Supporting Information for

Phenylcinnamides as novel antimitotic agents

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Biological Evaluation

Biological Materials. Purified bovine brain tubulin was a gift of Prof. Tim Mitchison.[1] Before use, polymerization-competent tubulin was repurified following Mitchison's polymerization/depolymerization cycling protocol and quantitated spectrophotometrically using $\varepsilon_{280\ nm}$ = 115,000 $M^{-1}cm^{-1}$.[1] MTS/PMS CellTiter 96 cell proliferation assay reagent was purchased from Promega (Madison, WI). Fetal bovine serum was purchased from Biomeda (Foster City, CA). FITC-conjugated mouse anti- α-tubulin antibody, Sulforhodamine B sodium salt, formaldehyde (37% solution in water), glutaraldehyde (50% aqueous solution, photographic grade) were purchased from Sigma-Aldrich (St. Louis, MO). Goat anti-mouse IgG, Alexa-Fluor 488 conjugate and propidium iodide were from Molecular Probes (Eugene, OR). Goat serum (10% solution) was from Invitrogen (Carlsbad, CA). Vectashield mounting medium was from Vector Laboratories (Burlingame, CA). RPMI-1640 cell culture medium was obtained from the UIUC School of Chemical Sciences Cell Media Facility. Microtiter plates (96-well, tissue culture-treated), microscope slides, No. 1 microscope coverslips, eppendorf tubes, and all other reagents were purchased from Fisher (Chicago, IL).

Cell Culture. HeLa and U-937 cell lines were purchased from American Type Culture Collection (Manassas, VA). HL-60 and HL-60/VCR cell lines were a generous gift from Dr. Russell J. Mumper. For all experiments, cell lines were cultured in RPMI-1640 supplemented with 10% FBS and 1% penicillin/streptomycin in tissue-culture treated flasks and Petri dishes and maintained at 37°C in a humidified 5% CO_2 incubator.

Flow Cytometric Analysis. HeLa cells were synchronized in G1/S following a standard double thymidine-block protocol.[2] Arrested cells were released by washing three times with thymidine-free medium, and immediately harvested by trypsinization, counted using a hemocytometer, and plated (1.5 x 10^6 per plate) in 10 cm cell-culture-treated Petri dishes containing 12 mL cell growth medium. 8H (25 μM), colchicine (100 nM), cycloheximide (2.5 μM) or an equal volume of DMSO vehicle as added and cells were incubated for 9 h at 37°C in a humidified 5% CO_2 incubator. Cells were rapidly harvested by scraping, washed with PBS pH 7.4,

and fixed for 10 min in 500 µL PBS pH 7.4 containing 3% formaldehyde in sealed 1.7 mL Eppendorf tubes in a 37°C water bath. After fixation, cells were incubated on ice for 1 min, centrifuged 5 min at 200 x g, and PBS was removed via aspiration. Cells were gently resuspended in 300 µL MeOH by dropwise addition of ice-cold 100% MeOH to a gently vortexed tube. Cell suspensions were incubated on ice for 30 min and stored overnight at -20 °C to permeabilize cells. Permeabilized cells were then washed twice with blocking solution (0.5% BSA in PBS pH 7.4) to remove MeOH, and resuspended in 500 µL blocking solution and incubated at 25 °C for 10 min. Blocking solution was removed by aspiration and cells were resuspended in 100 µL blocking solution containing mouse anti-phospho(Ser10) histone H3 antibody (Cell Signaling Technology) at 1:25 dilution and incubated for 30 min at 25 °C. A portion of the 8H-treated cells (0.5×10^6) were separated before treatment and were incubated in blocking solution alone as a control for nonspecific 2° antibody binding. After incubation, cells were washed 3 x 1 mL in blocking solution and were resuspended in 200 µL blocking solution containing goat anti-mouse IgG, Alexa-Fluor 488 conjugate (1:1000) and incubated for 30 min at 25 °C. After washing 3 x 1 mL in blocking solution, cells were resuspended in 200 µL PBS containing 100 µg/mL RNAse A and incubated for 30 min at 25 °C, at which point 100 µL propidium iodide (1 mg/mL in PBS) was added and incubated for an additional 30 min. Cells were washed 3 x 1 mL PBS, resuspended in 400 µL PBS and immediately analyzed on a BD Biosciences LSR II flow cytometer using a 488 nm excitation laser, monitoring green and red channels with 530 ± 15 nm and 695 ± 20 nm bandpass filters, respectively.

Assessment of Cell Viability via Sulforhodamine B Assay. Cytotoxicity against the HeLa cell line was assessed using the sulforhodamine B (SRB) assay in 96-well plate format using the optimized protocol of Vichai and Kirtikara.[3] Briefly, two µL of a dilution series of each compound dissolved in DMSO were added to 98 µL of appropriate growth medium at five replicates per concentration. Then, cells suspended in 100 µL growth medium at a concentration of 5×10^4cells/mL were added and the plate was incubated in a 5% CO_2 incubator 72 h at 37 °C. After 72 h, media was removed from the plates and cells were fixed by addition of 100 µL ice-cold 10% (w/v) trichloroacetic acid (TCA) in water and placed in a refrigerator. After overnight incubation, TCA was removed by washing plates four times with distilled water and plates were allowed to dry

at room temperature overnight. Sulforhodamine B, sodium salt (100 µL of 0.06% w/v solution dissolved in 1% acetic acid) was added to each well and the plates incubated at room temperature for 30 min, after which time unbound sulforhodamine B was removed by washing 4 times with 1% acetic acid. SRB bound to proteins was released by addition of 200 µL 10 mM Tris, pH 10.5, and absorbance of each well was measured at 510 nm on a Molecular Devices SpectraMax 384 plus plate reader after 30 min incubation at room temperature. Each plate contained internal positive (known cytotoxic compound at 100 µM) and negative (DMSO vehicle) controls for calibration of the percentage of cell death observed in each well, which was used to construct a dose-response curve and calculate an LC_{50}.

Assessment of Cell Viability via MTS Assay. Cytotoxicity of compounds against the U-937, HL-60, and HL-60/VCR cell lines was assessed using the MTS assay according to the manufacturer's specifications (Promega). Briefly, two µL of a dilution series of each compound dissolved in DMSO were added to 98 µL of appropriate growth medium at five replicates per concentration. Then, cells suspended in 100 µL growth medium at a concentration of 1 x 10^5 (U-937 and HL-60) or 1 x 10^5 (HL-60/VCR) cells/mL were added and the plate was incubated in a 5% CO_2 incubator at 37 °C. After 72 h, plates were removed and processed as per the MTS protocol, wherein a 20 µL solution of MTS ((3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt) and PMS (phenazine methosulfate) in PBS was added to each well, and plates were returned to a 5% CO_2 incubator until signal from vehicle-treated cells reached the upper end of the linear range of the assay (O.D. ~ 1.5, roughly 30 min for U-937 and HL-60 cell lines, up to 2 h for HL-60/VCR cell line). Absorbance of each well was measured at 490 nm on a Molecular Devices SpectraMax 384 plus plate reader. Each plate contained internal positive (known cytotoxic compound at 100 µM) and negative (DMSO vehicle) controls for calibration of the percentage of cell death observed in each well, which was used to construct a dose-response curve and calculate an LC_{50}.

Tubulin Polymerization Assay. Polymerization of tubulin was monitored by measuring absorbance at 340 nm in a 384-well plate Molecular Devices Spectramax 384 plus spectrophotometer (Sunnydale, CA) preheated to 37 °C. To a 0.5 mL polypropylene tube on ice was added 40 µL ice-cold 1.25X BRB-80 buffer (100 mM

PIPES/pH 6.8, 1.25 mM $MgCl_2$, 1.25 mM EGTA, 1.25 μM GTP (added from 100 mM stock immediately before use), 6.25% v/v glycerol). To individual aliquots of buffer was also added DMSO or compound in DMSO (DMSO 0.6 % final in buffer, paclitaxel and nocodazole at 10 μM, **8H** at 25 and 100 μM). To this solution was rapidly added 10 μL 15 mg/mL polymerization-competent tubulin in ice-cold 500 mM K-PIPES, pH 6.8, 0.5 mM $MgCl_2$, buffer, which had been thawed on ice immediately before use. Solutions were mixed rapidly on ice and then immediately transferred to a 384-well plate. Final concentrations of reagents in polymerization reaction were as follows: 80 mM PIPES/pH 6.8, 1.0 mM MgCl2, 1.0 mM EGTA, 1.0 μM GTP, 5% glycerol, 0.5% DMSO, 3 mg/mL tubulin. Turbidity at 340 nm corresponding to polymerization was assessed every 60 sec. for 60 min.

Laser Fluorescence Confocal Microscopy. For laser fluorescence confocal microscopy, HeLa cells were grown on nitric acid-washed No. 1 coverslips overnight in a 5% CO_2 incubator h at 37 °C. Compound in DMSO or DMSO vehicle alone (0.2% DMSO final) was added to the cells at 40 or 70% confluency, and further incubated for 6 h (70% confluent cells) or 16 h (40% confluent cells). Cells were washed briefly with BRB-80 and fixed for 10 min with 0.5% glutaraldehyde in BRB-80, then permeabilized for 15 min in 1% Triton X-100 in PBS. After washing three times in PBS/pH 8.0, unreacted aldehydes were reduced with three seven min incubations of 1 mg/mL $NaBH_4$ dissolved in PBS/pH 8 immediately before use. Cells were given 3 rinses with a solution of 0.1% Triton X-100 in PBS/pH 8.0 (PBST) and blocked 20 min in 10% goat serum. FITC-conjugated anti-α-tubulin was added at a 1:100 dilution in 10% goat serum and the cells incubated for 1 h, then washed 3 x 10 min in PBST. Goat anti-mouse IgG, Alexa-Fluor 488 conjugate was diluted 1:200 in 10% goat serum and incubated with cells for 1 h, then washed three times with PBST. Cells were incubated in PBS containing 10 μg/mL propidium iodide and 1 μg/mL RNAse A for 15 min, washed twice with PBS, once with dH_2O, and mounted onto microscope slides using 8 μL Vectashield mounting medium and sealed with colorless nail polish. Samples were visualized immediately on a Zeiss LSM 510 laser scanning confocal microscope, 63X oil DIC objective, 1.4 NA. Widefield images were acquired by moving the stage to 10-15 random locations on each slide, thereafter only adjusting the stage in the z-direction to bring a maximal number of cells into focus.

In silico prediction of biological properties. SMILES formulae for all new compounds were generated in Chemdraw (Cambridgesoft Corp. Cambridge, MA, USA). SMILES formulae for known antimitotics were downloaded from the PubChem database.[4] Values for calculated partition coefficient octanol/water ($C_{log}P$) and topological polar surface area (TPSA) were calculated using Daylight software (Daylight Chemical Information Systems, Inc., Aliso Viejo, CA, USA) which contains implementations of $C_{log}P$[5] (BioByte Corp., Claremont, CA, USA) and TPSA[6] algorithms. Predicted $_{log}BB$ was calculated using the formula $_{log}BB$ = -0.0148 TPSA + 0.152 $C_{log}P$ + 0.139.[5]

Chemistry

Method A. Two-step synthesis of phenylcinnamides. All reactions were run on a scale calculated to provide a theoretical yield of roughly 50 mg (~0.1-0.2 mmol) amide product. In an oven-dried microwave tube, a given carboxylic acid was dissolved in 2.0 mL anhydrous CH_2Cl_2. To the tube was added oxalyl chloride (2 eq.) and one drop of anhydrous *N,N*-dimethylformamide and the tube was stirred lightly capped at room temperature overnight. After acid chloride formation was complete as judged by TLC, all volatiles were evaporated to dryness under a steady stream of dry N_2. The resulting acid chloride was then immediately redissolved in 2.0 mL anhydrous MeCN. An amine (1.5 eq.) was added, followed by DIPEA (2.0 eq.). The solution was capped and the tube was heated in a CEM discover multimode reaction microwave, holding at 150 ºC for 90 minutes. After cooling, the reaction mixture was evaporated to dryness and the crude reaction mixture redissolved in 20 mL EtOAc and extracted 3 x 15 mL 1M HCl, 3 x 15 mL 1 M NaOH, then 1 x 15 mL brine. The organic layer was dried over $MgSO_4$, filtered and adsorbed onto silica gel. The silica was then loaded into a purification cartridge and the crude mixture was separated over a 0-100% hexanes-ethyl acetate gradient on an Isco Companion automated chromatography system.

Method B. One-step synthesis of phenylcinnamides. All reactions were run on a scale calculated to provide a theoretical yield of roughly 50 mg (~0.1-0.2 mmol) amide product. In an oven-dried microwave tube, a given

carboxylic acid was dissolved in 2.0 mL anhydrous MeCN. An amine (1.5 eq.) was added, followed by HATU (1.5 eq.) and DIPEA (2.0 eq.). The solution was capped and the tube was heated in a CEM discover multimode reaction microwave, holding at 150 ºC for 90 minutes. After cooling, the reaction mixture was evaporated to dryness and the crude reaction mixture redissolved in 20 mL EtOAc and extracted 3 x 15 mL 1M HCl, 3 x 15 mL 1 M NaOH, then 1 x 15 mL brine. The organic layer was dried over $MgSO_4$, filtered and adsorbed onto silica gel. The silica was then loaded into a purification cartridge and the crude mixture was separated over a 0-100% hexanes-ethyl acetate gradient on an Isco Companion automated chromatography system.

Yield and analytical HPLC purity analysis of library compounds

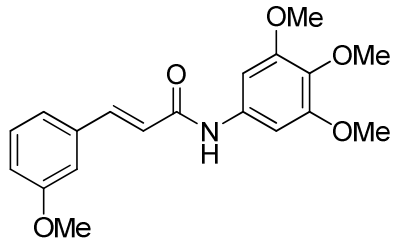
Compound	Yield (%)	Purity (%)	MW [M+H]	Calculated	Found	Method	MP (°C)
6	60	96	$C_{19}H_{19}NO_5F_3$	398.1205	398.1215	A	NA
7	51	97	$C_{17}H_{15}NO_3F_3$	338.0995	338.1004	A	NA
8	43	100	$C_{18}H_{20}NO_5$	330.1341	330.1335	A	195-198
9	77	95	$C_{18}H_{20}NO_4$	314.1392	314.1394	B	114-116
10	58	100	$C_{21}H_{26}NO_7$	404.1709	404.1709	A	81-83
11	19	97	$C_{20}H_{24}NO_6$	374.1604	374.1608	A	NA
12	58	100	$C_{19}H_{22}NO_5$	344.1498	344.1504	A	NA
13	20	98	$C_{19}H_{22}NO_5$	344.1498	344.1501	A	NA
14	16	100	$C_{18}H_{20}NO_4$	314.1392	314.1402	A	62-68
15	10	98	$C_{19}H_{22}NO_5$	344.1498	344.1505	A	126-128.5
16	88	100	$C_{19}H_{22}NO_5$	344.1498	344.1499	A	134-136
17	34	100	$C_{17}H_{18}NO_3$	284.1287	284.1291	A	137-139
18	15	95	$C_{18}H_{20}NO_5$	330.1341	330.1344	B	215-220
19	17	95	$C_{17}H_{18}NO_4$	300.1236	300.1239	B	NA
20	70	100	$C_{21}H_{22}NO_5$	368.1498	368.1508	A	NA
21	76	94	$C_{21}H_{24}NO_5$	370.1654	370.1664	A	NA
22	60	100	$C_{25}H_{25}NO_5$	420.1811	420.1798	A	144-146
23	21	97	$C_{18}H_{20}NO_4$	314.1392	314.1394	B	123-125
24	44	99	$C_{17}H_{18}NO_3$	284.1287	284.1289	A	93-97
25	39	98	$C_{17}H_{18}NO_3$	284.1287	284.1295	A	86-90
26	85	100	$C_{17}H_{18}NO_3$	284.1287	284.1296	A	137-138
27	33	95	$C_{16}H_{16}NO_2$	254.1181	254.1183	A	NA
28	30	100	$C_{18}H_{18}NO_4$	312.1236	312.1246	A	116-120
29	45	98	$C_{18}H_{20}NO_2$	282.1494	282.1503	A	140-142
30	39	98	$C_{18}H_{20}NO_2$	282.1494	282.1502	A	153-154
31	45	99	$C_{19}H_{24}NO_5$	346.1654	346.1661	B	NA
32	44	98	$C_{19}H_{24}NO_5$	346.1654	346.1643	A	NA
33	54	98	$C_{20}H_{24}NO_5$	358.1654	358.1665	A	123-125

NA = no melting point (oil).

NMR analysis of library members

Compound **8H**[7]

3-(3-Methoxy-phenyl)-N-(3,4,5-trimethoxy-phenyl)-acrylamide



NMR ^{1}H (500 MHz, $CDCl_3$) δ ppm 8.59 (br s, 1H) ppm 7.69 (d, 1H, J=15.5 Hz), 7.20 (t, 1H, J=7.9 Hz), 7.03 (s, 1H), 7.00 (d, 1H, J=7.7 Hz), 6.94 (s, 1H), 6.85 (dd, 1H, J=2.2 Hz, J=8.1 Hz), 6.68 (d, 1H, J=15.5 Hz), 3.80 (s, 3H) ppm 3.729 (s, 3H), 3.725 (s, 6H).

NMR ^{13}C (125 MHz, $CDCl_3$) δ ppm: 164.35, 159.73, 153.12, 141.92, 135.82, 134.67, 134.21, 129.76, 121.26, 120.24, 115.39, 113.19, 97.49, 60.86, 55.77, 55.08.

R_f=12mm/53mm (EtOAc/Hexanes=50/50);

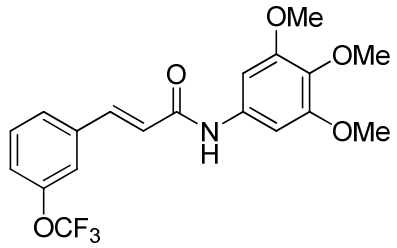
IR (thin film, cm^{-1}): 3313, 2938, 2836, 1663, 1607, 1546, 1507, 1452, 1432, 1411, 1332, 1290, 1234, 1210, 1128, 1044, 980, 913, 837, 776;

MS (FAB): 344.22(M+1, 57.77), 222.14(16.66), 195.11(10.18), 184.15(100), 168.13(59.86), 161.12(47.20);

HRMS (FAB): found: 344.1500 (M+1); calc. for $C_{19}H_{22}NO_5$: 344.149798

Compound **6**

(*E*)-3-(3-(trifluoromethoxy)phenyl)-*N*-(3,4,5-trimethoxyphenyl)acrylamide

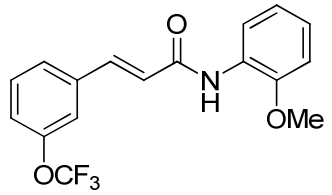


NMR ^{1}H (500 MHz, CD_3OD) δ ppm: 7.62 (d, 1H, J=15.78Hz), 7.54 (d, 1H, J=7.75Hz), 7.48 (m, 2H), 7.28 (d, 1H, J=8.17Hz), 7.05 (s, 2H), 6.78 (d, 1H, J=15.66Hz), 3.82 (s, 6H), 3.74 (s, 3H).

NMR ^{13}C (500 MHz, CD_3OD) δ ppm: 164.61, 153.34, 149.79, 139.58, 137.42, 135.11, 134.62, 130.54, 126.61, 123.18, 121.93, 119.75, 97.73, 60.04, 55.33.

Compound **7**

(*E*)-*N*-(2-methoxyphenyl)-3-(3-(trifluoromethoxy)phenyl)acrylamide

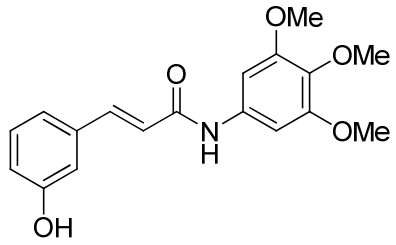


NMR ^{1}H (500 MHz, CD_3OD) δ ppm: 8.12 (dd, 1H, J=9.4 Hz, J=1.4 Hz), 7.63 (d, 1H, J=15.7 Hz), 7.59 (d, 1H, J=7.6 Hz), 7.53 (s, 1H), 7.49 (t, 1H, J=8.0 Hz), 7.29 (d, 1H, J=8.2 Hz), 7.09 (m, 2H), 7.02 (dd, 1H, J=8.2 Hz, J=1.1 Hz), 6.93 (td, 1H, J=7.7 Hz, J=1.1 Hz), 3.90 (s, 3H).

NMR ^{13}C (500 MHz, CD_3OD) δ ppm: 164.90, 150.14, 149.80, 139.59, 137.59, 130.52, 127.14, 126.73, 125.08, 123.42, 121.90, 121.87, 120.32, 119.79, 110.57, 55.07.

Compound **8**

(*E*)-3-(3-hydroxyphenyl)-*N*-(3,4,5-trimethoxyphenyl)acrylamide

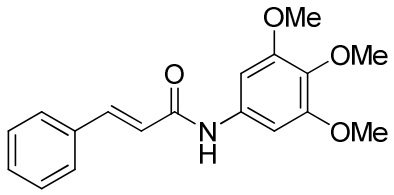


NMR ^{1}H (500 MHz, CD_3OD) δ ppm: 7.57 (d, 1H, J=15.6Hz), 7.21 (t, 1H, J=7.8Hz), 7.06 (s, 2H), 6.82 (d, 1H, J=7.9Hz), 6.69 (d, 1H, J=15.6Hz), 3.82 (s, 6H) 3.74 (s, 3H).

NMR ^{13}C (500 MHz, CD_3OD) δ ppm: 166.07, 158.54, 153.98, 142.45, 136.98, 135.90, 135.16, 130.49, 121.60, 120.03, 117.69, 114.77, 98.42, 60.75, 56.02.

Compound **9**

N-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)cinnamamide

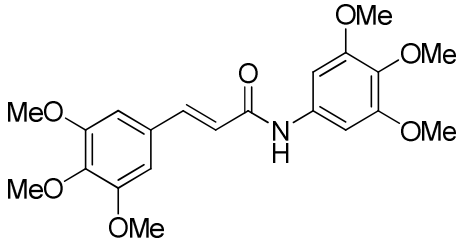


NMR ^{1}H (500 MHz, CD_3OD) δ ppm: 7.62 (d, 1H, J=15.7 Hz), 7.51 (m, 2H), 7.33 (m, 3H), 7.04 (s, 2H), 6.72 (d, 1H, J=15.7 Hz), 3.77 (s, 6H), 3.72 (s, 3H).

NMR ^{13}C (500 MHz, CD_3OD) δ ppm: 165.26, 153.31, 141.56, 135.23, 134.95, 134.47, 129.86, 128.82, 127.81, 121.11, 97.74, 60.11, 55.34.

Compound **10**

(E)-N,3-bis(3,4,5-trimethoxyphenyl)acrylamide

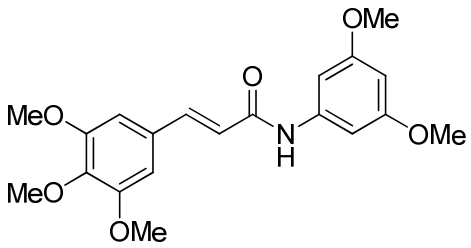


NMR ^{1}H (500 MHz, $(CD_3)_2CO$) δ ppm: 7.61 (d, 1H, J=15.5 Hz), 7.21 (s, 2H), 6.94 (s, 2H), 6.78 (d, 1H, J=15.5 Hz), 3.89 (s, 6H), 3.82 (s, 6H), 3.77 (s, 3H), 3.71 (s, 3H).

NMR ^{13}C (500 MHz, $(CD_3)_2CO$) δ ppm: 163.69, 153.95, 153.66, 141.02, 140.14, 135.82, 134.73, 130.85, 121.47, 105.48, 97.41, 59.99, 59.96, 55.79, 55.65.

Compound **11**

(*E*)-*N*-(3,5-dimethoxyphenyl)-3-(3,4,5-trimethoxyphenyl)acrylamide

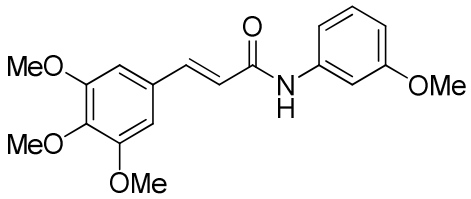


NMR ^{1}H (500 MHz, CD_3OD) δ ppm: 7.57 (d, 1H, J=15.6 Hz), 6.91 (s, 4H), 6.68 (d, 1H, J=15.6 Hz), 6.26 (s, 1H), 3.88 (s, 6H), 3.78 (m, 9H).

NMR ^{13}C (500 MHz, CD_3OD) δ ppm: 165.49, 161.30, 153.63, 141.67, 140.44, 139.75, 130.90, 120.51, 105.19, 98.20, 96.18, 60.00, 55.46, 54.56.

Compound **12**

(*E*)-*N*-(3-methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)acrylamide

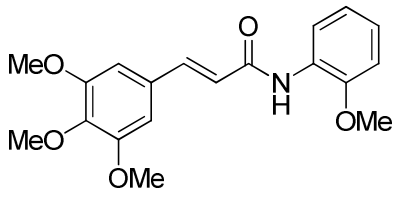


NMR ^{1}H (500 MHz, CD_3OD) δ ppm: 8.17 (d, 1H, J=7.9 Hz), 7.55 (d, 1H, J=15.6 Hz), 7.10 (m, 1H), 7.02 (m, 1H), 6.94 (m, 4H), 3.90 (s, 3H), 3.87 (s, 6H), 3.79 (s, 3H).

NMR ^{13}C (500 MHz, CD_3OD) δ ppm: 165.54, 153.59, 149.96, 141.55, 139.60, 131.04, 127.32, 124.84, 121.61, 120.80, 120.34, 110.53, 105.20, 60.00, 55.44, 55.09.

Compound **13**

(*E*)-*N*-(2-methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)acrylamide



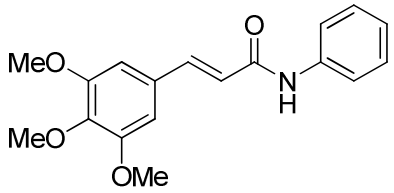
NMR ^{1}H (500 MHz, CD_3OD) δ ppm: 7.57 (d, 1H, J=15.6 Hz), 7.40 (s, 1H), 7.22 (t, 1H, J=8.1 Hz), 7.14 (d, 1H, J=8.1 Hz), 6.90 (s, 1H), 6.68 (d, 1H, J=15.5 Hz), 6.68 (dd, 1H, J=2.4 Hz, J=7.9 Hz), 3.87 (s, 6H), 3.79 (s,

6H).

NMR ^{13}C (500 MHz, CD_3OD) δ ppm: 165.48, 160.37, 153.63, 141.62, 139.96, 130.90, 129.40, 120.51, 112.14, 109.60, 105.78, 105.17, 59.99, 55.45, 54.48.

Compound **14**

(E)-N-phenyl-3-(3,4,5-trimethoxyphenyl)acrylamide

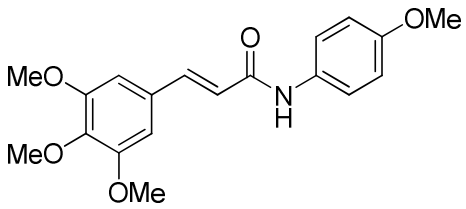


NMR ^{1}H (500 MHz, CD_3OD) δ ppm: 7.65 (d, 2H, J=8.2), 7.57 (d, 1H, J=15.6 Hz), 7.32 (t, 2H, J=8.3 Hz), 7.10 (t, 1H, J=7.4 Hz), 6.89 (s, 2H), 6.71 (d, 1H, J=15.6), 3.86 (s, 6H), 3.79 (s, 3H).

NMR ^{13}C (500 MHz, CD_3OD) δ ppm: 165.48, 153.61, 141.58, 139.70, 138.82, 130.91, 128.70, 124.11, 120.52, 120.00, 105.16, 60.01, 55.45.

Compound **15**

(*E*)-*N*-(4-methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)acrylamide

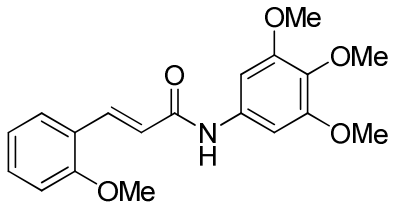


NMR ^{1}H (500 MHz, CD_3OD) δ ppm: 7.54 (m, 3H), 6.88 (m, 4H), 6.67 (d, 1H, J=15.6 Hz), 3.86 (s, 6H), 3.79 (s, 3H), 3.77 (s, 3H).

NMR ^{13}C (500 MHz, CD_3OD) δ ppm: 165.20, 156.78, 153.61, 141.14, 139.64, 131.82, 130.98, 121.63, 120.56, 113.83, 105.13, 60.00, 55.45, 54.67.

Compound **16**

(*E*)-3-(2-methoxyphenyl)-*N*-(3,4,5-trimethoxyphenyl)acrylamide

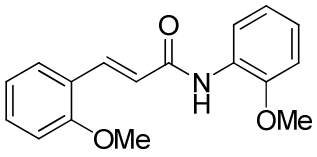


NMR ^{1}H (500 MHz, CD_3OD) δ ppm: 7.95 (d, 1H, J=15.8 Hz), 7.52 (d, 1H, J=6.8 Hz), 7.33 (t, 1H, J=7.1 Hz), 7.06 (s, 2H), 7.00 (d, 1H, J=8.3 Hz), 6.94 (t, 1H, J=7.5 Hz), 6.82 (d, 1H, J=15.8 Hz), 3.87 (s, 3H), 3.81 (s, 6H), 3.73 (s, 3H).

NMR ^{13}C (500 MHz, CD_3OD) δ ppm: 166.00, 158.57, 153.30, 137.03, 135.35, 134.42, 131.24, 128.49, 123.57, 121.38, 120.60, 111.24, 97.71, 60.05, 55.32, 54.85.

Compound **17**

(*E*)-*N*,3-bis(2-methoxyphenyl)acrylamide

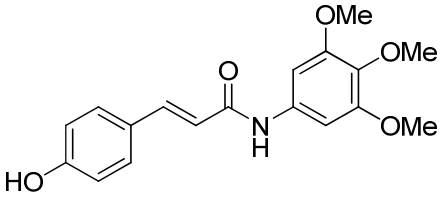


NMR ^{1}H (500 MHz, CD_3OD) δ ppm: 8.12 (d, 1H, J=7.7 Hz), 7.98 (d, 1H, J=15.8 Hz) 7.61 (d, 1H, J=7.5 Hz), 7.35 (t, 1H, J=7.1Hz), 7.10 (t, 1H, J=7.3 Hz), 6.98 (m, 5H), 3.90 (s, 6H).

NMR ^{13}C (500 MHz, CD_3OD) δ ppm: 166.16, 158.51, 150.24, 136.89, 131.20, 128.23, 127.28, 124.92, 123.71, 122.06, 121.32, 120.59, 120.29, 111.22, 110.57, 55.06, 54.86.

Compound **18**

(*E*)-3-(4-hydroxyphenyl)-*N*-(3,4,5-trimethoxyphenyl)acrylamide

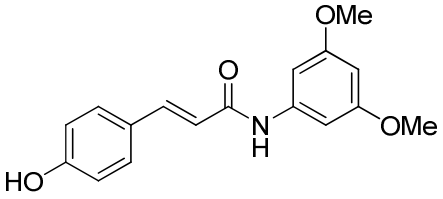


NMR ^{1}H (500 MHz, DMSO-d6) δ ppm: 9.92 (s br, 1H), 7.42 (m, 3H), 7.07 (s, 2H), 6.81 (d, 2H, J=8.5 Hz), 6.56 (d, 1H, J=15.6 Hz), 3.74 (s, 6H), 3.61 (s, 3H).

NMR ^{13}C (500 MHz, DMSO-d6) δ ppm: 164.56, 159.88, 153.40, 140.90, 136.30, 134.00, 130.18, 126.36, 119.23, 116.54, 97.52, 60.79, 56.35.

Compound **19**

(*E*)-*N*-(3,5-dimethoxyphenyl)-3-(4-hydroxyphenyl)acrylamide

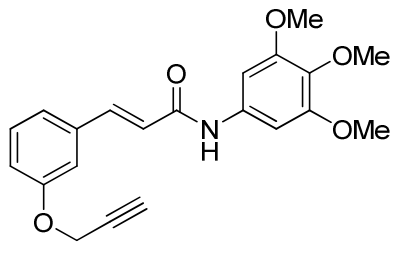


NMR ^{1}H (500 MHz, CD_3OD) δ ppm: 7.57 (d, 1H, J=15.6 Hz), 7.45 (d, 2H, J=8.5 Hz), 6.90 (dd, 1H, J=1.7 Hz, J=5.1 Hz), 6.81 (d, 2H, J=8.5 Hz), 6.57 (d, 1H, J=15.6 Hz), 6.25 (d, 1H, J=1.7 Hz), 5.48 (br s, 1H), 3.77 (s, 6H).

NMR ^{13}C (500 MHz, CD_3OD) δ ppm: 166.15, 161.28, 159.63, 141.90, 140.50, 129.60, 126.44, 117.53, 115.60, 98.20, 96.10, 54.55.

Compound **20**

(*E*)-3-(3-(prop-2-ynyloxy)phenyl)-*N*-(3,4,5-trimethoxyphenyl)acrylamide

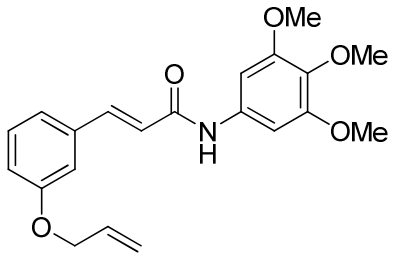


NMR ^{1}H (500 MHz, $CDCl_3$) δ ppm: 7.92 (s, 1H), 7.70 (d, 1H, J=15.5 Hz), 7.28 (dd, 1H, J=5.6 Hz, J=13.6 Hz), 7.11 (d, 1H, J=7.7 Hz), 7.08 (m, 1H), 6.98 (dd, 1H, J=2.6 Hz, J=8.5 Hz), 6.57 (d, 1H, J=15.5 Hz), 4.68 (d, 1H, J=2.4 Hz), 3.82 (s, 3H), 3.80 (s, 6H) 3.30 (s, 1H).

NMR ^{13}C (500 MHz, $CDCl_3$) δ ppm: 164.24, 158.05, 153.53, 142.21, 136.25, 134.61, 130.16, 121.63, 116.70, 114.43, 97.82, 78.48, 76.14, 61.22, 56.25, 46.79.

Compound **21**

(*E*)-3-(3-(allyloxy)phenyl)-*N*-(3,4,5-trimethoxyphenyl)acrylamide

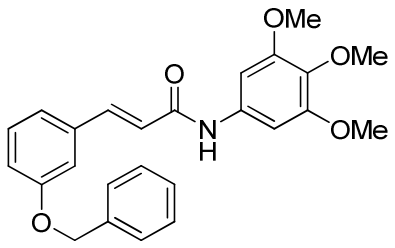


NMR ^{1}H (500 MHz, CD_3OD) δ ppm: 7.60 (d, 1H, J=15.6 Hz), 7.31 (t, 1H, J=7.8 Hz), 7.16 (m, 2H), 7.06 (s, 2H), 6.97 (dd, 1H, J=8.2 Hz, J=2.0 Hz), 6.73 (d, 1H, J=15.6 Hz), 6.07 (m, 1H), 5.42 (dd, 1H, J=1.4 Hz, J=17.3 Hz), 5.27 (dd, 1H, J=1.5 Hz, J=10.5 Hz), 4.58 (d, 2H, J=5.1 Hz), 3.83 (s, 6H), 3.76 (s, 3H)

NMR ^{13}C (500 MHz, CD_3OD) δ ppm: 165.250, 159.307, 153.329, 141.482, 136.363, 135.215, 134.515, 133.581, 129.826, 121.318, 120.464, 116.448, 116.351, 113.691, 97.686, 68.638, 60.032.

Compound **22**

(*E*)-3-(3-(benzyloxy)phenyl)-*N*-(3,4,5-trimethoxyphenyl)acrylamide

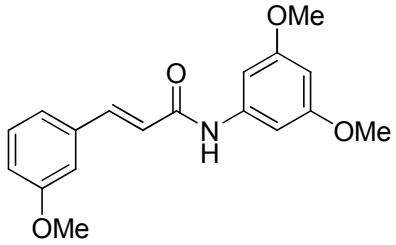


NMR ^{1}H (500 MHz, $CDCl_3$) δ ppm: 8.08 (br s, 1H), 7.92 (s, 1H), 7.70 (d, 1H, J=15.5 Hz), 7.36 (s, 1H), 7.08 (m, 1H), 7.00 (m, 1H), 6.57 (d, 1H, J=15.5 Hz), 5.04 (m, 1H), 3.82 (s, 3H), 3.81 (s, 3H), 3.80 (s, 3H).

NMR ^{13}C (500 MHz, $CDCl_3$) δ ppm: 169.03, 164.30, 159.31, 153.52, 142.36, 136.84, 136.23, 134.82, 134.65, 134.34, 130.16, 128.88, 128.35, 127.72, 121.44, 120.92, 119.14, 116.69, 114.73, 114.49, 112.90, 97.80, 80.64, 70.29, 61.21, 57.13, 56.23, 46.81.

Compound **23**

(E)-N-(3,5-dimethoxyphenyl)-3-(3-methoxyphenyl)acrylamide

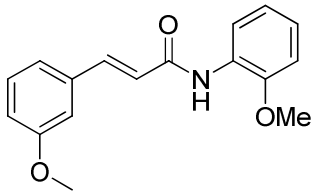


NMR ^{1}H (500 MHz, CD_3OD) δ ppm: 7.60 (d, 1H, J=15.6 Hz), 7.31 (t, 1H, J=7.9 Hz), 7.16 (d, 1H, J=7.6 Hz), 7.12 (s, 1H) ppm 6.95 (dd, 1H, J=2.5 Hz, J=8.2 Hz), 6.91 (d, 1H, J=2.2 Hz), 6.74 (d, 1H, J=15.6 Hz), 6.25 (t, 1H, J=2.2 Hz), 3.82 (s, 3H) 3.76 (s, 6H).

NMR ^{13}C (500 MHz, CD_3OD) δ ppm: 165.48, 153.61, 141.58, 139.70, 138.82, 130.91, 128.70, 124.11, 120.52, 120.00, 105.16, 60.01, 55.45.

Compound **24**

(*E*)-*N*-(2-methoxyphenyl)-3-(3-methoxyphenyl)acrylamide

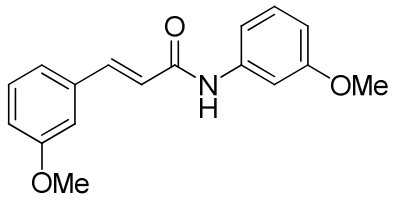


NMR ^{1}H (500 MHz, CD_3OD) δ ppm: 8.14 (d, 1H, J=7.8 Hz), 7.60 (d, 1H, J=15.6 Hz), 7.31 (t, 1H, 7.8 Hz), 7.17 (m, 2H), 7.11 (t, 1H, J=7.5 Hz), 6.97 (m, 4H), 3.90 (s, 3H), 3.82 (s, 3H).

NMR ^{13}C (500 MHz, CD_3OD) δ ppm: 165.51, 160.36, 150.14, 141.52, 136.50, 129.77, 124.97, 121.88, 121.56, 120.49, 120.31, 115.66, 112.57, 110.56, 55.07, 54.55.

Compound **25**

(*E*)-*N*,3-bis(3-methoxyphenyl)acrylamide

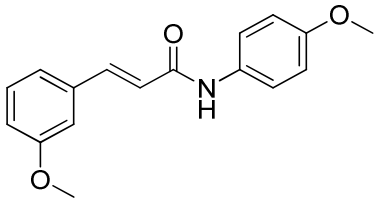


NMR ^{1}H (500 MHz, CD_3OD) δ ppm: 7.61 (d, 1H, J=15.7 Hz), 7.40 (s, 1H), 7.31 (t, 1H, J=7.9 Hz), 7.22 (t, 1H, J=8.1 Hz), 7.15 (m, 3H), 6.95 (dd, 1H, J=10.2 Hz, J=2.06Hz), 6.76 (d, 1H, J=15.6 Hz), 6.68 (dd, 1H, J=9.9 Hz, J=1.8 Hz), 3.82 (s, 3H), 3.79 (s, 3H).

NMR ^{13}C (500 MHz, CD_3OD) δ ppm: 165.41, 160.37, 141.58, 139.94, 136.38, 129.81, 129.40, 121.36, 120.29, 115.55, 112.79, 112.19, 109.66, 105.82, 54.55, 54.48.

Compound **26**

(E)-3-(3-methoxyphenyl)-N-(4-methoxyphenyl)acrylamide

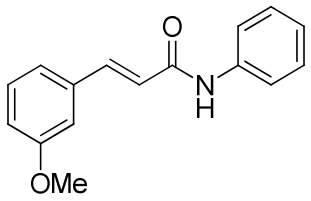


NMR ^{1}H (500 MHz, CD_3OD) δ ppm: 7.59 (d, 1H, J=15.7 Hz), 7.55 (dd, 2H, J=2.2 Hz, J=9.1 Hz), 7.31 (t, 1H, J=7.9 Hz), 7.17 (d, 1H, J=7.7 Hz), 7.13 (s, 1H), 6.97 (dd, 1H, J=10.3 Hz, J=2.1 Hz), 6.90 (m, 2H), 6.75 (d, 1H, J=15.7 Hz), 3.83 (s, 3H), 3.78 (s, 3H).

NMR ^{13}C (500 MHz, CD_3OD) δ ppm: 165.14, 160.38, 156.82, 141.12, 136.46, 131.79, 129.80, 121.66, 121.38, 120.24, 115.44, 113.83, 112.73, 54.66, 54.55.

Compound **27**

(*E*)-3-(3-methoxyphenyl)-*N*-phenylacrylamide

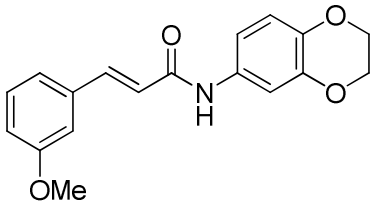


NMR ^{1}H (500 MHz, CD_3OD) δ ppm: 7.65 (d, 2H, J=7.7 Hz), 7.61 (d, 1H, J=15.7 Hz), 7.31 (m, 3H), 7.16 (d, 1H, J=7.6 Hz), 7.11 (m, 2H), 6.94 (dd, 1H, J=10.2 Hz, J=2.1 Hz), 6.77 (s, 1H, 15.7 Hz), 3.81 (s, 3H).

NMR ^{13}C (500 MHz, CD_3OD) δ ppm: 165.41, 160.36, 141.54, 138.79, 136.39, 129.81, 128.69, 124.14, 121.36, 120.29, 120.04, 115.54, 112.79, 54.56.

Compound **28**

(*E*)-*N*-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-3-(3-methoxyphenyl)acrylamide

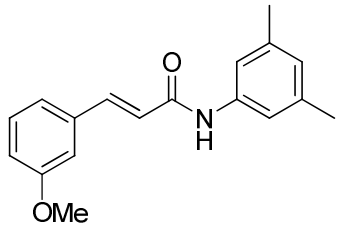


NMR ^{1}H (500 MHz, CD_3OD) δ ppm: 7.58 (d, 1H, J=15.7 Hz), 7.31 (m, 2H), 7.16 (d, 1H, J=7.7 Hz), 7.11 (s, 1H), 7.01 (dd, 1H, J=11.2 Hz, J=2.5 Hz), 6.95 (dd, 1H, J=10.3 Hz, J=2.1 Hz), 6.78 (d, 1H, J=8.7 Hz), 6.72 (d, 1H, J=15.7 Hz), 4.21 (m, 4H), 3.82 (s, 3H).

NMR ^{13}C (500 MHz, CD_3OD) δ ppm: 165.09, 160.37, 143.62, 141.17, 140.73, 136.44, 132.38, 129.79, 121.36, 120.24, 116.84, 115.46, 113.33, 112.73, 109.45, 64.54, 64.36, 54.55.

Compound **29**

(*E*)-*N*-(3,5-dimethylphenyl)-3-(3-methoxyphenyl)acrylamide

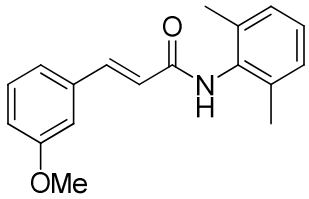


NMR ^{1}H (500 MHz, CD_3OD) δ ppm: 7.60 (d, 1H, J=15.6 Hz), 7.29 (m, 3H), 7.16 (d, 1H, J=7.5 Hz), 7.12 (s, 1H), 6.95 (d, 1H, J=8.1 Hz), 6.76 (m, 2H), 3.82 (s, 3H), 2.28 (s, 6H).

NMR ^{13}C (500 MHz, CD_3OD) δ ppm: 165.33, 160.36, 141.36, 138.53, 138.42, 136.43, 129.79, 125.75, 121.48, 120.28, 117.81, 115.50, 112.750, 54.547, 20.33.

Compound **30**

(*E*)-*N*-(2,6-dimethylphenyl)-3-(3-methoxyphenyl)acrylamide

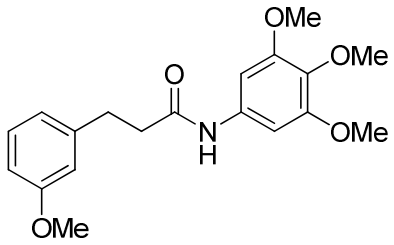


NMR ^{1}H (500 MHz, CD_3OD) δ ppm: 7.62 (d, 1H, J=15.7 Hz), 7.32 (t, 1H, J=7.8 Hz), 7.19 (d, 1H, J=7.6 Hz), 7.13 (m, 4H), 6.97 (dd, 1H, J=8.2 Hz, J=2.5 Hz), 6.87 (d, 1H, J=15.7 Hz), 3.83 (s, 3H), 2.22 (s, 6H).

NMR ^{13}C (500 MHz, CD_3OD) δ ppm: 166.07, 160.41, 141.63, 136.37, 135.61, 134.31, 129.86, 127.91, 127.24, 120.41, 120.30, 115.57, 112.75, 54.55, 17.30, 17.27.

Compound **31**

3-(3-methoxyphenyl)-N-(3,4,5-trimethoxyphenyl)propanamide

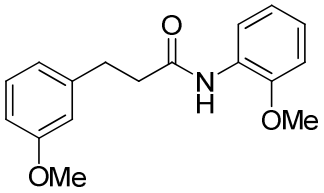


NMR ^{1}H (500 MHz, CD_3OD) δ ppm: 7.15 (t, 1H, J=8.2 Hz), 6.88 (s, 2H), 6.79 (m, 2H), 6.72 (m, 1H), 3.76 (s, 6H), 3.71 (s, 3H), 3.70 (s, 3H), 2.94 (t, 2H, J=7.5 Hz), 2.61 (t, 2H, J=7.5 Hz).

NMR ^{13}C (500 MHz, CD_3OD) δ ppm: 172.27, 160.07, 153.23, 142.51, 135.00, 129.32, 120.52, 113.92, 111.49, 97.82, 60.04, 55.30, 54.58, 54.36, 38.62, 31.63.

Compound **32**

N-(2-methoxyphenyl)-3-(3-methoxyphenyl)propanamide

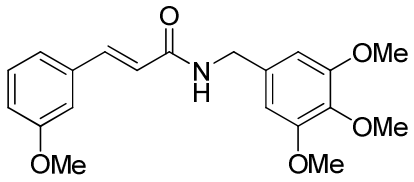


NMR ^{1}H (500 MHz, CD_3OD) δ ppm: 7.91 (dd, 1H, J=7.5 Hz, J=1.6 Hz), 7.16 (t, 1H, J=8.0 Hz), 7.06 (dt, 1H, J=1.6, J=8.2 Hz), 6.94 (dd, 1H, J=1.1Hz, J=8.2 Hz), 6.88 (dt, 1H, J=8.9, J=1.3 Hz), 6.81 (m, 2H), 6.73 (dd, 1H, J=1.6Hz, J=8.2Hz), 3.79 (s, 3H), 3.72 (s, 3H), 2.94 (t, 2H, J=7.4 Hz), 2.69 (t, 2H, J=8.0 Hz).

NMR ^{13}C (500 MHz, CD_3OD) δ ppm: 172.48, 160.09, 150.33, 142.51, 129.30, 126.93, 125.01, 122.34, 120.57, 120.23, 113.82, 111.60, 110.61, 55.00, 54.36, 38.41, 31.71.

Compound **33**

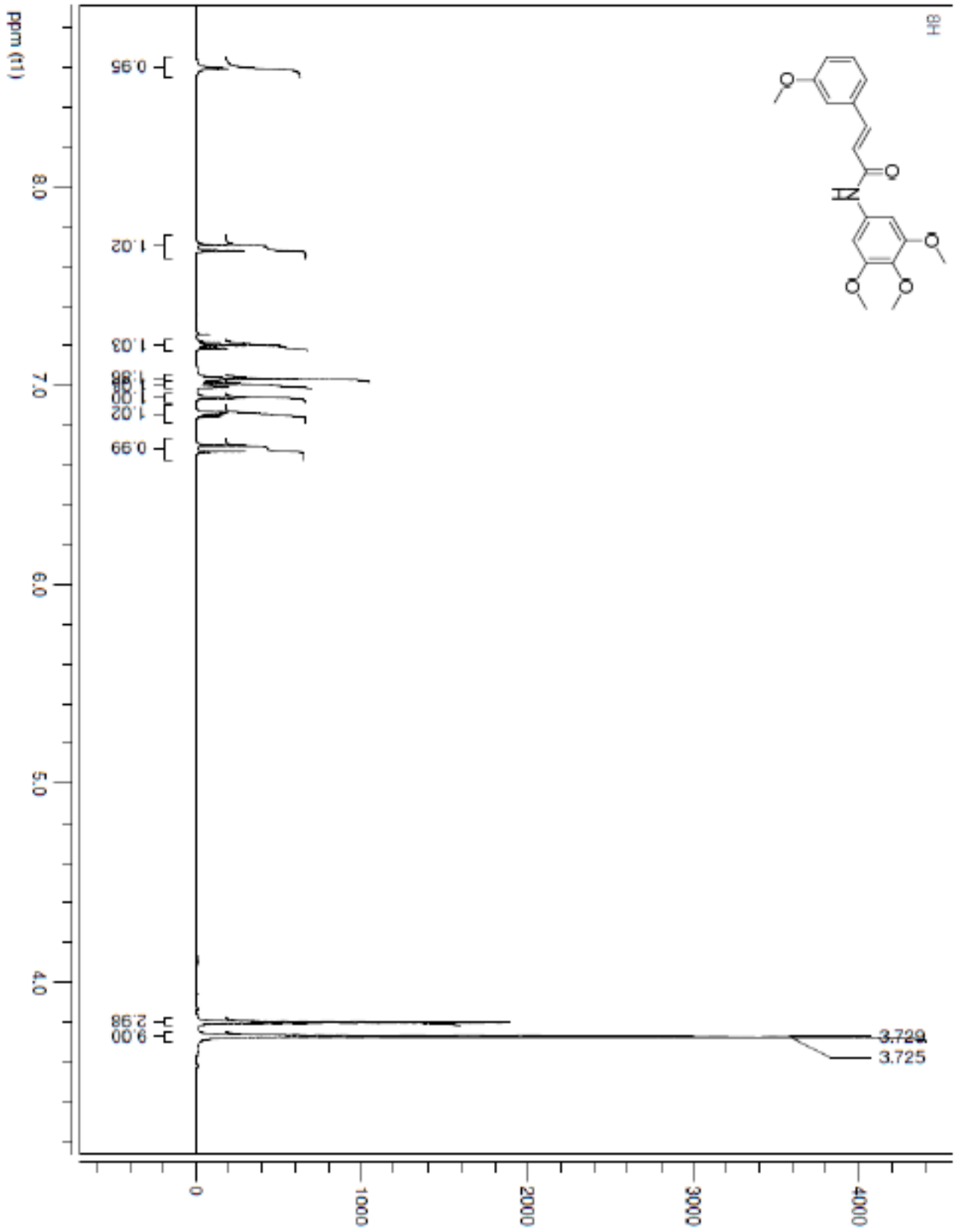
(*E*)-3-(3-methoxyphenyl)-*N*-(3,4,5-trimethoxybenzyl)acrylamide

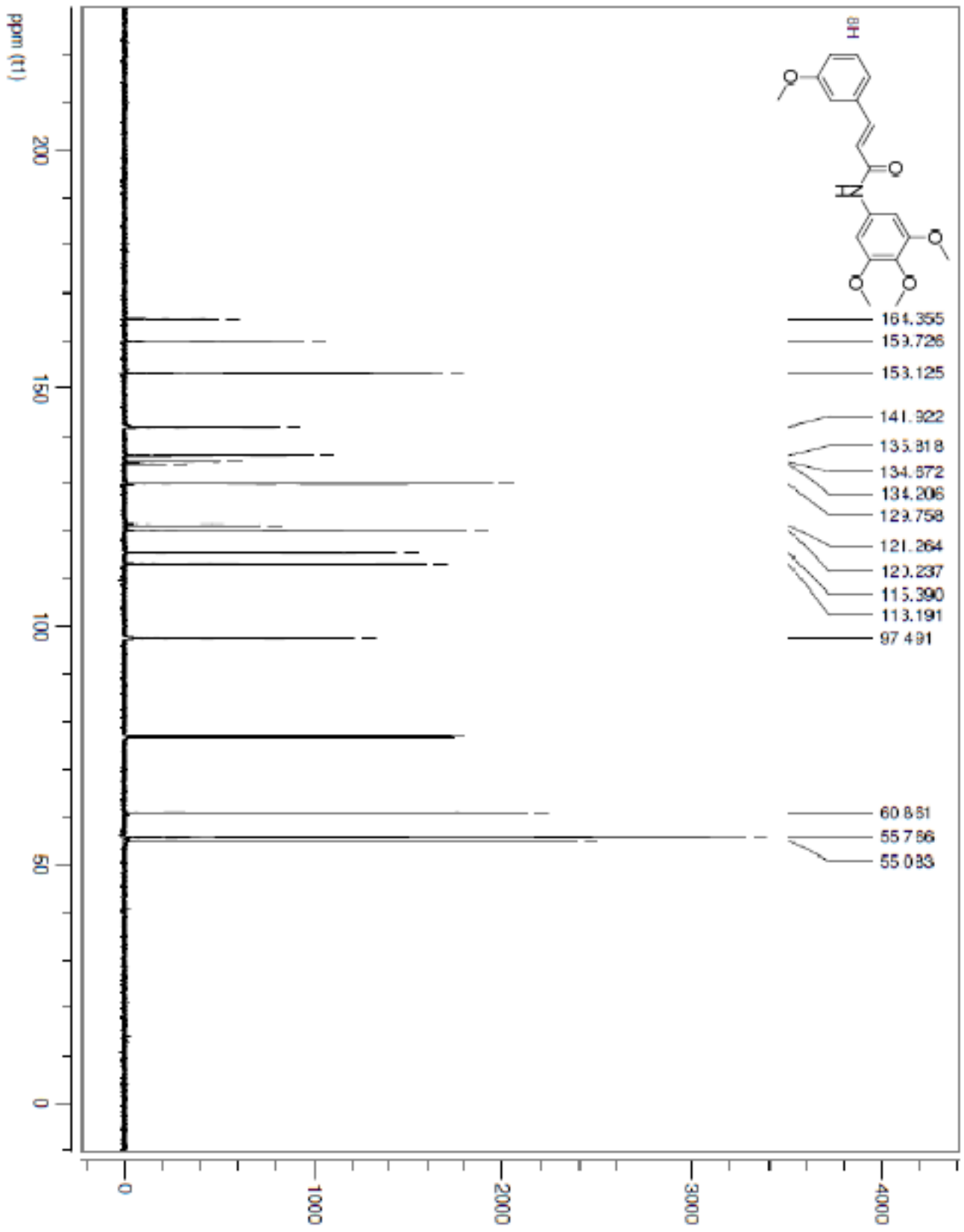


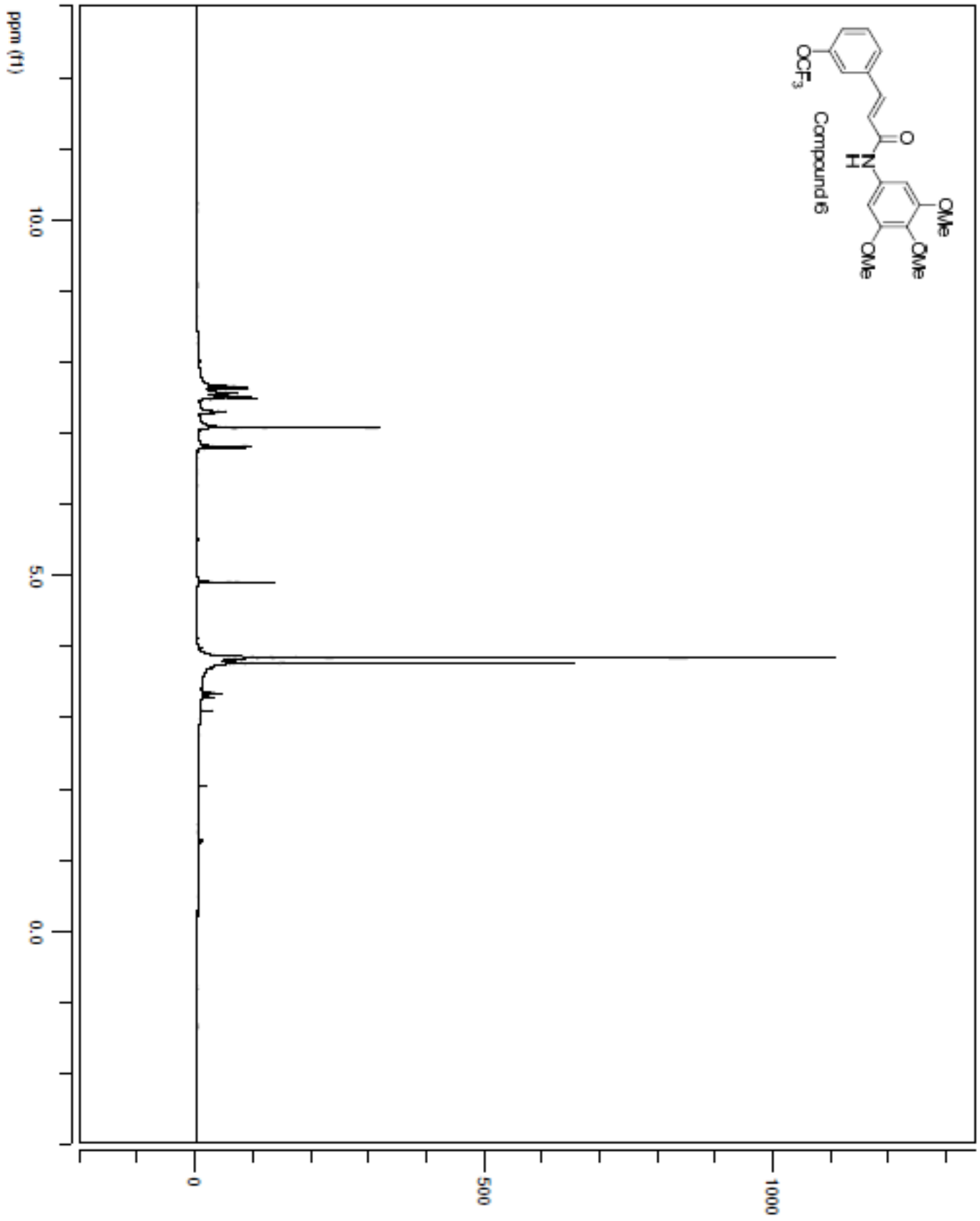
NMR ^{1}H (500 MHz, $(CD_3)_2O$) δ ppm: 7.80 (br s, 1H), 7.58 (d, 1H, J=15.7 Hz), 7.30 (d, 1H, J=7.9 Hz), 7.14 (m, 2H), 6.94 (dd, 1H, J=8.2 Hz, J=2.5 Hz), 6.78 (d, 1H, J=15.7 Hz), 6.67 (s, 2H), 4.47 (d, 2H, J=5.9 Hz), 3.81 (s, 3H), 3.79 (s. 6H), 3.70 (s, 3H).

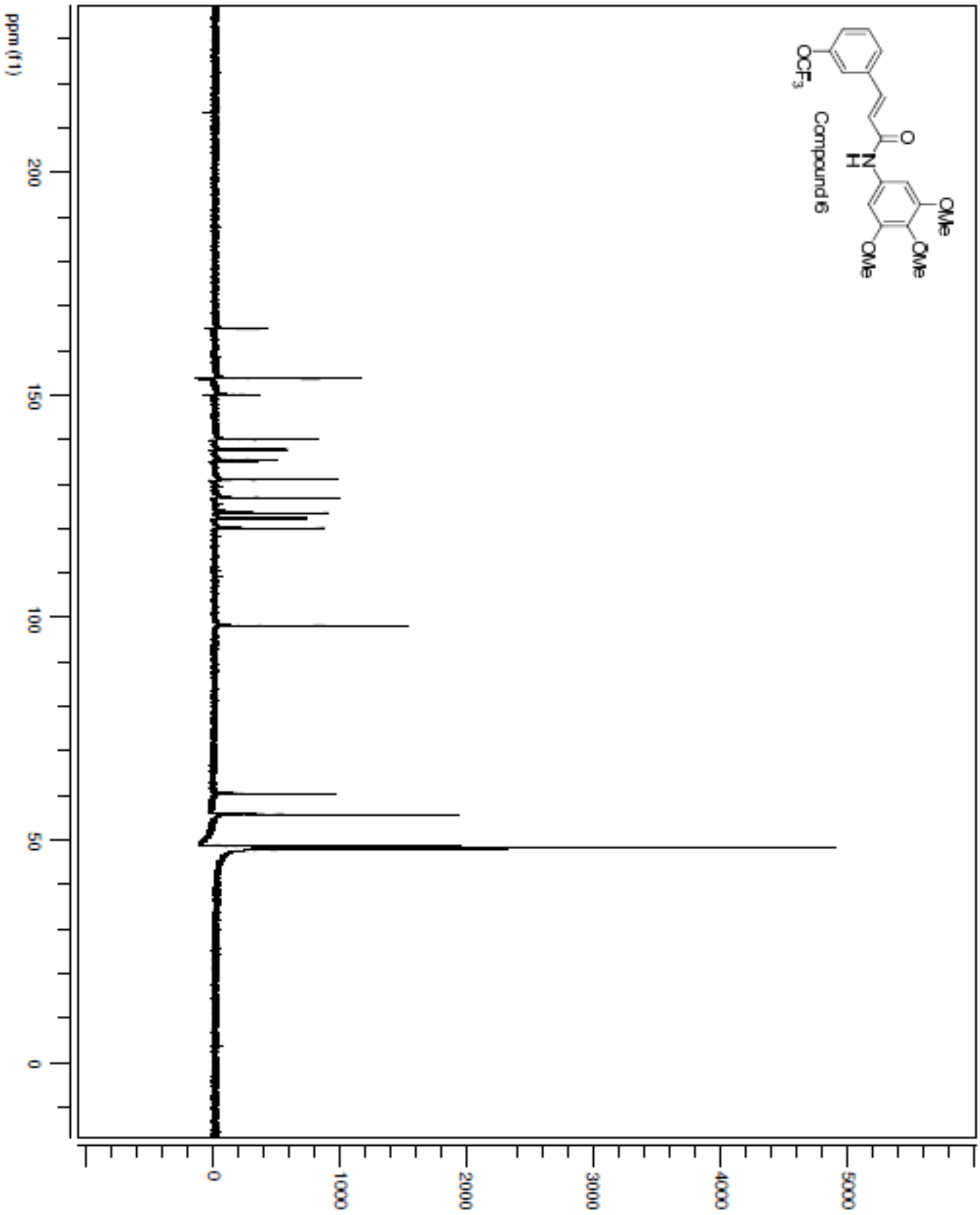
NMR ^{13}C (500 MHz, $(CD_3)_2O$) δ ppm: 165.32, 160.36, 153.72, 139.80, 137.59, 136.97, 135.27, 130.07, 122.41, 120.23, 115.39, 112.90, 105.32, 59.86, 55.73, 54.91, 43.41.

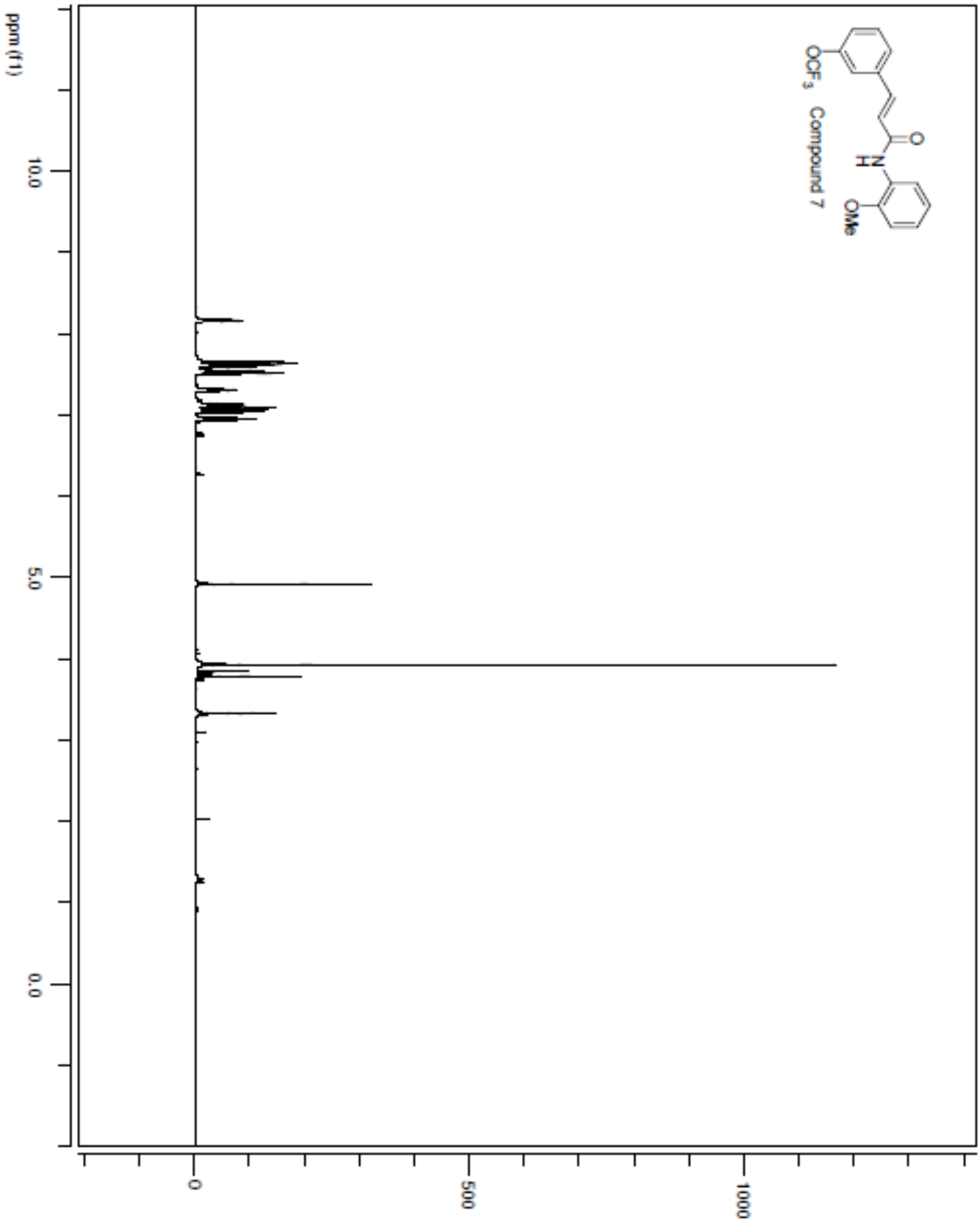
NMR Spectra for library compounds

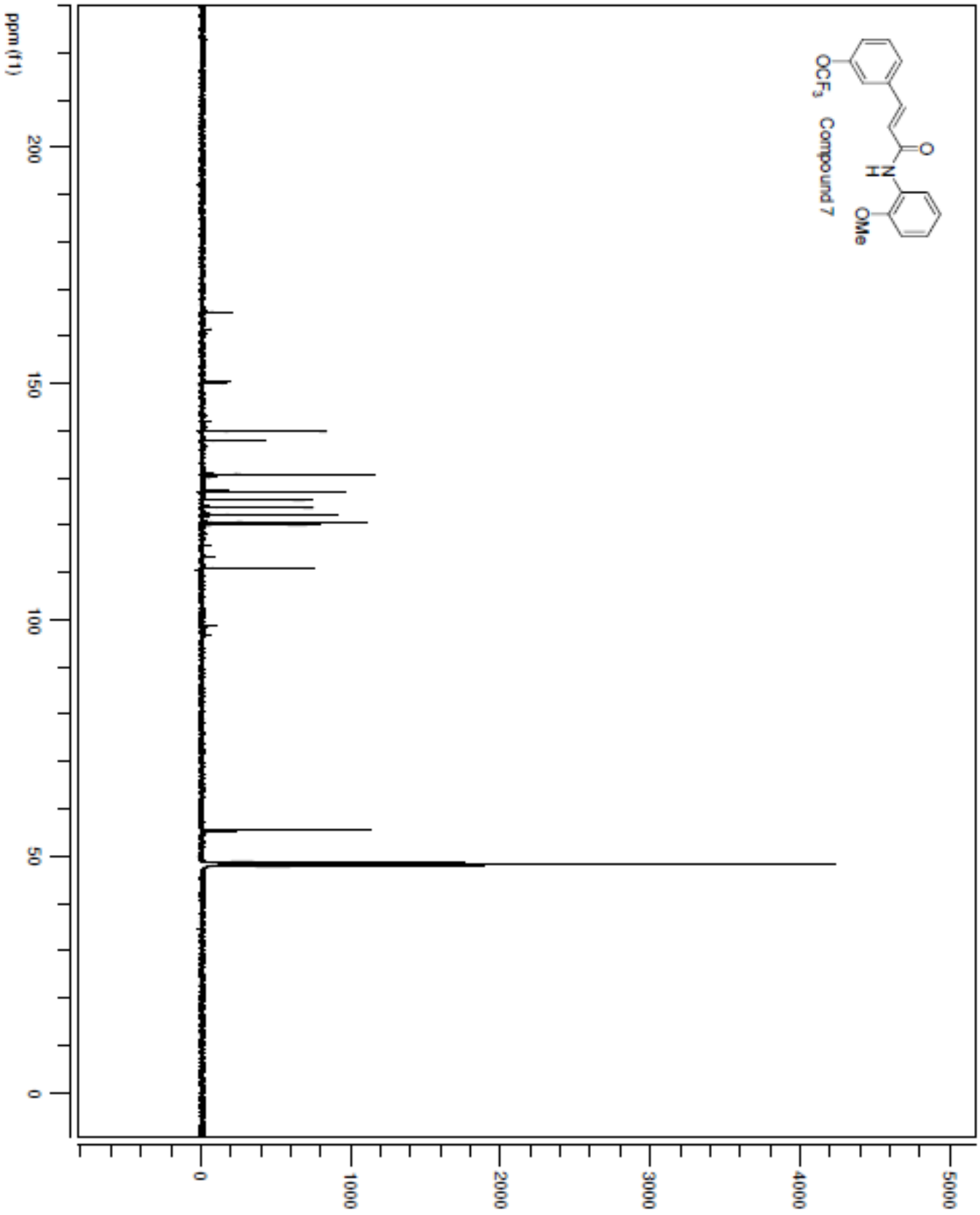


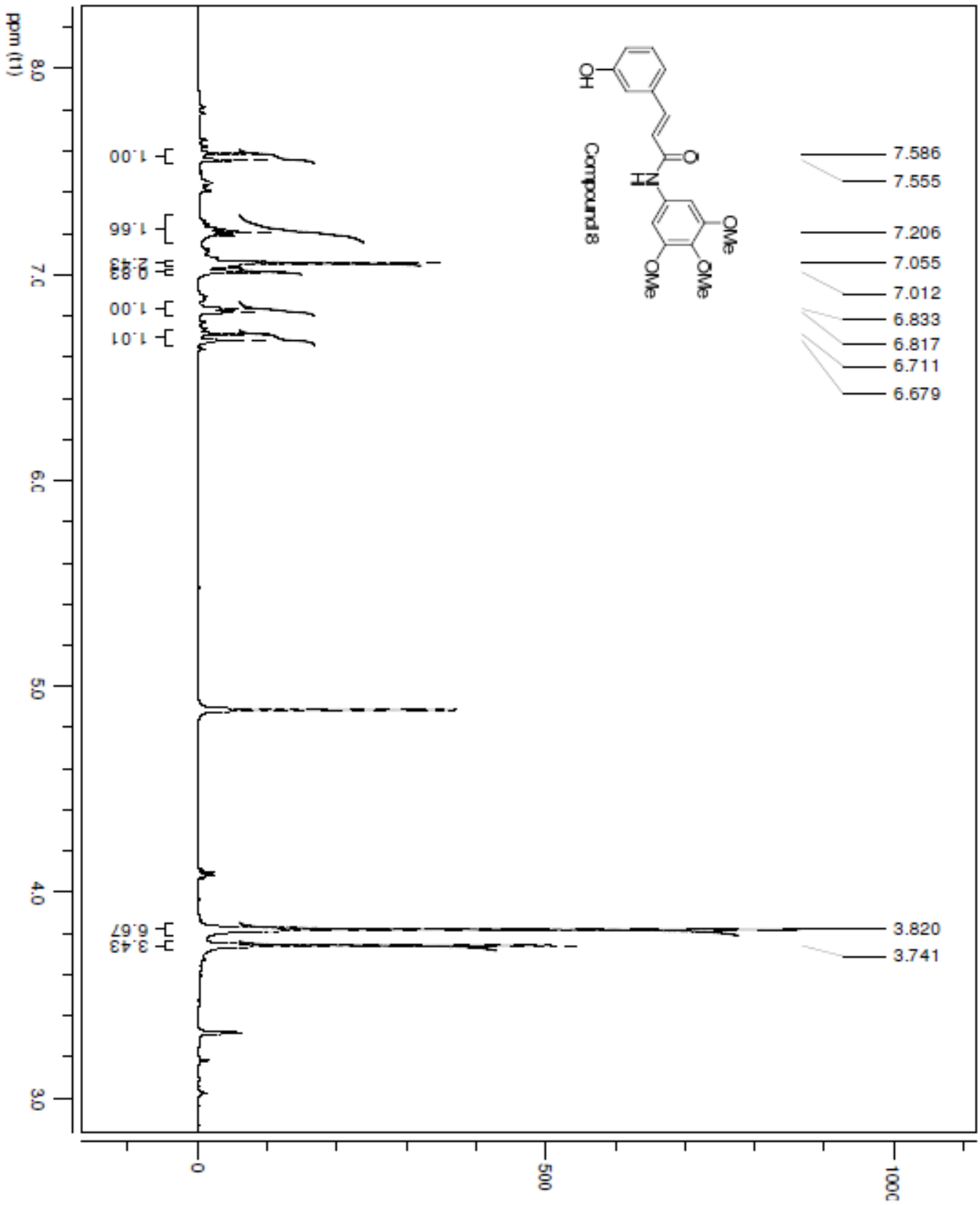

ppm (t1)
200
150
100
50
0
0
1000
2000
3000
4000
164.355
153.726
153.125
141.322
135.818
134.872
134.206
129.758
121.264
120.237
115.390
113.191
97.491
60.851
55.786
55.083

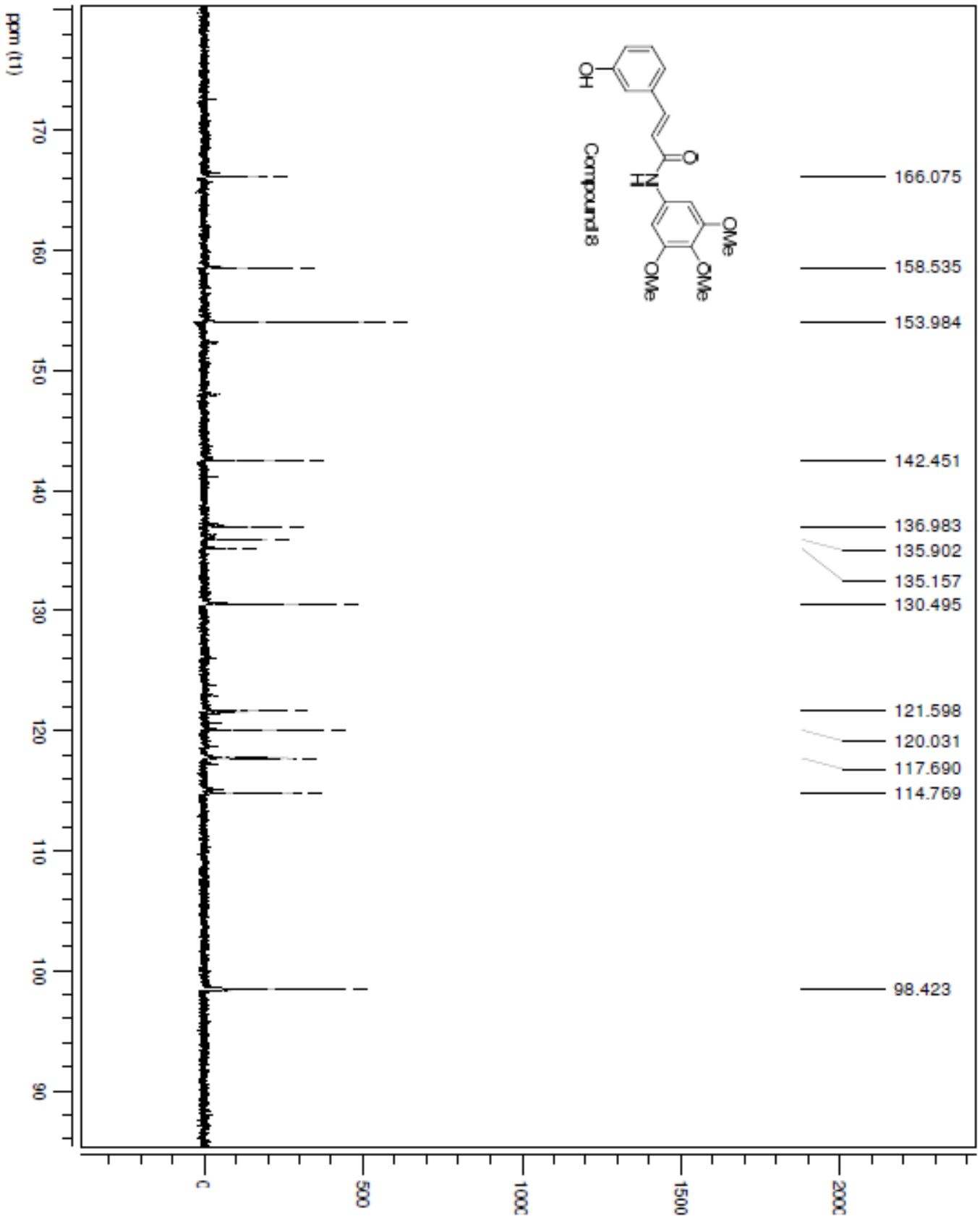

ppm (f1)
10.0
5.0
0.0
0
500
1000
OCF3
O
N
H
OMe
OMe
OMe
Compound 6

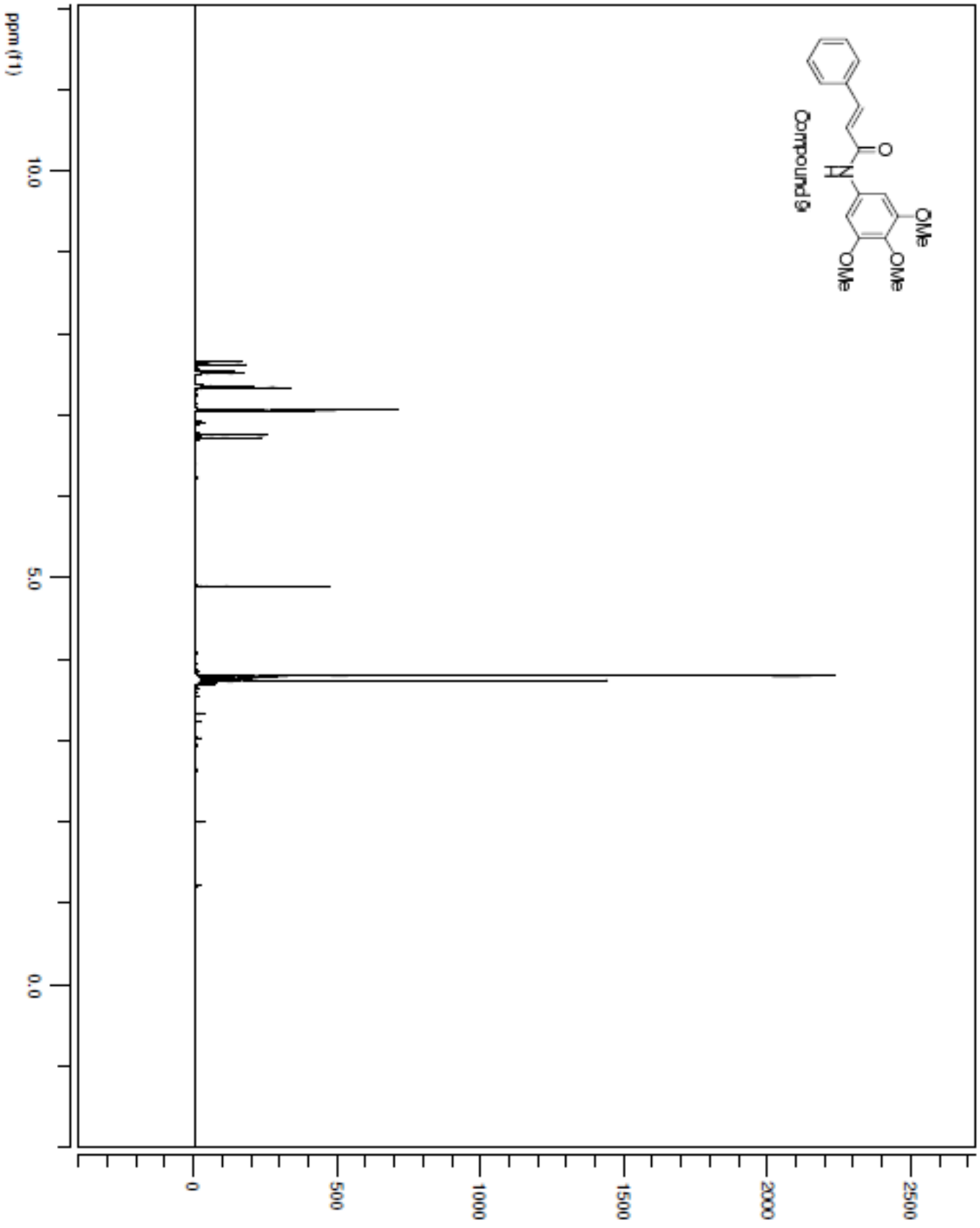

Compound 6
OCF3
O
N
H
OMe
OMe
OMe
ppm (f1)
0
50
100
150
200
0
1000
2000
3000
4000
5000

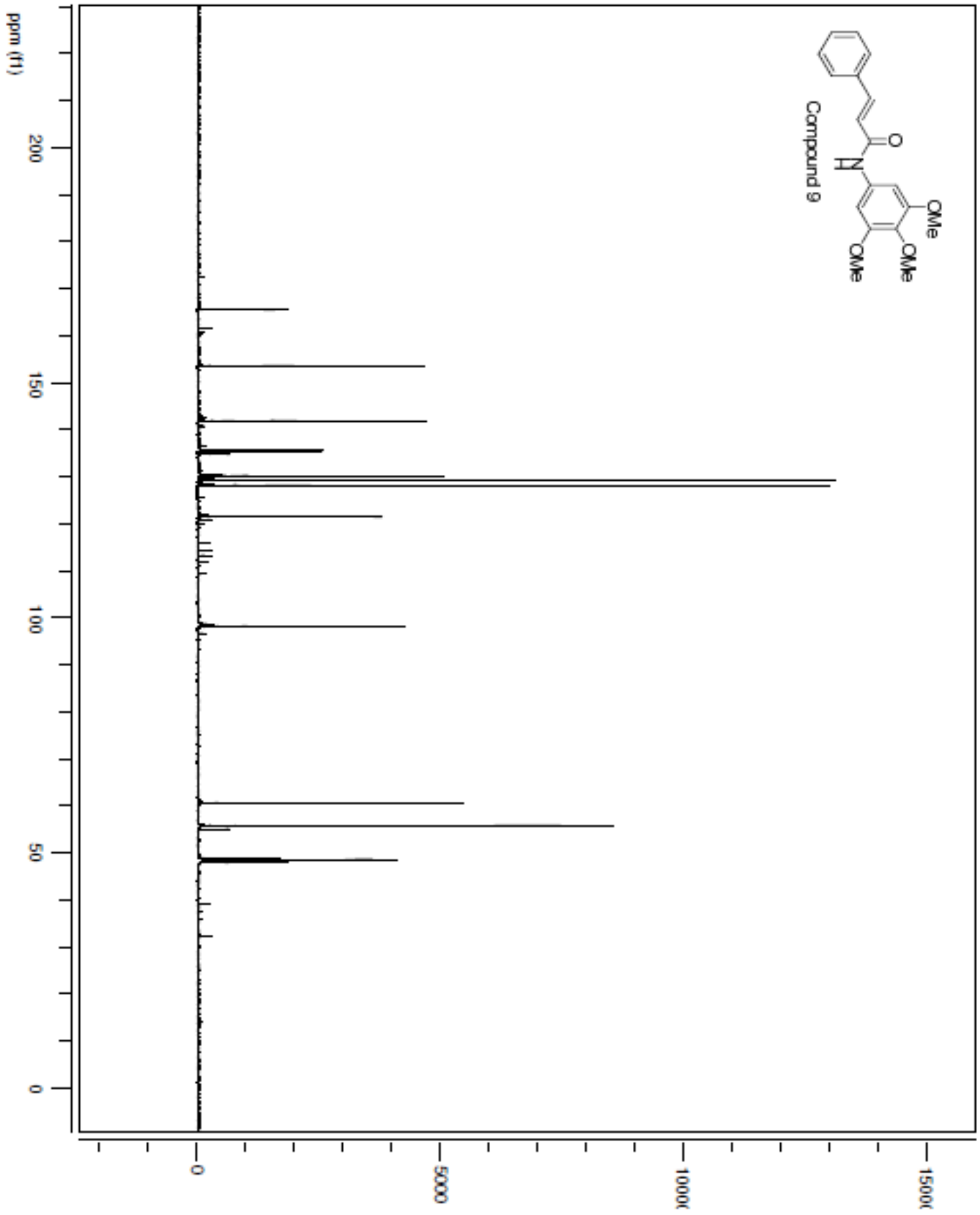

ppm (f1)
10.0
5.0
0.0
0
500
1000
OCF3
Compound 7
O
N
H
OMe

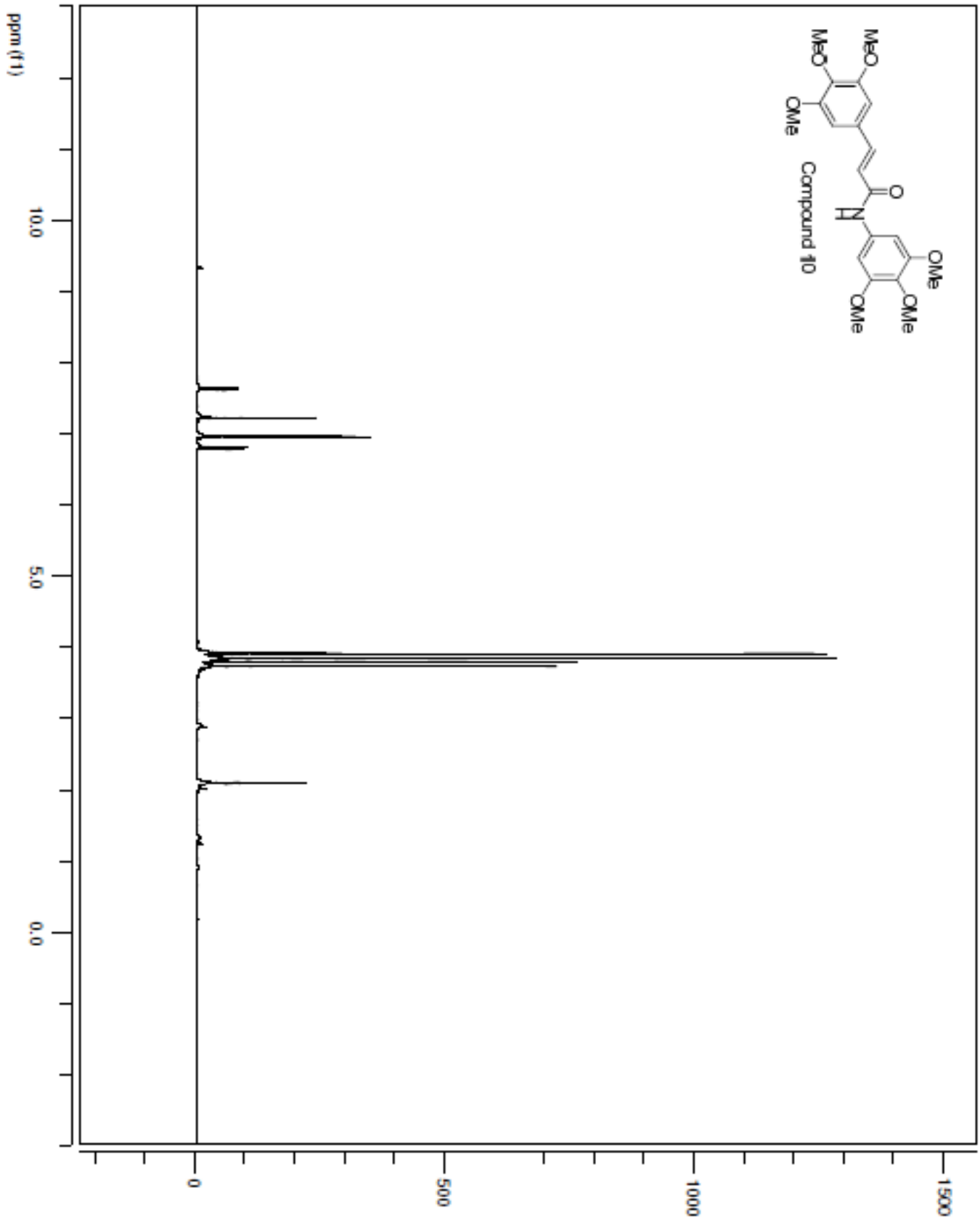

ppm (f1)
200
150
100
50
0
0
1000
2000
3000
4000
5000
OCF3
Compound 7
O
N
H
OMe

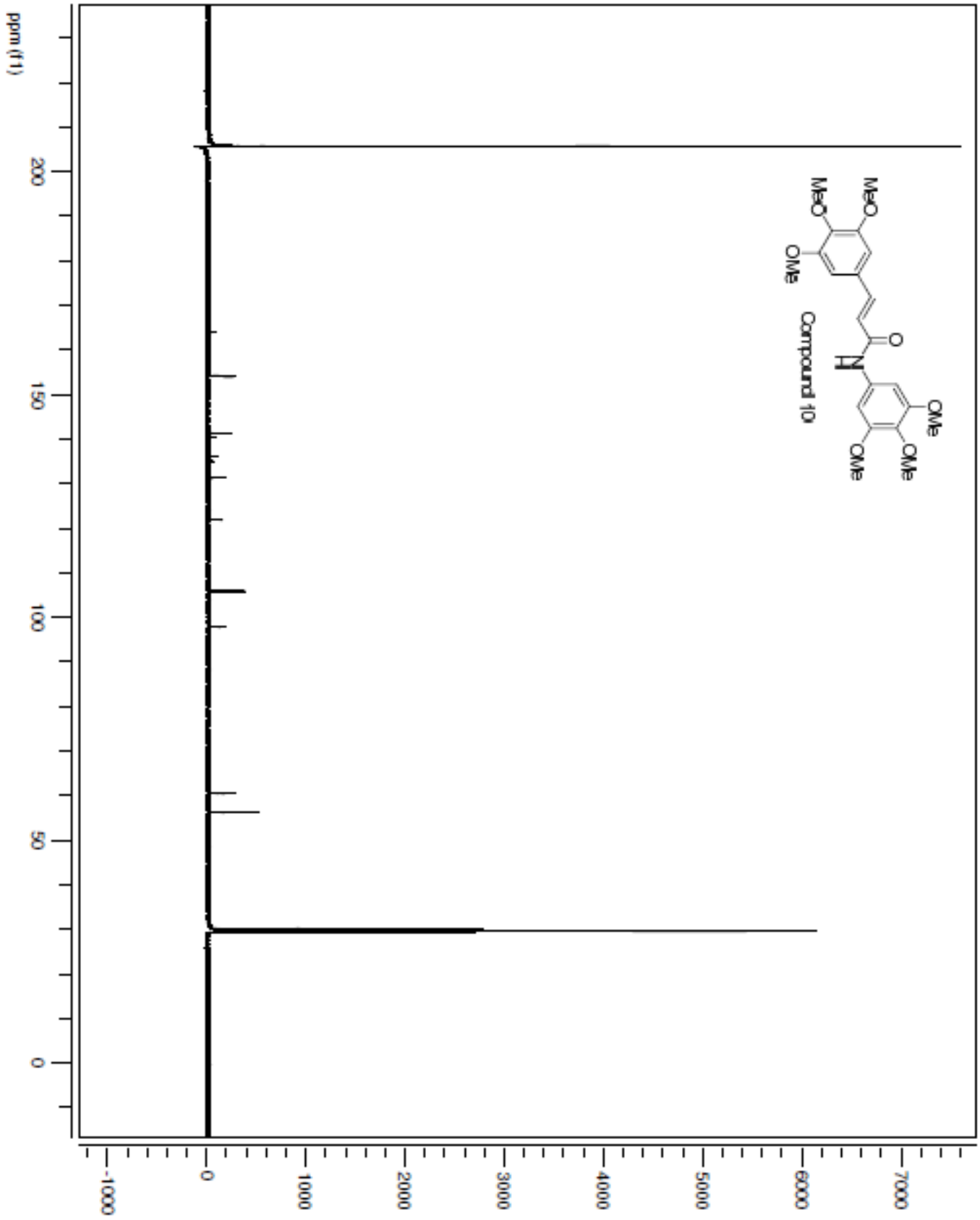

ppm (t1)
8.0
7.0
6.0
5.0
4.0
3.0
0
500
1000
1.00
1.66
2.43
0.89
1.00
1.01
6.67
3.43
Compound 8
OH
O
N
H
OMe
OMe
OMe
7.586
7.555
7.206
7.055
7.012
6.833
6.817
6.711
6.679
3.820
3.741

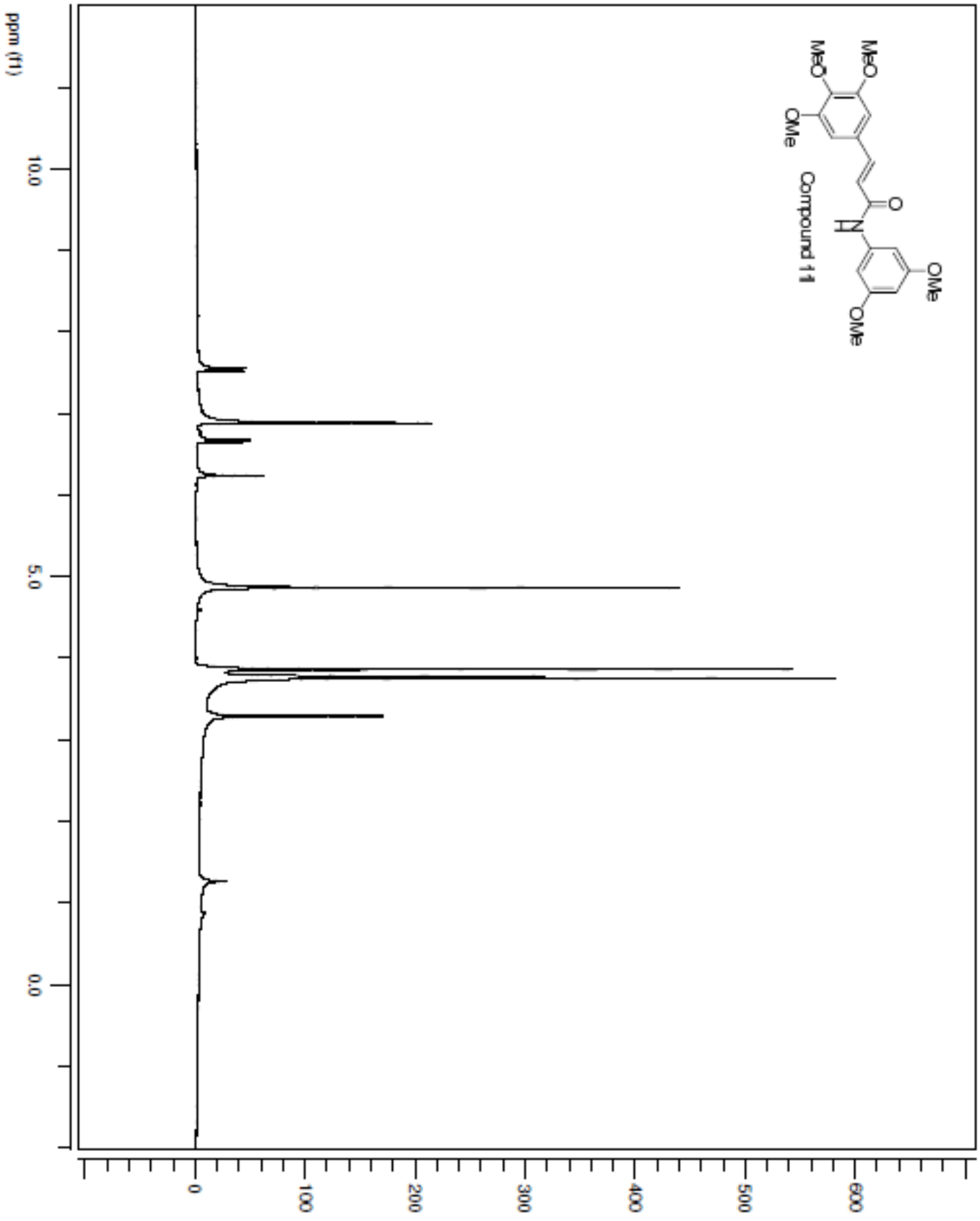

ppm (t1)
170
160
150
140
130
120
110
100
90
0
500
1000
1500
2000
OH
O
N
H
OMe
OMe
OMe
Compound 8
166.075
158.535
153.984
142.451
136.983
135.902
135.157
130.495
121.598
120.031
117.690
114.769
98.423

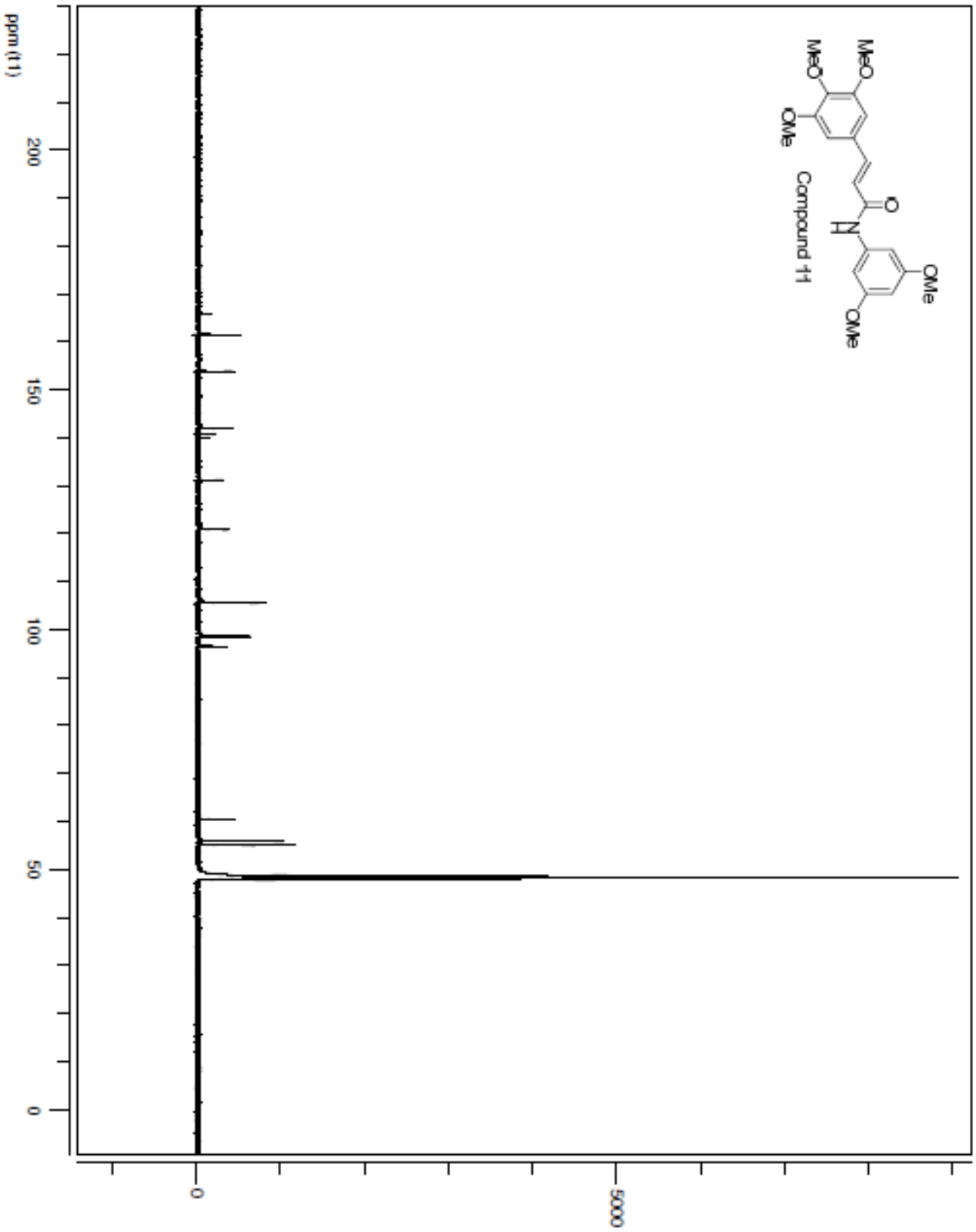

Compound 9
OMe
OMe
OMe
O
N
H
ppm (f1)
10.0
5.0
0.0
0
500
1000
1500
2000
2500

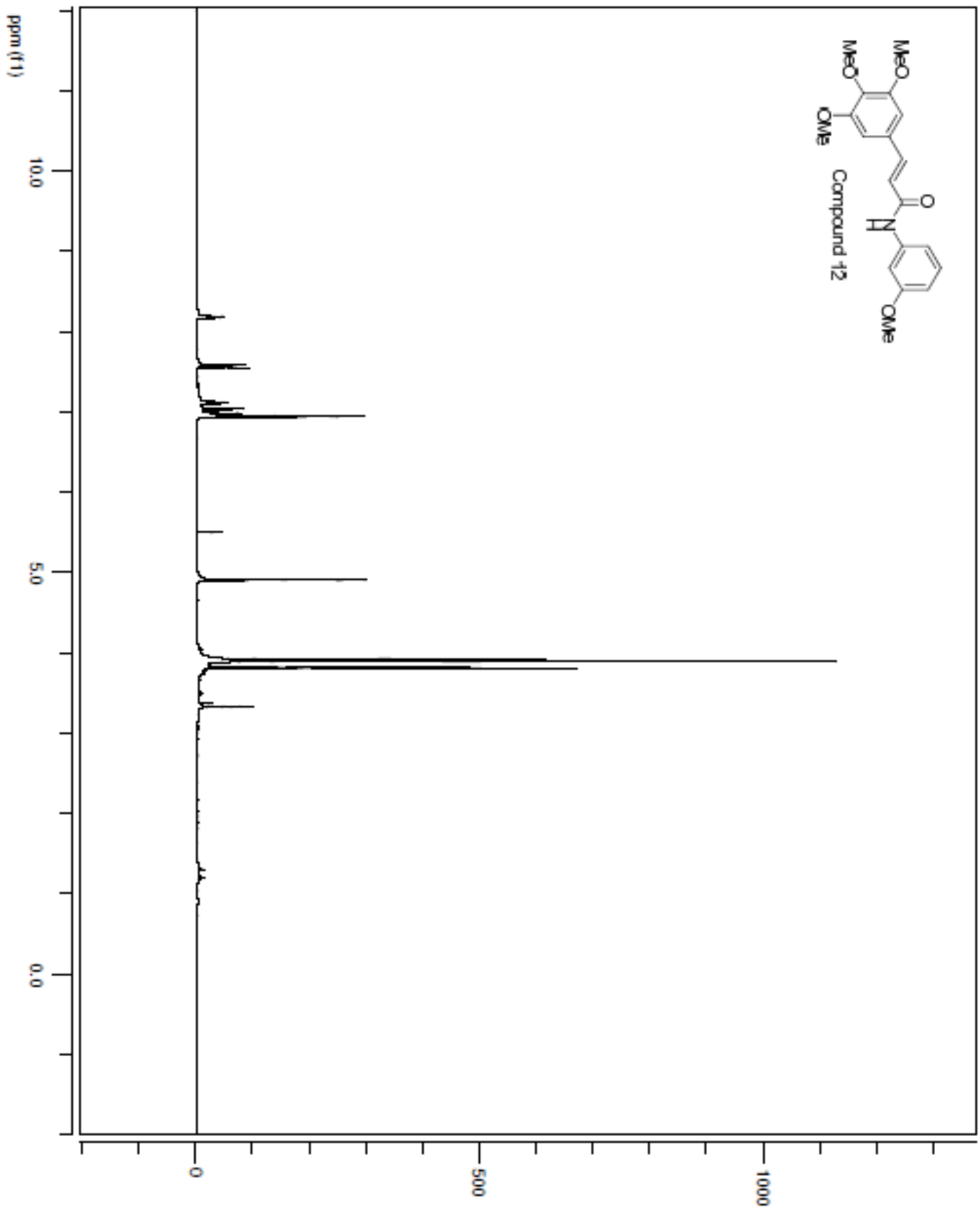

Compound 9
OMe
OMe
OMe
O
N
H
ppm (f1)
200
150
100
50
0
0
5000
10000
15000

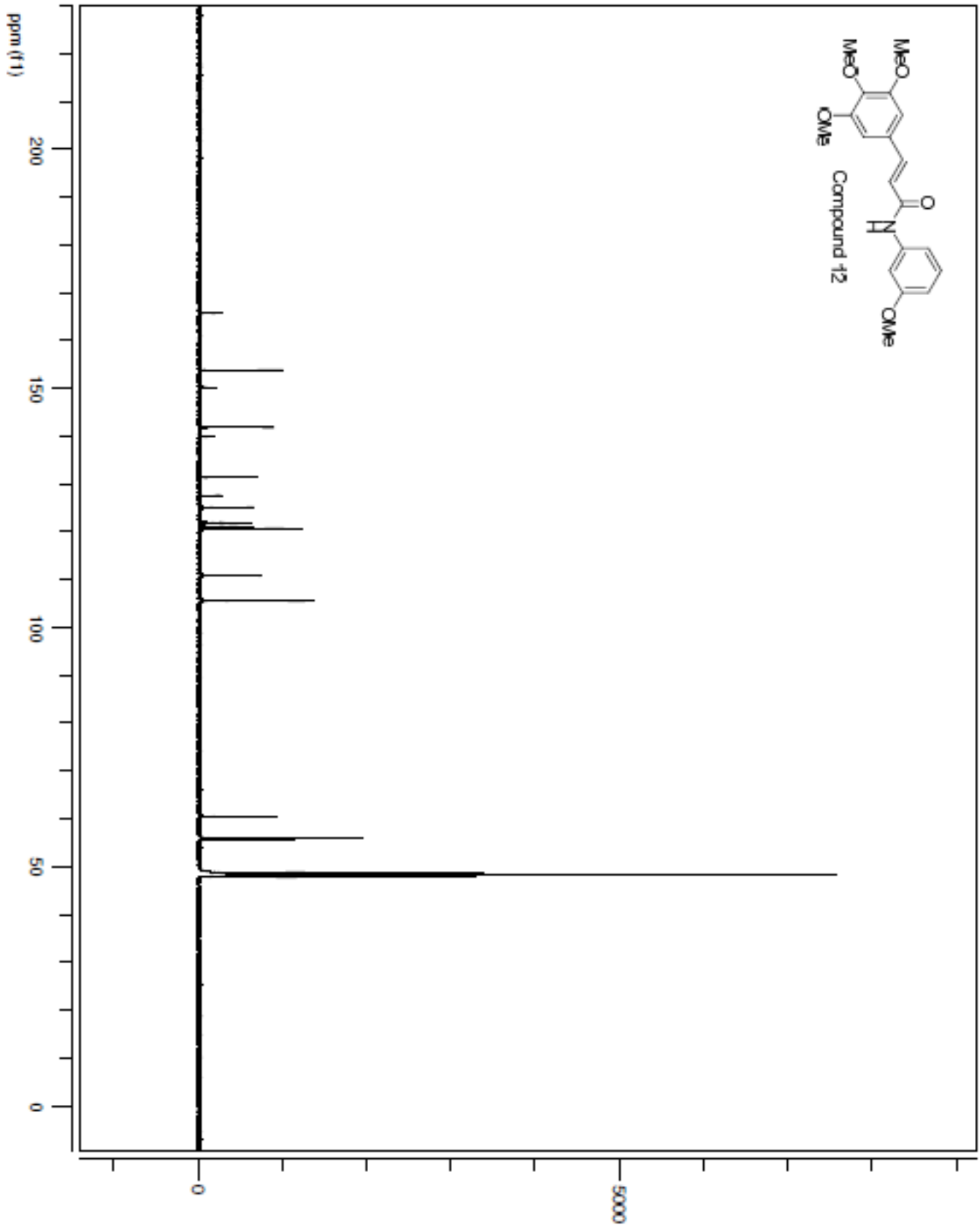

ppm (f1)
10.0
5.0
0.0
0
500
1000
1500
MeO
MeO
OMe
O
H
N
OMe
OMe
OMe
Compound 10

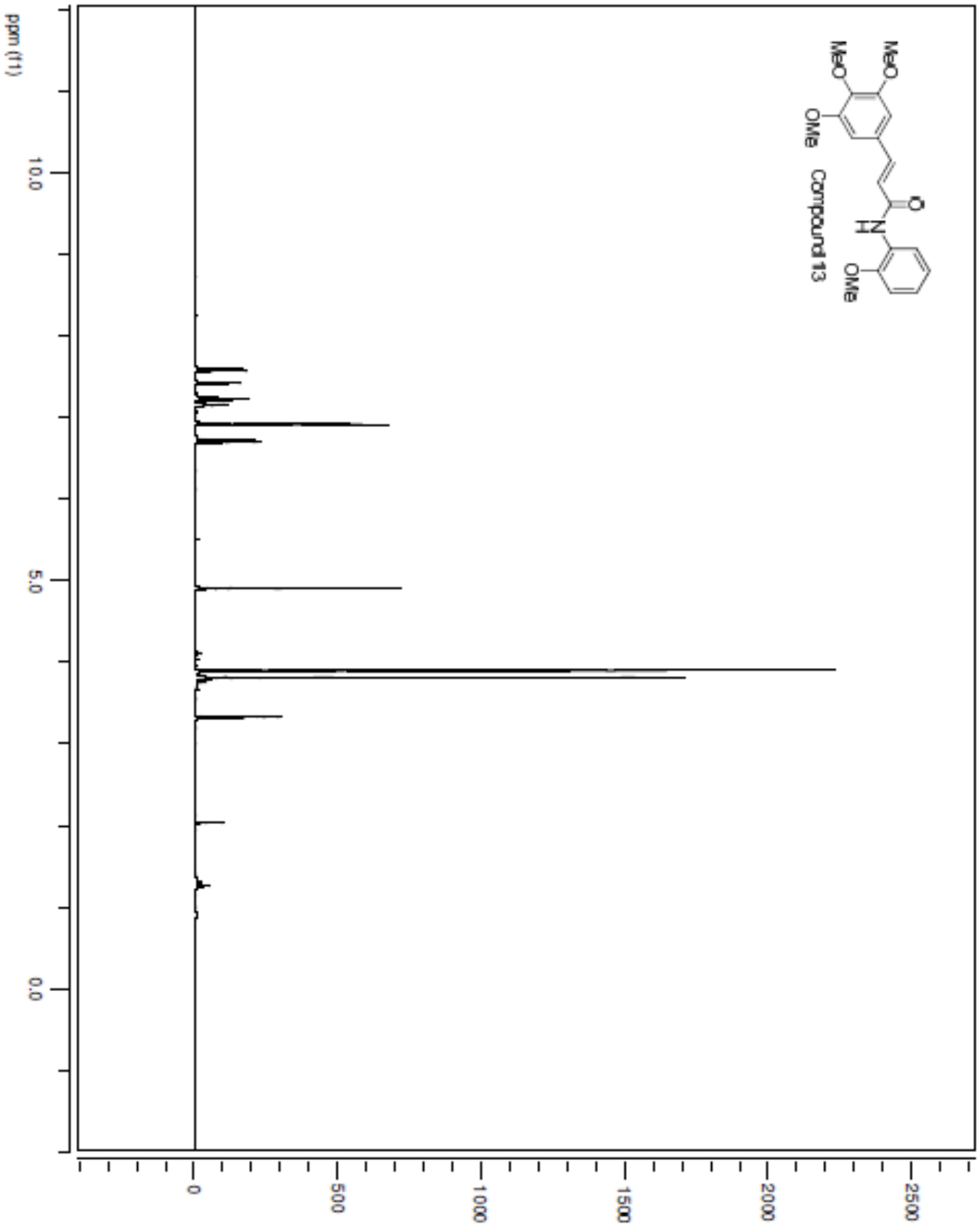

ppm (f1)
200
150
100
50
0
-1000
0
1000
2000
3000
4000
5000
6000
7000
MeO
MeO
OMe
O
N
H
OMe
OMe
OMe
Compound 10

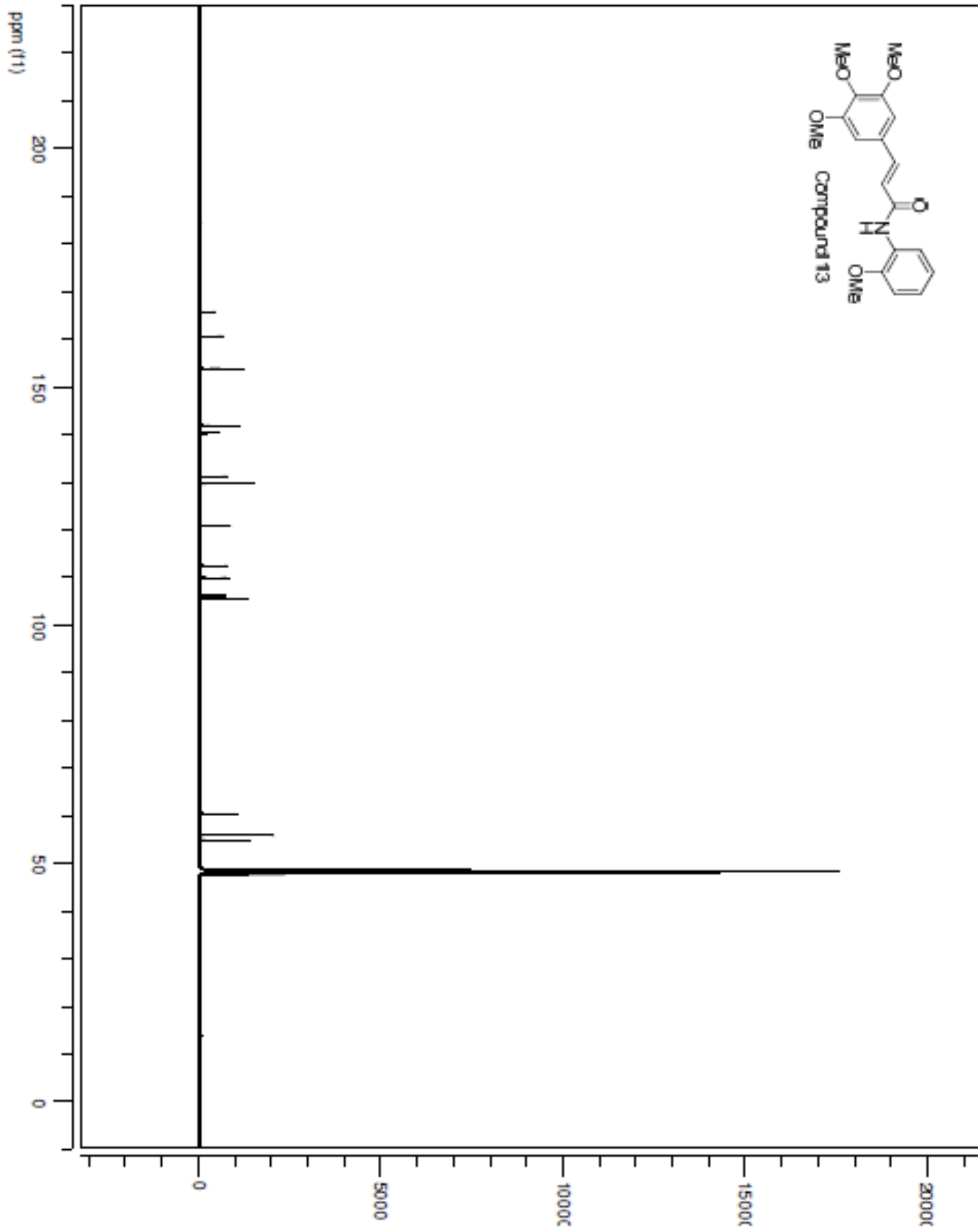

ppm (f1)
10.0
5.0
0.0
0
100
200
300
400
500
600
MeO
MeO
OMe
O
N
H
OMe
OMe
Compound 11

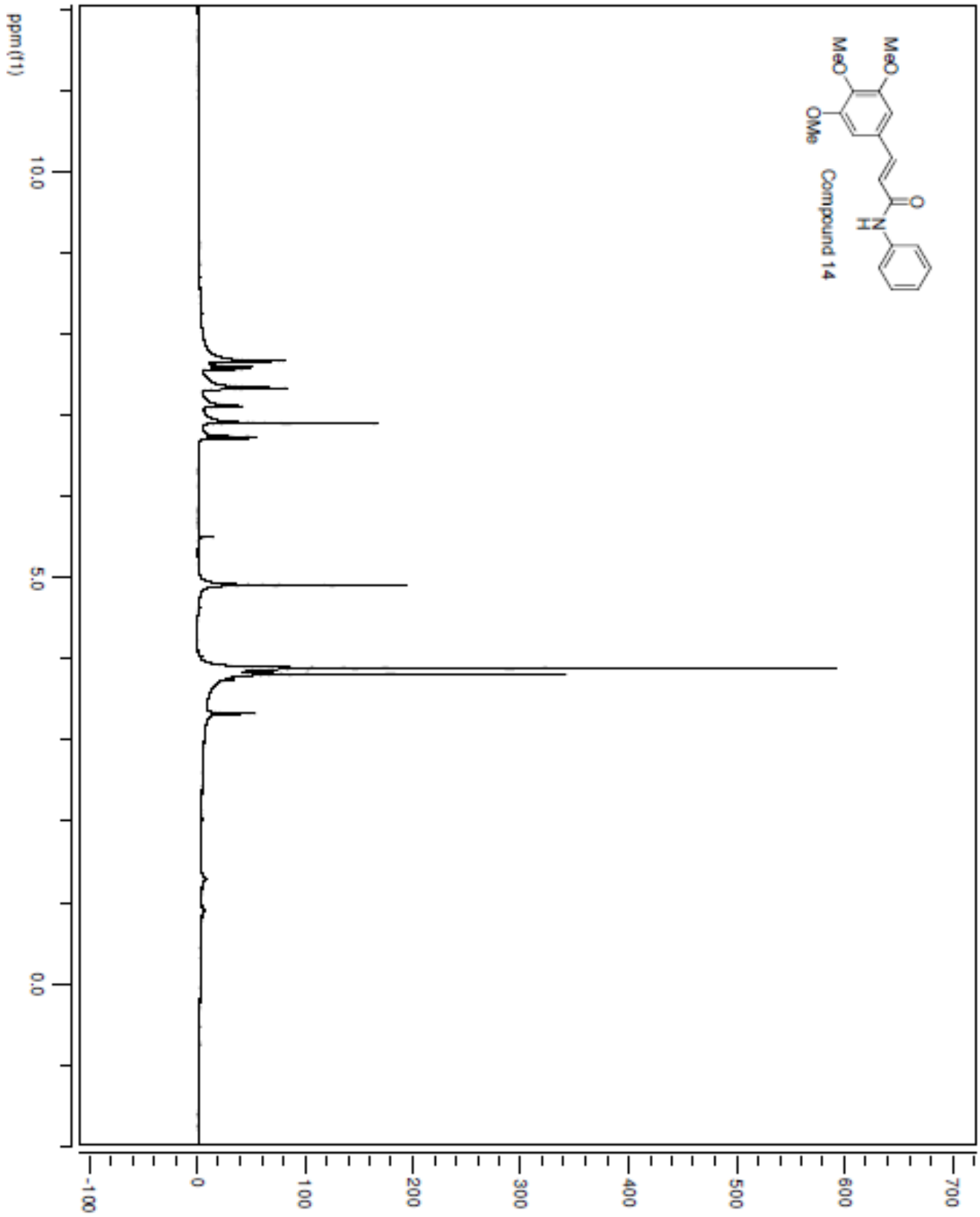

ppm (t1)
200
150
100
50
0
0
5000
MeO
MeO
OMe
O
N
H
OMe
OMe
Compound 11

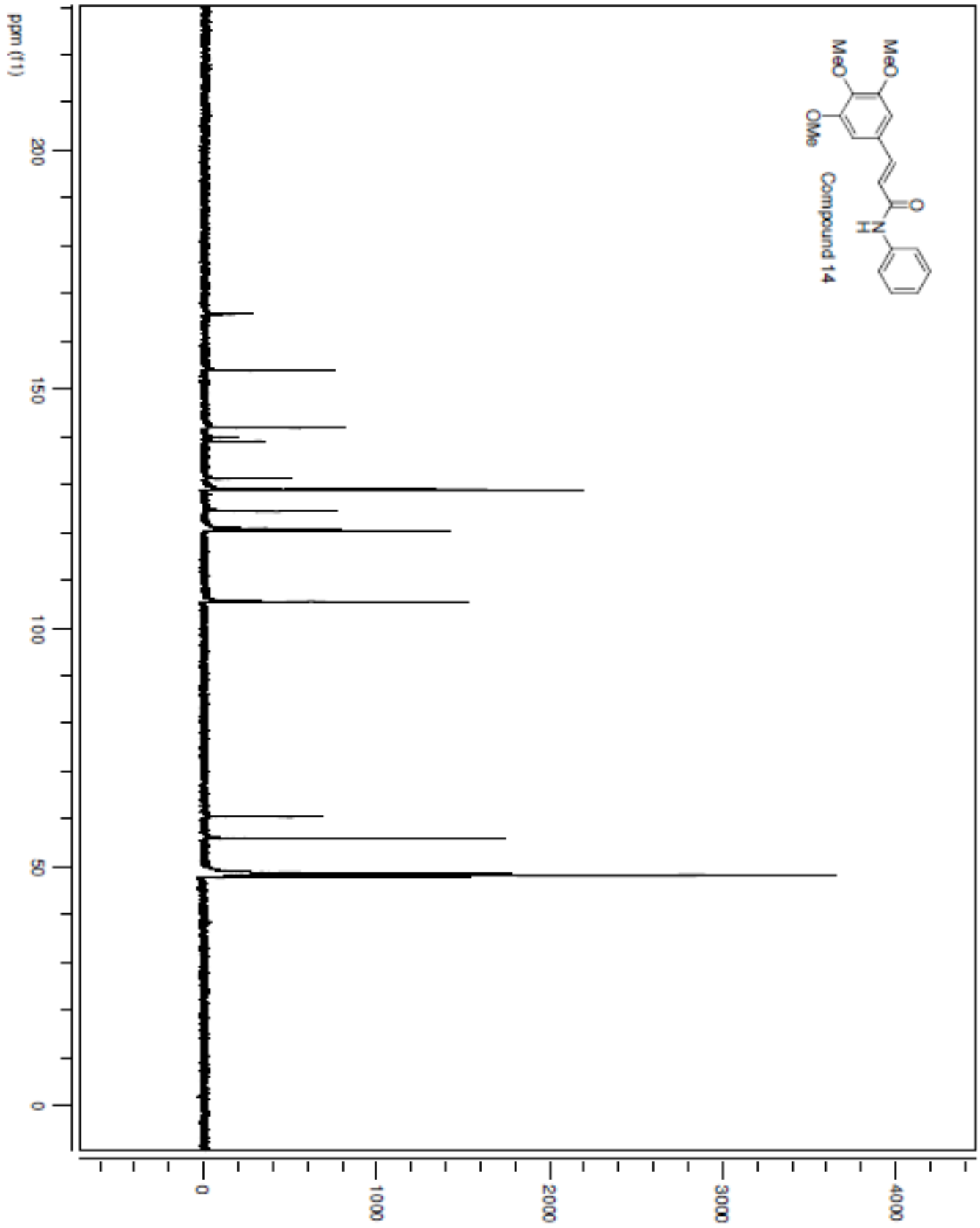

MeO
MeO
OMe
O
N
H
OMe
Compound 12
ppm (f1)
10.0
5.0
0.0
0
500
1000

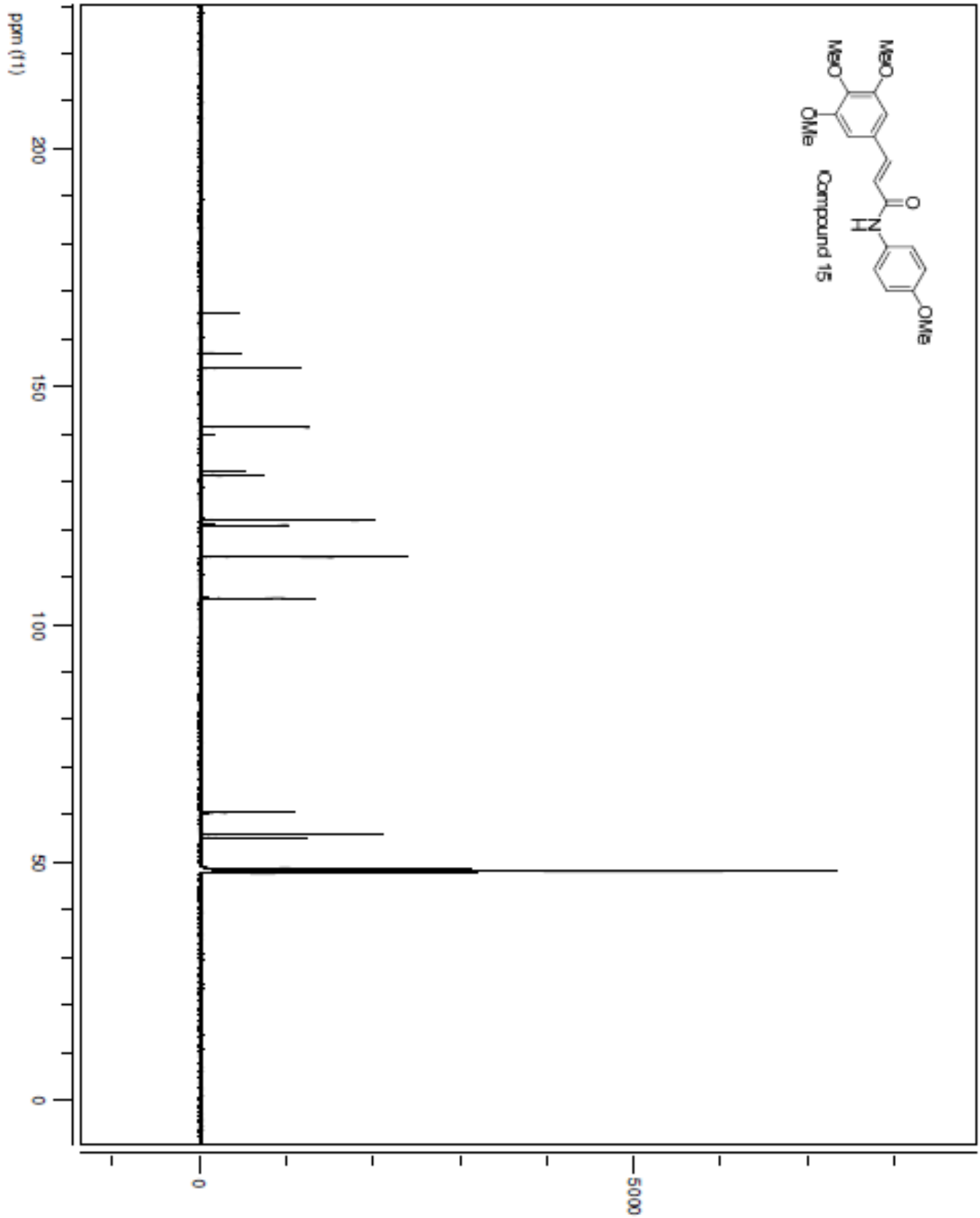

MeO
MeO
OMe
O
N
H
OMe
Compound 12
ppm (f1)
200
150
100
50
0
0
5000

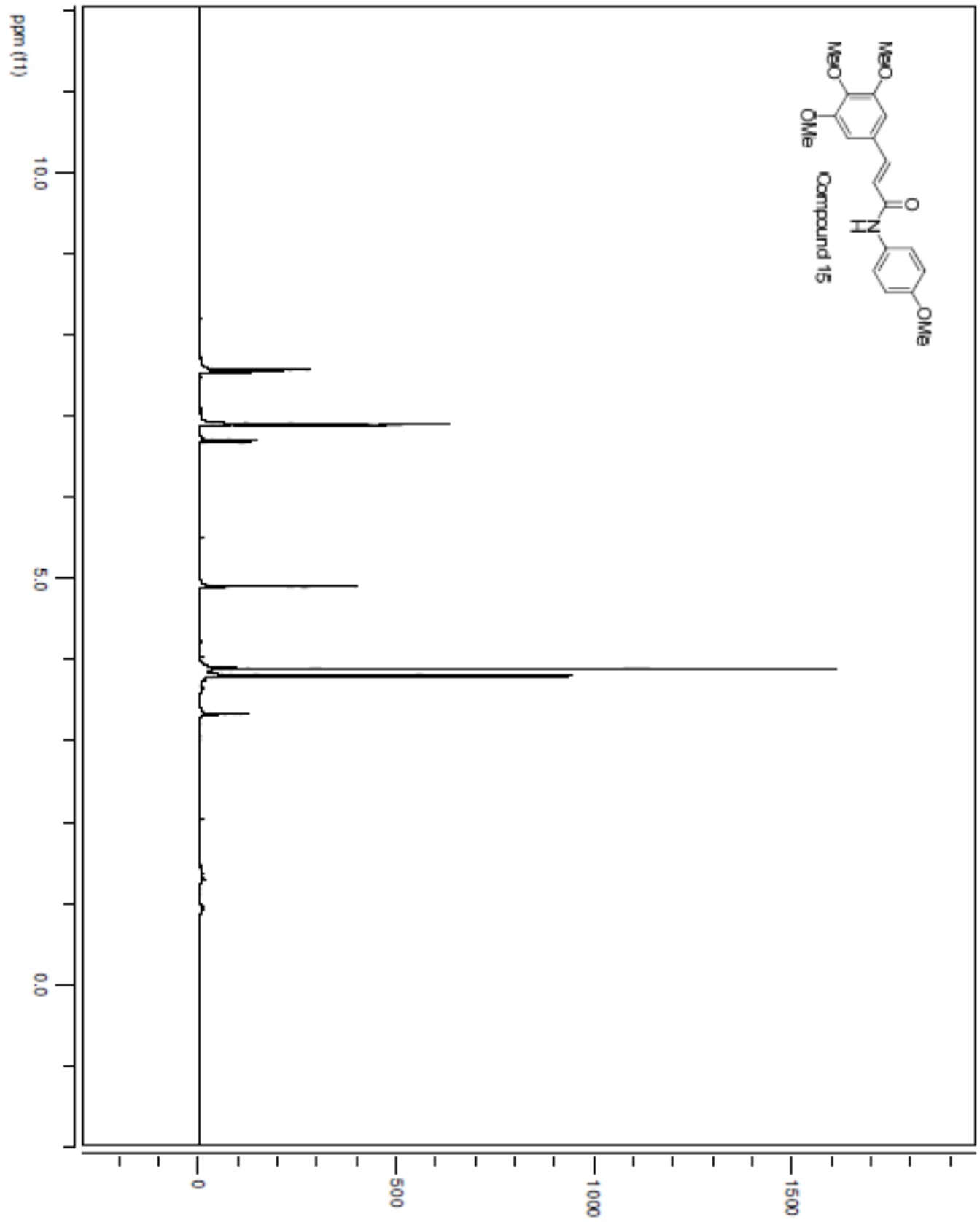

MeO
MeO
OMe
O
N
H
OMe
Compound 13
ppm (t1)
10.0
5.0
0.0
0
500
1000
1500
2000
2500

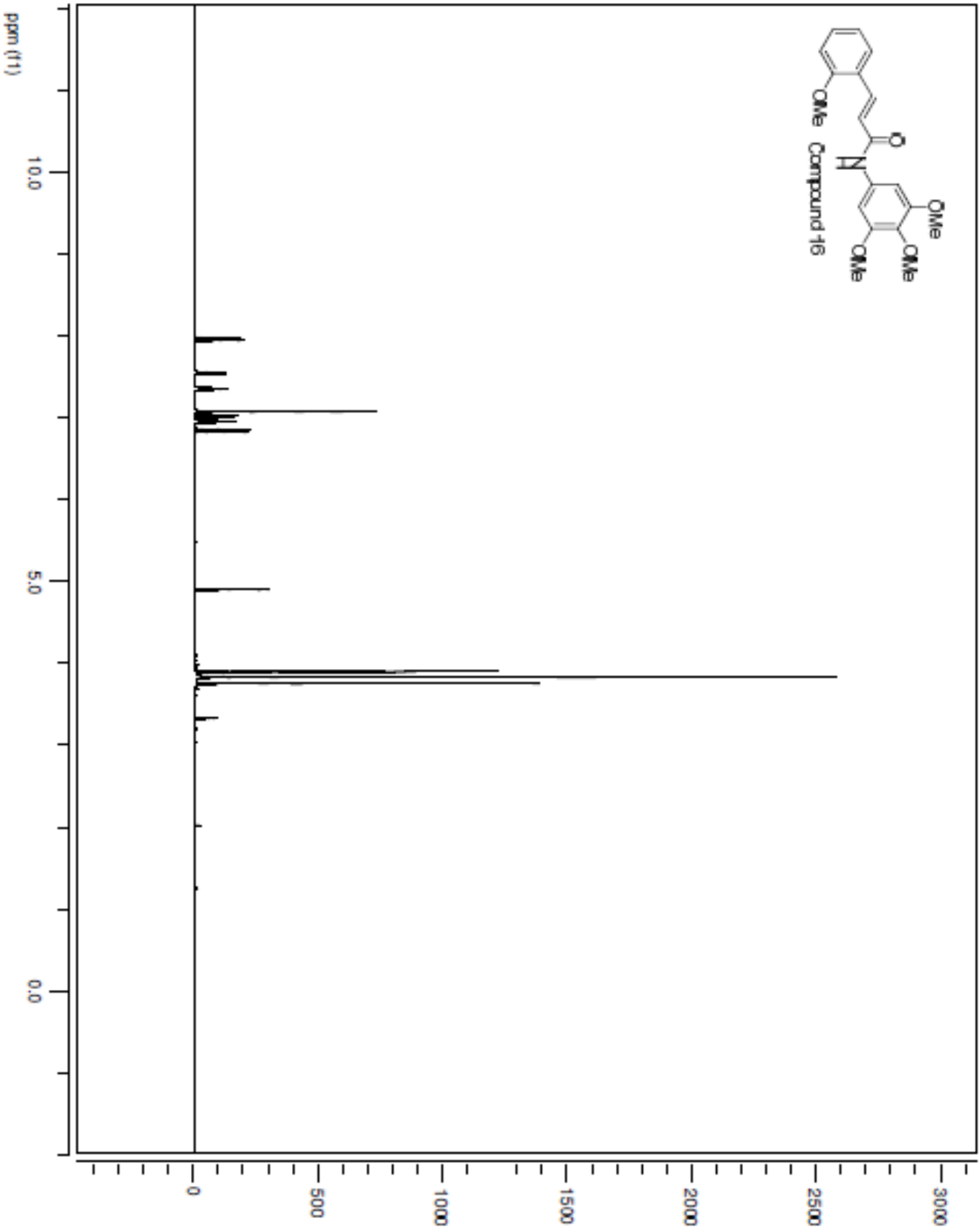

MeO
MeO
OMe
O
N
H
OMe
Compound 13
ppm (f1)
200
150
100
50
0
0
5000
10000
15000
20000

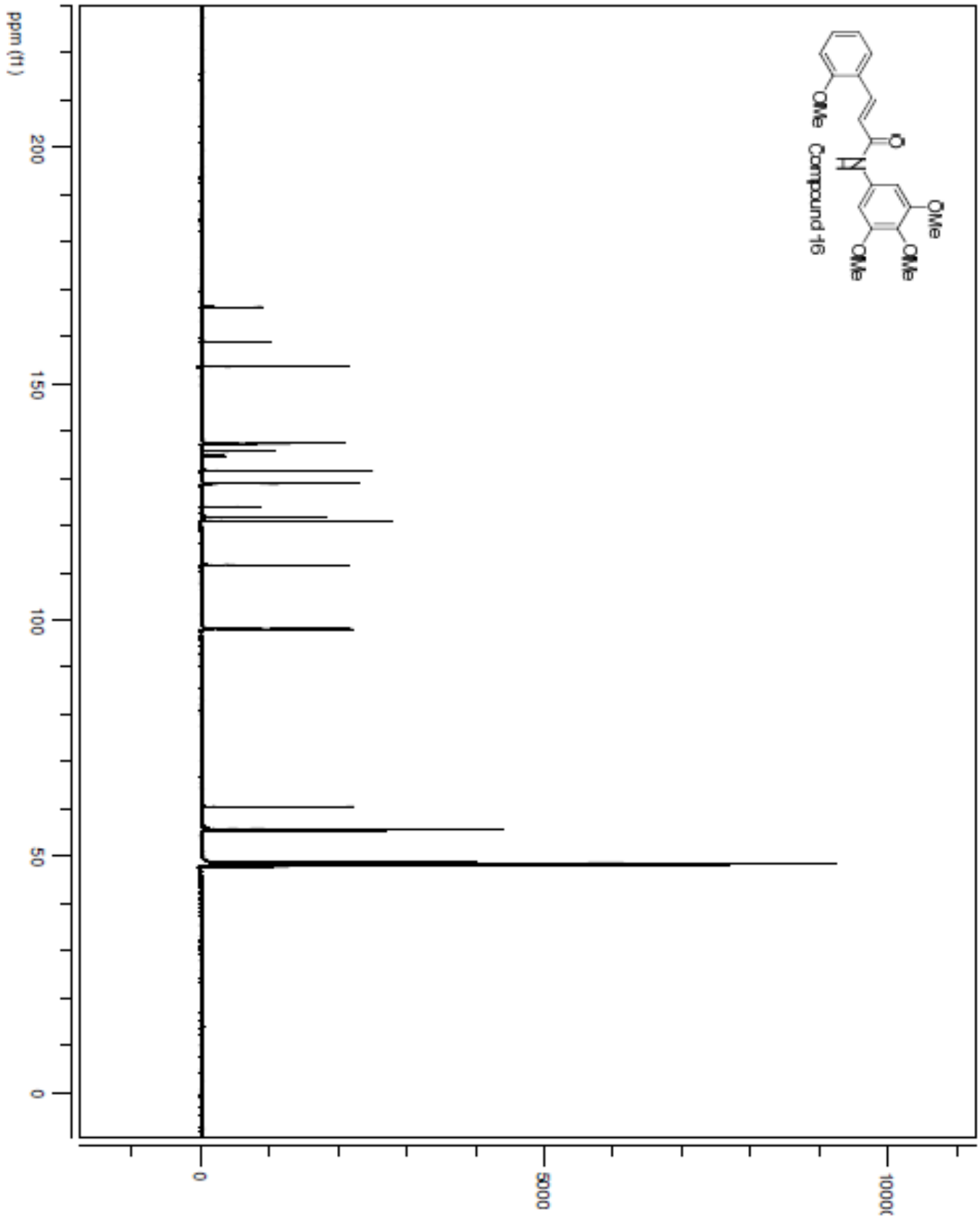

MeO
MeO
OMe
O
N
H
Compound 14
ppm (f1)
10.0
5.0
0.0
-100
0
100
200
300
400
500
600
700

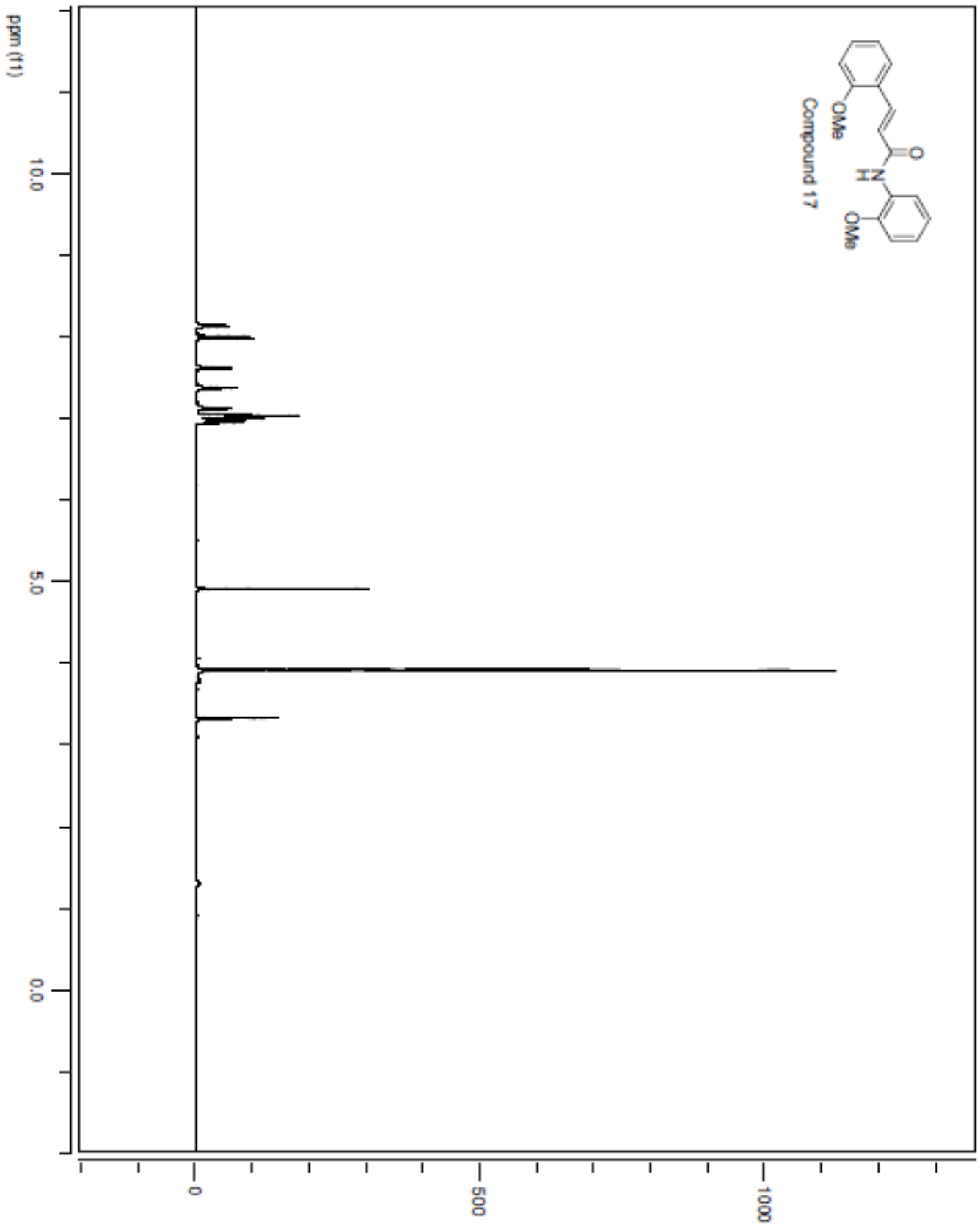

MeO
MeO
OMe
O
N
H
Compound 14
ppm (f1)
200
150
100
50
0
0
1000
2000
3000
4000

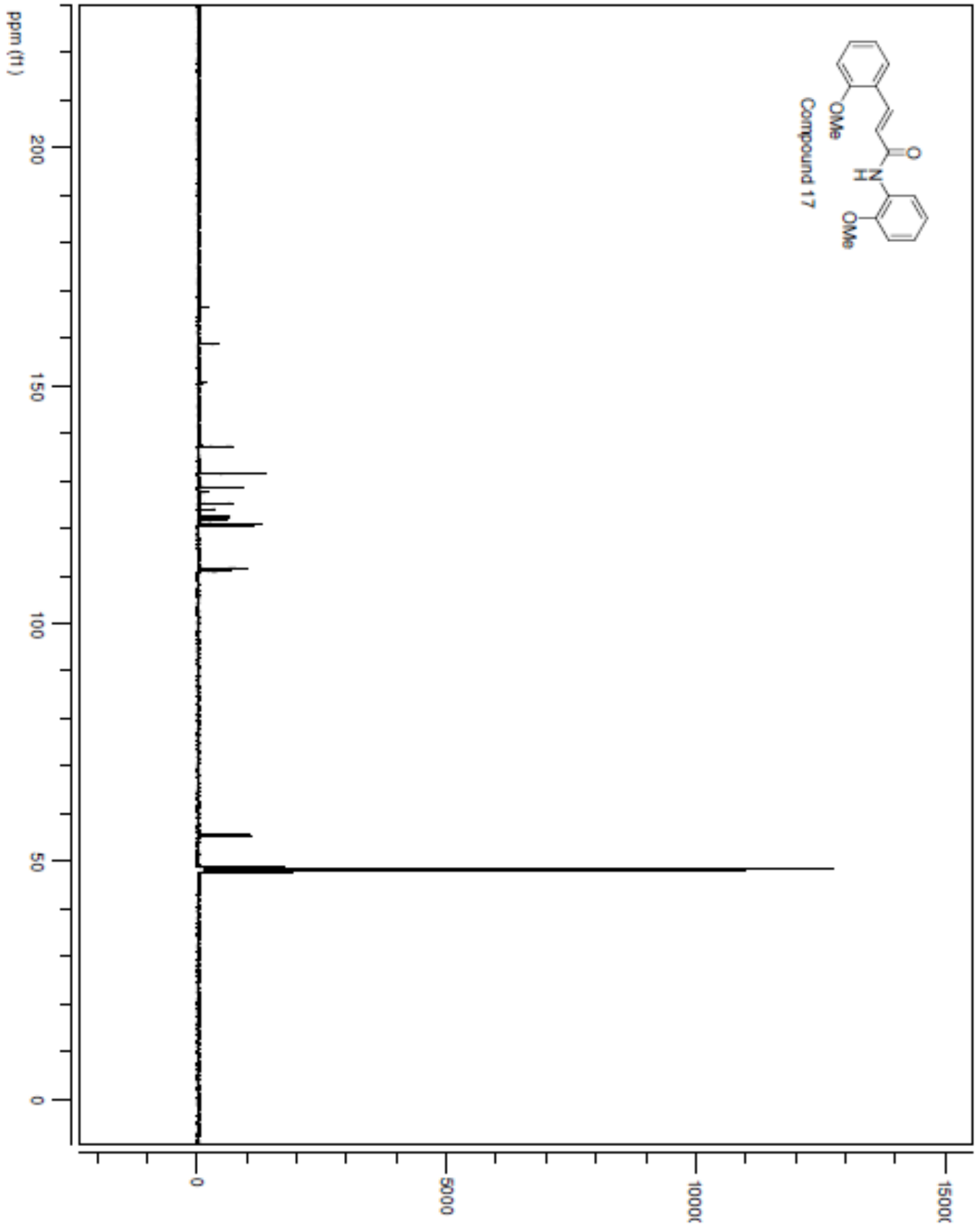

ppm (t1)
200
150
100
50
0
0
5000
Compound 15
MeO
MeO
OMe
O
N
H
OMe

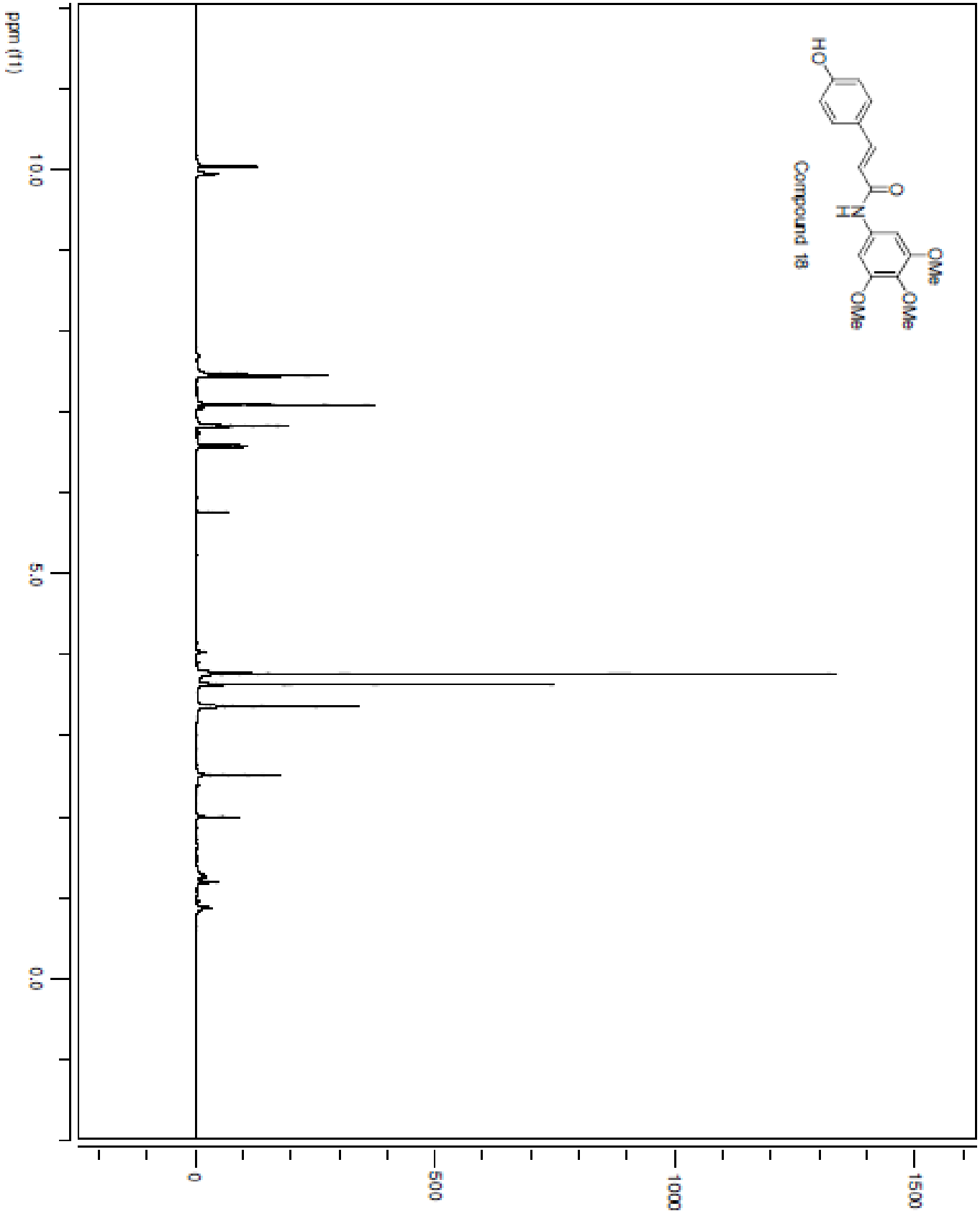

ppm (t1)
10.0
5.0
0.0
0
500
1000
1500
MeO
MeO
OMe
O
N
H
OMe
Compound 15

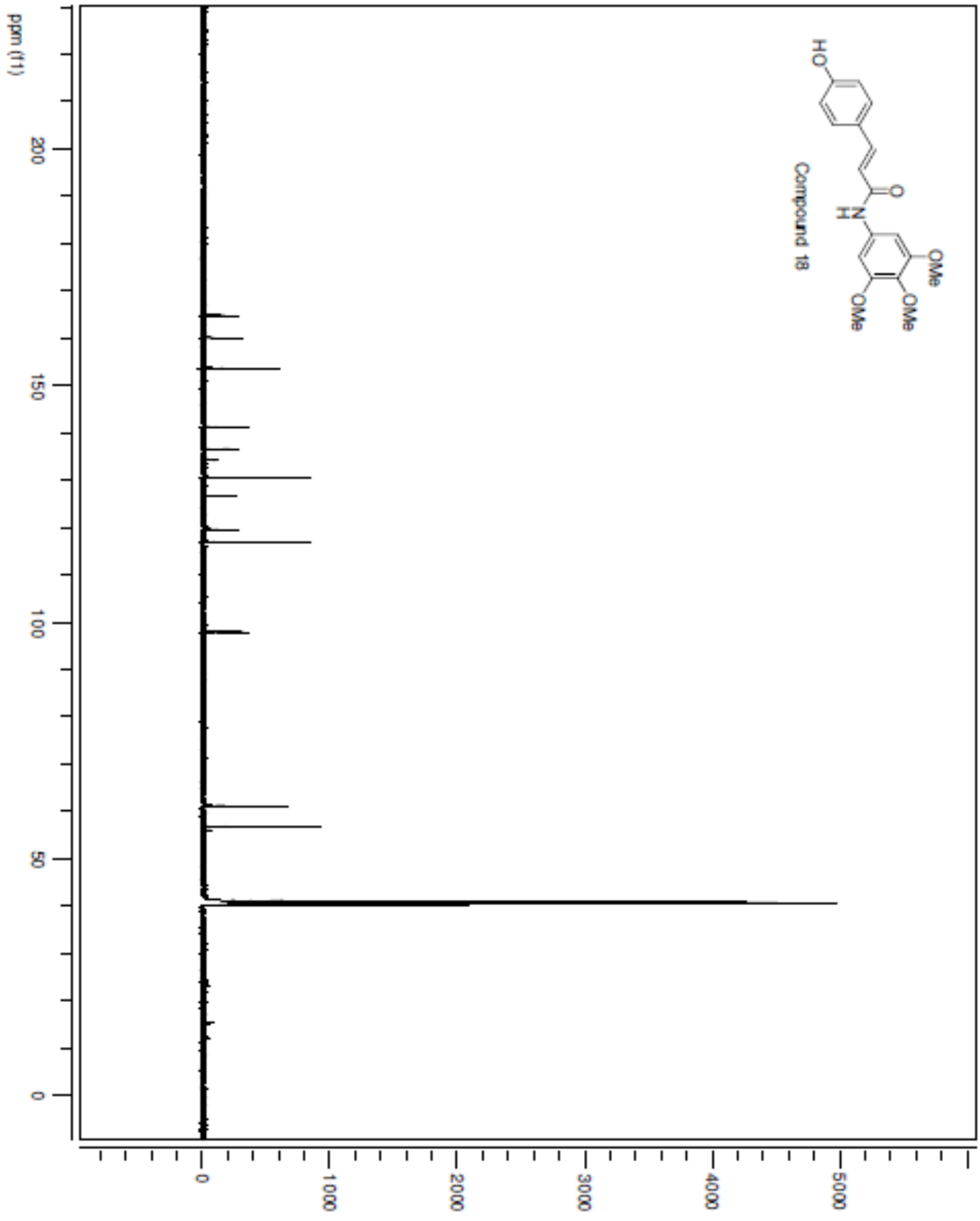

Compound 16
OMe
OMe
OMe
OMe
O
N
H
ppm (f1)
10.0
5.0
0.0
0
500
1000
1500
2000
2500
3000

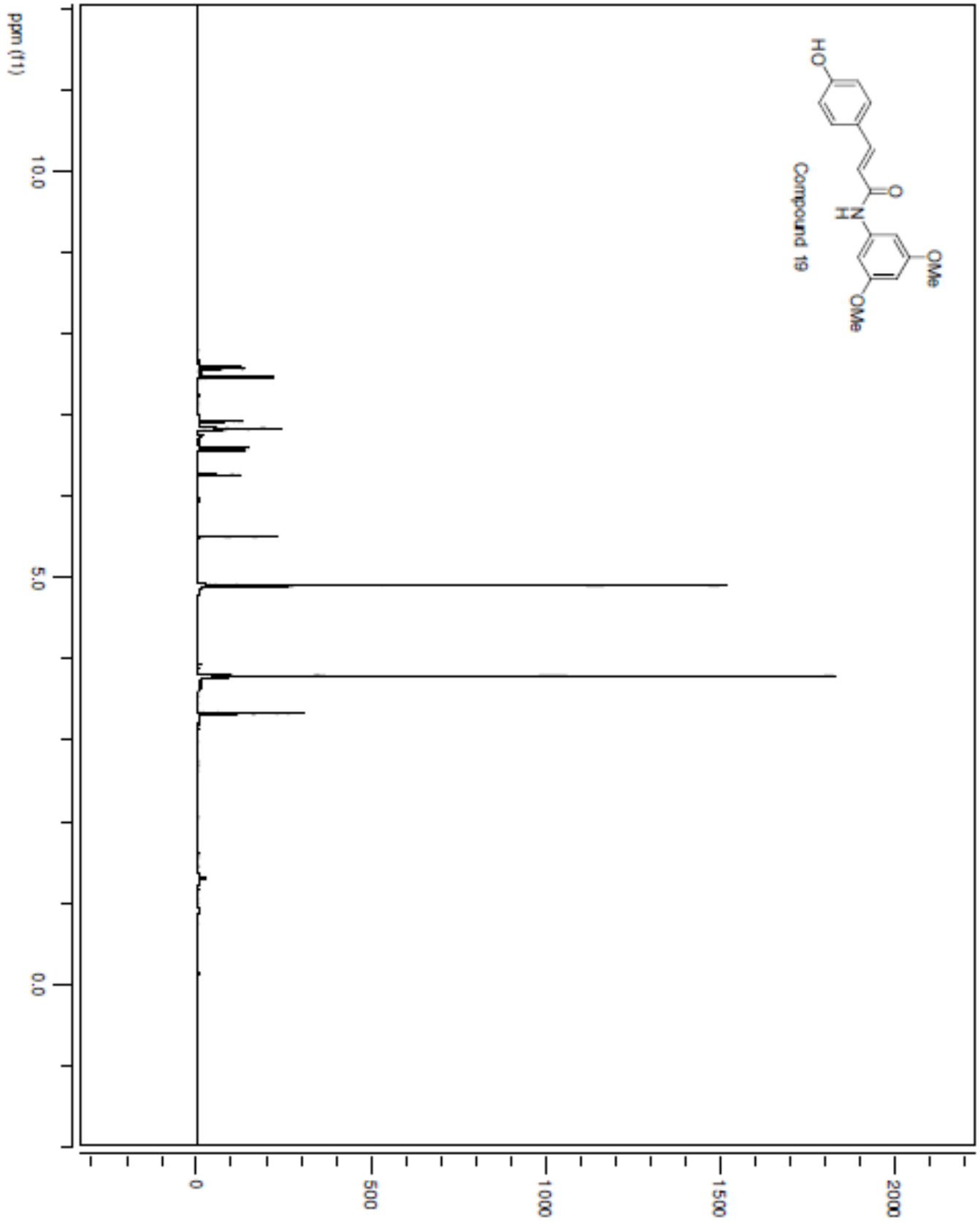

ppm (f1)
200
150
100
50
0
0
5000
10000
OMe
OMe
OMe
OMe
O
N
H
Compound 16

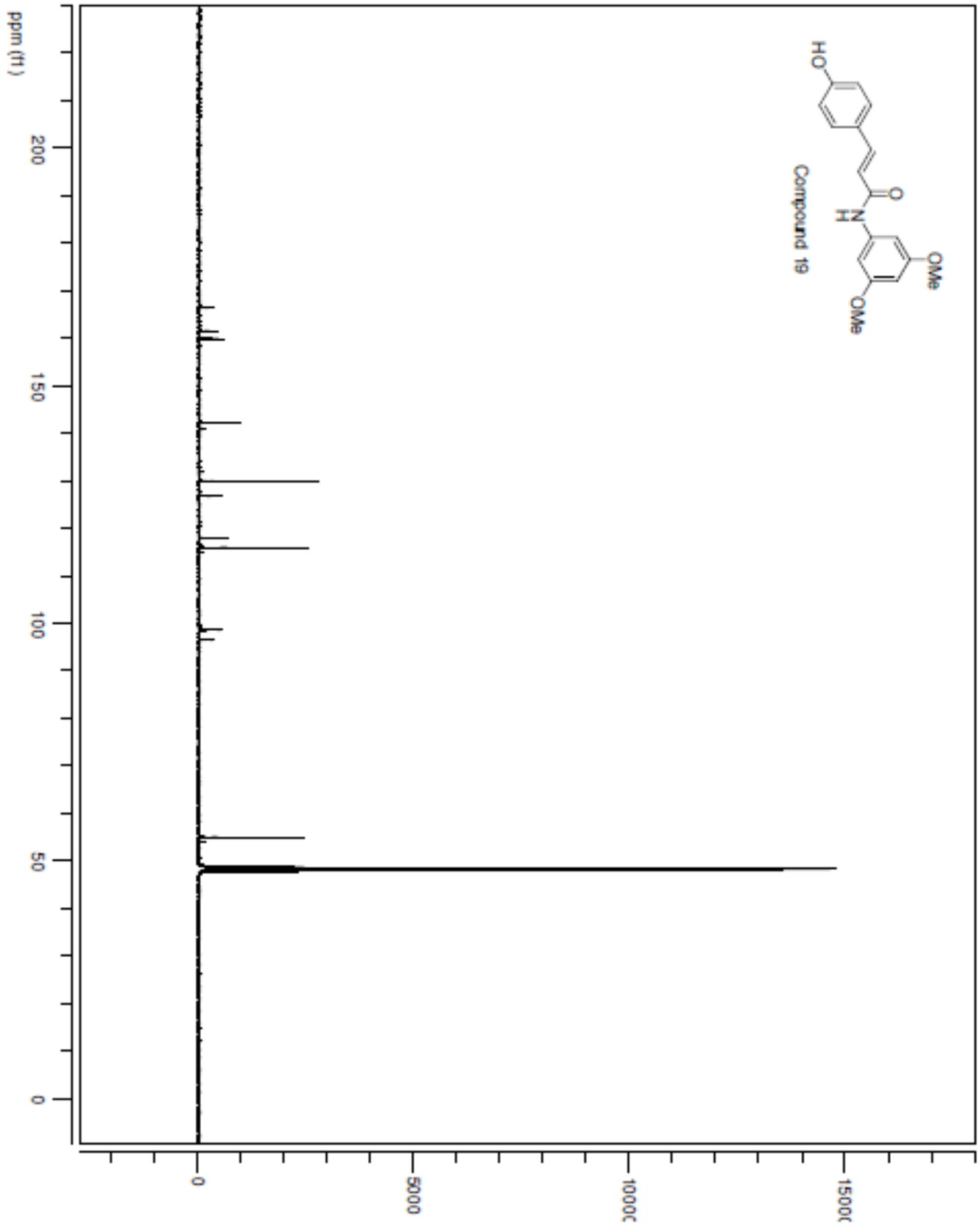

ppm (f1)
10.0
5.0
0.0
0
500
1000
OMe
O
N
H
OMe
Compound 17

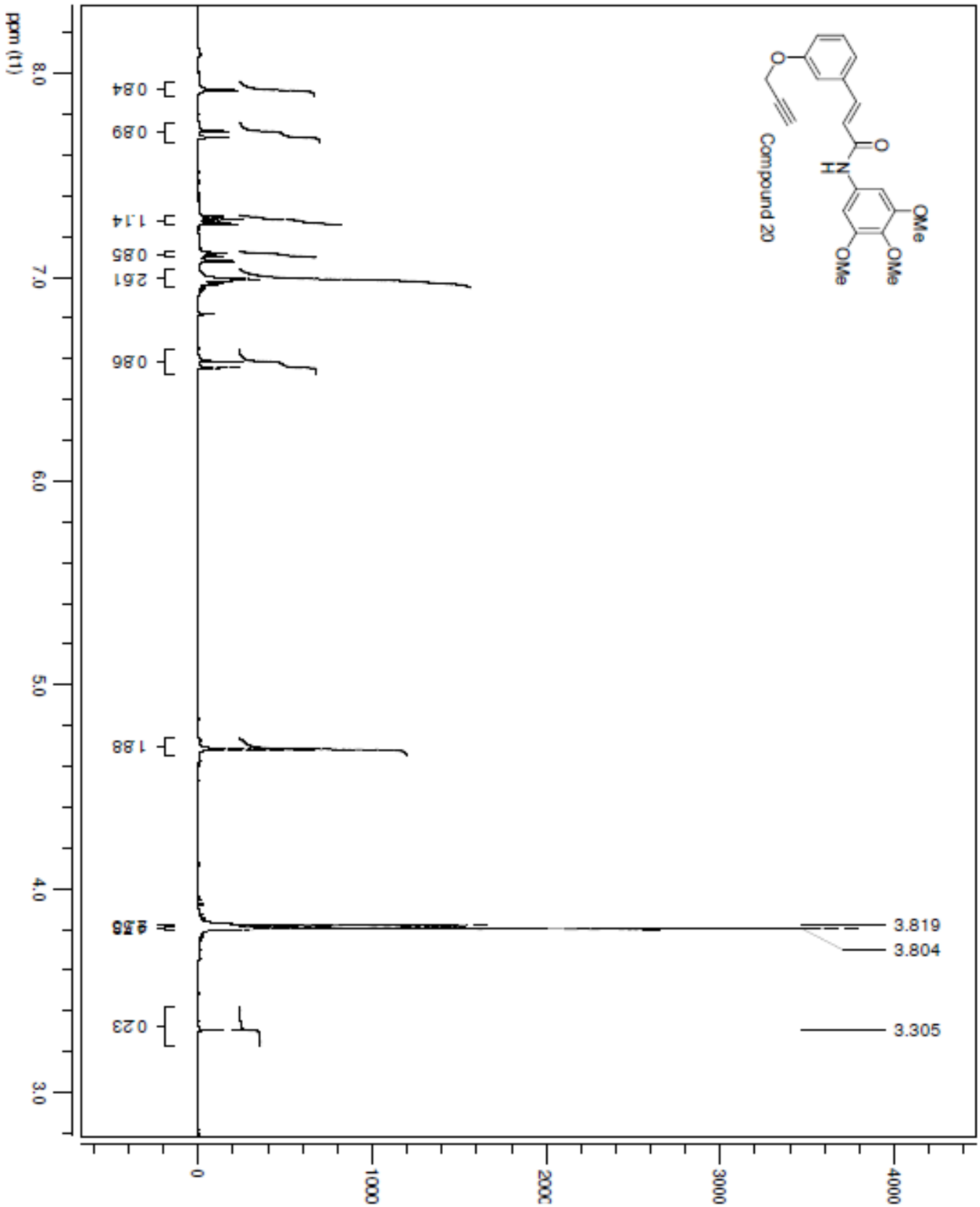

Compound 17
OMe
O
N
H
OMe
ppm (t1)
0
50
100
150
200
0
5000
1000
1500

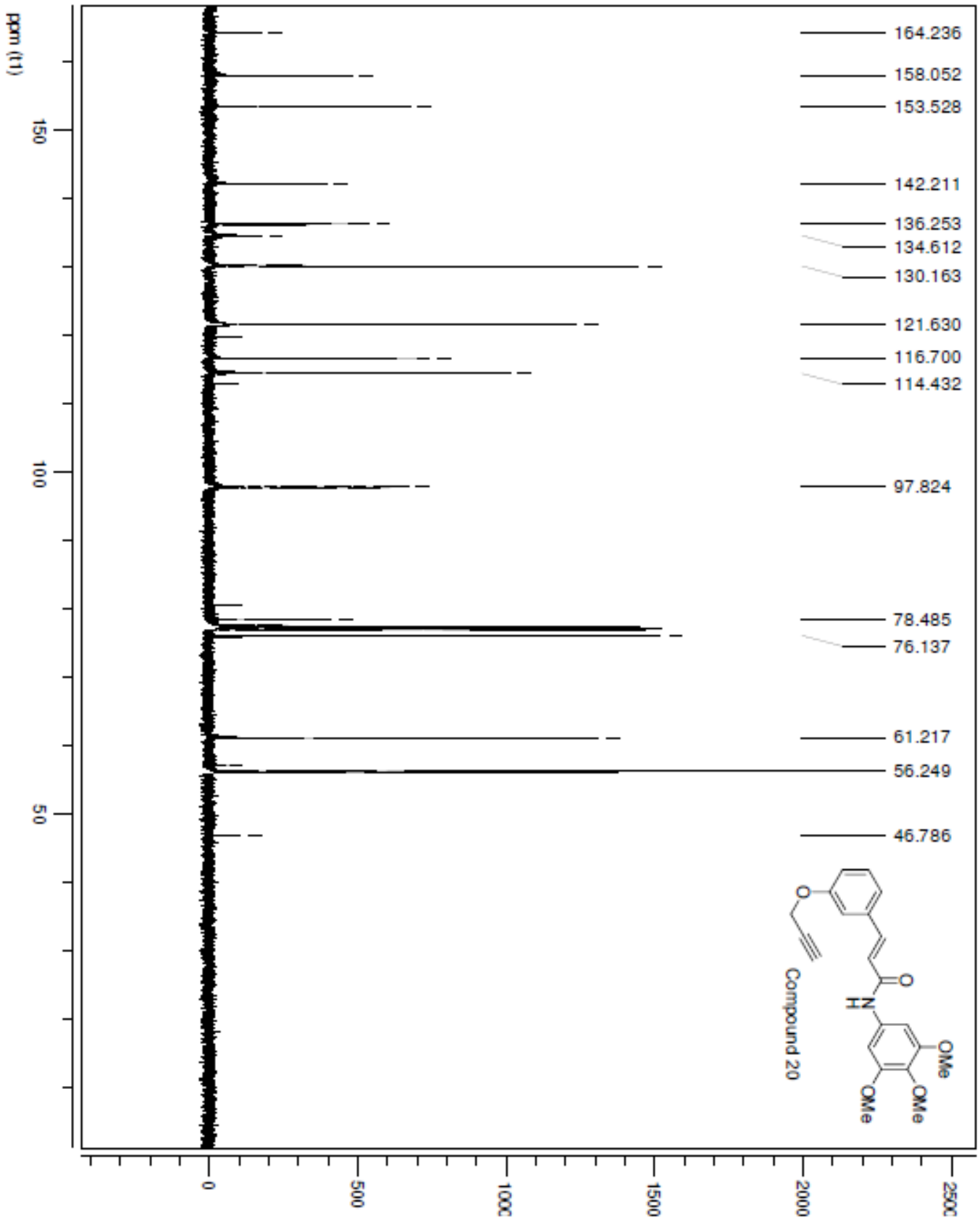

ppm (t1)
10.0
5.0
0.0
0
500
1000
1500
Compound 18
HO
O
N
H
OMe
OMe
OMe

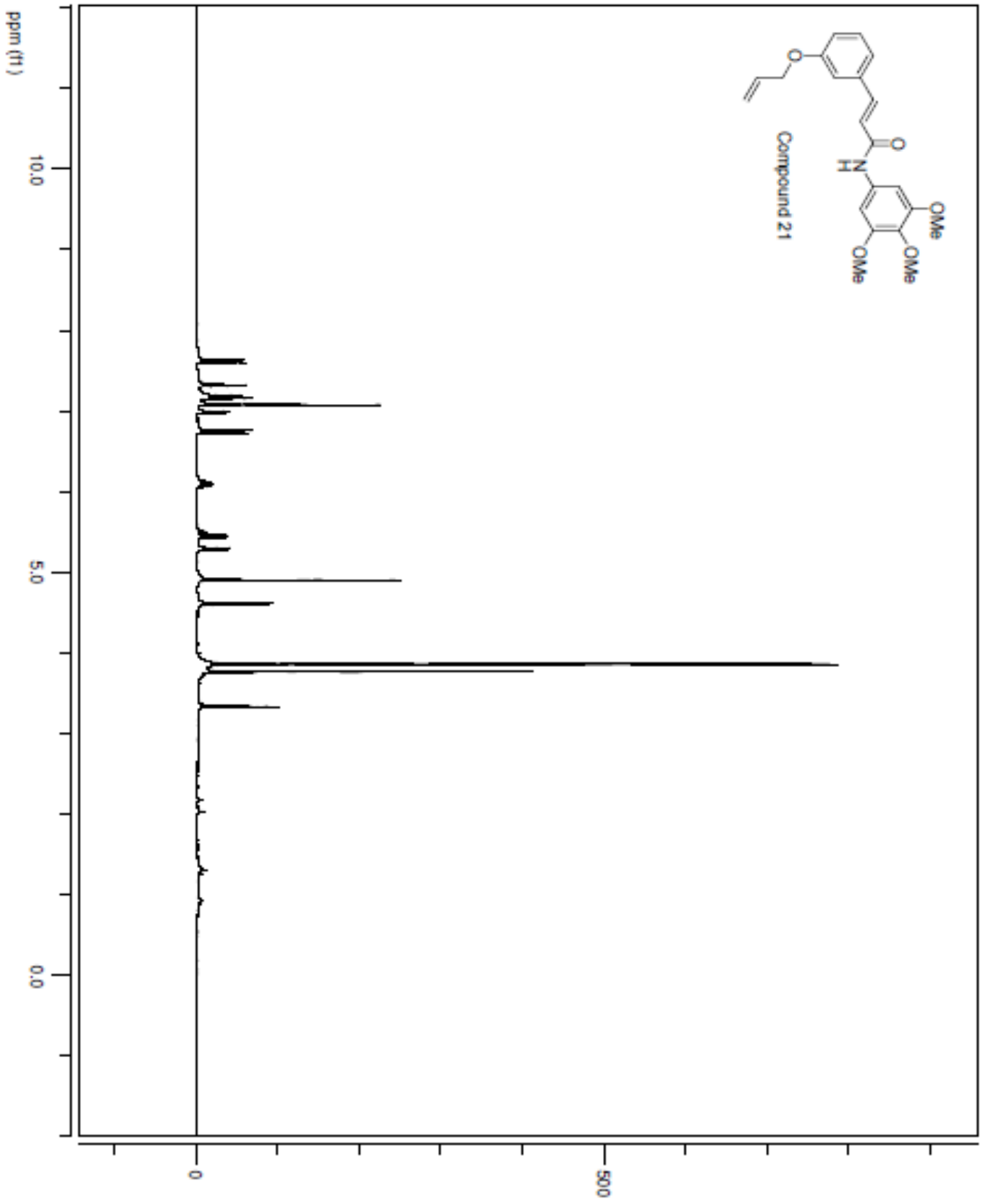

ppm (f1)
200
150
100
50
0
0
1000
2000
3000
4000
5000
HO
O
N
H
OMe
OMe
OMe
Compound 18

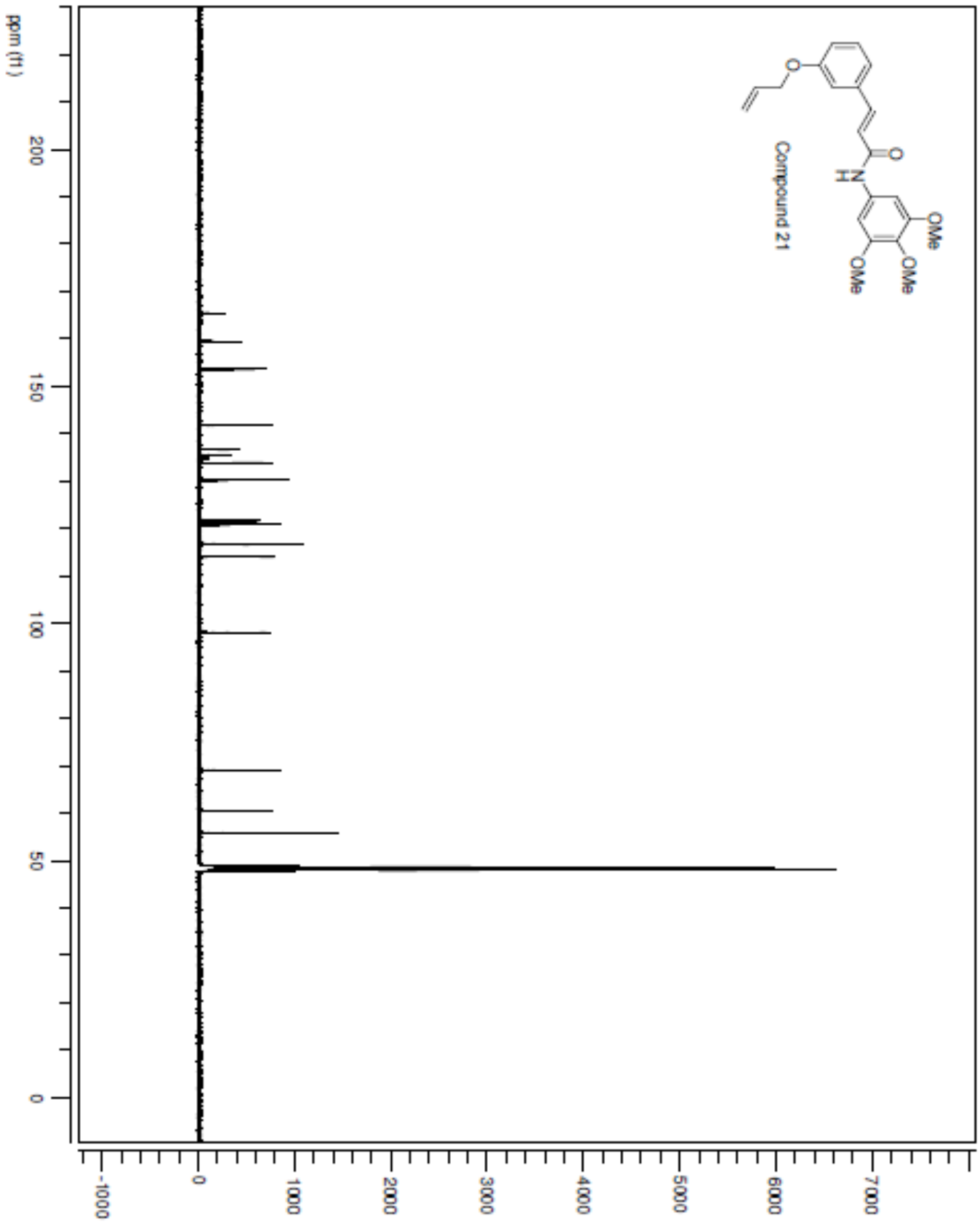

ppm (f1)
10.0
5.0
0.0
0
500
1000
1500
2000
HO
O
N
H
OMe
OMe
Compound 19

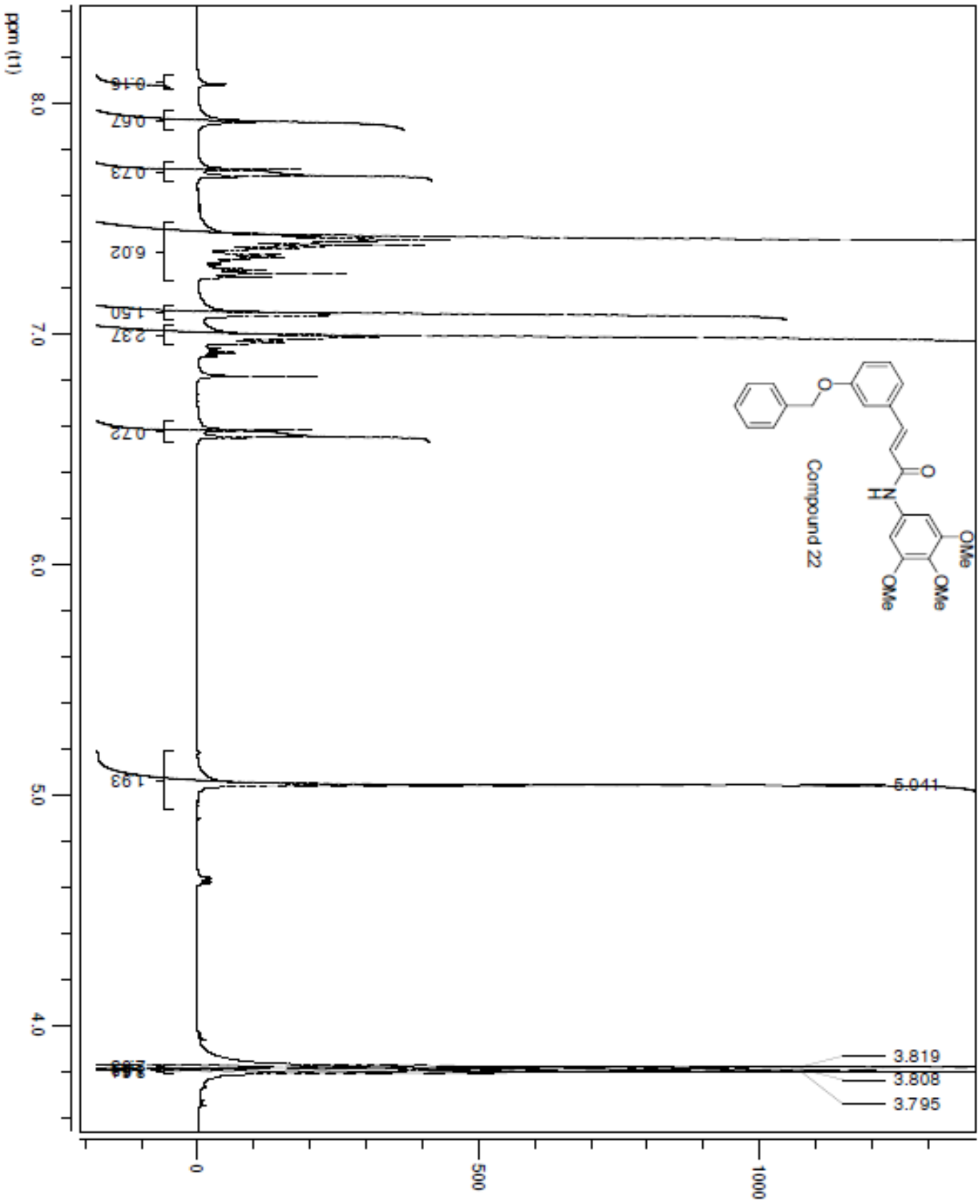

ppm (f1)
200
150
100
50
0
0
5000
10000
15000
HO
O
N
H
OMe
OMe
Compound 19

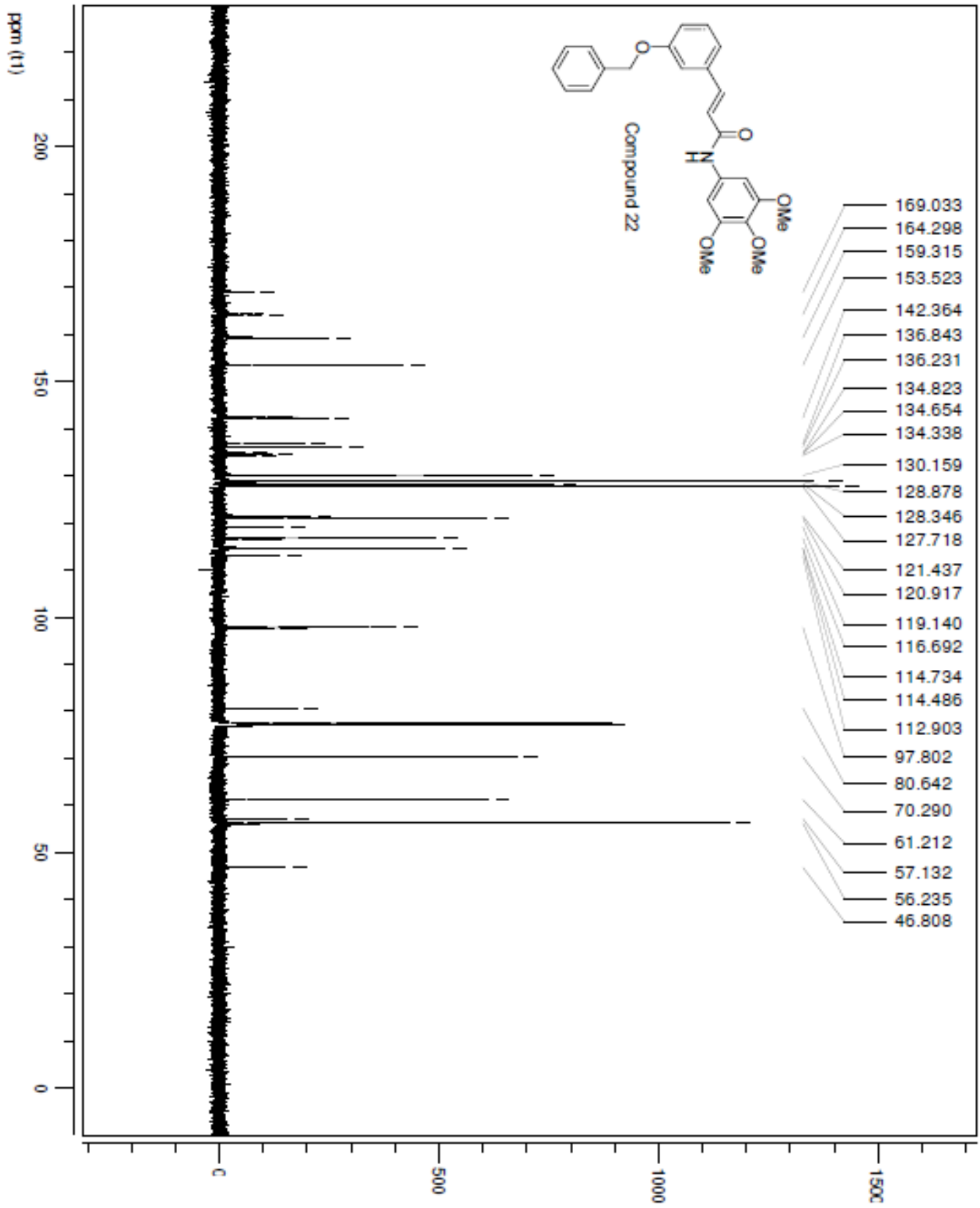

ppm (t1)
8.0
7.0
6.0
5.0
4.0
3.0
0
1000
2000
3000
4000
0.84
0.89
1.14
0.85
2.61
0.86
1.88
6.35
0.23
3.819
3.804
3.305
Compound 20
O
N
H
OMe
OMe
OMe

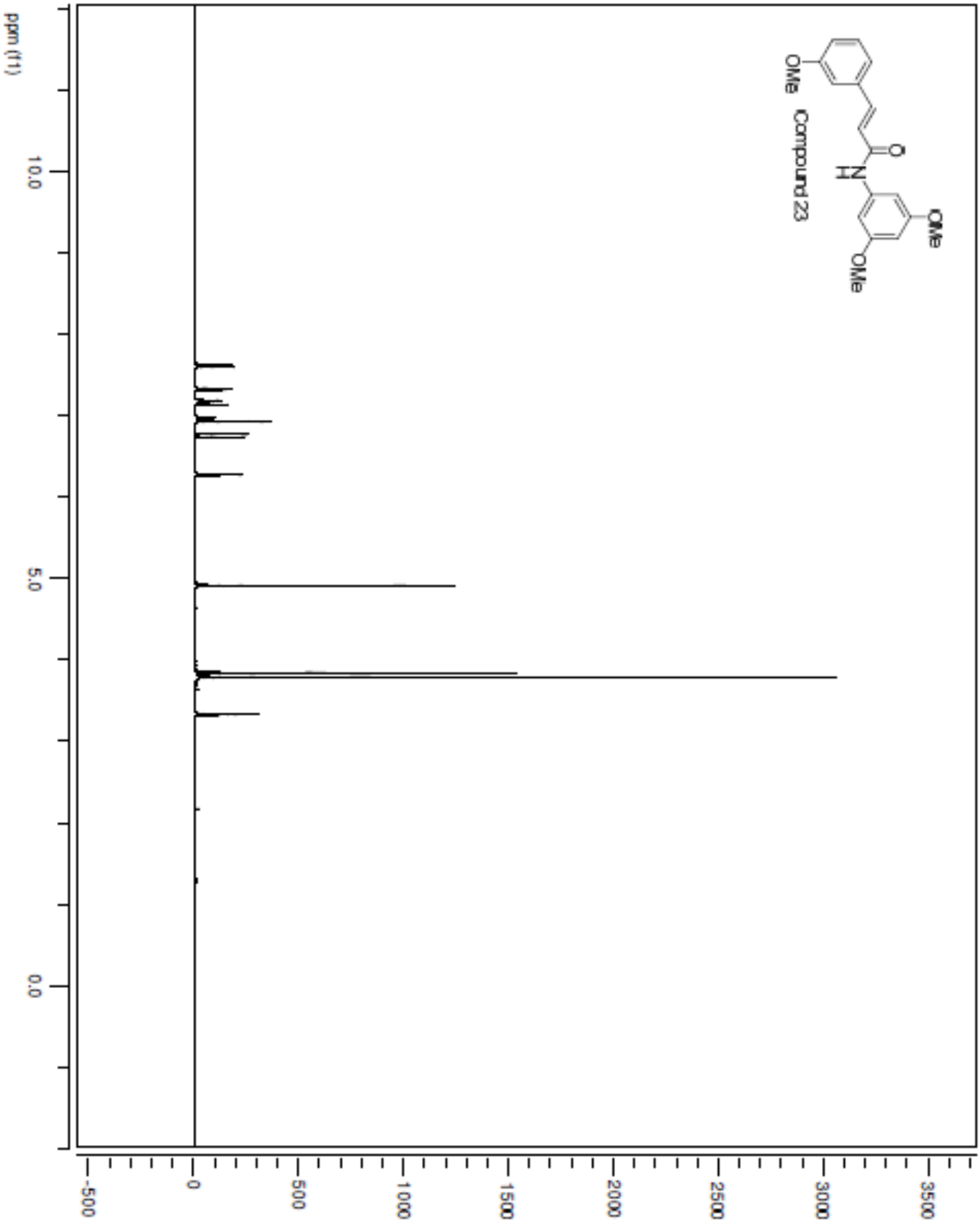

ppm (t1)
150
100
50
0
500
1000
1500
2000
2500
164.236
158.052
153.528
142.211
136.253
134.612
130.163
121.630
116.700
114.432
97.824
78.485
76.137
61.217
56.249
46.786
Compound 20
O
O
N
H
OMe
OMe
OMe

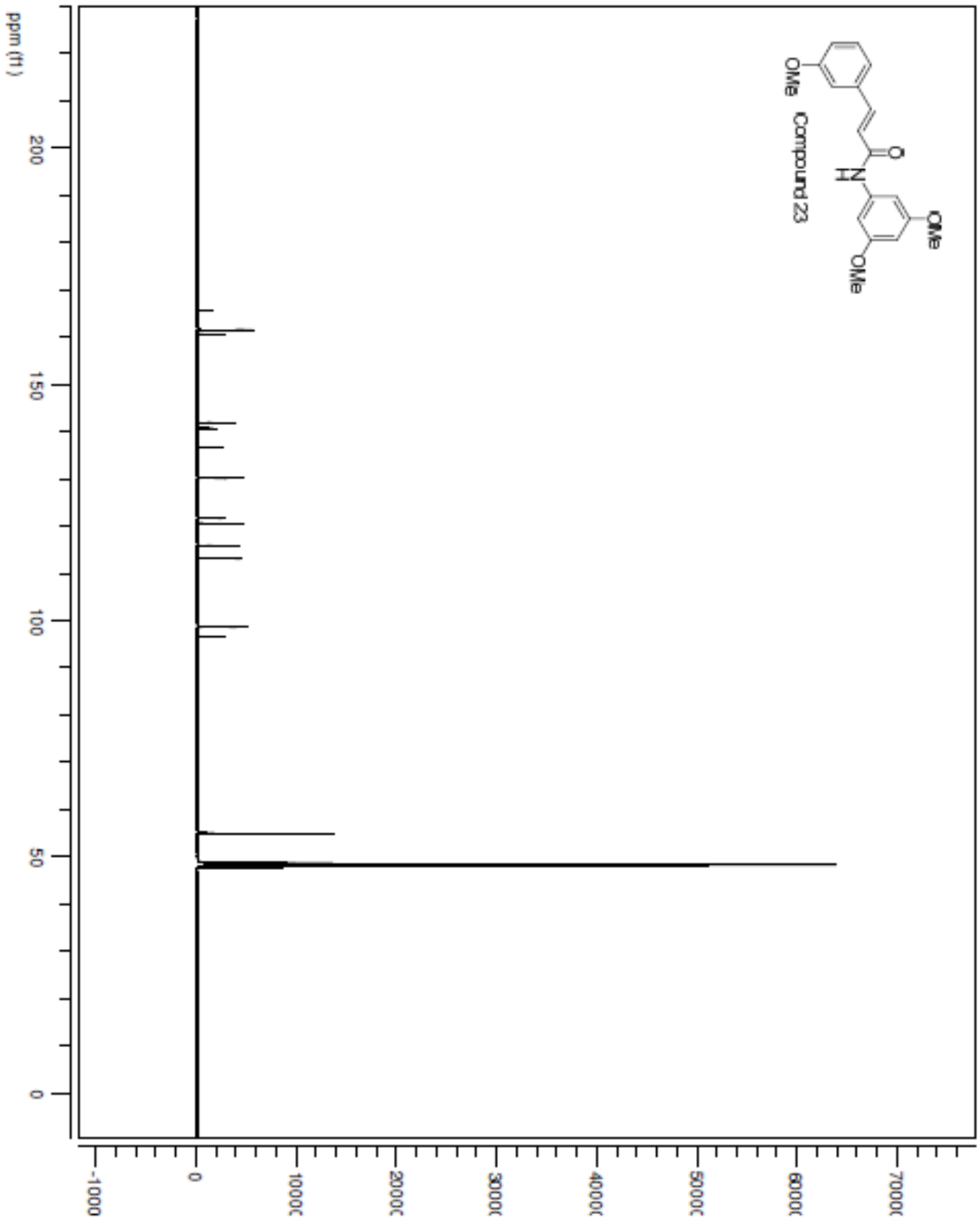

ppm (f1)
10.0
5.0
0.0
0
500
Compound 21
O
N
H
OMe
OMe
OMe

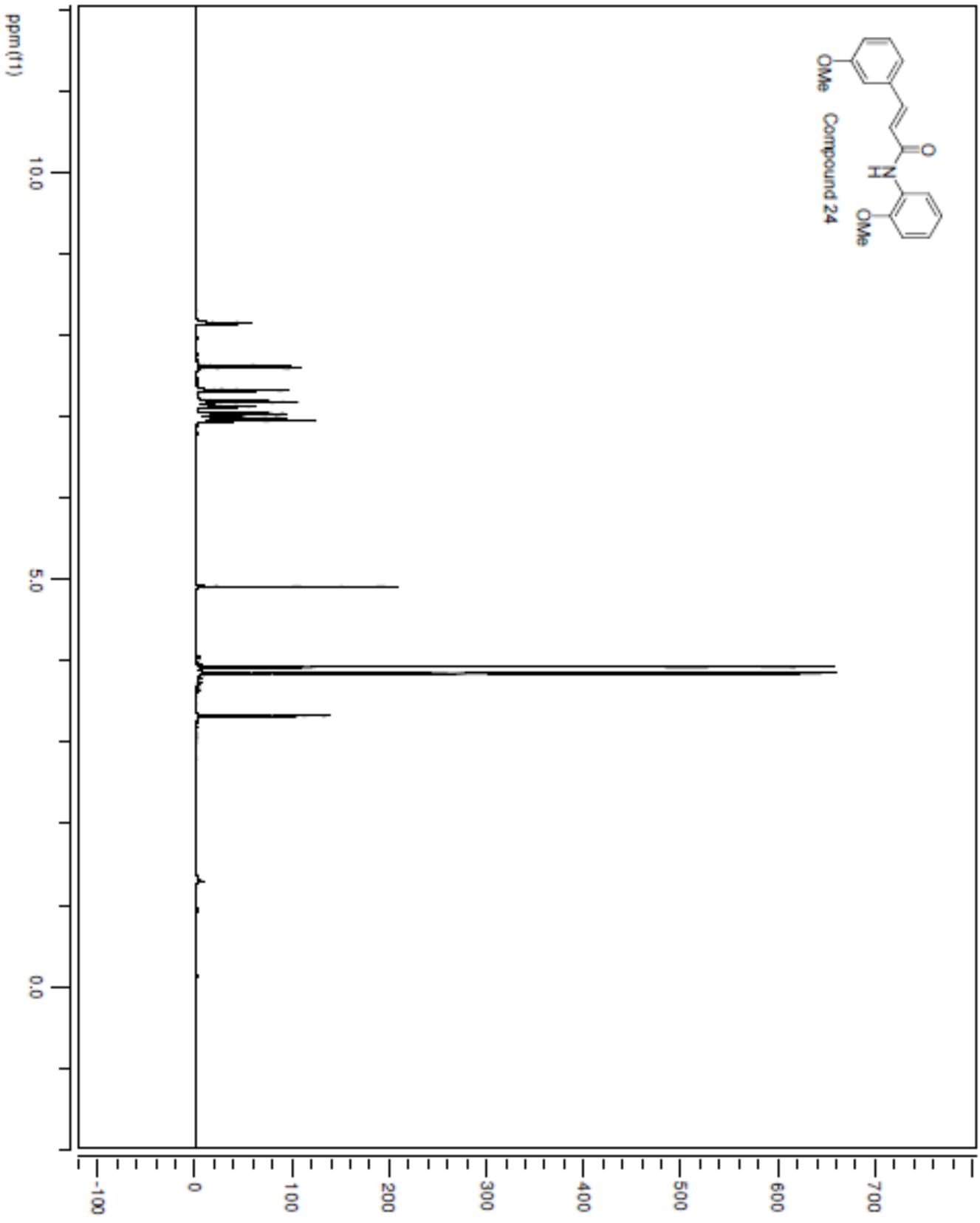

Compound 21
OMe
OMe
OMe
O
N
H
O
ppm (f1)
200
150
100
50
0
-1000
0
1000
2000
3000
4000
5000
6000
7000

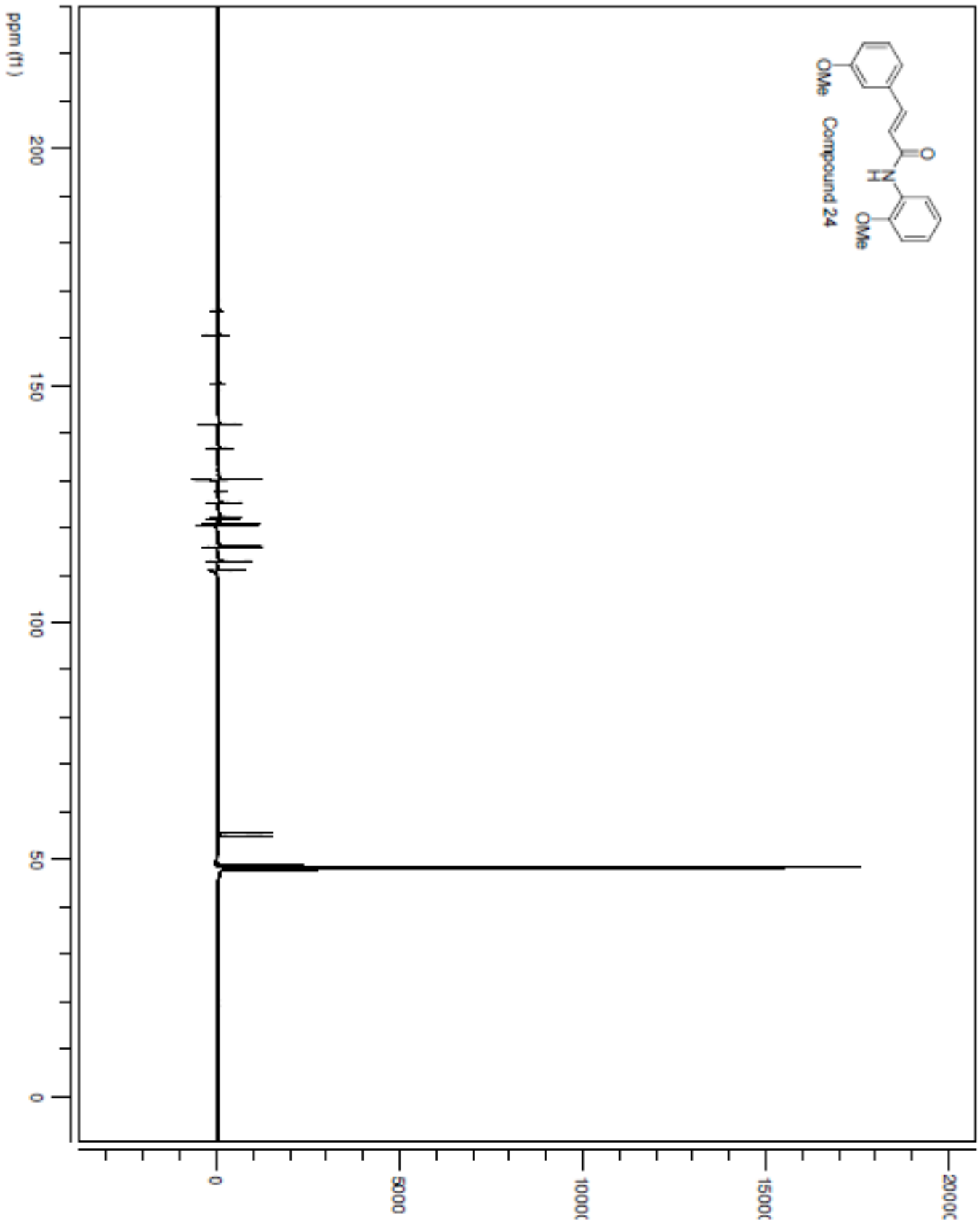

ppm (t1)
8.0
7.0
6.0
5.0
4.0
0.16
0.67
0.73
6.02
1.50
2.37
0.72
1.93
5.041
3.819
3.808
3.795
0
500
1000
Compound 22
O
N
H
OMe
OMe
OMe

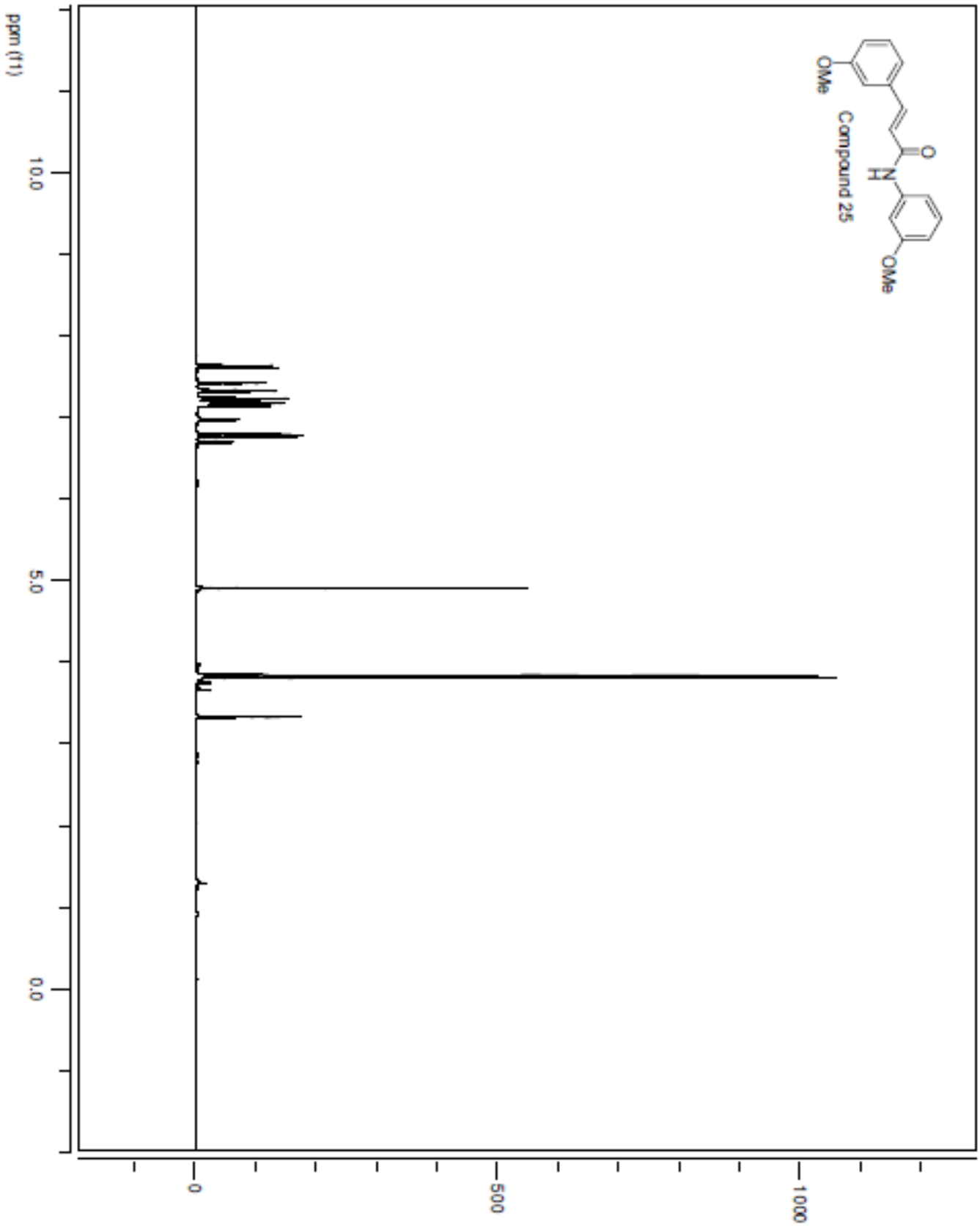

ppm (t1)
200
150
100
50
0
0
500
1000
1500
Compound 22
O
O
N
H
OMe
OMe
OMe
169.033
164.298
159.315
153.523
142.364
136.843
136.231
134.823
134.654
134.338
130.159
128.878
128.346
127.718
121.437
120.917
119.140
116.692
114.734
114.486
112.903
97.802
80.642
70.290
61.212
57.132
56.235
46.808

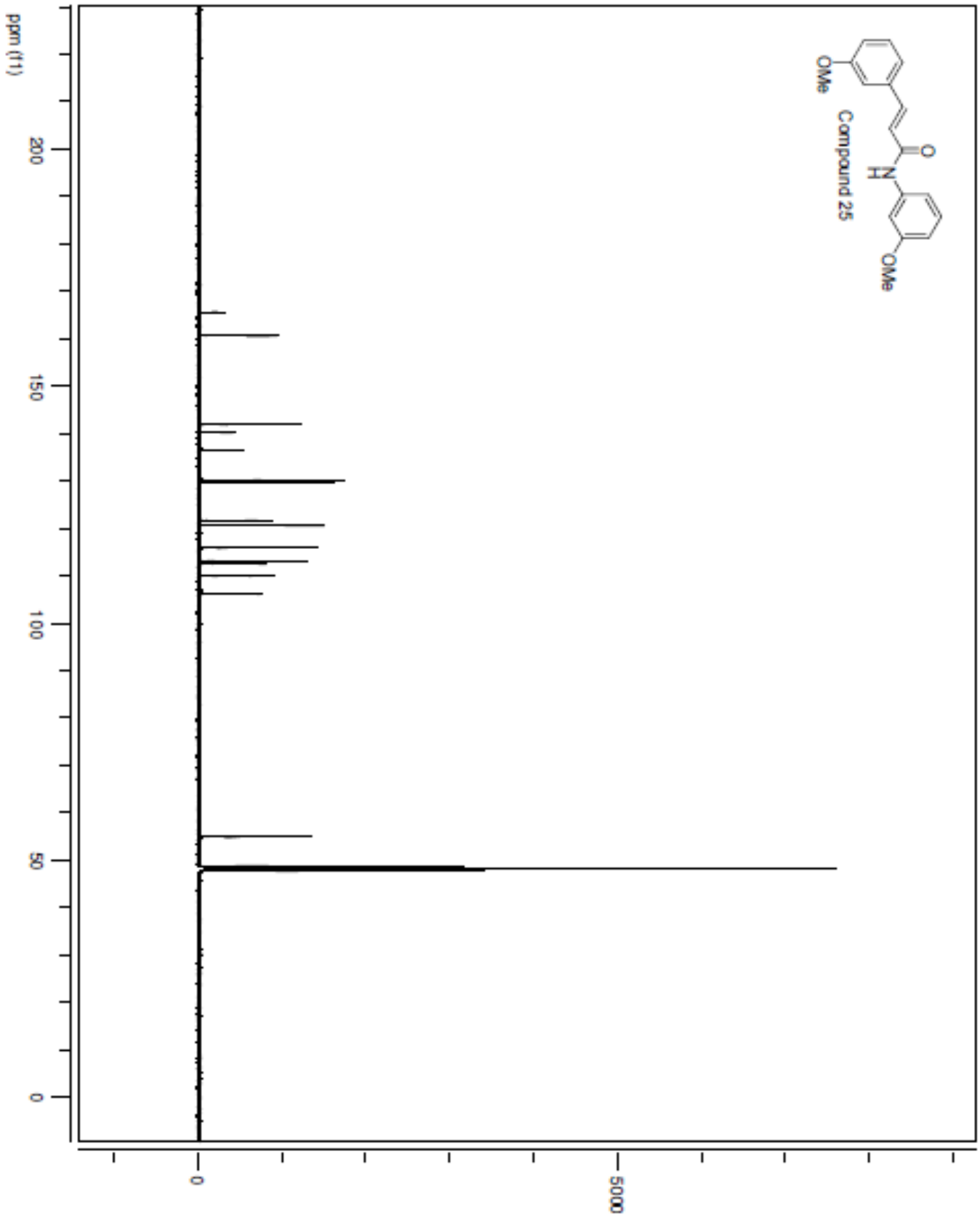

Compound 23
OMe
OMe
OMe
O
N
H
ppm (f1)
10.0
5.0
0.0
-500
0
500
1000
1500
2000
2500
3000
3500

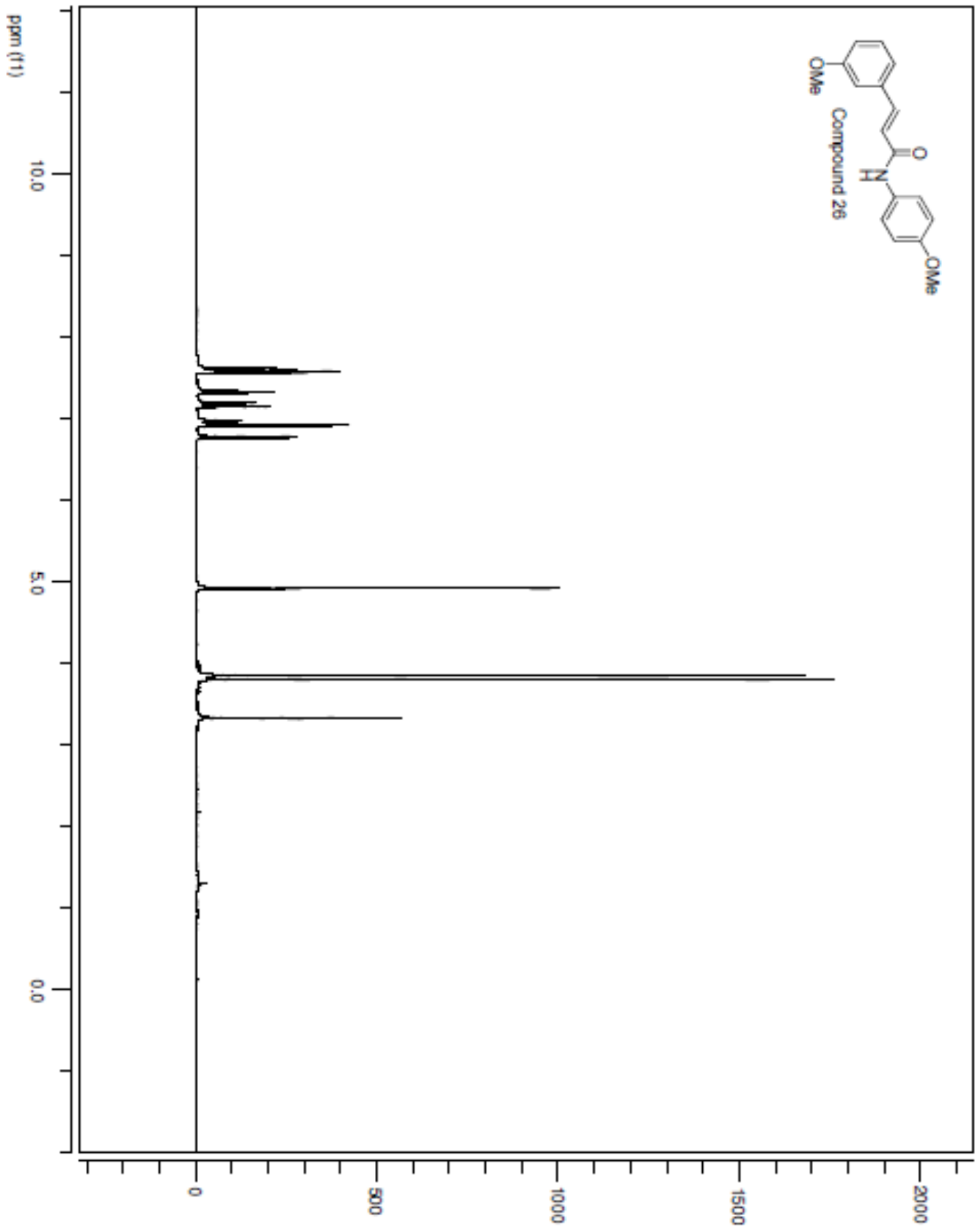

Compound 23
OMe
OMe
OMe
O
N
H
ppm (f1)
200
150
100
50
0
-1000
0
1000
2000
3000
4000
5000
6000
7000

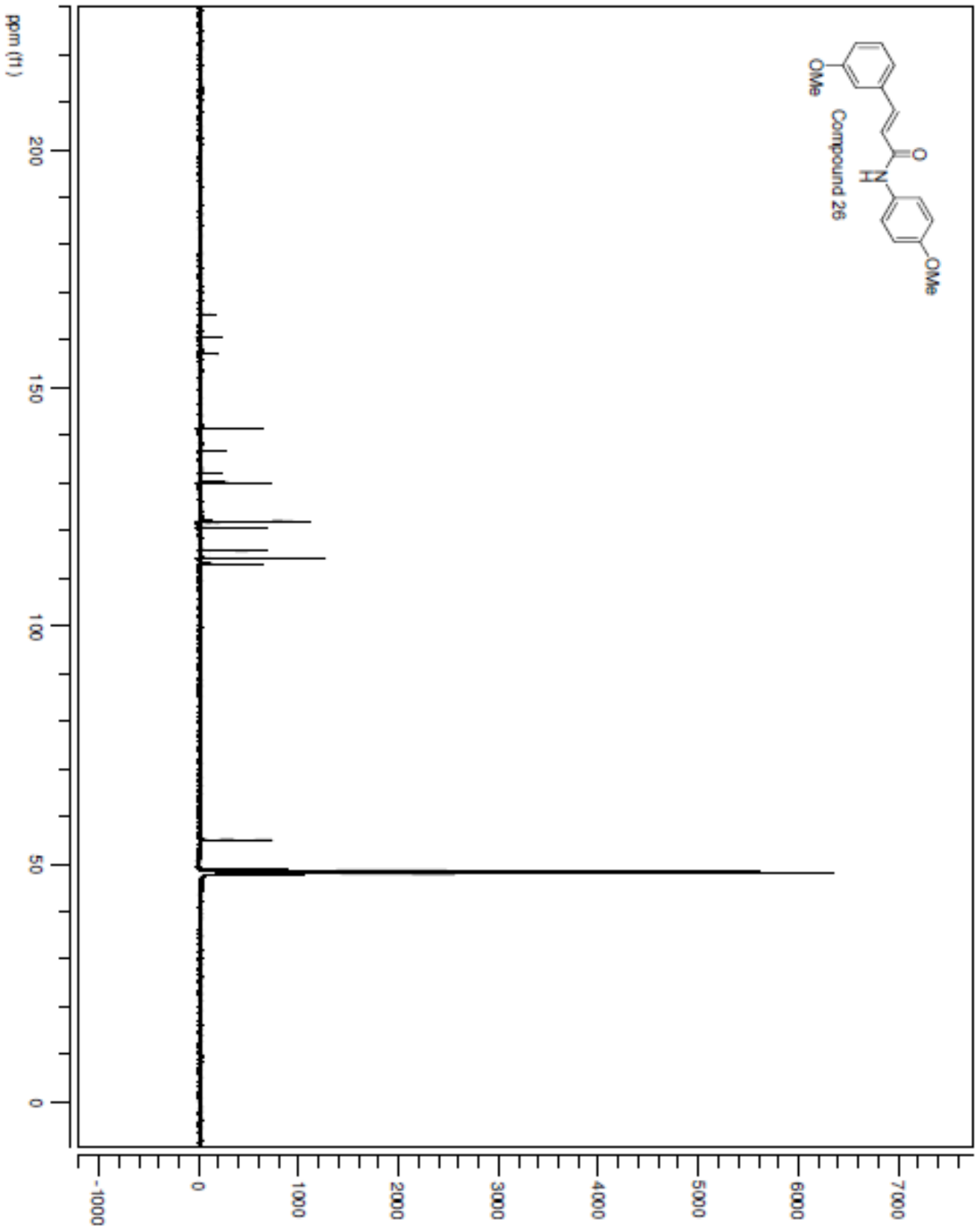

OMe
Compound 24
OMe
O
N
H
ppm (f1)
10.0
5.0
0.0
-100
0
100
200
300
400
500
600
700

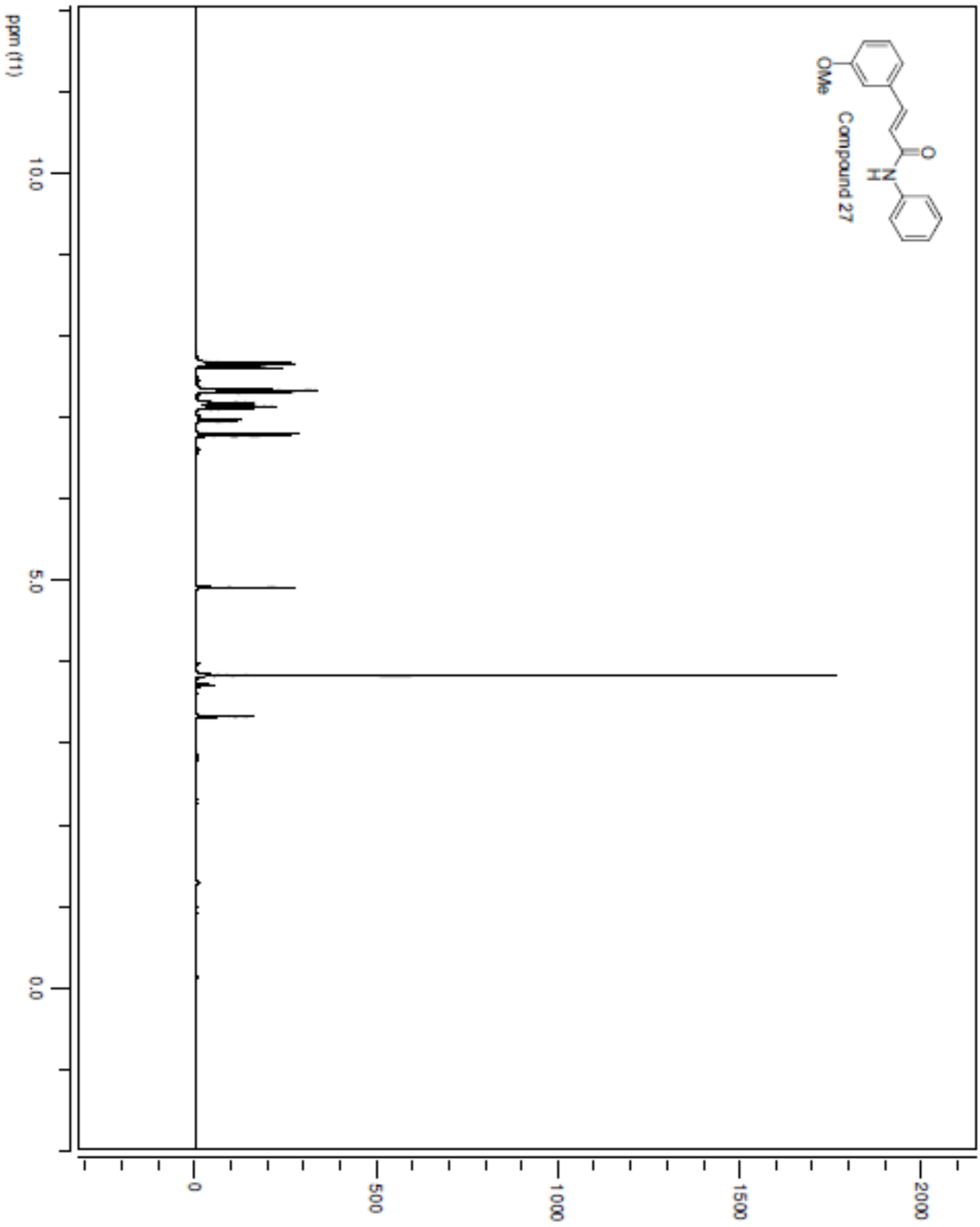

Compound 24
OMe
OMe
O
N
H
ppm (f1)
200
150
100
50
0
0
5000
10000
15000
20000

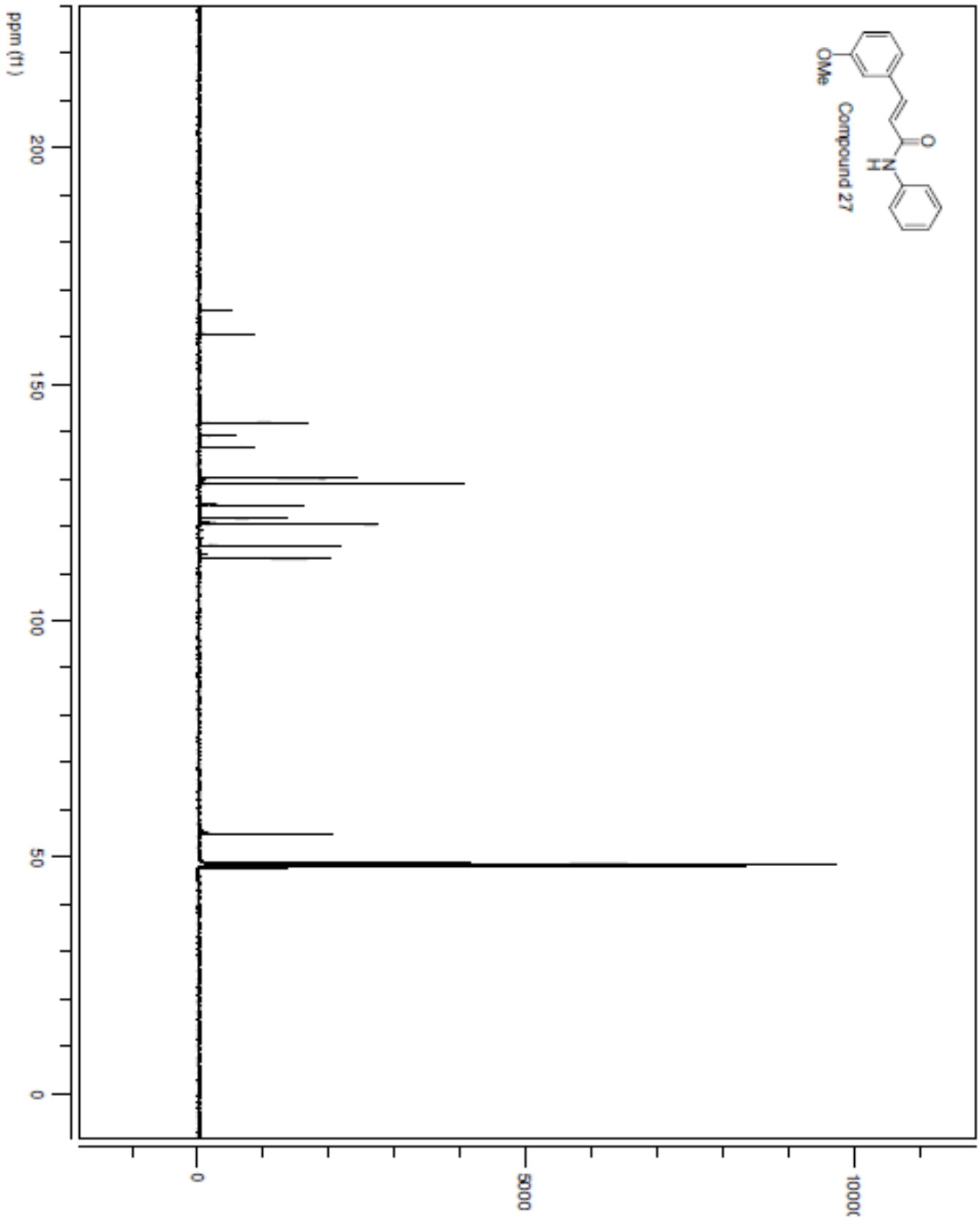

Compound 25
OMe
OMe
O
N
H
ppm (t1)
10.0
5.0
0.0
0
500
1000

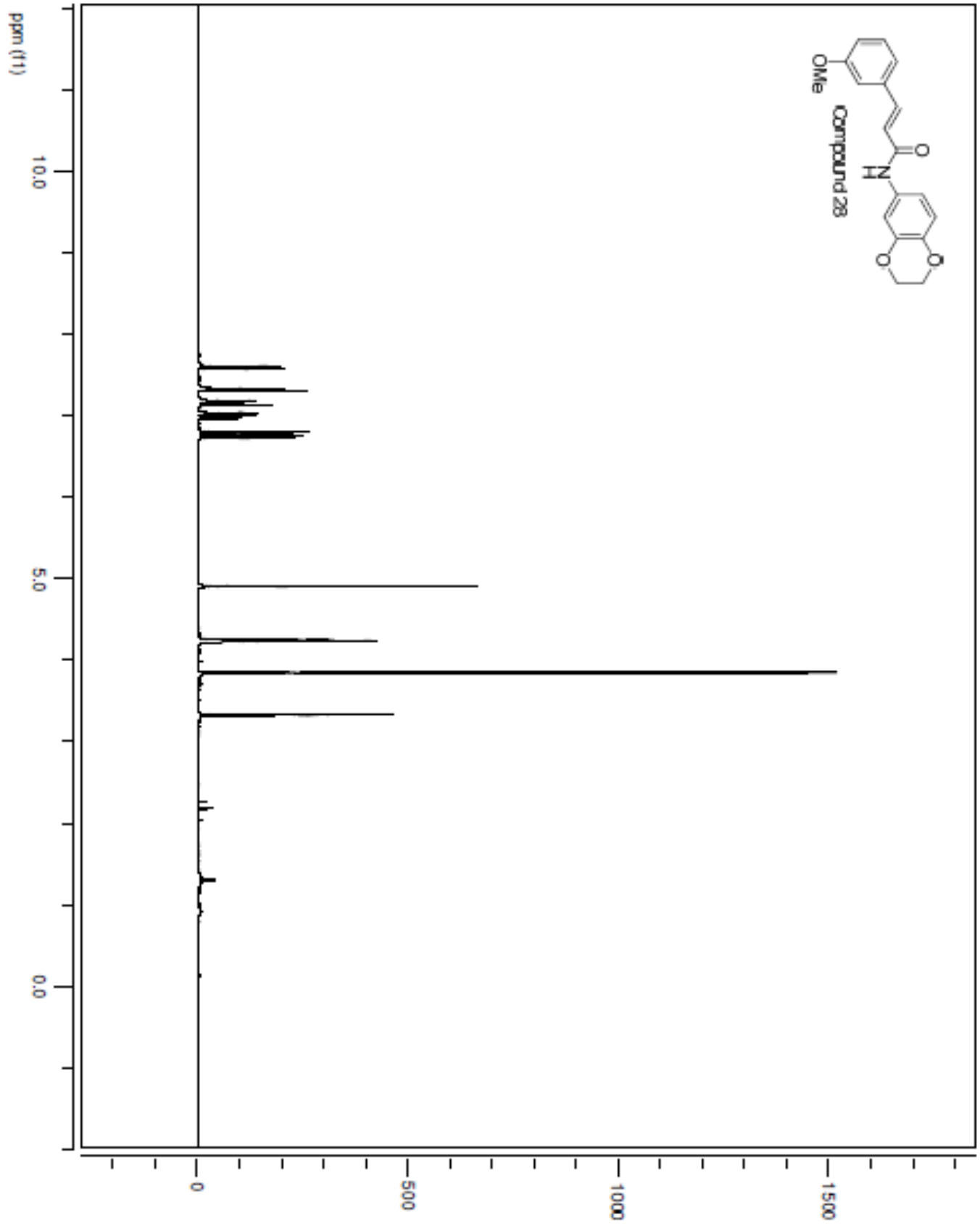

Compound 25
OMe
OMe
O
N
H
ppm (f1)
200
150
100
50
0
0
5000

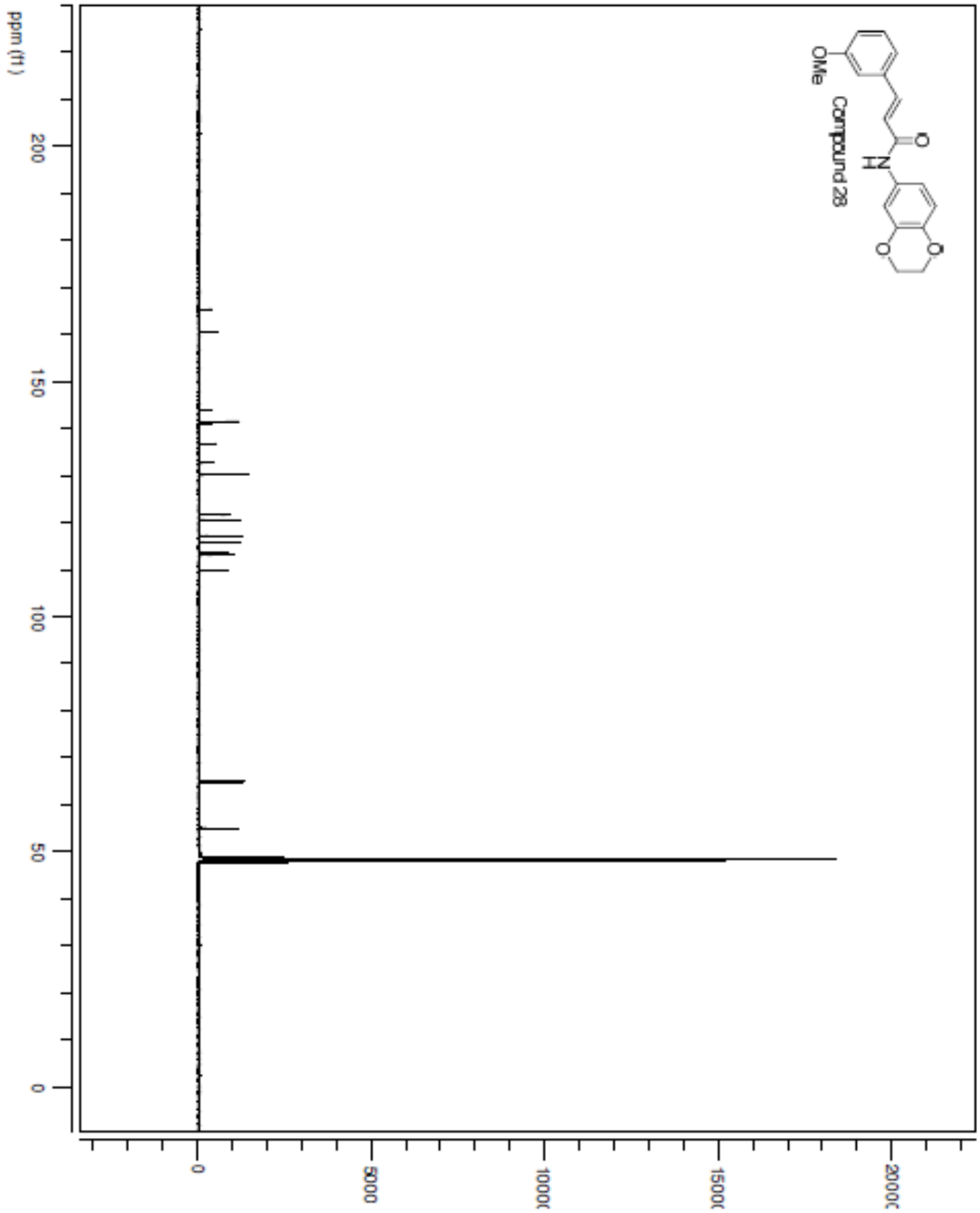

Compound 26
OMe
OMe
O
N
H
ppm (f1)
10.0
5.0
0.0
0
500
1000
1500
2000

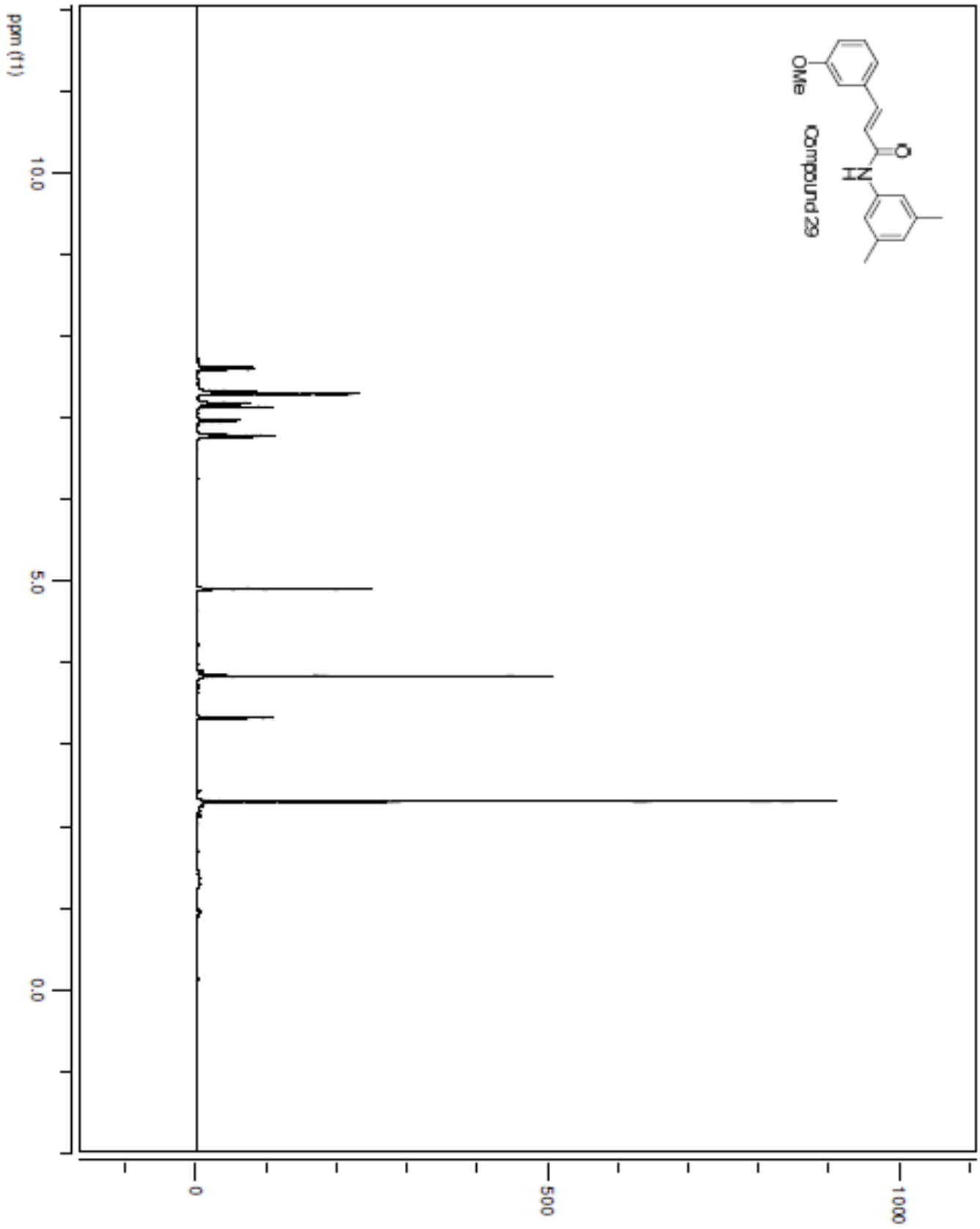

Compound 26
OMe
OMe
O
N
H
ppm (f1)
0
50
100
150
200
-1000
0
1000
2000
3000
4000
5000
6000
7000

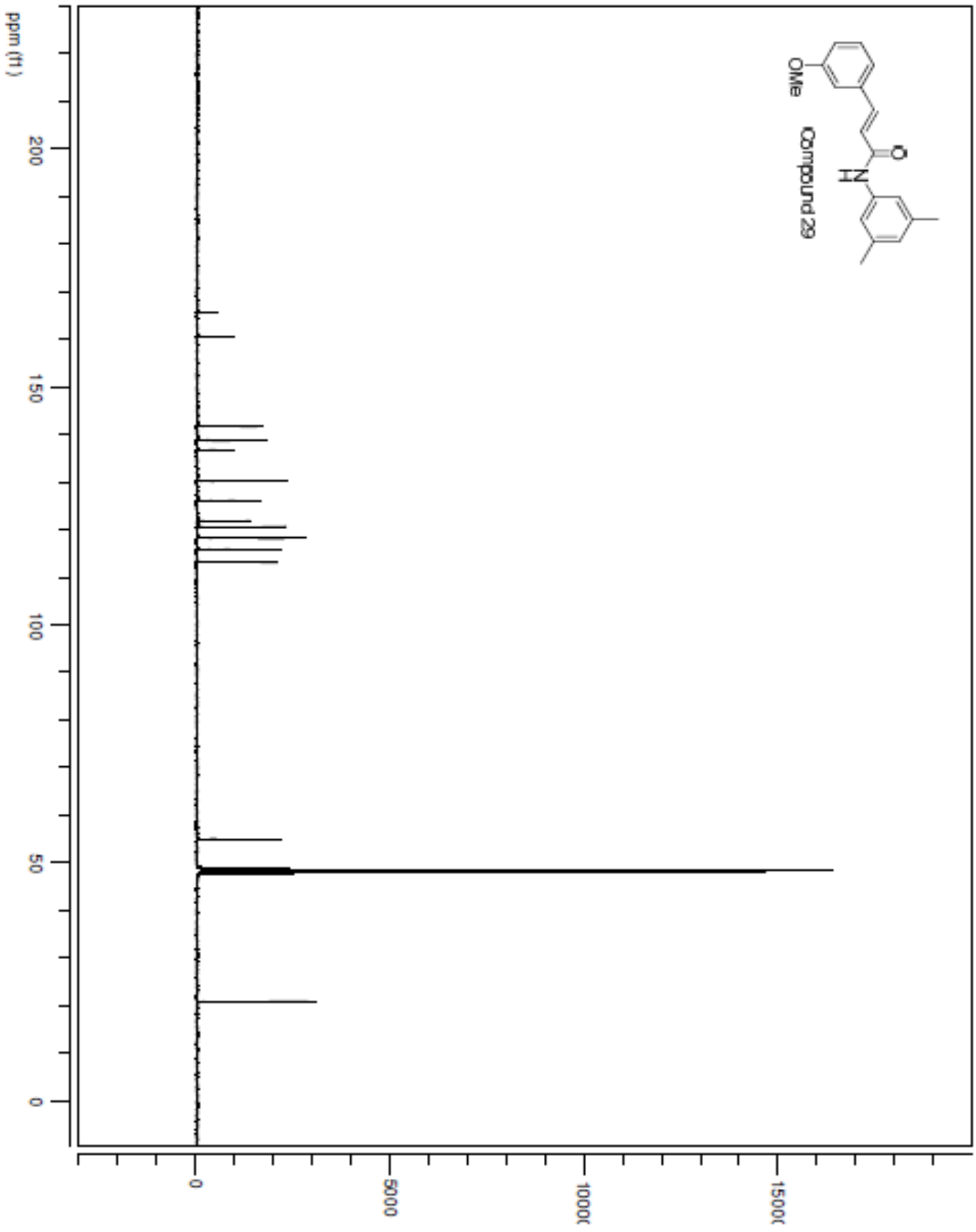

Compound 27
OMe
O
N
H
ppm (f1)
10.0
5.0
0.0
0
500
1000
1500
2000

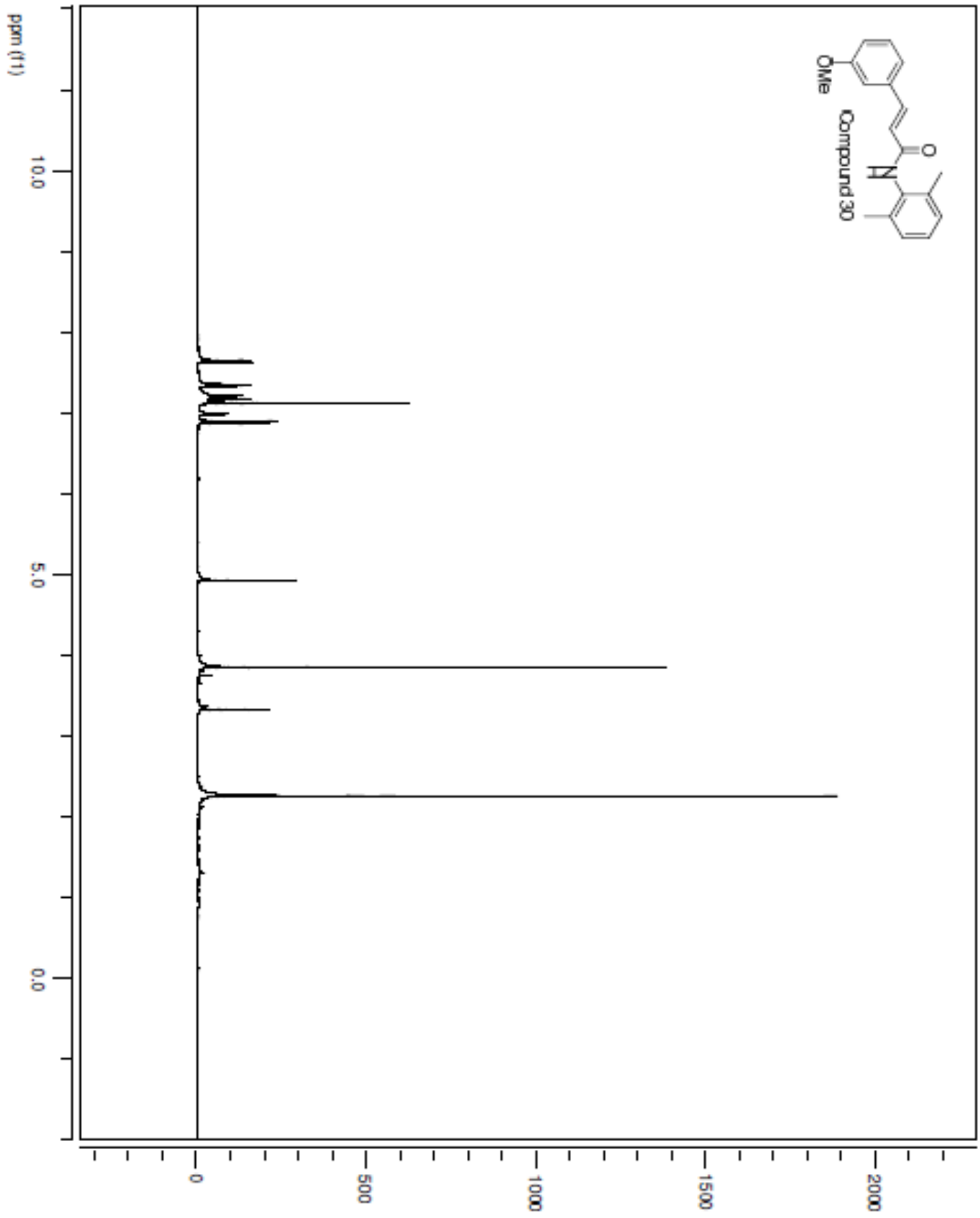

ppm (f1)
200
150
100
50
0
0
5000
1000
OMe
Compound 27
O
N
H

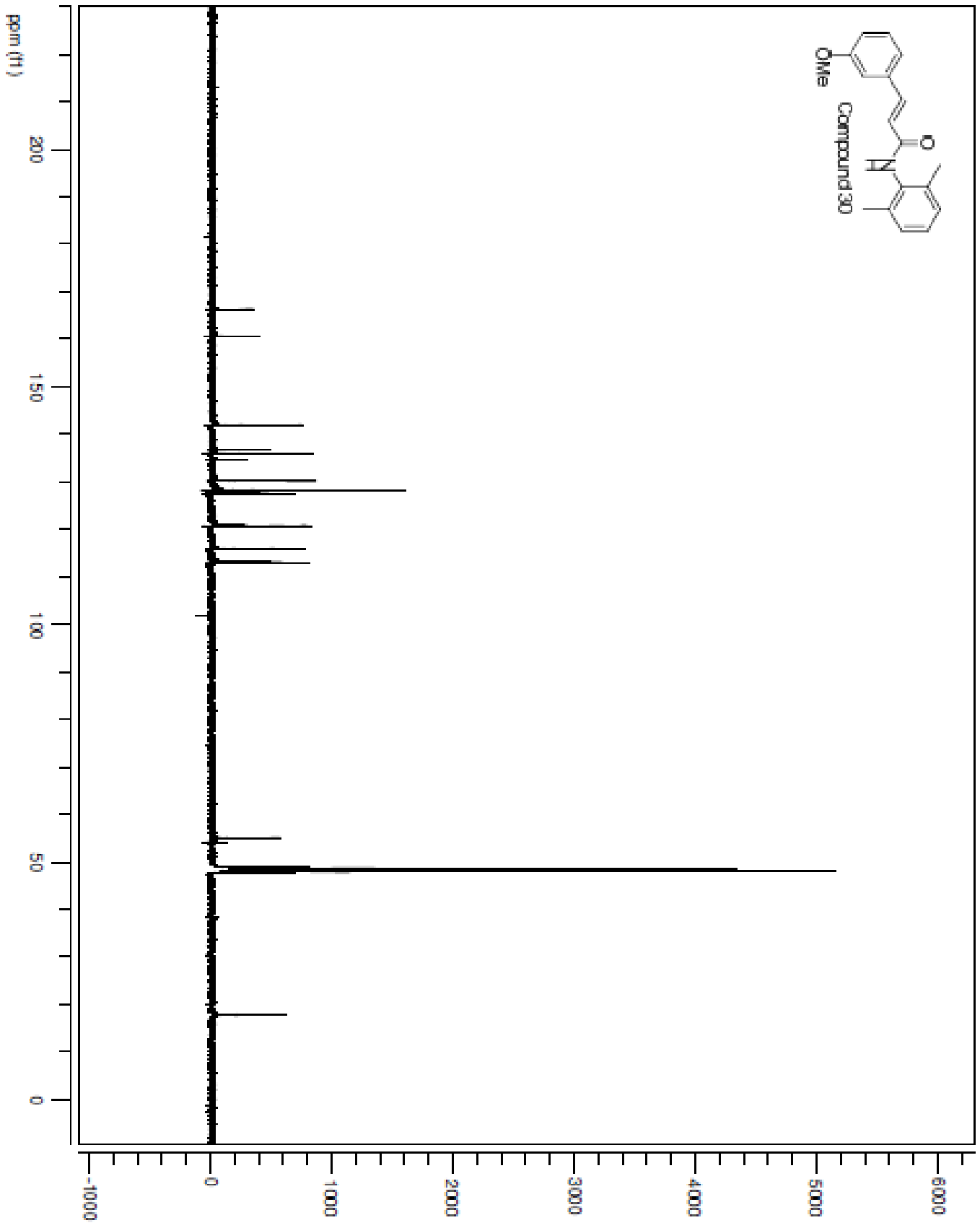

Compound 28
OMe
O
N
H
O
O
O
ppm (t1)
10.0
5.0
0.0
0
500
1000
1500

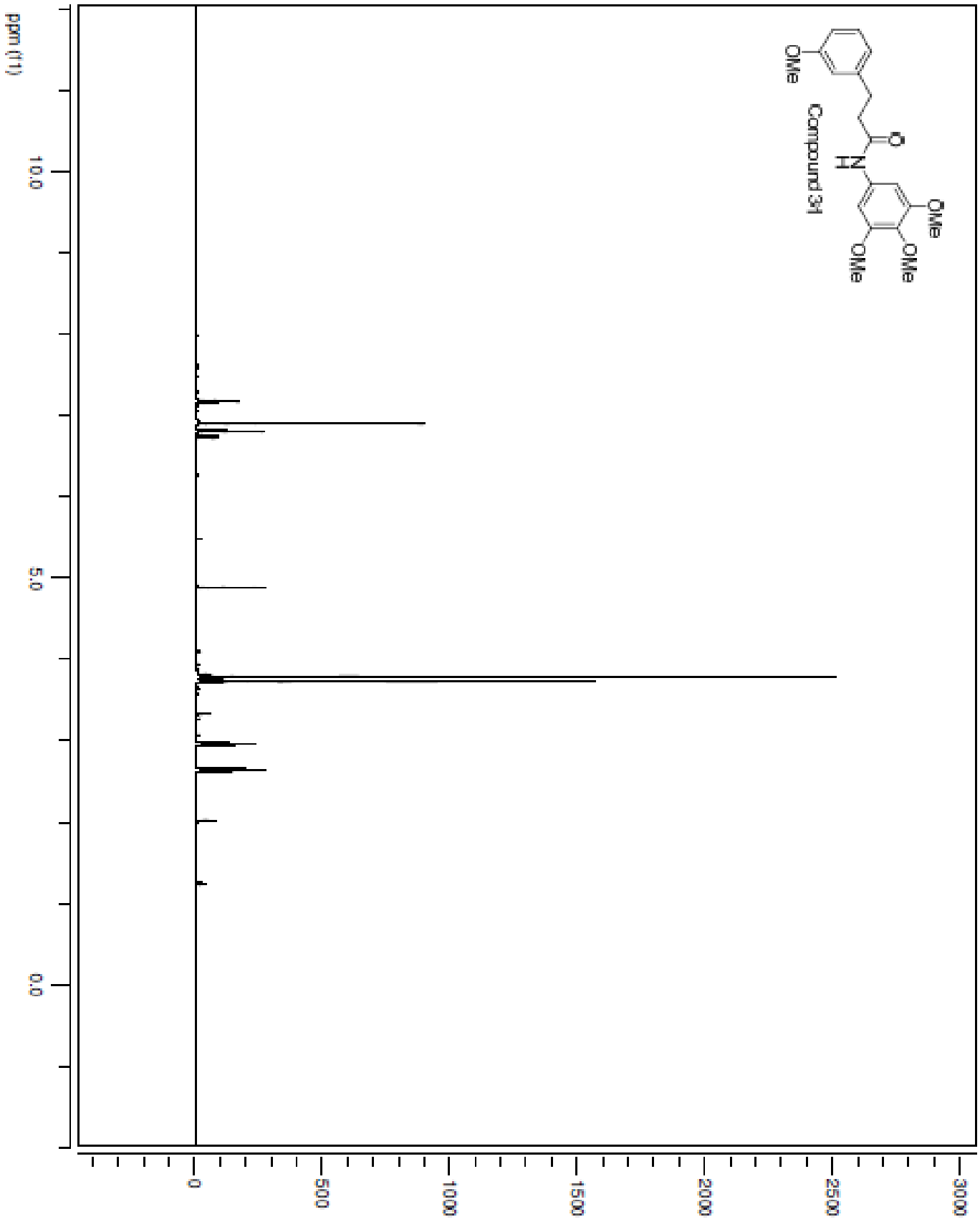

ppm (f1)
200
150
100
50
0
0
5000
10000
15000
20000
Compound 28
OMe
O
N
H
O
O

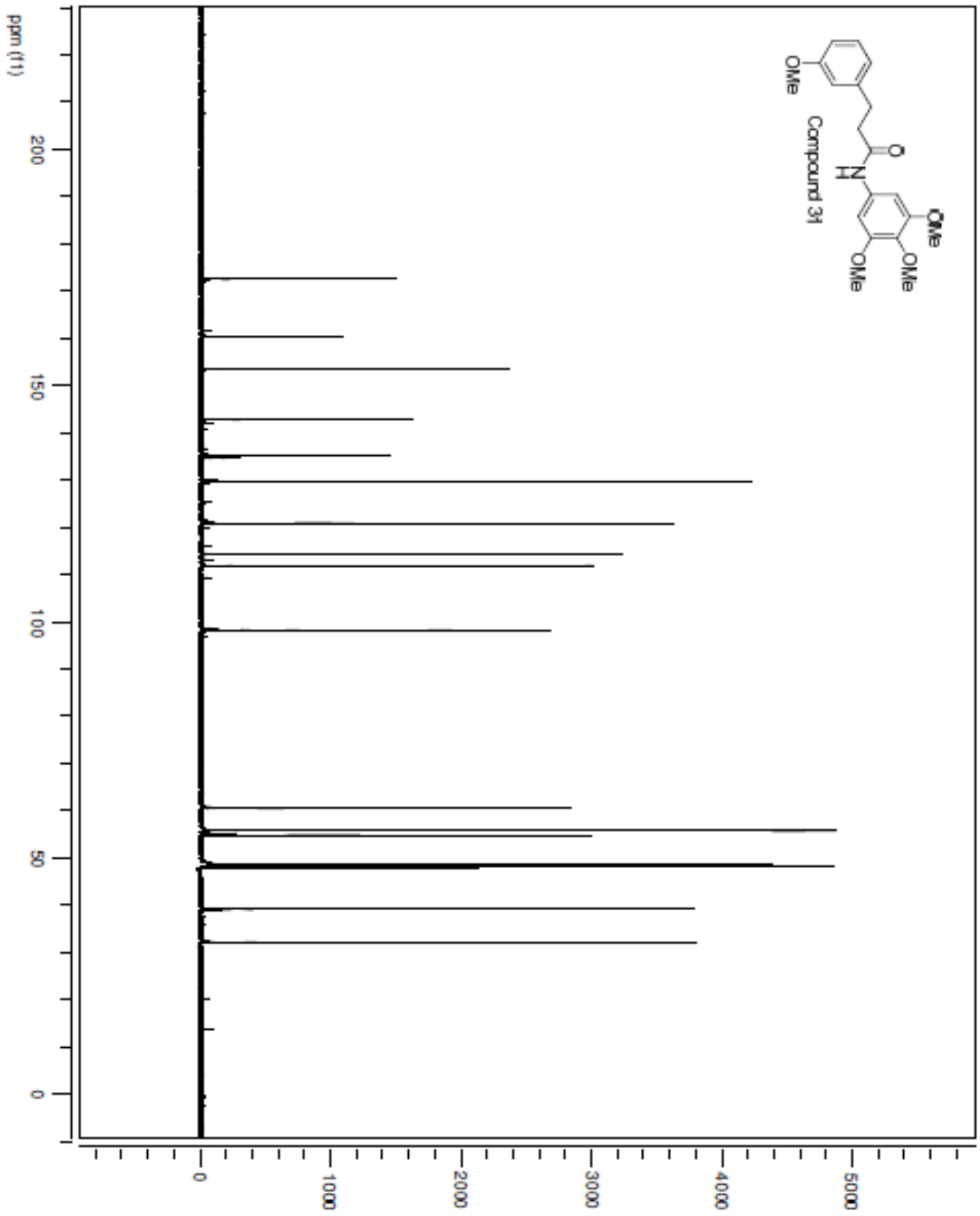

ppm (f1)
10.0
5.0
0.0
0
500
1000
OMe
O
N
H
Compound 29

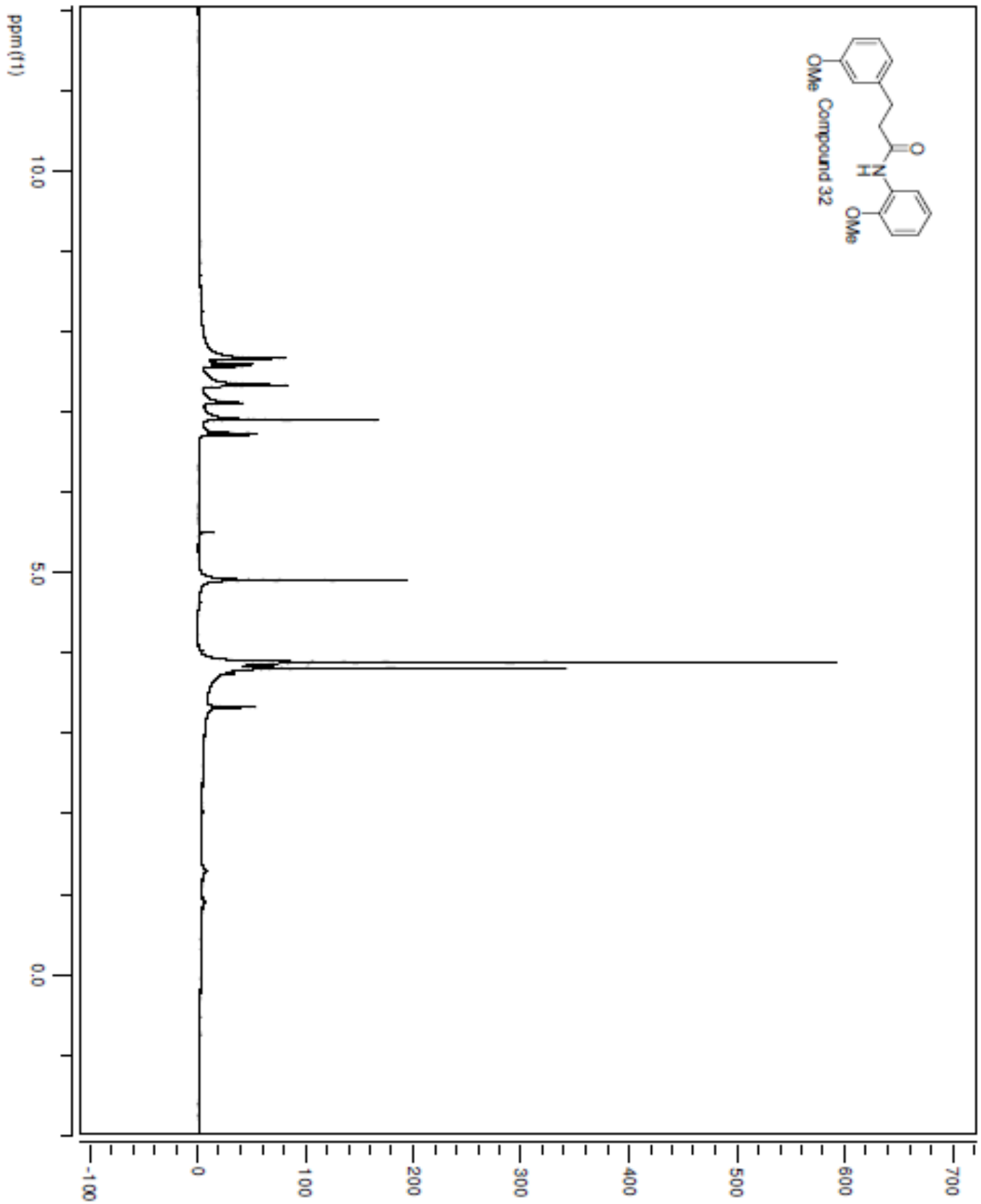

ppm (f1)
200
150
100
50
0
0
5000
1000(
1500(
OMe
Compound 29
O
N
H

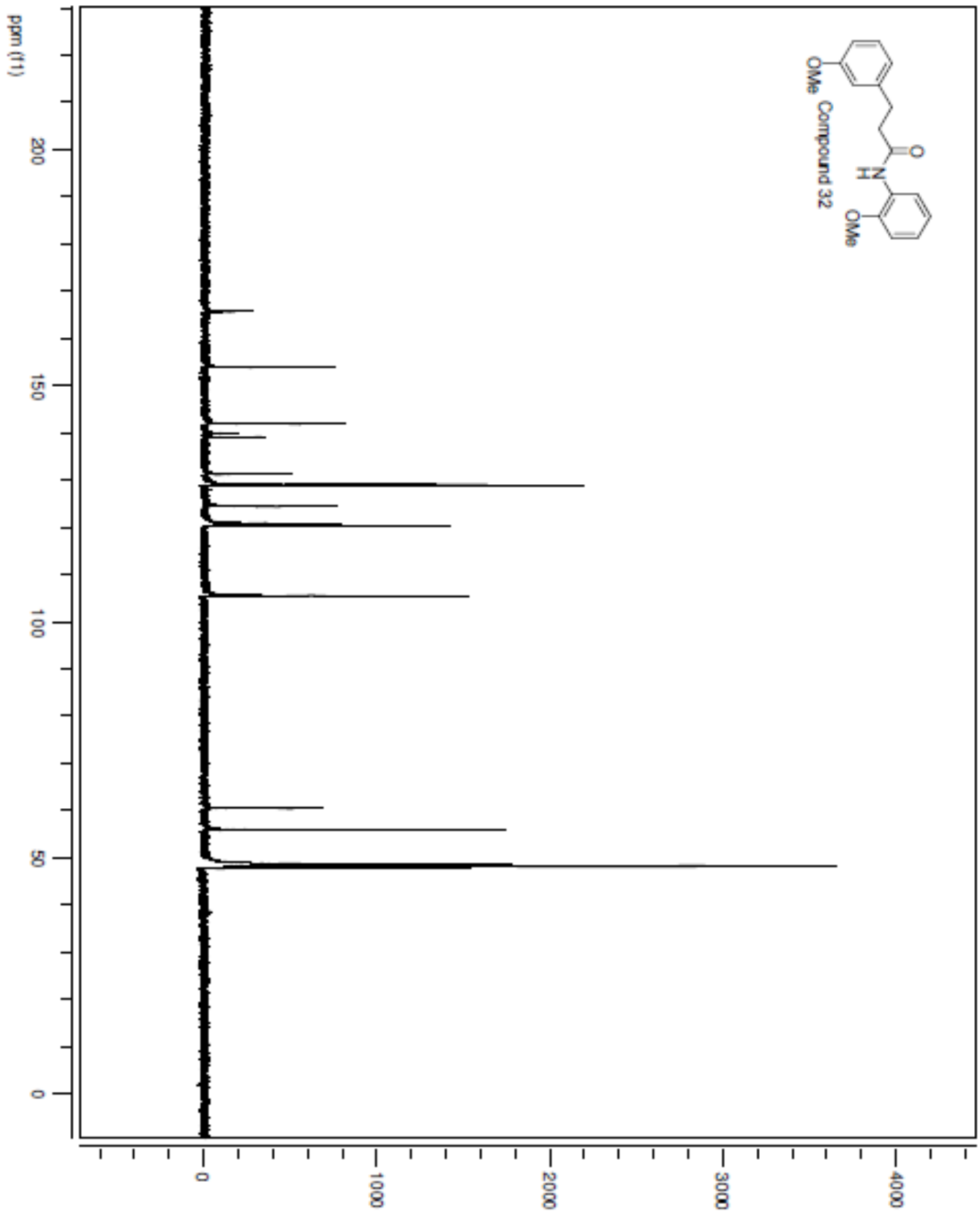

ppm (f1)
10.0
5.0
0.0
0
500
1000
1500
2000
OMe
O
N
H
Compound 30

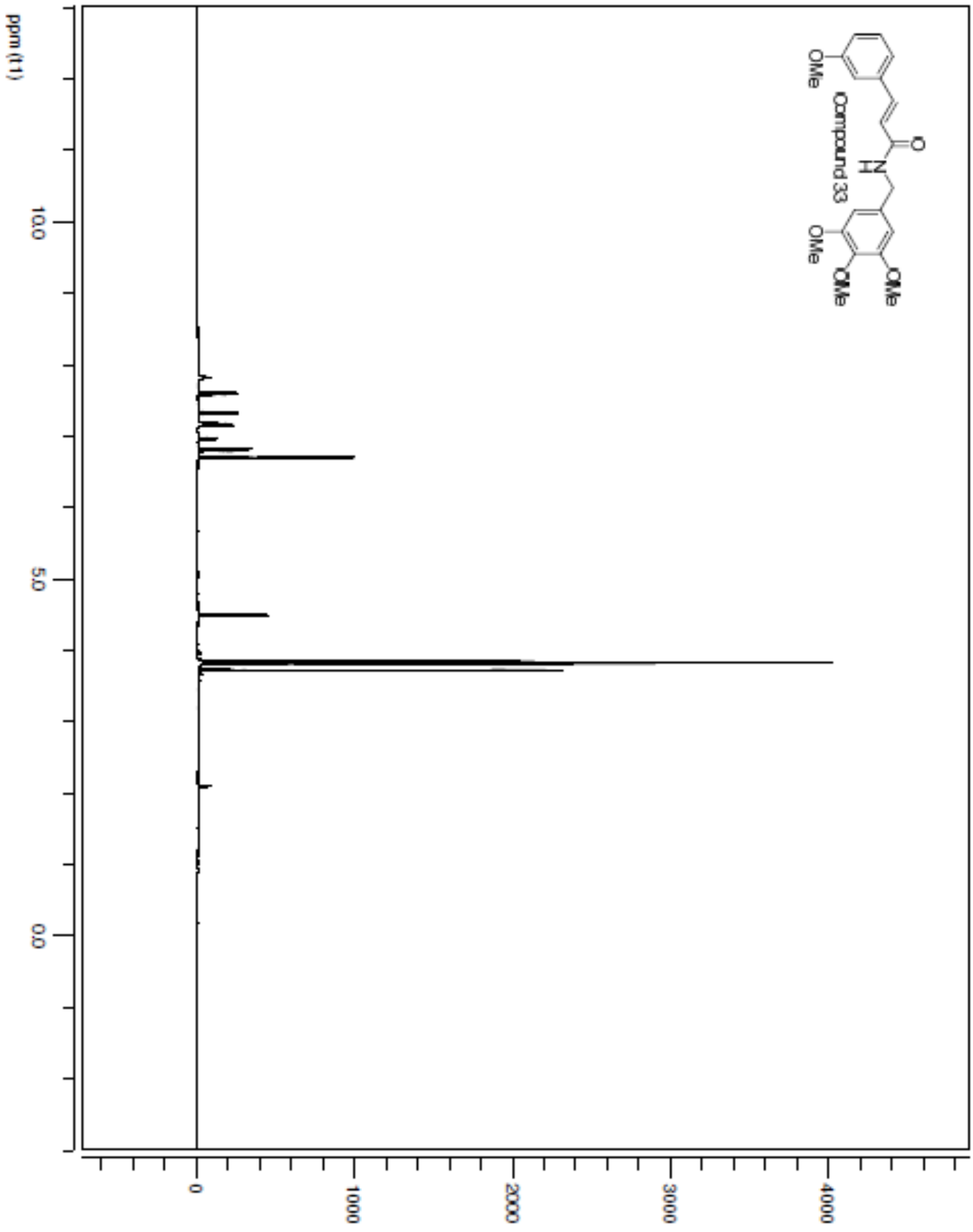

ppm (f1)
200
150
100
50
0
-1000
0
1000
2000
3000
4000
5000
6000
OMe
O
N
H
Compound 30

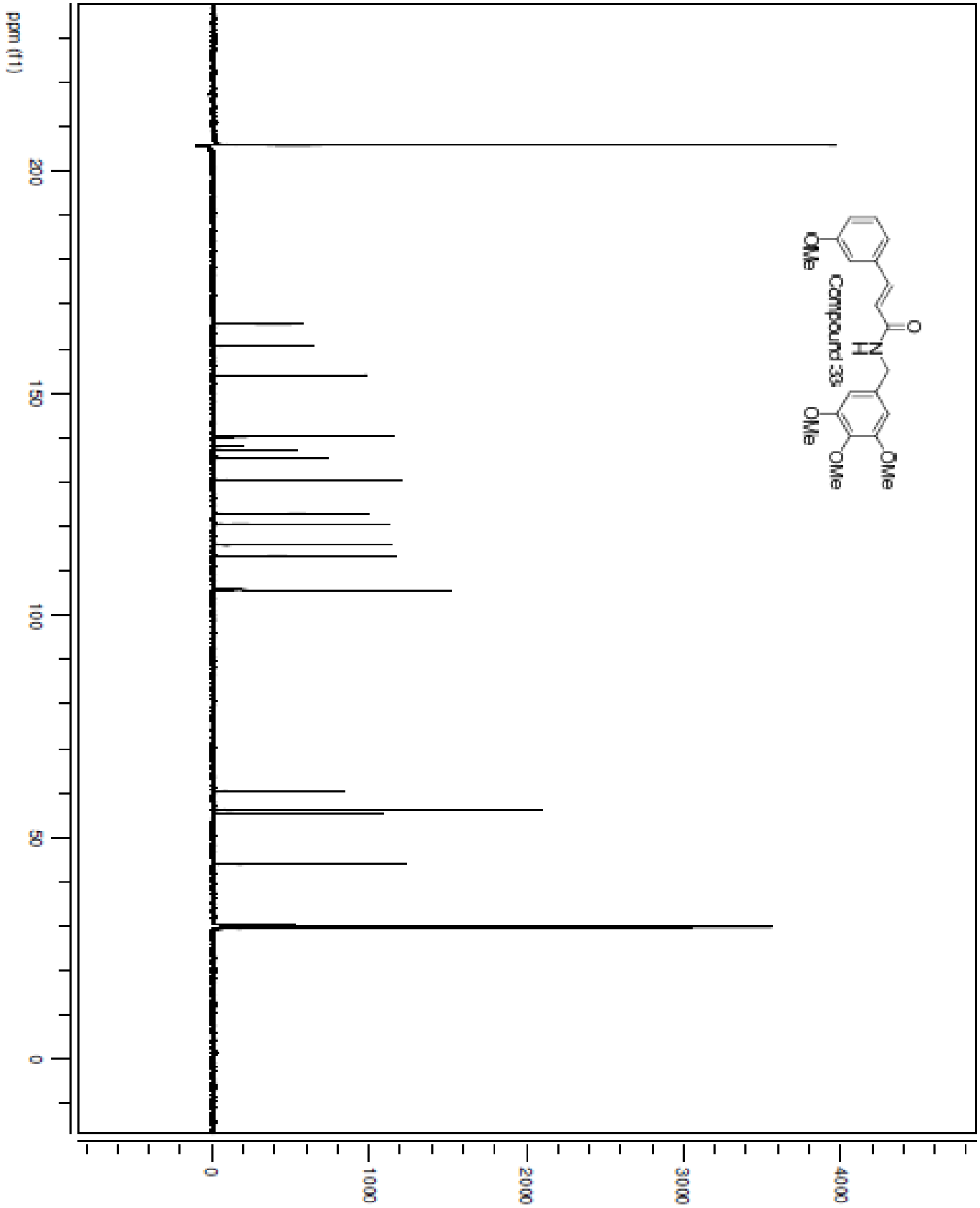

ppm (t1)
10.0
5.0
0.0
0
500
1000
1500
2000
2500
3000
Compound 34
OMe
OMe
OMe
OMe
H
N
O


Compound 31
OMe
OMe
OMe
OMe
O
N
H
ppm (f1)
200
150
100
50
0
0
1000
2000
3000
4000
5000


Compound 32
OMe
OMe
ppm (f1)
10.0
5.0
0.0
-100
0
100
200
300
400
500
600
700

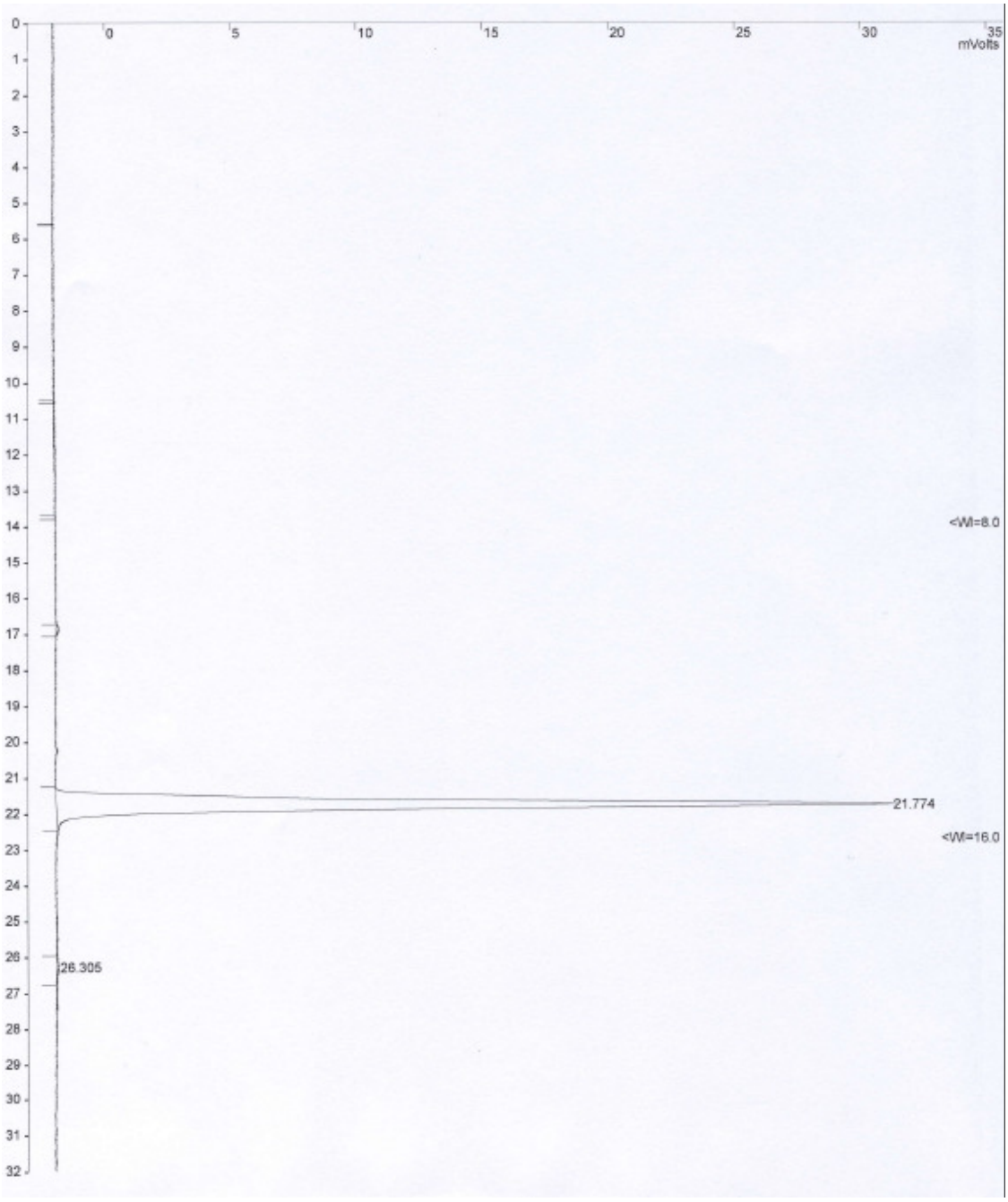

ppm (f1)
200
150
100
50
0
0
1000
2000
3000
4000
OMe
Compound 32
O
N
H
OMe


Compound 33
OMe
OMe
OMe
OMe
O
N
H
ppm (t1)
10.0
5.0
0.0
0
1000
2000
3000
4000

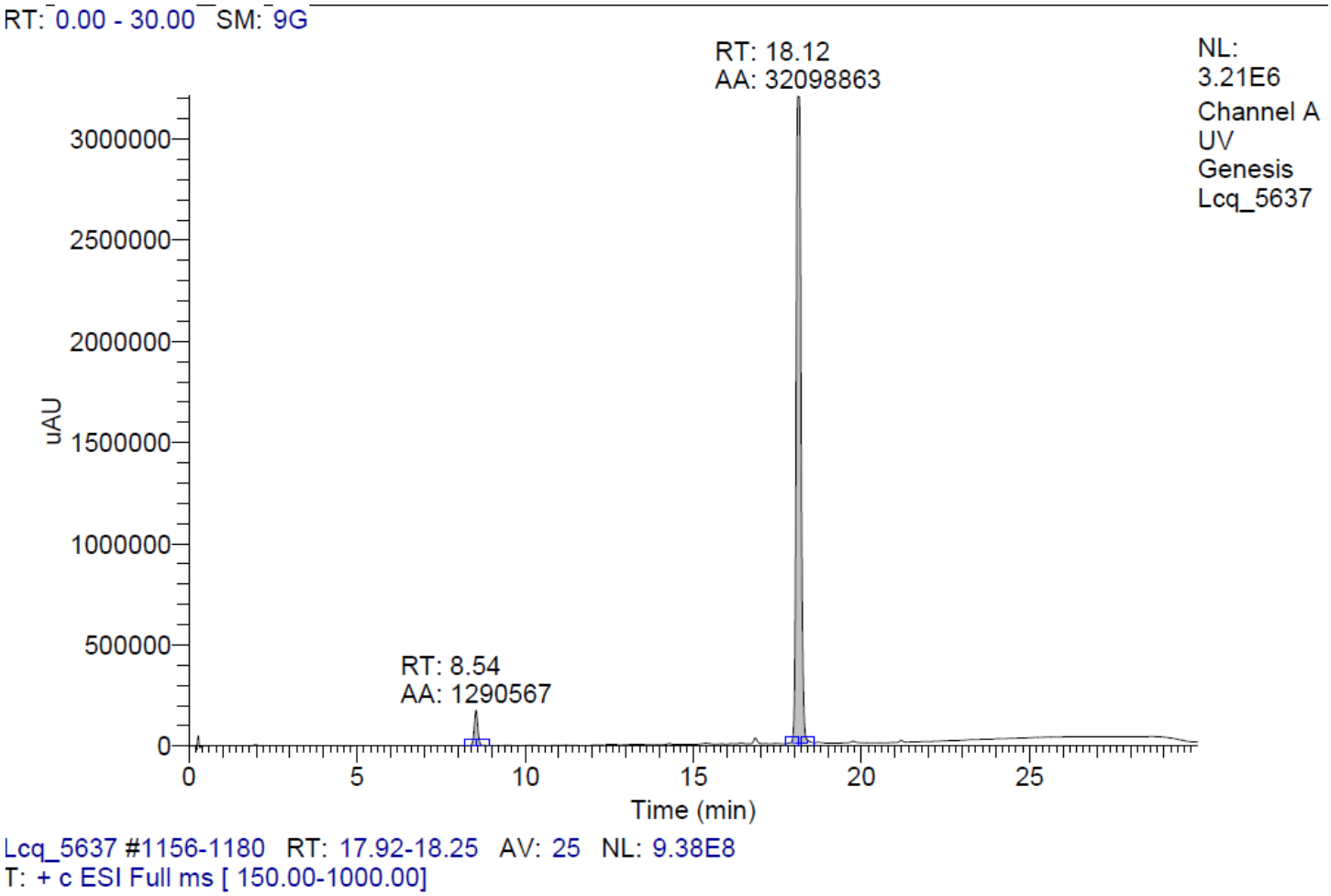

ppm (t1)
200
150
100
50
0
0
1000
2000
3000
4000
Compound 33
OMe
OMe
OMe
OMe
O
N
H

HPLC analysis of library compounds

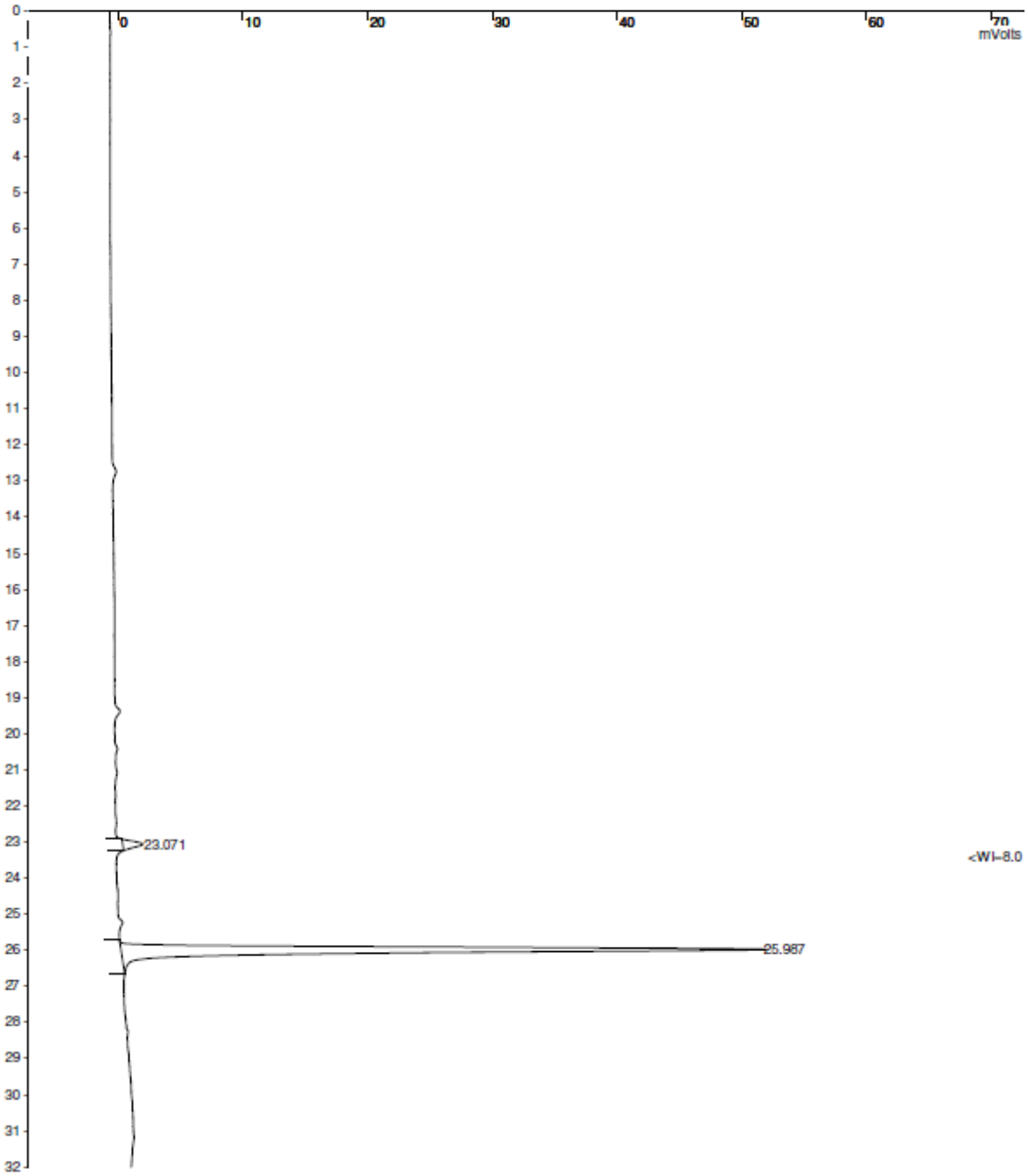
Compound 8H



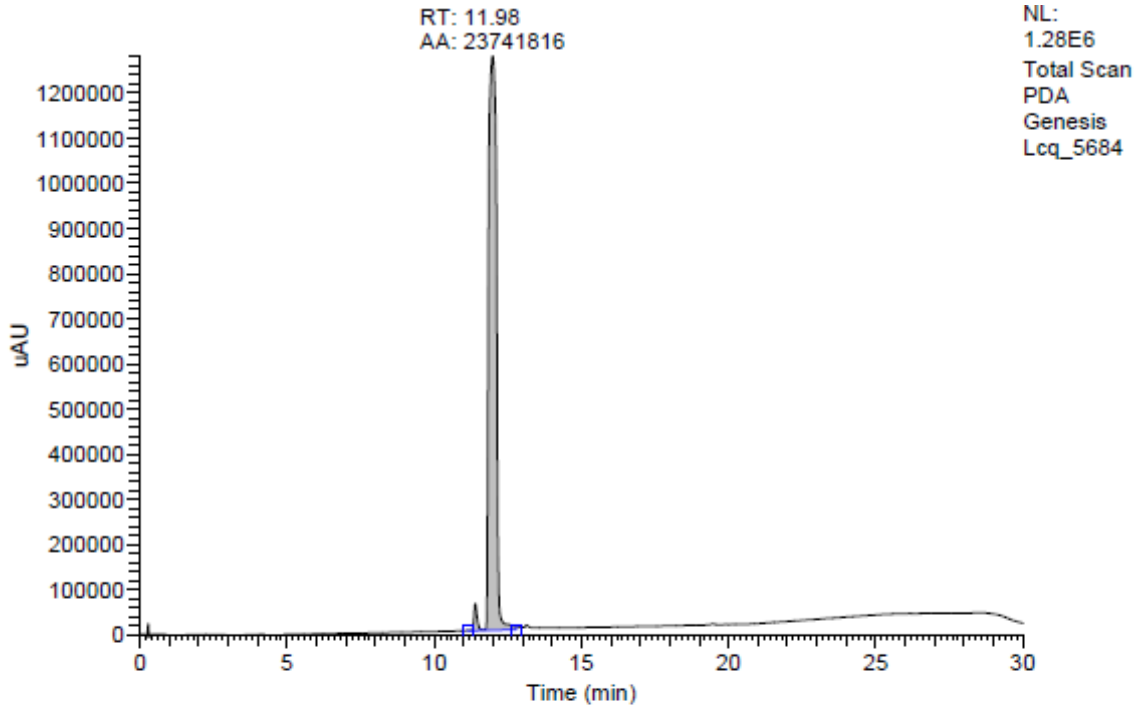
Compound 6


RT: 0.00 - 30.00 SM: 9G
RT: 18.12
AA: 32098863
NL:
3.21E6
Channel A
UV
Genesis
Lcq_5637
RT: 8.54
AA: 1290567
uAU
3000000
2500000
2000000
1500000
1000000
500000
0
0
5
10
15
20
25
Time (min)
Lcq_5637 #1156-1180 RT: 17.92-18.25 AV: 25 NL: 9.38E8
T: + c ESI Full ms [150.00-1000.00]

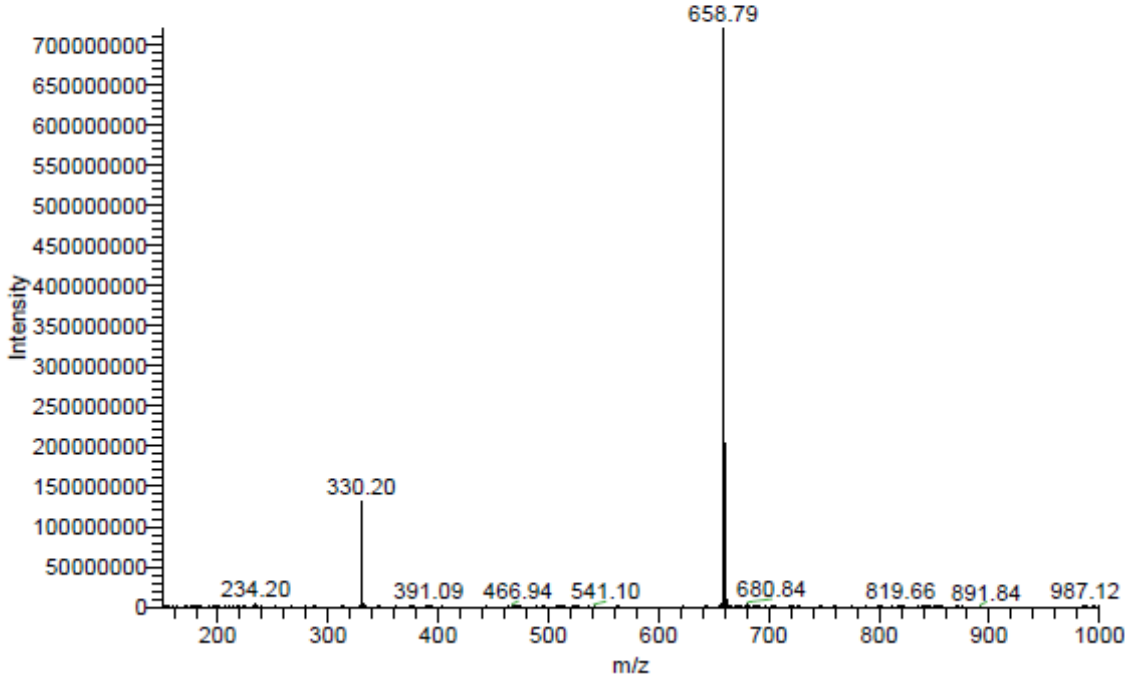
Compound 7



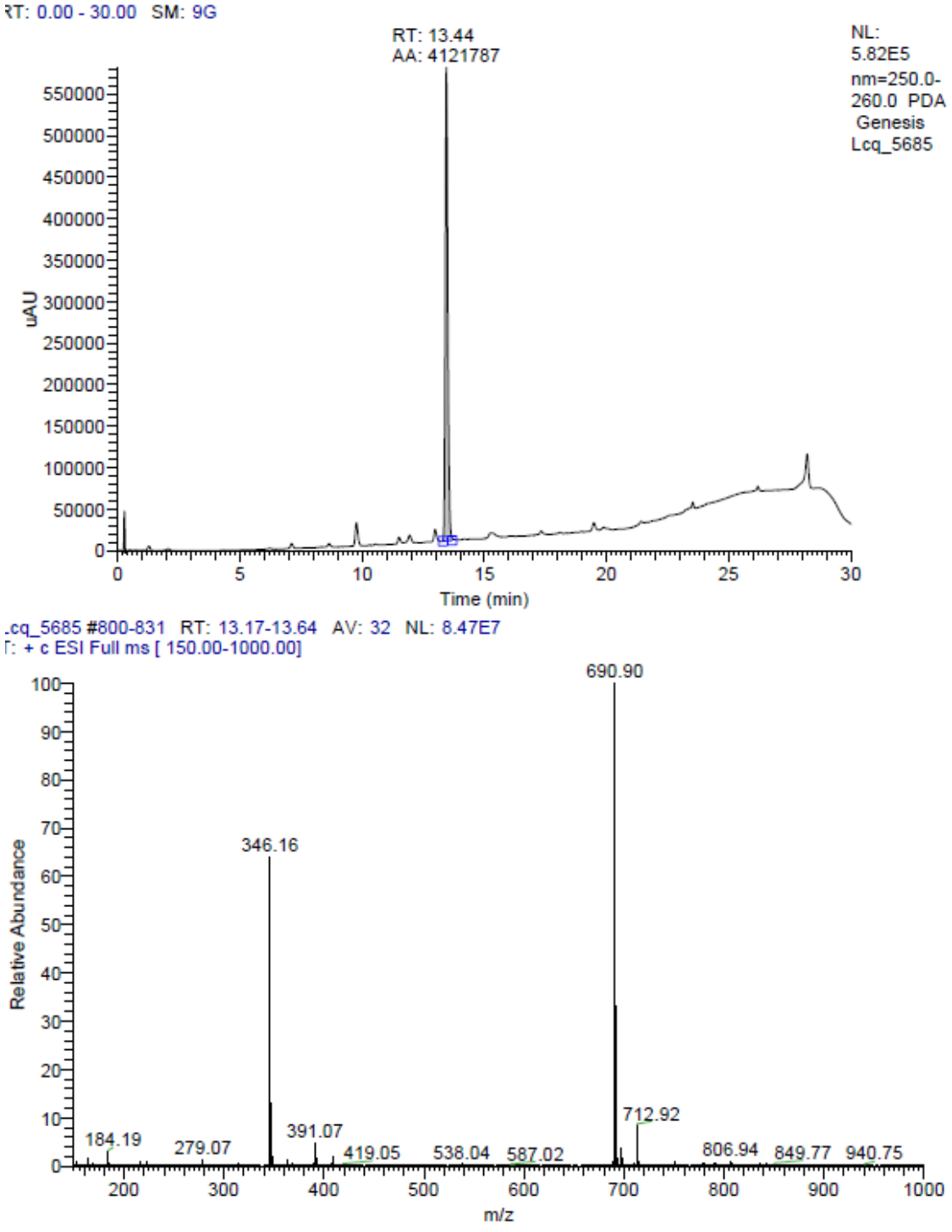
Compound 8



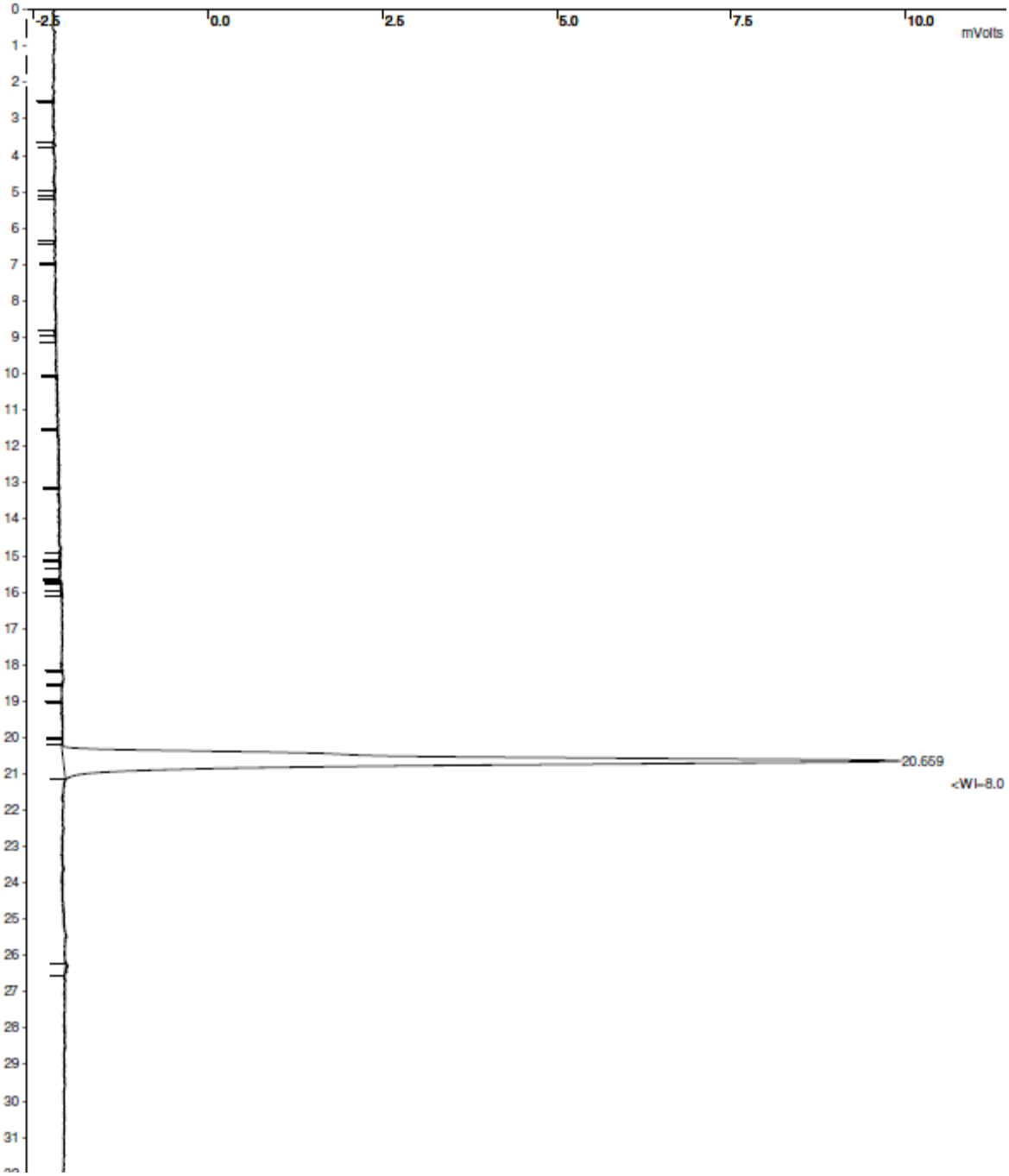
Lcq_5684 #675-810 RT: 11.10-13.05 AV: 136 NL: 7.20E8
T: + c ESI Full ms [150.00-1000.00]



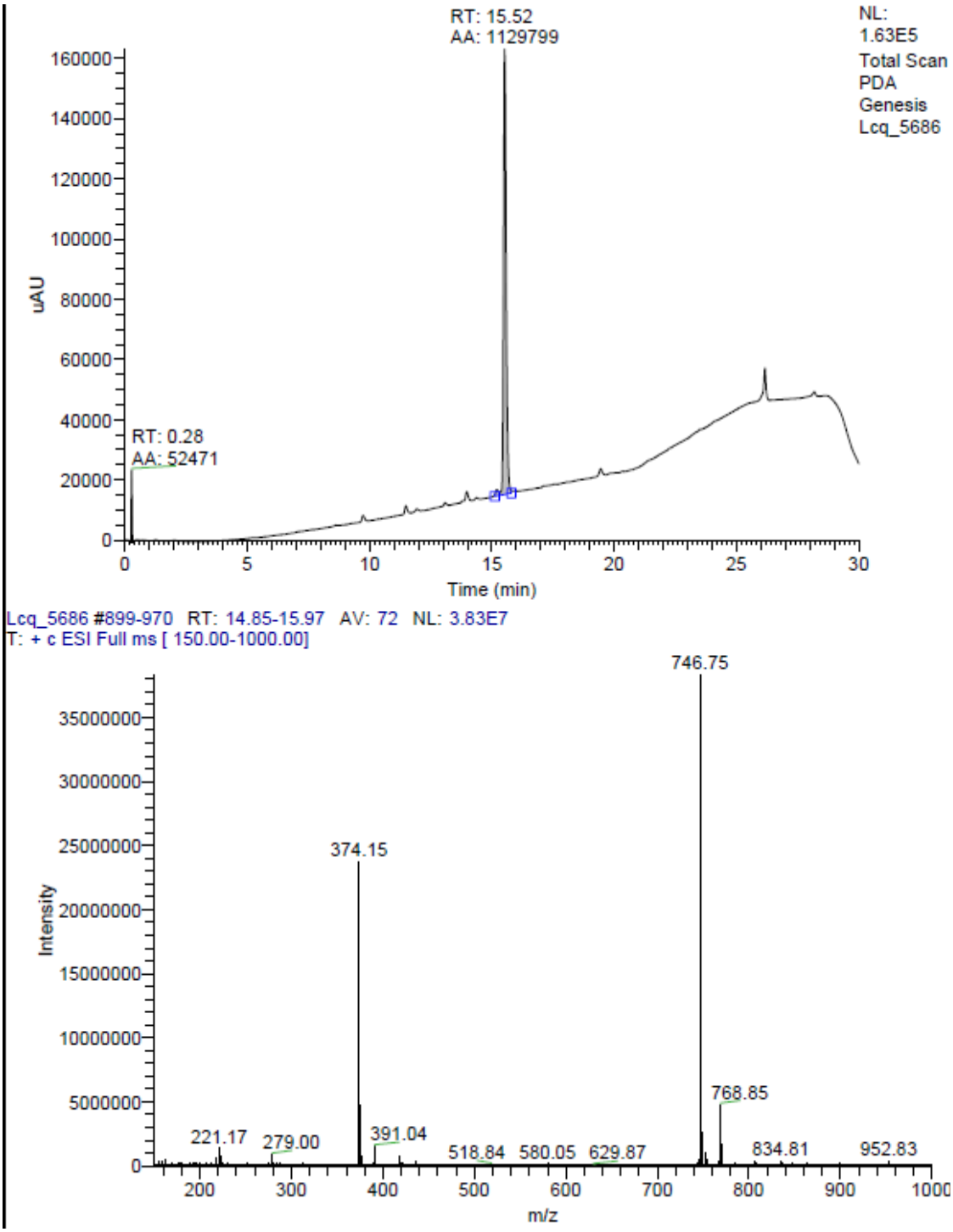
Compound 9


RT: 0.00 - 30.00 SM: 9G
RT: 13.44
AA: 4121787
NL:
5.82E5
nm=250.0-
260.0 PDA
Genesis
Lcq_5685
uAU
Time (min)
.cq_5685 #800-831 RT: 13.17-13.64 AV: 32 NL: 8.47E7
T: + c ESI Full ms [150.00-1000.00]
690.90
346.16
712.92
391.07
184.19
279.07
419.05
538.04
587.02
806.94
849.77
940.75
Relative Abundance
m/z

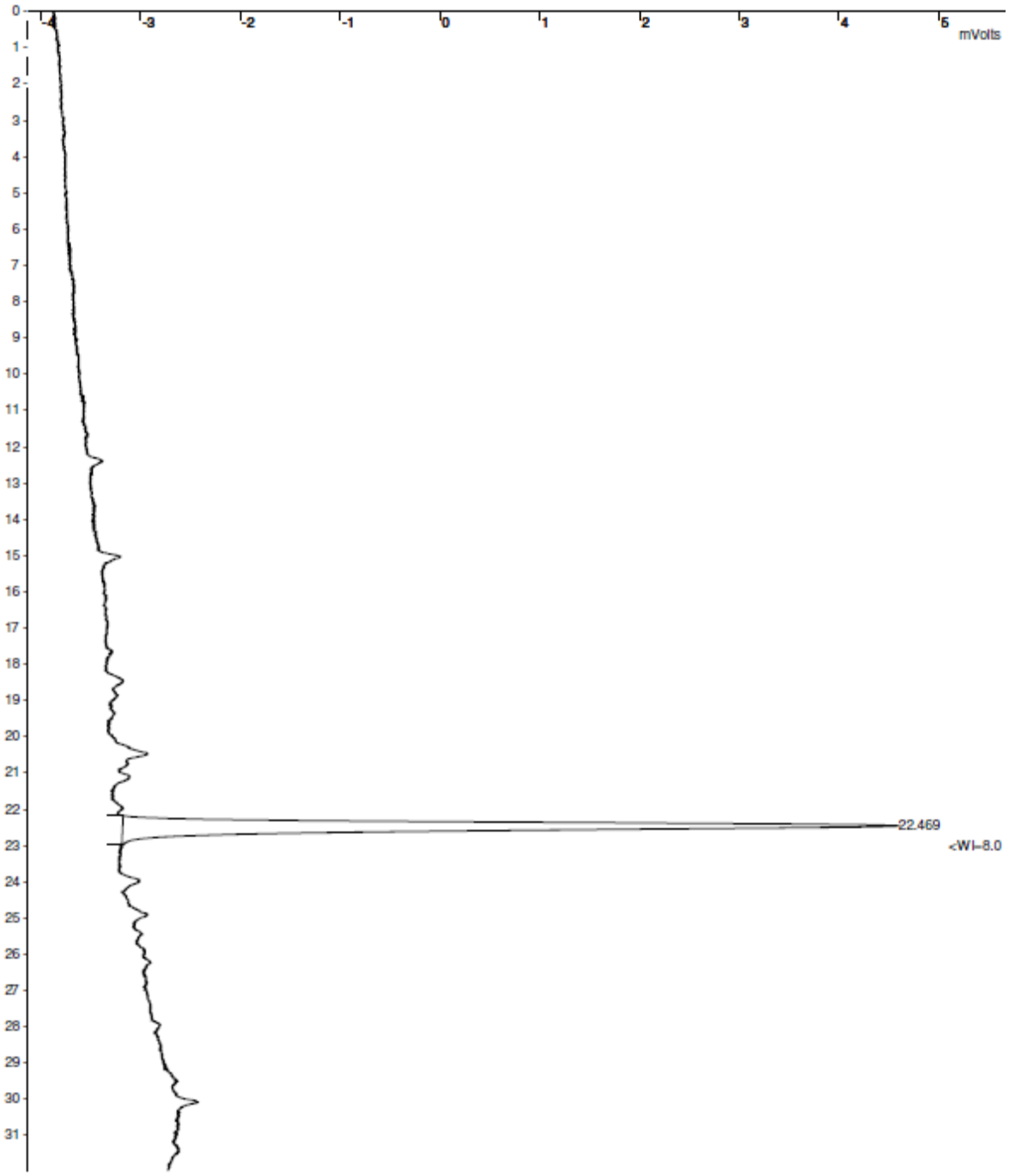
Compound 10



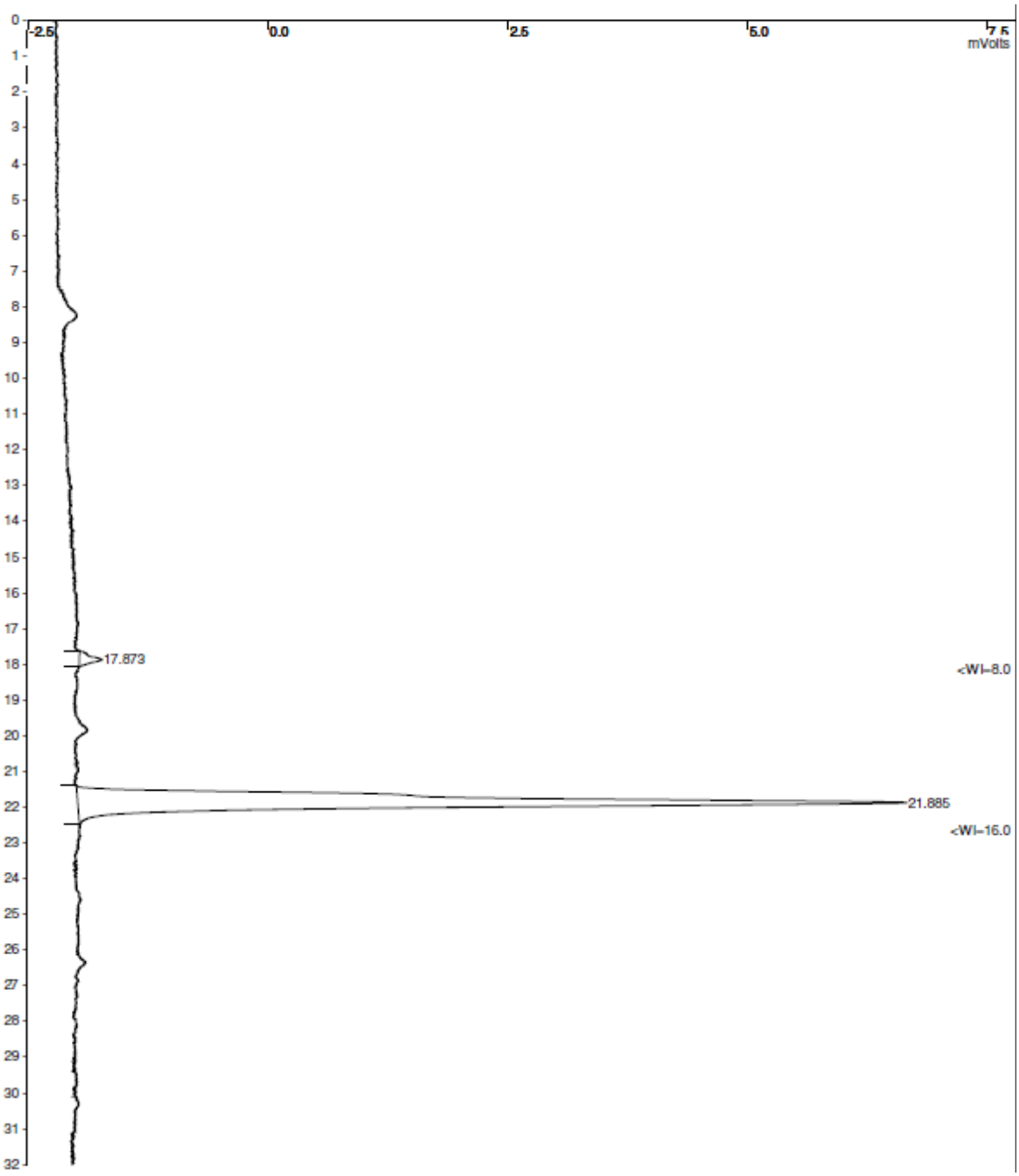
Compound 11


RT: 15.52
AA: 1129799
NL:
1.63E5
Total Scan
PDA
Genesis
Lcq_5686
uAU
RT: 0.28
AA: 52471
Time (min)
Lcq_5686 #899-970 RT: 14.85-15.97 AV: 72 NL: 3.83E7
T: + c ESI Full ms [150.00-1000.00]
Intensity
746.75
374.15
768.85
221.17
279.00
391.04
518.84
580.05
629.87
834.81
952.83
m/z

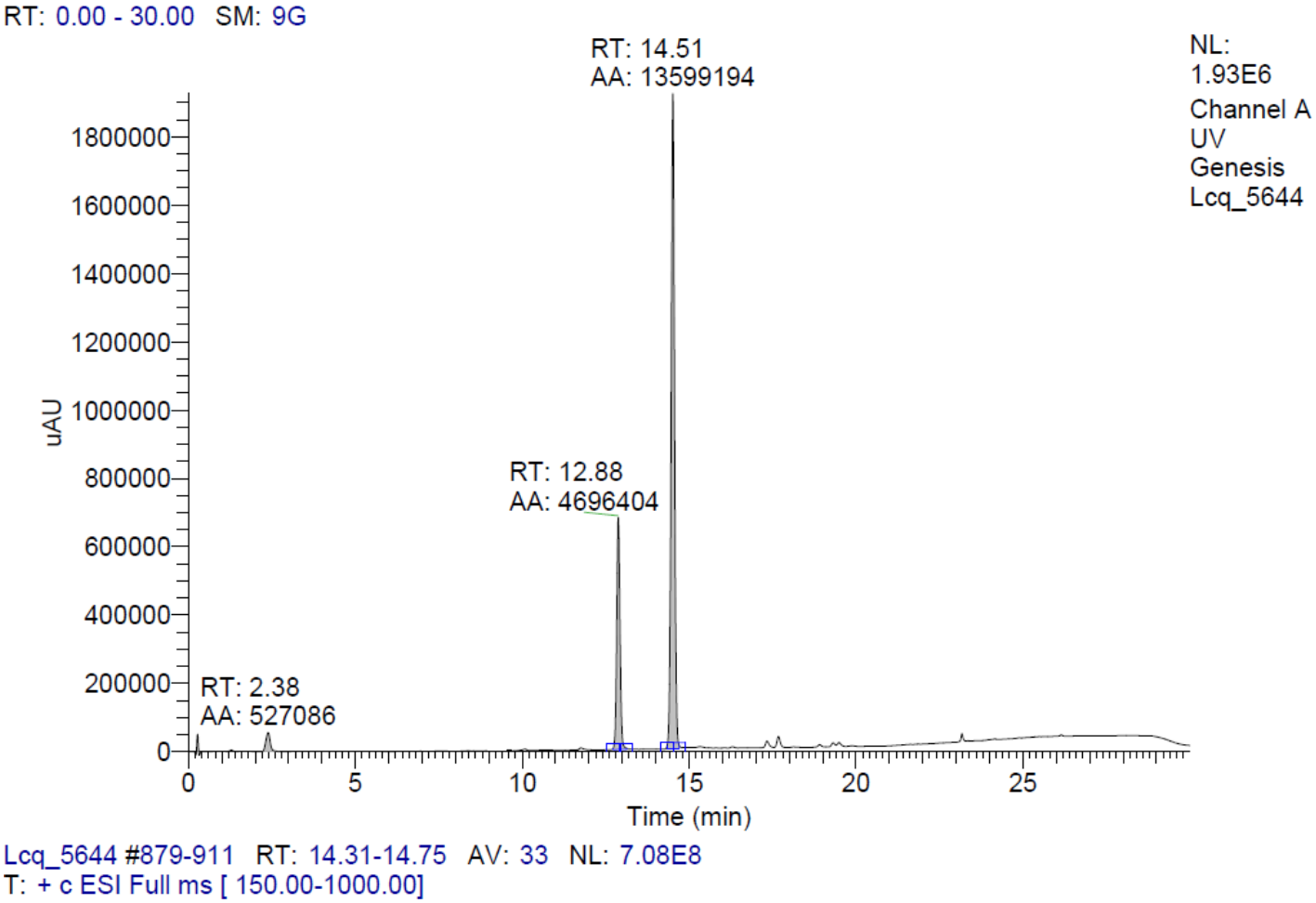
Compound 12



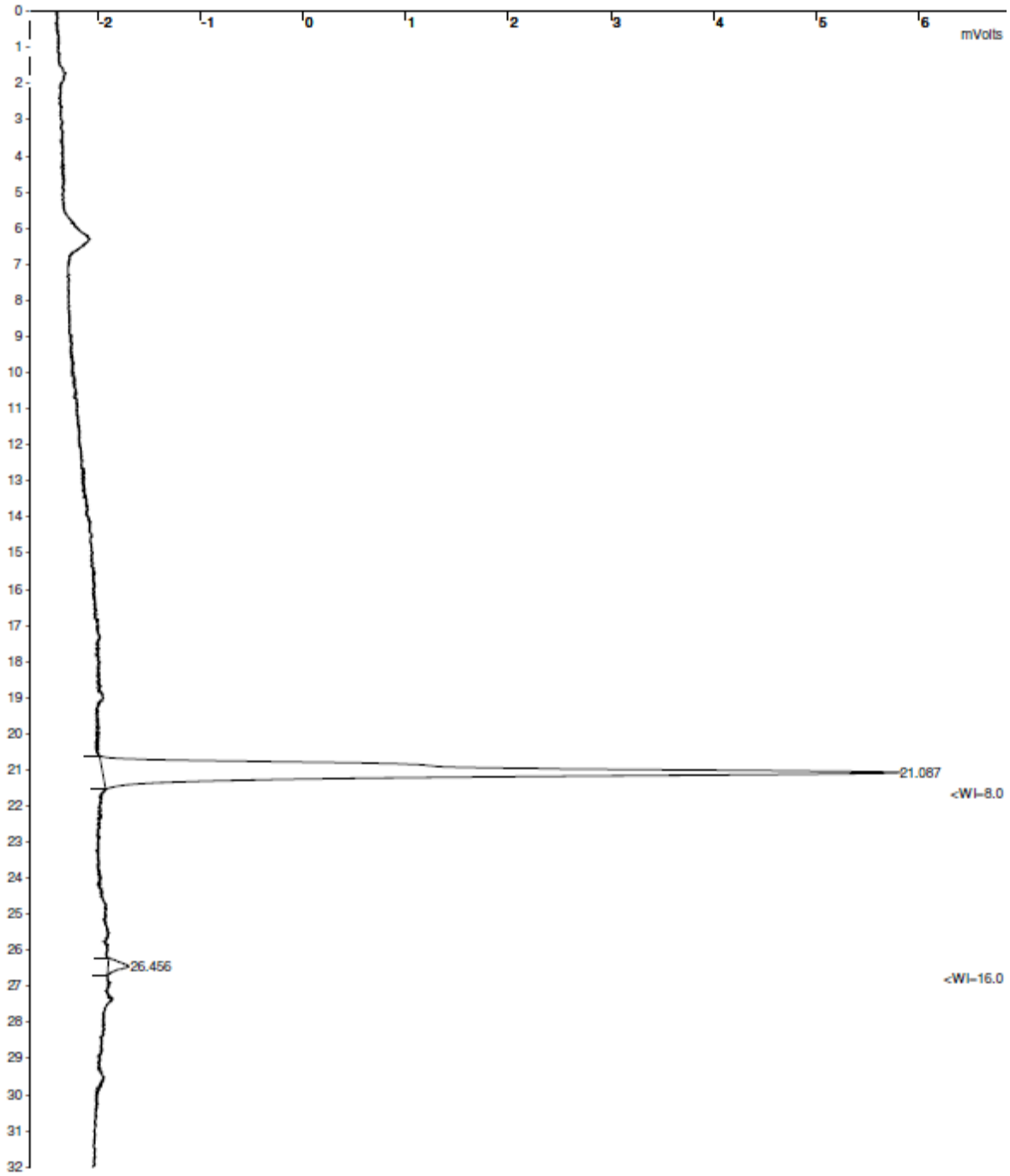
Compound 13



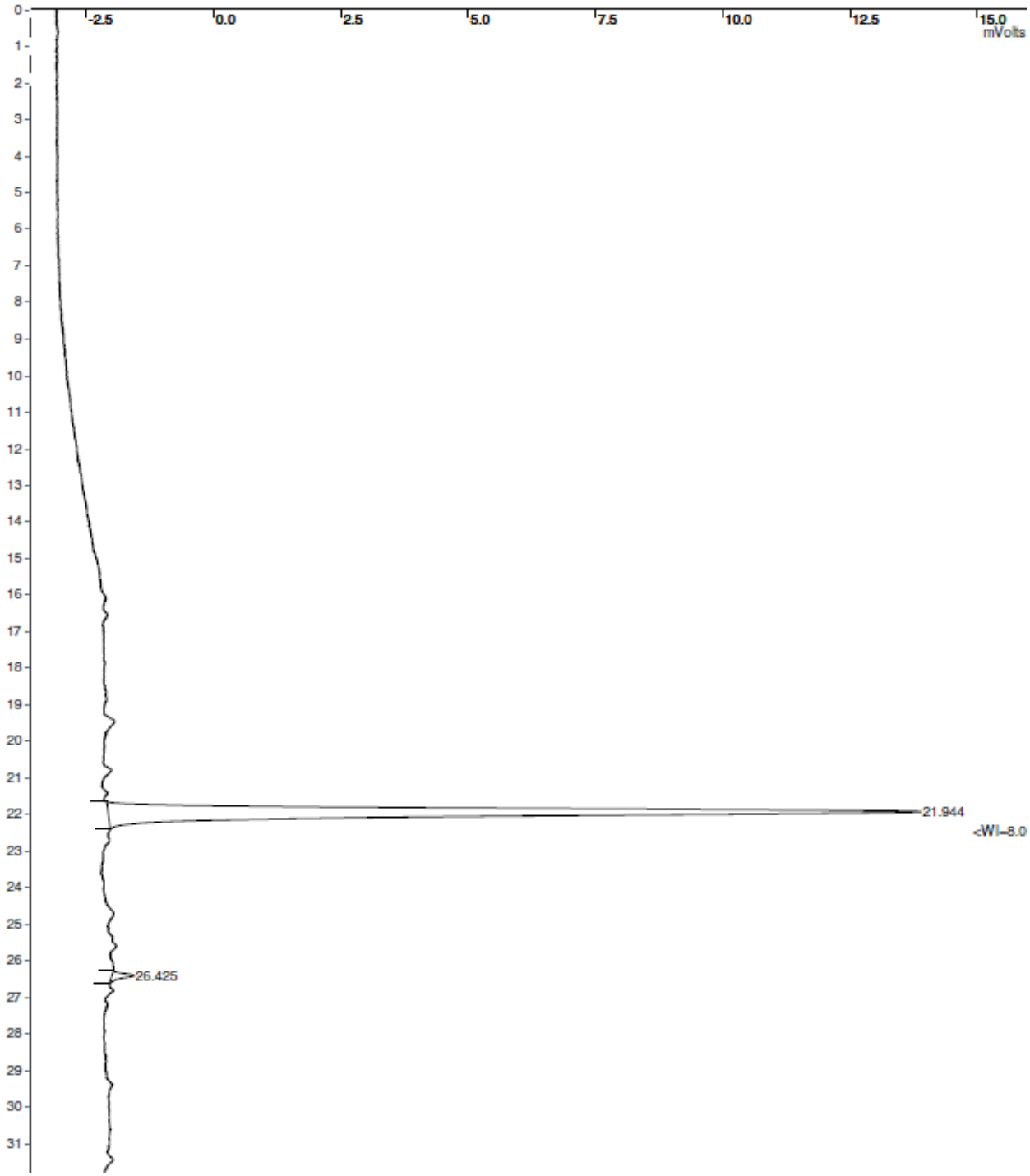
Compound 14


RT: 0.00 - 30.00 SM: 9G
RT: 14.51
AA: 13599194
NL:
1.93E6
Channel A
UV
Genesis
Lcq_5644
RT: 12.88
AA: 4696404
RT: 2.38
AA: 527086
uAU
Time (min)
Lcq_5644 #879-911 RT: 14.31-14.75 AV: 33 NL: 7.08E8
T: + c ESI Full ms [150.00-1000.00]

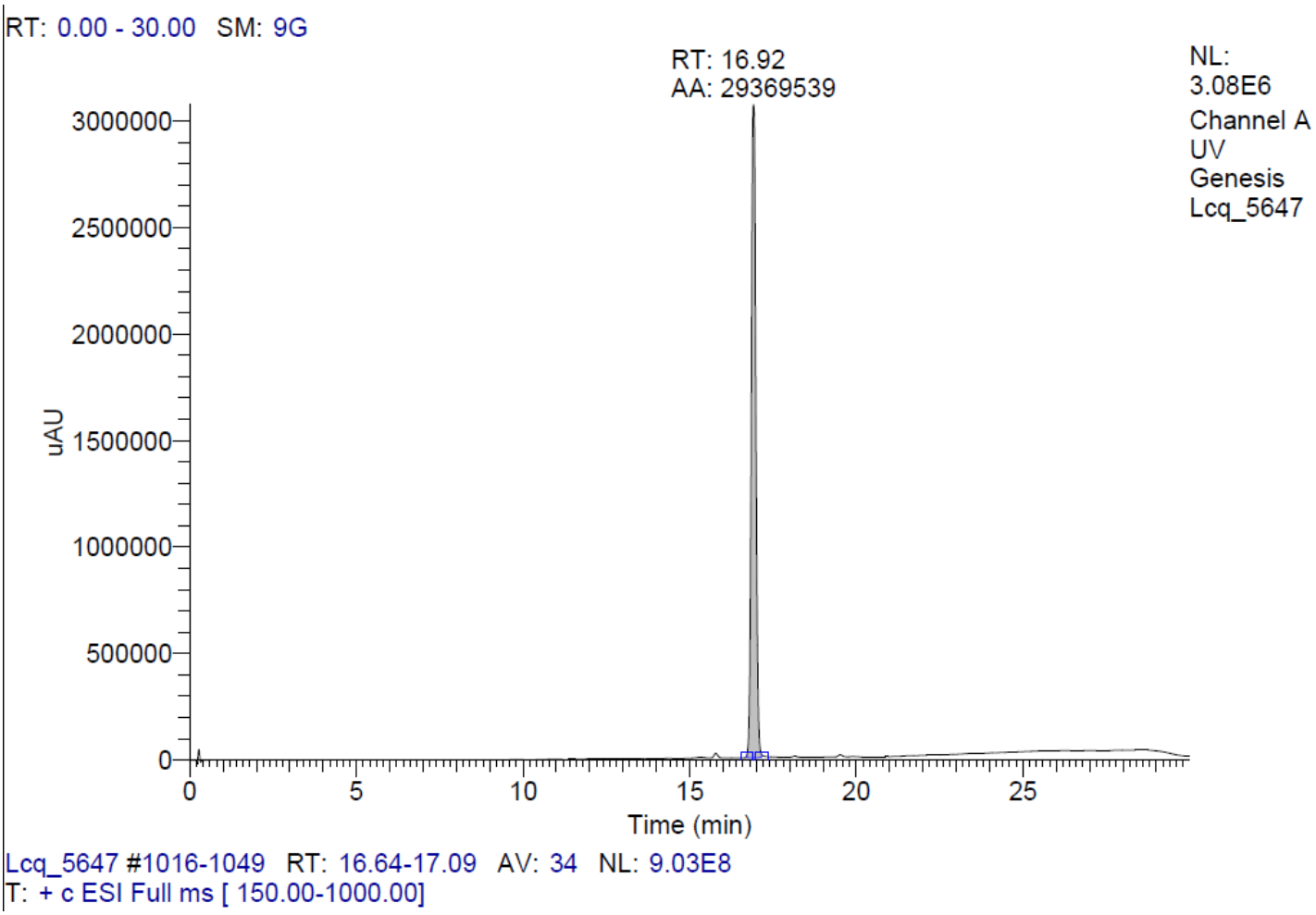
Compound 15



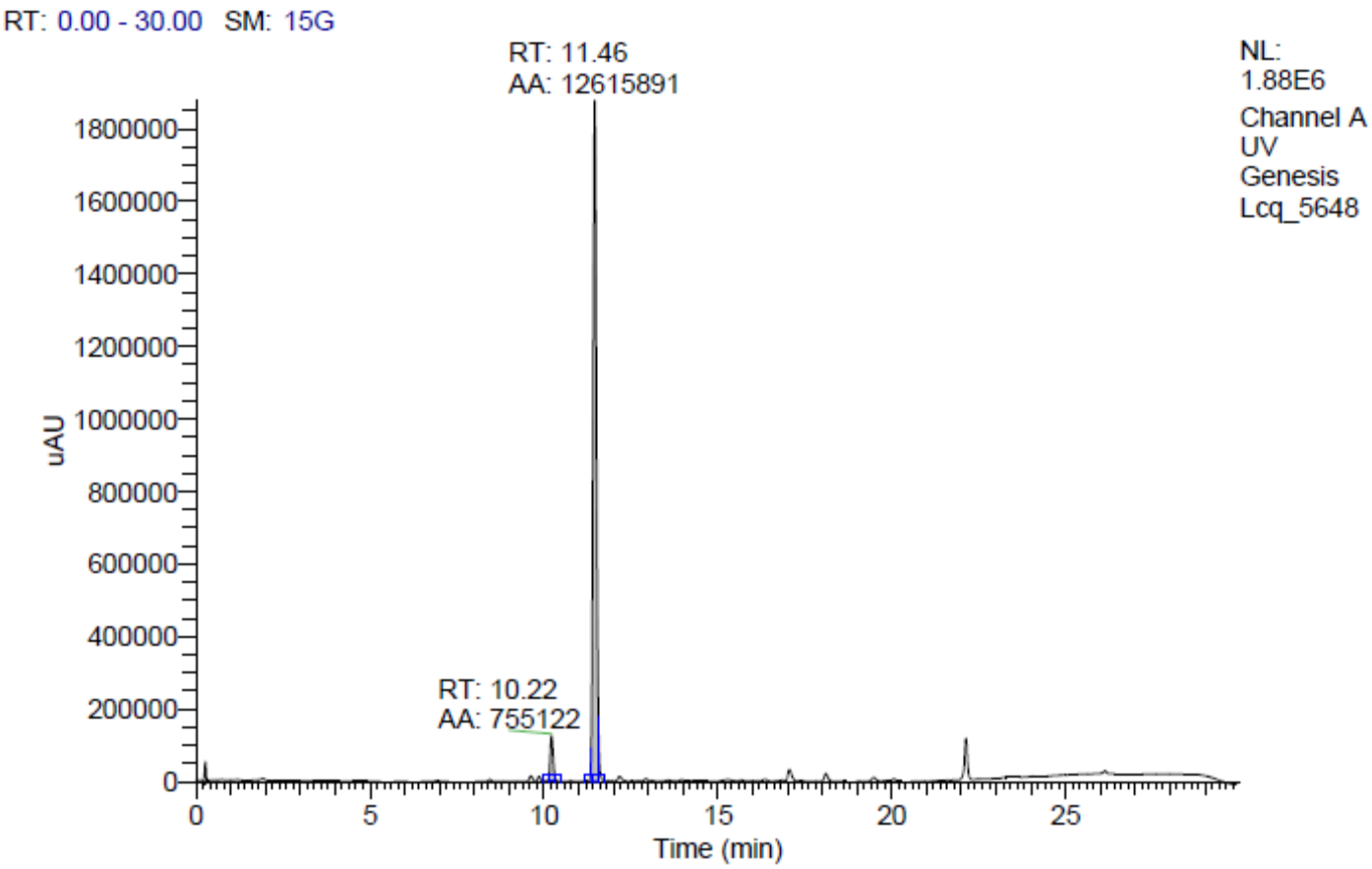
Compound 16



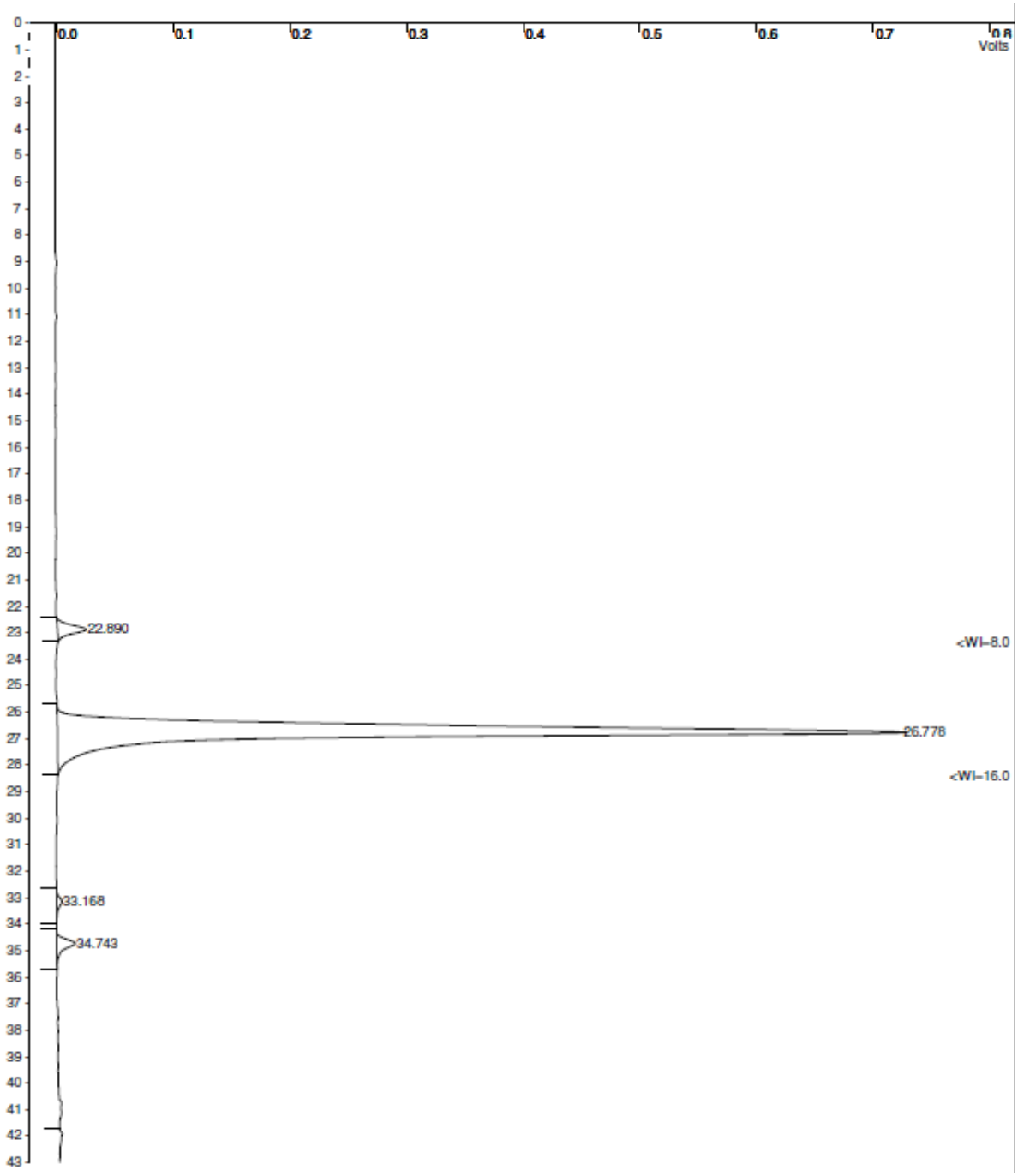
Compound 17


RT: 0.00 - 30.00 SM: 9G
RT: 16.92
AA: 29369539
NL:
3.08E6
Channel A
UV
Genesis
Lcq_5647
3000000
2500000
2000000
1500000
1000000
500000
0
uAU
0
5
10
15
20
25
Time (min)
Lcq_5647 #1016-1049 RT: 16.64-17.09 AV: 34 NL: 9.03E8
T: + c ESI Full ms [150.00-1000.00]

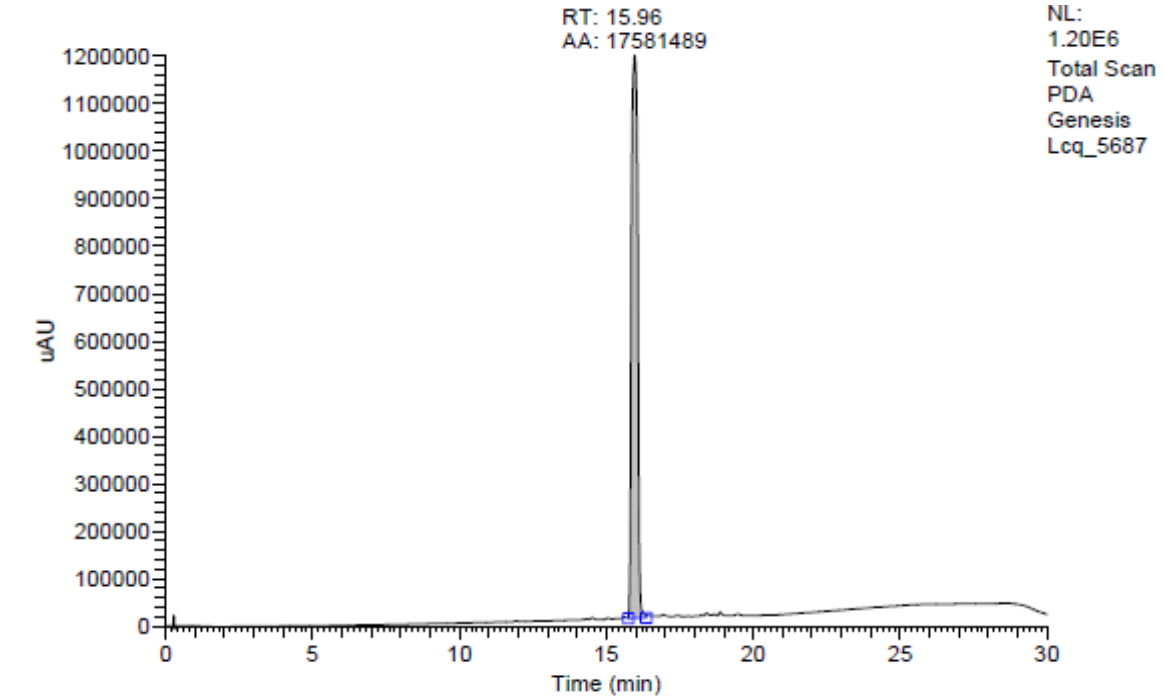
Compound 18


RT: 0.00 - 30.00 SM: 15G
RT: 11.46
AA: 12615891
NL:
1.88E6
Channel A
UV
Genesis
Lcq_5648
RT: 10.22
AA: 755122
uAU
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
0
5
10
15
20
25
Time (min)

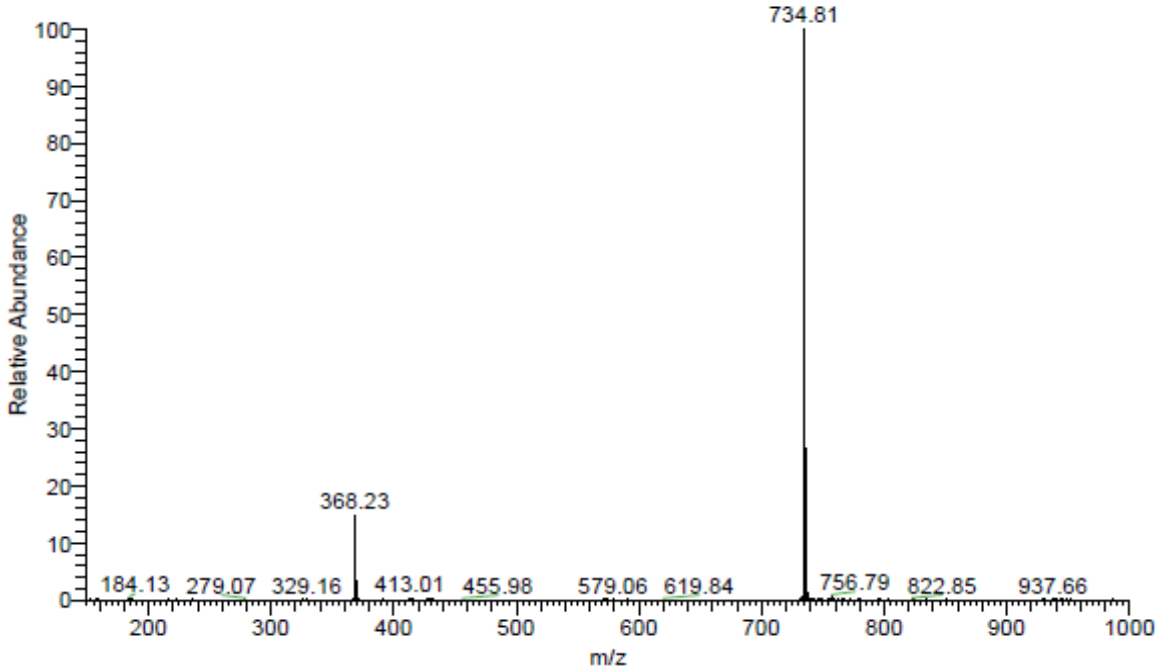
Compound 19


0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
Volts
22.690
26.778
33.168
34.743
<WI=8.0
<WI=16.0

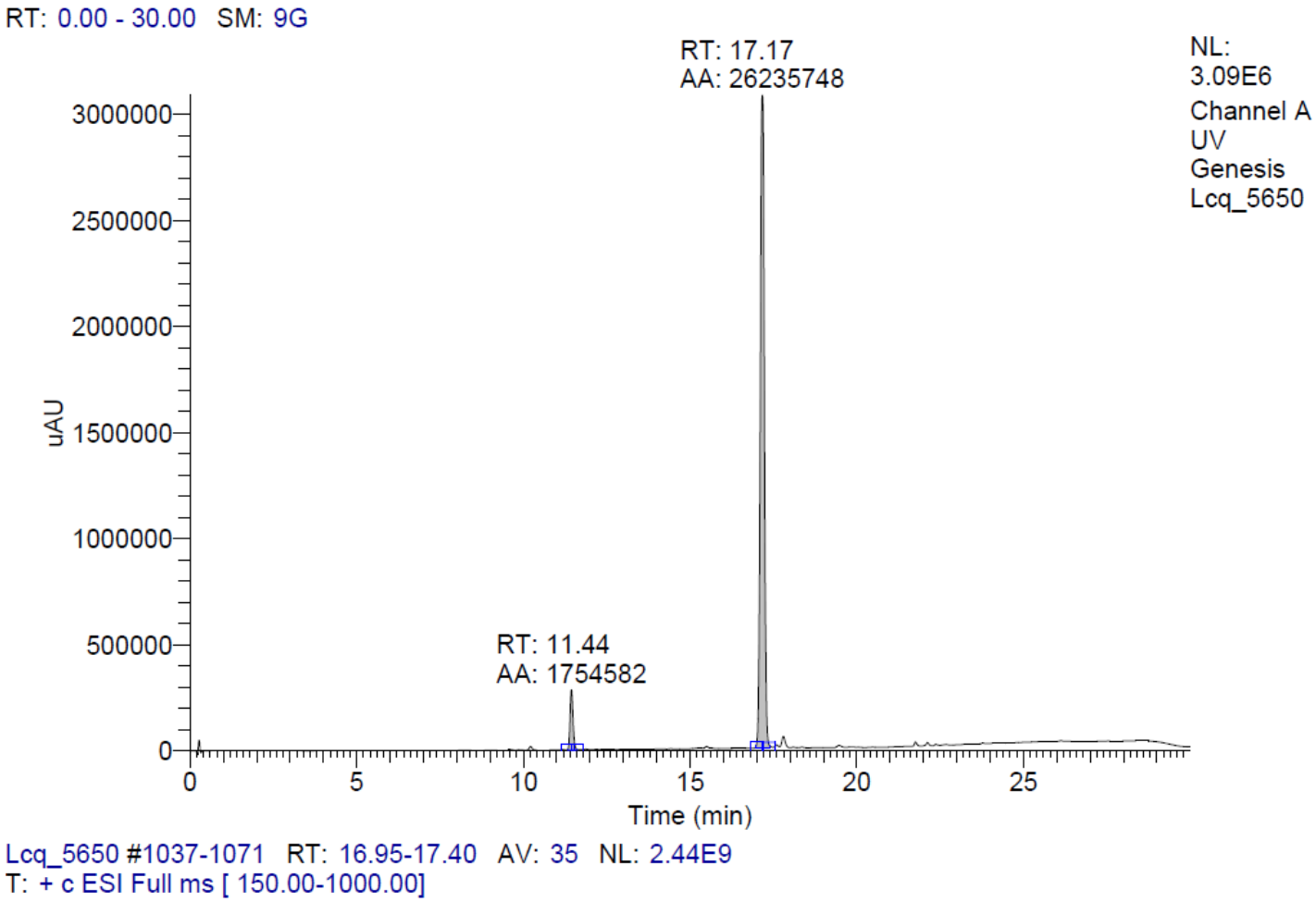
Compound 20



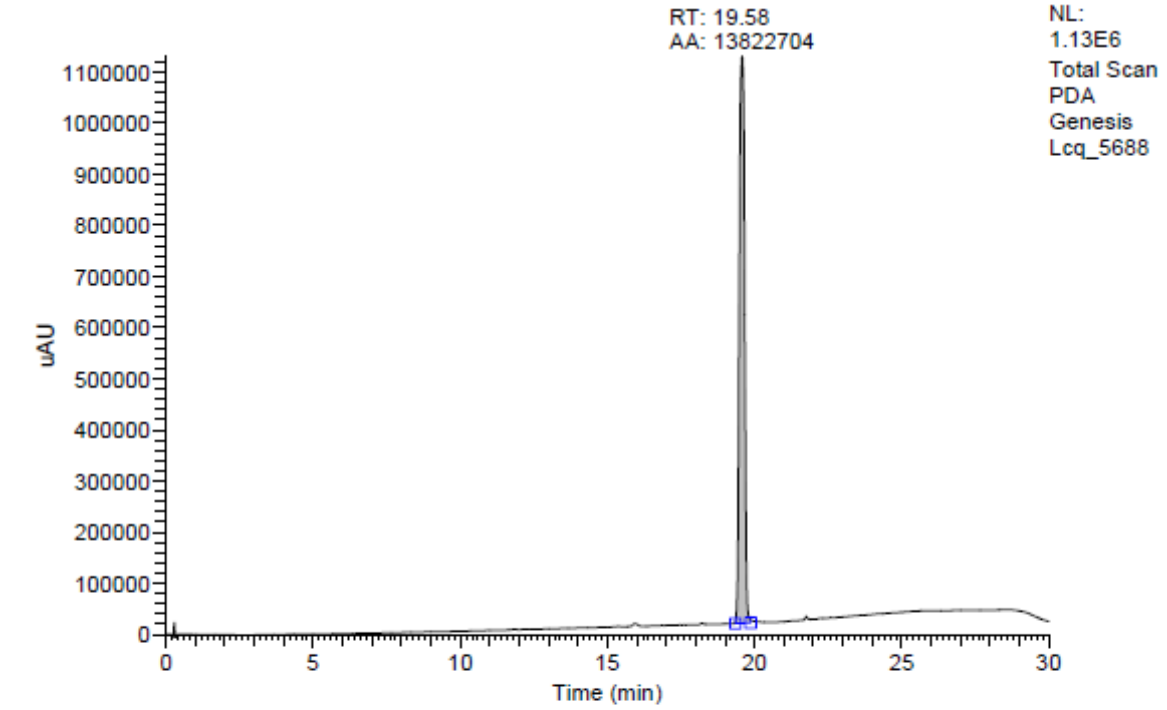
Lcq_5687 #947-987 RT: 15.64-16.20 AV: 41 NL: 2.87E9
T: + c ESI Full ms [150.00-1000.00]



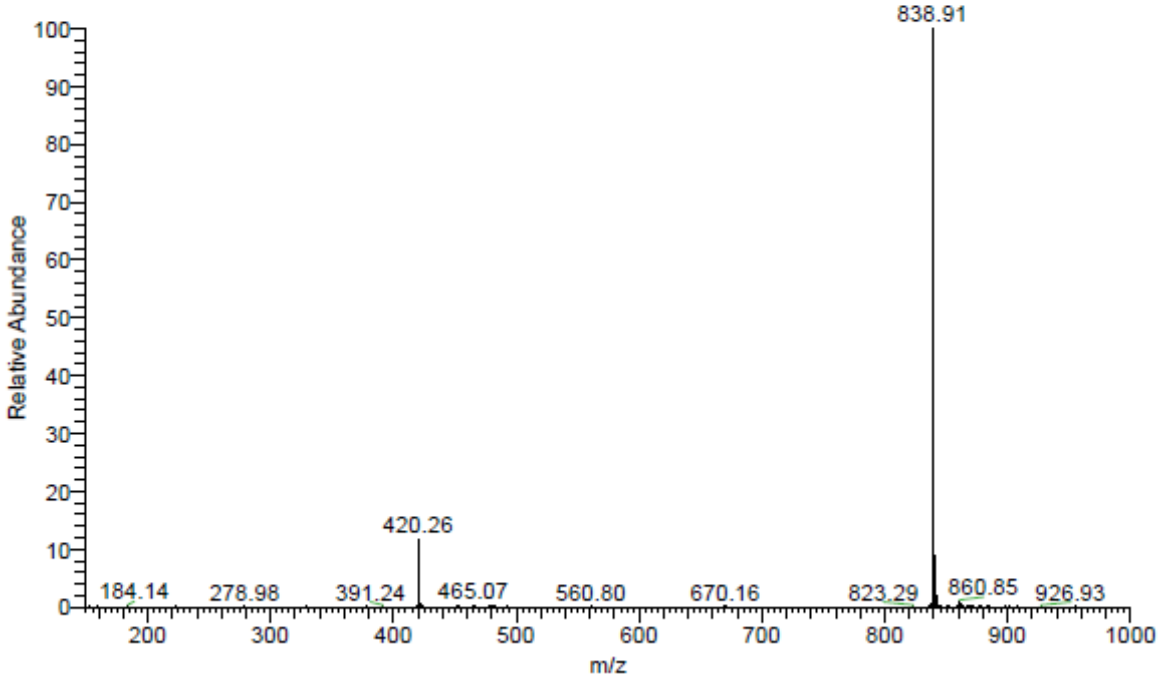
Compound 21


RT: 0.00 - 30.00 SM: 9G
RT: 17.17
AA: 26235748
NL:
3.09E6
Channel A
UV
Genesis
Lcq_5650
RT: 11.44
AA: 1754582
uAU
Time (min)
Lcq_5650 #1037-1071 RT: 16.95-17.40 AV: 35 NL: 2.44E9
T: + c ESI Full ms [150.00-1000.00]

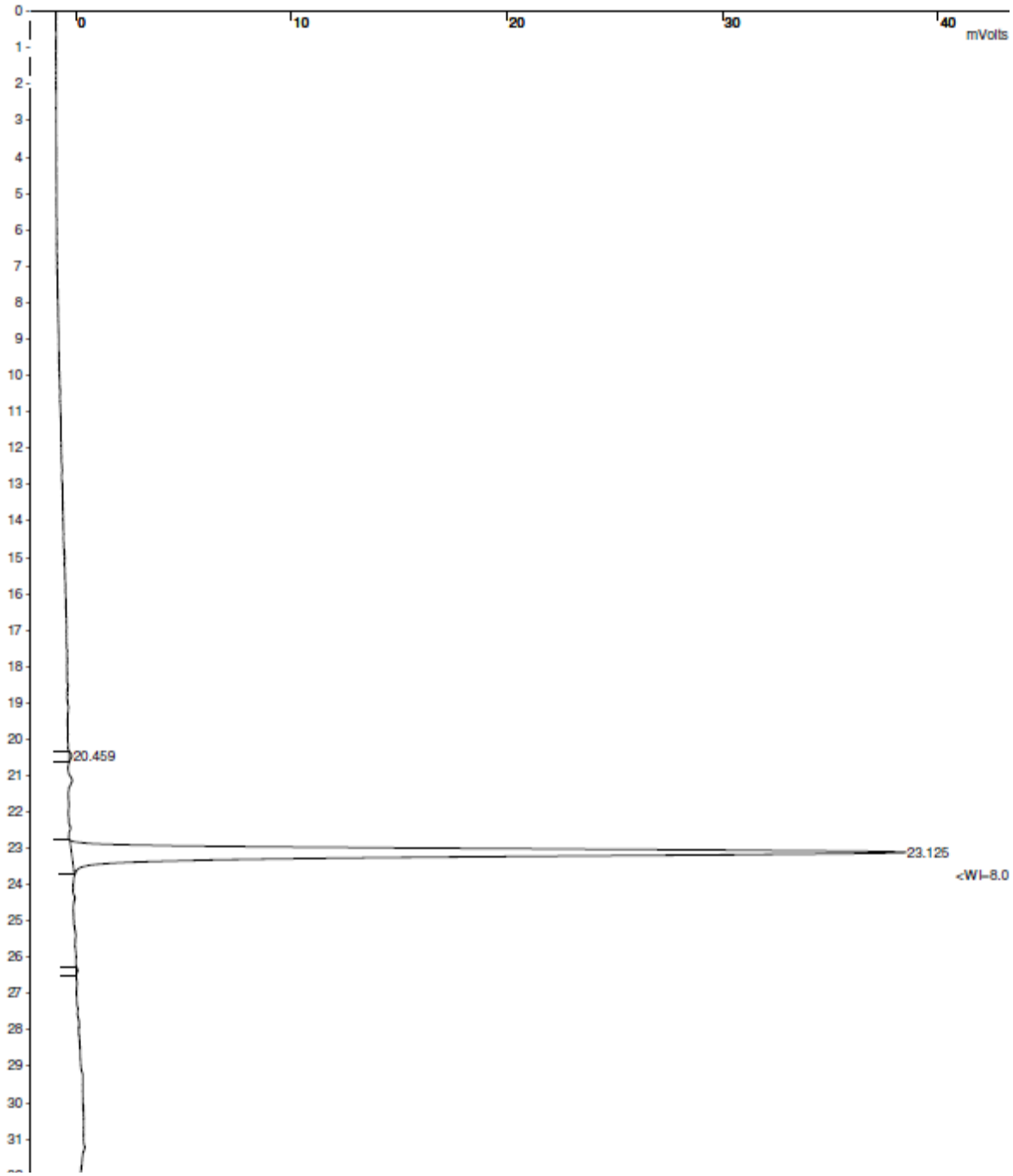
Compound 22



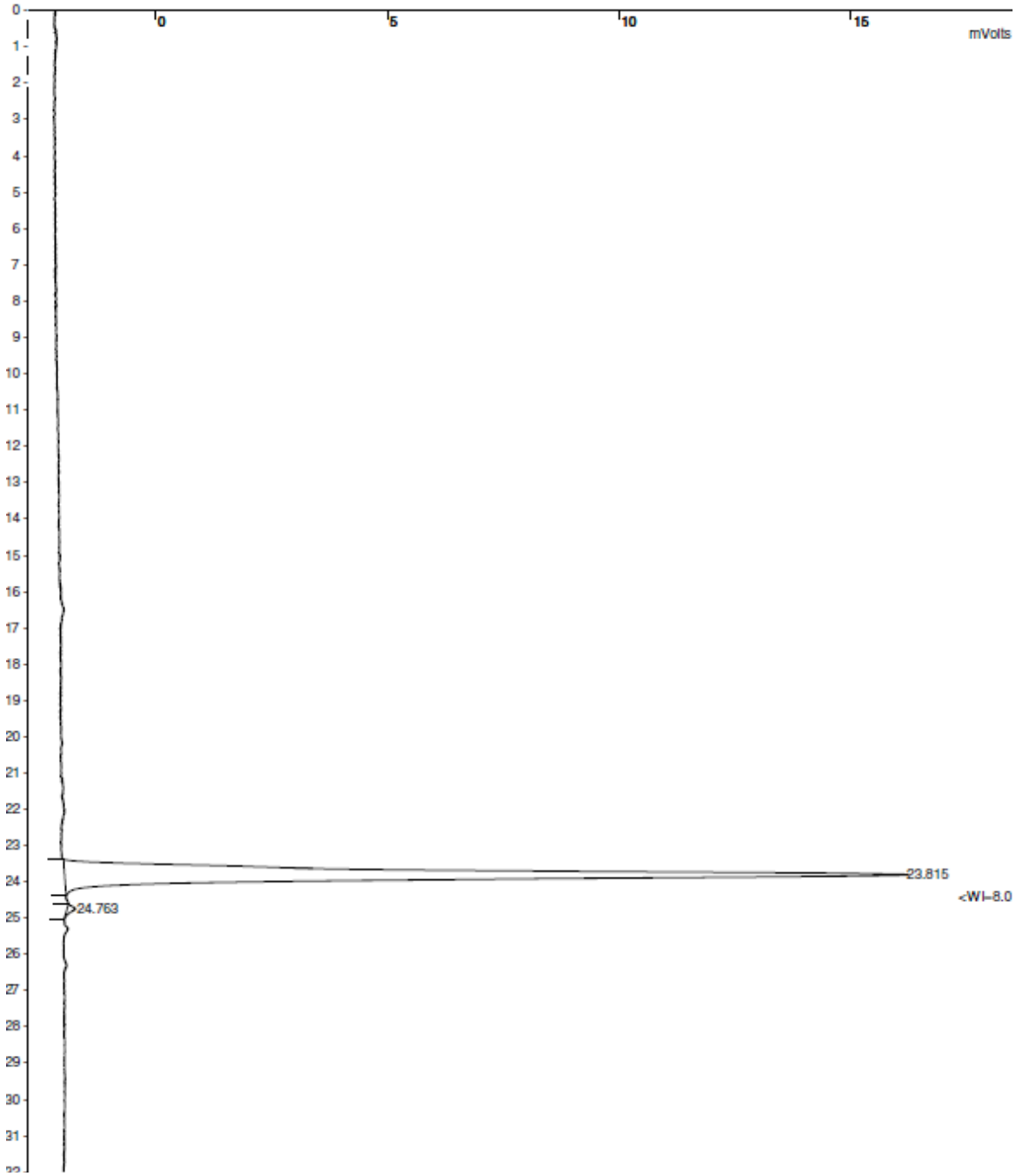
Lcq_5688 #1167-1205 RT: 19.28-19.80 AV: 39 NL: 1.76E9
T: + c ESI Full ms [150.00-1000.00]



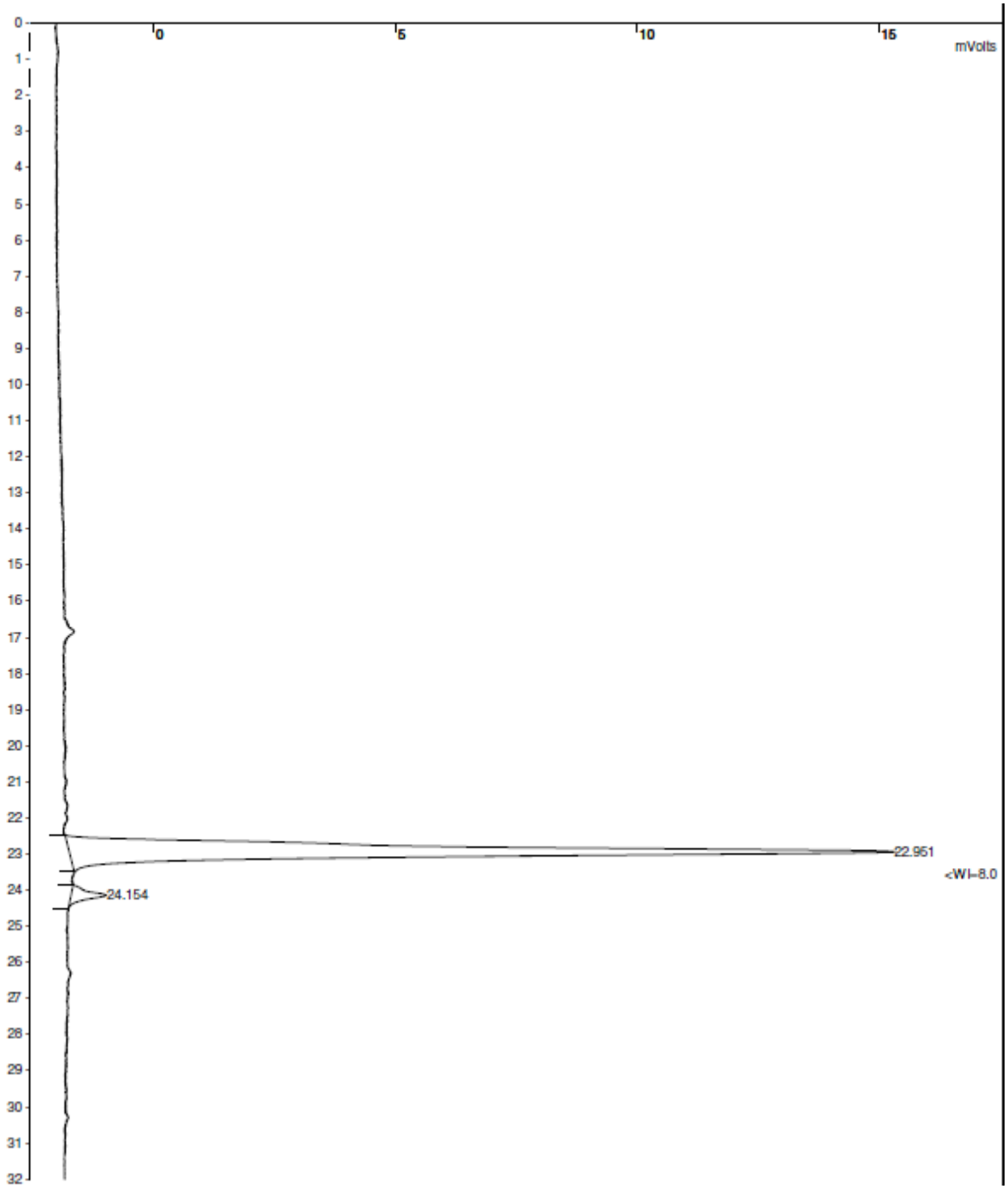
Compound 23



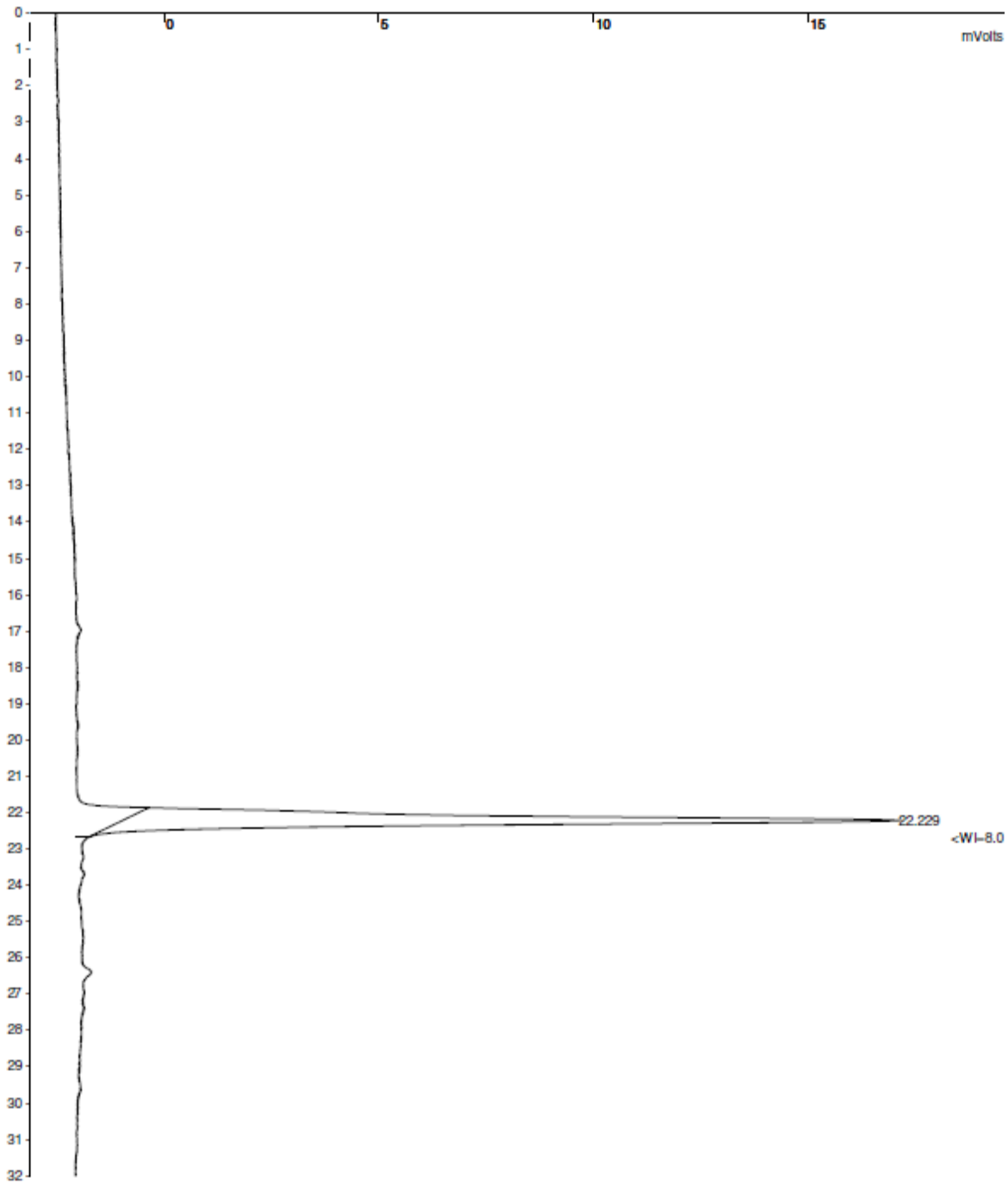
Compound 24



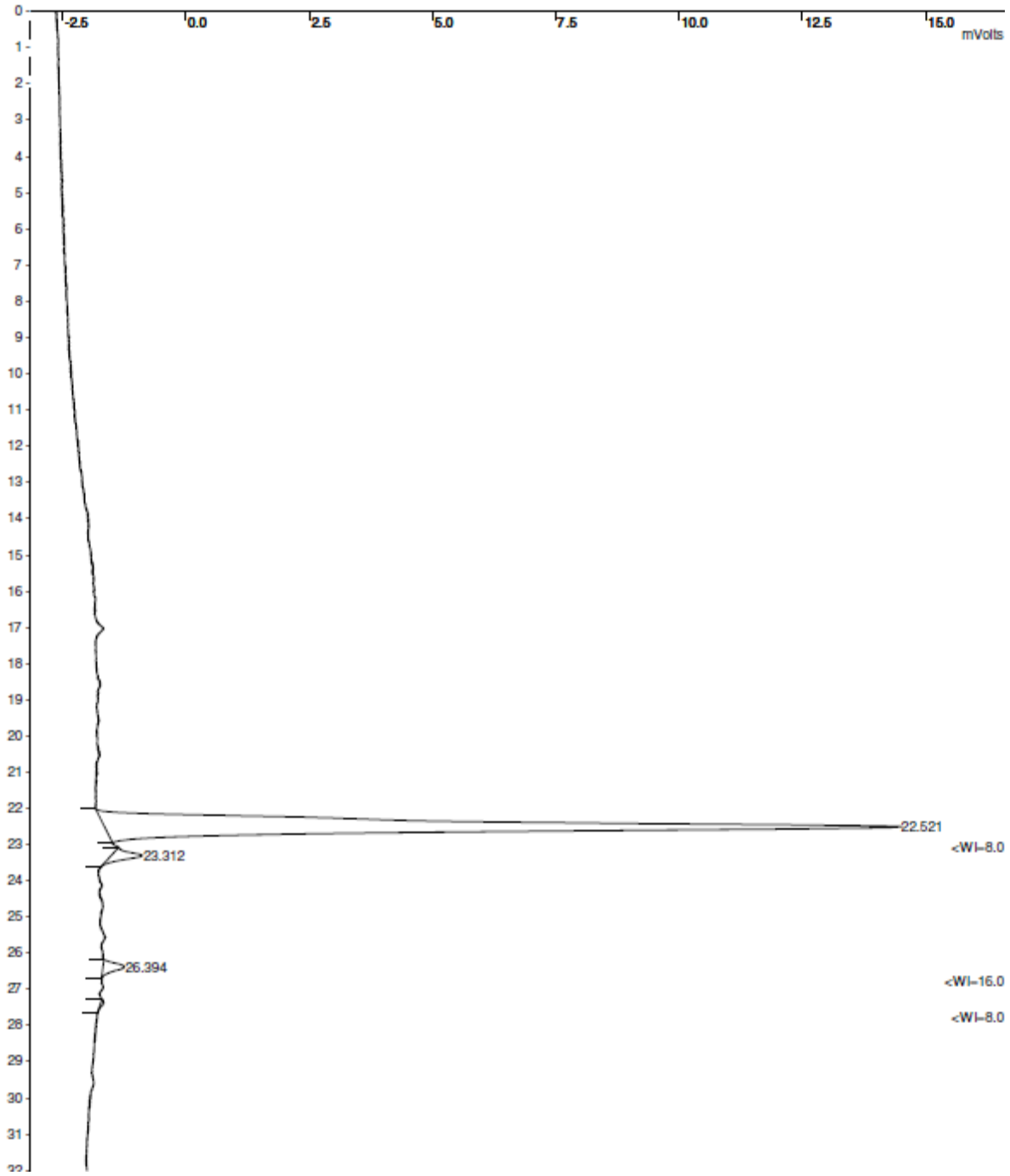
Compound 25



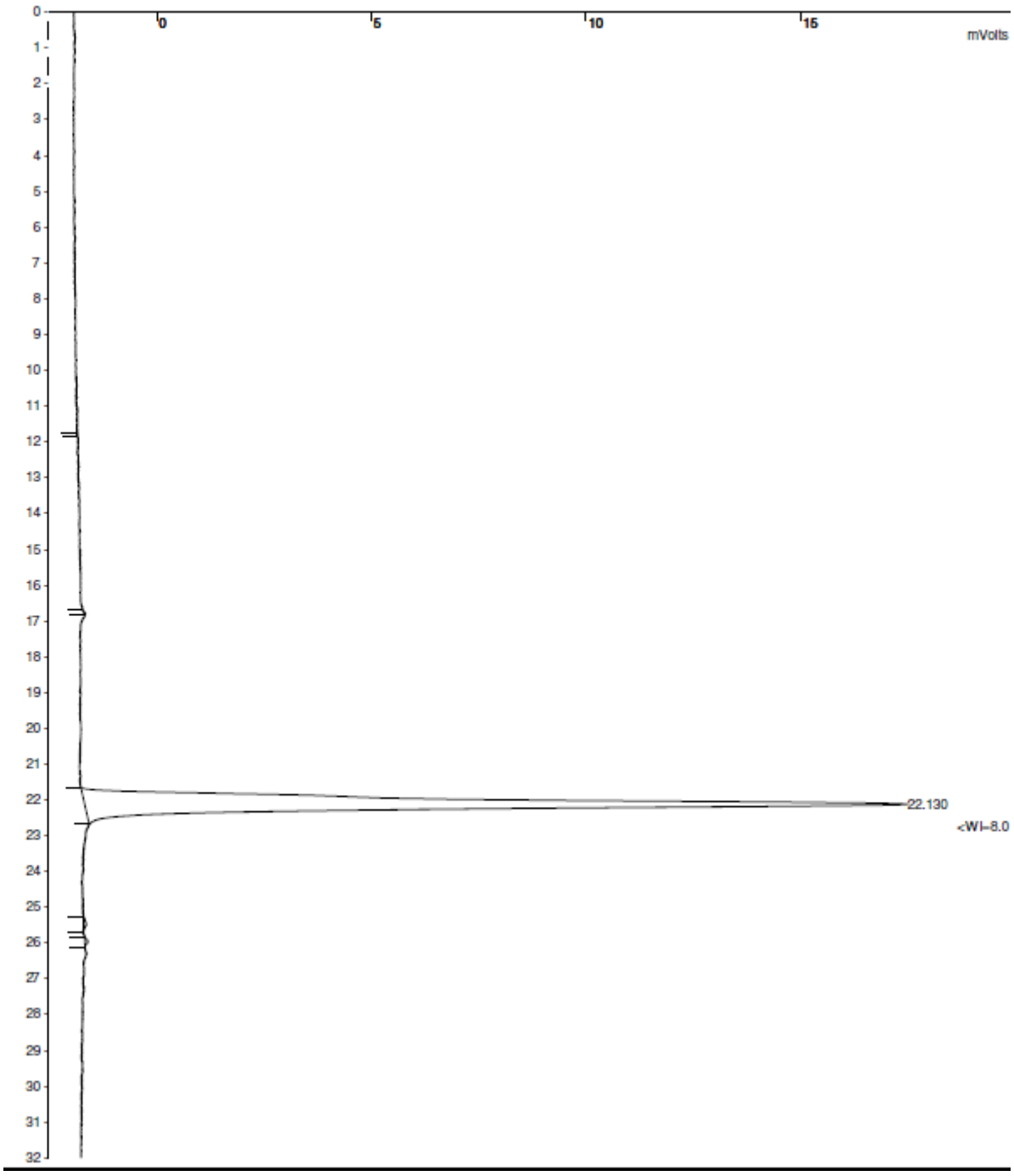
Compound 26



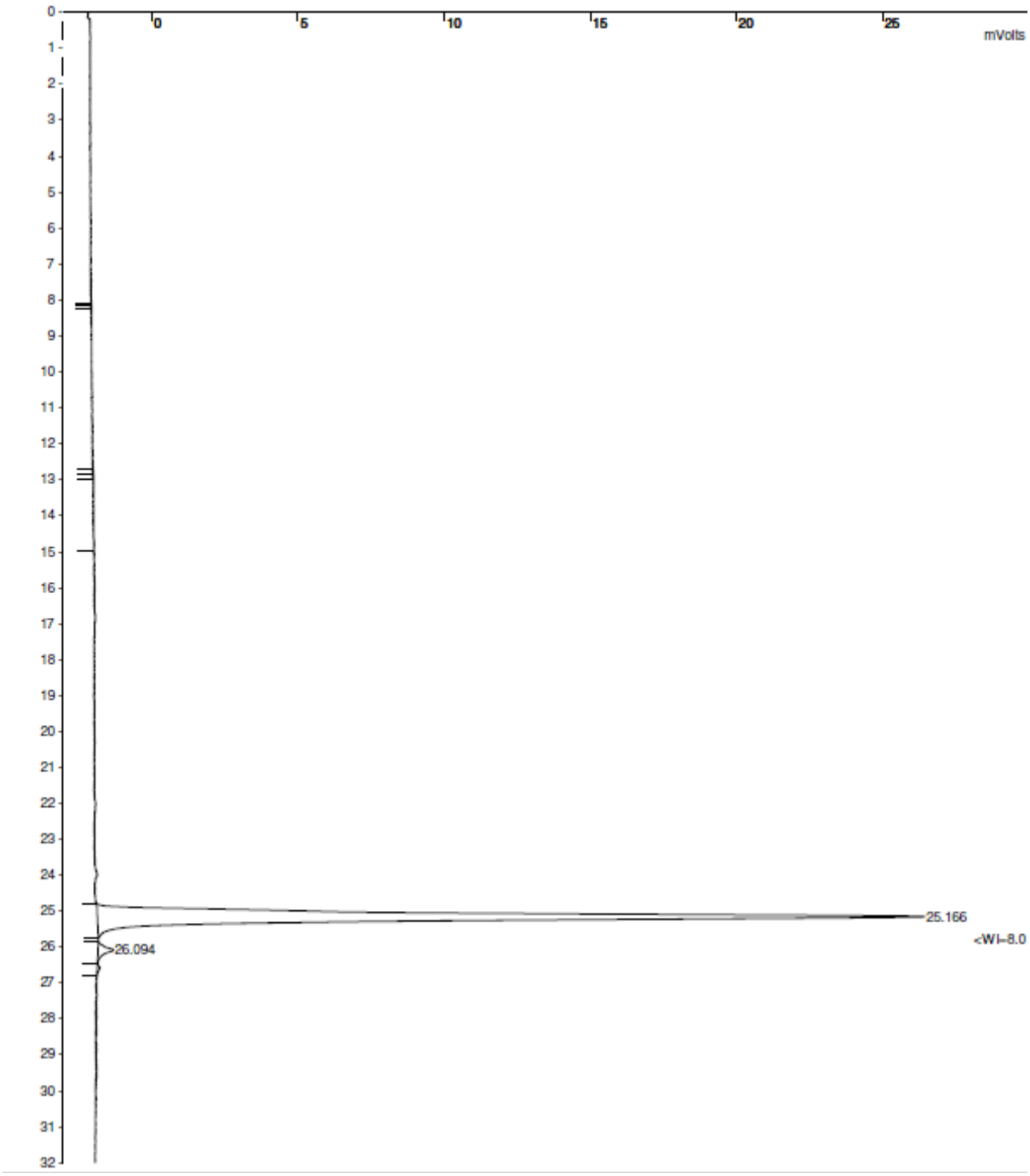
Compound 27


-2.5
0.0
2.5
5.0
7.5
10.0
12.5
15.0
mVolts
22.521
23.312
26.394
<WI=8.0
<WI=16.0
<WI=8.0

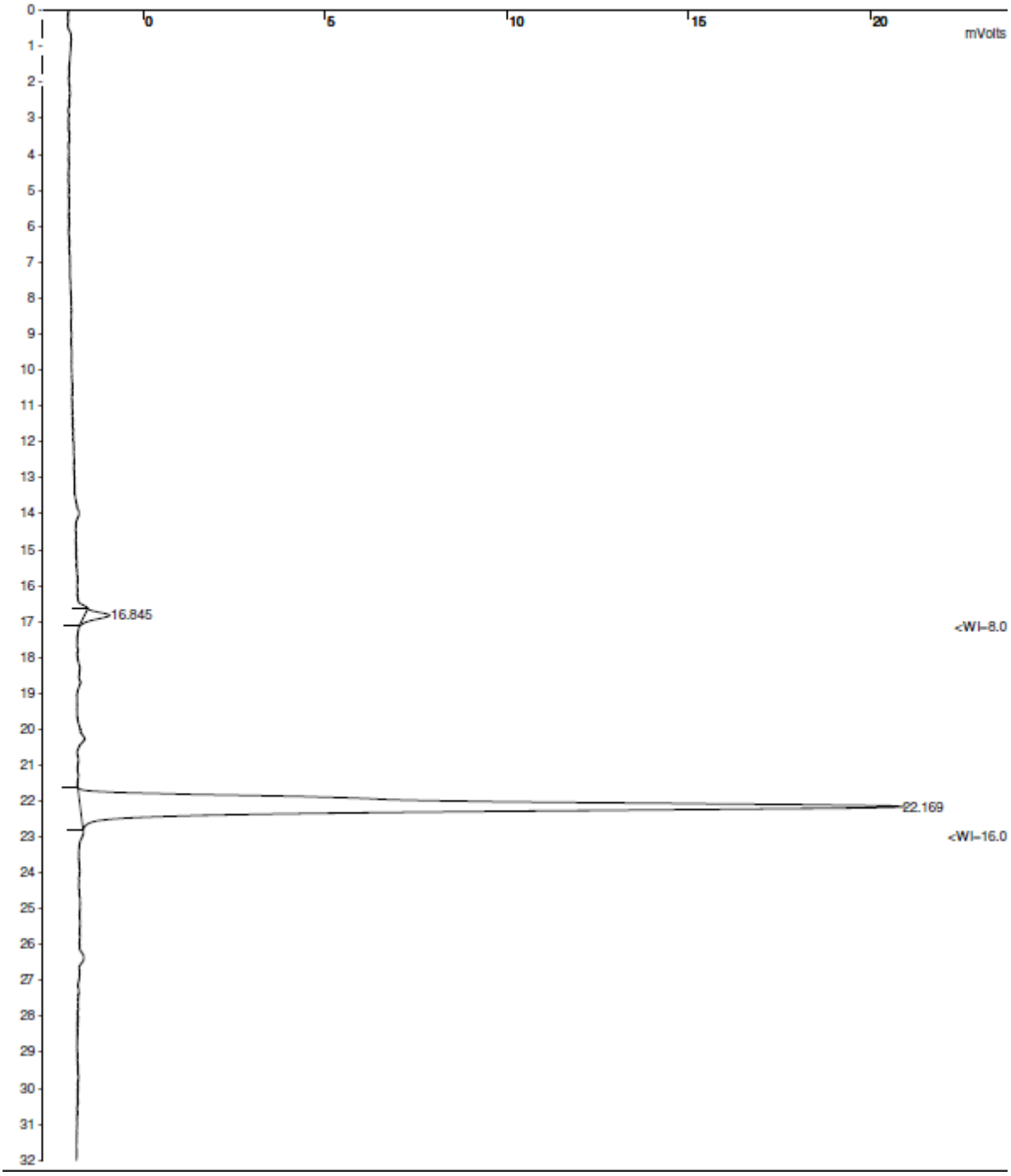
Compound 28



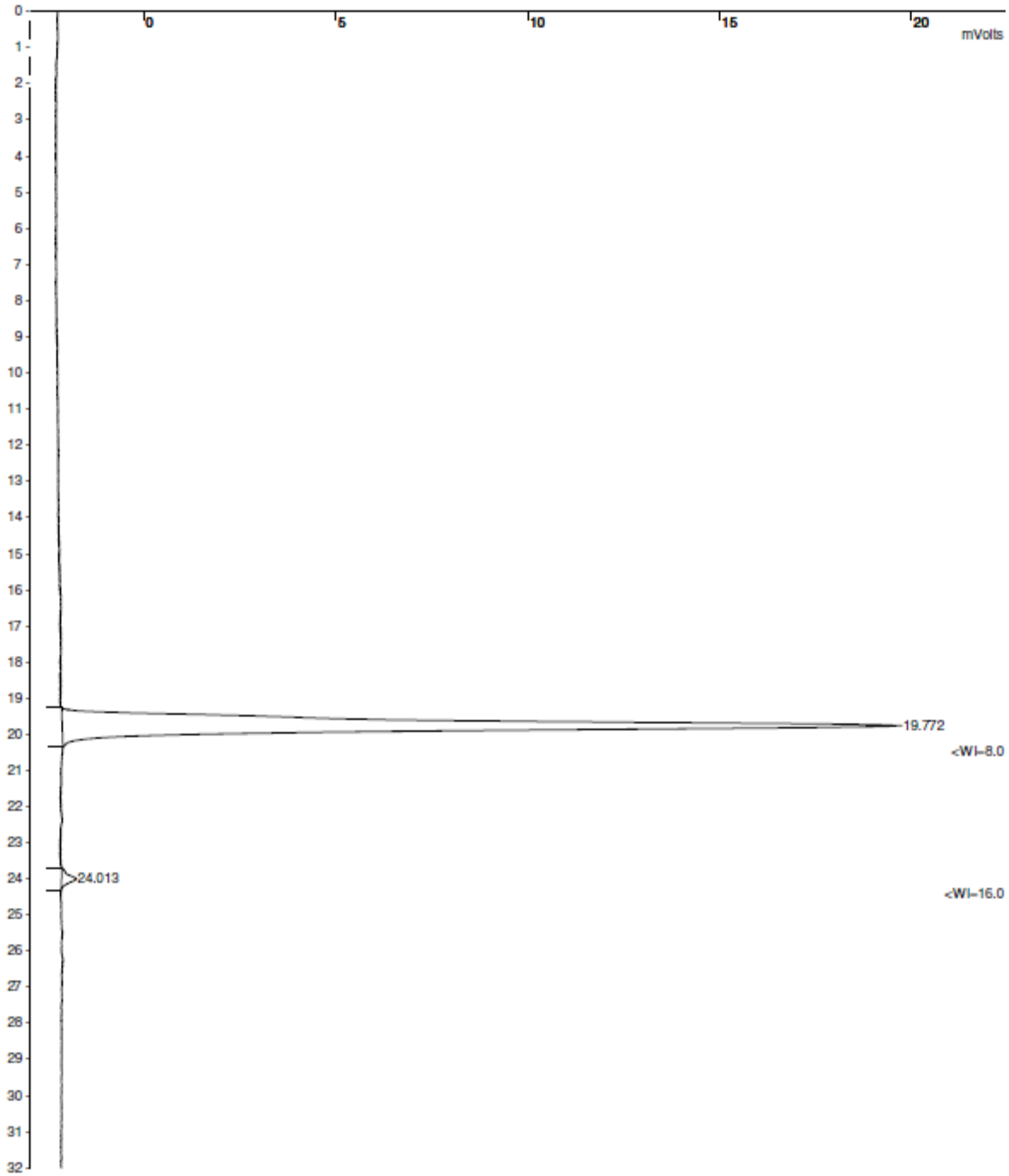
Compound 29



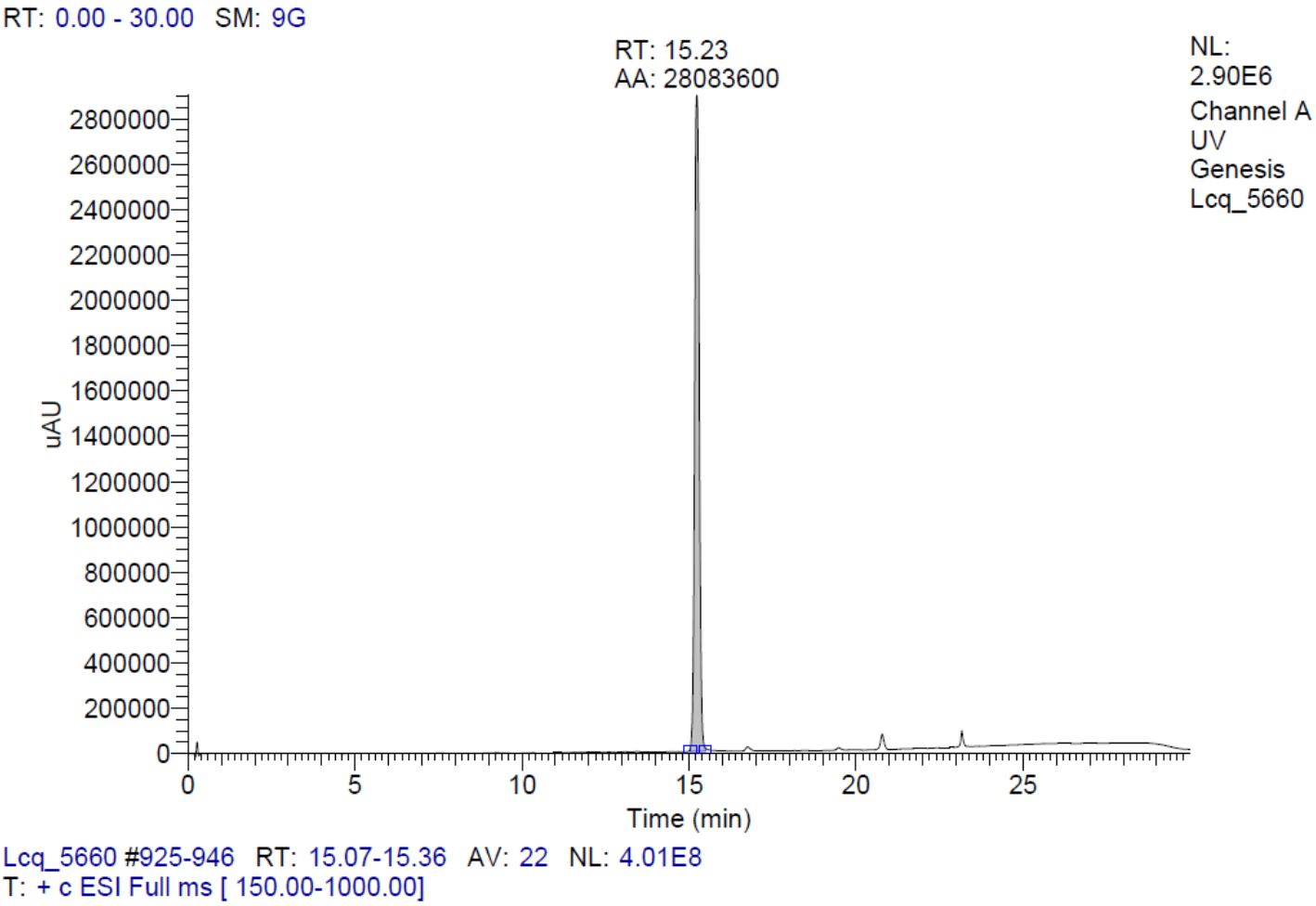
Compound 30



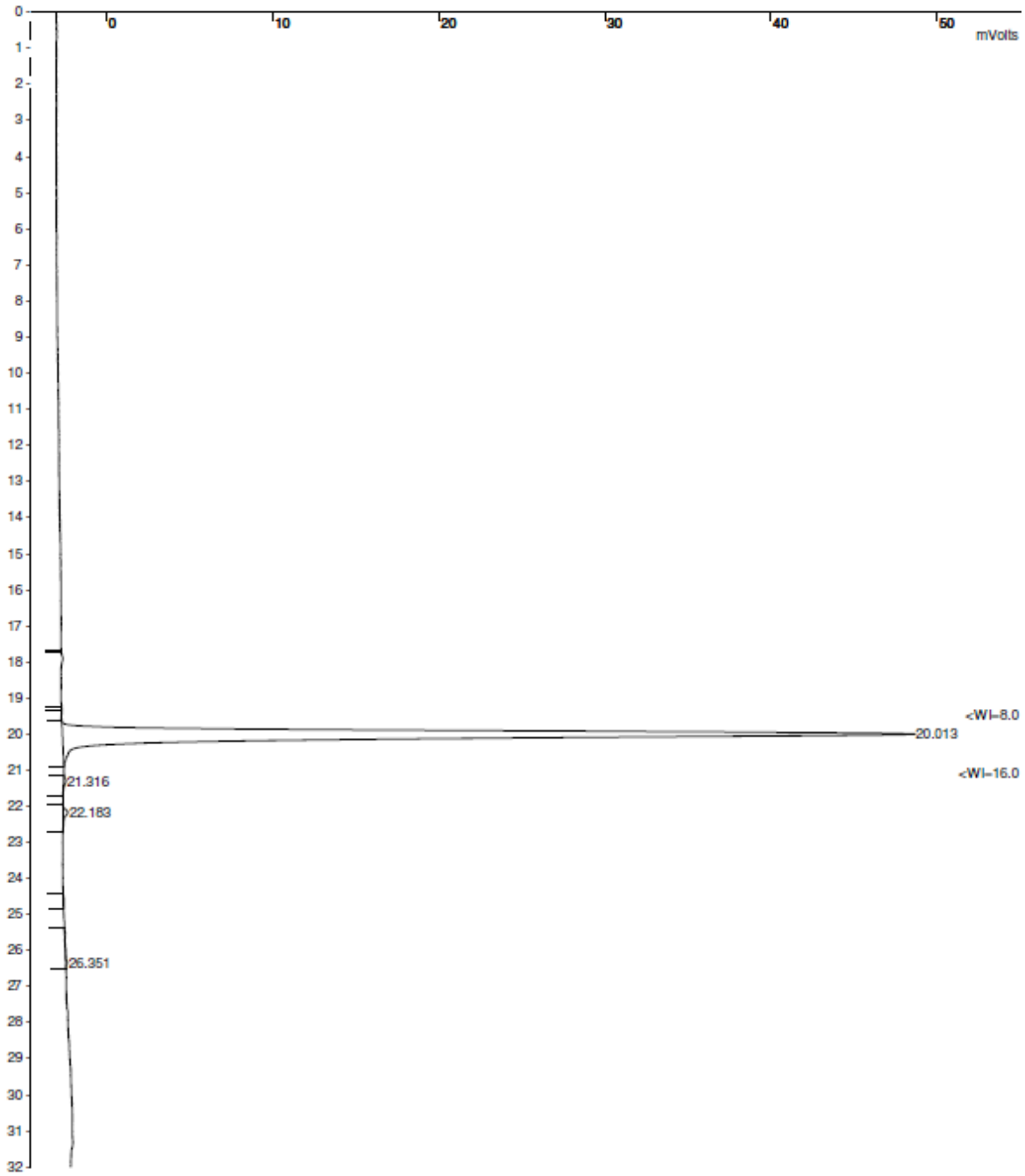
Compound 31



Compound 32


RT: 0.00 - 30.00 SM: 9G
RT: 15.23
AA: 28083600
NL:
2.90E6
Channel A
UV
Genesis
Lcq_5660
uAU
2800000
2600000
2400000
2200000
2000000
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
0
5
10
15
20
25
Time (min)
Lcq_5660 #925-946 RT: 15.07-15.36 AV: 22 NL: 4.01E8
T: + c ESI Full ms [150.00-1000.00]

Compound 33



SI References

1. Mitchison, T. J. Mitchison Lab Protocols. http://mitchison.med.harvard.edu/protocols.html.
2. Jackman, J.; O'Connor, P. M., Methods for Synchronizing Cells at Specific Stages of the Cell Cycle. *Curr. Protoc. Cell Biol.* **2003,** *Unit 8.3*, 11-14.
3. Vichai, V.; Kirtikara, K., Sulforhodamine B colorimetric assay for cytotoxicity screening. *Nat. Protocols* **2006,** *1* (3), 1112-1116.
4. National Center for Biotechnology Information. http://pubchem.ncbi.nlm.nih.gov/.
5. Norinder, U.; Haeberlein, M., Computational approaches to the prediction of the blood-brain distribution. *Adv. Drug Deliv. Rev.* **2002,** *54* (3), 291-313.
6. Ertl, P.; Rohde, B.; Selzer, P., Fast Calculation of Molecular Polar Surface Area as a Sum of Fragment-Based Contributions and Its Application to the Prediction of Drug Transport Properties. *Journal of Medicinal Chemistry* **2000,** *43* (20), 3714-3717.
7. Dothager, R. S.; Putt, K. S.; Allen, B. J.; Leslie, B. J.; Nesterenko, V.; Hergenrother, P. J., Synthesis and identification of small molecules that potently induce apoptosis in melanoma cells through G1 cell cycle arrest. *J. Am. Chem. Soc.* **2005,** *127* (24), 8686-96.