Identification from a Combinatorial Library of a Small Molecule that Selectively Induces Apoptosis in Cancer Cells

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Supporting Information

Materials and methods.

^{1}H and ^{13}C NMR spectra were recorded on Varian Unity400 (400 MHz ^{1}H, 100 MHz ^{13}C) and on Varian Unity500 (500 MHz ^{1}H, 125 MHz ^{13}C) spectrometers in deuterochloroform ($CDCl_3$) or deuteromethanol (CD_3OD). The data is reported as follows: chemical shifts in ppm (δ), multiplicities are indicated as s-singlet; d-doublet; t-triplet; q-quartet; m-multiplet, br - broad. Coupling constants, *J*, are reported in Hz. Infrared spectra were recorded on Perkin Elmer Spectrum BX spectrophotometer, referenced to polystyrene standard, and the peaks reported in cm^{-1}. Mass spectra were recorded by the University of Illinois Mass Spectroscopy Center, and the data reported in *m/e* (intensity to 100%). Analytical thin-layer chromatography was performed on Merk silica gel plated with F254 indicator. The plates were visualized by UV light, Iodine(I_2) and/or CAM stain. Optical rotations were obtained on Jasco DIP-360 Digital Polarimeter. Analytical chiral supercritical fluid chromatography (SFC) was performed on Berger Instruments SFC equipped with Diacel Chiracel OD column (250 x 4.5 mm), internal spectrophotometric detector operated at wavelength 220 nM. Solvents for extraction and chromatography were reagent grade and were used without further purification. Melting points were determined on a Thomas-Hoover Capillary Melting Point Apparatus and are uncorrected. The pH of solutions was measured by Aquemet™ Research AR15 pH meter equipped with Corning semimicrocombo electrode (#476156), which was standardized by 5 buffer solutions (pH=4.0±0.01, 6.0±0.01, 7.0±0.01, 9.0±0.01 and 10.0±0.01) prior to measurements.

Acids **A-G** were purchased from Aldrich and used as received. Acid **H** was prepared by Wadsworth-Emmons olefination following a procedure described in *Organic Synthesis*, CV 5, p. **547** from anisaldehyde, which was purchased from Aldrich. PS-carbodiimide resin was purchased from Argonaut Technologies (1101 Chess Dr. Foster City, CA 94404). *N*-Cbz-1-Aryl-2-aminoethanols were prepared by previously reported procedure.[1] Amines **16, 17, 18** were purchased from Aldrich.

General procedure for asymmetric aminohydroxylation of styrenes

As described for the synthesis of **[(*S*)-2-hydroxy-2-(4-acetoxy-phenyl)-ethyl]-carbamic acid benzyl ester (5)**:

In a 1000 mL round bottom three-neck flask, under an atmosphere of N_2, benzyl carbamate was dissolved (2.849 g, 18.6 mmol) in 120 mL of acetonitrile. The solution was cooled down in a water-ice bath (0-4 °C) and under efficient stirring a cold solution of potassium hydroxide (0.758 g KOH in 80 mL of water) was added. The solution was stirred 2 minutes, then a cold solution of tert-butyl hypochlorite (1.344 g, 12.4 mmol) in 20 mL of acetonitrile was added; the solution was then stirred for 10 min. A solution of potassium osmate (88.9 mg, 0.241 mmol) in 20 mL of cold H_2O was then added. After 2 min a solution of DHQ_2AQN (280.0 mg, 0.326 mmol) in 20 mL of CH_3CN was added, then a solution of 100 mL of cold acetonitrile was added and stirred for 3 min. Potassium phosphate buffer (240 mL, pH=7.57) was added, the cooling bath removed, and the solution allowed to stir for 5 min (the solution turned light green immediately). At this point the pH was checked and if necessary adjusted to pH=7.65±0.02 by addition of a monobasic sodium phosphate solution. A solution of styrene (1.005 mg, 6.2 mmol) in 40 mL of acetonitrile was then added in one portion. The reaction mixture was vigorously stirred at room temperature (21-23 °C). After 45-50 min the solution turns yellow, indicating completion (as verified by TLC disappearance of starting material).

The reaction mixture was cooled to 0-4 °C in an ice bath and 2.04 g of sodium sulfite in 20 mL of water was added. After 15 min of stirring the organic layer was separated and the aqueous layer was extracted with ethyl acetate (3x150 mL). The combined organic extracts were washed with water (2x100 mL) and brine (3x100 mL), and dried over anhydrous magnesium sulfate. Solvent evaporation resulted 4.186 g of amorphous crystals, which was subjected to flash

column chromatography (silica gel, 2:1=Hexane: EtOAc) to yield 1.410 g of **5** (71% isolated yield, 73%ee) as colorless crystals. The material was crystallized from EtOAc/hexane mixture (to a boiling suspension in hexanes (130 mL) EtOAc was added in portions until clear solution is obtained (37 mL)) to afford 0.380 g of colorless plate-like crystals (20%ee) after filtration. The solution obtained after filtration was evaporated and recrystallized as described above second time to yield 0.882 g (43% of theoretical yield) of needle-like crystals (>99%ee), 0.106 g of material remained in solution (>97%ee).

SFC (125psi CO_2, 12%, 3mL/min MeOH, Chiracel OD) 6.951 min (*R*), 7.384 (*S*) $[\alpha]^{25}_D$=29.64901 (c=.895, $CHCl_3$, $[\alpha]^{25}_D$=-16.7102 in MeOH at c=.31) (enantiomer, obtained with $DHQD_2AQN$ ligand, resulted $[\alpha]^{25}_D$=-29.6437 (c=.98, $CHCl_3$)).

The following compounds were synthesized according to the general protocol above on a 6.2 mmol scale; characterization data has been reported previously.[1] Yields below are of the single regioisomer after chromatography, and the enatiomeric ratios were determined after crystallization, as described above.

Table S1. Products of aminohydroxylation. Characterization is as described in reference 1.

	% Yield from aminohydroxylation	er after crystallization	$[\alpha]^{25}$
5 (HO, NHCBz, OAc)	71 %	>99:1	29.64901 (c=.895, $CHCl_3$), 16.7102 (c=.31, MeOH)
5a (HO, NHCbz)	59 %	>99:1	
5b (HO, NHCBz, OCH_3)	65 %	96.3:3.7	26.38912 (c=.52, $CHCl_3$),
5c (HO, NHCBz, OAc)	73 %	>99:1	-29.64347 (c=.895, $CHCl_3$)

General procedure for *O*-methylation of *N*-Cbz-2-amino-1-phenyl-ethanols

As described for the synthesis of **[(*S*)-2-methoxy-2-(4-acetoxy-phenyl)-ethyl]-carbamic acid benzyl ester (6)**:

An oven dried 50 mL round bottom flask, equipped with septum and Teflon coated magnetic stir bar, was charged with **5** (329.5 mg, 1 mmol), evacuated for 15 min at 0.01 mm Hg and flushed with dry N_2. After repeating the cycle three times, freshly distilled THF (15 mL) was added via syringe and the solution cooled in a dry ice-acetone bath (-78 ^oC external, -74 ^oC internal) for 10 minutes. A solution of *n*-butylithium (1.26 mL, 2.02 mmol, 1.6 M in hexanes)

was added via syringe and after stirring for 1 min methyltriflate (237 μL, 344 mg, 2.1 mmol) was added, and the mixture stirred for 1.5 h at -74 °C. The reaction mixture was poured onto 100 mL of cold 1% HCl and extracted with ether (4x30 mL), washed with 5% sodium bicarbonate and brine (2x30 mL), and dried over anhydrous magnesium sulfate. Solvent evaporation resulted in 329.1 mg of an oily substance, which was subjected to column chromatography (silica gel, 4:1=Hexane: EtOAc) to yield 246 mg (71%) of colorless oil **6**.

Yields for methylation reactions:

HO NHCbz, OAc (**5**) → *n*-BuLi, MeOTf, -78 °C, 71 % → MeO NHCbz, OAc (**6**)

HO NHCbz (**5a**) → *n*-BuLi, MeOTf, -78 °C, 89 % → MeO NHCbz (**6a**)

HO NHCbz, OCH_3 (**5b**) → *n*-BuLi, MeOTf, -78 °C, 90 % → MeO NHCbz, OCH_3 (**6b**)

HO NHCbz, OAc (**5c**) → *n*-BuLi, MeOTf, -78 °C, 72 % → H_3CO NHCbz, OAc (**6c**)

Analytical data for alkylated carbamates.

Acetic acid 4-(2-benzyloxycarbonylamino-1-(*S*)-methoxy-ethyl)-phenyl ester (6):

$C_{19}H_{21}NO_5$
Exact Mass: 343.14
Mol. Wt.: 343.37
C, 66.46; H, 6.16; N, 4.08; O, 23.30

Synthesized by general procedure for methylation, above.

HMR ^{1}H (400 MHz, $CDCl_3$) δ ppm:

7.35 (m, 6H); 7.31 (d, 2H, J1=8.6 Hz); 7.08 (d, 2H, J1=8.5 Hz); 5.30 (br. s, 1H); 5.11 (d, 2H, J1=2.7 Hz); 4.28 (dd, 1H, J1=8.6 Hz, J2=3.7 Hz); 3.53 (ddd, 1H, J1=7.8 Hz, J2=4.0 Hz, J3=12.0 Hz); 3.23 (s, 3H); 3.21 (ddd overlaid with singlet, 1H, J1=9.7 Hz, J2=5.1 Hz, J3=13.9 Hz); 2.29 (s, 3H).

NMR ^{13}C (125 MHz, $CDCl_3$) δ ppm:

169.3; 156.3; 150.4; 136.5; 136.4; 128.4; 128.0; 127.6; 121.6; 81.9; 66.6; 56.8; 47.2; 21.0.

R$_f$ =9mm/50mm (EtOAc/Hexanes=25/75); colorless oil
IR (thin film, cm^{-1}): 3342, 3065, 3034, 2984, 2936, 2899, 2825, 1755, 1722, 1606, 1537, 1505, 1455, 1370, 1216, 1201, 1165, 1109, 1075, 1016, 912, 849, 776
MS (FAB): 344.09(M+1, 66.55); 312.09(M-31, 100.00); 268.09(44.44); 222.05(20.64); 179.06(22.31); 164.08(11.89); 155.00(16.87); 152.00(25.53); 137.05(46.16); 118.98(55.43)
HRMS (FAB): 344.1500($C_{19}H_{22}NO_5$, M+1; calc. 344.149798)
SFC (125psi CO_2; 15%, 3mL/min MeOH, Chiracel OD): 3.672 min (R), 3.865 min (S), 1.11:98.89 er, 97.78% ee.

(2-Methoxy-2-phenyl-ethyl)-carbamic acid benzyl ester (6a):

$C_{17}H_{19}NO_3$
Exact Mass: 285.14
Mol. Wt.: 285.34
C, 71.56; H, 6.71; N, 4.91; O, 16.82

Synthesized by general procedure for methylation, above.

HMR ^{1}H (400 MHz, $CDCl_3$) δ ppm: 7.37 (m, 6H); 7.32 (m, 4H); 5.28 (br. s, 1H); 5.12 (d, 2H, J1=2.0 Hz); 4.29 (dd, 1H, J1=8.5 Hz, J2=3.6 Hz); 3.57 (ddd, 1H, J1=7.8 Hz, J2=3.8 Hz, J3=12.0 Hz); 3.28 (ddd overlaid with singlet, 1H, J1=8.7 Hz, J2=4.1 Hz); 3.25 (s, 3H).
NMR ^{13}C (125 MHz, $CDCl_3$) δ ppm: 168.5; 156.3; 138.9; 136.5; 128.5; 128.5; 128.1; 128.1; 126.6; 82.5; 66.6; 56.7; 47.2.
R$_f$ =20mm/50mm (EtOAc/Hexanes=25/75); colorless oil
IR (Thin film, cm^{-1}) 3339, 3063, 3032, 2982, 2936, 2884, 2825, 1714, 1537, 1519, 1455, 1249, 1145, 1109, 1065, 756
MS (FAB): 286.09(M+1, 100.00); 254.07(M-31, 51.24); 210.1(23.17); 164.05(27.7); 134.97(29.82); 118.96(42.44);
HRMS (FAB): 286.1450($C_{17}H_{20}NO_3$, M+1; calc 286.144319)

[2-Methoxy-2-(4-methoxy-phenyl)-ethyl]-carbamic acid benzyl ester (6b):

$C_{18}H_{21}NO_4$
Exact Mass: 315.15
Mol. Wt.: 315.36
C, 68.55; H, 6.71; N, 4.44; O, 20.29

Synthesized by general procedure for methylation, above.

HMR ^{1}H (400 MHz, $CDCl_3$) δ ppm:
7.37 (m, 5H); 7.22 (d, 2H, J1=8.4 Hz); 6.89 (d, 2H, J1=8.6 Hz); 5.21 (br. s, 1H); 5.11 (d, 2H, J1=1.1 Hz); 4.22 (dd, 1H, J1=8.4 Hz, J2=3.9 Hz); 3.81 (s, 3H); 3.53

(ddd, 1H, J1=7.8 Hz, J2=3.9 Hz, J3=12.2 Hz); 3.27 (ddd overlaid with singlet, 1H, J1=8.6 Hz, J2=4.0 Hz, J3=13.3 Hz).

NMR ^{13}C (125 MHz, $CDCl_3$) δ ppm:

159.5; 156.3; 136.5; 130.9; 128.5; 128.1; 127.9; 114.0; 82.0; 66.7; 56.5; 55.2; 47.2.

R_f =14mm/50mm (EtOAc/Hexanes=25/75); colorless oil

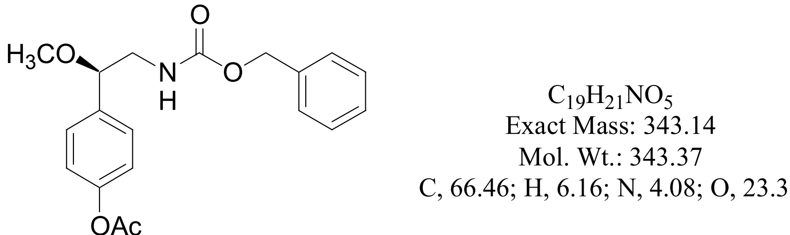
$[\alpha]^{25}_D$=26.638912 (c=.895, $CHCl_3$)

IR (thin film, cm^{-1}): 3377, 3002, 2937, 2840, 1708, 1611, 1523, 1513, 1463, 1444, 1365, 1308, 1262, 1235, 1173, 1113, 1078, 1027, 986, 826, 760

MS (FAB): 316.09(M+1, 8.04); 284.07(M-31, 100.00); 240.1(14.64); 194.06(17.08); 151.06(39.63); 134.99(23.88); 118.97(34.28)

HRMS (FAB): 316.1553($C_{18}H_{22}NO_4$, M+1, calc. 316.154883)

Acetic acid 4-(2-benzyloxycarbonylamino-1-(*S*)-methoxy-ethyl)-phenyl ester (6c):



Synthesized by general procedure for methylation, above.

HMR ^{1}H (400 MHz, $CDCl_3$) δ ppm:

7.36 (m, 5H); 7.31 (d, 2H, J1=6.6 Hz); 7.08 (d, 2H, J1=8.6 Hz); 5.22 (br. s, 1H); 5.11 (d, 2H, J1=1.3 Hz); 4.28 (dd, 1H, J1=8.5 Hz, J2=4.0 Hz); 3.54 (ddd, 1H, J1=7.8 Hz, J2=4.0 Hz, J3=12.0 Hz); 3.24 (s, 3H); 3.21 (ddd overlaid with singlet, 1H, J1=9.7 Hz, J2=5.4 Hz, J3=13.9 Hz); 2.30 (s, 3H).

NMR ^{13}C (100 MHz, $CDCl_3$) δ ppm:

169.4; 156.3; 150.4; 136.5; 136.4; 128.5; 128.1; 127.7; 121.7; 82.0; 66.7; 56.9; 47.3; 21.1.

R_f =9mm/50mm (EtOAc/Hexanes=25/75); colorless oil

$[\alpha]^{25}_D$=-29.6437 (c=.98, $CHCl_3$)

IR (thin film, cm^{-1}): 3342, 3065, 3034, 2984, 2937, 2899, 2825, 1755, 1722, 1606, 1531, 1505, 1455, 1370, 1217, 1203, 1165, 1110, 1074, 1016, 912, 849, 776

MS (FAB): 344.09(M+1, 56.86); 312.05(M-31, 100.00); 268.09(60.67); 222.05(20.90); 179.05(33.58); 167.02(18.52); 165.00(18.90); 155.00(36.59); 151.97(42.26); 137.05(62.70); 118.98(72.01)

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

SFC (125psi CO_2; 8%, 3mL/min MeOH, Chiracel OD): 7.83 min (R), >99% ee.

Procedure and Analytical data for hydrolysis of 6 and 6c:

[2-(4-Hydroxy-phenyl)-2-(*S*)-methoxy-ethyl]-carbamic acid benzyl ester (6d):

To a solution of **6** (160 mg, 0.46 mmol) in EtOH (5 mL) 0.568 g of a freshly prepared solution of potassium hydroxide (253.2 mg in 5.013 g H_2O, 0.045 M) was added. The mixture

was stirred at 23-25 °C for 1h (completion monitored by TLC) and neutralized with 0.1% HCl (neutral by pH indicator paper). Solvent was evaporated *in vacuo* and the residue dissolved in ethyl acetate and flash filtered through a pad of silica gel to remove inorganic impurities. The silica gel was washed with ethyl acetate, fractions combined and solvent evaporated *in vacuo* to afford 136 mg (97%) of **6d**.

6d

$C_{17}H_{19}NO_4$
Exact Mass: 301.13
Mol. Wt.: 301.34
C, 67.76; H, 6.36; N, 4.65; O, 21.24

HMR ^{1}H (500 MHz, $CDCl_3$) δ ppm:
7.46 (br. s, 1H); 7.35 (m, 5H); 7.11 (d, 2H, J1=8.3 Hz); 6.83 (d, 2H, J1=8.3 Hz); 5.47 (br. s, 1H); 5.13 (s, 2H); 4.21 (dd, 1H, J1=8.6 Hz, J2=3.7 Hz); 3.53 (ddd, 1H, J1=7.7 Hz, J2=3.9 Hz, J3=12.1 Hz); 3.27 (ddd overlaid with singlet, 1H, J1=8.7 Hz, J2=4.1 Hz, J3=13.3 Hz); 3.20 (s, 3H).
NMR ^{13}C (125 MHz, $CDCl_3$) δ ppm:
156.8; 156.4; 136.2; 129.8; 128.4; 128.1; 128.0; 127.9; 115.5; 82.0; 66.9; 56.4; 47.2.
R_f =14mm/50mm (EtOAc/Hexanes=25/75);

[2-(4-Hydroxy-phenyl)-2-(*R*)-methoxy-ethyl]-carbamic acid benzyl ester (6e):

To a solution of **6c** (132 mg, 0.35 mmol) in EtOH (5 mL) 0.469 g of a freshly prepared solution of potassium hydroxide (253.2 mg in 5.013 g H_2O, 0.045M, 1.1eq.) was added. The mixture was stirred at 23-25 °C for 1h (completion monitored by TLC) and neutralized with 0.1% HCl (neutral by pH indicator paper). Solvent was evaporated *in vacuo* and the residue dissolved in ethyl acetate and flash filtered through a pad of silica gel to remove inorganic impurities. The silica gel was washed with ethyl acetate, fractions combined and solvent evaporated *in vacuo* to afford 107 mg (92%) of **6e**.

6e

$C_{17}H_{19}NO_4$
Exact Mass: 301.13
Mol. Wt.: 301.34
C, 67.76; H, 6.36; N, 4.65; O, 21.24

HMR ^{1}H (400 MHz, $CDCl_3$) δ ppm:
7.35 (m, 5H); 7.17 (br. s, 1H); 7.10 (d, 2H, J1=8.3 Hz); 6.82 (d, 2H, J1=8.3 Hz); 5.42 (br. s, 1H); 5.13 (s, 2H); 4.20 (dd, 1H, J1=8.6 Hz, J2=3.7 Hz); 3.52 (ddd, 1H, J1=7.7 Hz, J2=3.7 Hz, J3=12.1 Hz); 3.26 (ddd overlaid with singlet, 1H, J1=8.7 Hz, J2=4.1 Hz, J3=13.3 Hz); 3.20 (s, 3H).
NMR ^{13}C (100 MHz, $CDCl_3$) δ ppm: 156.8; 156.3; 136.2; 130.0; 128.5; 128.2; 128.1; 128.0; 115.5; 82.1; 66.9; 56.4; 47.3.
R_f =14mm/50mm (EtOAc/Hexanes=25/75);

Compounds **6f** and **6g** (see Table S2, below) were obtained by the hydrolysis of **5** and **5c**, respectively. This hydrolysis was performed exactly as described for **6d**, above.

General procedure for deprotection of Cbz group

As described the synthesis of ***(S)*-4-(2-Amino-1-methoxy-ethyl)-phenol (9):**

5% Pd/C (48 mg, 2.4 mg Pd, 1.5 wt.%) was loaded in a 25 mL tube-shaped flask, equipped with a magnetic stirrer, a hydrogen balloon and a vacuum outlet. The flask was evacuated for 5 min at 0.001 mm Hg and flushed with H_2. After repeating the cycle 4 times the catalyst was suspended in EtOH (4 mL) and **6d** (136.0 mg, 0.45 mmol), was added as a solution in EtOH (2 mL) and the mixture was stirred under hydrogen atmosphere for 8h. Upon completion (monitored by TLC by disappearance of the starting material), the solution was filtered with celite. The celite was washed with EtOH (3x3 mL) and solvent was removed in vacuum and the residue dried at 20-23 ^oC for 1h at 0.01 mm Hg to give 73.6 mg of a crystalline substance (Table S2). The presence of the amine was verified by ninhydrin stain and the product was used in the next step for coupling. Due to relative instability of the amine products, they were immediately coupled to the appropriate acid, and were not characterized. For the library synthesis, all Cbz deprotections were performed in parallel, and the amines were then used immediately to create the library.

Table S2. Synthesis of amine building blocks by deprotection of carbamates.

5% Pd/C, EtOH

1 atm H_2, RT

Entry	Carbamate	Loaded, mg	Pd, wt%	Amine product	yield, %
1.	**6a**	160.0	1.4	**8**	54.3%
2.	**5a**	132.5	1.51	**12**	88%
3.	**6d**	136	1.39	**9**	97%
4.	**6f**	144	1.42	**13**	85%
5.	**6e**	98	1.46	**11**	85%
6.	**6g**	142.1	1.49	**15**	87%
7.	**6b**	132	1.48	**10**	94%
8.	**5b**	134	1.46	**14**	86%

Procedure and Analytical data for amides (^{1}H and ^{13}C spectra follow)

Amides were prepared by both solution and solid phase couplings of appropriate amine and acid building blocks. Solution phase reaction was generally used for scale up reactions, but required more tedious chromatography to purify the products. The ^{1}H and ^{13}C spectra are below.

3-(4-Hydroxy-3-(*S*)-methoxy-phenyl)-N-[2-(4-hydroxy-phenyl)-2-methoxy-ethyl]-acrylamide (1, 9-D): METHOD 1—solid-phase coupling. An oven dried 15 mL round bottom flask, equipped with a Teflon-coated magnetic stir bar, was charged with PS-CDI (45 mg, 0.0576 mmol, 2eq. 1.28 mmol/g), evacuated for 15 min at 0.01 mm Hg and flushed with dry N_2. After repeating the cycle three times, freshly distilled THF (5 mL) was added via syringe followed by a solution of ferulic acid (6.0 mg, 0.031 mmol, 1.1 eq) in THF (0.4 mL). Compound **9** (4.7 mg, 0.028 mmol, 1 eq.) was added as solution in THF (0.5 mL) and the mixture is stirred at 20-22 ^{o}C for 6-8h. The beads were filtered off and washed with THF (3x1 mL). The organic washes were combined and solvent evaporated to afford 9.1 mg of oily substance, which was subjected to column chromatography (silica gel, 1:7=Hexane:EtOAc) to yield 3.3 mg (37 %) of **9-D** as an amorphous substance.

3-(4-Hydroxy-3-(*S*)-methoxy-phenyl)-N-[2-(4-hydroxy-phenyl)-2-methoxy-ethyl]-acrylamide (1, 9-D): METHOD 2—solution-phase coupling. An oven dried 25 mL round bottom flask, equipped with a Teflon coated magnetic stir bar, was evacuated for 15 min at 0.01 mm Hg and flushed with dry N_2. After repeating the cycle three times, the flask was charged with dicyclohexylcarbodiimide (19.5 mg, 0.095 mmol, as a solution in THF, 2.1 mL) followed by a solution of **D** (18.3 mg, 0.095 mmol per 2.1 mL) in THF (2.1 mL). Amine **9** (15.1 mg, 0.09 mmol) was added as a solution in THF/DMF (8:1, 2.1 mL) and the mixture was stirred at 20-22 ^{o}C for 10 h. Upon completion (monitored by TLC, by disappearance of the amine) solvent evaporation resulted 52.1 mg of a viscous oil. The mixture then was subjected to column chromatography (silica gel, 1:4=Hexane: EtOAc) to yield 21.4 mg (71%) of **9-D** as a light-yellow viscous oil.

$C_{19}H_{21}NO_5$
Exact Mass: 343.14
Mol. Wt.: 343.37
C, 66.46; H, 6.16; N, 4.08; O, 23.30

NMR ^{13}C (125 MHz, CD_3OD) δ ppm:

7.43 (d, 1H, J1=15.9 Hz); 7.17 (app. dt, 2H, J1=6.4 Hz, J2=1.7 Hz); 7.12 (d, 1H, J1=1.6 Hz); 7.03 (dd, 1H, J1=8.1 Hz, J2=1.7 Hz); 6.79 (d, 1H, J1=1.7 Hz); 6.79 (d, 1H, J1=2.4 Hz); 6.78 (d, 1H, J1=1.9 Hz); 6.47 (d, 1H, J1=15.9 Hz); 4.24 (dd, 1H, J1=8.6 Hz, J2=4.5 Hz); 3.87 (s, 3H); 3.52 (dd, 1H, J1=13.7 Hz, J2=4.5 Hz); 3.40 (dd, 1H, J1=13.7 Hz, J2=8.6 Hz); 3.20 (s, 3H).

NMR ^{13}C (125 MHz, CD_3OD) δ ppm:

169.2; 158.6; 149.9; 149.3; 142.3; 131.4; 129.2; 128.3; 127.1; 123.3; 118.7; 116.5; 116.3; 111.5; 83.3; 56.8; 56.4; 47.1.

R*f* =14mm/50mm (EtOAc/Hexanes=75/25);
$[\alpha]^{25}_{D}$= -28.8805 (c=1.23, MeOH)
IR (KBr, cm^{-1}): 3398, 2937, 2826, 1654, 1648, 1596, 1515, 1458, 1271, 1254, 1209, 1032, 837
MS (FAB): 344.2(M+1), 312.1(M-31)
HRMS (FAB): 344.1500($C_{19}H_{22}NO_5$, M+1; calc. 344.149798)
MS (ESI) (m/z): 708.85(2M+Na, 6.60), 343.84(M+1, 100.00), 312.05(M-31, 43.11)
UV (PDA; λ_{max}, nm): 225, 295, 320;

3-(4-Hydroxy-3-methoxy-phenyl)-*N*-(2-hydroxy-2-phenyl-ethyl)-acrylamide (12-D): An oven dried 50 mL round bottom flask, equipped with a Teflon coated magnetic stir bar, was evacuated for 15 min at 0.01 mm Hg and flushed with dry N_2. After repeating the cycle three times, the flask was charged with dicyclohexylcarbodiimide (59.3 mg, 0.287 mmol, as a solution in THF, 5 mL) followed by a solution of **D** (53.4 mg, 0.275 mmol) in THF (5 mL). Amine **12** (34.3 mg, 0.25 mmol) was added as solution in THF/DMF (8:1, 5 mL) and the mixture was stirred at 20-22 ^{o}C for 10h. Upon completion (monitored by TLC by disappearance of the amine) solvent evaporation resulted 146.1 mg of viscous oily substance. The mixture then was subjected to column chromatography (silica gel, 1:4=Hexane: EtOAc) to yield 32.1 mg (42%) of **12-D** as a viscous oil.

HO, O, N, H, OCH_3, OH

$C_{18}H_{19}NO_4$
Exact Mass: 313.13
Mol. Wt.: 313.35
C, 68.99; H, 6.11; N, 4.47; O, 20.42

HMR ^{1}H (CD_3OD) δ ppm:
7.43 (d, 1H, J1=15.6 Hz); 7.40 (d, 1H, J1=1.5 Hz); 7.38 (s, 1H); 7.31 (app. t, 2H, J1=7.8 Hz); 7.24 (app. tt, 1H, J1=7.2 Hz, J2=2.2 Hz); 7.10 (d, 1H, J1=2.0 Hz); 7.02 (dd, 1H, J1=8.0 Hz, J2=1.7 Hz); 6.78 (d, 1H, J1=8.2 Hz); 6.45 (d, 1H, J1=15.7 Hz).
NMR ^{13}C (125 MHz, CD_3OD) δ ppm:
169.5; 149.9; 149.3; 144.0; 142.3; 129.4; 128.6; 128.2; 127.2; 123.3; 118.5; 116.4; 111.5; 73.7; 56.3; 48.4.
R*f* =15.5mm/50mm (EtOAc/Hexanes=75/25);
MS (FAB): 314.1(M+1)
HRMS (FAB): 314.1393($C_{18}H_{20}NO_4$, M+1; calc. 314.139233)
MS (ESI) (m/z): 313.93(M+1, 100.00), 296.15(M-17, 7.54)
UV (PDA; λ_{max}, nm): 220, 295, 320;

***N*-[2-(*S*)-Hydroxy-2-(4-hydroxy-phenyl)-ethyl]-3-(4-hydroxy-3-methoxy-phenyl)-acrylamide (13-D):** An oven dried 50 mL round bottom flask, equipped with a Teflon coated magnetic stir bar, was evacuated for 15 min at 0.01 mm Hg and flushed with dry N_2. After repeating the cycle three times, the flask was charged with dicyclohexylcarbodiimide (59.3 mg, 0.287 mmol, as a solution in THF, 5 mL) followed by a solution of **D** (53.4 mg, 0.275 mmol) in THF (5 mL). Amine **13** (38.3 mg, 0.25 mmol) was added as solution in THF/DMF (8:1, 5 mL) and the mixture was stirred at 20-22 ^{o}C for 10h. Upon completion (monitored by TLC by

disappearance of the amine) solvent evaporation resulted 147 mg of a viscous oily substance. The crude reaction mixture then was subjected to column chromatography (silica gel, 1:4=Hexane: EtOAc) to yield 45 mg (56%) of **13-D** as a viscous oil.

$C_{18}H_{19}NO_5$
Exact Mass: 329.13
Mol. Wt.: 329.35
C, 65.64; H, 5.81; N, 4.25; O, 24.29

HMR ^{1}H (CD_3OD) δ ppm:

7.42 (d, 1H, J1=15.6 Hz); 7.20 (app. dt, 2H, J1=8.4 Hz); 7.09 (d, 1H, J1=1.7 Hz); 7.01 (dd, 1H, J1=8.3 Hz, J2=2.0 Hz); 6.75 (app. t, 3H, J1=8.5 Hz); 6.45 (d, 1H, J1=15.8 Hz); 4.71 (dd, 1H, J1=7.7 Hz, J2=4.8 Hz); 3.85 (s, 3H); 3.53 (dd, 1H, J1=13.3 Hz, J2=4.8 Hz); 3.43 (dd, 1H, J1=13.5 Hz, J2=7.8 Hz).

NMR ^{13}C (125 MHz, CD_3OD) δ ppm:

169.4; 158.1; 149.8; 149.2; 142.2; 134.7; 128.5; 128.2; 123.3; 118.6; 116.4; 116.1; 111.5; 73.4; 56.3; 48.4.

R_f =6mm/50mm (EtOAc/Hexanes=75/25); amorphous crystals

$[\alpha]^{25}_D$= -37.5557 (c=1.376, MeOH)

IR (KBr, cm^{-1}): 3338, 3276, 2938, 2875, 1654, 1599, 1559, 1517, 1449, 1430, 1369, 1253, 1167, 1127, 1075, 1030, 966, 835;

MS (FAB): 330.1(M+1)

HRMS (FAB): 330.1335($C_{18}H_{20}NO_5$, M+1; calc. 330.134148)

MS (ESI) (m/z): 712.04(10.07), 329.8(M+1, 100.00), 312.04(M-17, 87.74)

UV (PDA; λ_{max}, nm): 230, 295, 320;

***N*-[2-(*R*)-Hydroxy-2-(4-hydroxy-phenyl)-ethyl]-3-(4-hydroxy-3-methoxy-phenyl)-acrylamide (15-D):** An oven dried 50 mL round bottom flask, equipped with a Teflon coated magnetic stir bar, was evacuated for 15 min at 0.01 mm Hg and flushed with dry N_2. After repeating the cycle three times, the flask was charged with dicyclohexylcarbodiimide (59.3 mg, 0.287 mmol, as solution in THF, 5 mL) followed by a solution of **D** (53.4 mg, 0.275 mmol) in THF (5 mL). Amine **15** (38.3 mg, 0.25 mmol) was added as solution in THF/DMF (8:1, 5 mL) and the mixture was stirred at 20-22 ^{o}C for 10 h. Upon completion (monitored by TLC by disappearance of the amine) solvent evaporation resulted 149.1 mg of a viscous oily substance. The crude reaction mixture then was subjected to column chromatography (silica gel, 1:4=Hexane: EtOAc) to yield 32.0 mg (40%) of **15-D** as a viscous oil.

$C_{18}H_{19}NO_5$
Exact Mass: 329.13
Mol. Wt.: 329.35
C, 65.64; H, 5.81; N, 4.25; O, 24.29

HMR ^{1}H (CD_3OD) δ ppm:

7.43 (d, 1H, J1=15.9 Hz); 7.20 (app. dt, 2H, J1=8.9 Hz); 7.09 (d, 1H, J1=1.9 Hz); 7.01 (dd, 1H, J1=8.2 Hz, J2=1.5 Hz); 6.75 (app. t, 3H, J1=7.6 Hz); 6.45 (d, 1H, J1=15.7 Hz); 4.72 (dd, 1H, J1=7.8 Hz, J2=4.9 Hz); 3.84 (s, 3H); 3.54 (dd, 1H, J1=13.4 Hz, J2=4.9 Hz); 3.44 (dd, 1H, J1=13.7 Hz, J2=7.9 Hz).

NMR ^{13}C (CD_3OD) δ ppm:

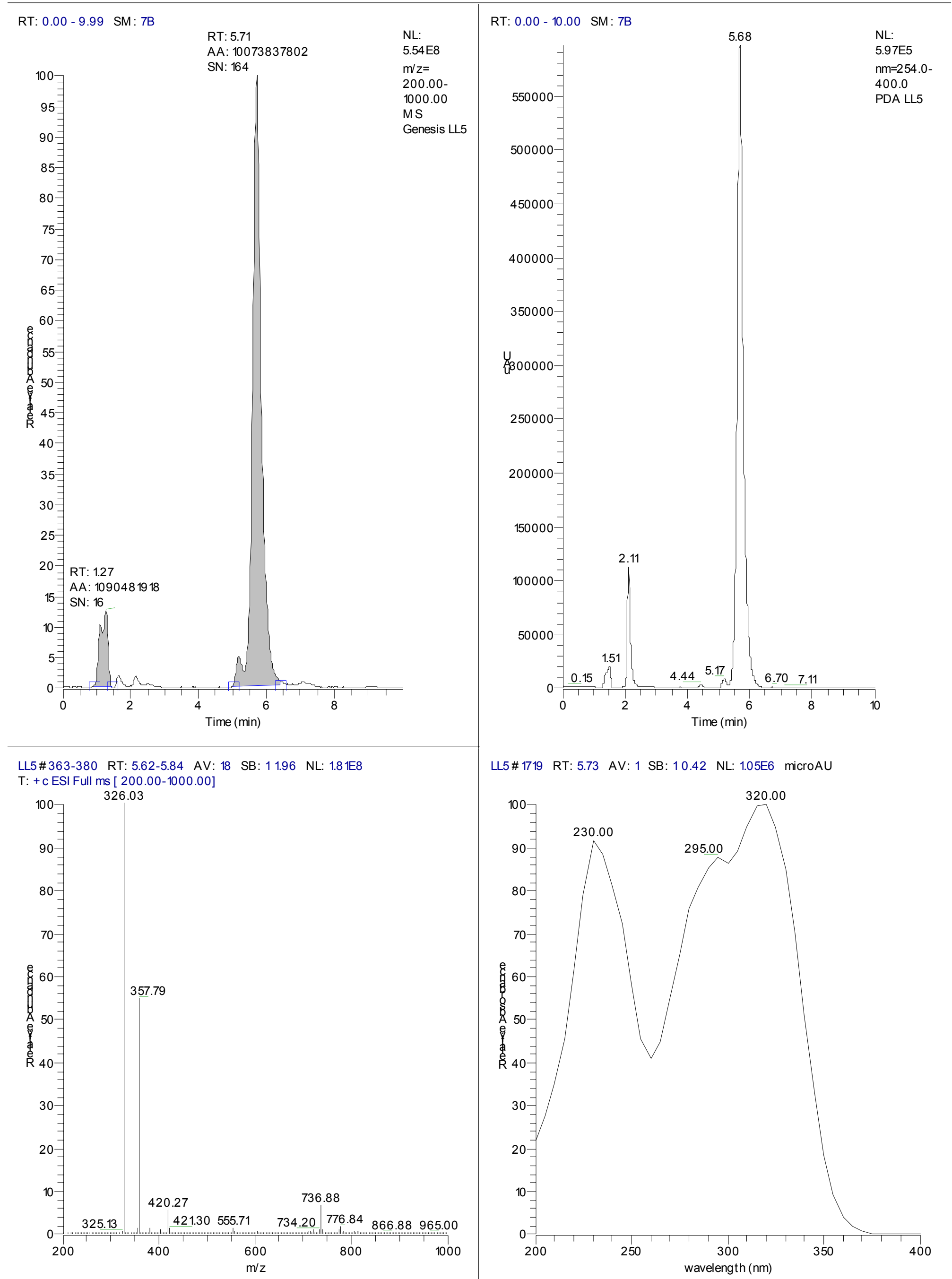
169.4; 158.1; 149.8; 149.2; 142.2; 134.7; 128.4; 128.2; 123.3; 118.6; 116.4; 116.1; 111.5; 73.4; 56.3; 48.3.

R_f =22mm/50mm (EtOAc/Hexanes=75/25); amorphous crystals
MS (FAB): 330.1(M+1)
HRMS (FAB): 330.1338($C_{18}H_{20}NO_5$, M+1; calc. 330.134148)
MS (ESI) (m/z): 712.02(16.97), 329.81(M+1, 98.90), 312.02(M-17, 100.00)
UV (PDA; λ_{max}, nm): 230, 295, 320;

Procedure for parallel synthesis of combinatorial library:

PS-carbodiimide beads (Argonaut Technologies Inc., 110 µm, 1.37 mmol/g) were loaded (using the bead loading block; see Figure S2) into 96 deep well plate (well volume 1 mL, 22 mg/well, 0.03 mol/well, see Figure S2) and suspended in freshly distilled THF (0.25 mL/well). Solutions of corresponding acid building blocks **A-H** (0.3 mL, 0.0153 mmol/well) were added in each column, followed by solutions of corresponding amine building blocks **8-18** (0.3 mL, 0.015 mmol/well) added to each row. The 96-well plate was capped with a Teflon lid and rotated (60-80 rpm) for 48 h on a mechanical stirrer so that the beads are freely moving in the solution. Upon completion the beads were filtered off (through a Pasteur pipettes fitted with a cotton plug) and washed with THF (3x0.3 mL). Removal of the solvent *in vacuo* afforded amide products in 85% average yield. The library was analyzed by TLC and LC-MS. LC-MS was conducted on all 88 members of the library using a reversed-phase C18 column, acetonitrile/H_2O containing 0.1% formic acid. Two representative LC-MS analyses are shown in Figure S1, below. The mass spectrometer was a Finnigan LCQ decaXP equipped with a Surveyor autosampler, a Surveyor PDA detector (200-400 nm) and a mass selective detector. The purity of each compound in the library, and the mass observed for each compound in the library is listed in the Table S3, below. Table S4 list the purity data alone.

Figure S1. Representative LC/MS analysis of 2 compounds from the library.



D:\030807\D4
8/7/2003 9:27:32 PM
RT: 0.00 - 9.99 SM: 7B
RT: 3.78
AA: 5230900520
SN: 150
NL:
3.64E8
m/z=
200.00-
1000.00
MS
Genesis D4
RT: 1.27
AA: 648702154
SN: 92
Relative Abundance
Time (min)
RT: 0.00 - 10.00 SM: 7B
3.76
NL:
6.79E5
nm=254.0-
400.0
PDA D4
uAU
0.94
1.51
2.76
5.32
8.06
8.35
Time (min)
D4 #245 RT: 3.79 AV: 1 SB: 11.99 NL: 2.07E8
T: + c ESI Full ms [200.00-1000.00]
314.04
206.16
317.14
489.86
626.79
680.07
803.19
992.97
Relative Abundance
m/z
D4 #1137 RT: 3.79 AV: 1 SB: 10.42 NL: 1.16E6 microAU
320.00
295.00
230.00
Relative Absorbance
wavelength (nm)

Table S3. Characterization of library members.

Mw		151.21	137.18	167.21	153.18	137.18	167.21	153.18	121.18	181.23	167.21	151.21			
		8	**12**	**11**	**15**	**17**	**9**	**13**	**16**	**10**	**14**	**18**	empty		
148.16	**A**	96.0	49.0	74.0	86.0	84.0	78.0	96.0	96.0	92.0	20.0	95.0		LC/MS purity	
		282.0	267.9	297.8	283.9	268.1	297.8	283.9	252.1	311.8	297.9	282.1		M+1	
								266		280	280.0			M-17 or	M-31
		275	220 275	225 275	275	220 275	225 275	275	270	275	220 275	275		UV	
164.16	**B**	45.0	87.0	70.0	87.0	91.0	95.0	92.0	86.7	96.0	87.0	99.0		LC/MS purity	
		297.9	283.9	313.8	299.8	284.1	313.8	299.8	268.00	327.0	313.8	298.0		M+1	
				282.1	282.0		282.1	282.1			296.0			M-17 or	M-31
		240 295	240 295	240 295	240 295	240 290	240 295	240 295	230 295 300	230 295	225 295	225 290		UV	
180.16	**C**	97	92.0	78.0	88.0	94.0	91.0	87.0	97.5	94.0	92.0	98.0		LC/MS purity	
		313.96	299.9	329.8	315.8	300.1	329.8	315.8	284.07	343.8	329.8	314.1		M+1	
		282.08	282.1	298.0			298.0	298.0						M-17 or	M-31
		245 295 320	245 295 320	245 295 320	245 295 320	245 295 320	245 295 320	245 295 320	240 295 320	230 295 320	230 295 320	235 295 320		UV	

Table S3. Characterization of library members (continued).

194.18	**D**	59.0	94.0	76.0	94.0	93.0	94.0	95.0	95.7	83.0	94.0	97.0		LC/MS purity	
		327.4	313.9	343.8	329.8	314.1	343.8	329.8	298.1	357.8	343.8	328.1		M+1	
			296.1	312.0	312.1		312.0	312.1			326.7			M-17 or	M-31
		235 295 320	245 295 320	245 295 320	245 295 320	245 295 320	245 295 320	245 295 320	235 295 315	230 295 320	230 295 320	230 295 320		UV	
194.18	**E**	87.0	82.0	93.0	81.0	79.0	85.0	85.0	70.6	86.0	64.0	82.0		LC/MS purity	
		328.0	314.0	343.9	329.9	314.1	343.9	329.9	298.1	357.0	326.0	328.0		M+1	
			296.1	312.0	312.1		312.1				310.1			M-17 or	M-31
		240 295 320	245 295 320	245 295 320	245 290 320	245 290 320	245 295 320	245 295 320	240 290 320	230 290 320	240 280 320	225 290 320		UV	
238.24	**F**	50.0	77.0	79.0	86.0	94.2	89.0	84.0	70.1	31.0	63.0	61.0		LC/MS purity	
		372.0	357.9	387.8	373.8	358.0	387.8	373.8	342.0	401.9	387.8	372.0		M+1	
					536.1			356.0		370.0	370.0			M-17 or	M-31
		245 295	230 300	230 300	240 300	230 300	235 300	240 300	225 275	225 275	230 300	240 300		UV	
224.21	**G**	64.0	94.0	79.0	91.0	91.0	93.0	87.0	95.0	92.0	93.0	94.0		LC/MS purity	

Table S3. Characterization of library members (continued).

		358.0	343.9	373.9	359.8	344.0	373.9	359.8	328.0	387.8	373.8	358.1		M+1	
			326.1	342.1	342.0		342.0				356			M-17 or	M-31
		240 320	245 320	245 320	245 320	245 320	245 320	245 320	240 320	240 320	240 320	235 320		UV	
178.18	**H**	92.0	87.0	76.0	81.0	74.0	87.0	96.0	90.0	91.0	10.0	82.0		LC/MS purity	
		311.9	297.9	327.8	313.8	298.0	327.8	313.8	282.1	341.8	327.8	312.1		M+1	
					296.0					310.0	310.0			M-17 or	M-31
		240 275	230 275	230 275	275.0	225 275	230 275	240 275	275	275	275	275		UV	

Table S4. Purity of the library members as assessed by LC-MS.

						% Purity, by LC/MS						
Mw		151.21	137.18	167.21	153.18	137.18	167.21	153.18	121.18	181.23	167.21	151.21
		8	**12**	**11**	**15**	**17**	**9**	**13**	**16**	**10**	**14**	**18**
148.16	**A**	96.0	49.0	74.0	86.0	84.0	78.0	96.0	96.0	92.0	20.0	95.0
164.16	**B**	45.0	87.0	70.0	87.0	91.0	95.0	92.0	86.7	96.0	87.0	99.0
180.16	**C**	97.0	92.0	78.0	88.0	94.0	91.0	87.0	97.5	94.0	92.0	98.0
194.18	**D**	59.0	94.0	76.0	94.0	93.0	94.0	95.0	95.7	83.0	94.0	97.0
194.18	**E**	87.0	82.0	93.0	81.0	79.0	85.0	85.0	70.6	86.0	64.0	82.0
238.24	**F**	50.0	77.0	79.0	86.0	94.2	89.0	84.0	70.1	31.0	63.0	61.0
224.21	**G**	64.0	94.0	79.0	91.0	91.0	93.0	87.0	95.0	92.0	93.0	94.0
178.18	**H**	92.0	87.0	76.0	81.0	74.0	87.0	96.0	90.0	91.0	10.0	82.0

Structures of ligands for AA reaction

Dihydroquinyl(DHQ)

Dihydroquinidyl(DHQD)

DHQO ODHQ O O

$(DHQ)_2$-AQN
CLogP = 10.091

DHQDO ODHQD O O

$(DHQD)_2$-AQN
CLogP = 10.091

O N C N

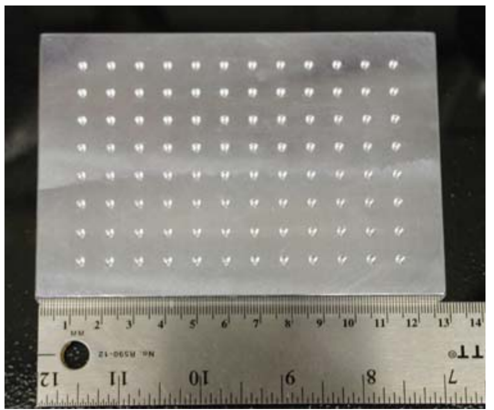
PS-CDI
1.37mmol/g

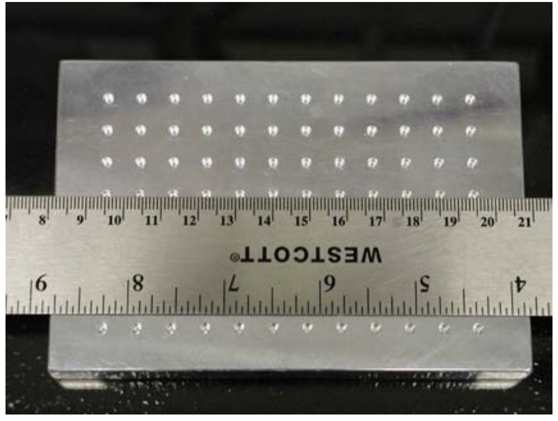
(7)

Figure S2. Apparatus for parallel synthesis. The devices below were constructed in the machine shop housed in the School of Chemical Sciences, at the University of Illinois, Urbana-Champaign.

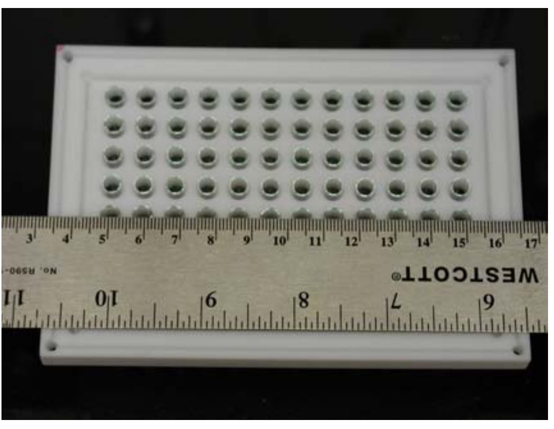
Use of Loading Plate, 96 deep-well plate with tubes, and Lid: The Loading Plate was used to deliver a defined amount of beads to each well of the deep-well synthesis tubes. The PS-CDI beads were poured over the Loading Plate, the excess was scrapped off, and the holes were then aligned with the holes of the 96-deep-well plate with tubes. Simple inversion (followed by gentle tapping) delivered the beads into the wells. After loading the appropriate acids and amines, the Lid was placed onto the tubes and the entire apparatus was connected to an overhead stirrer for rotation.

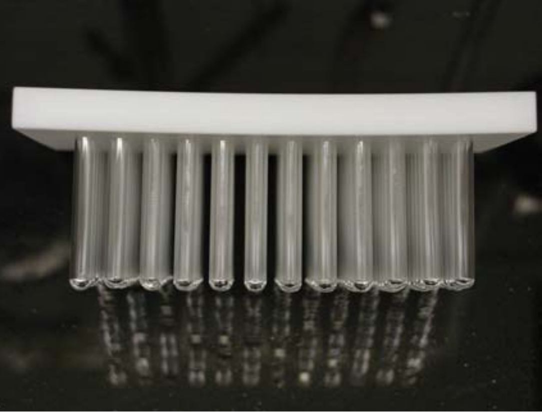
1. **Loading Plate**: made in the 96 well format from aluminum, each well (3x6 mm) delivers 10.8-11.3 mg of PS-CDI beads.



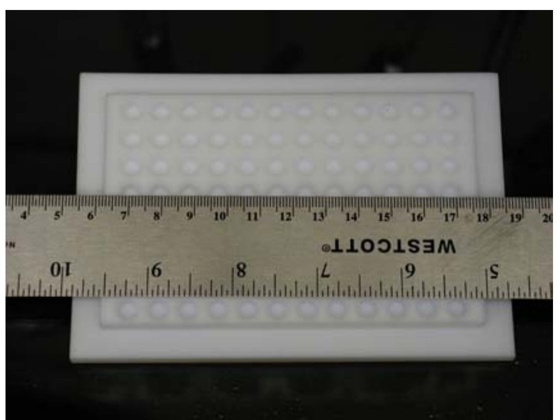


2. **Positioning plate and 96 deep-well plate**: made in the 96 well format from Teflon, holes 4.95 mm diameter, accommodates 6x50 mm glass inserts, Fisher Scientific #14958-A, volume 1ml)





3. **Lid**: made in the 96 well format from Teflon, allows tightly seal the glass inserts to assure no leaking from the insert.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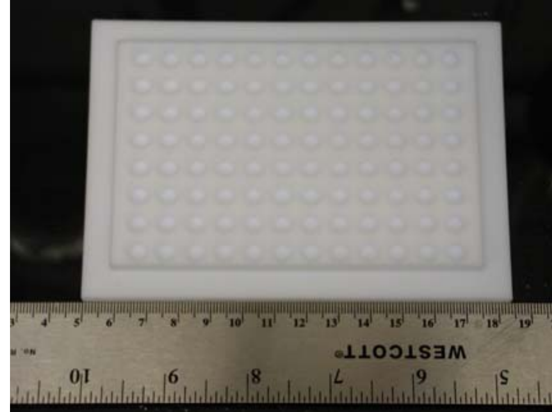
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Protocols for Biological Assays

General Cell Culture Conditions:

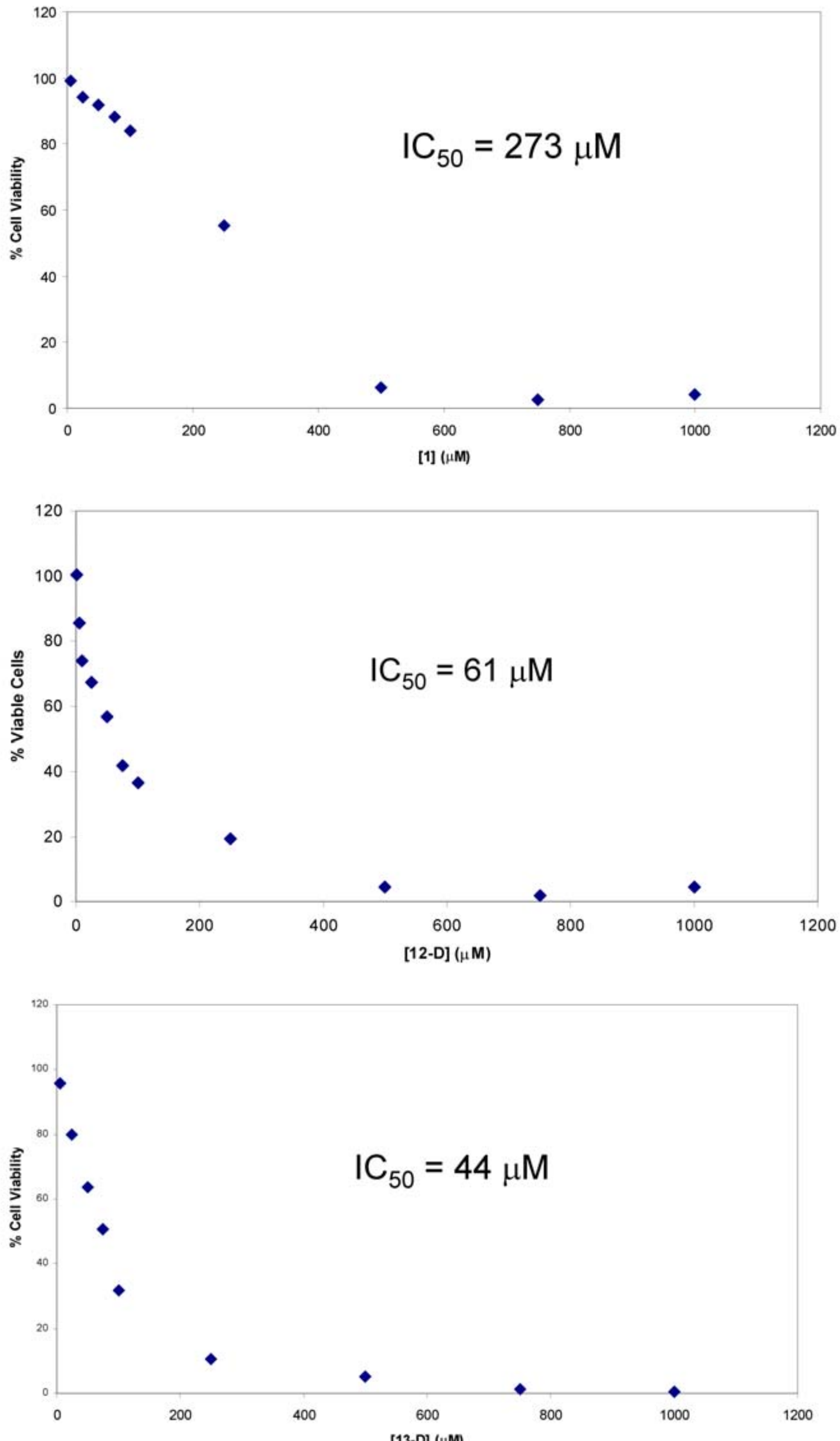
U-937 and HL-60 cell lines were grown in RPMI 1640 supplemented with 10% FBS and incubated at 37 ^{O}C in a 5% CO_2, 95% air atmosphere and were split every two to three days as necessary.

High-throughput Cell Death Assay on Library Members:

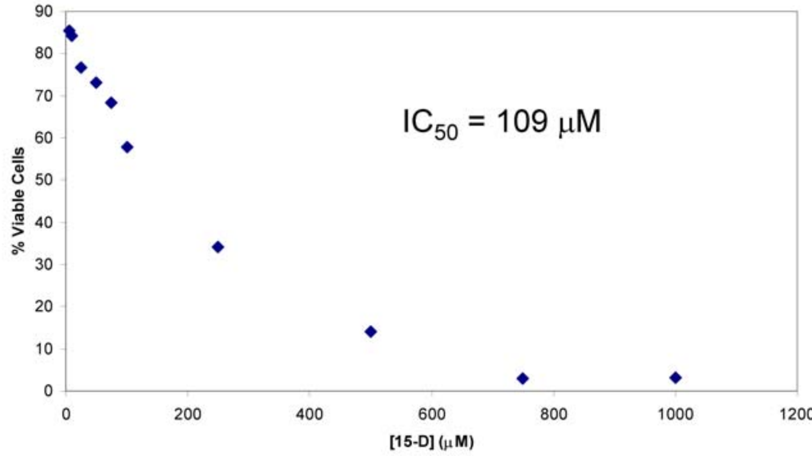
U-937 and HL-60 cells from cell culture were harvested by centrifugation at 250 x g for 5 min. Cells were then resuspended in RPMI 1640 + 10% FBS, counted using a hemocytometer and diluted so that 20,000 cells were seeded into each well of a Corning 96-well flat bottom microtiter plate (Fisher, Chicago IL). Media was then added to bring the total volume of each well to 100 μL. Test compounds were transferred into the wells using a 96-pin transfer apparatus (V & P Scientific, San Diego CA) that transfers 0.2 μL of compound. The compounds were made up as 50 mM stock solutions in EtOH, so one transfer gave a final concentration of 100 μM. Controls were performed in which only EtOH (containing no compound) was pin-transferred into wells containing cells. The cells were incubated with the compounds for 24 hours, and then cell death was quantitated. This quantitation was performed by addition of 20 μL of the MTS/PMS CellTiter 96 Cell Proliferation Assay reagent (Promega, Madison WI) to each well. The plates were incubated at 37 ^{O}C for approximately one hour until the colored product formed and the absorbance was then measured at 490 nm in a Spectra Max Plus 384 plate reader (Molecular Devices, Sunnyvale CA).

Determination of IC_{50} Values for 1, 12-D, 13-D, and 15-D

U-937 and HL-60 cells from cell culture were harvested by centrifugation at 250 x g for 5 min. Cells were then resuspended in RPMI 1640 + 10% FBS, counted using a hemocytometer and diluted so that 10,000 cells were seeded in to each well of a Corning 96-well flat bottom microtiter plate (Fisher, Chicago IL). Media was then added to bring the total volume of each well to 100 μL. Each compound was weighed and then diluted with EtOH to make a 100 mM stock solution. The compounds were added at 9 or more different concentration in 1 μL of EtOH. After a 72 h incubation (37 ^{O}C in a 5% CO_2, 95% air atmosphere) cell death was quantitated by addition of 20 μL of the MTS/PMS CellTiter 96 Cell Proliferation Assay reagent (Promega, Madison WI) to each well. The plates were incubated at 37 ^{O}C for approximately 1 hour until the colored product formed and the absorbance was then measured at 490 nm in a Spectra Max Plus 384 plate reader (Molecular Devices, Sunnyvale CA). The IC_{50} was taken as the concentration that caused 50% cell death. The graphs used to determine the IC_{50} values are below:



120
100
80
60
40
20
0
% Cell Viability
IC_{50} = 273 μM
0
200
400
600
800
1000
1200
[1] (μM)
120
100
80
60
40
20
0
% Viable Cells
IC_{50} = 61 μM
0
200
400
600
800
1000
1200
[12-D] (μM)
120
100
80
60
40
20
0
% Cell Viability
IC_{50} = 44 μM
0
200
400
600
800
1000
1200
[13-D] (μM)



Caspase-3 Activity Assay:

The amount of caspase-3 like protease activity was determined by the amount of Ac-DEVD-pNA (*N*-acetyl-Asp-Glu-Val-Asp *p*-nitroanilide) cleaved per minute by cell lysates. To accomplish this, 100 μM of **13-D** was added to cell culture flasks containing 50 mL of 10 x 10^6 U-937 cells/mL at 72, 48, 36, 24, 12 and 0 hours before harvesting. Cells were harvested by centrifugation, counted and diluted with RPMI 1640 media to a concentration of 4 x 10^6 cells/mL. 100 μL of the diluted cells were added to the wells of a 96-well plate in quadruplicate. The plate was then spun at 1000 x g for 5 minutes to pellet the cells. The cells were washed with 100 μL of PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4, pH 7.4) and resuspended in 150 μL of ice cold Caspase Assay Buffer (50mM HEPES, 100mM NaCl, 10mM DTT, 0.1mM EDTA, 0.1% CHAPS and 10% Glycerol, pH 7.4). Each well was then sonicated to lyse the cells. 90 μL of cell lysate was transferred from each well into a new plate. Caspase Assay Buffer was added to wells as a control. Ac-DEVD-pNA (Sigma, St. Louis MO) was added into each well to give a final concentration of 200 μM. The plate was then read every 2 minutes at 405 nm for 2 hours in a Spectra Max Plus 384 plate reader (Molecular Devices, Sunnyvale CA). The slope of the linear portion for each compound was then determined and any cleavage of the substrate in the control wells was subtracted out. The amount of Ac-DEVD-pNA cleaved in pmol/min was then calculated and plotted.

Analysis of Mitochondria Depolarization by Flow Cytometry:

The depolarization of the mitochondrial membrane was measured by the fluorescence emitted by the JC-9 dye (Molecular Probes, Eugene OR). 100 μM of **13-D** or 10 μM etoposide in 1 μL of EtOH were added to cell culture flasks containing 50 mL of 10 x 10^6 U-937 cells/mL. After growth for 72 hours (37 ^{O}C in a 5% CO_2, 95% air atmosphere) the cells were harvested by centrifugation, counted, and diluted to 1 x 10^6 cells/mL in RPMI 1640 media. 10 μg of the JC-9 dye was added to 1 x 10^6 cells in 1 mL and incubated at room temperature for 10 min. Cells were washed twice with PBS and resuspended in a final volume of 500 μL PBS. The fluorescence intensity of each cell was determined by flow cytometry at 525nm (channel 1 green) and 675nm (channel 4 red). 50,000 cells were analyzed in each experiment. The data was then analyzed using Summit Software (Cytomation, Fort Collins CO) and the number of cells within the upper left region, viable cells, was determined.

Splenocyte Toxicity Assay:

Splenocytes were isolated from the spleen of a 7-month old male C57Black/6 mouse and suspended in 1 mL RPMI 1640 + 10% FBS + 2.5 μg/mL concanavalin A. These cells were counted and diluted so that 2.5 x 10^5 cells were seeded in to each well containing a total of 200 μL of media. Various concentrations of compound **13-D** in 1 μL of EtOH were added and the plates were then incubated at 37 ^{O}C in a 5% CO_2, 95% air atmosphere for 72 hours. 1 μL of EtOH was added to separate control wells. After 72 hours of incubation, 20 μL of the MTS/PMS CellTiter 96 Cell Proliferation Assay reagent (Promega, Madison WI) was added to each well. The plates were incubated at 37 ^{O}C for approximately two hours until the colored product formed and the absorbance was then measured at 490 nm in a Spectra Max Plus 384 plate reader (Molecular Devices, Sunnyvale CA).

T-cell Isolation and Toxicity Assay:

Splenocytes were isolated from the spleen of a 3-month old male C57Black/6 mouse and suspended in 1mL RPMI 1640 + 10% FBS. Erythrocytes were selectively lysed and the T cells were highly enriched by using a mouse erythrocyte lysing kit and a mouse T cell enrichment column (R & D Systems, Minneapolis MN). FITC labeled anti-TCR antibodies were used to determine the purity of T cells in pre- and post-column samples. The T cells were enriched from approximately 35% to 90% of the total cell population (see Figure S5, S6 below). The purified T cells were then counted and diluted so that 2.5 x 10^5 cells were seeded in to each well of a 96-well plate containing 200 μL of media. These cells were either stimulated to grow (by addition of ConA to a concentration of 2.5 μg/mL) or left untreated. Various concentrations of compound **13-D** in 1 μL of EtOH or 1 μL of EtOH as a control were added and the plates were incubated at 37 ^{O}C in a 5% CO_2, 95% air atmosphere for 72 hours. After the 72 hours of incubation, 200 μL of cells were diluted in 300 μL PBS. Propidium iodide was then added to a concentration of 1 μg/mL. Cells were then incubated at room temperature for 5 minutes and analyzed by flow cytometry. The fluorescene intensity of each cell was determined at 620 nm (channel 3) and at least 50,000 cells were analyzed for each experiment (Figure S7, S8, below). The data was then analyzed using Summit Software (Cytomation, Fort Collins CO) and the number of viable cells was determined. Non-concanavalin A stimulated T cells were also treated with **13-D**, and viable cells in these samples were determined by PI staining (as above). In both the ConA stimulated and non-stimulated experiments, no difference was observed between **13-D** treated and non-treated control cells.

Figure S5.
Pre-column T cells -- FITC labeled anti-TCR antibodies were used to determine the purity of T cells**:**

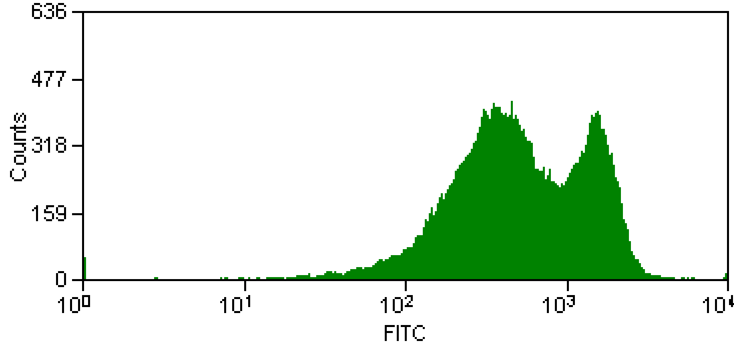


Figure S6.
Post-column T cells -- FITC labeled anti-TCR antibodies were used to determine the purity of T cells**:**

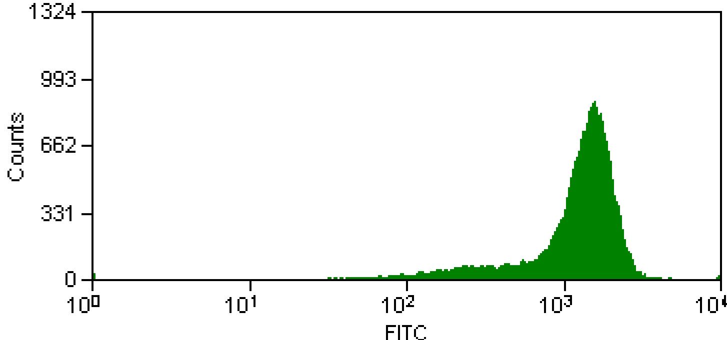
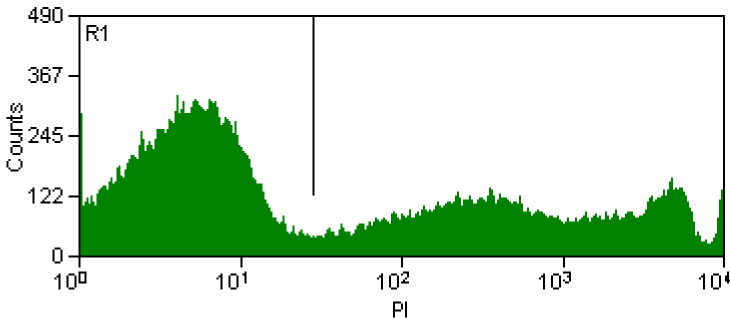


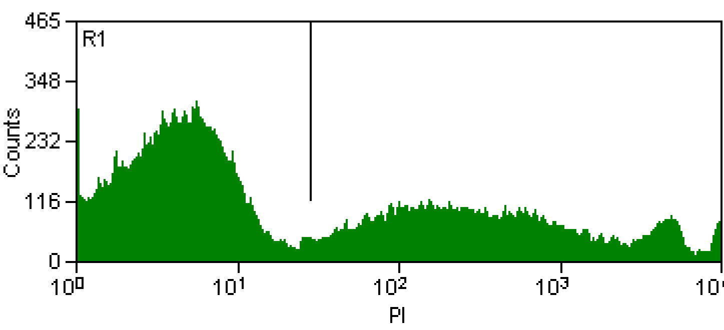
Figure 3B of the text is an overlay of Figures S7 and S8, below.

Figure S7.
Concanavalin A stimulated T cells:



<u>Viable Cells (R1) = 86.4%</u>

Figure S8.
Concanavalin A stimulated T cells + 500 µM 13-D:

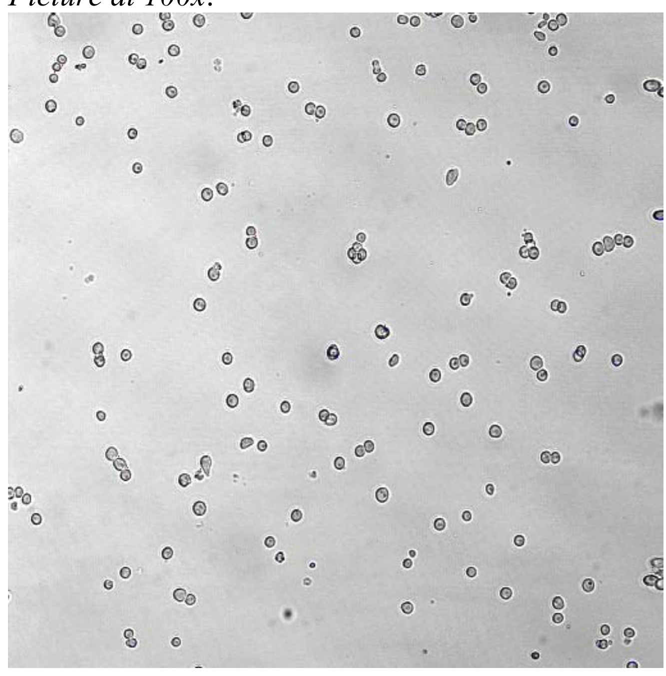


<u>Viable Cells (R1) = 87.4%</u>

Microscopy:

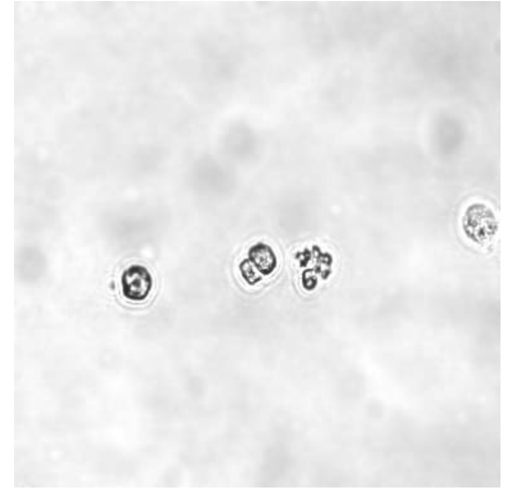
U-937 cells from cell culture were harvested by centrifugation at 250x*g* for 5 minutes. Cells were resuspended in RPMI 1640 + 10% FBS, counted using a hemocyometer and diluted so that 20,000 cells were seeded in to each well of a 96-well plate. Media was then added to bring the total volume of each well to 100μL. Compound **13-D** was added to make a final concentration of 100 μM. Pictures of the cells were taken at various times using a Carl Zeiss confocal microscope (Carl Zeiss, Thornwood NY).

Picture at 100x:



Picture at 100x:

Picture at 400x:



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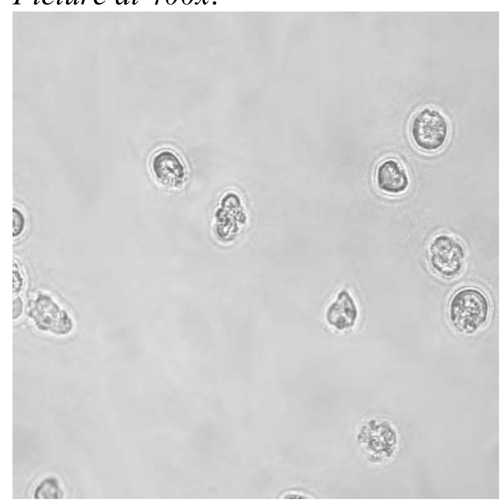

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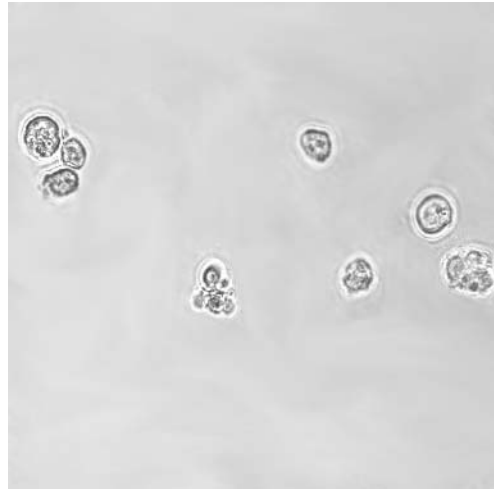

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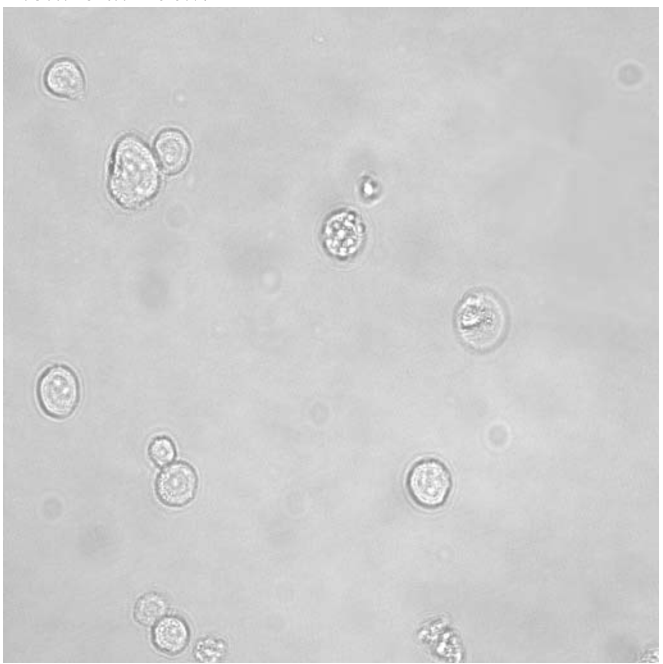
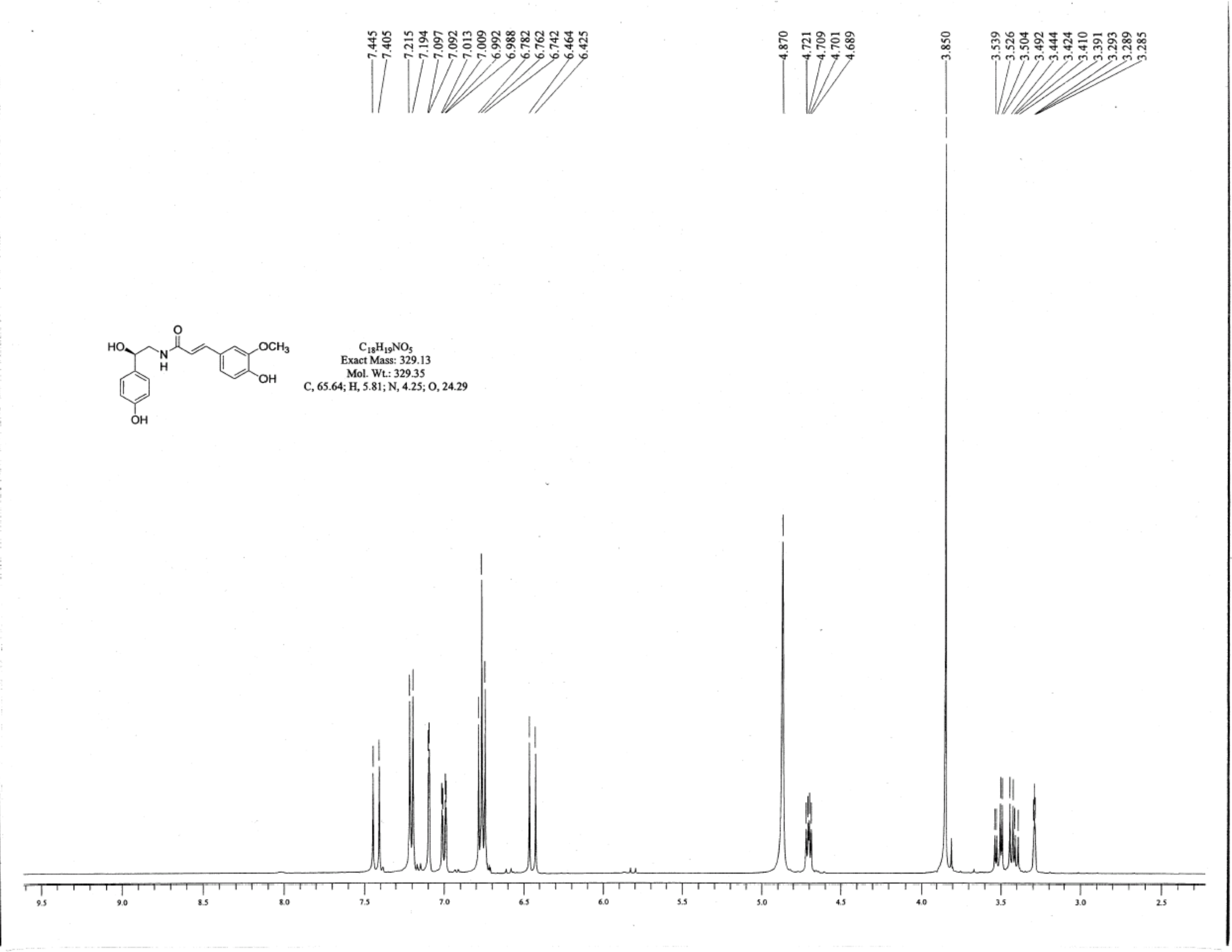


References

1. Nesterenko, V.; Byers, J. T.; Hergenrother, P. J. *Org. Lett.* **2003**, *5*, 281-284.



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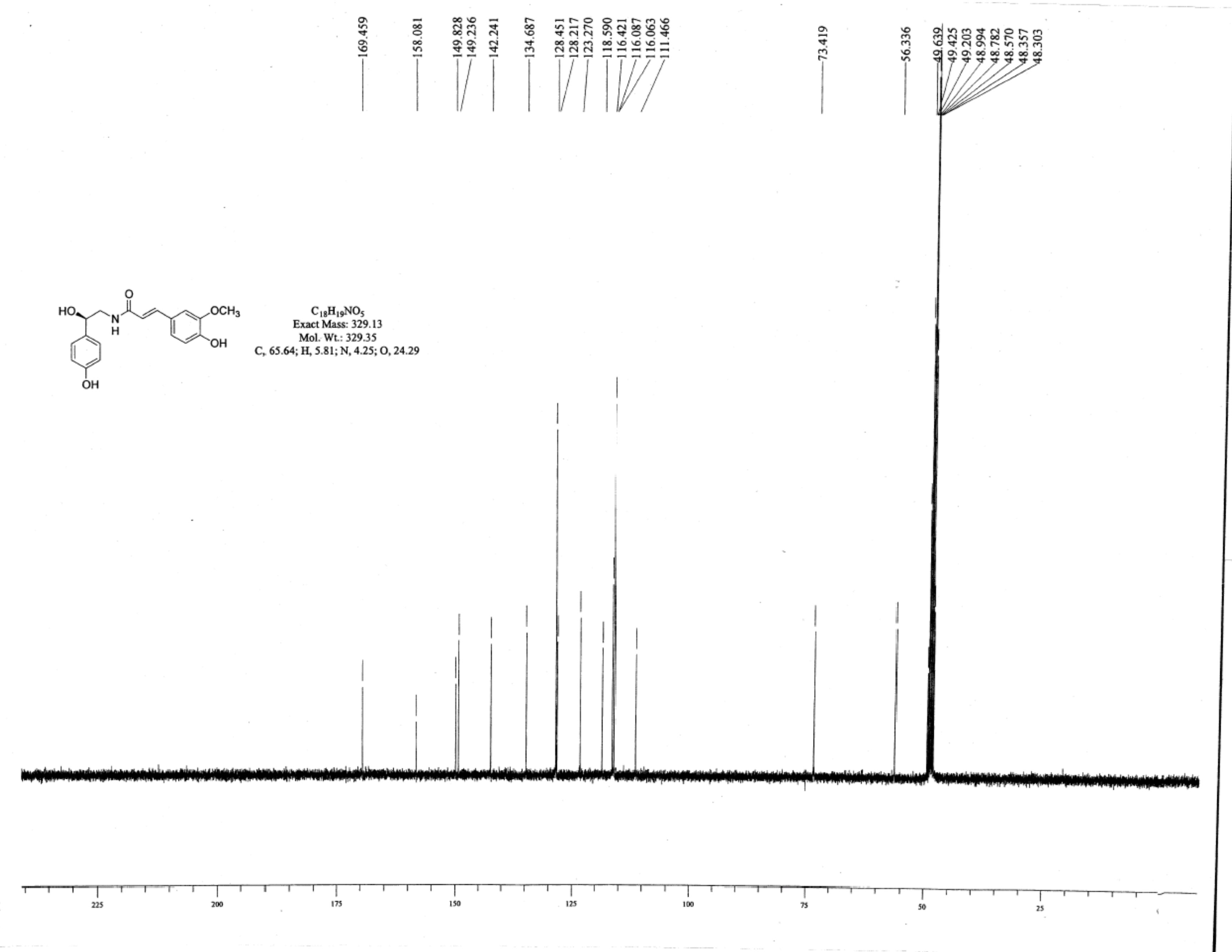
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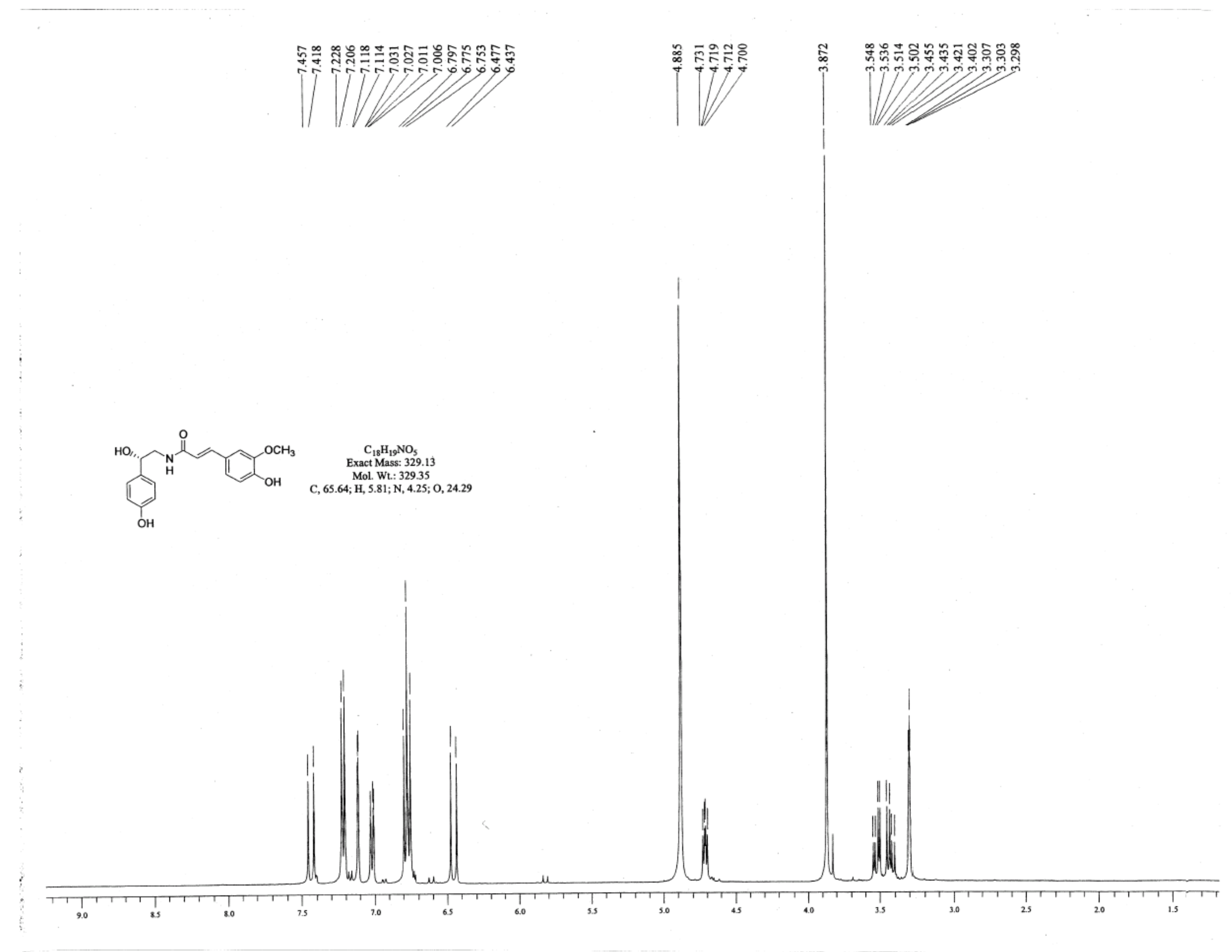
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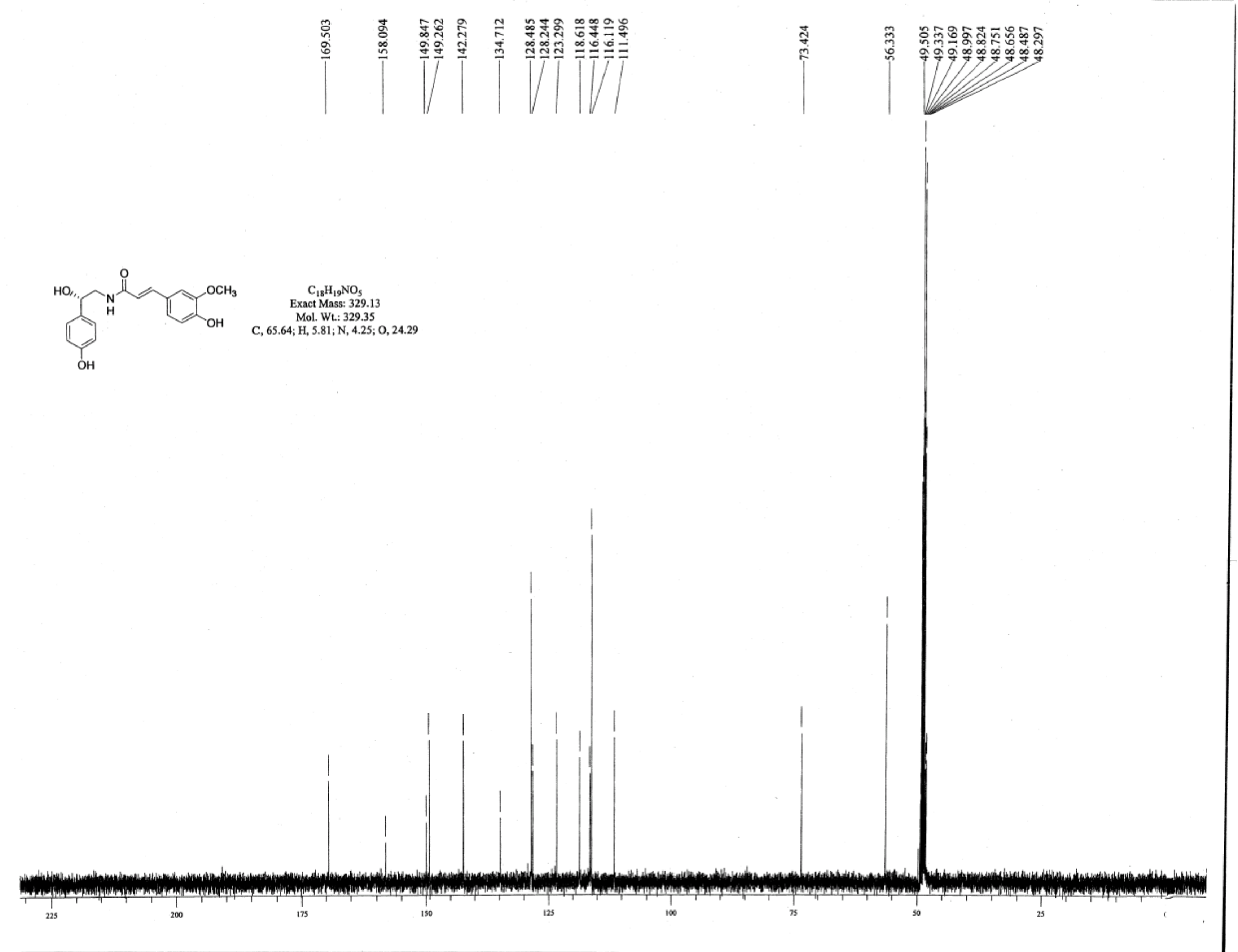

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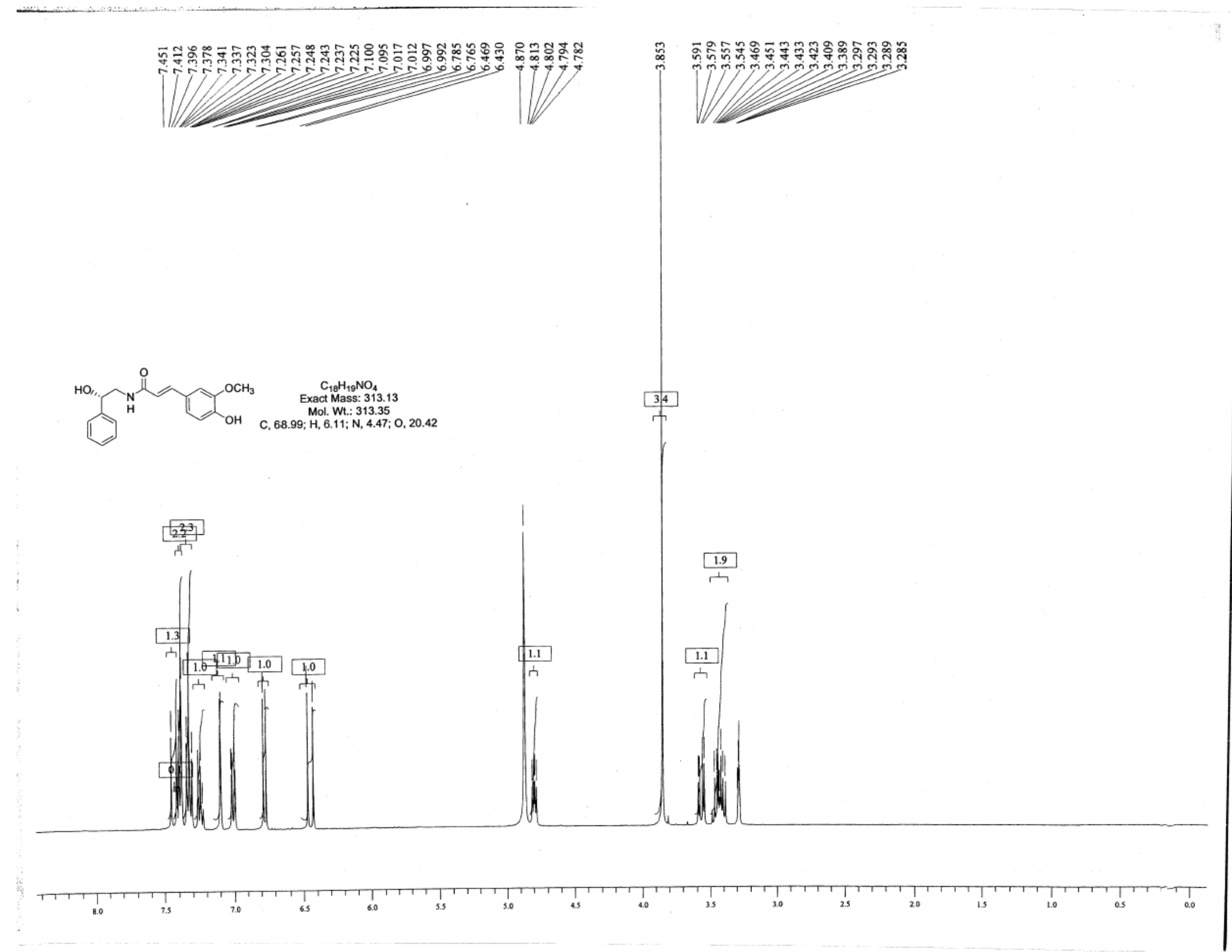

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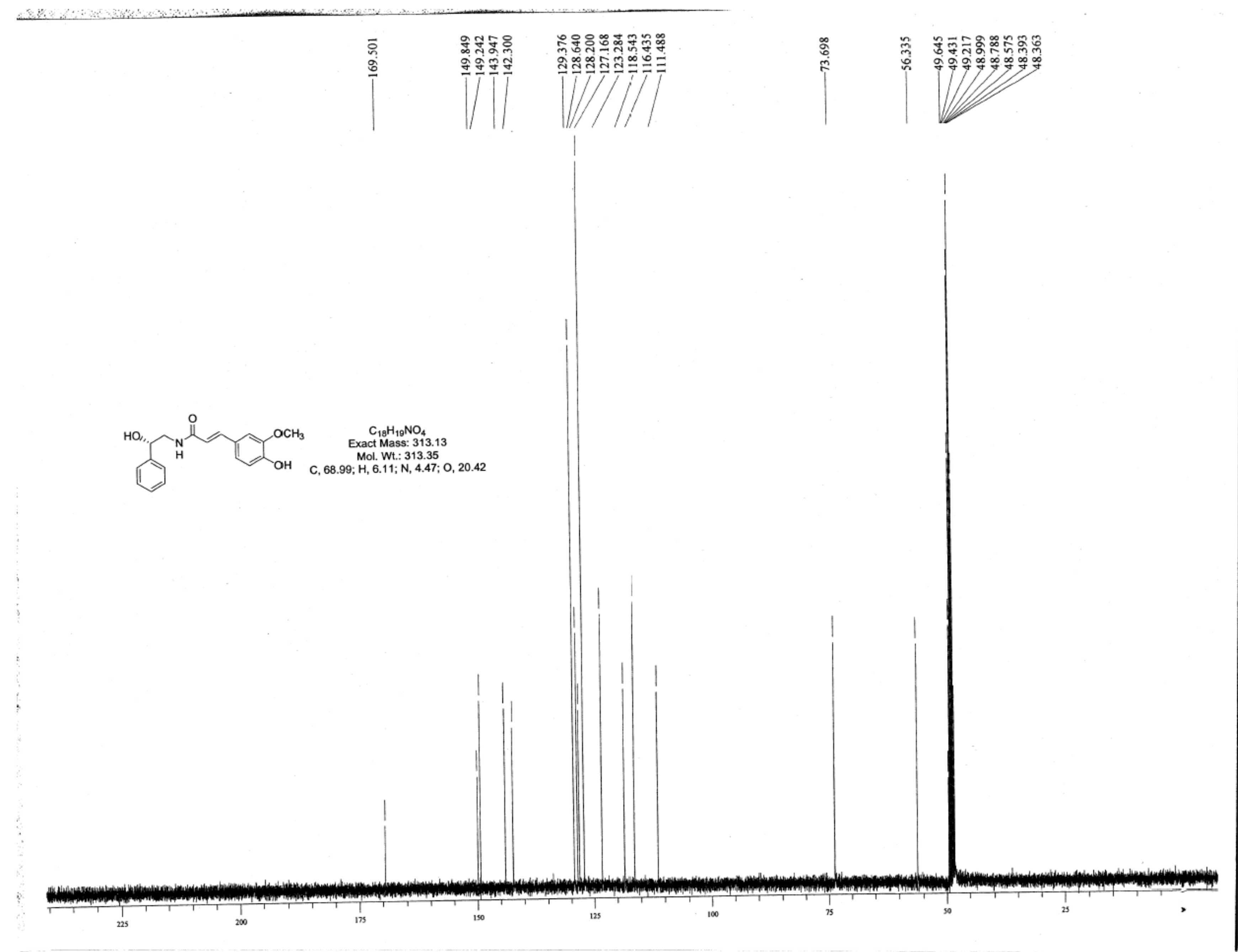
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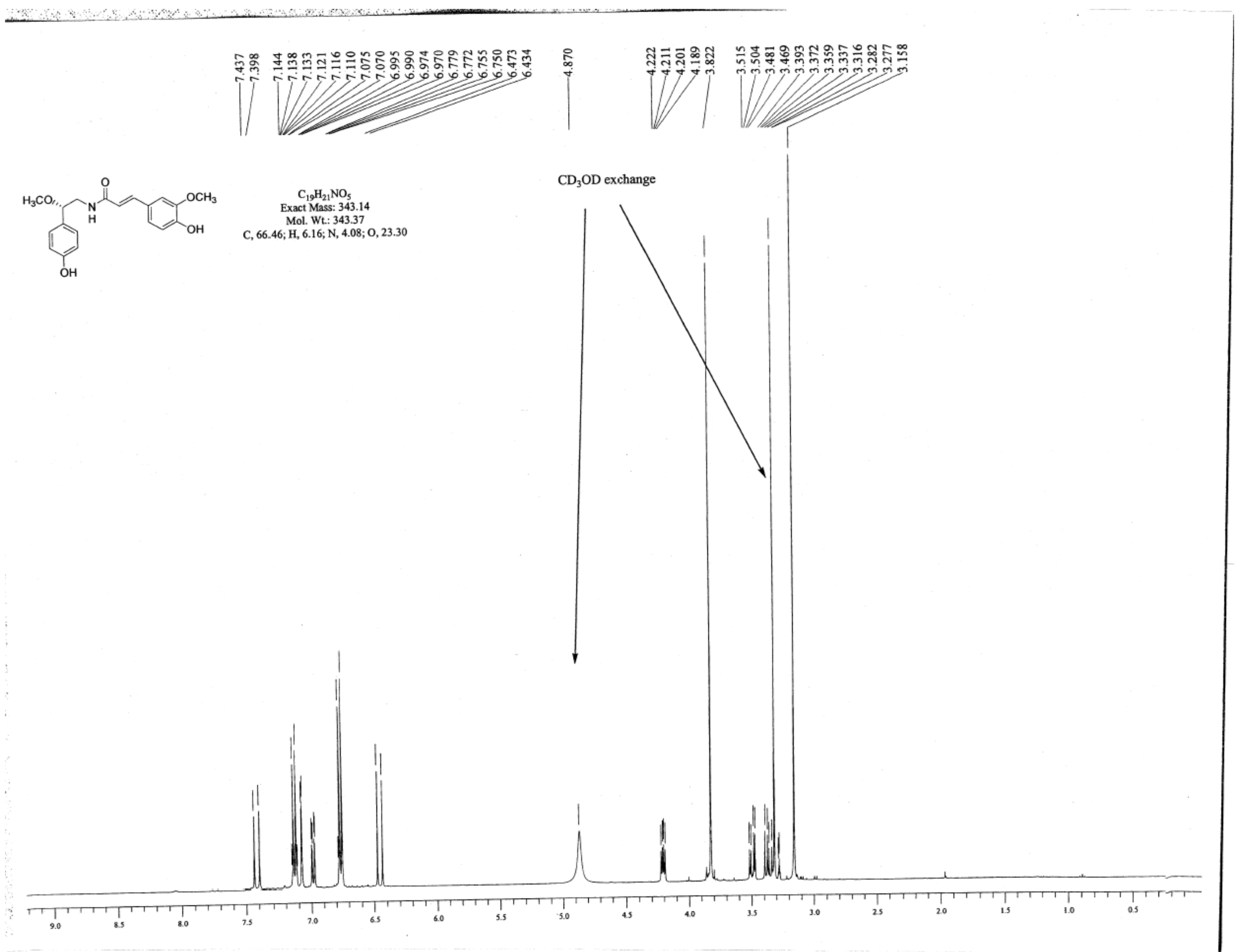

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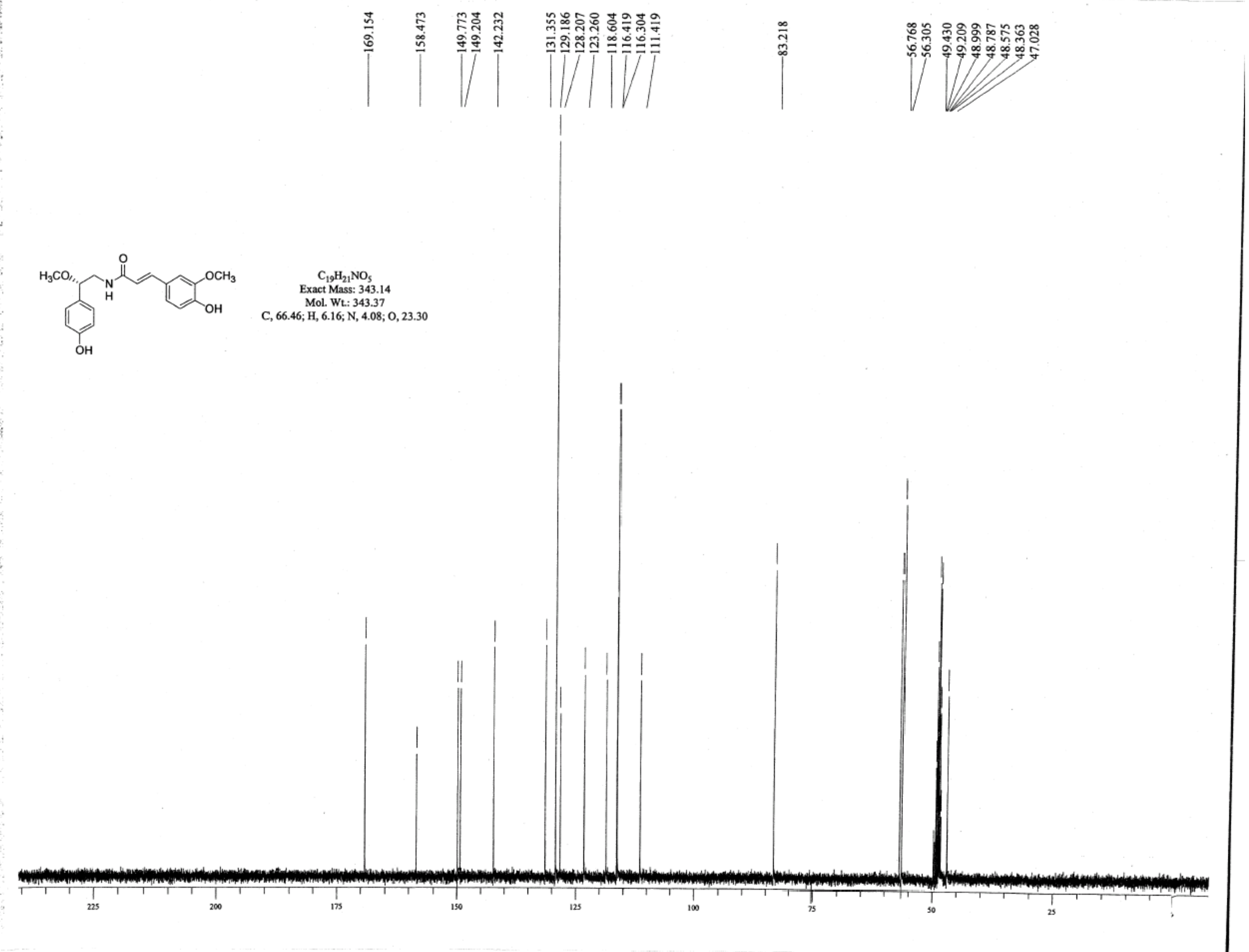
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