SUPPLEMENTARY MATERIAL

Evaluation of structural effects on 5-HT_{2A} receptor antagonism by aporphines: identification of a new aporphine with 5-HT_{2A} antagonist activity

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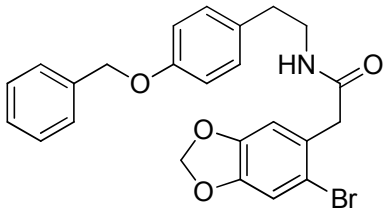
General Experimental Procedures

All glass apparatus were oven dried prior to use. A CEM Discover microwave reactor was used to carry out all direct arylation reactions. ^{1}H NMR and ^{13}C NMR spectra were recorded using Bruker DPX-500 spectrometer (operating at 500 MHz for ^{1}H; 125 MHz respectively for ^{13}C) using $CDCl_3$ as solvent. Tetramethylsilane (δ 0.00 ppm) served as an internal standard in ^{1}H NMR and $CDCl_3$ (δ 77.0 ppm) in ^{13}C NMR as solvent unless stated otherwise. Reactions were monitored by TLC with Whatman Flexible TLC silica gel G/UV 254 precoated plates (0.25 mm). TLC plates were visualized by UV (254 nm) and by staining with vanillin spraying reagent (2 gm vanillin in 1 L of 10% H_2SO_4) followed by heating. Flash column chromatography was performed with silica gel 60 (EMD Chemicals, 230-400 mesh, 0.04-0.063 μm particle size). All chemicals and reagents were obtained from Sigma-Aldrich and Fischer Scientific (USA) and were used without further purification.

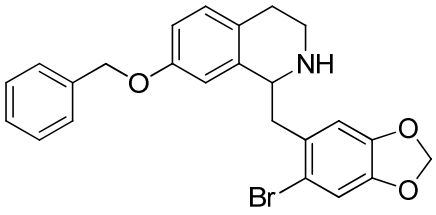
Synthesis of Compound 6



6

A solution of acid **5** (5.1 mmol) and 1,1′-carbonyldiimidazole (CDI, 4.6 mmol) in anhydrous tetrahydrofuran (THF, 40 mL) was stirred at 0 °C for 1.5 h and then at room temperature for 1 h. The mixture was cooled in an ice-bath and stirred for 1 h. 4-methoxyphenethylamine **4** (4.6 mmol) was then added and the solution was stirred at 0 °C for 4 h and stirred overnight at room temperature. The reaction mixture was evaporated under reduced pressure and the residue was dissolved in ethyl acetate and extracted with 1 N HCl (25 mL), washed sequentially with water (50 mL), saturated $NaHCO_3$ solution (25 mL), water (50 mL), and finally with brine (50 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was crystallized from ethyl acetate/diethylether to furnish amide **6** as a white solid (80% yield).

Synthesis of Compound 7

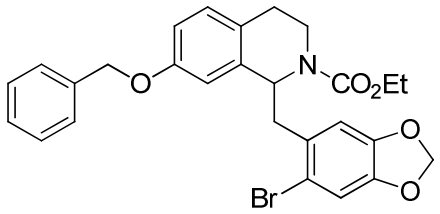


7

To a stirred solution of amide **6** (2.2 mmol) and pyridine (0.8 mL) in dichloromethane (DCM, 7 mL) was added trifluoromethanesulfonic acid dropwise at -78 ^{o}C under argon atmosphere. The reaction mixture was stirred at -78 ^{o}C for

30 minutes and warmed to room temperature. The reaction mixture was transferred in to a separatory funnel and washed with aqueous HCl (10 mL, 1M) followed by brine (15 mL). The DCM layer was dried over anhydrous Na_2SO_4, filtered and evaporated to dryness to yield a crude imine, which was used further without any purification. To a magnetically stirred ice-cooled solution of this crude imine in a mixture of dry methanol (MeOH, 20 mL) and dry DCM (20 mL), was added powdered sodium borohydride ($NaBH_4$, 11.5 mmol) in three portions over 10 min. The reaction mixture was stirred at 0 °C for 2 h. The mixture was diluted with water and extracted with DCM (3 × 20 mL). The combined organic layer was washed with brine (10 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The crude product was purified by flash column chromatography over deactivated silica gel using 0.7% MeOH/DCM as eluant to furnish pure **7** as a white solid (88% yield).

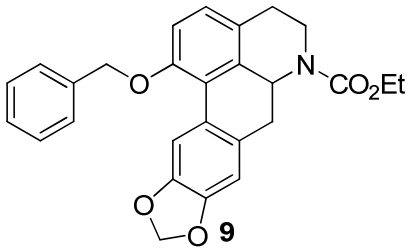
Synthesis of Compound 8



8

To a solution of compound **7** (1 mmol) dissolved in dry DCM (25 mL) was added ethyl chloroformate (1 mmol), and potassium carbonate (1.63 mmol) at room temperature. The reaction mixture was stirred at room temperature for 12 h, quenched with saturated NH_4Cl solution (10 mL), and extracted with DCM (3 × 20 mL). The organic layer was washed with water (10 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resultant crude product, was purified via column chromatography over silica gel using ethyl acetate/hexanes (20:80) as eluant. This furnished **8** as a viscous oil (85% yield).

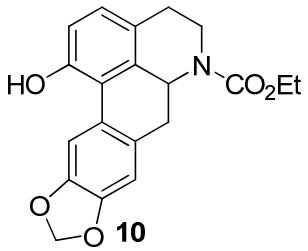
Synthesis of Compound 9



9

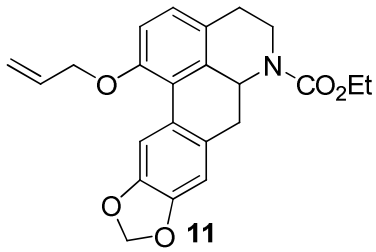
In a 5 mL microwave reaction vial, compound **8** (0.1 mmol), $Pd(OAc)_2$ (0.02 mmol), di-*tert*-butyl(methyl)phosphonium tetrafluoroborate, (0.04 mmol) and K_2CO_3 (0.30 mmol) were added and dissolved in Argon-purged anhydrous DMSO (0.5 mL). The mixture was irradiated in a CEM Discover microwave reactor for 6 min at 130 °C with the power level at 300 W. After cooling to room temperature, the reaction mixture was loaded directly onto a deactivated silica gel column and eluted with 10% ethyl acetate/hexanes to furnish compound **9** as a white solid (50% yield).

Synthesis of Compound 10



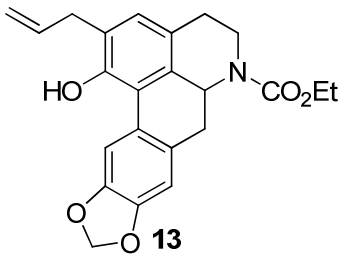
To a solution of compound **9** (1 mmol) in dry methanol (10 mL) was added 10% Pd/C (25 mg) under inert atmosphere. The reaction was purged with H_2 and stirred under a balloon of H_2 for 8 h. 10% Pd/C was filtered through a celite pad and the filtrate was evaporated to give the corresponding phenol **10** as a white solid (95% yield).

Synthesis of Compound 11



To a stirred solution of phenol (1 mmol) in acetonitrile (10 mL) was added solid K_2CO_3 (2 mmol) and potassium iodide (2 mmol) at room temperature. The resulting mixture was stirred for 5 min and then allyl bromide (1.5 mmol) was added and refluxed for 6 h. The solvent was evaporated and the resulting solid dissolved in water and extracted with dichloromethane twice (20 mL each). The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography using 12.5% ethyl acetate/hexanes (60-70% yield).

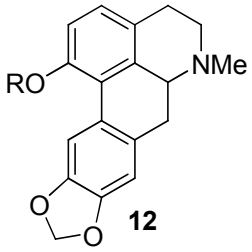
Synthesis of Compound 13



In a 5mL microwave reaction vial, compound **11** (0.**5** mmol) was dissolved in *N,N*-diethylaniline (2 mL). The mixture was irradiated in the CEM Discover microwave reactor for 6 min at 215 ^{o}C with the power level at 300 W. After cooling to room temperature, the reaction mixture was washed with 1N HCl (5 mL) and extracted with DCM (10 mL). The crude

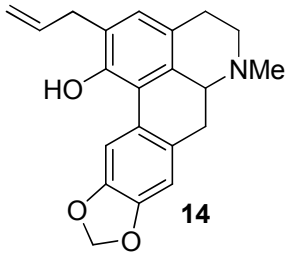
mixture was then loaded directly onto a silica gel column and eluted with 10% acetone/hexanes to furnish compound **13** (90% yield).

General procedure for LAH reduction: Synthesis of Compounds 12a, 12b and 14



12

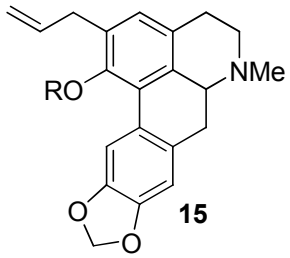
12a: R=H
12b: R=allyl



14

To a stirred suspension of LAH (10 mmol) in dry THF at 0 °C under inert atmosphere was added a solution of the carbamate (**10**, **11** or **13**; 1 mmol) in THF (5 mL) dropwise. After stirring at room temperature for 30 min, the reaction mixture was heated to reflux for 3 h and then cooled to 0 °C. The mixture was then quenched by dropwise addition of water (0.5 mL) followed by addition of 10% aqueous NaOH (1 mL). The reaction mixture was filtered through a pad of celite and the residue washed with THF (5 mL). The filtrate was evaporated under reduced pressure to provide the crude product, which was re-dissolved in DCM. The organic layer was washed sequentially with water (10 mL) and brine (10 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel with gradient elution in 5% MeOH/DCM (50% yield for **12a** from **10**; 60 % yield for **12b** from **11**; 54% yield for **14** from **13**).

Synthesis of Compounds 15a-15c



15

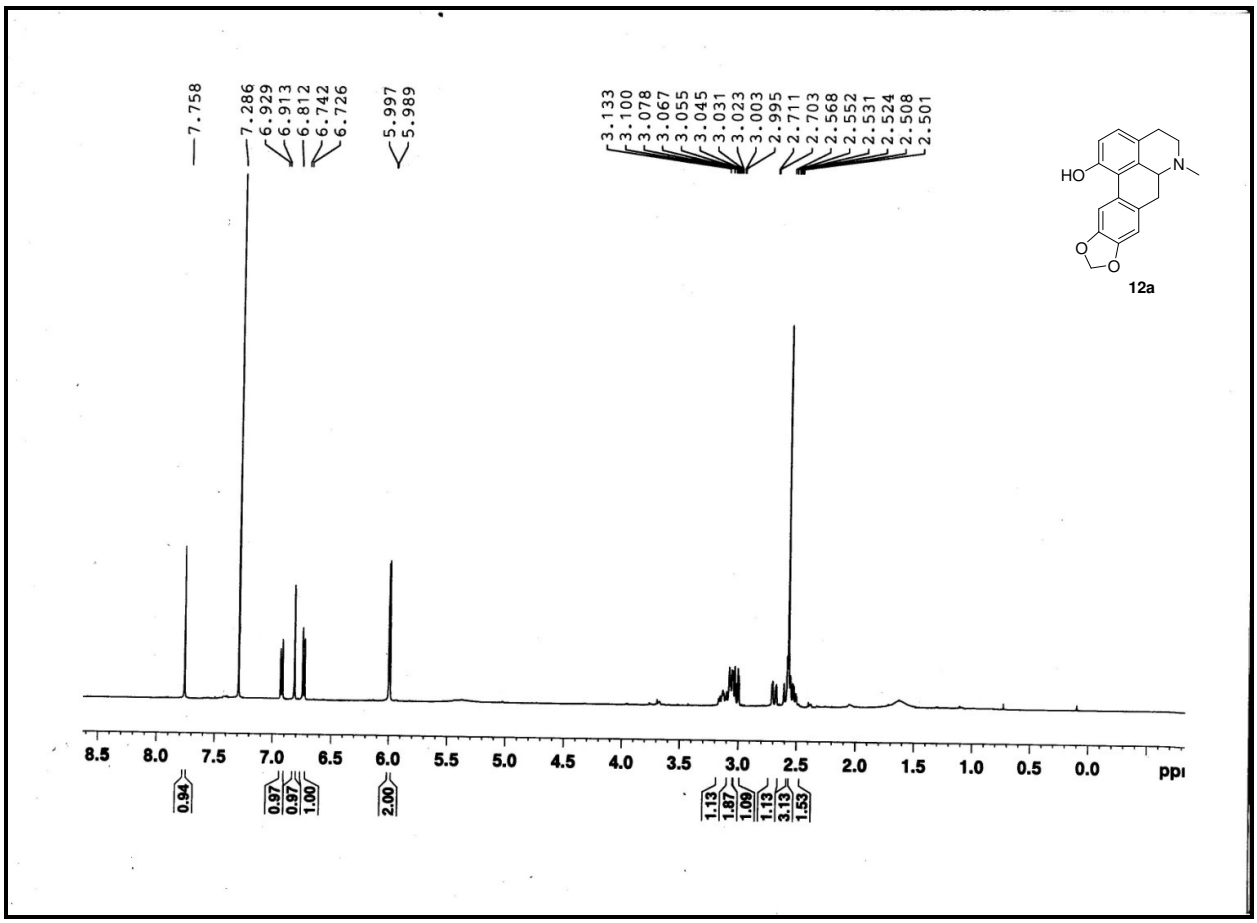
15a: R = methyl
15b: R = allyl
15c: R=cyclopropylmethyl

To a stirred solution of allylated phenol **13** (1 mmol) in acetone (10 mL) was added solid K_2CO_3 (2 mmol) and potassium iodide (2 mmol) at room temperature. The mixture was stirred for 5 min and the corresponding alkyl bromide (1.5 mmol) was added and refluxed for 7 h. The solvent was evaporated and the resulting solid dissolved in water and extracted with dichloromethane twice (20 mL each). The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography eluting in 10-30% ethyl acetate/hexanes. The resulting crude product was added to a suspension of LAH (10 mmol) in THF (25 mL) at 0 ^{o}C and heated at reflux for 6 h. After the usual workup (see general procedure for LAH reduction above), the crude compound was purified via flash

column chromatography on a silica gel column, eluted in 5-10% MeOH/DCM to furnish compounds **15 a-c** (Yield 50 - 60%).

Receptor Assays

All analogs were screened at 10 μM in multi-well format for intrinsic (agonist) and antagonist activity at the human 5-HT_{2A} receptor using FLIPR-based (Molecular Devices, Sunnydale, CA) functional assays that detect receptor-mediated mobilization of internal calcium with a calcium sensitive fluorescent dye. Compounds that showed no intrinsic activity in the functional assay and inhibited the increase in basal fluorescence elicited by the EC_{80} of 5-HT by at least 50%, had their K_e (apparent affinity in a functional assay) determined. K_e values were determined by running an 8-point half-log 5-HT concentration response curve in the presence and absence of a single concentration of antagonist. EC_{50} values were calculated for 5-HT (A) and 5-HT + test compound (A'), and these used to calculate the test compound K_e using the formula: $K_e = [L]/(DR-1)$, where [L] equals the concentration of test compound in the assay and DR equals the dose ratio or A'/A. A similar set of assays was performed for the α_{1A}-adrenergic receptor.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2.703
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2.531
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2.508
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N
O
O
12a
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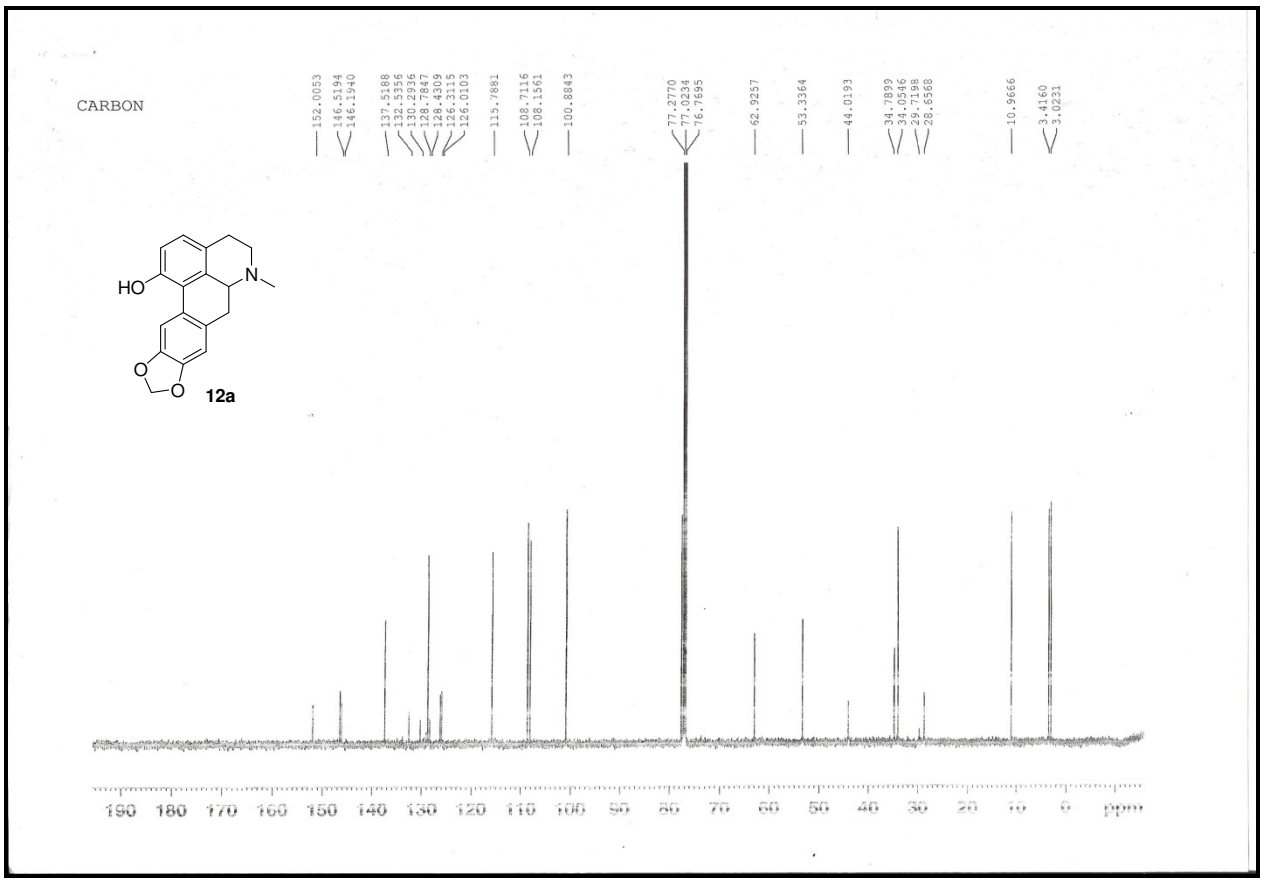
1.53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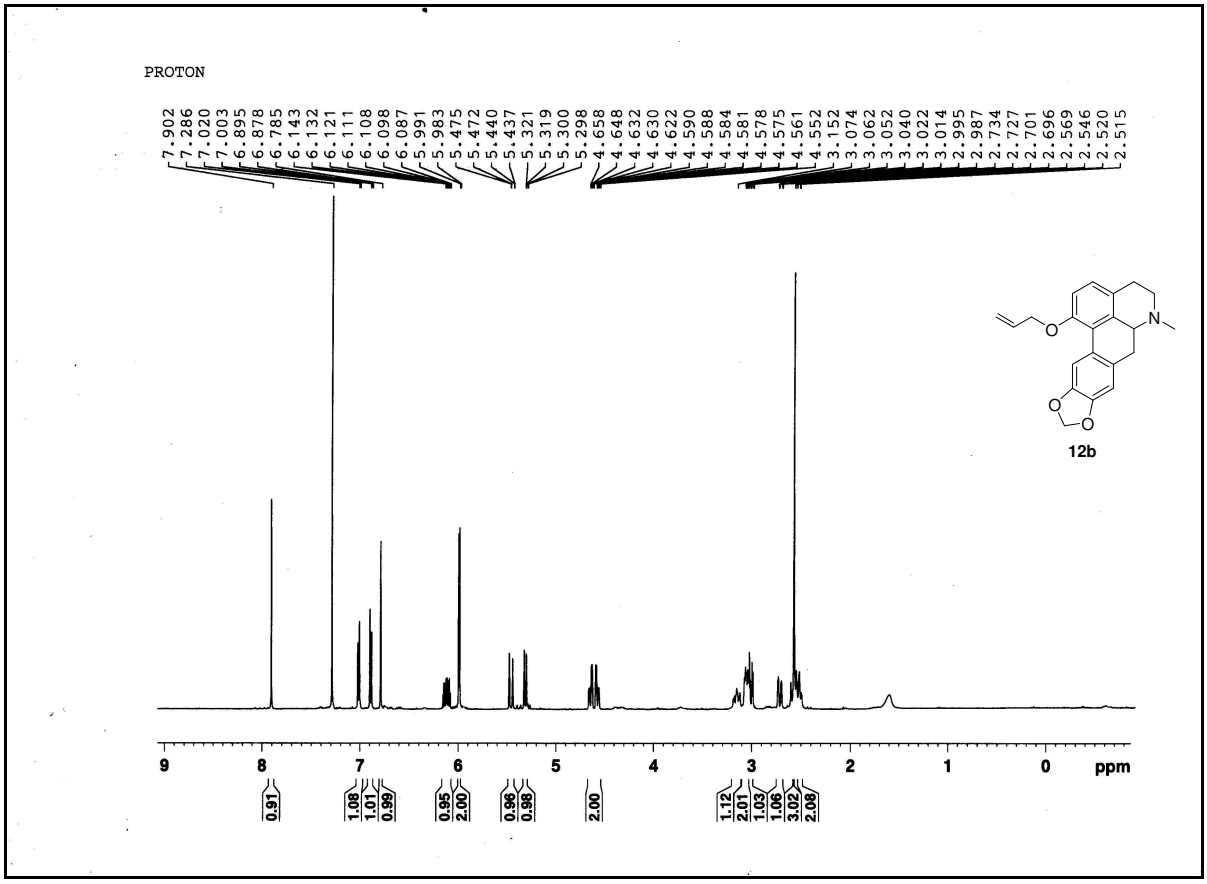
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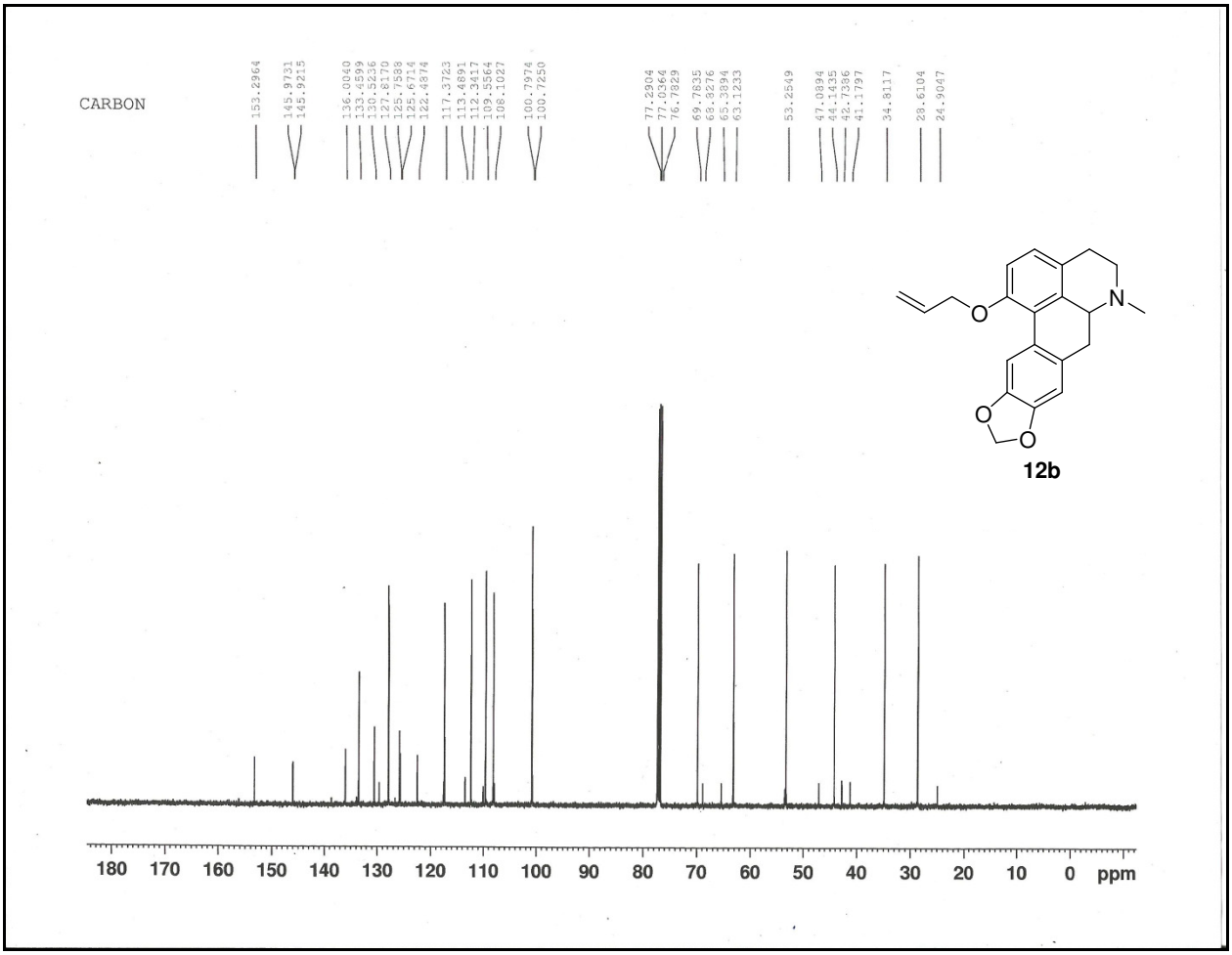
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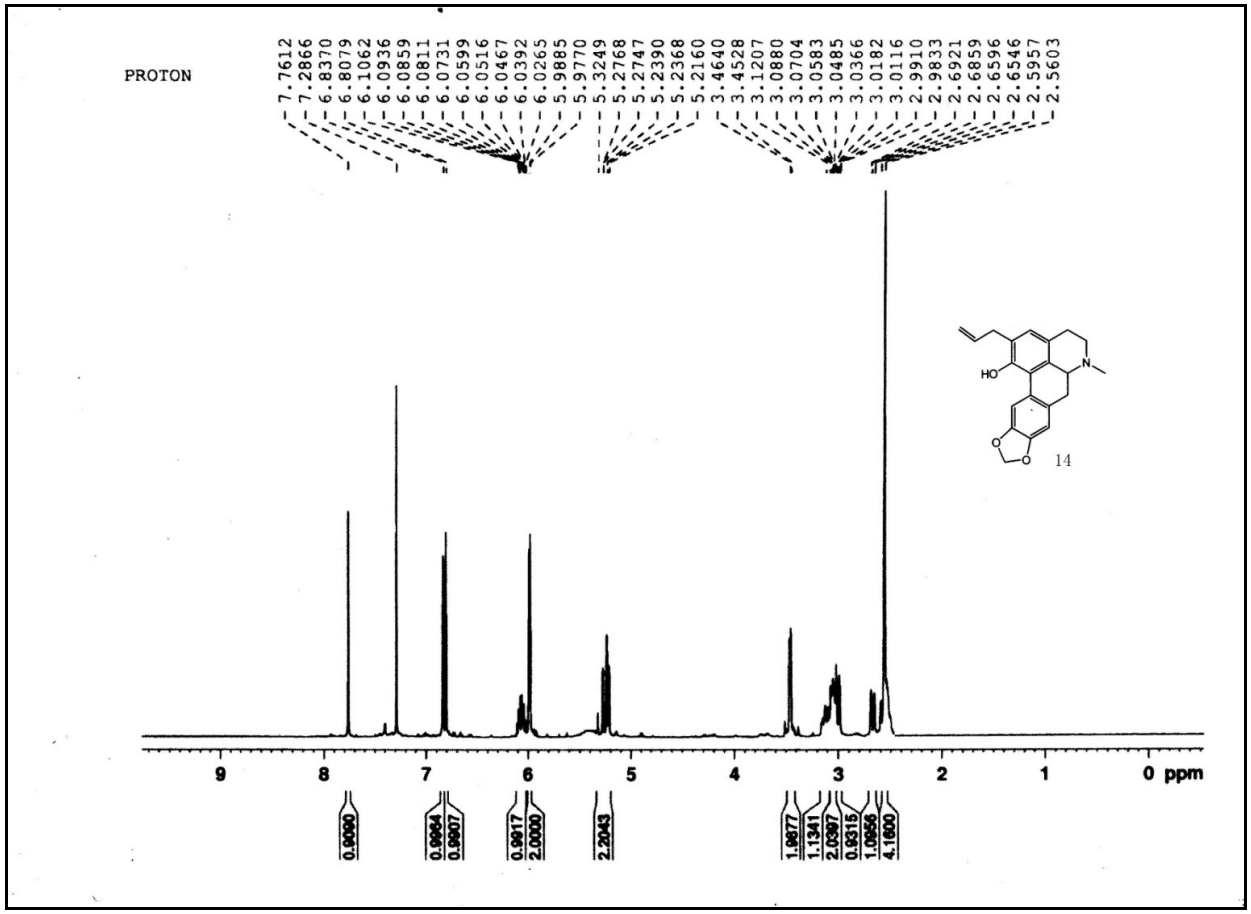
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6
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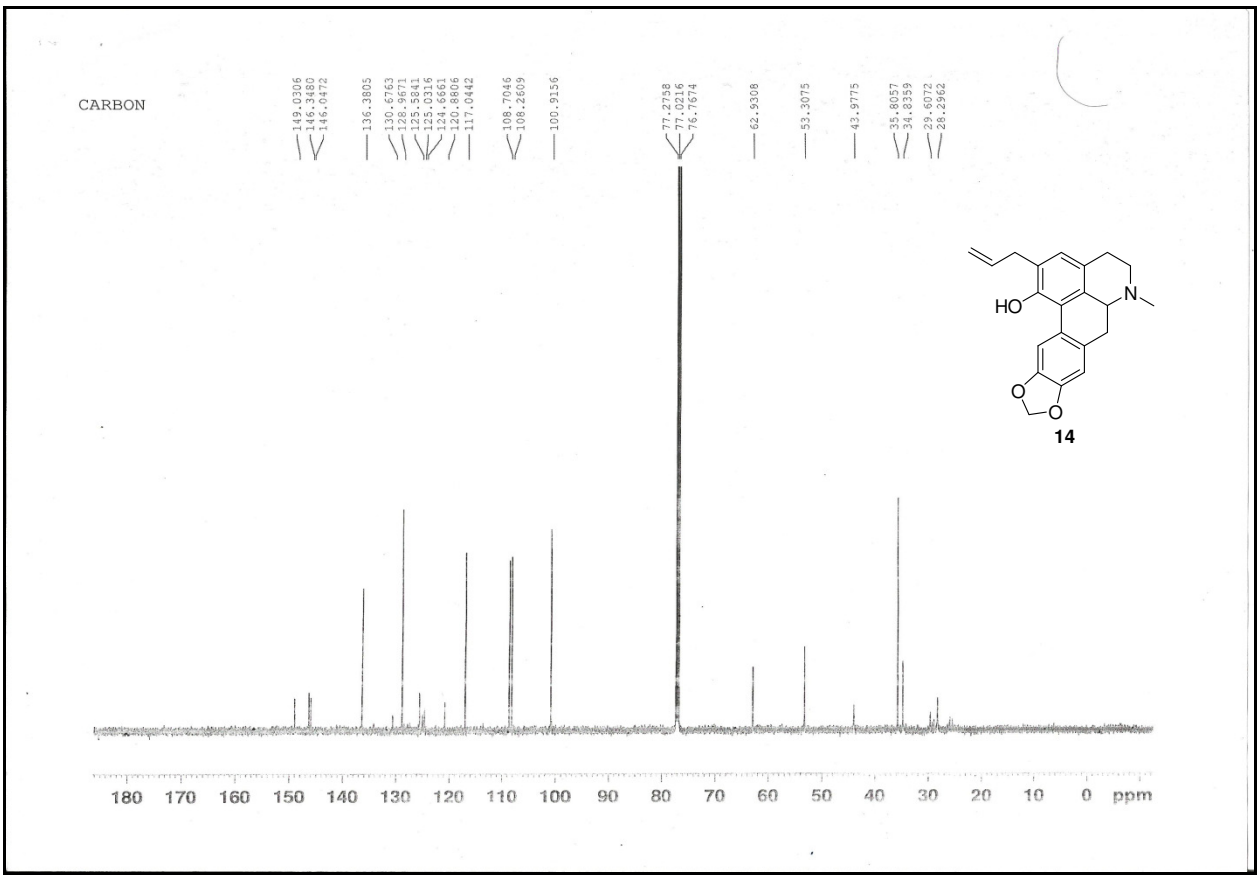
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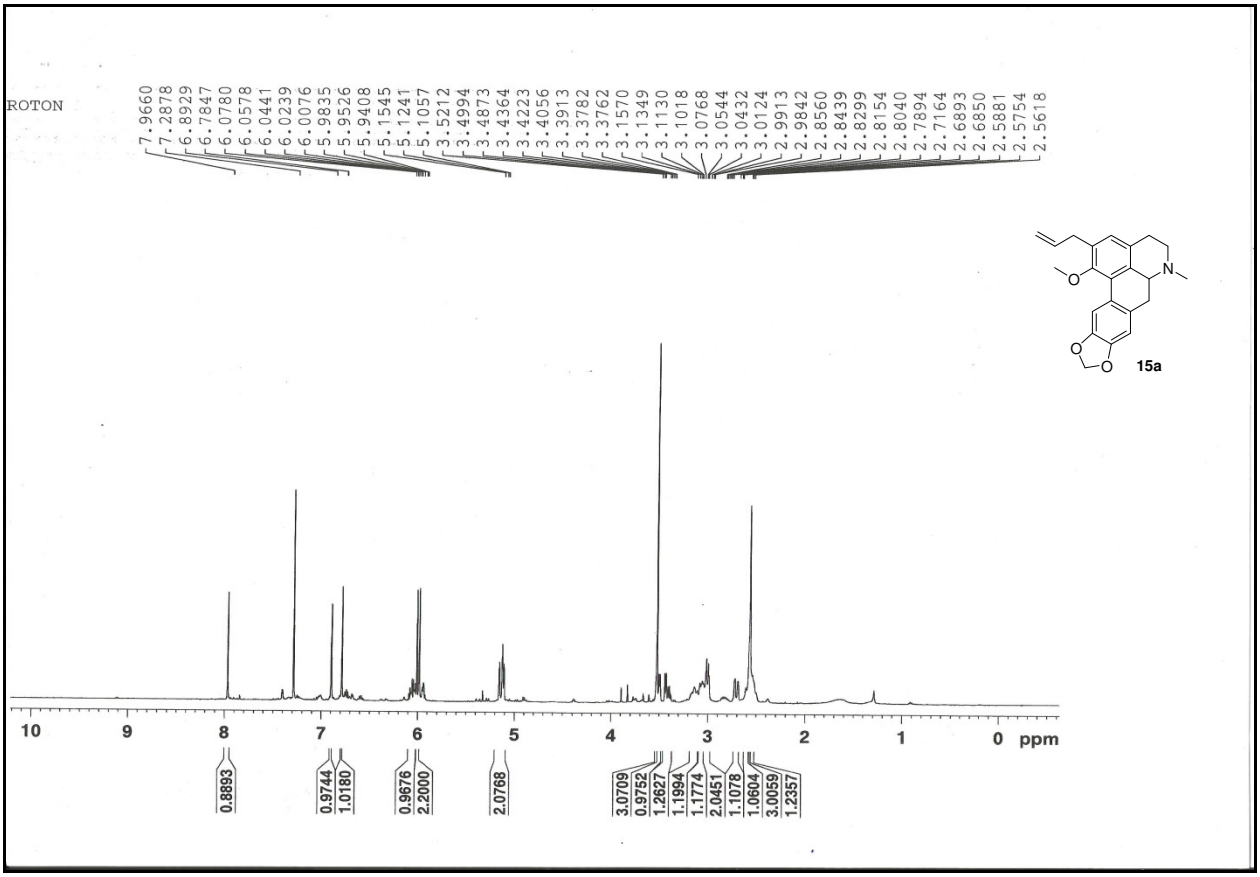
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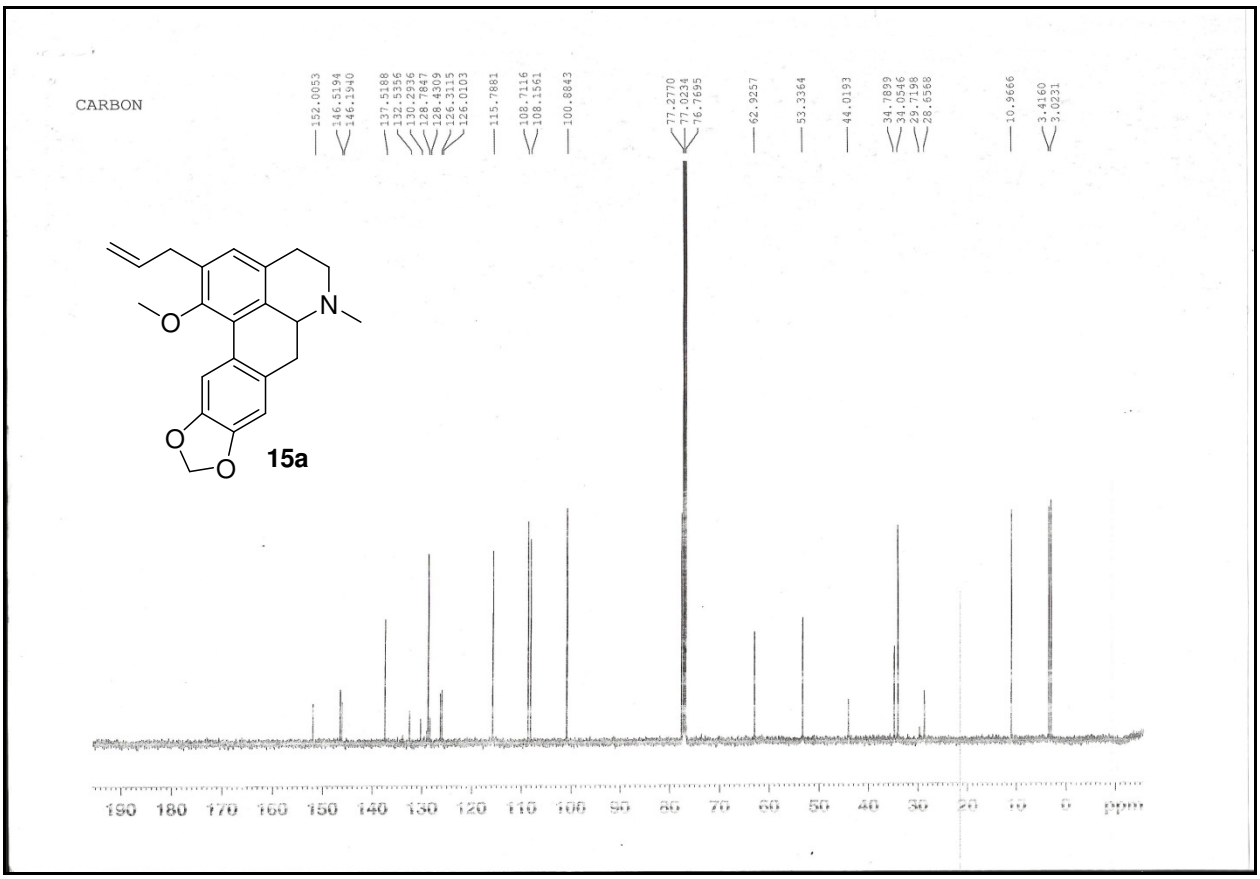
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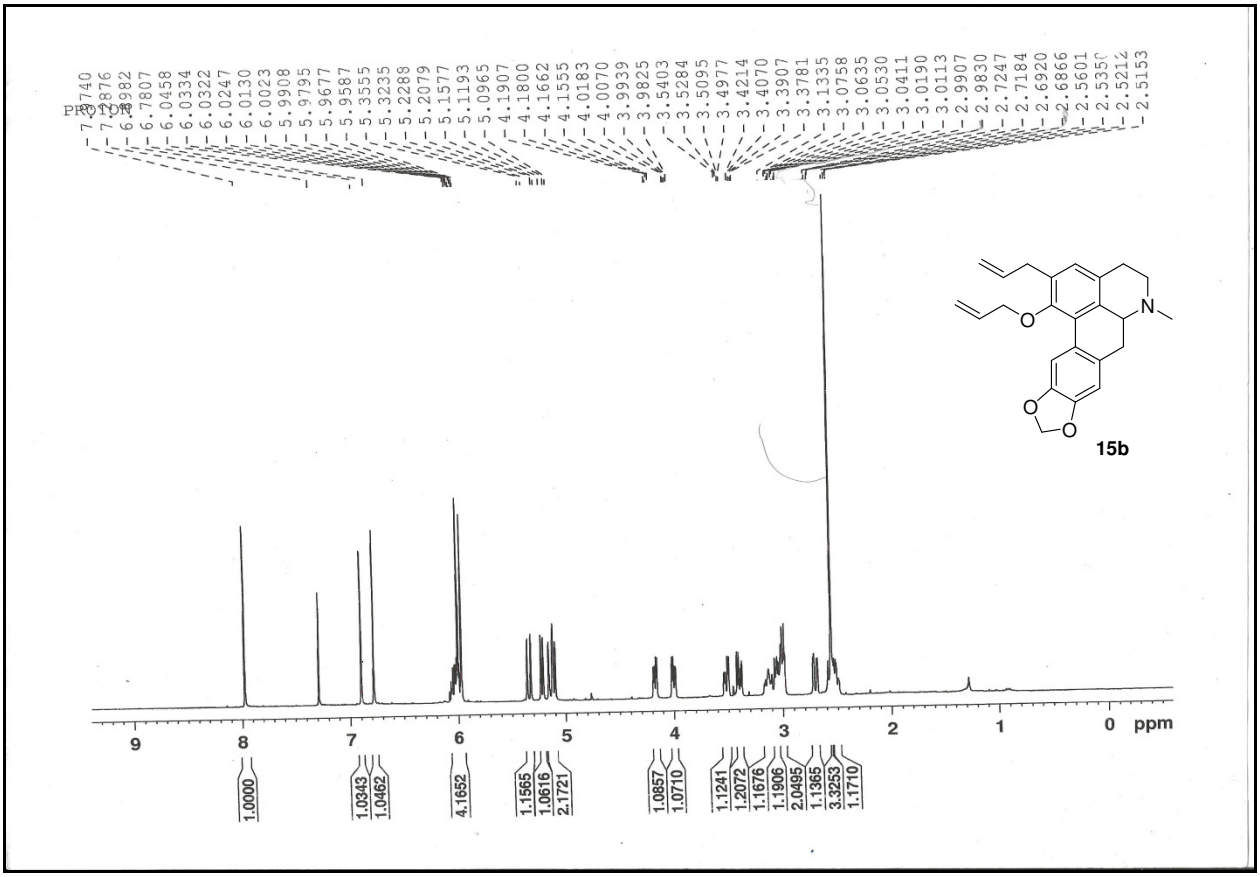
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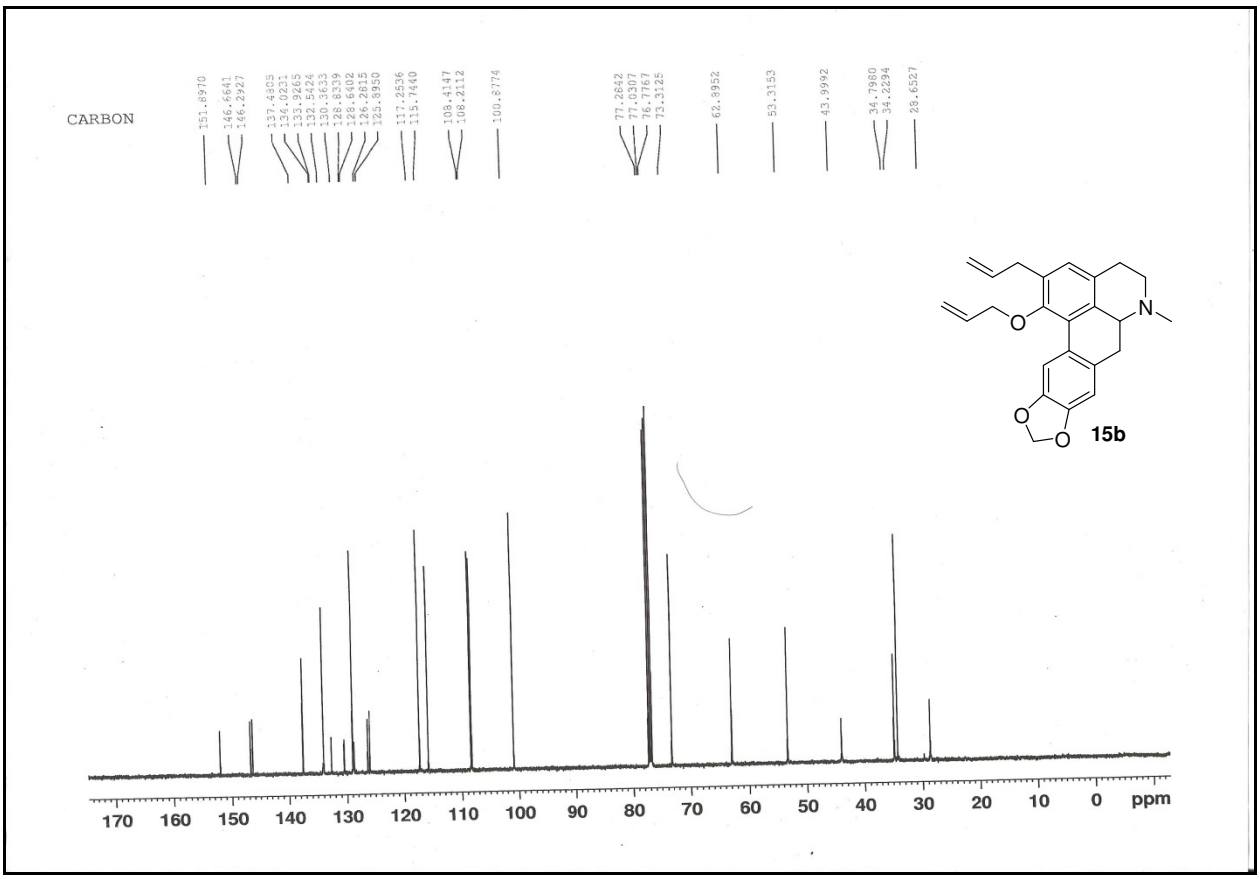
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43.9992
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34.2294
28.6527
O
N
O
O
O
15b
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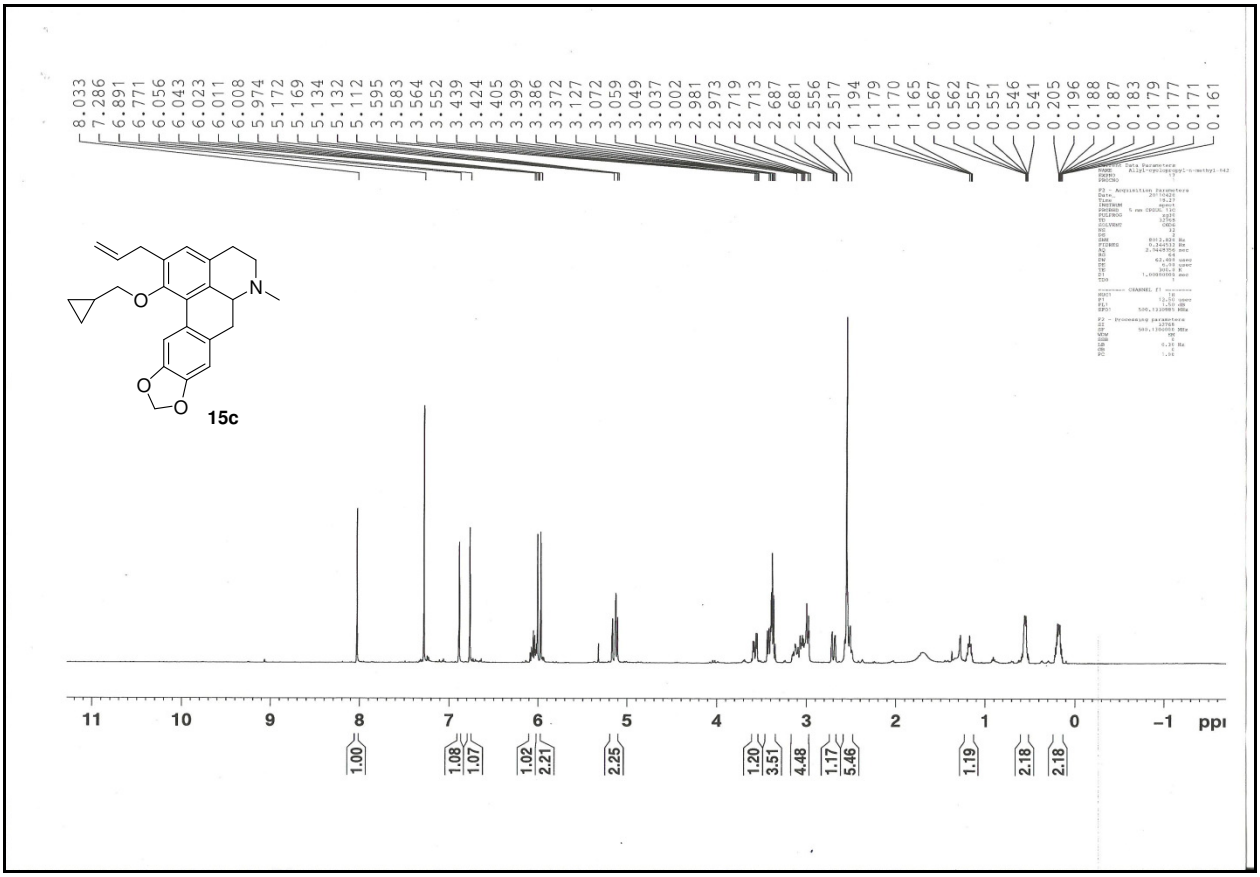
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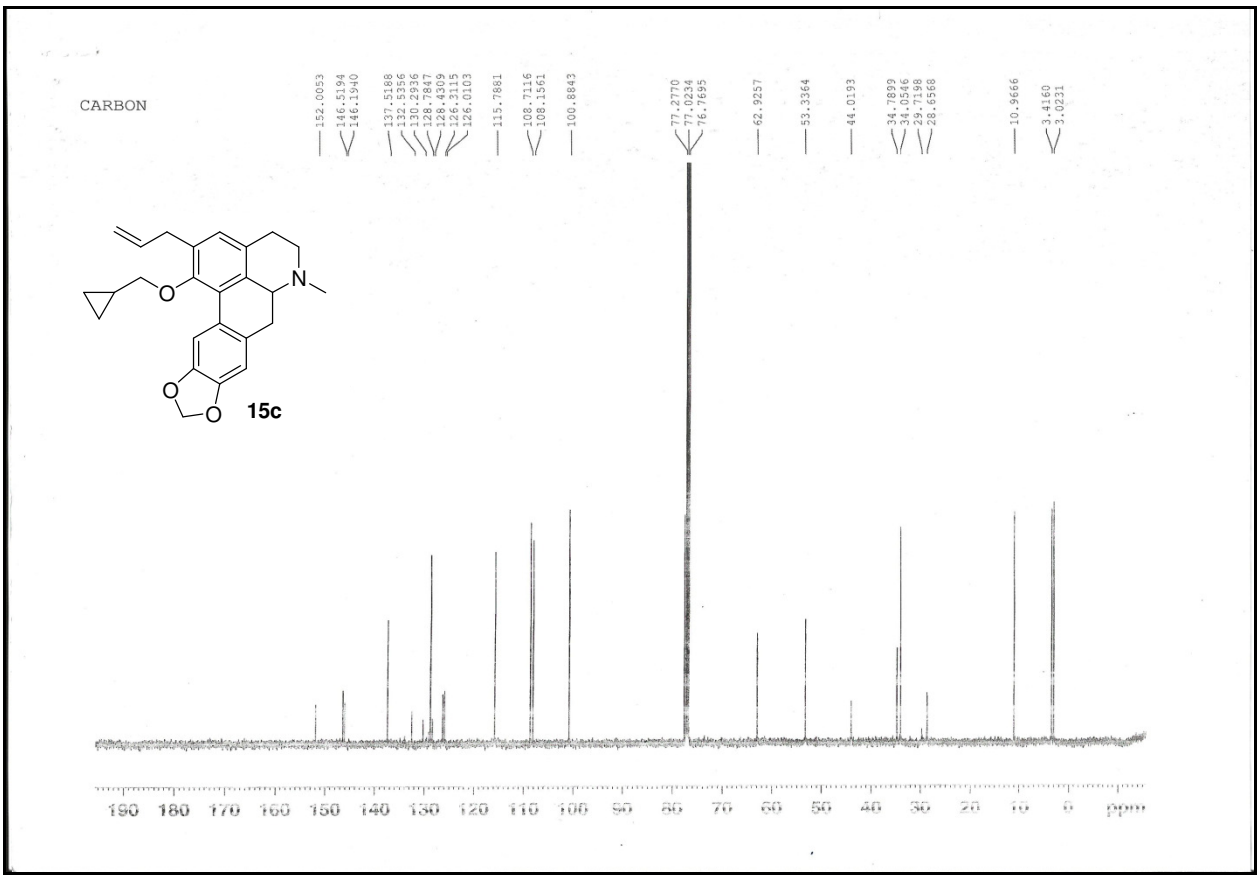
5.134
5.132
5.112
3.595
3.583
3.564
3.552
3.439
3.424
3.405
3.399
3.386
3.372
3.127
3.072
3.059
3.049
3.037
3.002
2.981
2.973
2.719
2.713
2.687
2.681
2.556
2.517
1.194
1.179
1.170
1.165
0.567
0.562
0.557
0.551
0.546
0.541
0.205
0.196
0.188
0.187
0.183
0.179
0.177
0.171
0.161
15c
11
10
9
8
7
6
5
4
3
2
1
0
−1
ppm
1.00
1.08
1.07
1.02
2.21
2.25
1.20
3.51
4.48
1.17
5.46
1.19
2.18
2.18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130.2936
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115.7881
108.7116
108.1561
100.8843
77.2770
77.0234
76.7695
62.9257
53.3364
44.0193
34.7899
34.0546
29.7198
28.6568
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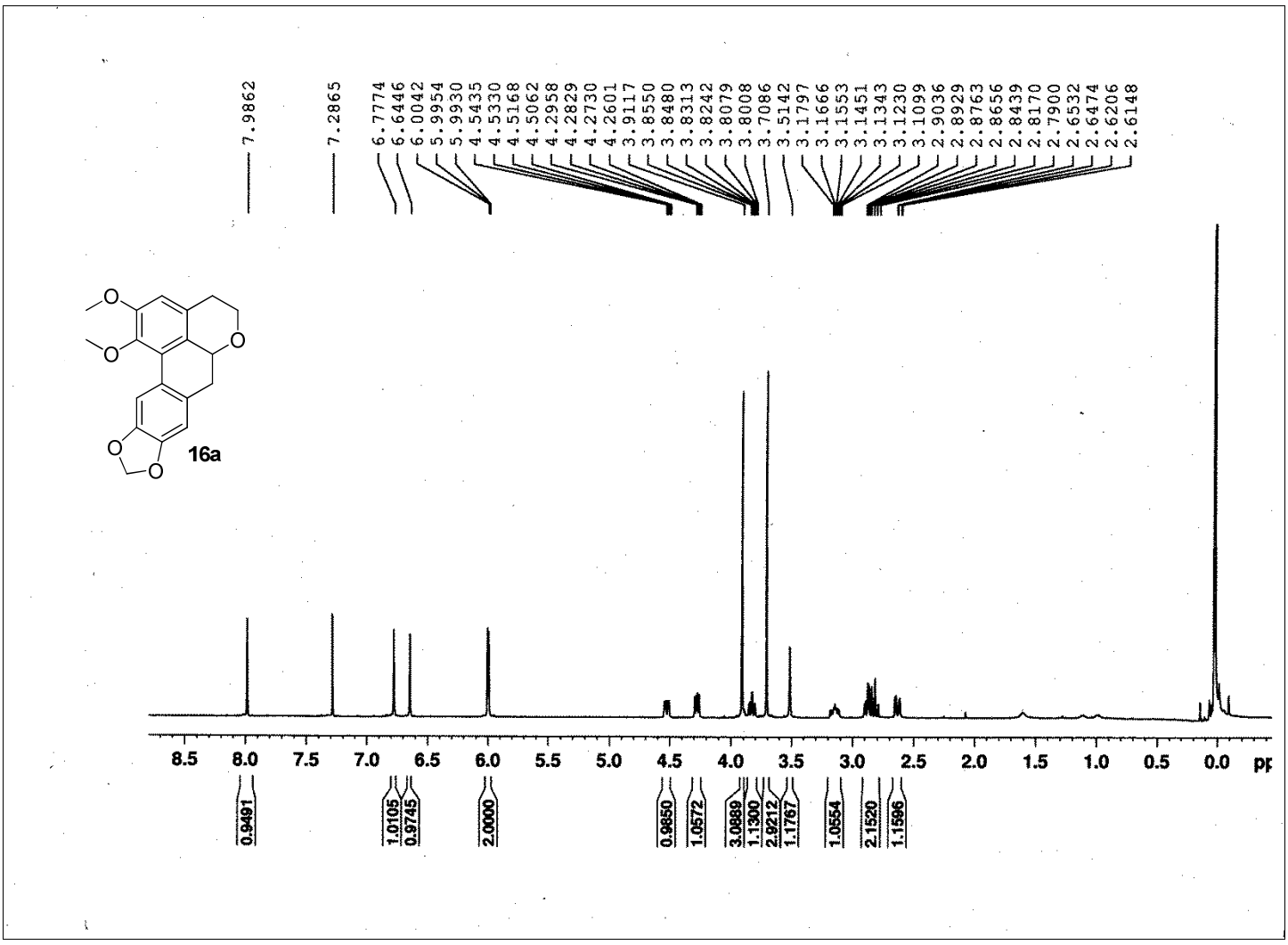
3.4160
3.0231
O
N
O
O
15c
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm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6.7774
6.6446
6.0042
5.9954
5.9930
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4.5330
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4.5062
4.2958
4.2829
4.2730
4.2601
3.9117
3.8550
3.8480
3.8313
3.8242
3.8079
3.8008
3.7086
3.5142
3.1797
3.1666
3.1553
3.1451
3.1343
3.1230
3.1099
3.9036
2.8929
2.8763
2.8656
2.8439
2.8170
2.7900
2.6532
2.6474
2.6206
2.6148
16a
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
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1.0105
0.9745
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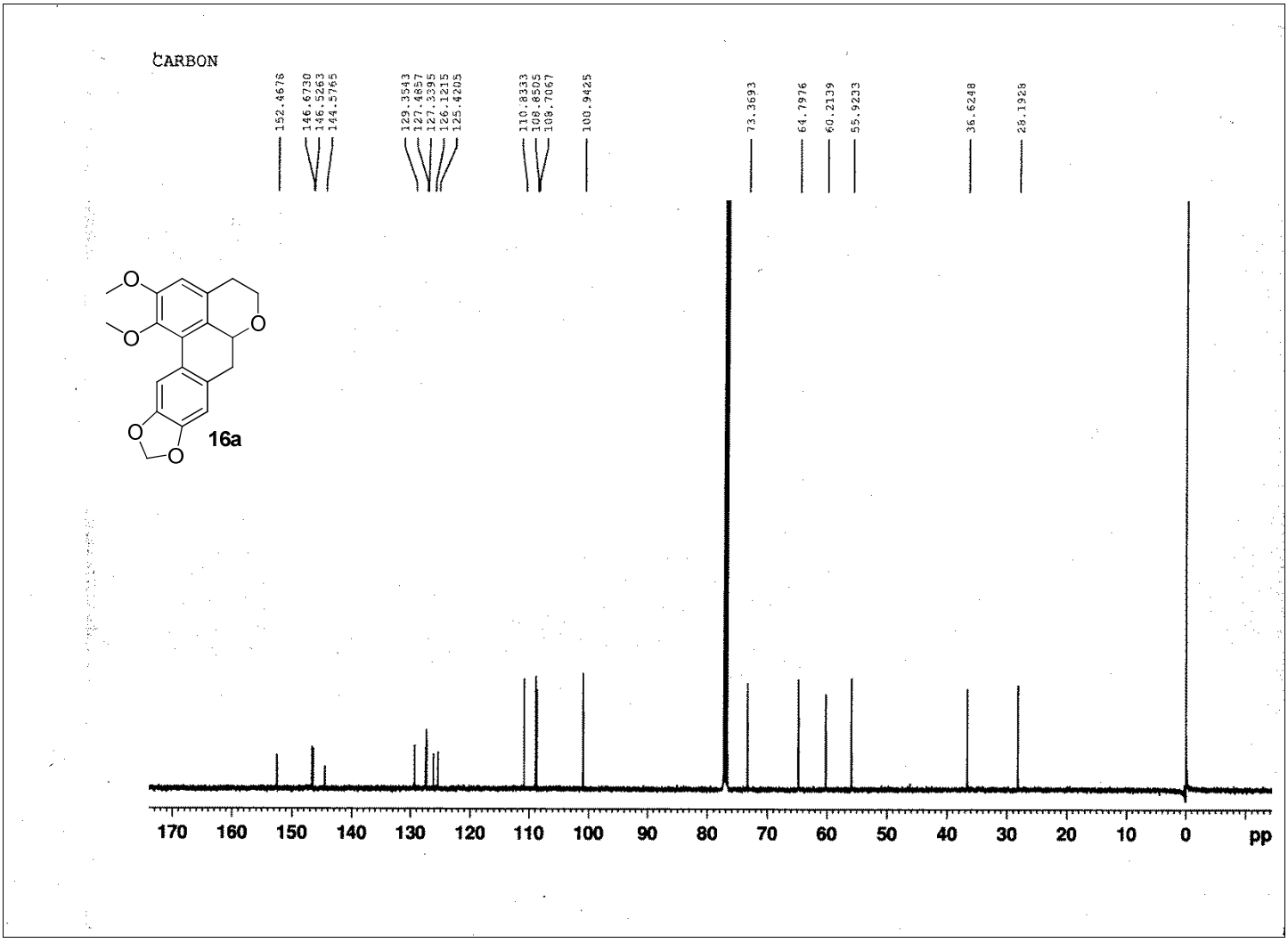
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127.4857
127.3395
126.1215
125.4205
110.8333
108.8505
108.7067
100.9425
73.3693
64.7976
60.2139
55.9233
36.6248
28.1928
16a
170
160
150
140
130
120
110
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90
80
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50
40
30
20
10
0
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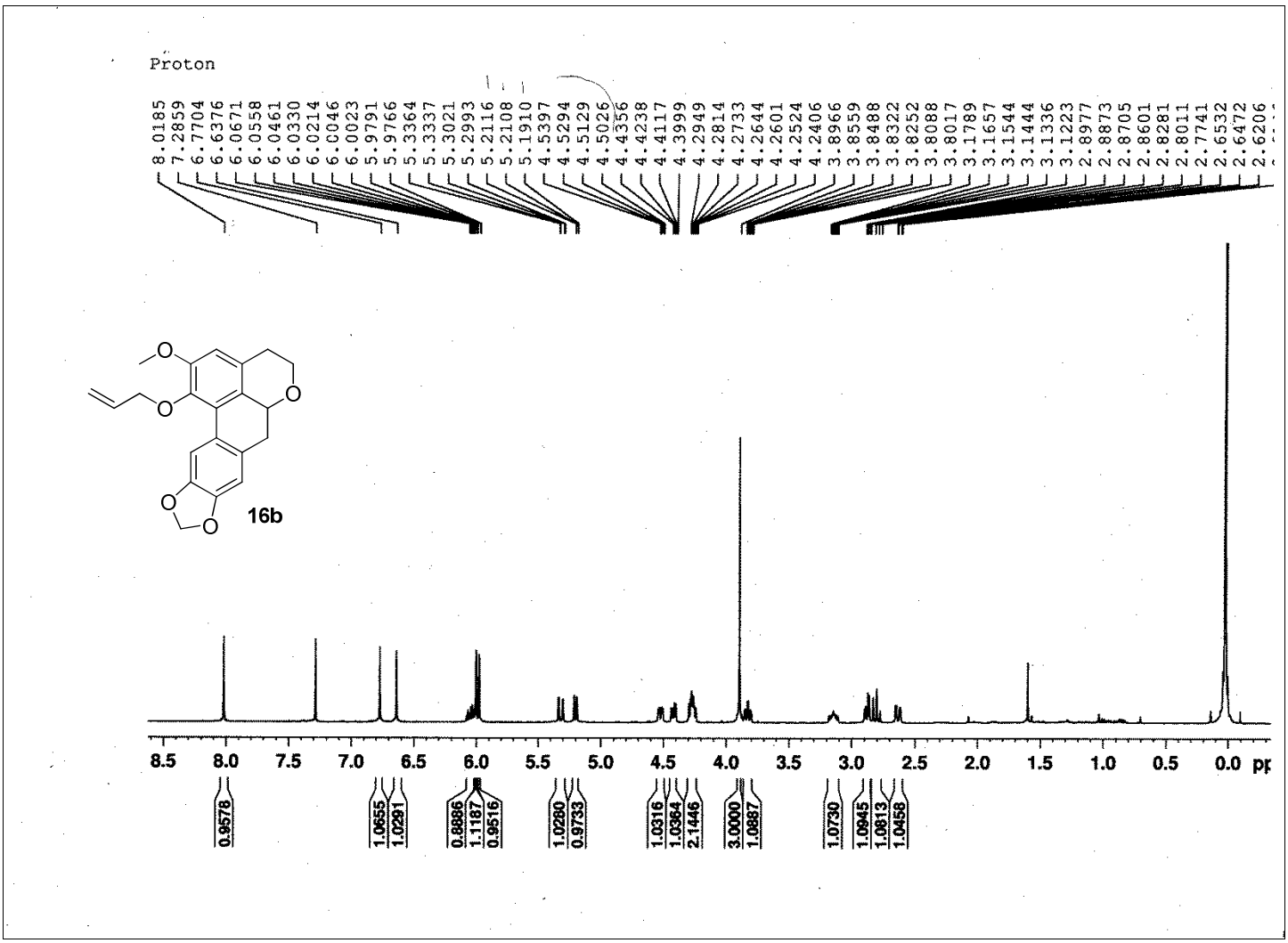
Proton
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6.0023
5.9791
5.9766
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5.2993
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5.2108
5.1910
4.5397
4.5294
4.5129
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4.4356
4.4238
4.4117
4.3999
4.2949
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4.2733
4.2644
4.2601
4.2524
4.2406
3.8966
3.8559
3.8488
3.8322
3.8252
3.8088
3.8017
3.1789
3.1657
3.1544
3.1444
3.1336
3.1223
2.8977
2.8873
2.8705
2.8601
2.8281
2.8011
2.7741
2.6532
2.6472
16b
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.9578
1.0655
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0.9516
1.0280
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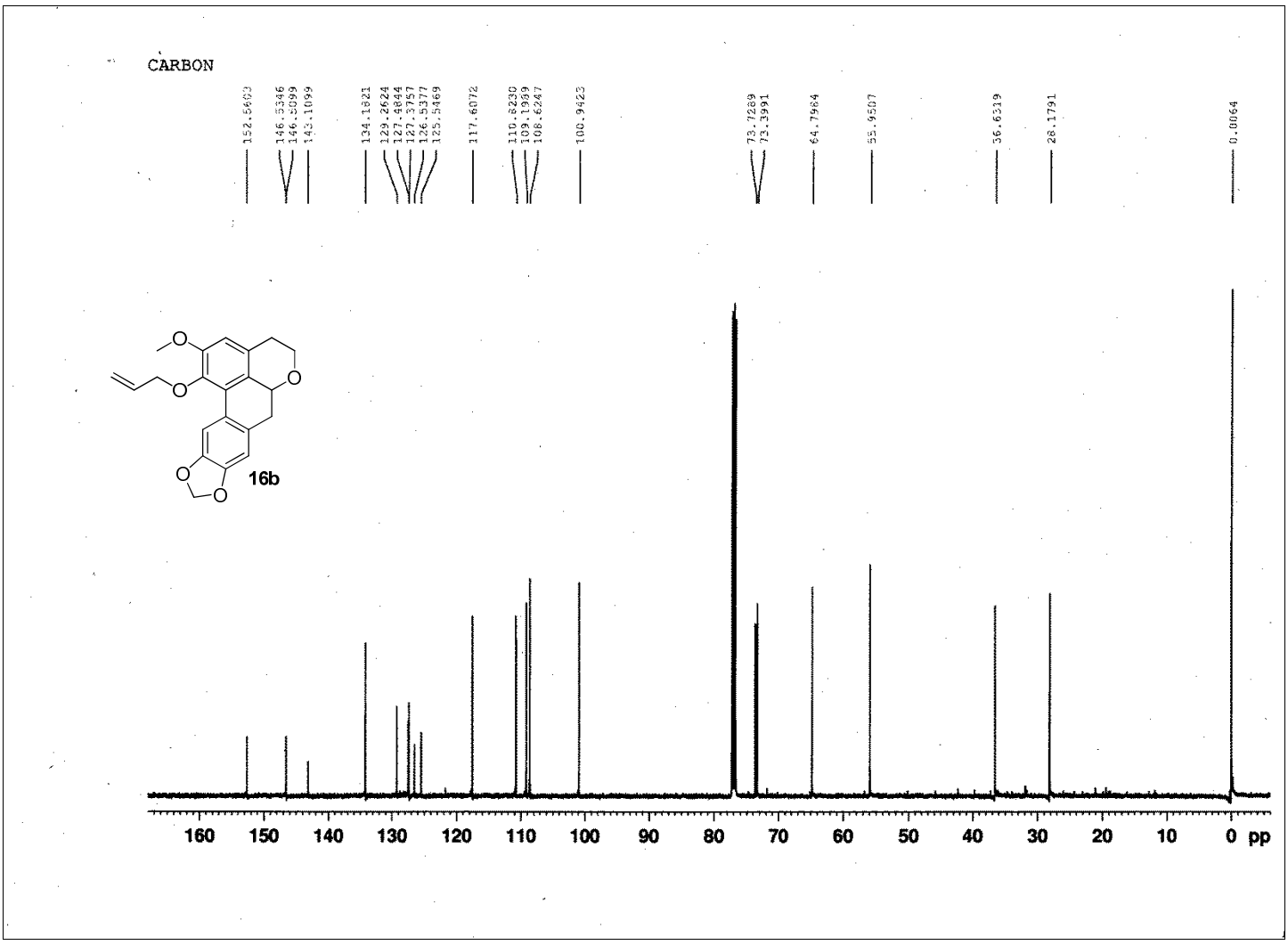
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127.3757
126.5377
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117.6072
110.8230
109.1959
108.6247
100.9423
73.7289
73.3991
64.7964
55.9507
36.6319
28.1791
0.0064
16b
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0 pp