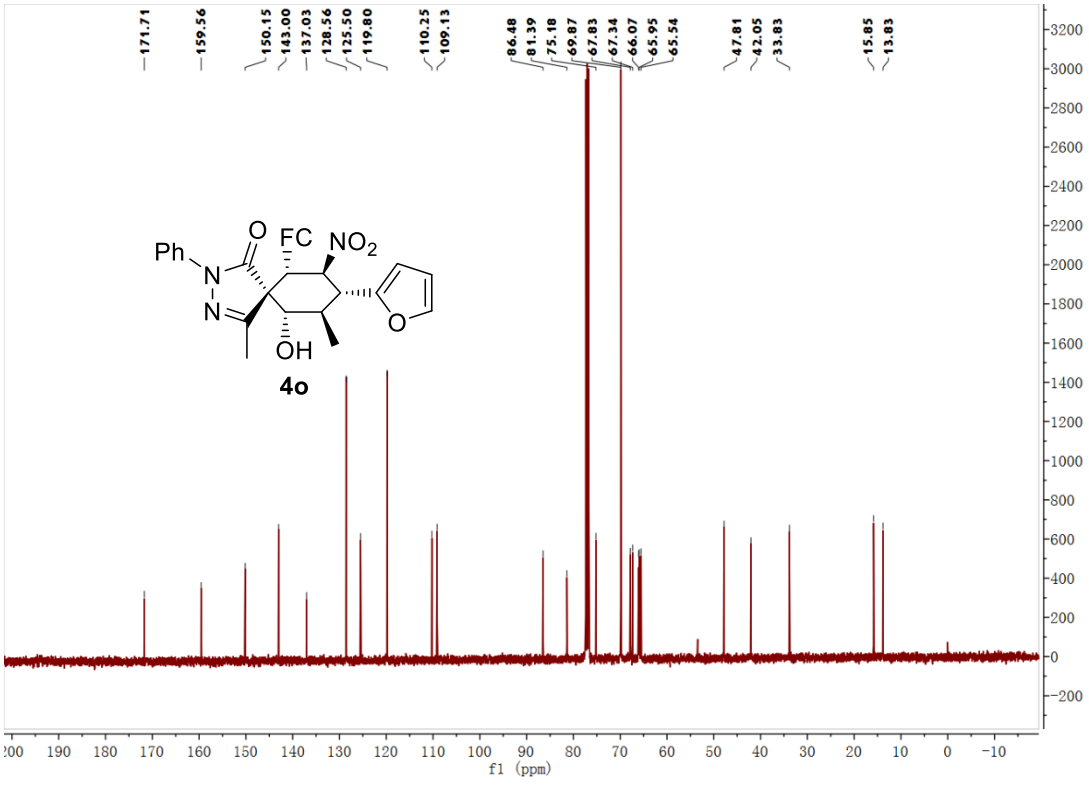


<Peak Table>

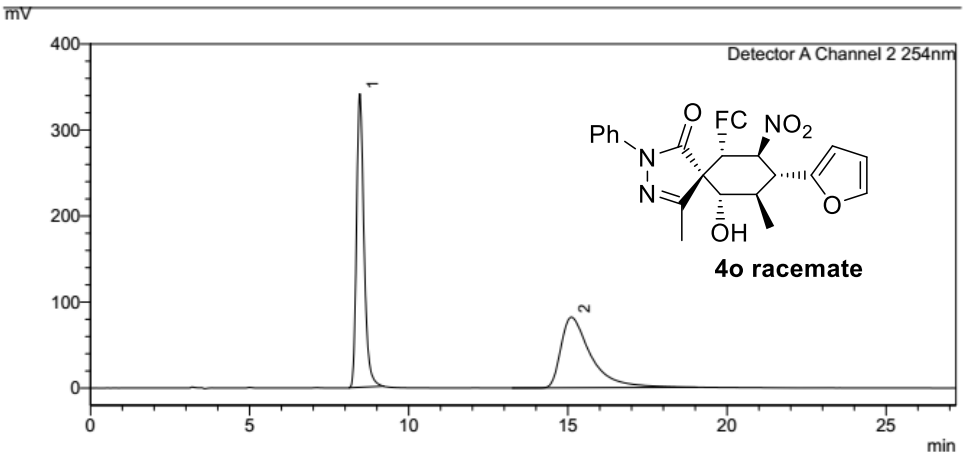
Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	10.590	123944	5716	3.065	3.065
2	15.557	3919735	77290	96.935	96.935
Total		4043680	83006		100.000


7.483 7.479 7.474 7.461 7.458 7.449 7.445 7.303 7.297 7.284 7.281 7.263 7.144 7.129 7.125 6.335 6.330 6.327 6.322 6.243 6.235 5.841 5.813 5.785 4.147 4.143 4.138 4.134 4.029 4.018 4.007 4.004 4.001 3.997 3.841 3.837 3.832 3.828 3.667 3.651 3.642 3.626 3.517 3.489 3.118 3.090 2.273 1.908 1.891 0.906 0.890
O
FC
NO2
Ph
N
N
OH
O
4o
2.97 1.99 0.97 0.92 0.93 1.00 0.97 6.96 0.98 0.98 0.97 1.01 1.07 2.97 0.94 3.00
f1 (ppm)


171.71 159.56 150.15 143.00 137.03 128.56 125.50 119.80 110.25 109.13 86.48 81.39 75.18 69.87 67.83 67.34 66.07 65.95 65.54 47.81 42.05 33.83 15.85 13.83
O
FC
NO2
Ph
N
N
OH
O
4o
f1 (ppm)

<Chromatogram>

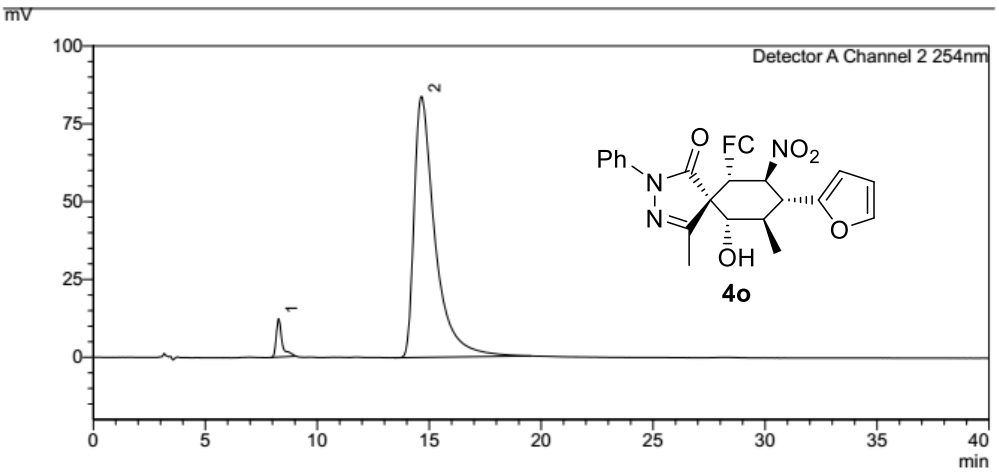


<Peak Table>

Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	8.462	5568008	341318	50.965	50.965
2	15.113	5357178	82057	49.035	49.035
Total		10925186	423375		100.000

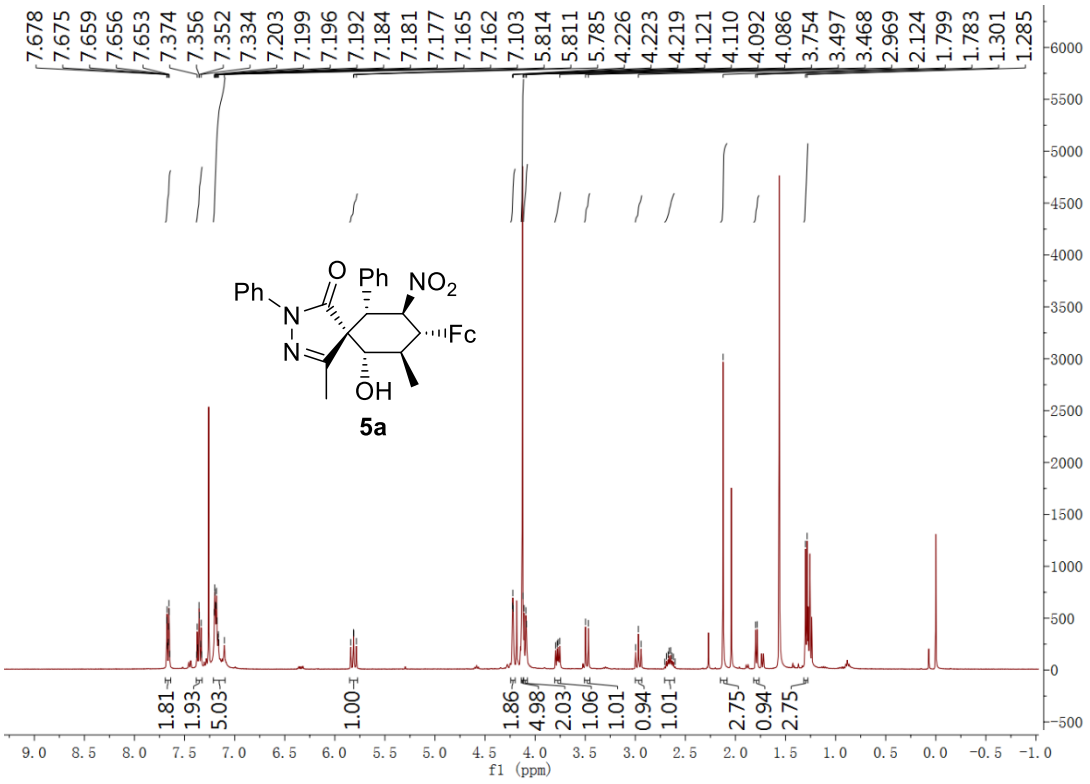
<Chromatogram>



<Peak Table>

Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	8.275	226216	12341	3.984	3.984
2	14.653	5451548	83870	96.016	96.016
Total		5677763	96211		100.000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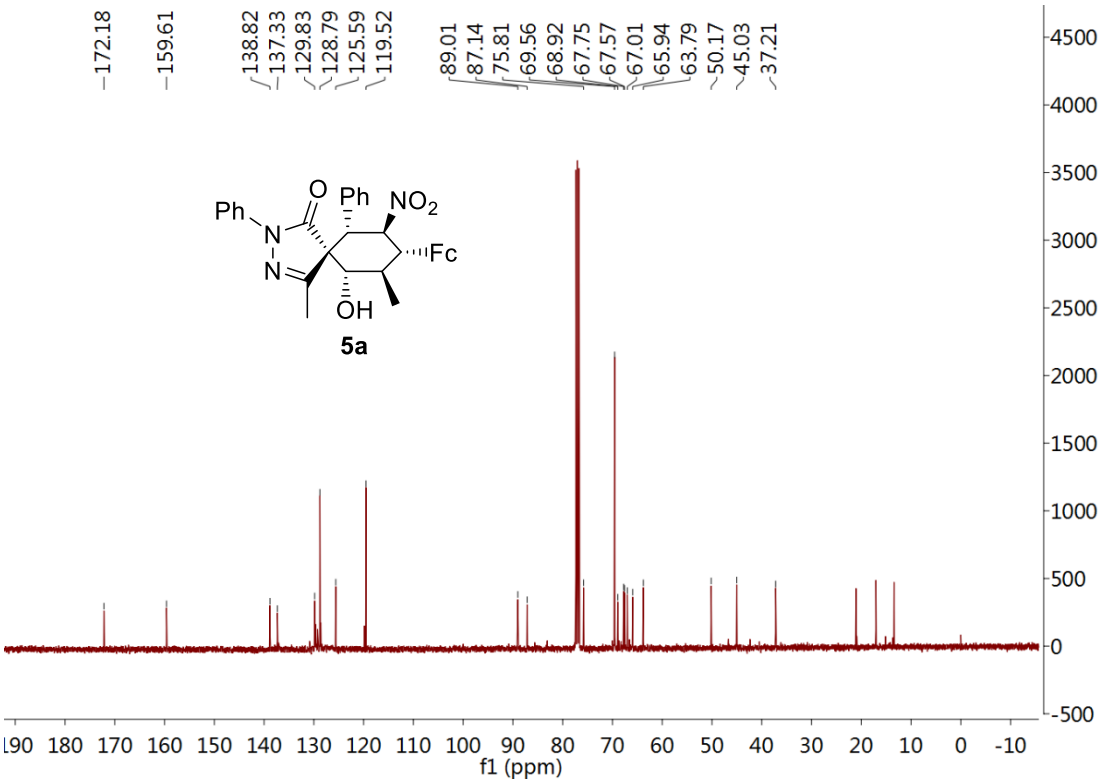
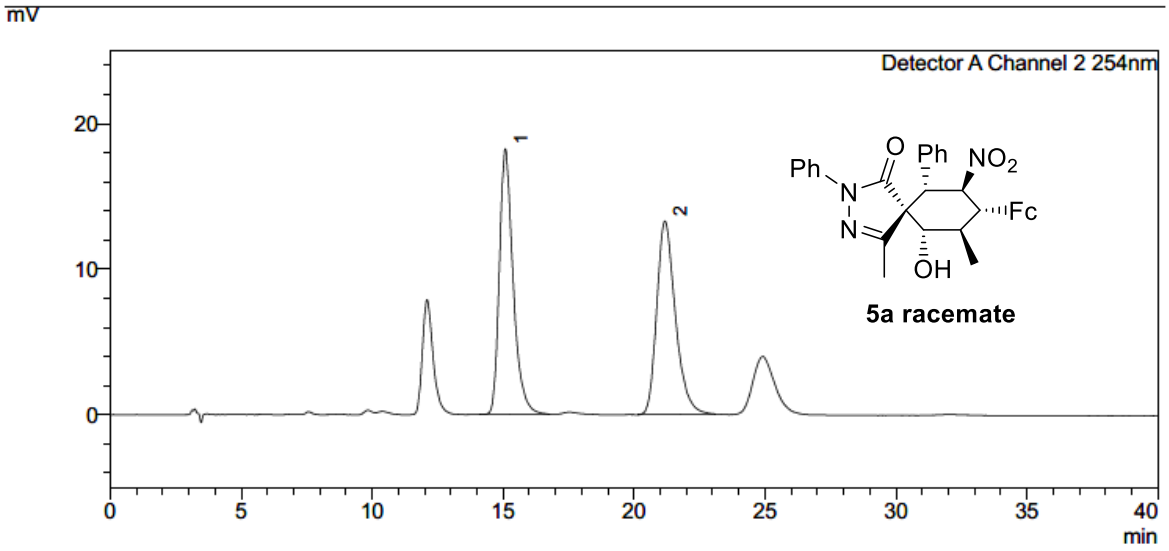
7.659
7.656
7.653
7.374
7.356
7.352
7.334
7.203
7.199
7.196
7.192
7.184
7.181
7.177
7.165
7.162
7.103
5.814
5.811
5.785
4.226
4.223
4.219
4.121
4.110
4.092
4.086
3.754
3.497
3.468
2.969
2.124
1.799
1.783
1.301
1.285
Ph
O
Ph
NO2
N
N
Fc
OH
5a
1.81
1.93
5.03
1.00
1.86
4.98
2.03
1.06
1.01
0.94
1.01
2.75
0.94
2.75
f1 (ppm)


172.18
159.61
138.82
137.33
129.83
128.79
125.59
119.52
89.01
87.14
75.81
69.56
68.92
67.75
67.57
67.01
65.94
63.79
50.17
45.03
37.21
Ph
O
Ph
NO2
N
N
Fc
OH
5a
f1 (ppm)

<Chromatogram>

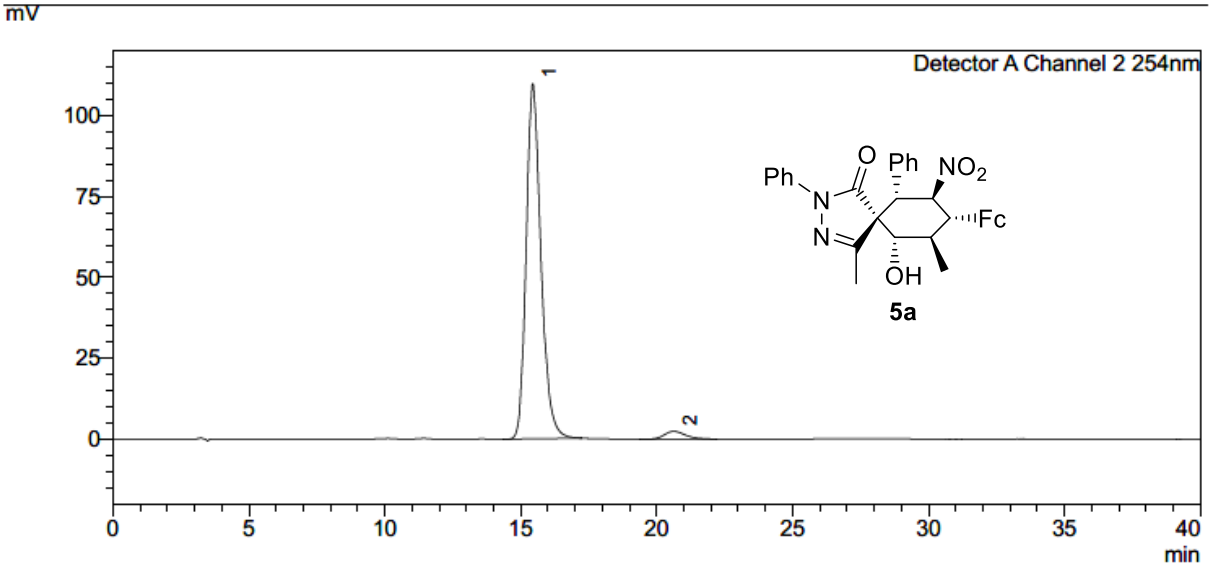


<Peak Table>

Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	15.079	663408	18247	50.285	50.285
2	21.176	655901	13288	49.715	49.715
Total		1319308	31536		100.000

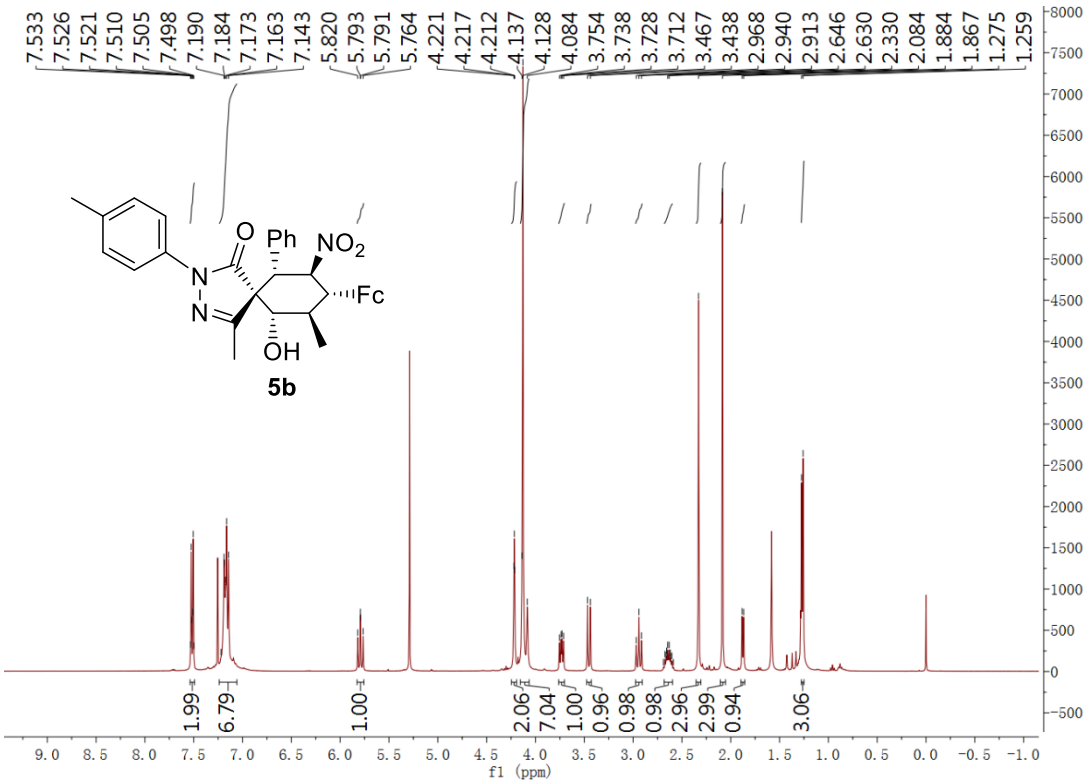
<Chromatogram>

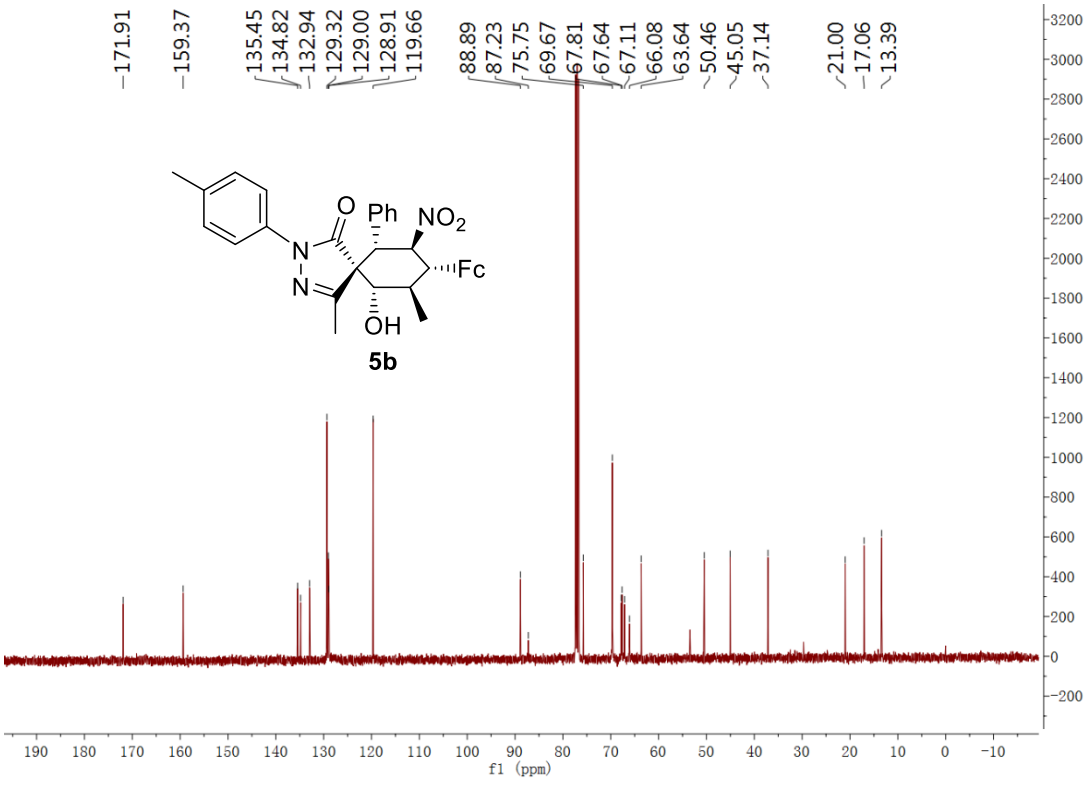


<Peak Table>

Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	15.439	4130184	109762	97.002	97.002
2	20.620	127668	2390	2.998	2.998
Total		4257852	112152		100.000

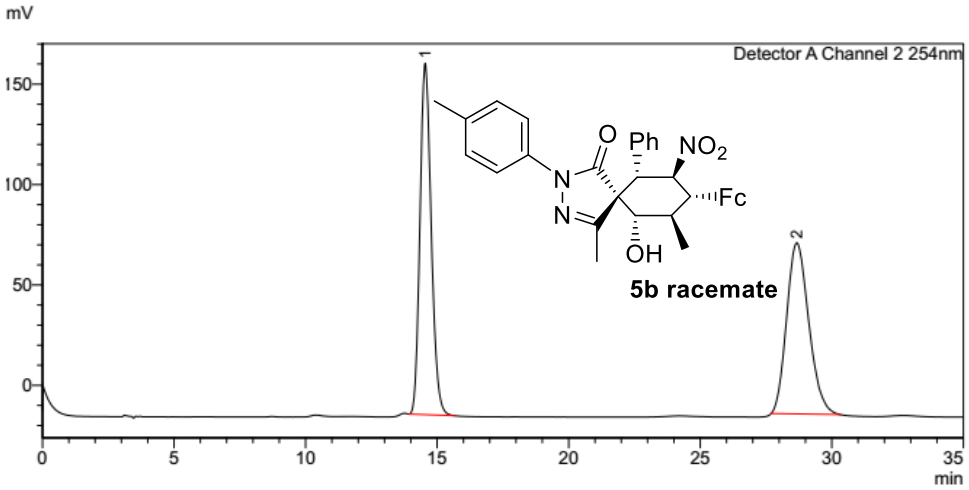

7.533
7.526
7.521
7.510
7.505
7.498
7.190
7.184
7.173
7.163
7.143
5.820
5.793
5.791
5.764
4.221
4.217
4.212
4.137
4.128
4.084
3.754
3.738
3.728
3.712
3.467
3.438
2.968
2.940
2.913
2.646
2.630
2.330
2.084
1.884
1.867
1.275
1.259
O
Ph
NO2
N
N
Fc
OH
5b
1.99
6.79
1.00
2.06
7.04
1.00
0.96
0.98
0.98
2.96
2.99
0.94
3.06
f1 (ppm)


171.91
159.37
135.45
134.82
132.94
129.32
129.00
128.91
119.66
88.89
87.23
75.75
69.67
67.81
67.64
67.11
66.08
63.64
50.46
45.05
37.14
21.00
17.06
13.39
O
Ph
NO2
N
N
Fc
OH
5b
f1 (ppm)

Peak Analysis Report

Detector A Channel 2 254nm

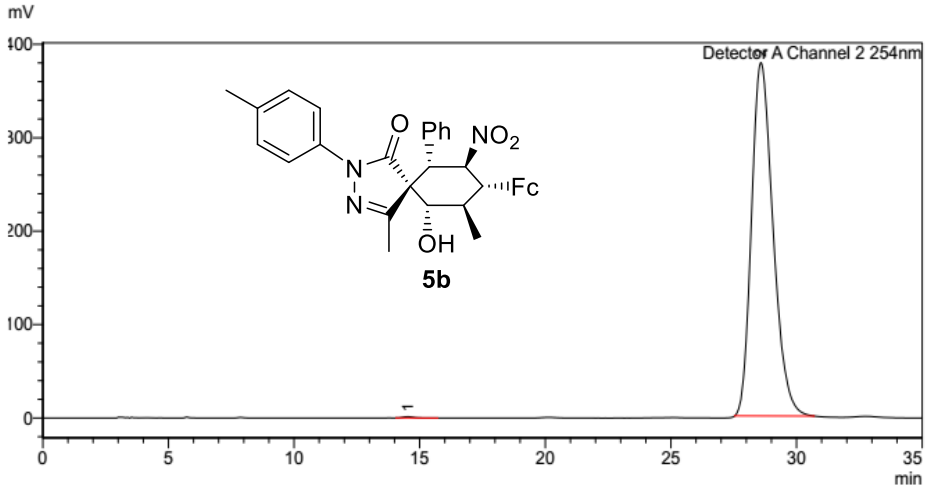
No.	Ret. Time	Height (mAu)	Area (mAu*min)	Rel. Area (%)
1	14.545	174881	5181606	50.999
2	28.669	85194	4978536	49.001
Total		260076	10160143	100.000

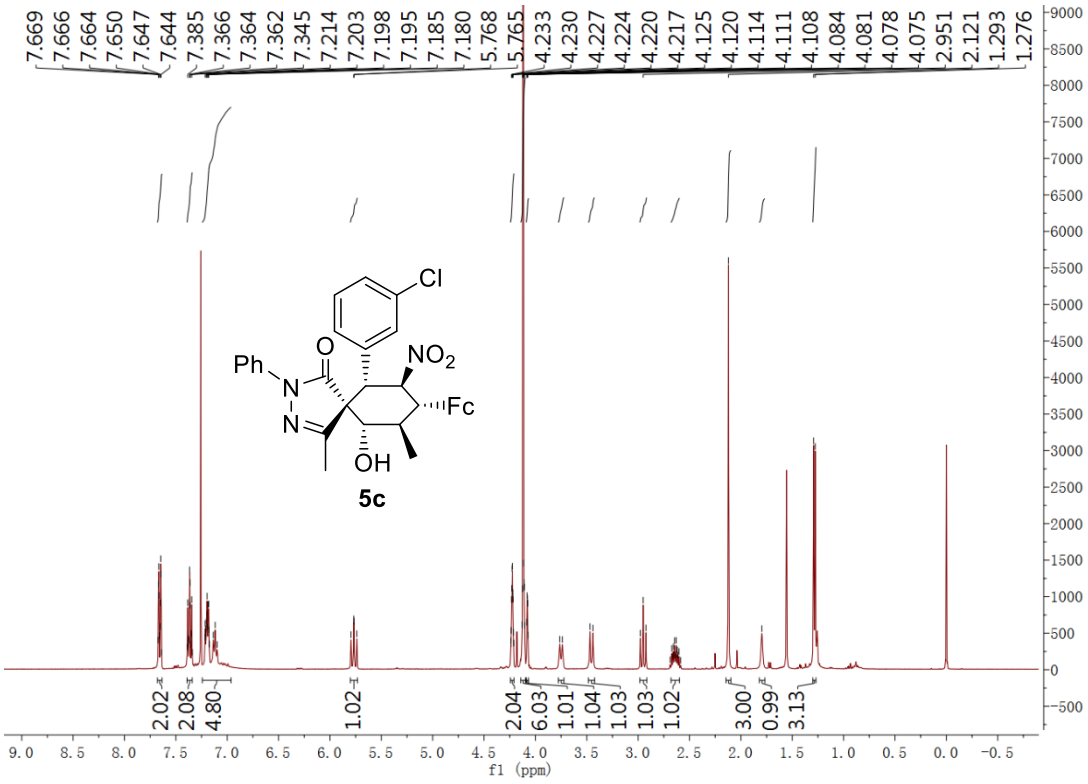


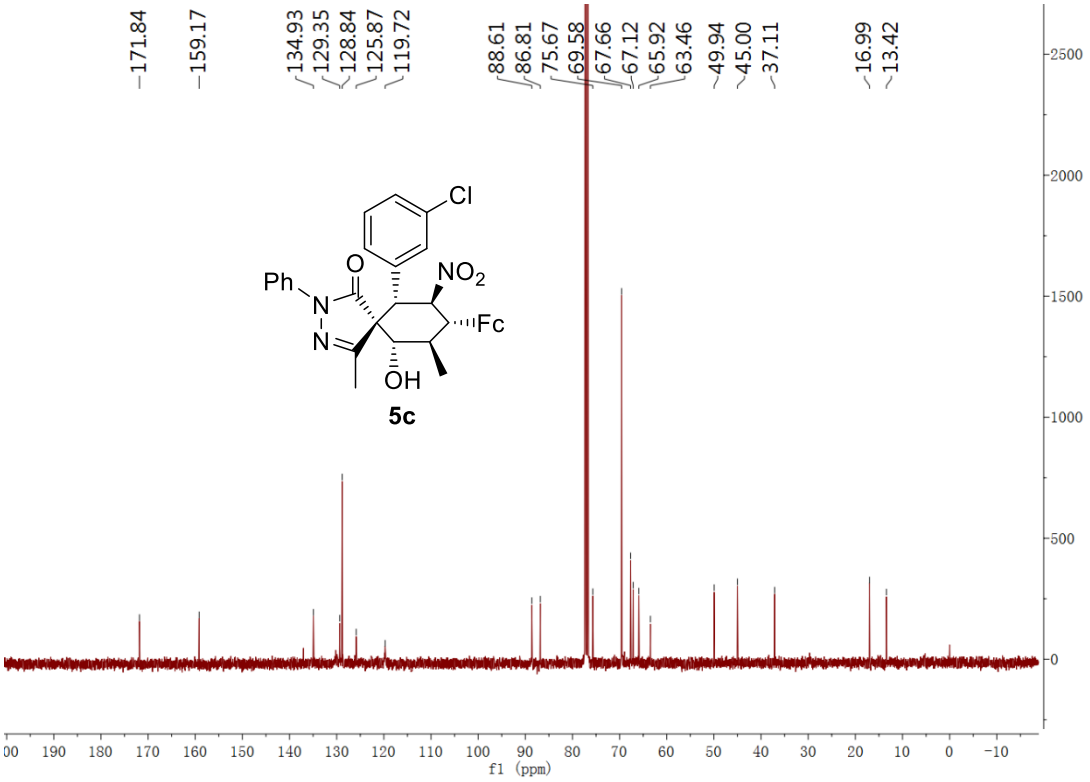
Peak Analysis Report

Detector A Channel 2 254nm

No.	Ret. Time	Height (mAu)	Area (mAu*min)	Rel. Area (%)
1	14.522	1354	38693	0.171
2	28.583	378138	22540514	99.829
Total		379492	22579207	100.000

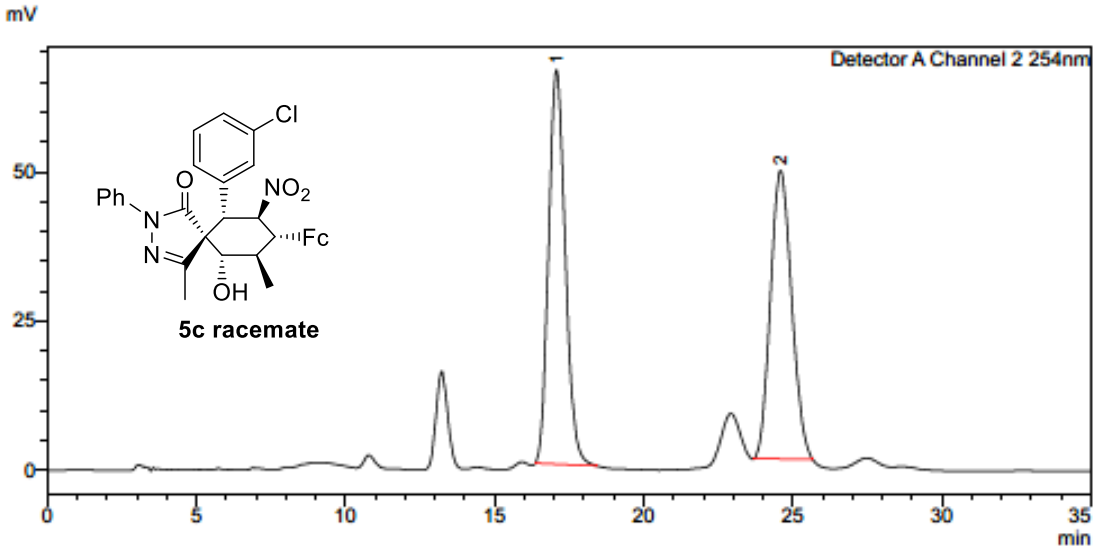



7.669
7.666
7.664
7.650
7.647
7.644
7.385
7.366
7.364
7.362
7.345
7.214
7.203
7.198
7.195
7.185
7.180
5.768
5.765
4.233
4.230
4.227
4.224
4.220
4.217
4.125
4.120
4.114
4.111
4.108
4.084
4.081
4.078
4.075
2.951
2.121
1.293
1.276
Cl
O
NO2
Ph
N
N
Fc
OH
5c
2.02
2.08
4.80
1.02
2.04
6.03
1.01
1.04
1.03
1.03
1.02
3.00
0.99
3.13
f1 (ppm)


171.84
159.17
134.93
129.35
128.84
125.87
119.72
88.61
86.81
75.67
69.58
67.66
67.12
65.92
63.46
49.94
45.00
37.11
16.99
13.42
Cl
O
NO2
Ph
N
N
Fc
OH
5c
f1 (ppm)

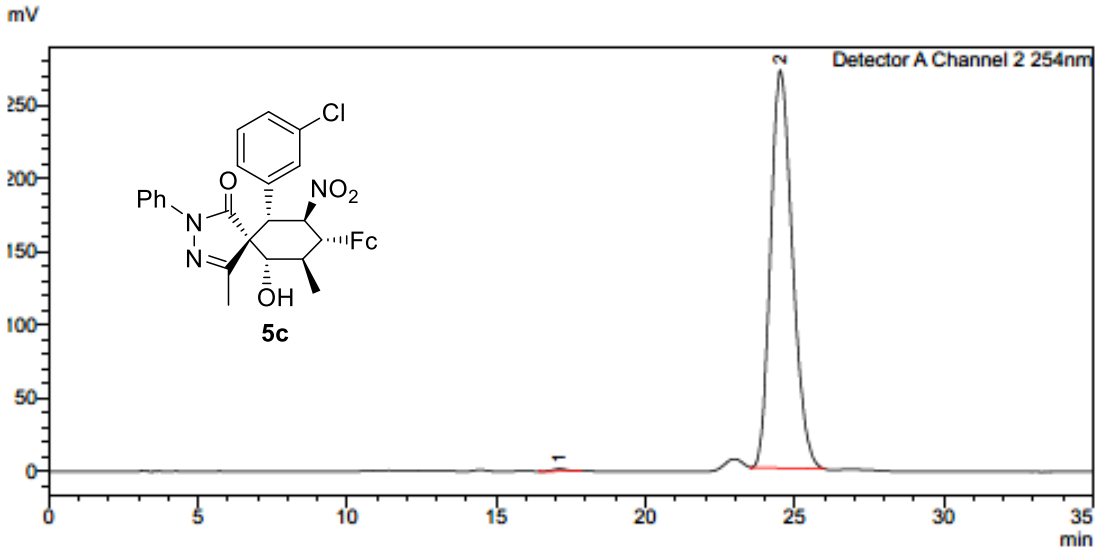
Detector A Channel 2 254nm

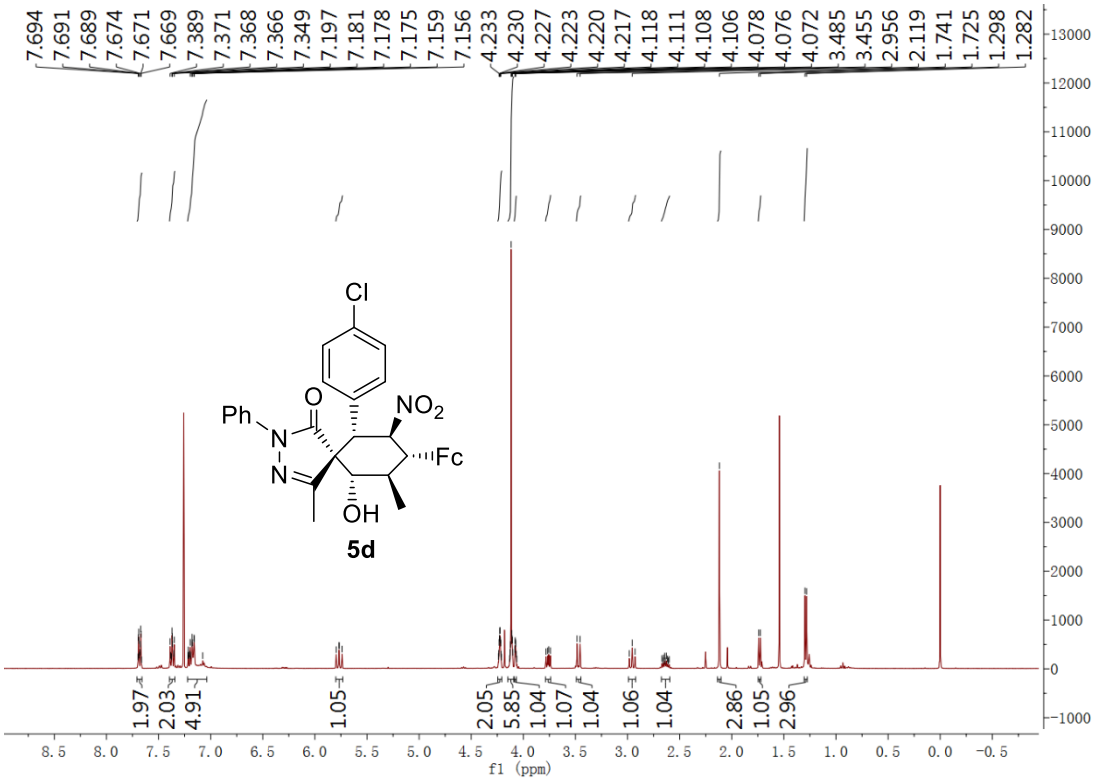
No.	Ret. Time	Height (mAu)	Area (mAu*min)	Rel. Area (%)
1	17.075	66028	2533715	51.573
2	24.588	48313	2379142	48.427
Total		114341	4912857	100.000

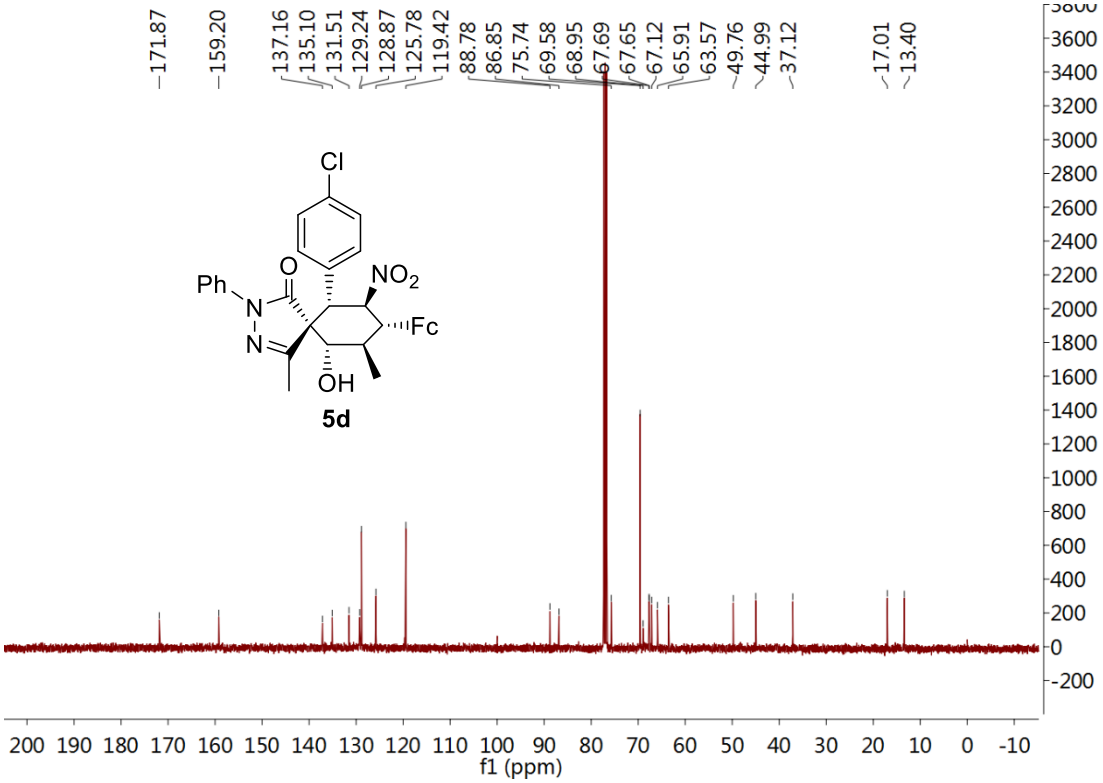


Detector A Channel 2 254nm

No.	Ret. Time	Height (mAu)	Area (mAu*min)	Rel. Area (%)
1	17.111	1529	48693	0.352
2	24.521	271038	13788775	99.648
Total		272567	13837469	100.000

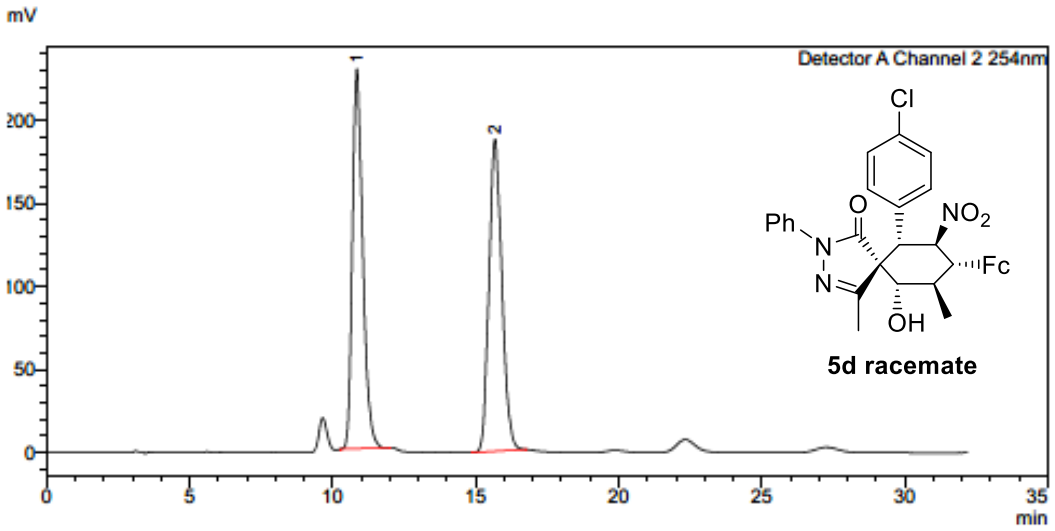



7.694
7.691
7.689
7.674
7.671
7.669
7.389
7.371
7.368
7.366
7.349
7.197
7.181
7.178
7.175
7.159
7.156
4.233
4.230
4.227
4.223
4.220
4.217
4.118
4.111
4.108
4.106
4.078
4.076
4.072
3.485
3.455
2.956
2.119
1.741
1.725
1.298
1.282
Cl
Ph
N
N
O
NO2
Fc
OH
5d
1.97
2.03
4.91
1.05
2.05
5.85
1.04
1.07
1.04
1.06
1.04
2.86
1.05
2.96
f1 (ppm)


171.87
159.20
137.16
135.10
131.51
129.24
128.87
125.78
119.42
88.78
86.85
75.74
69.58
68.95
67.69
67.65
67.12
65.91
63.57
49.76
44.99
37.12
17.01
13.40
Cl
Ph
N
N
O
NO2
Fc
OH
5d
f1 (ppm)

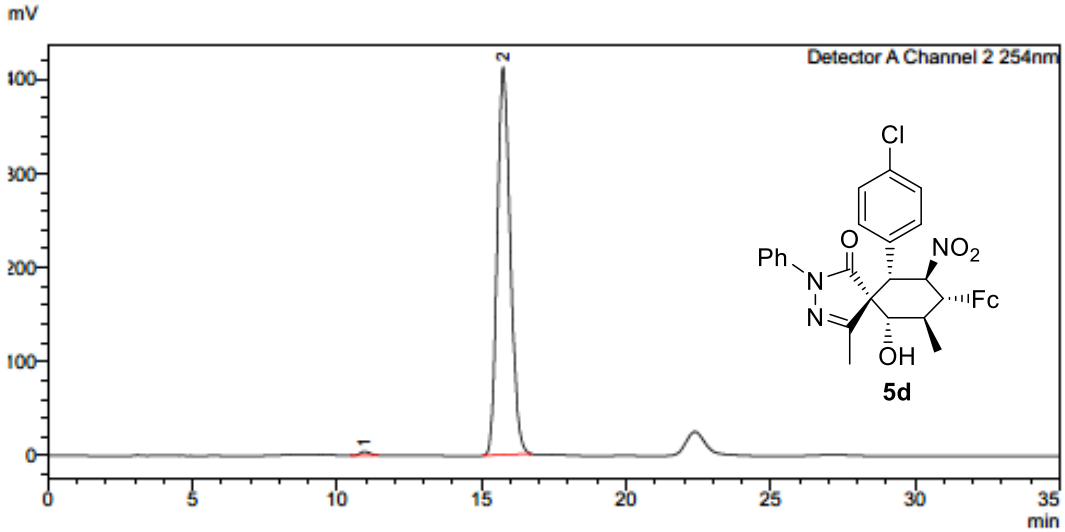
Detector A Channel 2 254nm

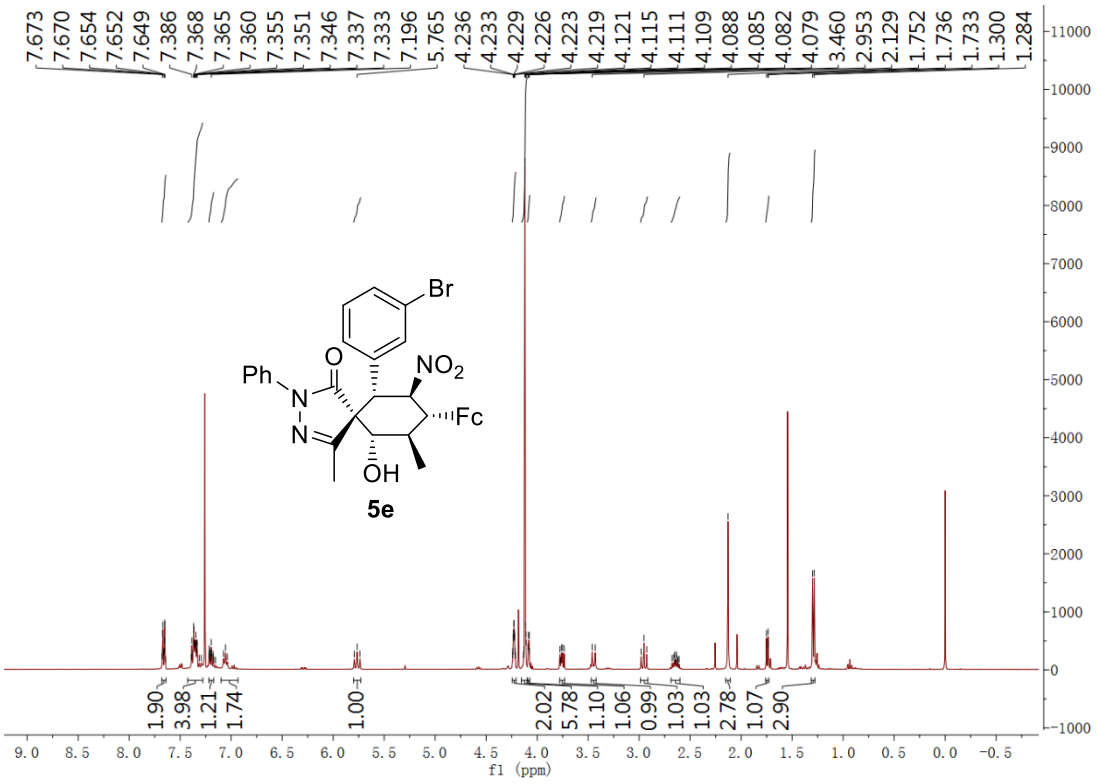
No.	Ret. Time	Height (mAu)	Area (mAu*min)	Rel. Area (%)
1	10.848	228649	5759421	49.503
2	15.678	187857	5875182	50.497
Total		416507	11634603	100.000

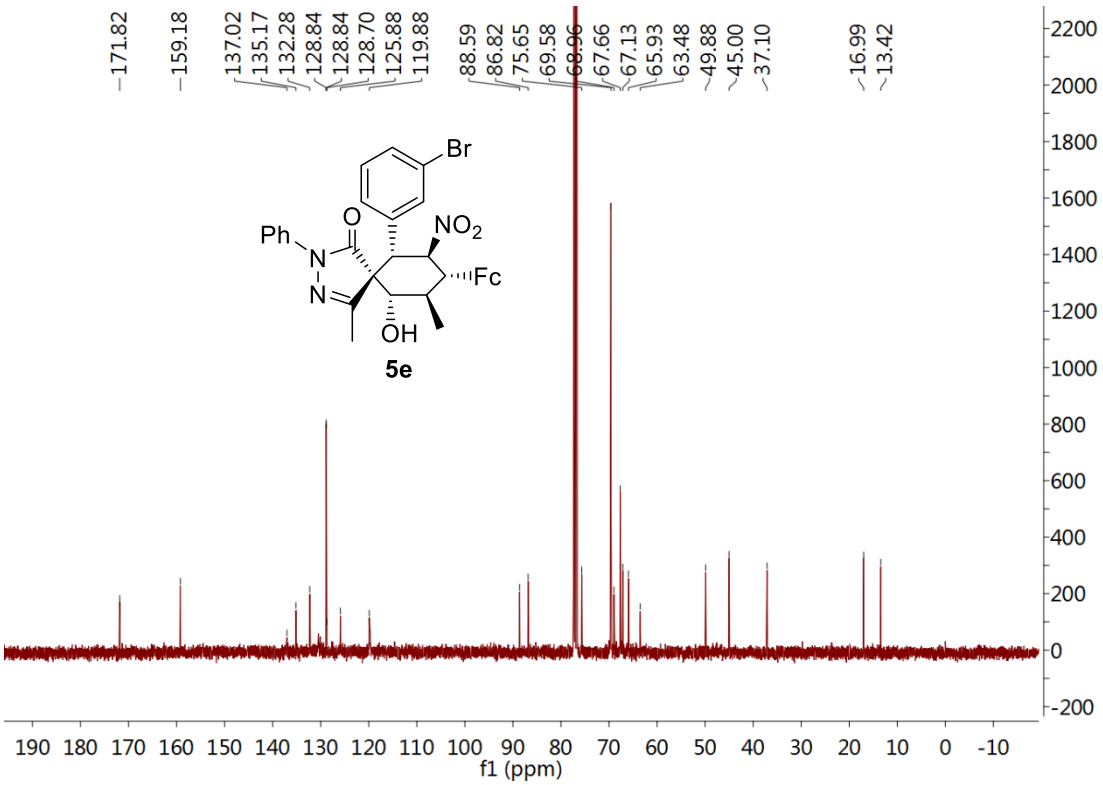


Detector A Channel 2 254nm

No.	Ret. Time	Height (mAu)	Area (mAu*min)	Rel. Area (%)
1	10.955	4184	97596	0.755
2	15.751	411768	12826520	99.245
Total		415952	12924116	100.000

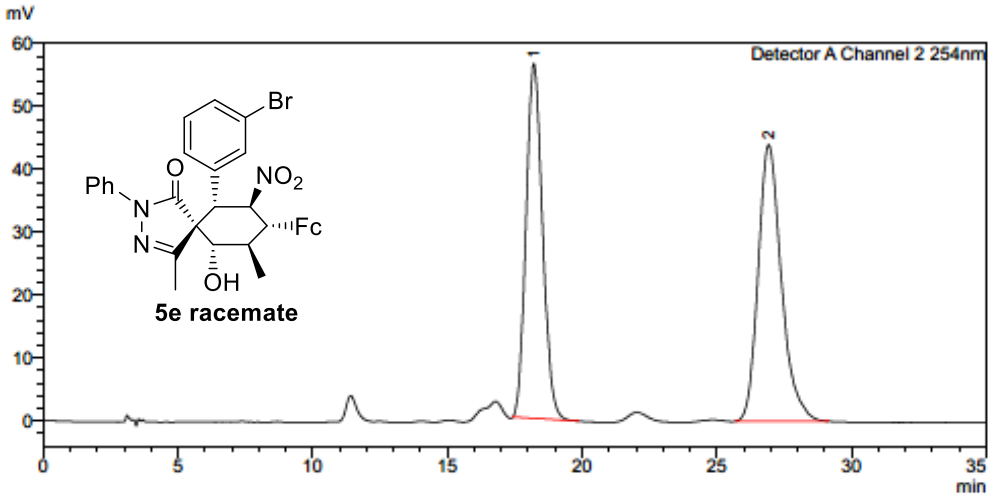



7.673
7.670
7.654
7.652
7.649
7.386
7.368
7.365
7.360
7.355
7.351
7.346
7.337
7.333
7.196
5.765
4.236
4.233
4.229
4.226
4.223
4.219
4.121
4.115
4.111
4.109
4.088
4.085
4.082
4.079
3.460
2.953
2.129
1.752
1.736
1.733
1.300
1.284
Br
O
Ph
N
N
NO2
Fc
OH
5e
1.90
3.98
1.21
1.74
1.00
2.02
5.78
1.10
1.06
0.99
1.03
1.03
2.78
1.07
2.90
f1 (ppm)


171.82
159.18
137.02
135.17
132.28
128.84
128.84
128.70
125.88
119.88
88.59
86.82
75.65
69.58
68.96
67.66
67.13
65.93
63.48
49.88
45.00
37.10
16.99
13.42
Br
O
Ph
N
N
NO2
Fc
OH
5e
f1 (ppm)

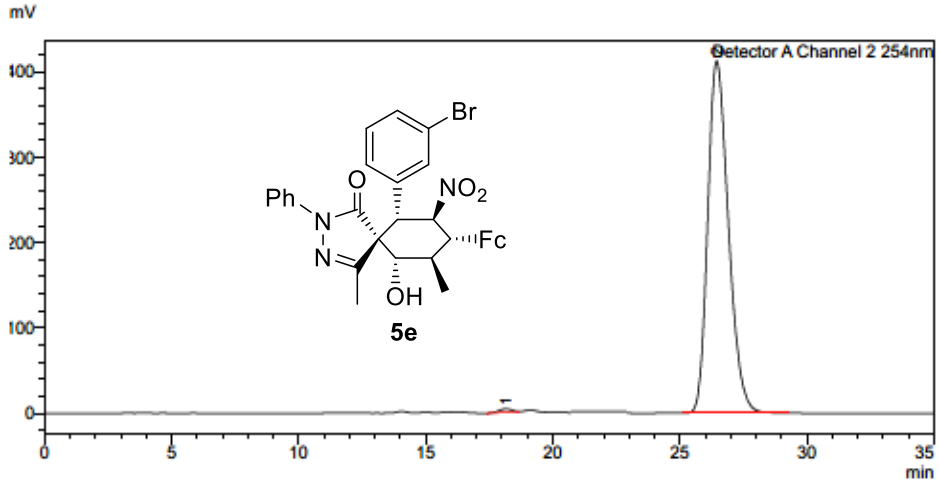
Detector A Channel 2 254nm

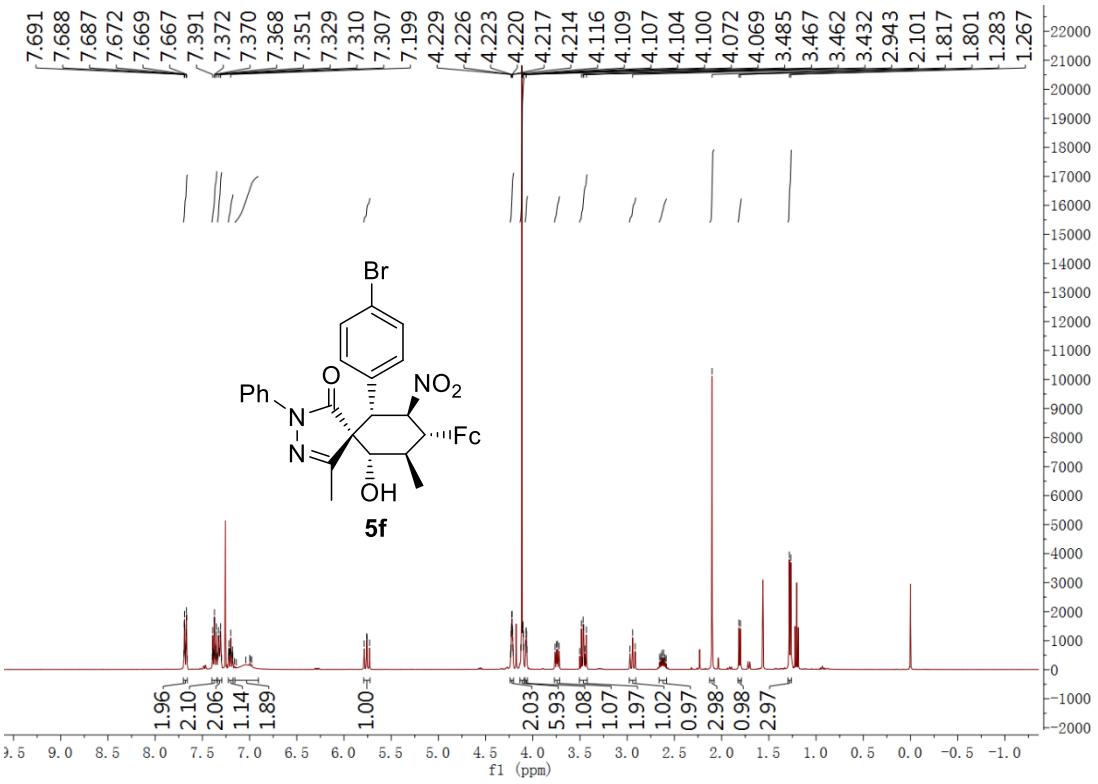
No.	Ret. Time	Height (mAu)	Area (mAu*min)	Rel. Area (%)
1	18.209	56402	2381819	47.562
2	26.928	43964	2625972	52.438
Total		100366	5007791	100.000

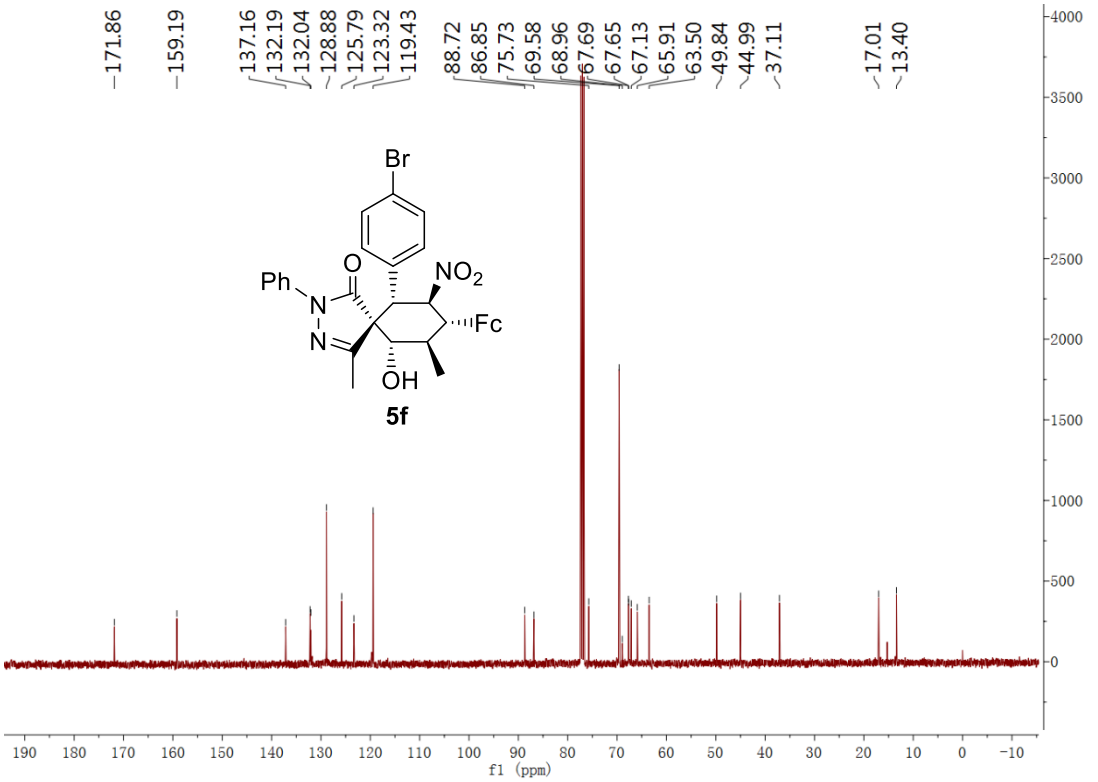


Detector A Channel 2 254nm

No.	Ret. Time	Height (mAu)	Area (mAu*min)	Rel. Area (%)
1	18.155	4100	138508	0.590
2	26.450	412732	23318272	99.410
Total		416833	23456780	100.000

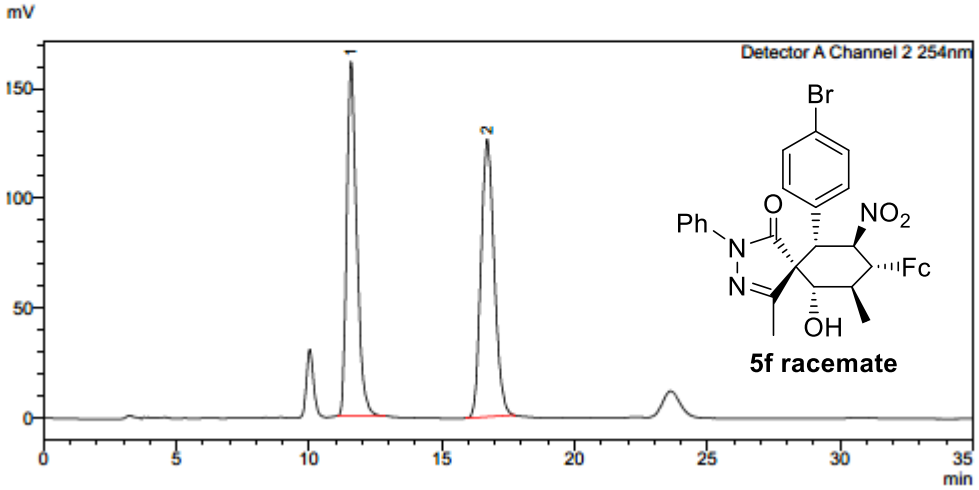



7.691
7.688
7.687
7.672
7.669
7.667
7.391
7.372
7.370
7.368
7.351
7.329
7.310
7.307
7.199
4.229
4.226
4.223
4.220
4.217
4.214
4.116
4.109
4.107
4.104
4.100
4.072
4.069
3.485
3.467
3.462
3.432
2.943
2.101
1.817
1.801
1.283
1.267
Br
O
Ph
N
N
NO2
Fc
OH
5f
1.96
2.10
2.06
1.14
1.89
1.00
2.03
5.93
1.08
1.07
1.97
1.02
0.97
2.98
0.98
2.97
f1 (ppm)


171.86
159.19
137.16
132.19
132.04
128.88
125.79
123.32
119.43
88.72
86.85
75.73
69.58
68.96
67.69
67.65
67.13
65.91
63.50
49.84
44.99
37.11
17.01
13.40
Br
O
Ph
N
N
NO2
Fc
OH
5f
f1 (ppm)

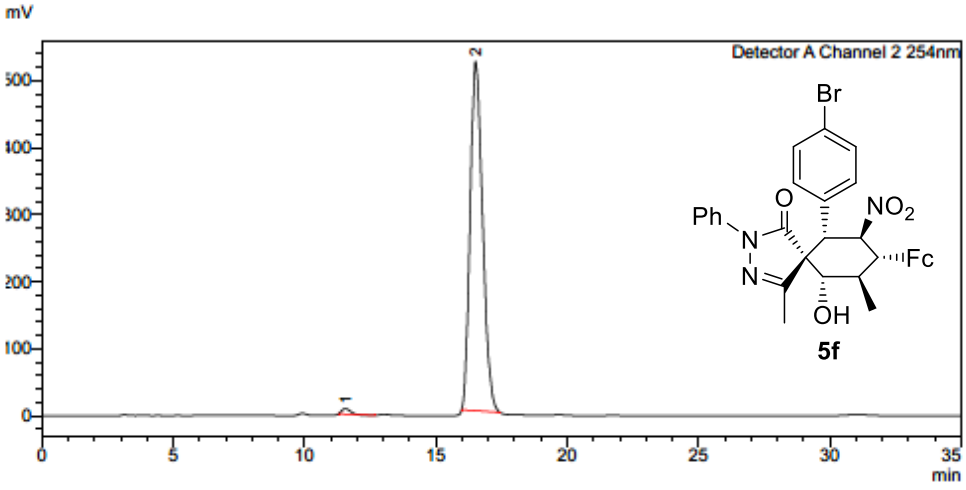
Detector A Channel 2 254nm

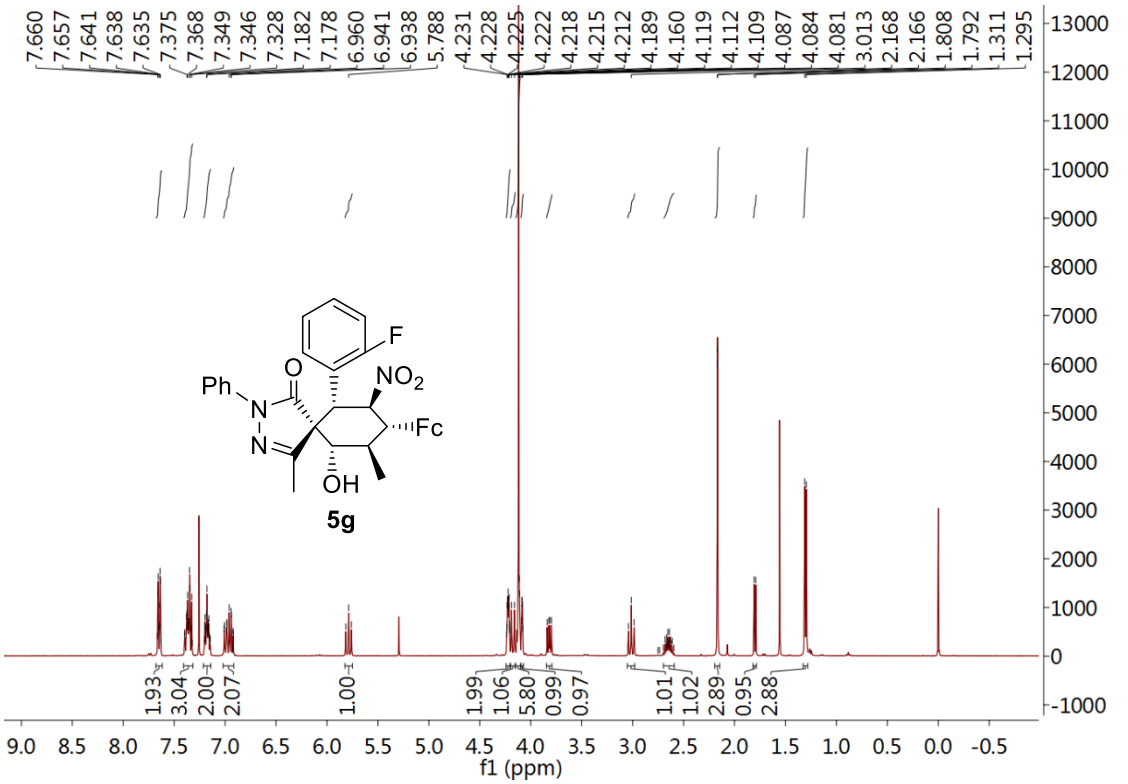
No.	Ret. Time	Height (mAu)	Area (mAu*min)	Rel. Area (%)
1	11.579	161089	4168699	48.896
2	16.710	126259	4356951	51.104
Total		287349	8525650	100.000

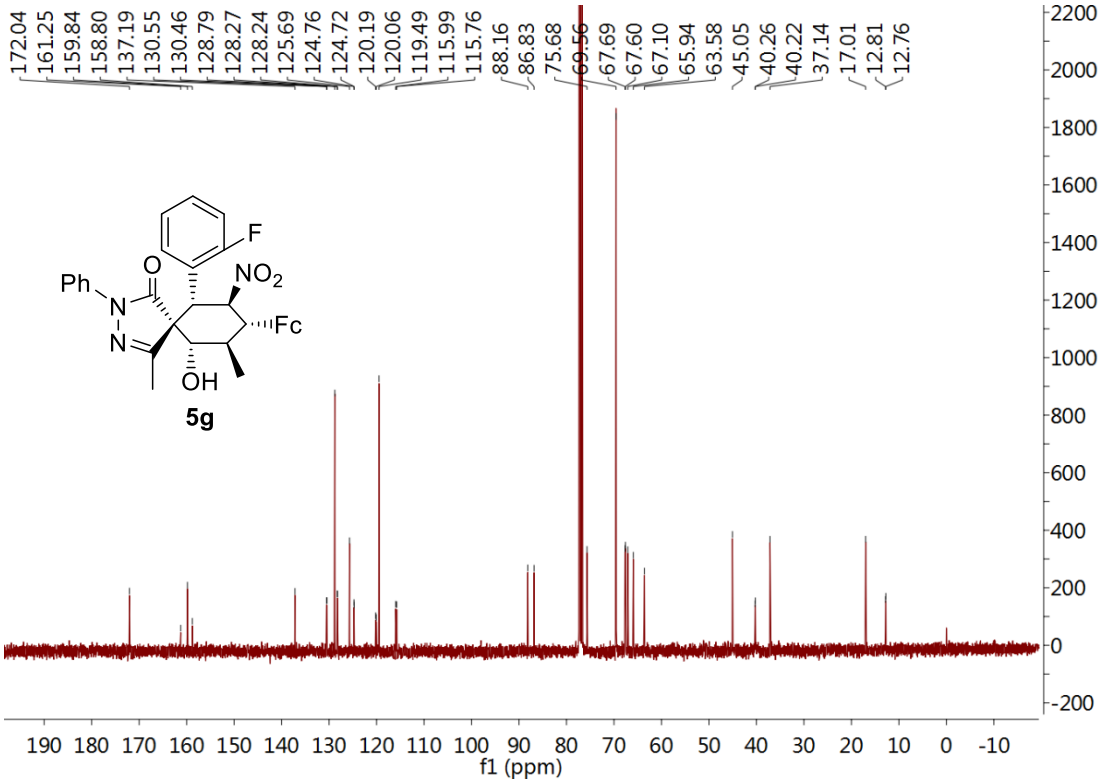


Detector A Channel 2 254nm

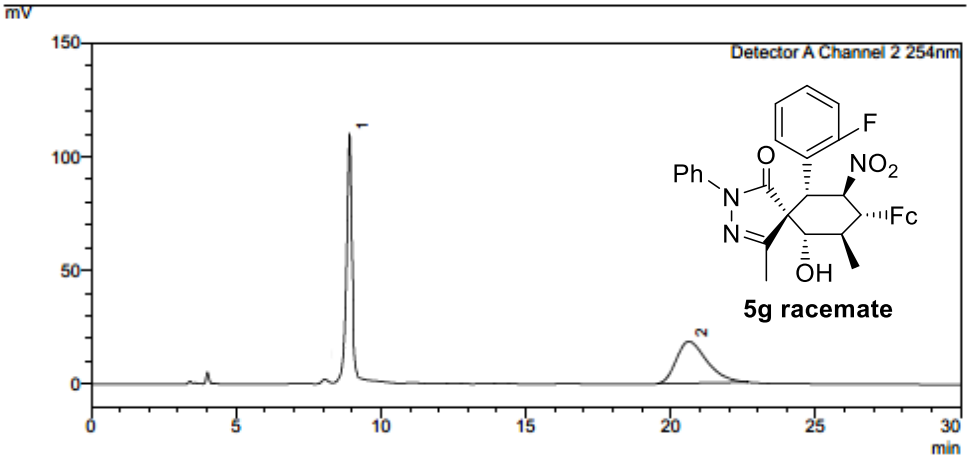
No.	Ret. Time	Height (mAu)	Area (mAu*min)	Rel. Area (%)
1	11.557	8350	162267	0.942
2	16.515	521059	17068853	99.058
Total		529408	17231120	100.000




7.660 7.657 7.641 7.638 7.635 7.375 7.368 7.349 7.346 7.328 7.182 7.178 6.960 6.941 6.938 5.788 4.231 4.228 4.225 4.222 4.218 4.215 4.212 4.189 4.160 4.119 4.112 4.109 4.087 4.084 4.081 3.013 2.168 2.166 1.808 1.792 1.311 1.295
F
O
Ph
N
N
NO2
Fc
OH
5g
1.93 3.04 2.00 2.07 1.00 1.99 1.06 5.80 0.99 0.97 1.01 1.02 2.89 0.95 2.88
f1 (ppm)


172.04 161.25 159.84 158.80 137.19 130.55 130.46 128.79 128.27 128.24 125.69 124.76 124.72 120.19 120.06 119.49 115.99 115.76 88.16 86.83 75.68 69.56 67.69 67.60 67.10 65.94 63.58 45.05 40.26 40.22 37.14 17.01 12.81 12.76
F
O
Ph
N
N
NO2
Fc
OH
5g
f1 (ppm)

<Chromatogram>

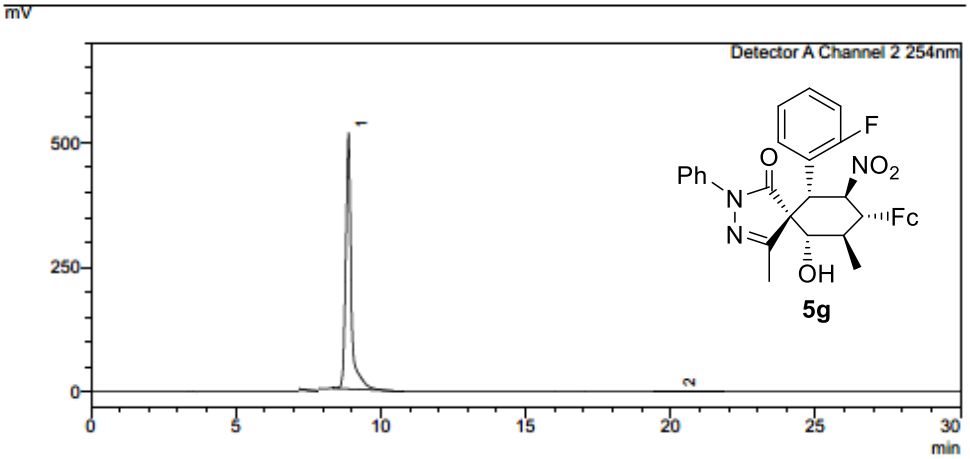


<Peak Table>

Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	8.920	1185509	97975	46.802	46.802
2	20.629	1347506	18361	53.198	53.198
Total		2533015	116336		100.000

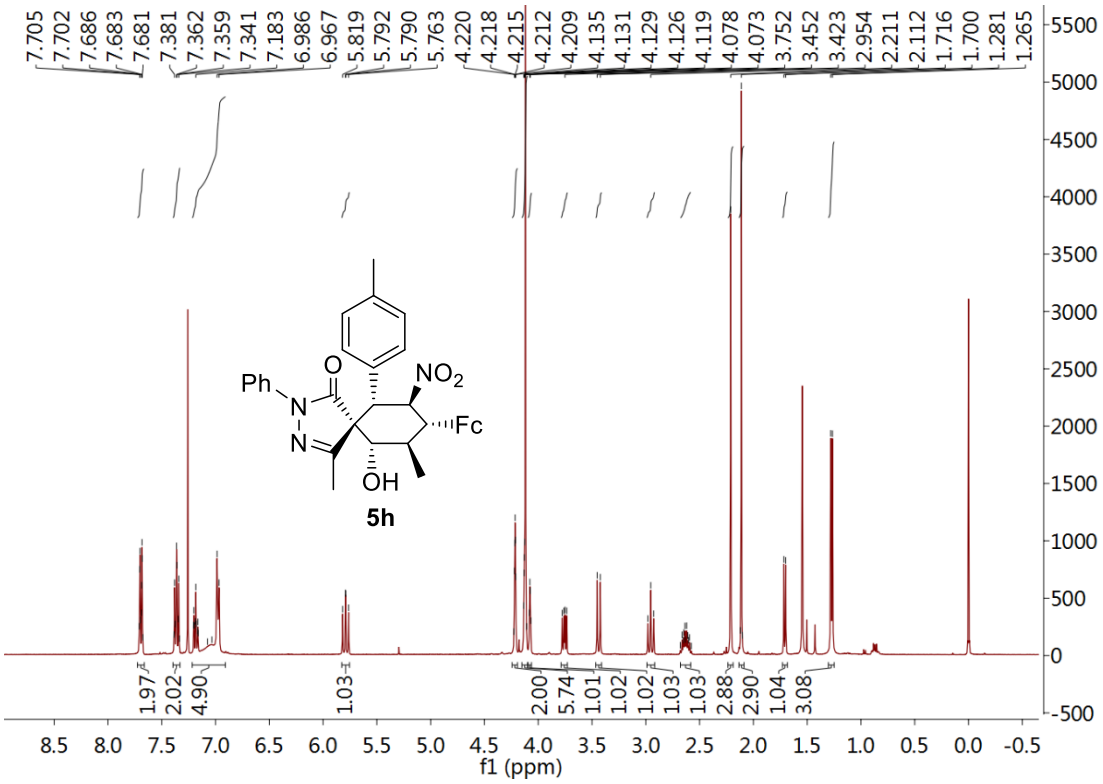
<Chromatogram>

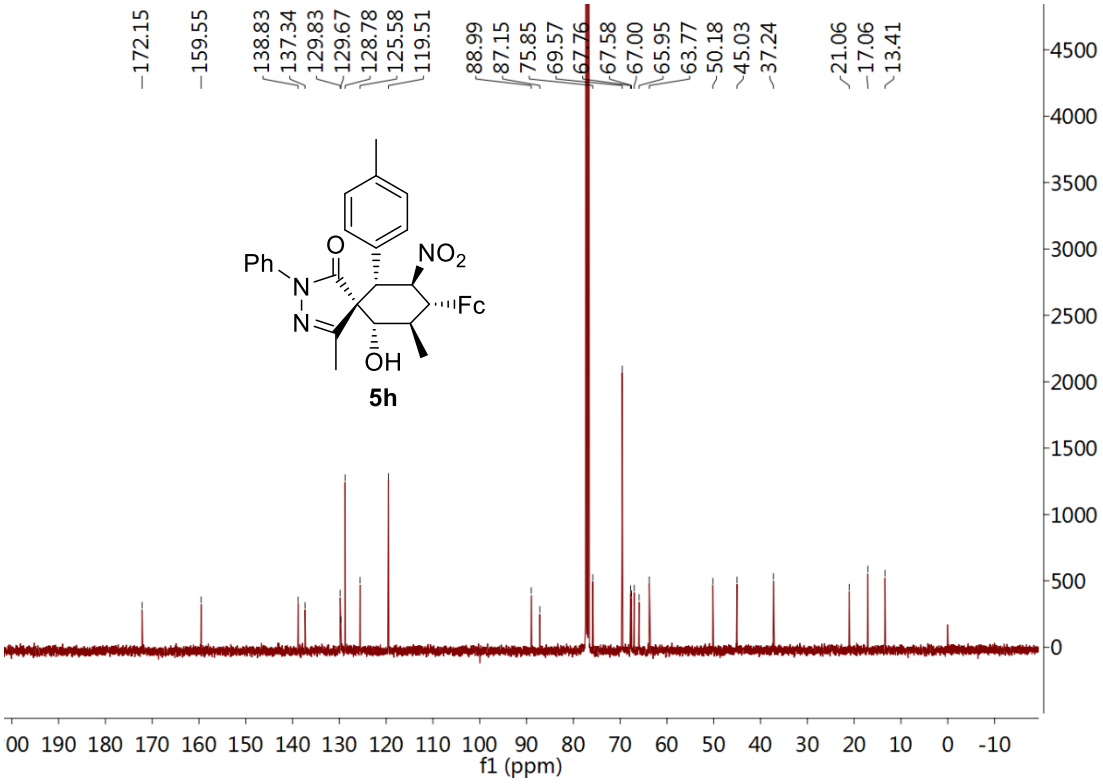


<Peak Table>

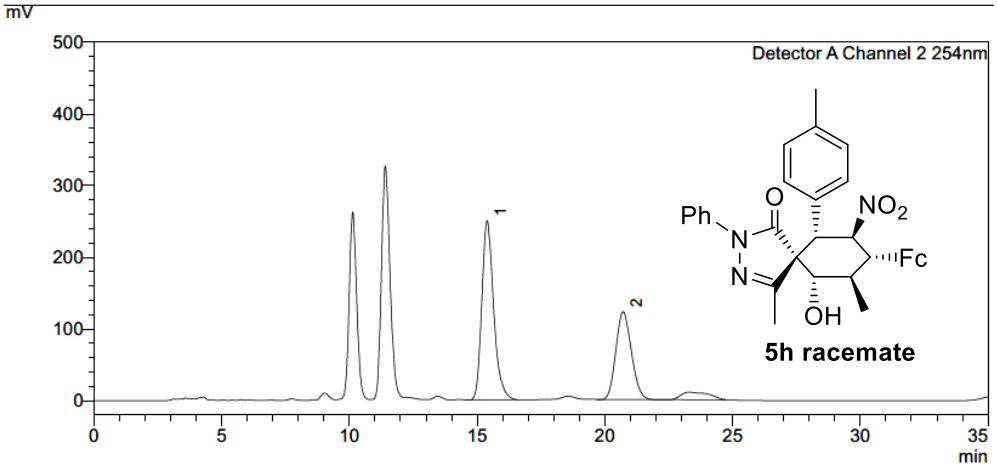
Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	8.890	6813290	514017	99.936	99.936
2	20.221	4342	55	0.064	0.064
Total		6817631	514072		100.000


7.705
7.702
7.686
7.683
7.681
7.381
7.362
7.359
7.341
7.183
6.986
6.967
5.819
5.792
5.790
5.763
4.220
4.218
4.215
4.212
4.209
4.135
4.131
4.129
4.126
4.119
4.078
4.073
3.752
3.452
3.423
2.954
2.211
2.112
1.716
1.700
1.281
1.265
Ph
N
N
O
NO2
Fc
OH
5h
1.97
2.02
4.90
1.03
2.00
5.74
1.01
1.02
1.02
1.03
1.03
2.88
2.90
1.04
3.08
f1 (ppm)


172.15
159.55
138.83
137.34
129.83
129.67
128.78
125.58
119.51
88.99
87.15
75.85
69.57
67.76
67.58
67.00
65.95
63.77
50.18
45.03
37.24
21.06
17.06
13.41
Ph
N
N
O
NO2
Fc
OH
5h
f1 (ppm)

<Chromatogram>



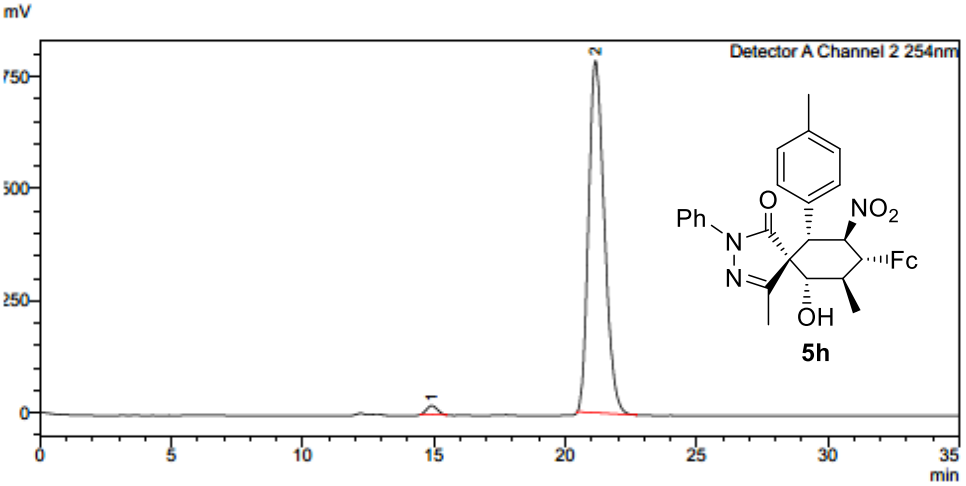
<Peak Table>

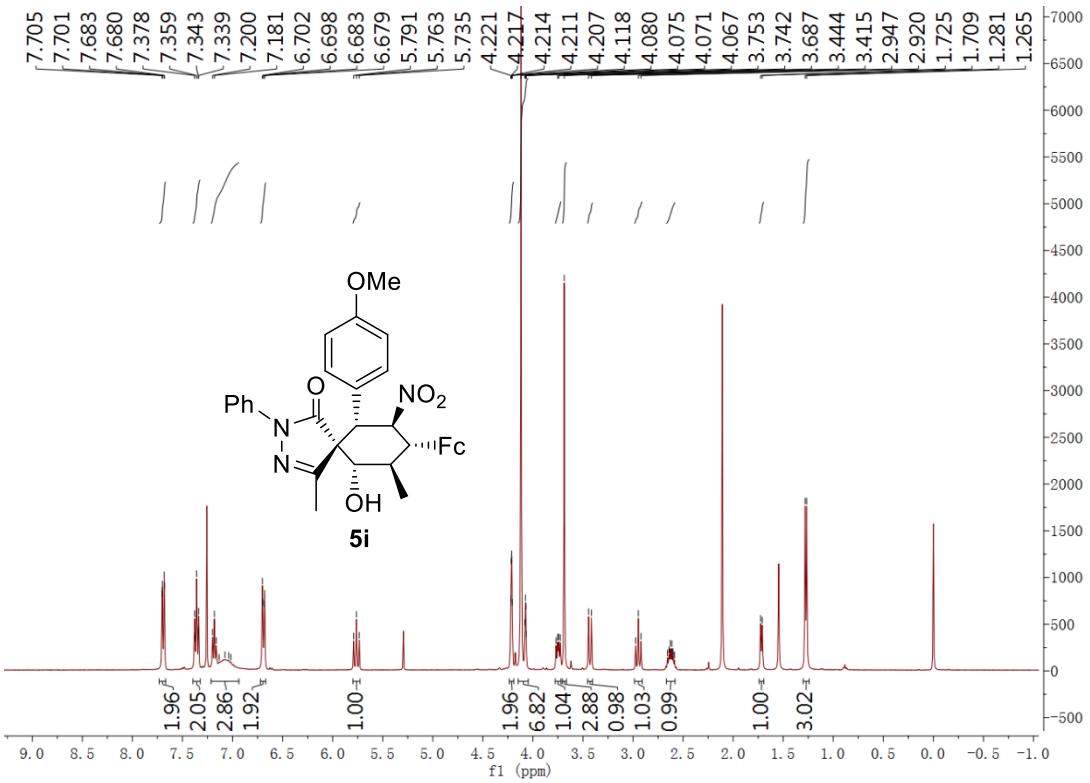
Detector A Channel 2 254nm

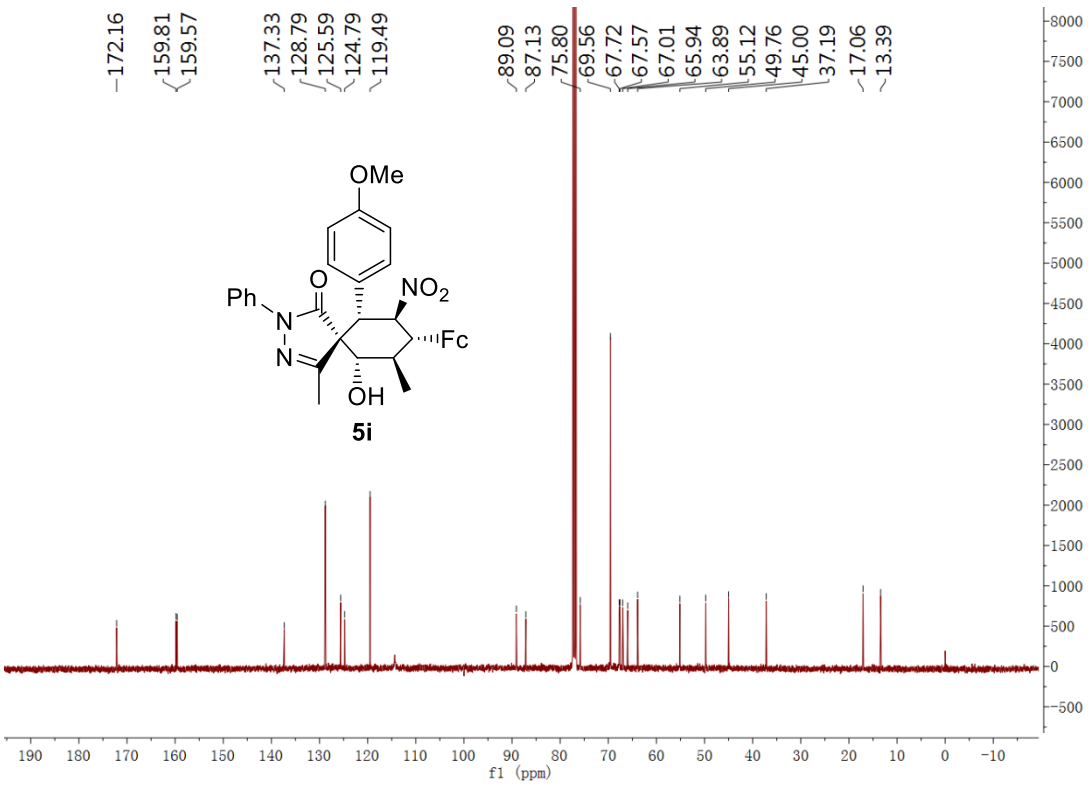
Peak#	Ret. Time	Area	Height	Conc.	Area%
1	15.394	8045622	250034	57.522	57.522
2	20.715	5941365	122587	42.478	42.478
Total		13986987	372621		100.000

Detector A Channel 2 254nm

No.	Ret. Time	Height (mAu)	Area (mAu*min)	Rel. Area (%)
1	14.917	19699	548960	1.650
2	21.154	785684	32711437	98.350
Total		805383	33260397	100.000

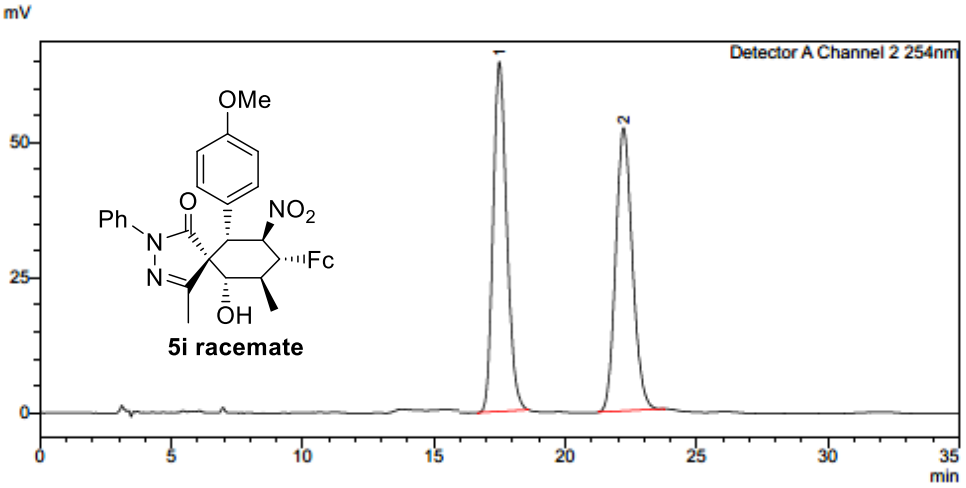



7.705
7.701
7.683
7.680
7.378
7.359
7.343
7.339
7.200
7.181
6.702
6.698
6.683
6.679
5.791
5.763
5.735
4.221
4.217
4.214
4.211
4.207
4.118
4.080
4.075
4.071
4.067
3.753
3.742
3.687
3.444
3.415
2.947
2.920
1.725
1.709
1.281
1.265
OMe
Ph
N
N
O
NO2
Fc
OH
5i
1.96
2.05
2.86
1.92
1.00
1.96
6.82
1.04
2.88
0.98
1.03
0.99
1.00
3.02
f1 (ppm)


172.16
159.81
159.57
137.33
128.79
125.59
124.79
119.49
89.09
87.13
75.80
69.56
67.72
67.57
67.01
65.94
63.89
55.12
49.76
45.00
37.19
17.06
13.39
OMe
Ph
N
N
O
NO2
Fc
OH
5i
f1 (ppm)

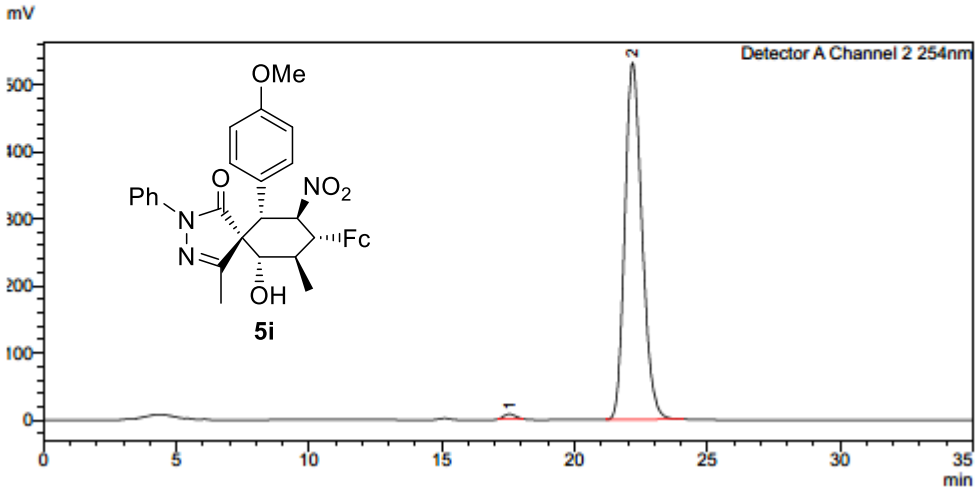
Detector A Channel 2 254nm

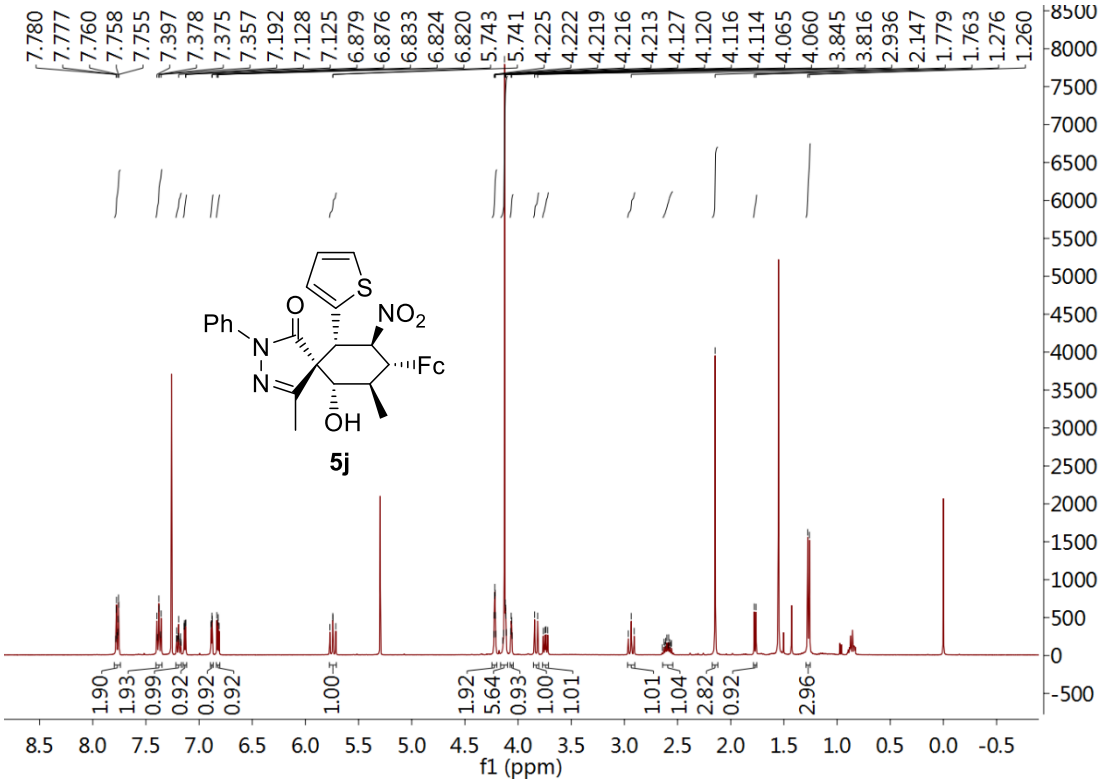
No.	Ret. Time	Height (mAu)	Area (mAu*min)	Rel. Area (%)
1	17.493	64706	2329282	50.155
2	22.220	52358	2314849	49.845
Total		117064	4644131	100.000

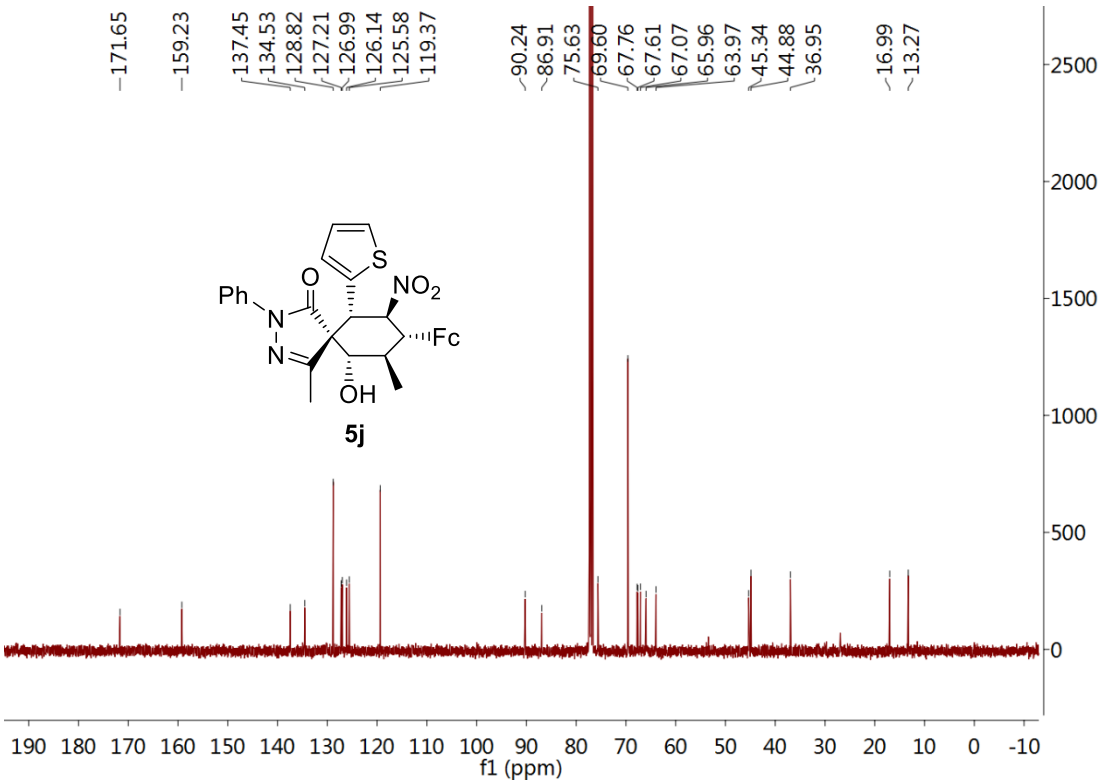


Detector A Channel 2 254nm

No.	Ret. Time	Height (mAu)	Area (mAu*min)	Rel. Area (%)
1	17.544	7017	203568	0.845
2	22.186	531335	23898685	99.155
Total		538351	24102253	100.000

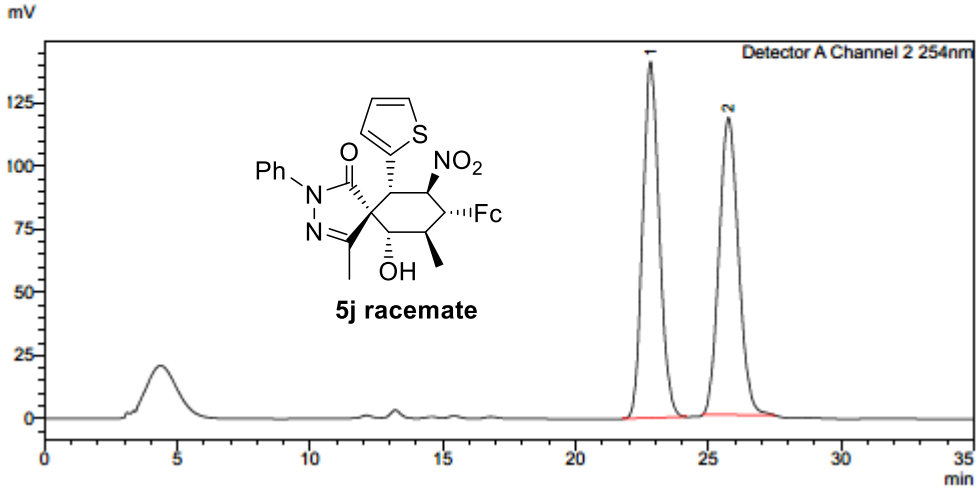



7.780 7.777 7.760 7.758 7.755 7.397 7.378 7.375 7.357 7.192 7.128 7.125 6.879 6.876 6.833 6.824 6.820 5.743 5.741 4.225 4.222 4.219 4.216 4.213 4.127 4.120 4.116 4.114 4.065 4.060 3.845 3.816 2.936 2.147 1.779 1.763 1.276 1.260
1.90 1.93 0.99 0.92 0.92 0.92 1.00 1.92 5.64 0.93 1.00 1.01 1.01 1.04 2.82 0.92 2.96
f1 (ppm)
Ph
N
N
O
S
NO2
Fc
OH
5j


171.65 159.23 137.45 134.53 128.82 127.21 126.99 126.14 125.58 119.37 90.24 86.91 75.63 69.60 67.76 67.61 67.07 65.96 63.97 45.34 44.88 36.95 16.99 13.27
f1 (ppm)
Ph
N
N
O
S
NO2
Fc
OH
5j

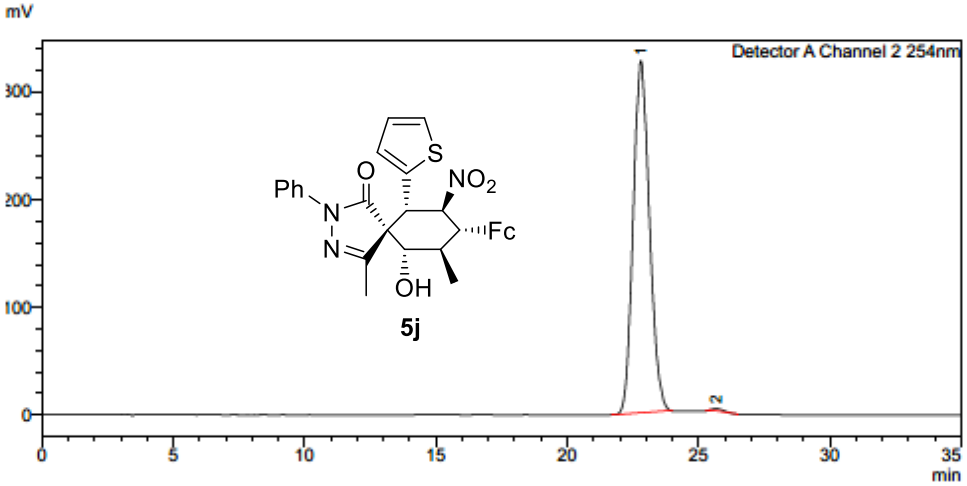
Detector A Channel 2 254nm

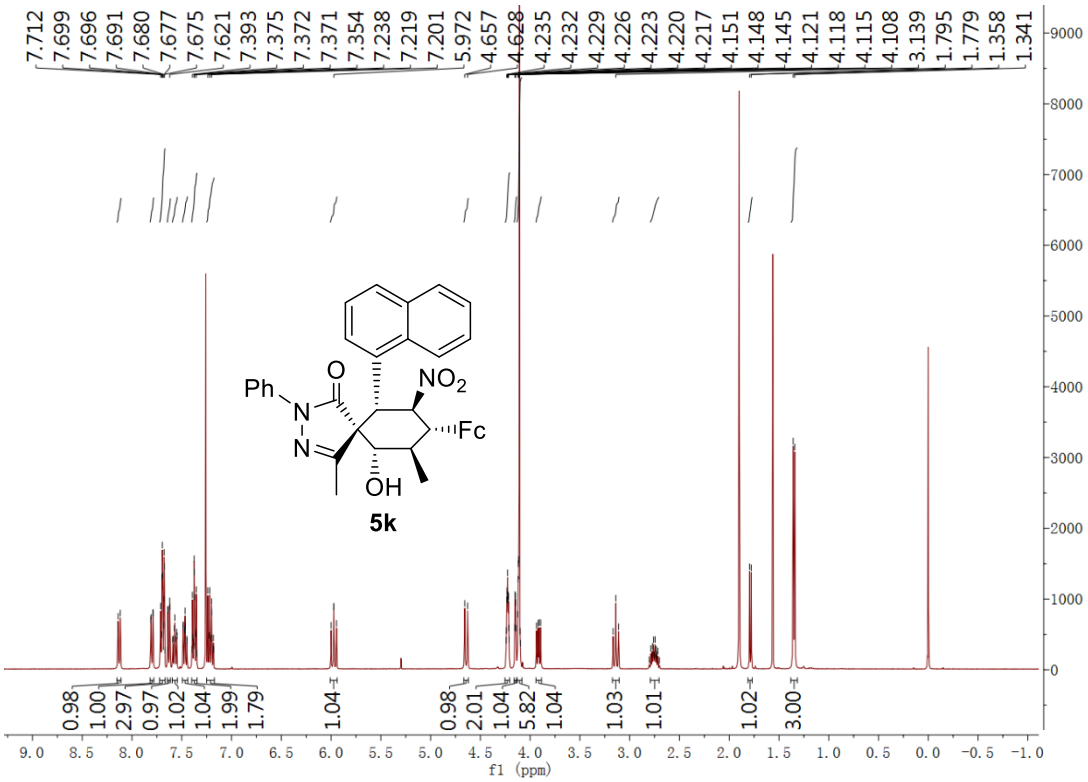
No.	Ret. Time	Height (mAu)	Area (mAu*min)	Rel. Area (%)
1	22.825	140983	6101571	50.541
2	25.757	117677	5970905	49.459
Total		258660	12072476	100.000



Detector A Channel 2 254nm

No.	Ret. Time	Height (mAu)	Area (mAu*min)	Rel. Area (%)
1	22.803	326941	14070994	99.656
2	25.664	1834	48624	0.344
Total		328775	14119618	100.000

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7.691
7.680
7.677
7.675
7.621
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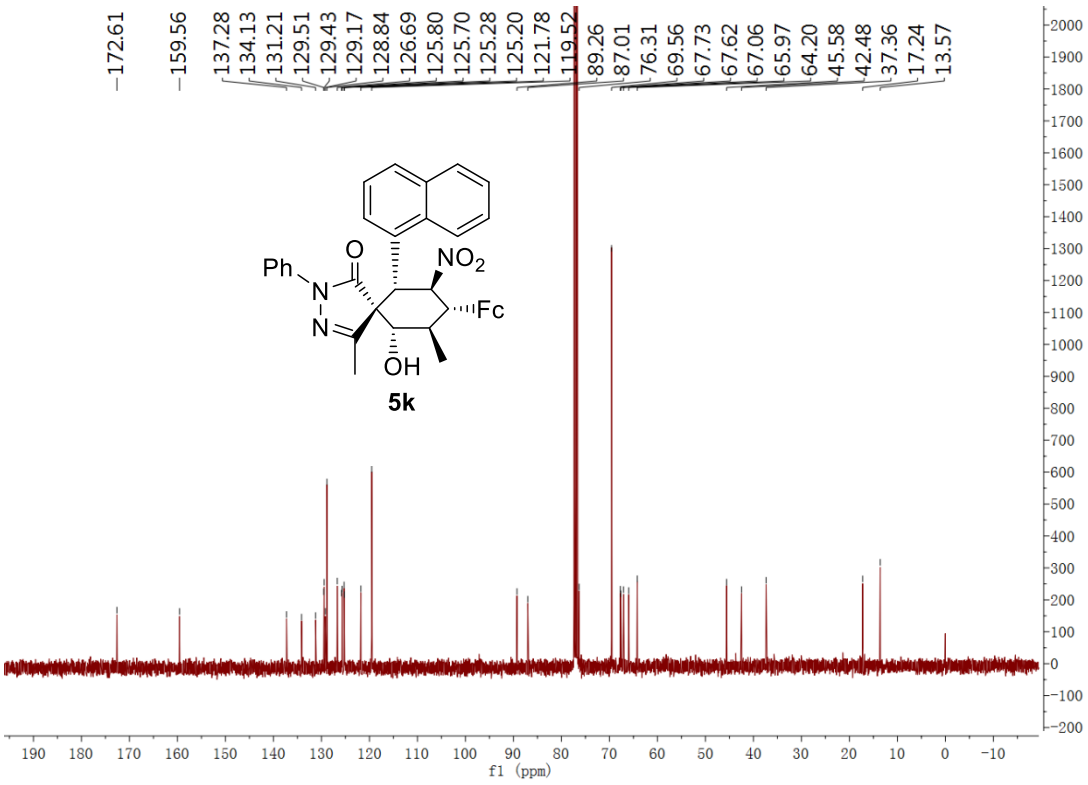
7.375
7.372
7.371
7.354
7.238
7.219
7.201
5.972
4.657
4.628
4.235
4.232
4.229
4.226
4.223
4.220
4.217
4.151
4.148
4.145
4.121
4.118
4.115
4.108
3.139
1.795
1.779
1.358
1.341
Ph
O
N
N
NO2
Fc
OH
5k
0.98
1.00
2.97
0.97
1.02
1.04
1.99
1.79
1.04
0.98
2.01
1.04
5.82
1.04
1.03
1.01
1.02
3.00
f1 (ppm)


172.61
159.56
137.28
134.13
131.21
129.51
129.43
129.17
128.84
126.69
125.80
125.70
125.28
125.20
121.78
119.52
89.26
87.01
76.31
69.56
67.73
67.62
67.06
65.97
64.20
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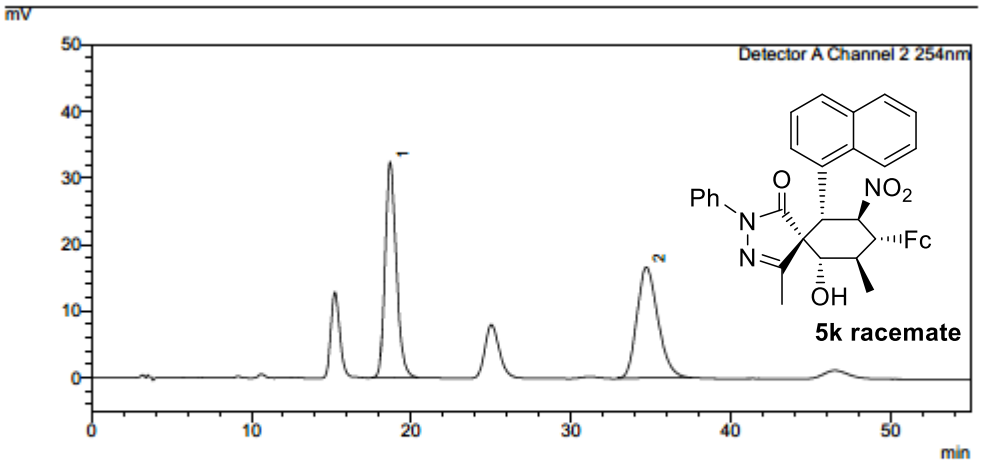
13.57
Ph
O
N
N
NO2
Fc
OH
5k
f1 (ppm)

<Chromatogram>



<Peak Table>

Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	18.686	1561365	32389	50.680	50.680
2	34.719	1519490	16643	49.320	49.320
Total		3080855	49032		100.000

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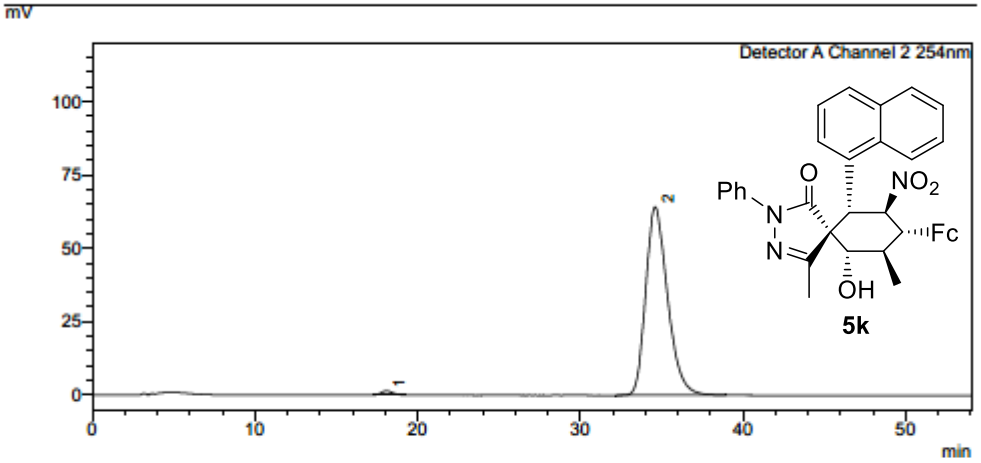
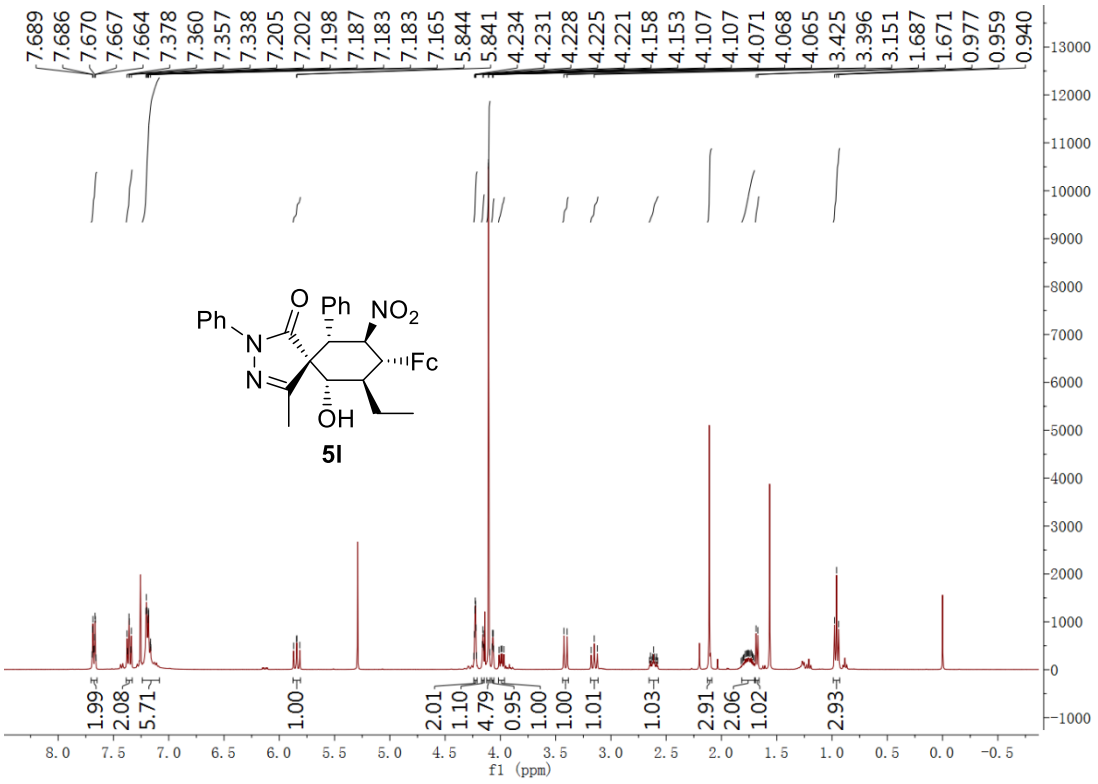


<Peak Table>

Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	18.094	66853	1407	1.105	1.105
2	34.610	5984236	64148	98.895	98.895
Total		6051088	65554		100.000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7.202
7.198
7.187
7.183
7.183
7.165
5.844
5.841
4.234
4.231
4.228
4.225
4.221
4.158
4.153
4.107
4.107
4.071
4.068
4.065
3.425
3.396
3.151
1.687
1.671
0.977
0.959
0.940
Ph
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Ph
NO2
N
N
Fc
OH
5l
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2.08
5.71
1.00
2.01
1.10
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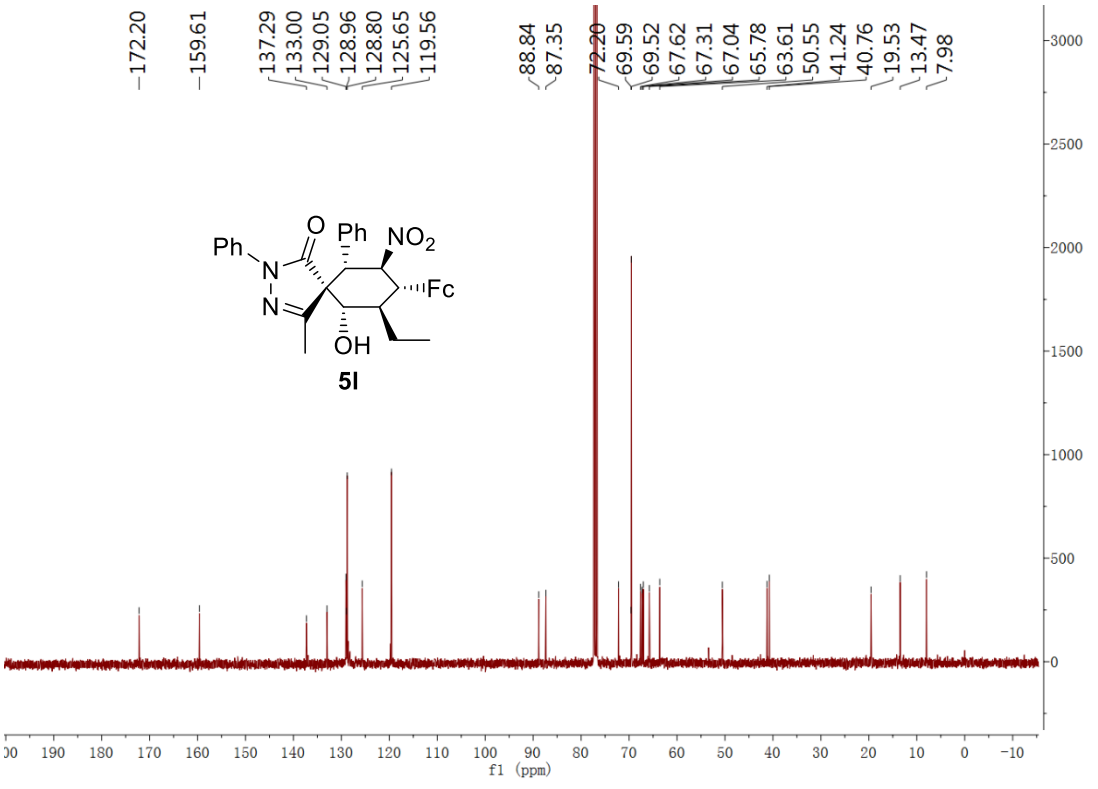
1.00
1.00
1.01
1.03
2.91
2.06
1.02
2.93
f1 (ppm)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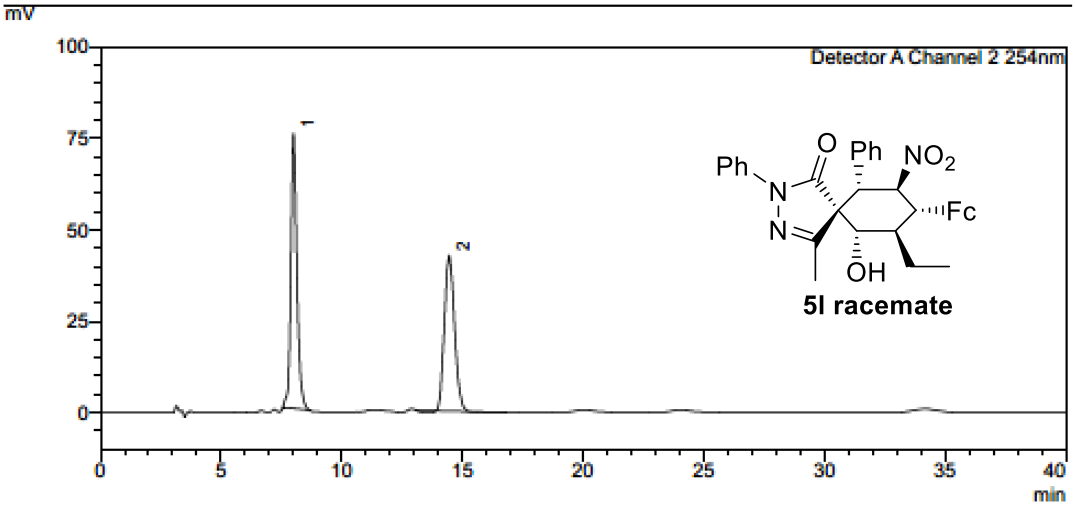
133.00
129.05
128.96
128.80
125.65
119.56
88.84
87.35
72.20
69.59
69.52
67.62
67.31
67.04
65.78
63.61
50.55
41.24
40.76
19.53
13.47
7.98
Ph
O
Ph
NO2
N
N
Fc
OH
5l
f1 (ppm)

<Chromatogram>

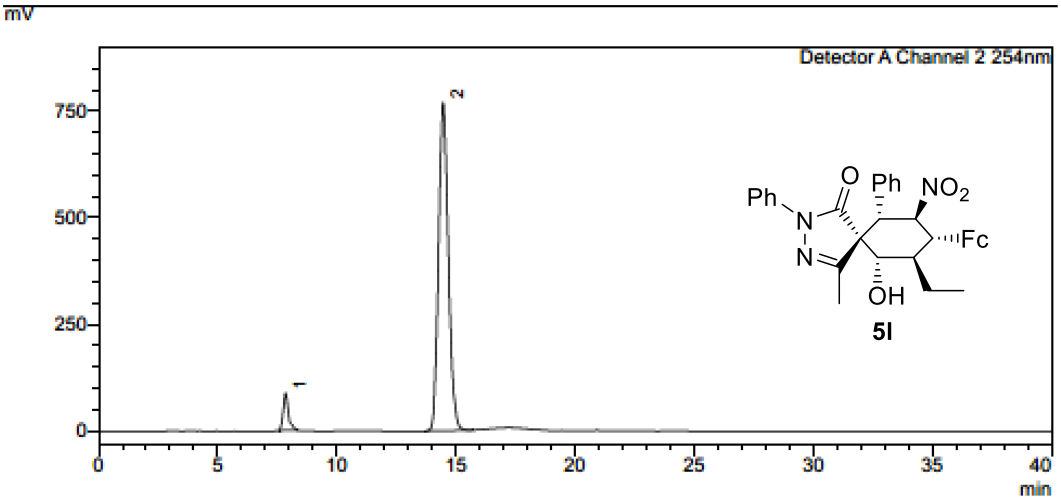


<Peak Table>

Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	8.002	1321233	75342	52.206	52.206
2	14.449	1209596	42582	47.794	47.794
Total		2530829	117924		100.000

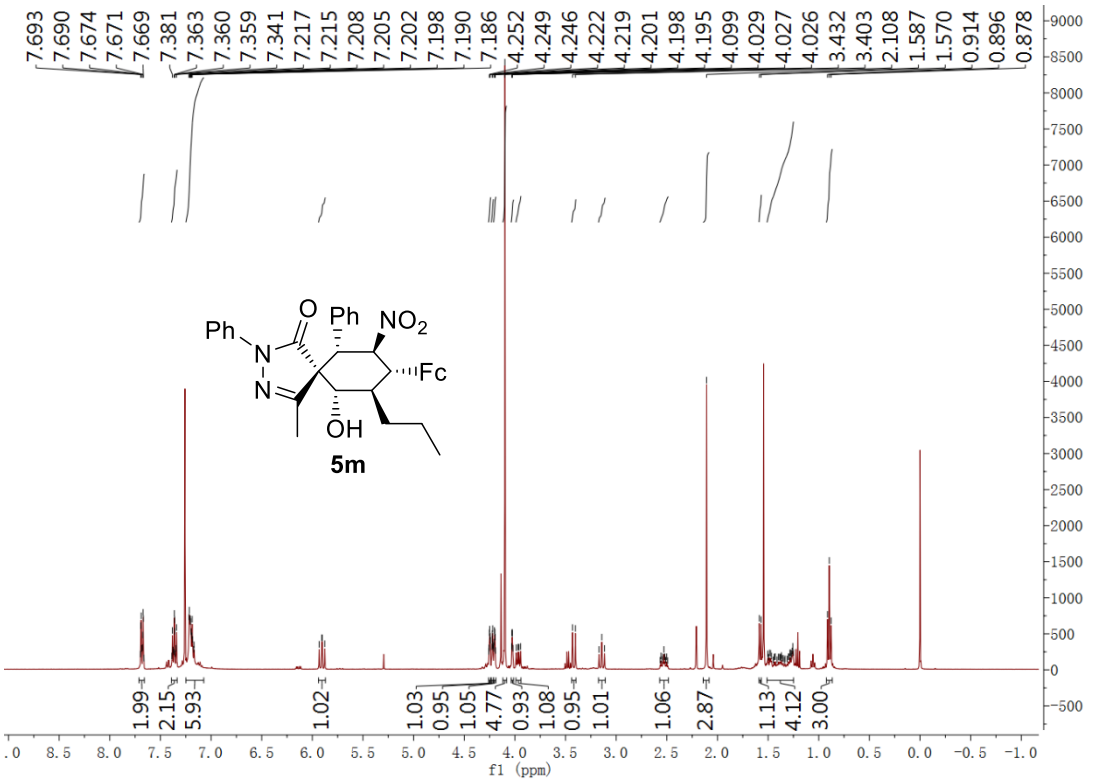
<Chromatogram>



<Peak Table>

Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	7.945	933575	32608	4.069	4.069
2	14.460	22010035	768765	95.931	95.931
Total		22943610	801373		100.000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7.671
7.669
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7.360
7.359
7.341
7.217
7.215
7.208
7.205
7.202
7.198
7.190
7.186
4.252
4.249
4.246
4.222
4.219
4.201
4.198
4.195
4.099
4.029
4.027
4.026
3.432
3.403
2.108
1.587
1.570
0.914
0.896
0.878
Ph
O
Ph
NO2
N
N
Fc
OH
5m
1.99
2.15
5.93
1.02
1.03
0.95
1.05
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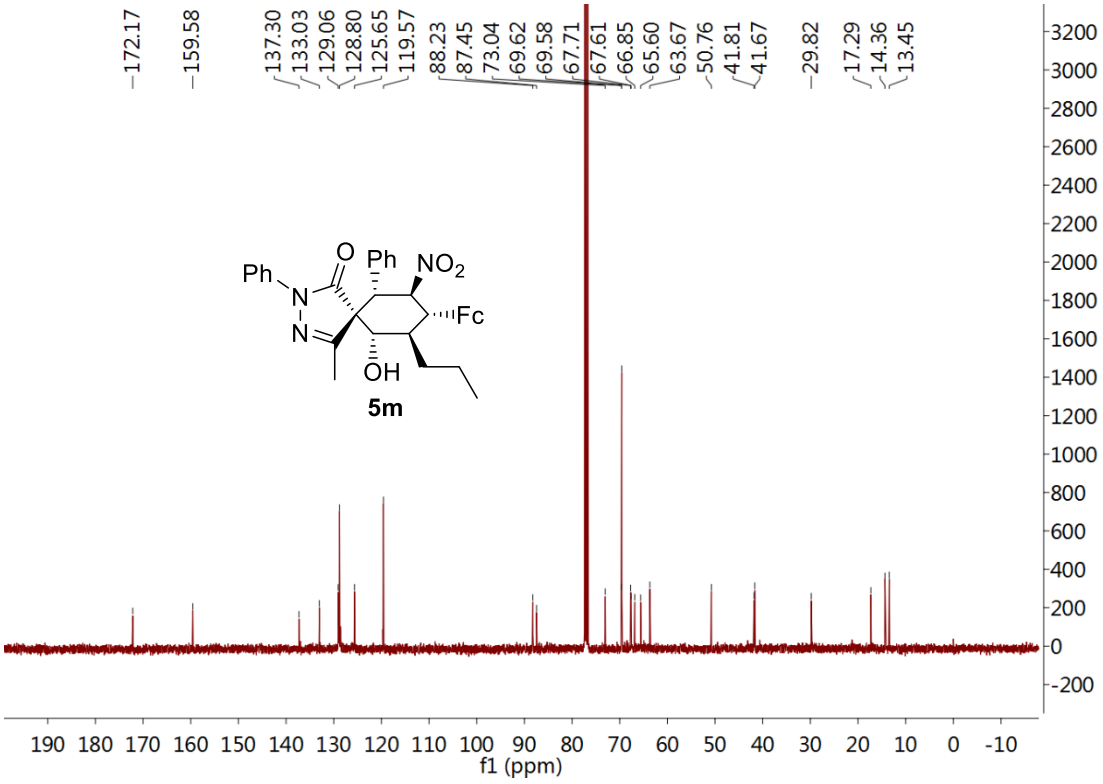
0.93
1.08
0.95
1.01
1.06
2.87
1.13
4.12
3.00
f1 (ppm)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133.03
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88.23
87.45
73.04
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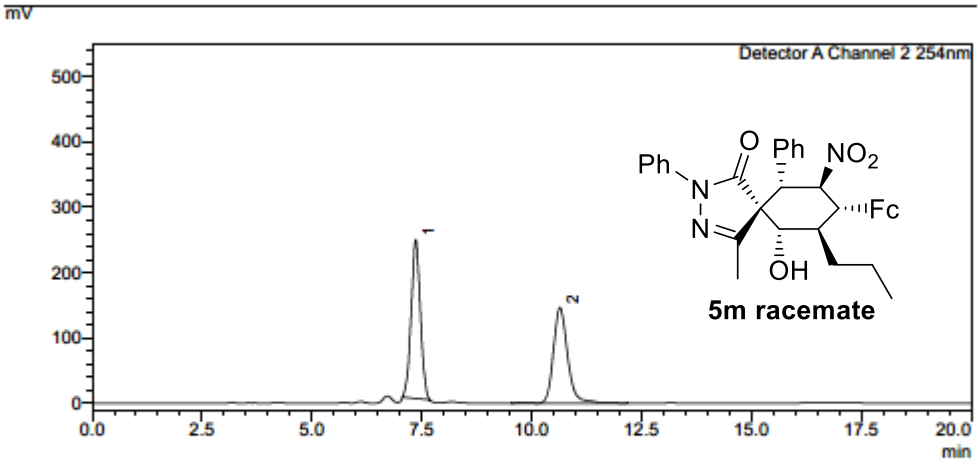
67.61
66.85
65.60
63.67
50.76
41.81
41.67
29.82
17.29
14.36
13.45
Ph
O
Ph
NO2
N
N
Fc
OH
5m
f1 (ppm)

<Chromatogram>

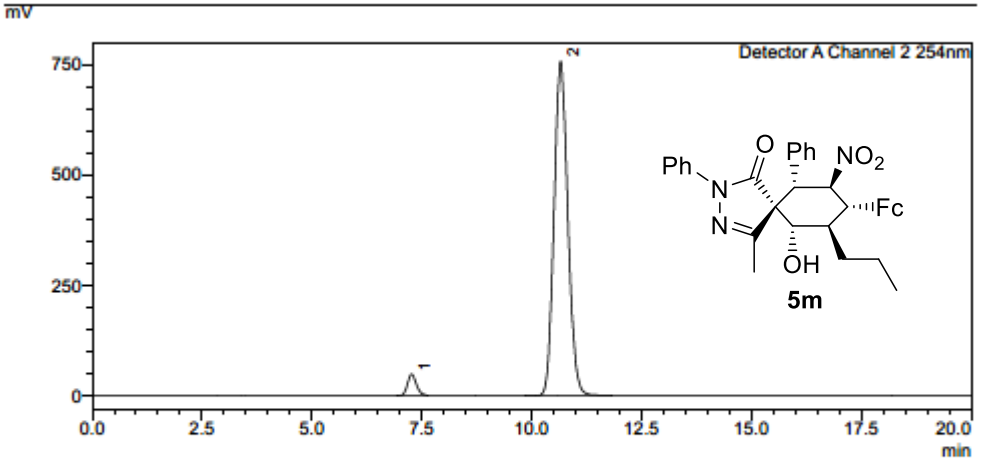


<Peak Table>

Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	7.361	3509524	242947	51.472	51.472
2	10.644	3308832	146539	48.528	48.528
Total		6818356	389486		100.000

<Chromatogram>



<Peak Table>

Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.	Area%
1	7.267	668395	48029	3.889	3.889
2	10.659	16520480	758013	96.111	96.111
Total		17188875	806042		100.000

5. RalA mRNA and protein expression in pancreatic cancer tissues (Figure S1)

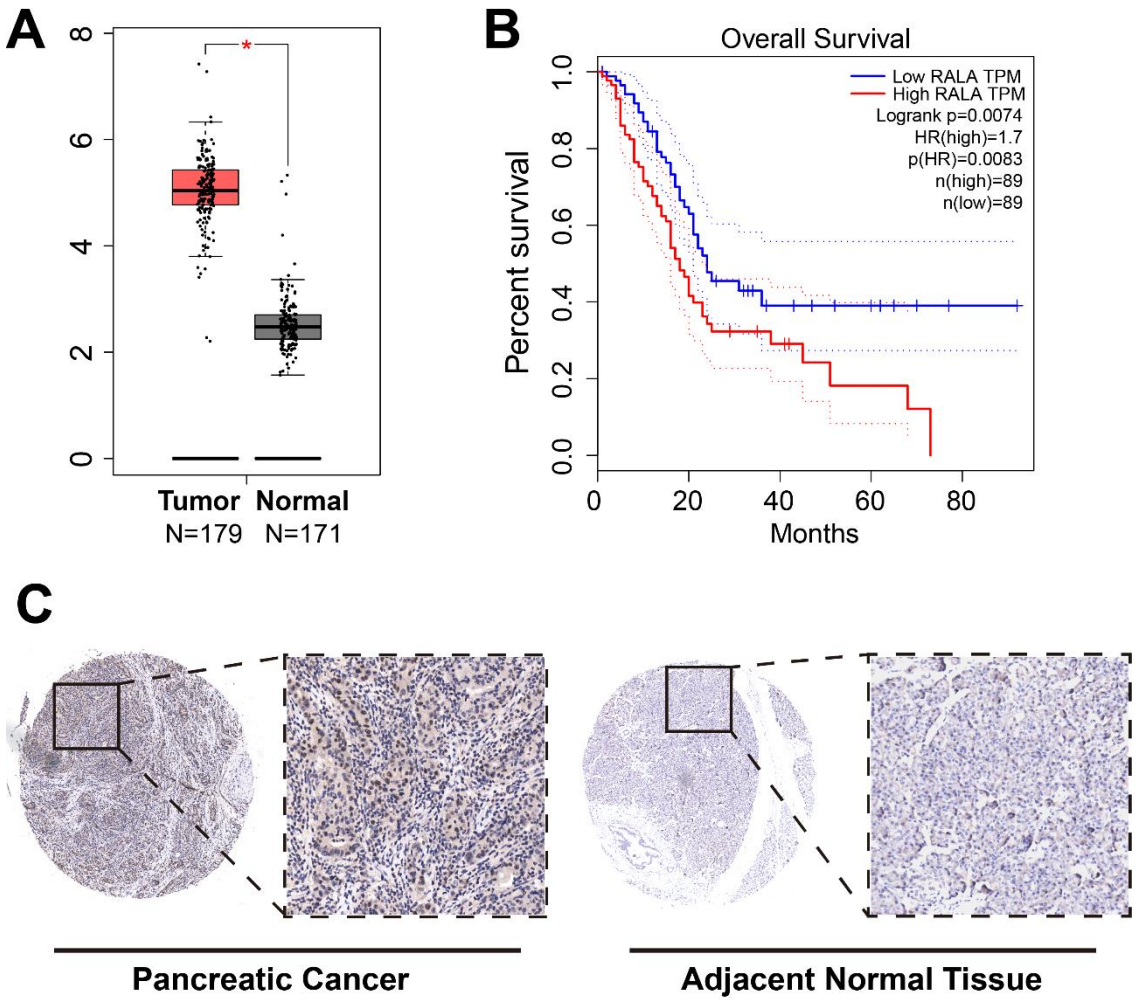


Figure S1. (A) Scatter plot of RalA expression in pancreatic cancer and adjacent normal tissues; (B) Kaplan-Meier plots revealed an association between increased RalA levels and reduced overall survival; (C) The immunohistochemistry staining of RalA protein in pancreatic cancer and adjacent normal tissues (×100).

6. Preliminary screening of the anti-proliferative activity (Table S2) [a,b]

Compound	IC_{50} Values (μM)		
	RalA	PANC-1	HPAF-II
4a	40.41	19.75	28.29
4b	24.95	17.51	26.4
4c	>50	36.04	49.42
4d	>50	32.05	41.32
4e	38.28	27.04	35.52
4f	>50	20.48	46.89
4g	37.18	30.46	38.47
4h	46.46	20.49	38.22
4i	>50	40.88	35.33
4j	>50	37.09	47.44
4k	29.82	19.94	28.42
4l	35.27	17.93	27.82
4m	>50	37.47	32.48
4n	23.6	18.03	16.97
4o	42.5	25.98	29.06
5a	9.03	7.12	11.93
5b	1.2	1.58	4.79
5c	6.17	3.81	9.14
5d	10.9	8.62	11.1
5e	1.32	4.54	6.31
5f	2.92	5.47	6.58
5g	7.55	7.14	12.77
5h	3.98	14.23	13.12
5i	11.54	5.07	13.03
5j	3.58	7.58	11.17
5k	22.64	14.22	22.67
5l	11.27	6.09	12.58
5m	8.19	8.04	11.76
5n	5.94	3.46	12.17
5o	12.86	7.15	15.31

[a] The enzymatic IC_{50} values were determined from HTRF based assays for RalA. The cytotoxicity IC_{50} values were determined by using MTT methods; [b] Each compound was tested in triplicate; the data are presented as the mean values.

7. The superposition of binding conforms of 9e and p53 peptide (Figure S2)

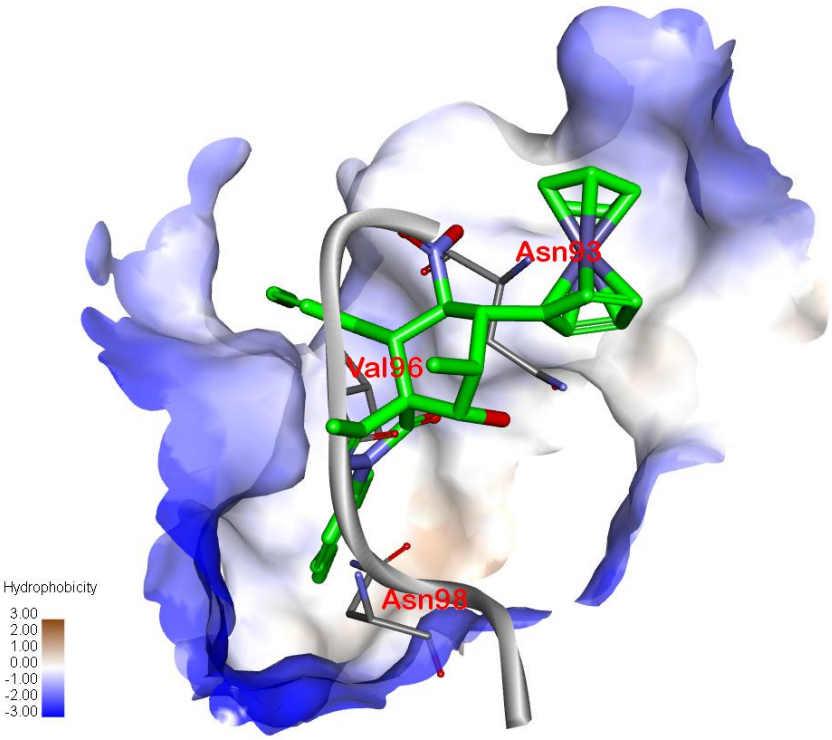


Figure S2. The potential binding modes of 5b to RalA. The binding mode of C3 exoenzyme peptide to RalA was shown in gray as a reference.

8. The intracellular ROS induced by 5b and 5b' (Figure S3)

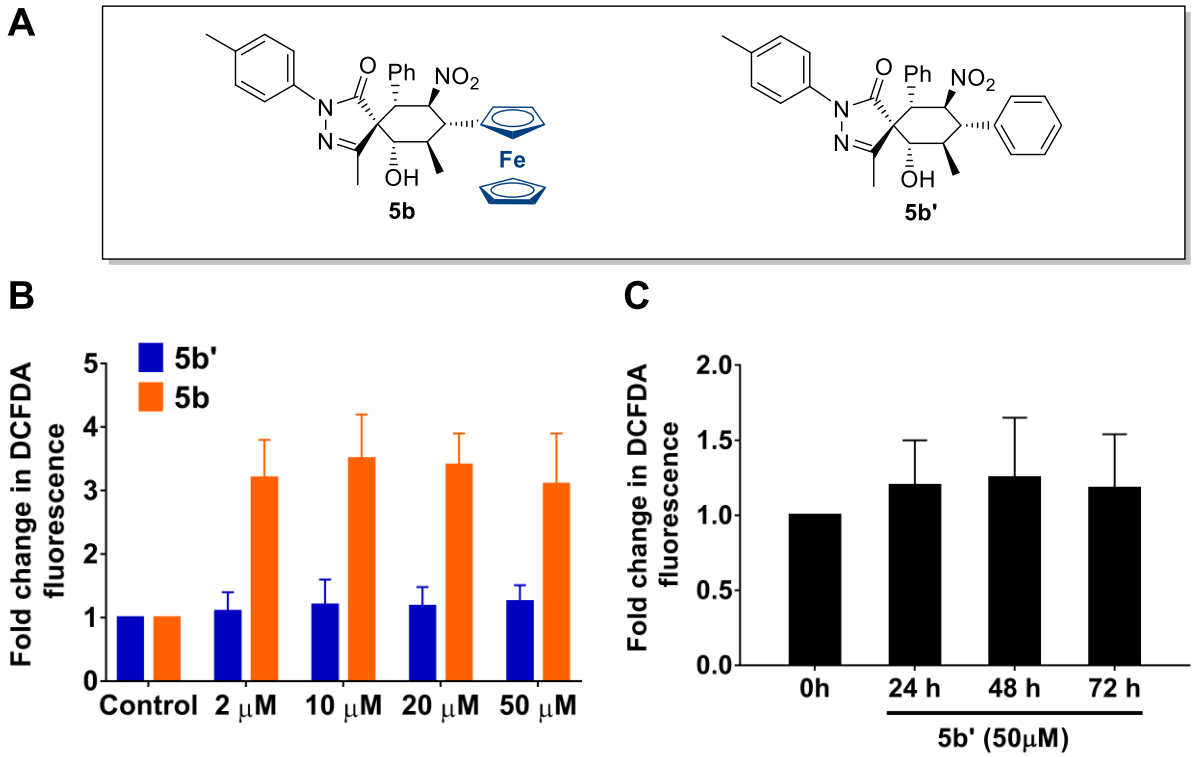


Figure S3. (A) Chemical structures of **5b** and **5b'**; (B) The changes in fluorescence of the ROS probe DCFDA in PANC-1 cells treated with compound **5b** or **5b'** in different concentration; (C) The changes in fluorescence of the ROS probe DCFDA in PANC-1 cells treated with 50 μM compound **5b'** under different incubation times.