

Discovery of a Neuroprotective Chemical, (*S*)-*N*-(3-(3,6-dibromo-9*H*-carbazol-9-yl)-2-fluoropropyl)-6-methoxypyridin-2-amine [(-)-P7C3-S243] with Improved Drug-Like Properties

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Supplementary Information

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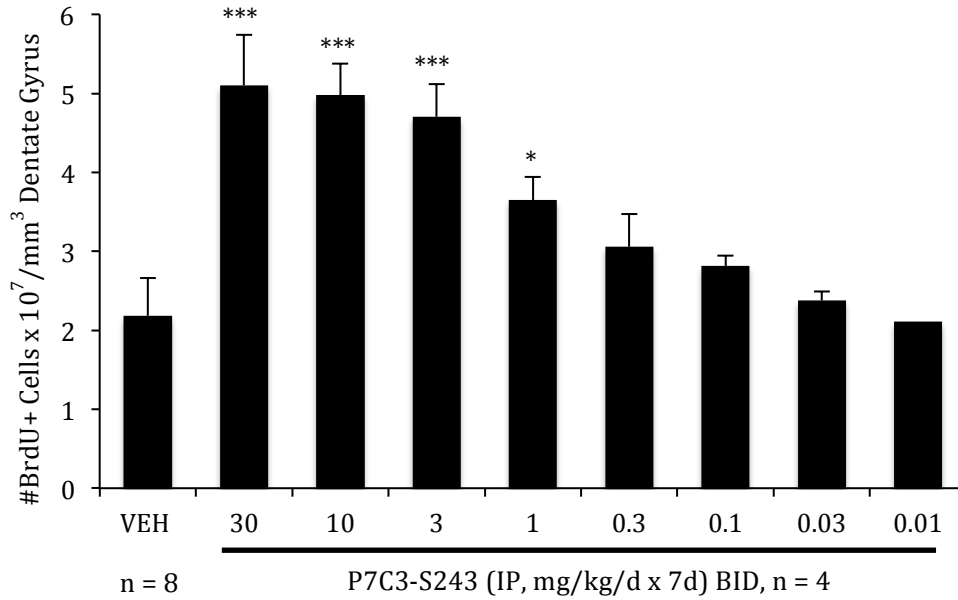


Figure S1. Adult mice were administered a bolus of BrdU (150 mg/kg) to label newborn neurons, and 24 hours later, twice-daily IP treatment was begun with vehicle or compound for 7 days. At the end of the 7 days, the number of surviving BrdU-labeled neurons was quantified. Importantly, under the conditions of this experiment, newborn neurons were labeled prior to exposure to drug, so any increase in number of neurons can be attributed to neuroprotective activity rather than proliferative effects. P7C3-S243 showed protection down to 1 mg/kg/day dose. Data shown are mean +/- SEM. P values are shown for comparison to VEH treated group, and were obtained by one-way ANOVA with Dunnet's post-hoc test, using GraphPad Prism 5.0. Asterisks indicate $p < 0.01$ (*) or $p < 0.0001$ (***)

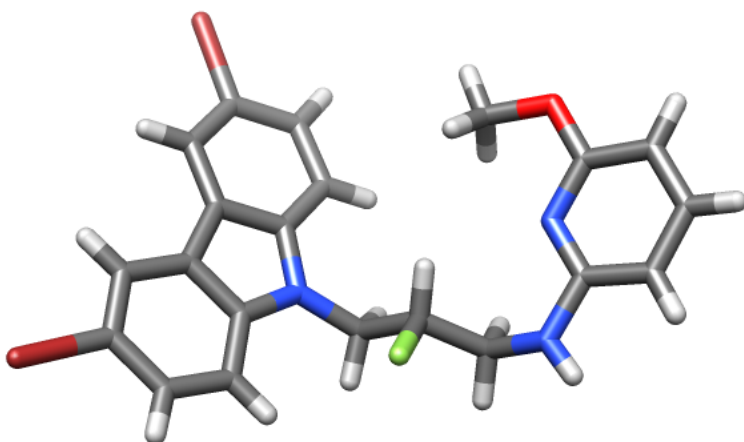


Fig S2. X-ray crystal structure of (-)-(S)-P7C3-S243

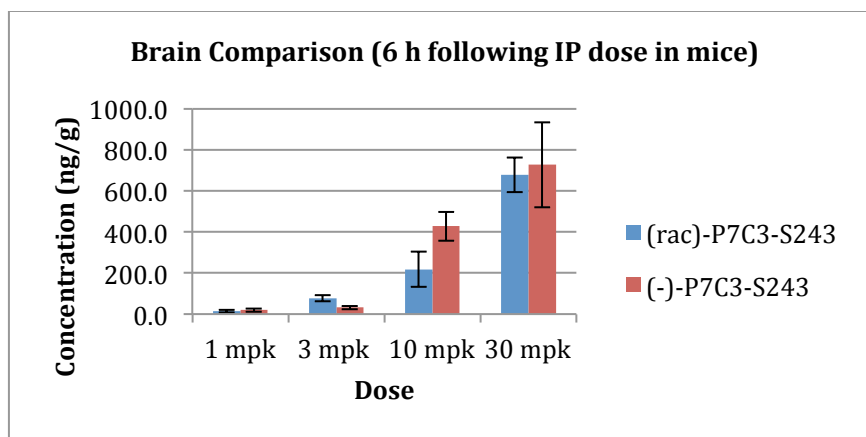
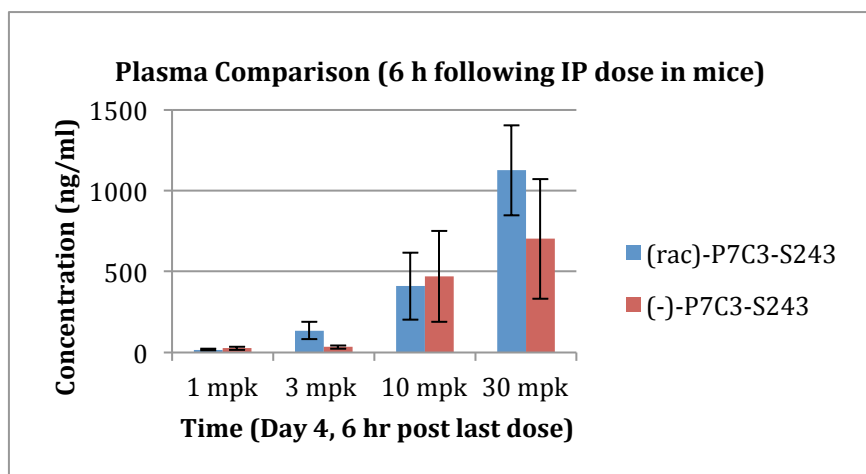
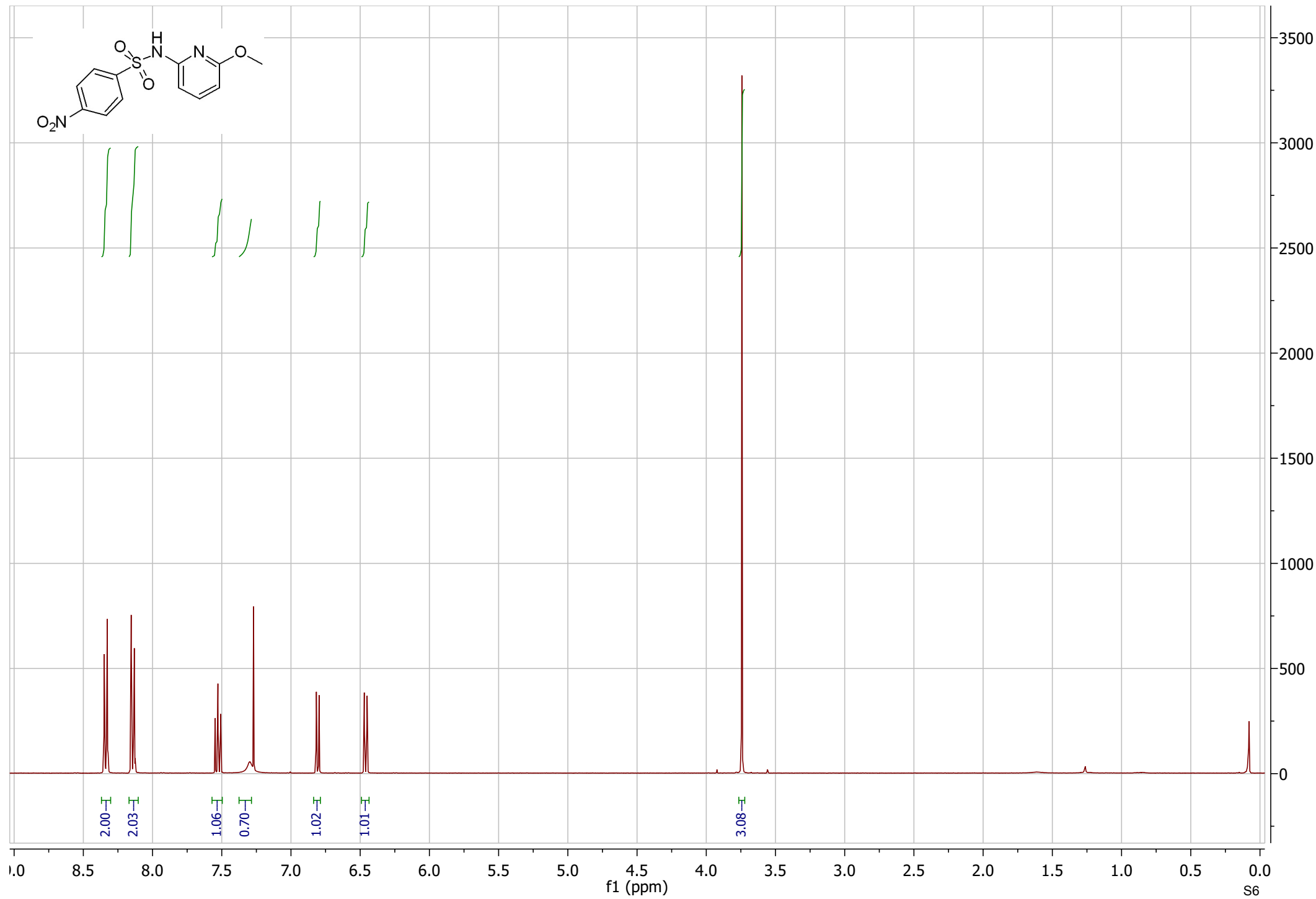
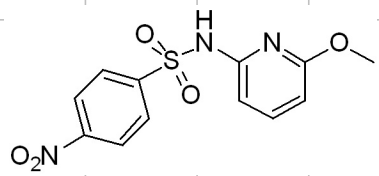


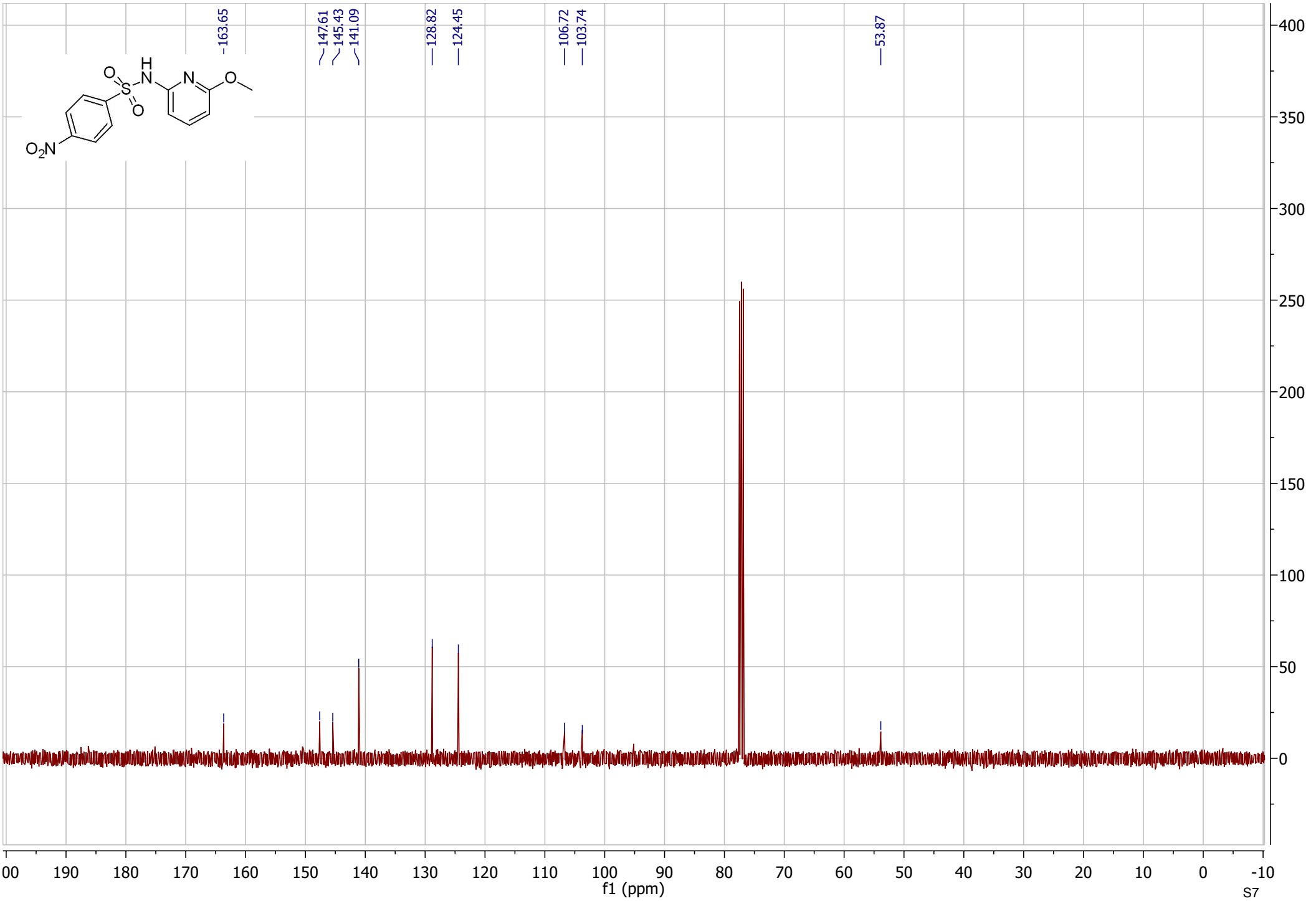
Figure S3. Comparison of brain levels of optically active and racemic 15 (P7C3-S243) 6 h after an IP dose at the indicated amounts (mpk = mg/kg). No difference is observed between racemic and optically active compound.

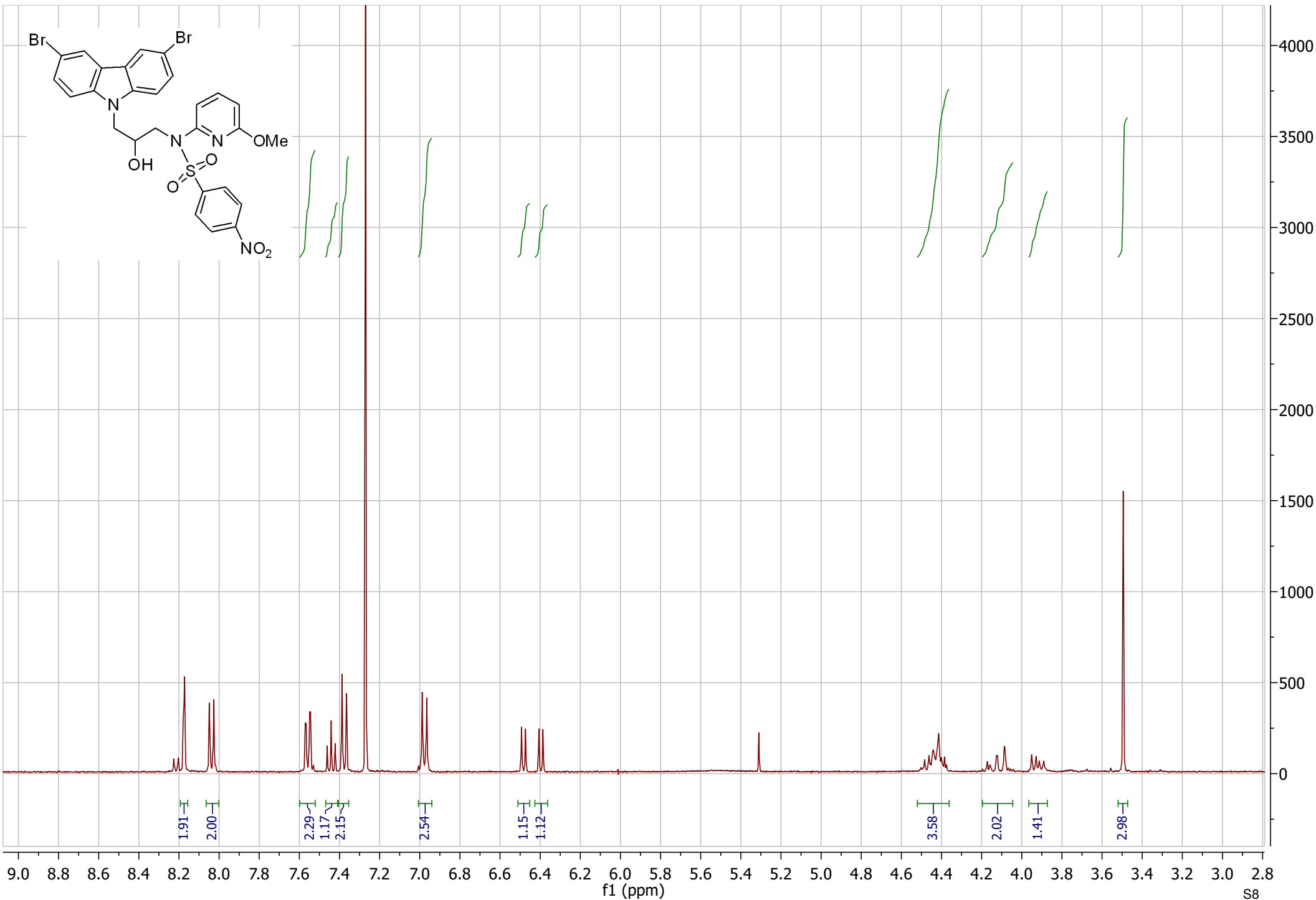
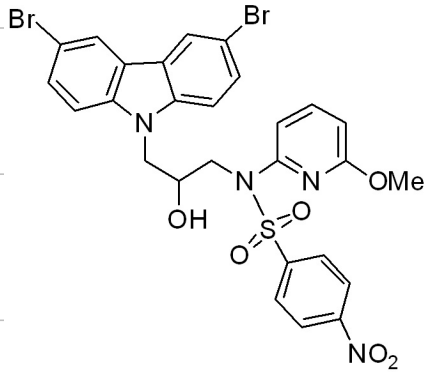
Table S1. Results from Psychoactive Drug Screening Program. Values represent Ki in nM in competition assays. Empty cells indicate Ki > 10,000 nM in dose-response experiments or <50% inhibition when tested at 10,000 nM.

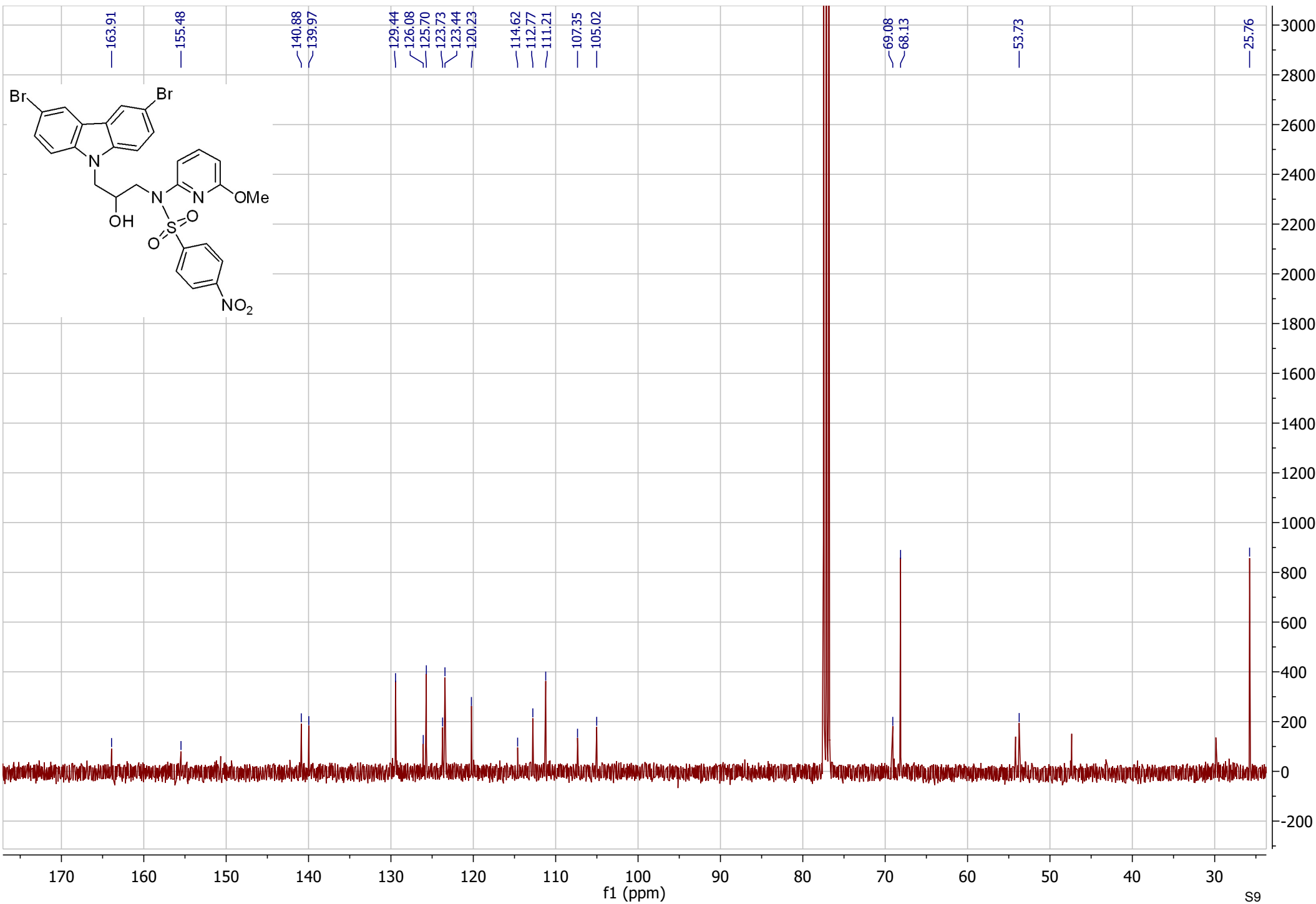
| | PDSP Code: | 29845 | 29846 | 29848 | 29849 | |
|---------------------------|----------------------|----------------------|----------|----------|----------|--|
| | Cmpd Name: | P7C3 | P7C3 A20 | (-)-S243 | (+)-S243 | |
| Receptor Code | Receptor | Ki (nM) | | | | |
| 5-HT1A | Serotonin receptors | | | | | |
| 5-HT1B | | | | | | |
| 5-HT1D | | 2595 | 2334 | | | |
| 5-ht1e | | | | | | |
| 5-HT2A | | | | | | |
| 5-HT2B | | | | | 4327 | |
| 5-HT2C | | | | | | |
| 5-HT3 | | | | | | |
| 5-ht5a | | | | | | |
| 5-HT6 | | | | | | |
| 5-HT7 | | | | | 1857 | |
| Alpha1A | | Adrenergic receptors | | | | |
| Alpha1B | | | | | | |
| Alpha1D | | | | | | |
| Alpha2A | | | | | | |
| Alpha2B | | | | | | |
| Alpha2C | > 8606 | | | | | |
| Beta1 | | | | | | |
| Beta2 | | | | | | |
| Beta3 | | | | | | |
| AMPA | Glutamate receptor | | | | | |
| KA | | | | | | |
| BZP Rat Brain Site | GABA receptor | > 8246 | | | | |
| GABAA | | | | | | |
| D1 | Dopamine receptor | | | | | |
| D3 | | | | | | |
| D4 | | | | | | |
| D5 | | | | | | |
| DAT | | Transporters | | | | |
| NET | 226 | | 1075 | | | |
| SERT | | | | | | |
| DOR | Opiate receptors | | | | | |
| KOR | | | | | | |
| MOR | | | | > 8231 | | |
| H1 | Histamine receptor | | | | | |
| H3 | | | | | | |
| M1 | Muscarinic receptors | | | | | |
| M2 | | | | | | |

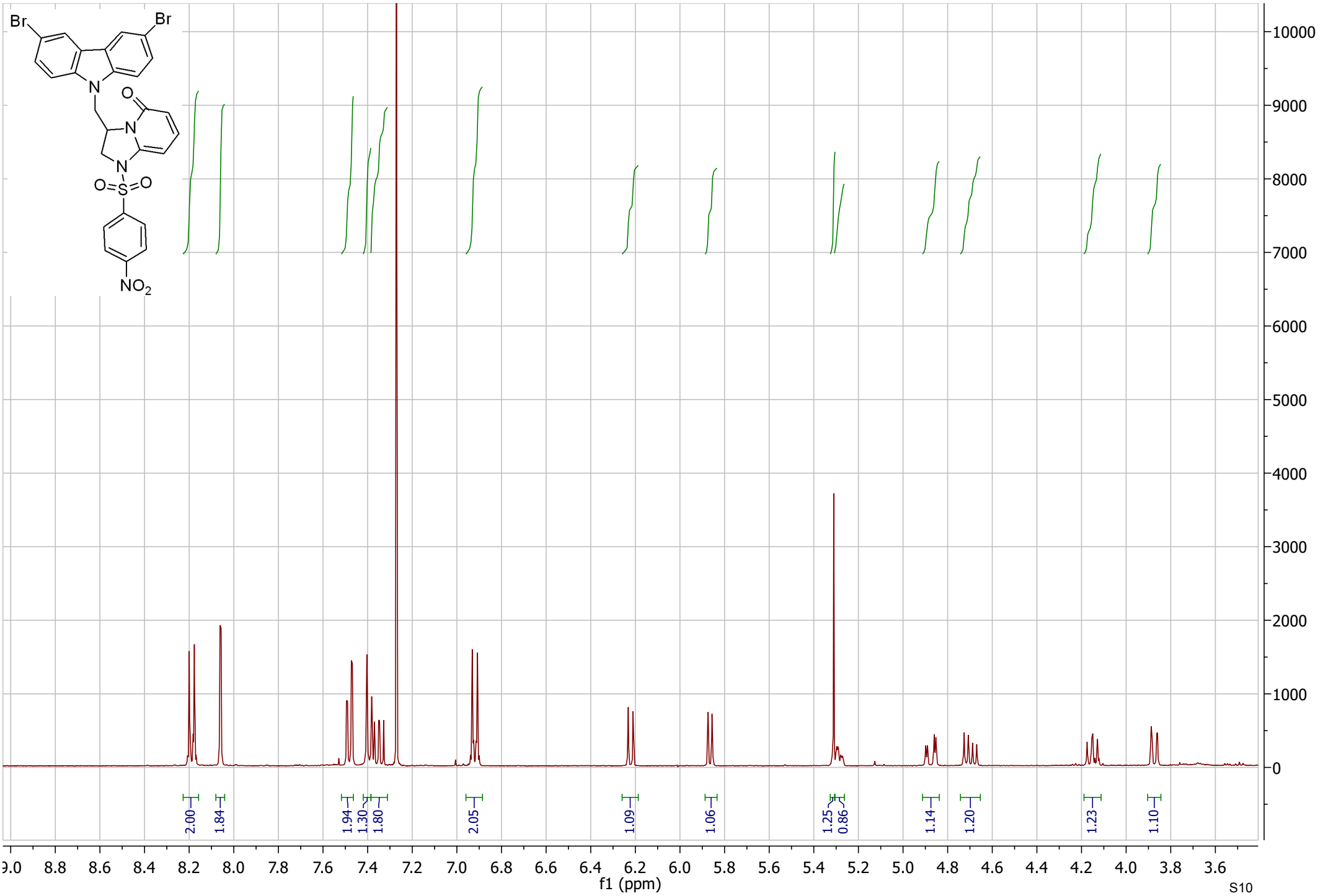
| | | | | | |
|----------------|------------------------------------|--------|------------|-----|-----|
| M3 | | | | | |
| M4 | | | | | |
| M5 | | > 8049 | | | |
| NMDA | | | | | |
| PBR | Peripheral benzodiazepine receptor | 2352 | 1972 ± 823 | 350 | 675 |
| Sigma 1 | | | | | |
| Sigma 2 | | 7985 | | | |

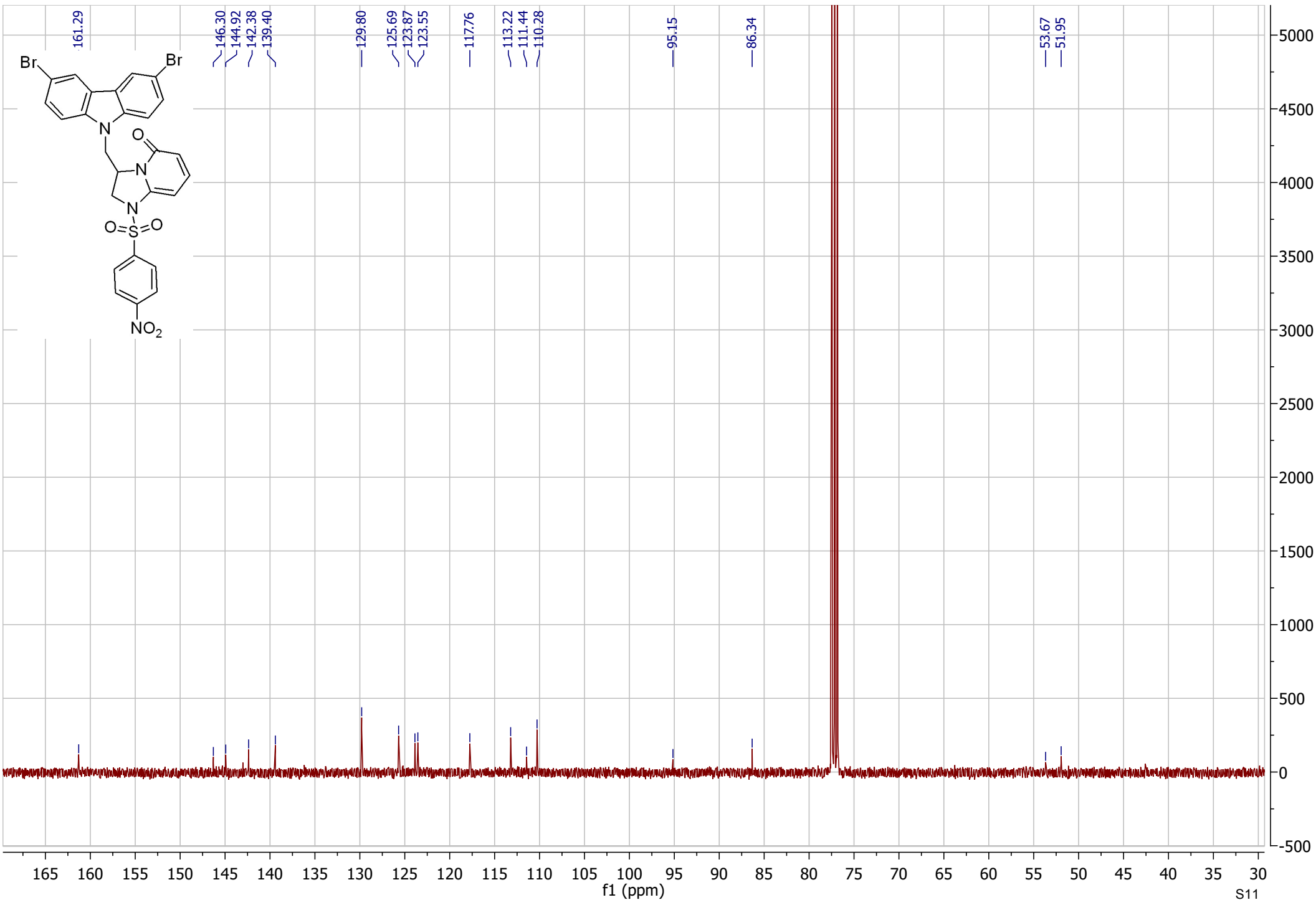


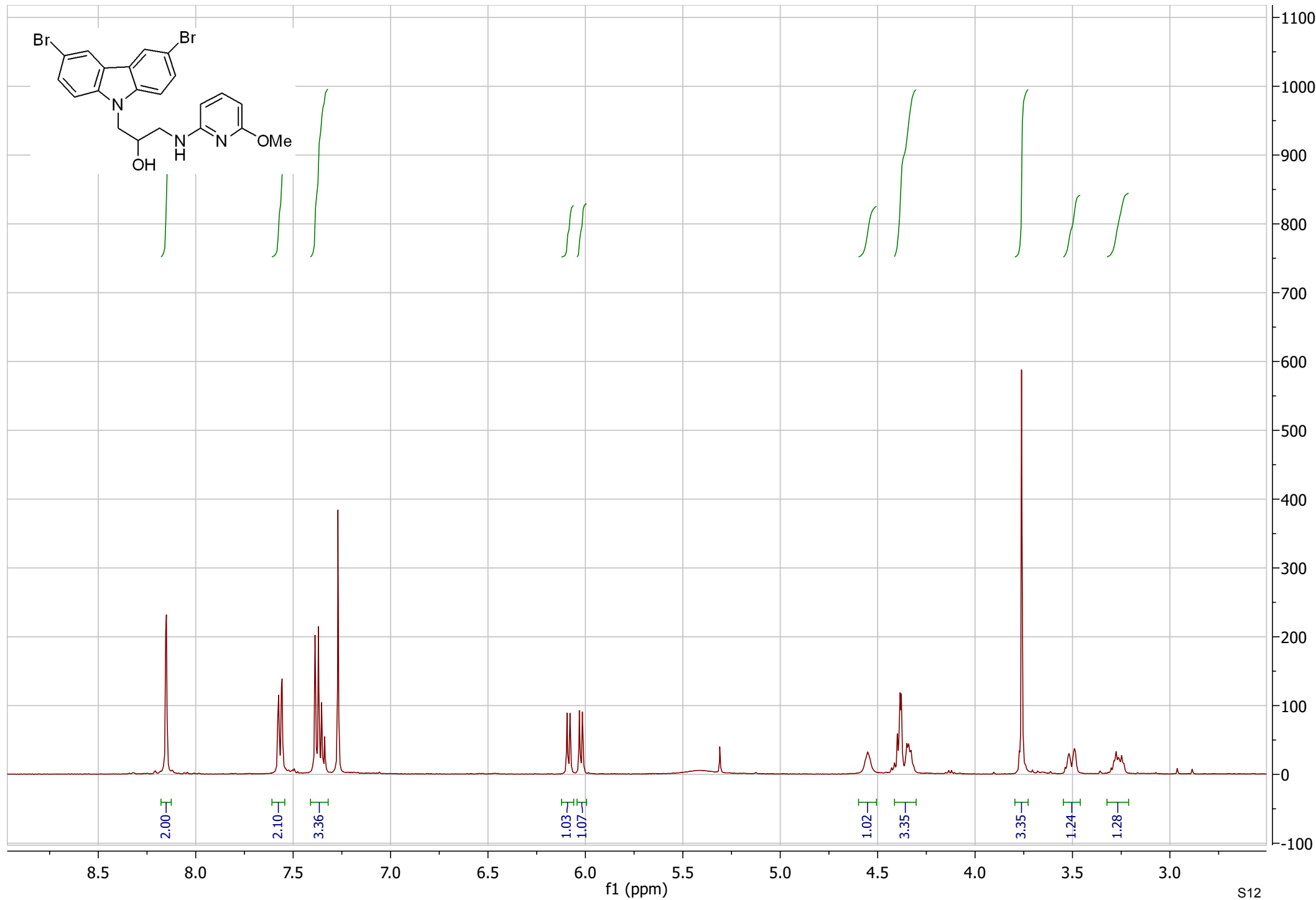
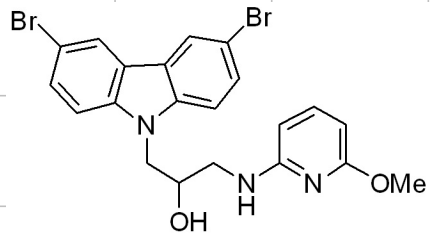


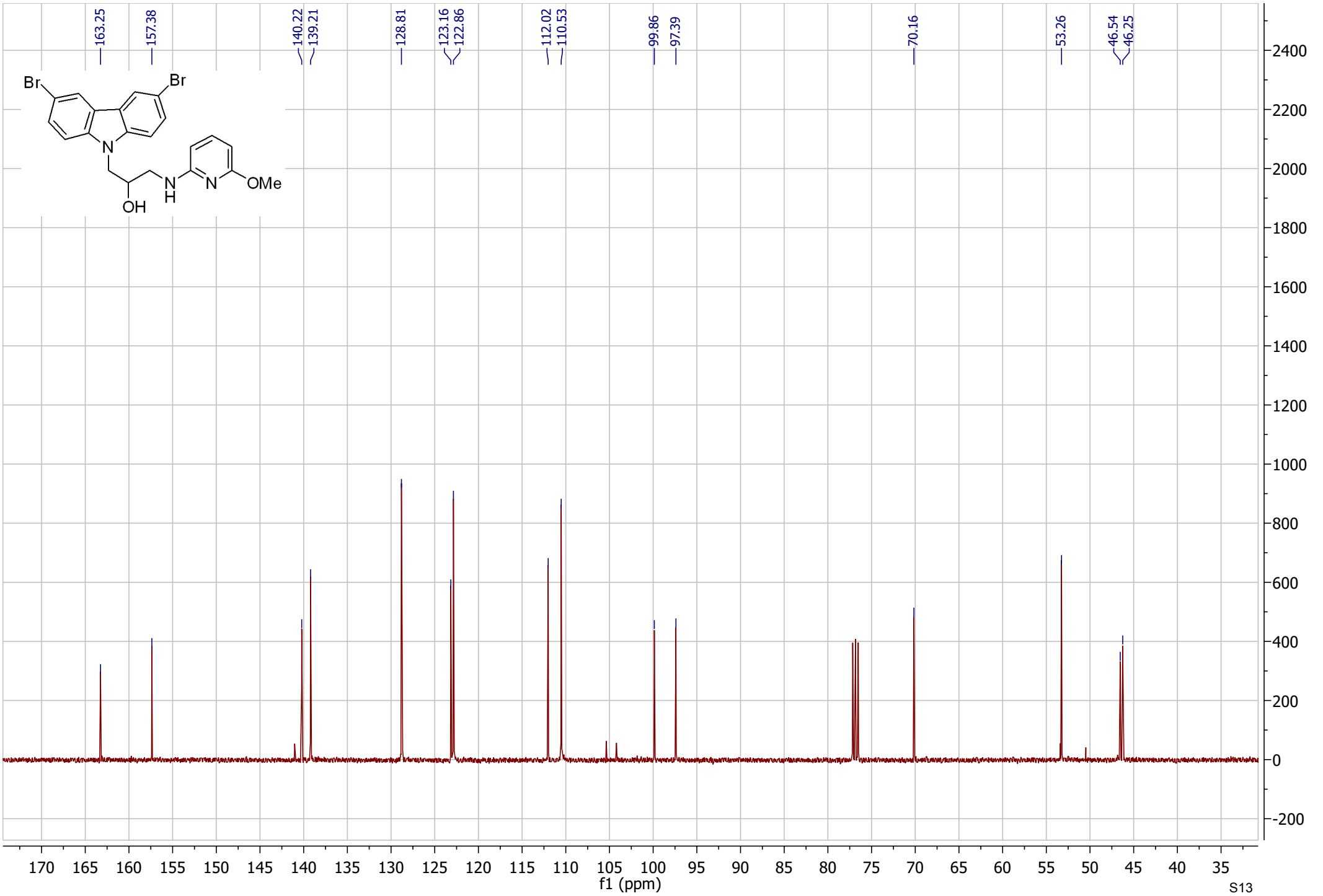


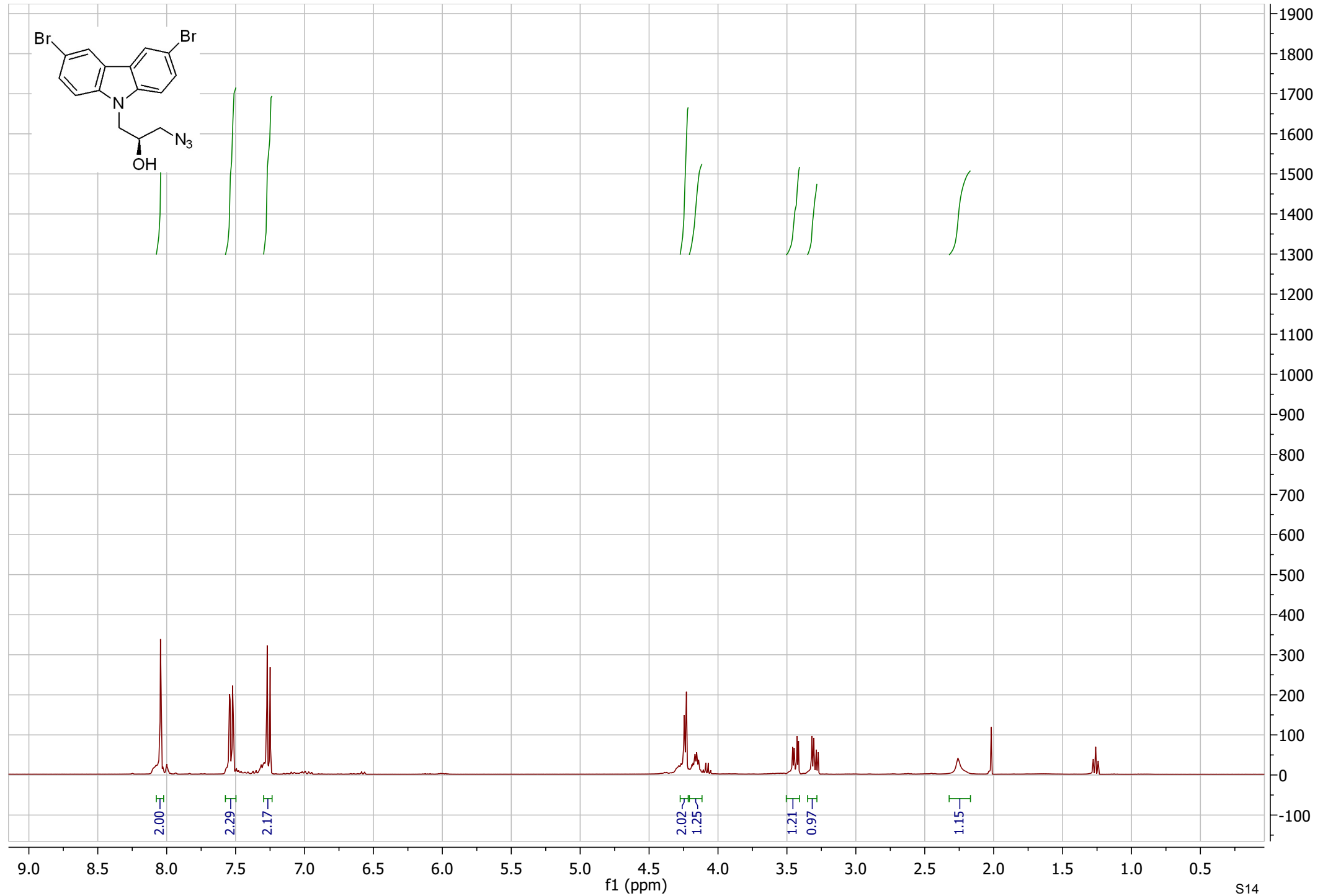
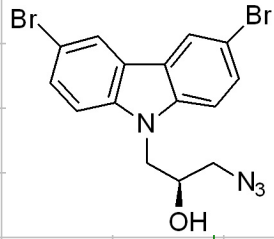


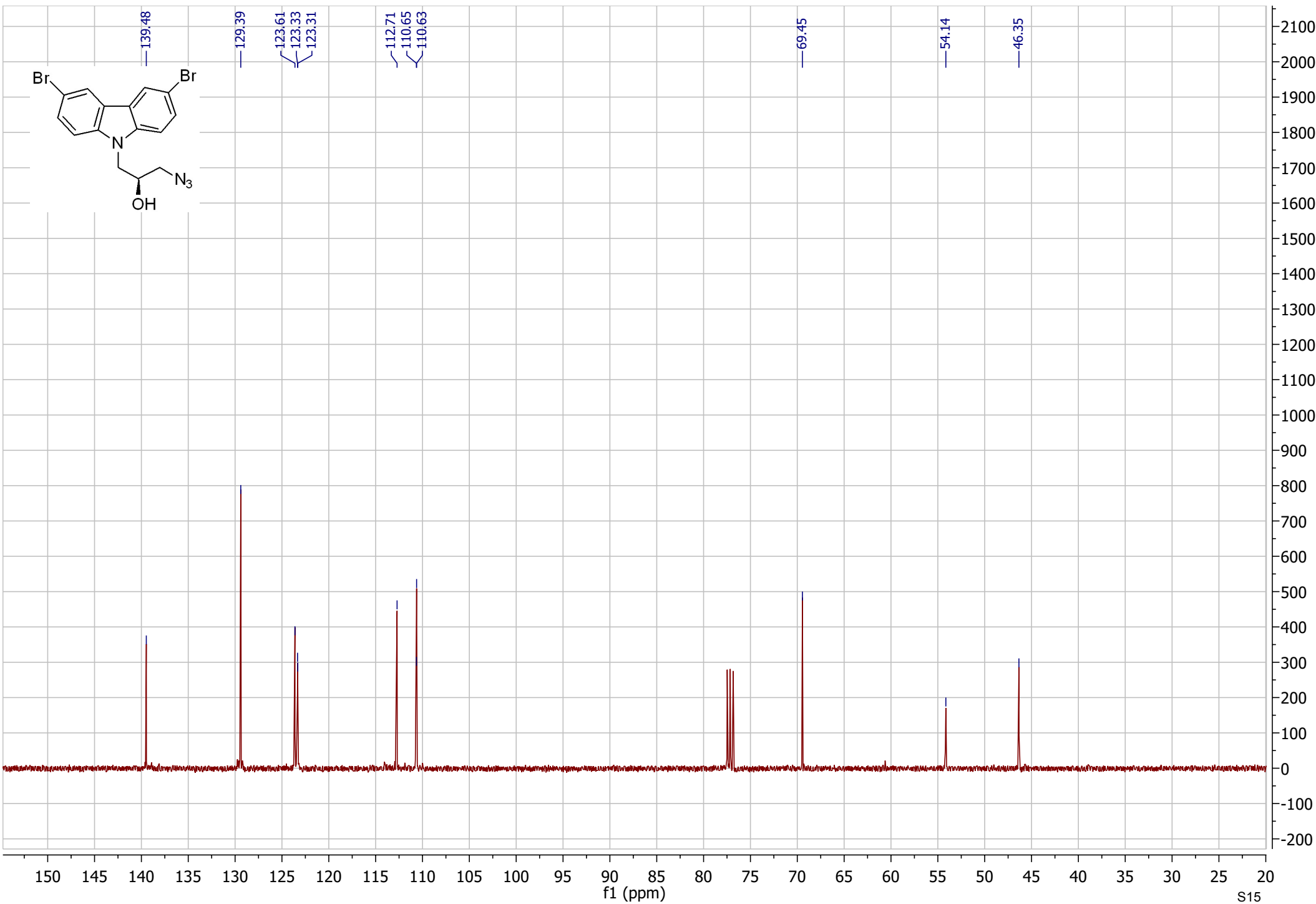
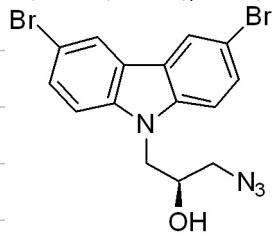


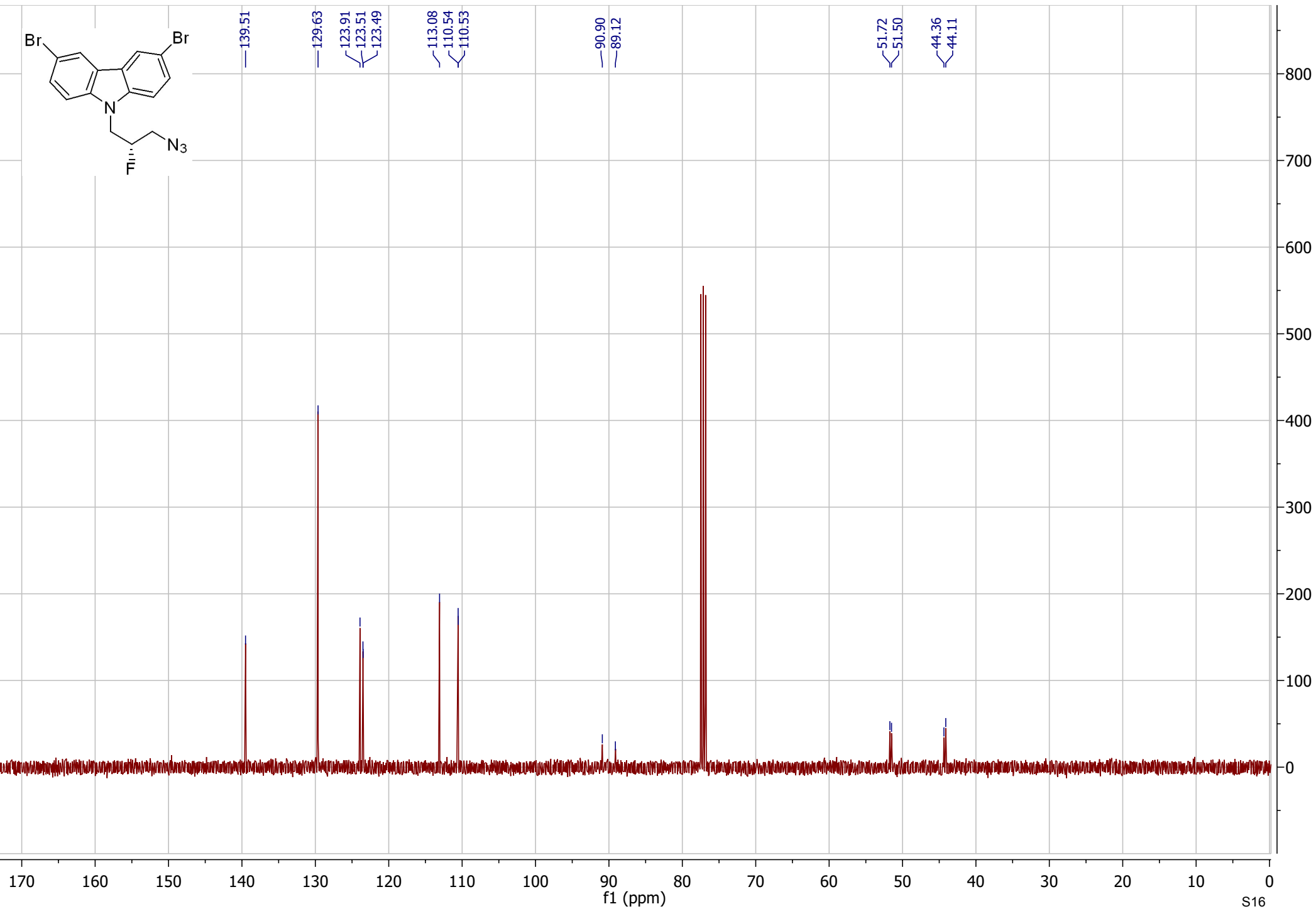
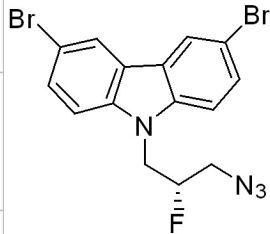


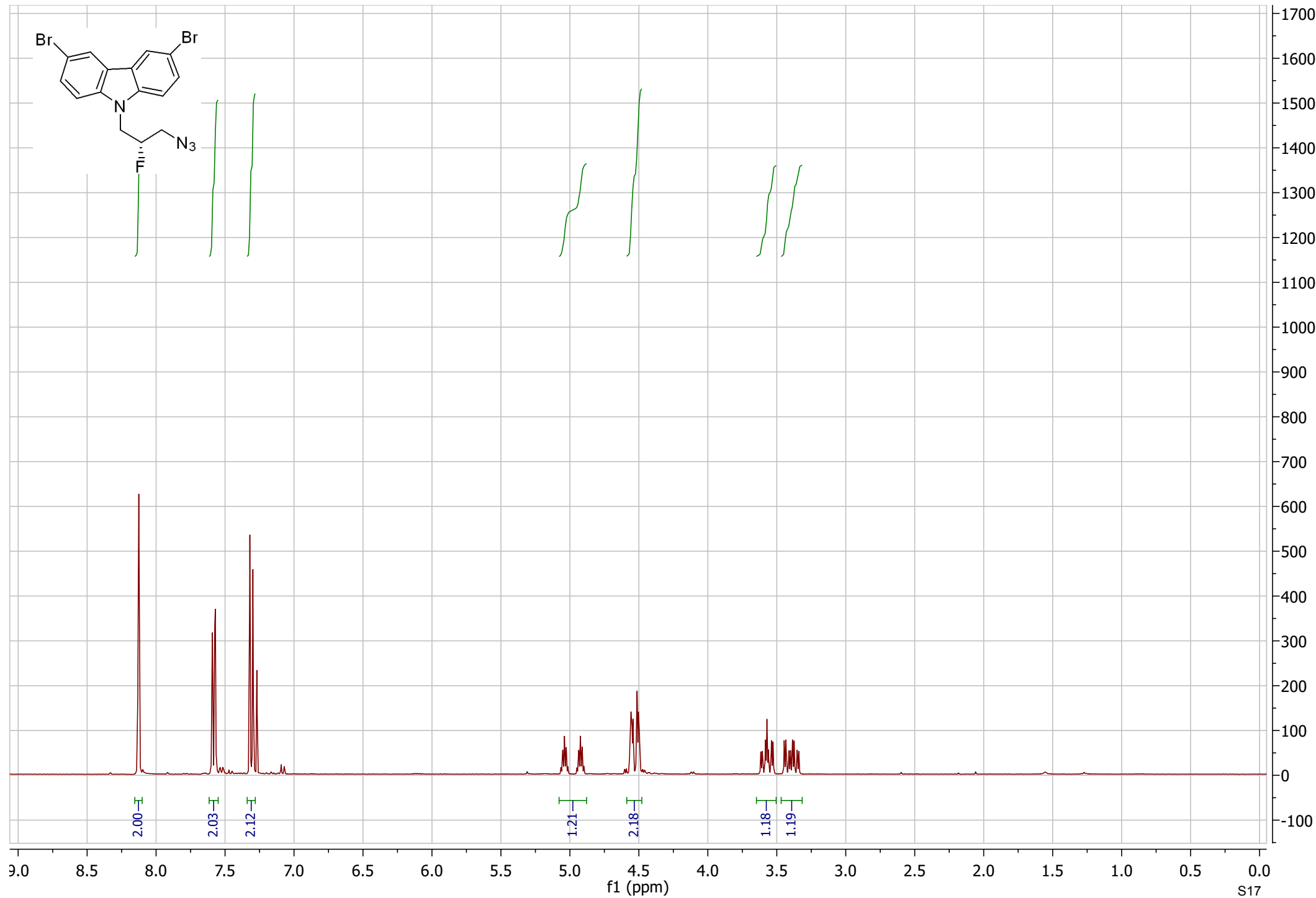
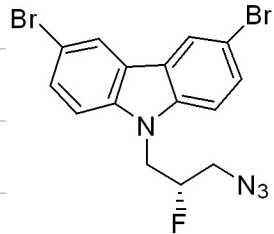


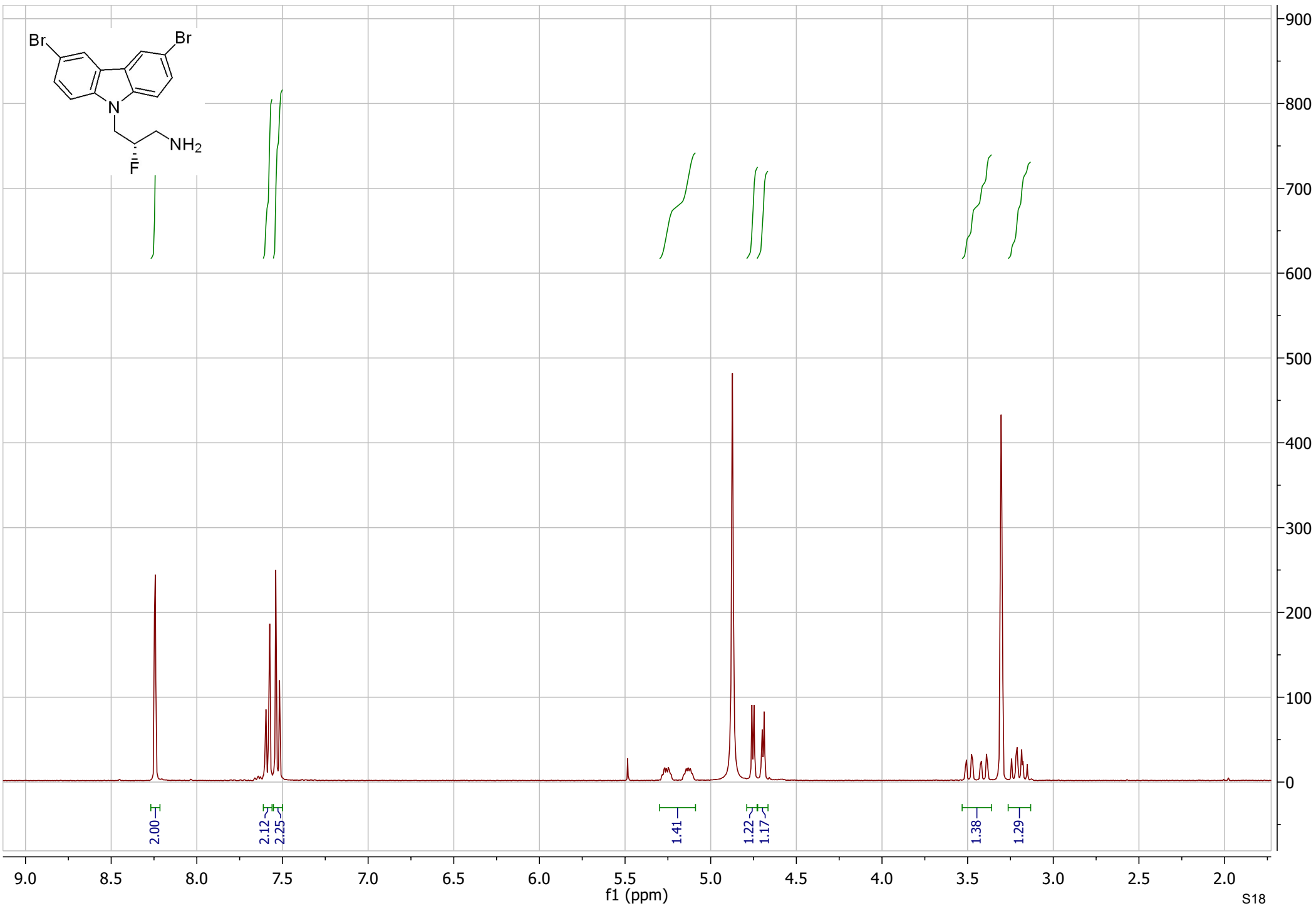
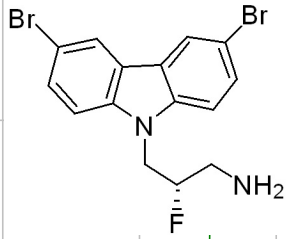


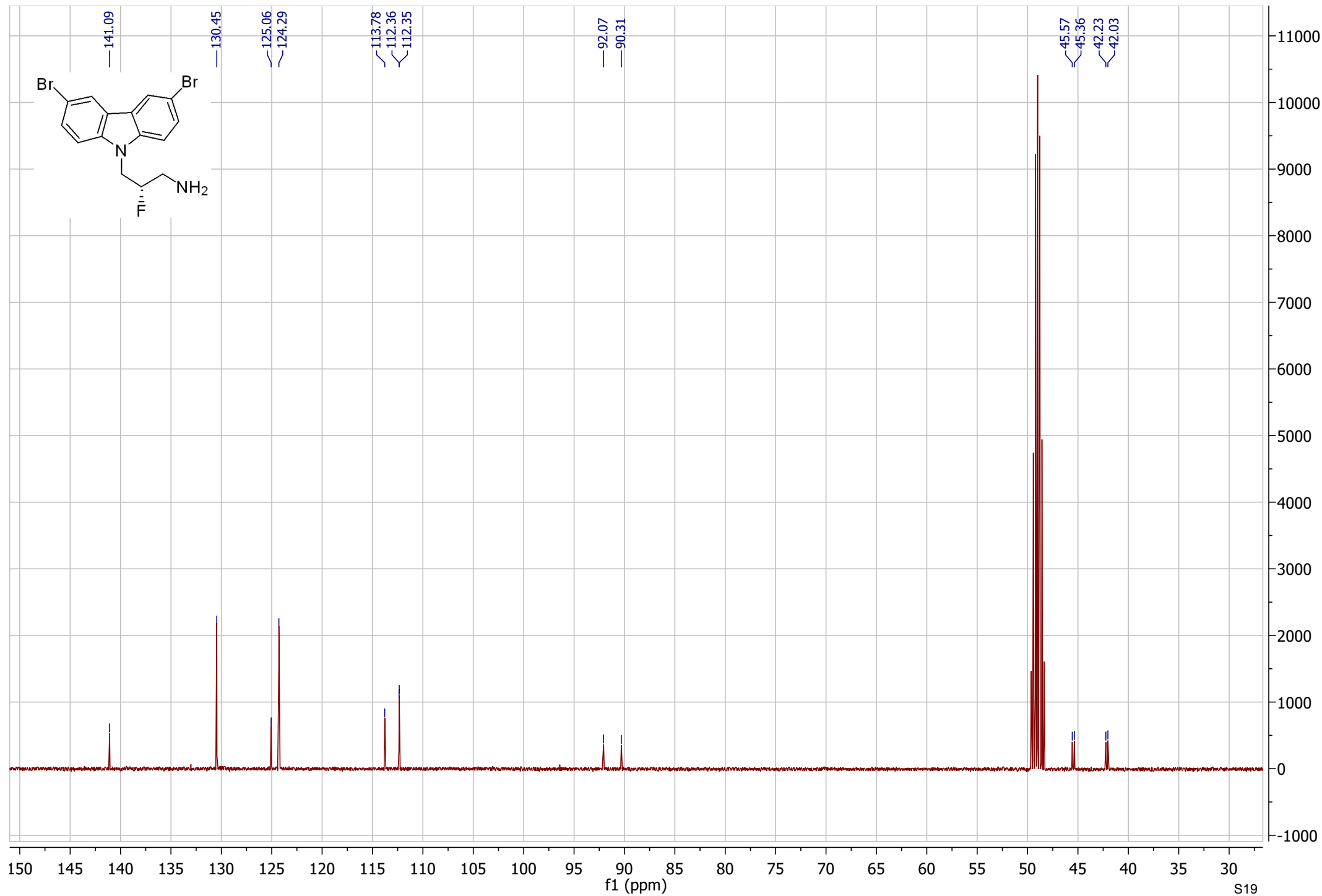
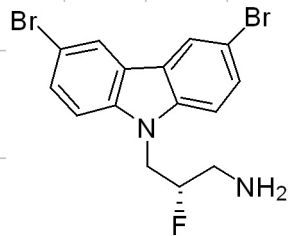


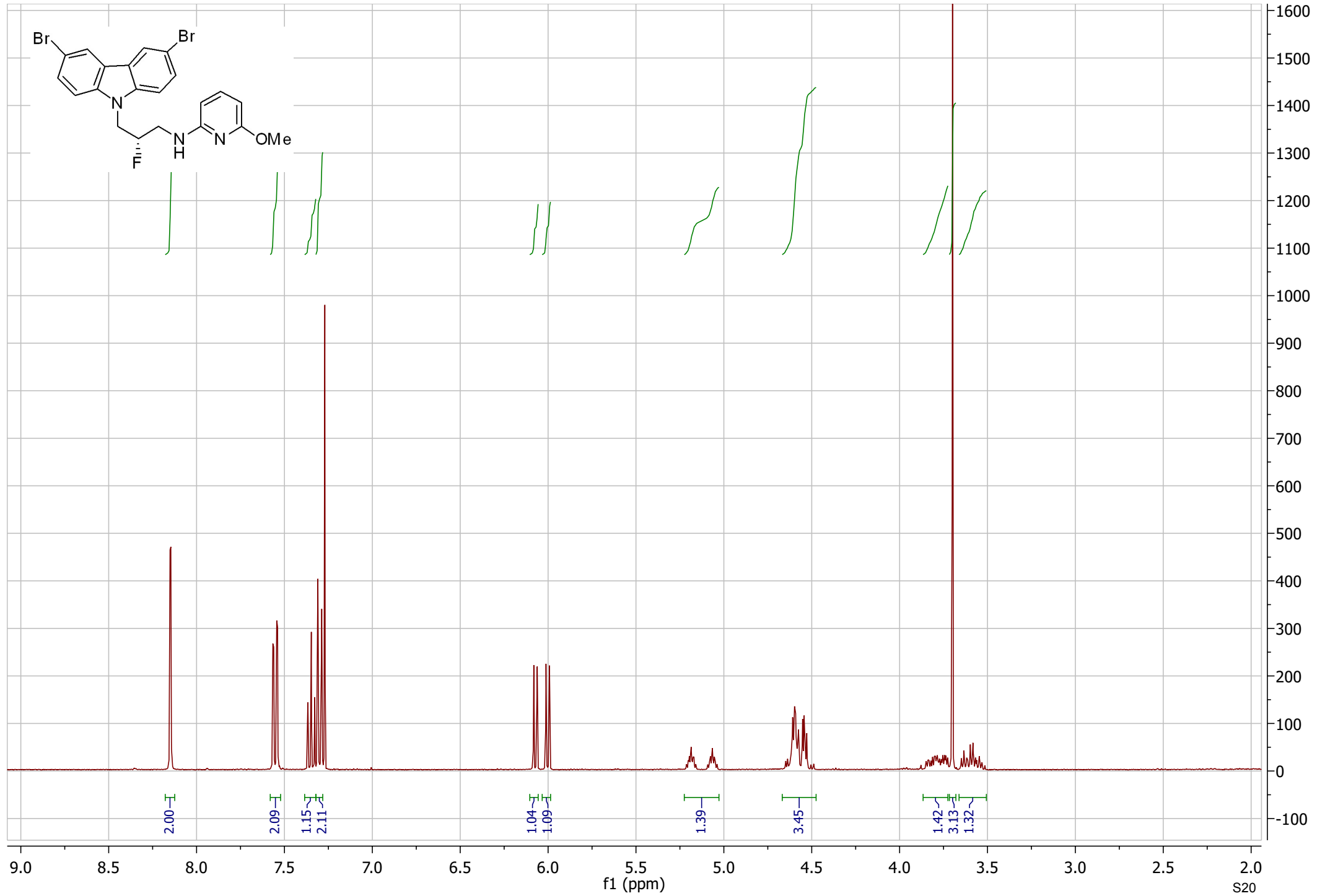
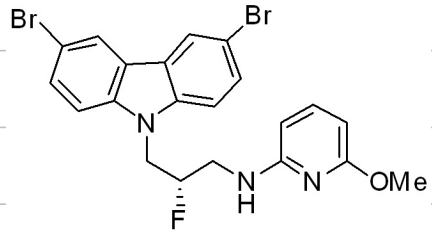


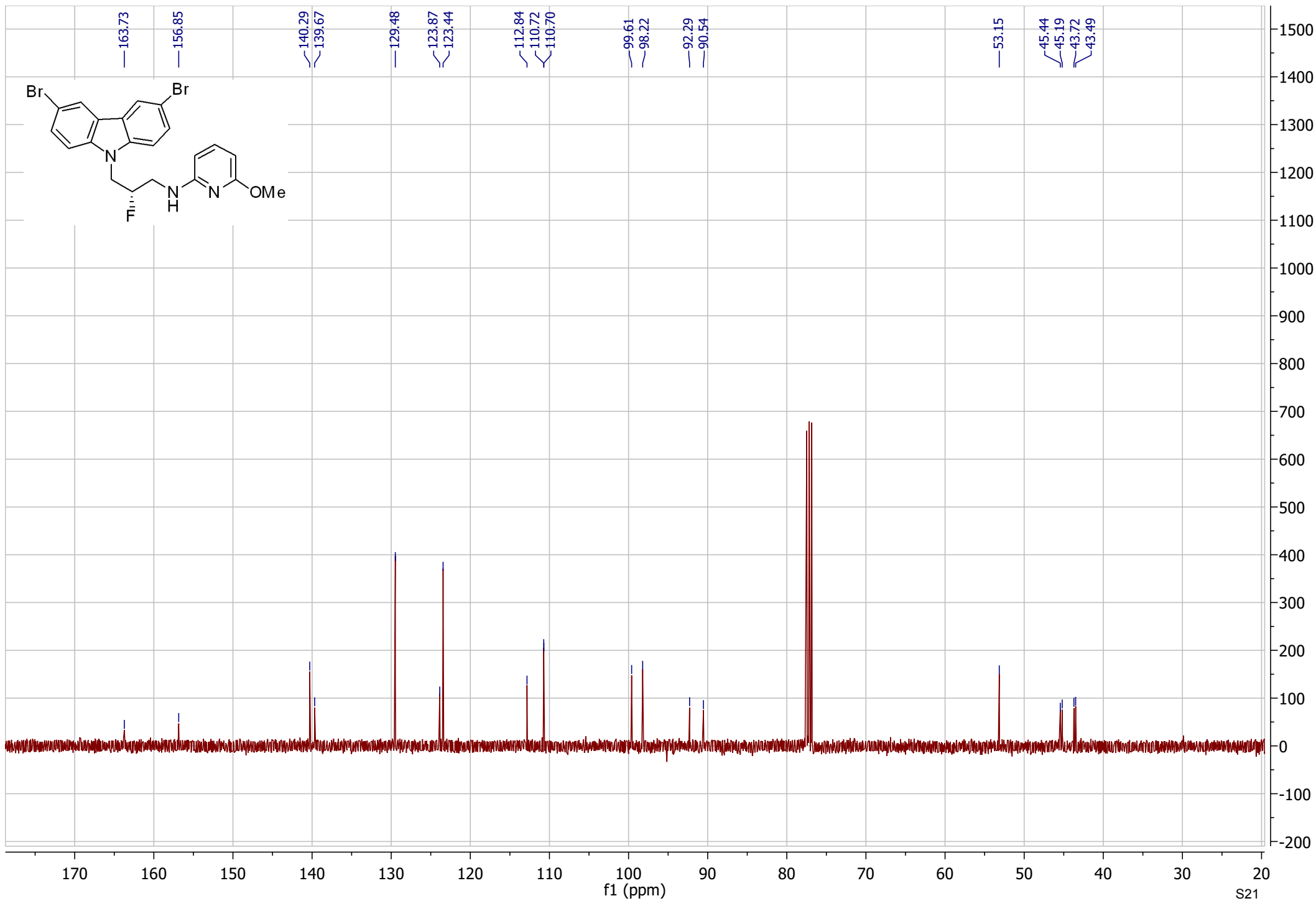
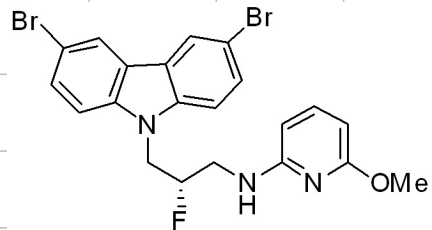


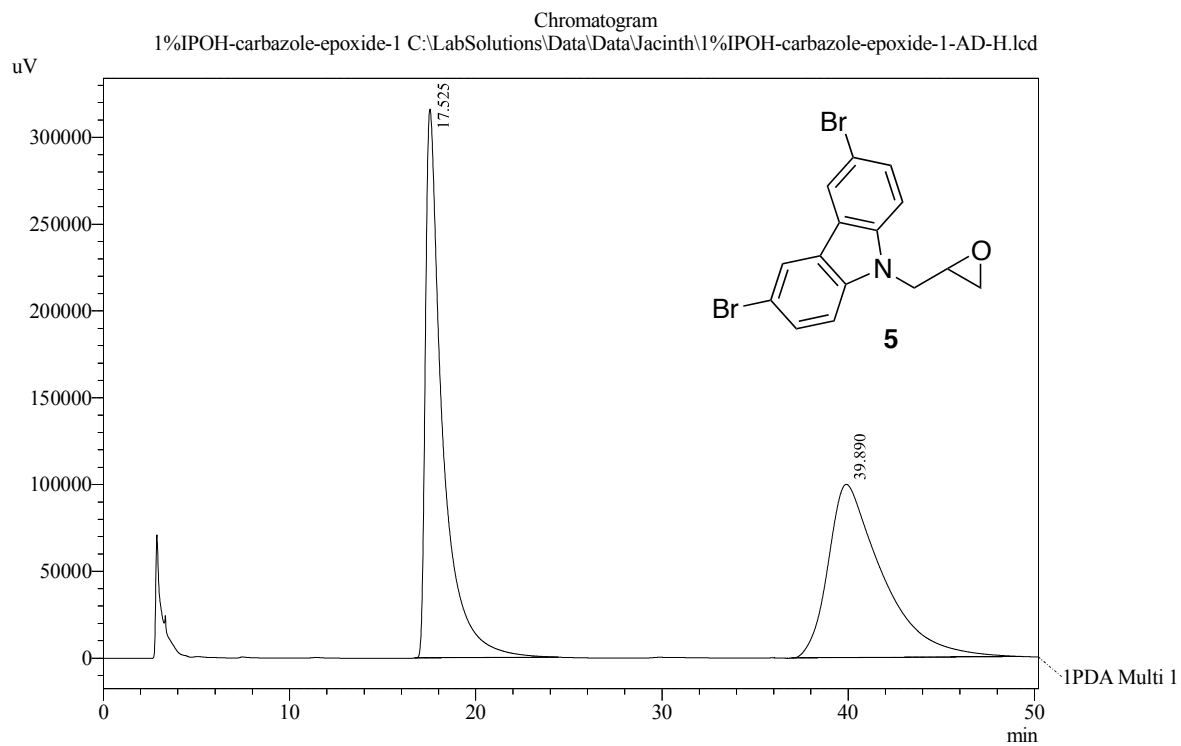












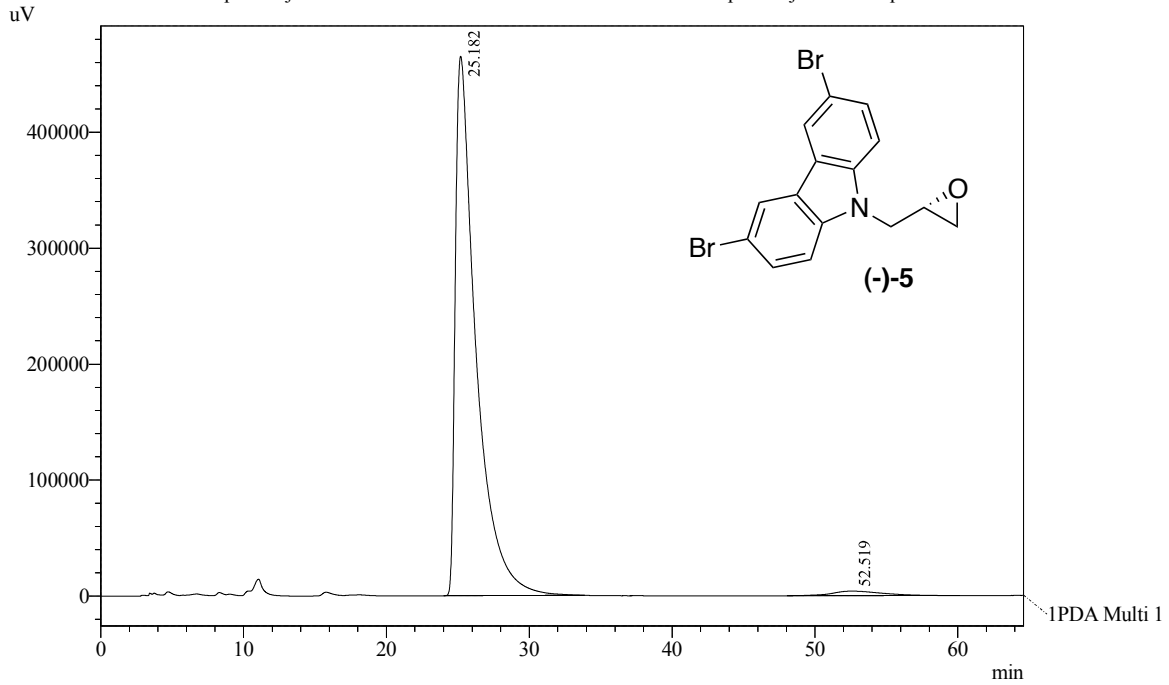
1 PDA Multi 1 / 254nm 4nm

PeakTable

PDA Ch1 254nm 4nm

| Peak# | Ret. Time | Area | Height | Area % |
|-------|-----------|----------|--------|---------|
| 1 | 17.525 | 20321801 | 316194 | 50.146 |
| 2 | 39.890 | 20203501 | 99840 | 49.854 |
| Total | | 40525302 | 416035 | 100.000 |

Chromatogram
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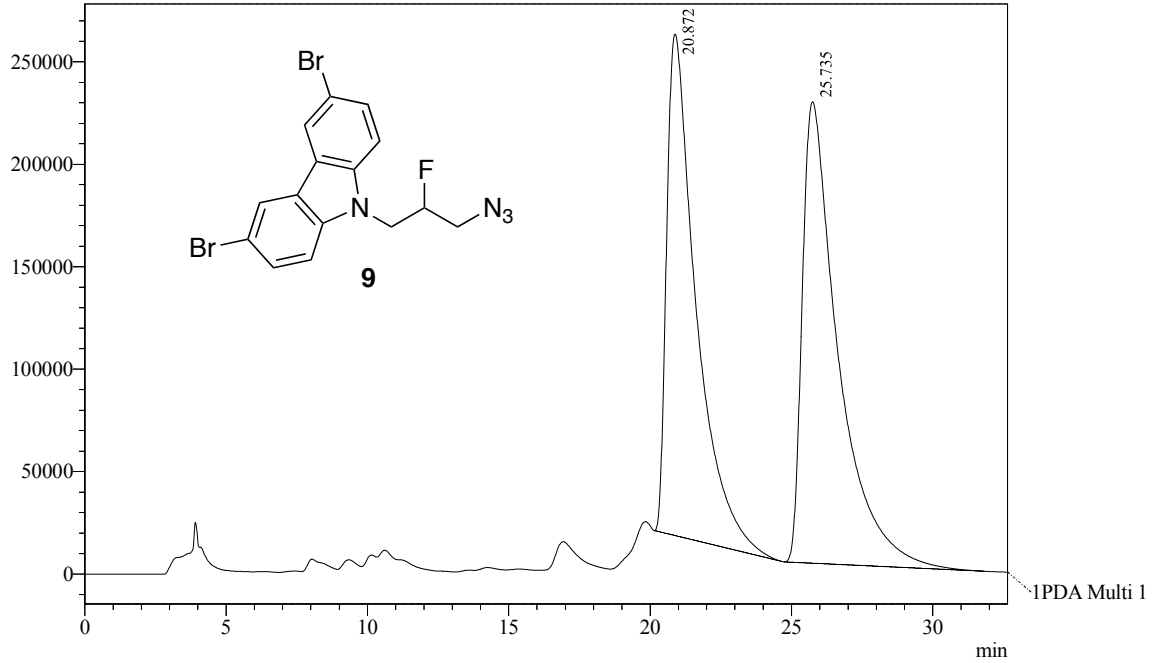
1 PDA Multi 1 / 254nm 4nm

PeakTable

PDA Ch1 254nm 4nm

| Peak# | Ret. Time | Area | Height | Area % |
|-------|-----------|----------|--------|---------|
| 1 | 25.182 | 46808223 | 465163 | 97.943 |
| 2 | 52.519 | 983006 | 3951 | 2.057 |
| Total | | 47791229 | 469114 | 100.000 |

Chromatogram
half%IPOH-fluorazide-jn-200-142-racemate-1 C:\LabSolutions\Data\Data\Jacinth\half%IPOH-fluorazide-jn-200-142-racemate-1-AD-H.lcd
uV



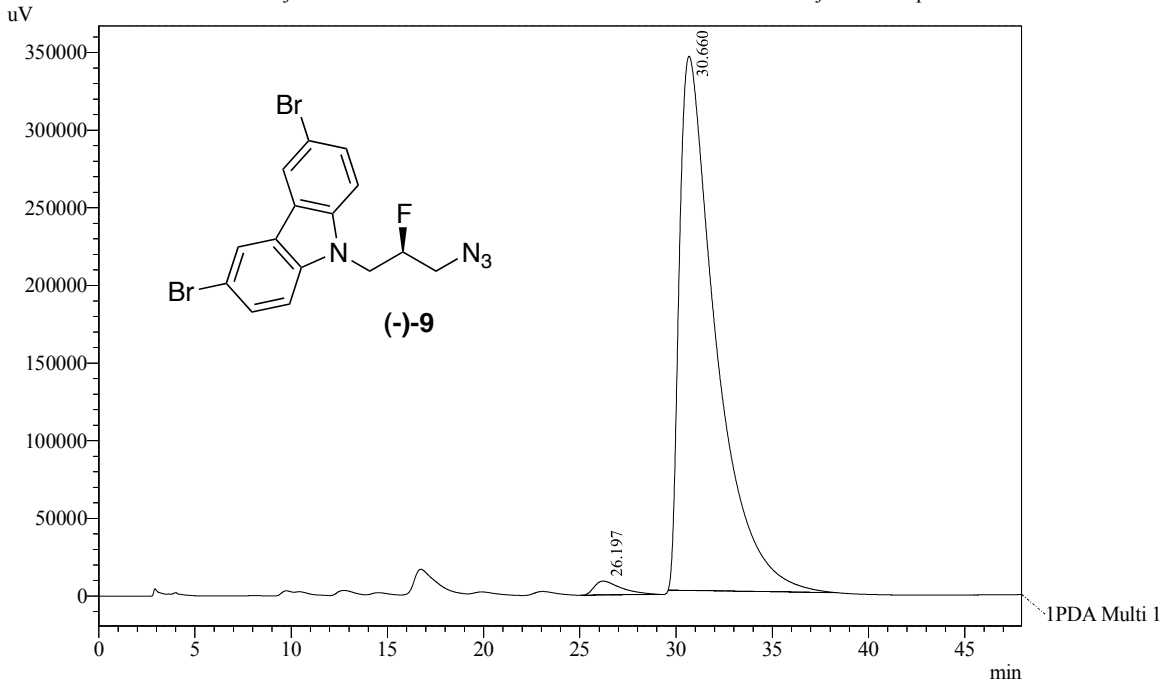
1 PDA Multi 1 / 254nm 4nm

PeakTable

PDA Ch1 254nm 4nm

| Peak# | Ret. Time | Area | Height | Area % |
|-------|-----------|----------|--------|---------|
| 1 | 20.872 | 17694311 | 244750 | 47.158 |
| 2 | 25.735 | 19826767 | 225140 | 52.842 |
| Total | | 37521078 | 469891 | 100.000 |

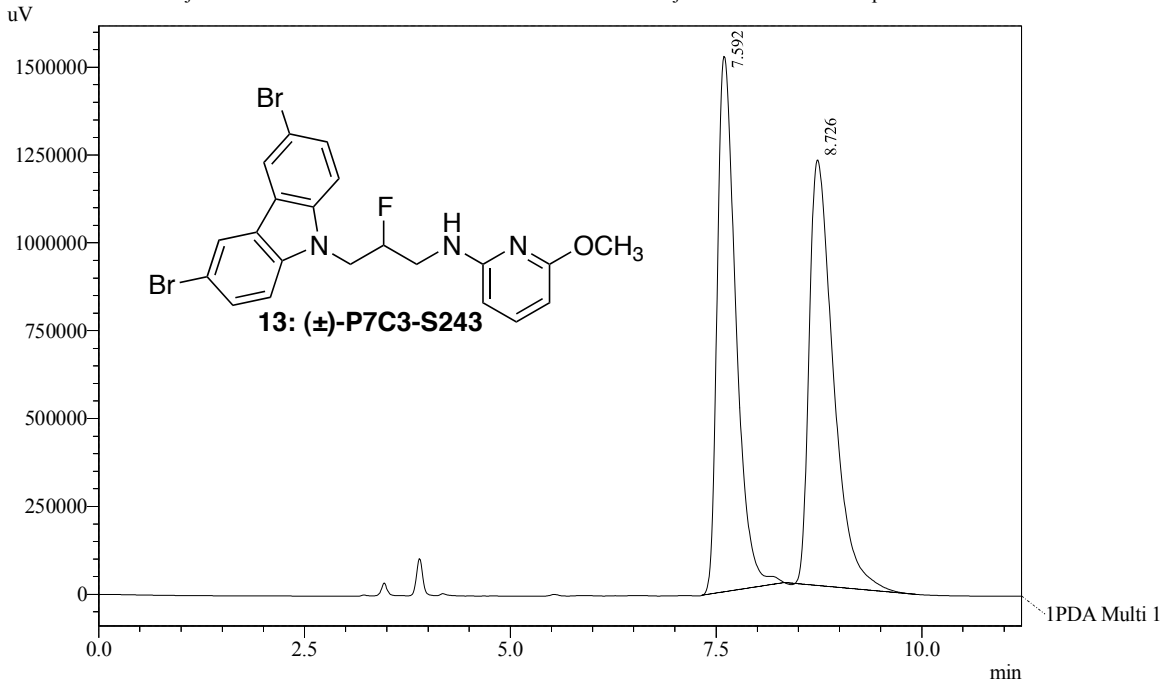
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PeakTable

PDA Ch1 254nm 4nm

| Peak# | Ret. Time | Area | Height | Area % |
|-------|-----------|----------|--------|---------|
| 1 | 26.197 | 889619 | 9048 | 1.920 |
| 2 | 30.660 | 45447952 | 343951 | 98.080 |
| Total | | 46337571 | 352999 | 100.000 |

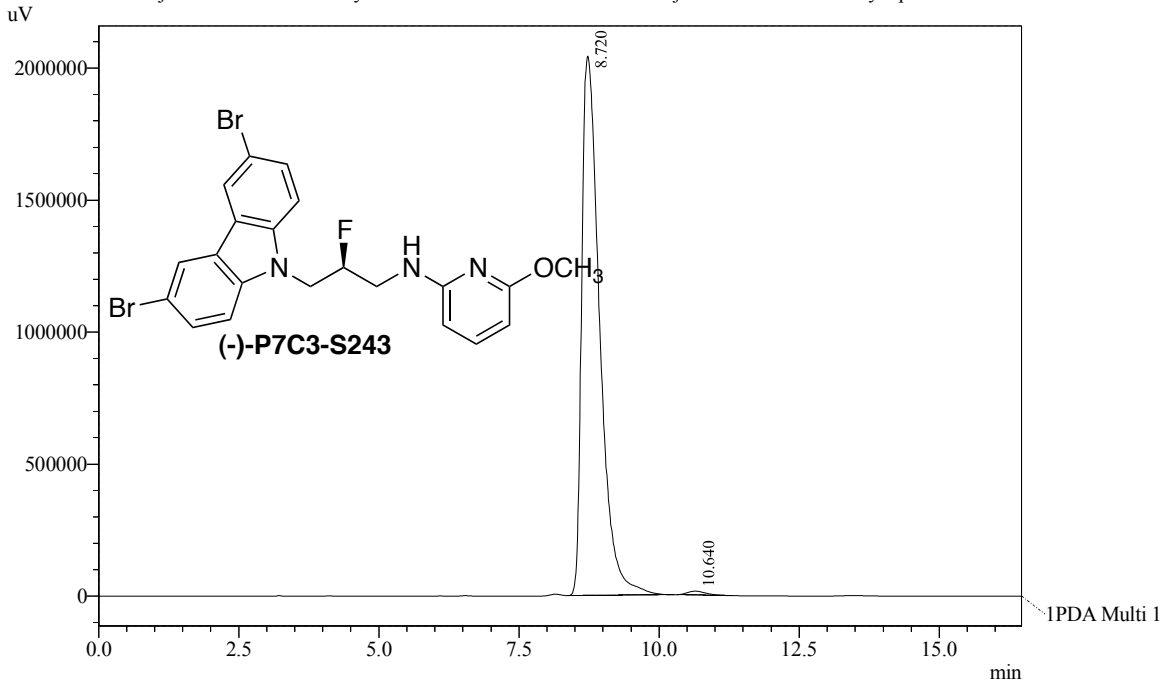


1 PDA Multi 1 / 254nm 4nm

PeakTable

PDA Ch1 254nm 4nm

| Peak# | Ret. Time | Area | Height | Area % |
|-------|-----------|----------|---------|---------|
| 1 | 7.592 | 24279294 | 1524959 | 49.520 |
| 2 | 8.726 | 24749758 | 1211408 | 50.480 |
| Total | | 49029052 | 2736367 | 100.000 |



1 PDA Multi 1 / 254nm 4nm

PeakTable

PDA Ch1 254nm 4nm

| Peak# | Ret. Time | Area | Height | Area % |
|-------|-----------|----------|---------|---------|
| 1 | 8.720 | 45635033 | 2042657 | 99.256 |
| 2 | 10.640 | 341903 | 14661 | 0.744 |
| Total | | 45976936 | 2057317 | 100.000 |