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Supporting Information

Structure-Based Design of Potent Non-Peptide MDM2 Inhibitors

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I. Chemistry

Elemental analyses were performed by the Department of Chemistry of the University of Michigan, Ann Arbor, MI. Where molecular formulas are given, elemental compositions were found to be within 0.4% of the theoretical values unless otherwise noted. Optical rotations were determined at 589 nm at 25 ^{o}C on a Perkin-Elmer 241 polarimeter (in $CHCl_3$). Single-crystal X-ray analysis was performed at the Naval Research Laboratory, Washington, DC. ^{1}H NMR spectra were recorded at 300 MHz and ^{13}C NMR spectra were recorded at 75 MHz on a Bruker AVANCE300 spectrometer. All NMR spectra were obtained in $CDCl_3$ and results were recorded as parts per million (ppm) downfield from tetramethylsilane (TMS).The following abbreviations are used for multiplicity of NMR signals: s = singlet, d = doublet, t = triplet. q = quartet, m = multiplet, dd = double doublet, dt = double triplet, dq = double quartet, br = broad. All starting materials, solvents and silica gel were purchased from Aldrich, Fisher, or Lancaster and were used without further purification.

General method for synthesis of 3-E-benzylidene-1, 3-dihydro-6-chloro-indol-2-one analogues.

To a solution of 6-chlorooxindole (1.67 g, 10.0 mmol) in 60 mL CH_2Cl_2-CH_3CN (1 : 1), substituted benzaldehyde (10.0 mmol) and KF-Al_2O_3 (10 g) were added. After 10 min at room temperature, the solvent was removed *in vacuo,* and the residues together with the flask was placed in a microwave oven and cooked for 5 min (60 ~ 80 W). Extraction was carried out with 150 mL CH_3CN, the solid was filtered off and the solvent was removed *in vacuo* to yield the crude product which was used without further purification.

General method for synthesis of (*2'R, 3S, 4'R, 5'R*) 6-Chloro-2'-isobutyl-2-oxo-4'-phenyl-1,2-dihydro-spiro[indole-3,3'-pyrrolidine]-5'-carboxylic acid dimethylamide analogues.

Under argon, to a 100mL flask with stir bar was added (2S, 3R)-2,3,5,6-tetrahydro-2,3-diphenyl-1,4-oxazin-6-one (**4**) (1.0 g, 3.96 mmol), 3-E-benzylidene-6-chloro-1,3-dihydro-indol-2-one (**2)** (4.75 mmol), 2 g freshly activated 4 Å molecular sieves, aldehyde (**3**) (4.75 mmol) and 50 mL toluene. The mixture was heated to 70^{o}C and kept that temperature for 5 hour. The mixture was cooled to room temperature and the molecular sieves were filtered off. The solvent was removed *in vacuo* and the residue was purified by chromatography to yield the 1,3-dipolar product.

The obtained 1,3-dipolar product (2.0 mmol) was dissolved in 4M dimethylamine in THF (5 mL) and the resulting solution was stirred at room temperature overnight. The solvent was removed *in vacuo* and the residue was purified by chromatography to yield compound **5.**

(*1"R, 2"S, 2'R, 3'R, 3S, 4'R*) 6-Chloro-4'-phenyl-1'-(2-hydroxy-1,2-diphenyl-ethyl)-2'-isobutyl-2-oxo-1,2-dihydro-spiro[indole-3,3'-pyrrolidine]-5'-carboxylic acid dimethylamide (5a). $[\alpha]_D^{25}$ -81.9 (c, 0.3, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 8.25 (br, 1H), 7.73 (d, J = 7.88 Hz, 1H), 7.42 ~ 6.93 (m, 16H), 6.78 (s, 1H), 5.20 (s, 1H), 4.64 (d, J = 10.07 Hz, 1H), 4.59 (s, 1H), 4.52 (d, J = 3.13 Hz, 1H), 4.17 ~ 4.10 (m, 1H), 3.50 (d, J = 10.83 Hz, 1H), 2.86 (s, 3H), 2.64 (dd, J = 12.45, 13.20 Hz, 1H), 1.95 (s, 3H), 1.73 ~ 1.65 (m, 1H), 1.13 ~ 1.07 (m, 1H), 0.85 (d, J = 6.38 Hz, 3H), 0.54 (d, J = 6.08 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.43, 174.25, 140.95, 140.55, 135.38, 133.91, 133.77, 131.39, 130.81, 129.78, 127.96, 127.81, 127.58, 126.58, 125.90, 125.36, 122.19, 110.52, 75.52, 75.10, 73.58, 64.71, 60.59, 58.45, 57.52, 37.34, 36.54, 36.30, 29.56, 28.12.

(*1"R, 2"S, 2'R, 3'R, 3S, 4'R*) 6-Chloro-4'-(3-chloro-phenyl)-1'-(2-hydroxy-1,2-diphenyl-ethyl)-2'-isobutyl-2-oxo-1,2-dihydro-spiro[indole-3,3'-pyrrolidine]-5'-carboxylic acid dimethylamide (5b). $[\alpha]_D^{25}$ -76.0 (c, 0.2 $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 8.15 (br, 1H), 7.75 (d, J = 7.91 Hz, 1H), 7.21 ~ 6.86 (m, 14H), 6.82 (d, J = 7.82 Hz, 1H), 6.73 (s, 1H), 5.18 (s, 1H), 4.69 (d, J = 10.28 Hz, 1H), 4.51 (d, J = 3.45 Hz, 1H), 4.32 (br, 1H), 4.22 ~ 4.11 (m, 1H), 3.48 (d, J = 10.99 Hz, 1H), 2.90 (s, 3H), 2.57 (dd, J = 12.53, 13.24 Hz, 1H), 2.06 (s, 3H), 1.85 ~ 1.56 (m, 1H), 1.10 ~ 0.95 (m, 1H), 0.84 (d, J = 6.41 Hz, 3H), 0.47 (d, J = 6.90 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.06, 173.83, 140.80, 140.61, 136.27, 135.04, 134.06, 133.86, 130.81, 130.71, 129.54, 129.23, 128.09, 127.87, 127.62, 127.38, 126.70, 125.80, 125.54, 122.39, 110.60, 73.92, 72.51, 72.23, 62.55, 60.41, 56.75, 39.23, 36.59, 26.43, 23.39, 21.03.

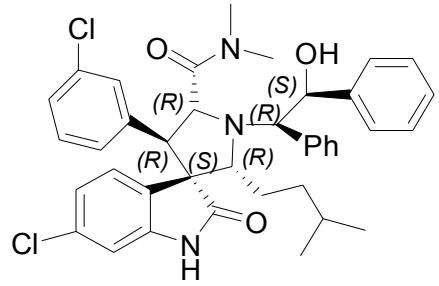
(*1"R, 2"S, 2'R, 3'R, 3S, 4'R*) 6-Chloro-4'-(4-chloro-phenyl)-1'-(2-hydroxy-1,2-diphenyl-ethyl)-2'-isobutyl-2-oxo-1,2-dihydro-spiro[indole-3,3'-pyrrolidine]-5'-carboxylic acid dimethylamide (5c). $[\alpha]_D^{25}$ -93.3 (c, 0.2 $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.80 (br, 1H), 7.74 (d, J = 8.09 Hz, 1H), 7.34 ~ 6.90 (m, 14H), 6.91 (d, J = 8.41 Hz, 1H), 6.78 (s, 1H), 5.18 (s, 1H), 4.65 (d, J = 9.89 Hz, 1H), 4.51 (d, J = 3.54 Hz, 1H), 4.36 (br, 1H), 4.18 ~ 4.10 (m, 1H), 3.48 (d, J = 10.96 Hz, 1H), 2.89 (s, 3H), 2.59 (dd, J = 12.39, 12.77 Hz, 1H), 2.05 (s, 3H), 1.70 ~ 1.60 (m, 1H), 1.10 ~ 1.00 (m, 1H), 0.85 (d, J = 6.28 Hz, 3H), 0.49 (d, J = 6.10 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.72, 173.98, 140.71, 140.59, 135.08, 134.02, 133.80, 132.58, 130.86, 130.70, 128.25, 127.87, 127.62, 126.70, 125.80, 125.56, 122.42, 110.50, 74.54, 72.64, 72.19, 62.52, 60.59, 60.41, 56.67, 39.31, 36.58, 26.44, 23.38, 21.06.

(*1"R, 2"S, 2'R, 3'R, 3S, 4'R*) 6-Chloro-4'-(3-chloro-phenyl)-2'-(2,2-dimethyl-propyl)-1'-(2-hydroxy-1,2-diphenyl-ethyl)-2-oxo-1,2-dihydro-spiro[indole-3,3'-pyrrolidine]-5'-carboxylic acid

dimethylamide (5d). $[\alpha]_D^{25}$ -92.7 (c, 0.6 $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.85 (br, 1H), 7.45 (d, J = 8.10 Hz, 2H), 7.41~ 6.72 (m, 14H), 6.68 (d, J = 7.72 Hz, 1H), 5.43 (d, J = 3.24 Hz, 1H), 4.84 (br, 1H), 4.50 (d, J = 3.55 Hz, 1H), 4.38 (d, J = 10.46 Hz, 1H), 3.98 (d, J = 10.46 Hz, 1H), 3.65 (d, J = 9.00 Hz, 1H), 2.97 (dd, J = 9.00 Hz, 12.00 Hz, 1H), 2.86 (s, 3H), 1.94 ~ 1.85 (m, 1H), 1.93 (s, 3H), 0.79 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.32, 140.84, 135.61, 135.32, 134.20, 133.71, 130.97, 129.51, 129.08, 128.16, 128.02, 127.60, 127.41, 126.48, 125.89, 125.17, 122.41, 110.48,74.85, 73.82, 72.00, 62.31, 60.94, 60.41, 57.92, 42.19, 36.69, 30.31, 29.68.

(*1"R, 2"S, 2'R, 3'R, 3S, 4'R*) 6-Chloro-4'-(3-chloro-phenyl)-1'-(2-hydroxy-1,2-diphenyl-ethyl)-2'-propyl-2-oxo-1,2-dihydro-spiro[indole-3,3'- pyrrolidine]-5'-carboxylic acid dimethylamide (5e) $[\alpha]_D^{25}$ -73.9 (c, 0.3 $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 8.27 (br, 1H), 7.66 (d, J = 8.01 Hz, 1H), 7.63 (d, J = 6.97 Hz, 1H), 7.42 (d, J = 6.99 Hz, 1H), 7.32 (d, J = 7.26 Hz, 1H), 7.28 ~ 6.57 (m, 13H), 5.14 (s, 1H), 4.60, (d, J = 10.06 Hz, 1H), 4.58 ~ 4.48 (m, 1H), 4.48 (d, J = 3.32 Hz, 1H), 4.17 ~ 4.10 (m, 1H), 3.37 (d, J = 10.11 Hz, 1H), 2.87 (s, 3H), 2.56 ~ 2.40 (m,1H), 1.99 (s, 3H), 2.00 ~ 1.88 (m, 1H), 1.10 ~ 0.87 (m, 2H), 0.27 ~ 0.72 (m, 3H).

(*1"R, 2"S, 2'R, 3'R, 3S, 4'R*) 6-Chloro-4'-(3-chloro-phenyl)-1'-(2-hydroxy-1,2-diphenyl-ethyl)-2'-(3-methyl-butyl)-2-oxo-1,2-dihydro-spiro[indole-3,3'-pyrrolidine]-5'-carboxylic acid dimethylamide (5f).



$[\alpha]_D^{25}$ -85.6 (c, 0.4 $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 8.68 (br, 1H), 7.63 (d, J = 7.77 Hz, 1H), 7.28 ~ 6.81 (m, 16H), 5.13 (s, 1H), 4.59 (d, J = 10.12 Hz, 1H), 4.54 (s, 1H), 4.46 (d, J = 2.94 Hz, 1H), 4.12 (d, J = 10.10 Hz, 1H), 3.31 (d, J = 10.26 Hz, 1H), 2.85 (s, 3H), 2.60 ~ 2.45 (m, 1H), 1.99 (s, 3H), 1.95 ~ 1.84 (m, 1H), 1.48 ~ 1.42 (m, 1H), 0.95 ~ 0.82 (m, 1H), 0.77 (t, J = 5.50 Hz, 6H), 0.70 ~ 0.60 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.58, 173.82, 140.98, 140.53, 136.12, 135.21, 134.03, 133.76, 130.75, 129.43, 129.16, 128.04, 127.81, 127.59, 127.32, 126.64, 125.86, 125.30, 122.29, 110.75, 75.18, 74.99, 73.10, 62.68, 60.39, 56.99, 37.22, 36.57, 36.42, 29.19, 28.08, 22.61.

At 0°C, to a solution of compound **5** (2.0 mmol) in CH_2Cl_2-MeOH (10 mL, 1 : 1), $Pb(OAc)_4$ (1.34 g, 3.0 mmol) was added. And the reaction was stirred at 0°C for 5 ~ 10 min, the solution was filtered through a short silica gel column. The solvent was removed *in vacuo* and the residue was purified by chromatography to yield the product.

(*2'R, 3S, 4'R, 5'R*) 6-Chloro-2'-isobutyl-2-oxo-4'-phenyl-1,2-dihydro-spiro[indole-3,3'-pyrrolidine]-5'-carboxylic acid dimethylamide (1a). $[\alpha]_D^{25}$ 24.7 (c, 0.8 $CHCl_3$); ^{1}HNMR (300 MHz, $CDCl_3$), δ 8.20 (br, 1H), 7.38 ~ 7.01 (m, 5H), 6.80 (d, J = 1.86 Hz, 1H), 6.66 (dd, J = 1.91, 8.10 Hz, 1H), 6.32 (d, J = 8.13 Hz, 1H), 4.63 (d, J = 7.12 Hz, 1H), 3.94 (d, J = 7.18 Hz, 1H), 3.65 ~ 3.55 (m, 1H), 2.97 (s, 3H), 2.75 (s, 3H), 1.76 ~ 1.51 (m, 2H), 0.99 ~ 0.88 (m, 1H), 0.82 (d, J = 6.63 Hz, 3H), 0.78 (d, J = 6.52 Hz, 3H); ^{13}CNMR (75 MHz, $CDCl_3$), δ 180.54, 170.75, 142.16, 138.68, 133.34, 128.78, 128.63, 128.43, 128.30, 127.56, 127.11, 125.85, 121.67, 109.86, 68.75, 64.59, 63.72, 59.87, 38.58, 37.14, 36.23, 25.85, 23.49, 21.74; EI/MS, 426 (M^++ 1); HRMS $C_{24}H_{29}ClN_3O_2$ ($[M+H]^+$) required 426.1948, found 426.1937. Anal. Calcd. For $C_{24}H_{28}ClN_3O_2$: C, 67.67; H, 6.63; N, 9.87; found: C, 67.91; H, 6.82; N, 9.56.

(*2'R, 3S, 4'R, 5'R*) 6-Chloro-4'-(3-chloro-phenyl)-2'-isobutyl-2-oxo-1,2-dihydro-spiro[indole-3,3'-pyrrolidine]-5'-carboxylic acid dimethylamide (1b) $[\alpha]_D^{25}$ 50.0 (c, 0.3 $CHCl_3$); ^{1}HNMR (300 MHz, $CDCl_3$), δ 9.30 (br, 1H), 7.27 ~ 6.95 (m, 4H), 6.88 (s, 1H), 6.73 (d, J = 8.00 Hz, 1H), 6.47 (d, 8.01 Hz, 1H), 4.61 (d, J = 7.66 Hz, 1H), 4.00 (d, J = 7.64 Hz, 1H), 3.58 ~ 3.54 (m, 1H), 2.97 (s, 3H), 2.88 (s, 3H), 1.65 ~ 1.45 (m, 2H), 0.98 ~ 0.91 (m, 1H), 0.78 (d, J = 6.63 Hz, 3H), 0.76 (d, J = 6.53 Hz, 3H); ^{13}CNMR (75 MHz, $CDCl_3$), δ 180.98, 170.23, 142.44, 140.40, 134.35, 133.58, 129.77, 128.68, 127.64, 126.94, 125.64, 121.75, 110.36, 68.84, 64.08, 63.59, 59.50, 38.54, 37.22, 36.21, 25.78, 23.38, 21.72; EI/MS, 460 (M^++1); HRMS $C_{24}H_{28}Cl_2N_3O_2$ ($[M+H]^+$) required 460.1559, found 460.1552. Anal. Calcd. For $C_{24}H_{27}Cl_2N_3O_2$: C, 62.61; H, 5.91; N, 9.13; found: C, 62.96; H, 6.19; N, 8.88.

(*2'R, 3S, 4'R, 5'R*) 6-Chloro-4'-(4-chloro-phenyl)-2'-isobutyl-2-oxo-1,2-dihydro-spiro[indole-3,3'-pyrrolidine]-5'-carboxylic acid dimethylamide (1c). $[\alpha]_D^{25}$ 68.0 (c, 0.3 $CHCl_3$); ^{1}HNMR (300 MHz, $CDCl_3$), δ 8.98 (br, 1H), 7.21 (d, J = 8.18 Hz, 2H), 7.09 (d, J = 8.17 Hz, 2H), 6.86 (s, 1H), 6.74 (d, J = 7.75 Hz, 1H), 6.46 (d, J = 7.78 Hz, 1H), 4.59 (d, J = 7.63 Hz, 1H), 3.99 (d, J = 7.64 Hz, 1H), 3.56 (m, 1H), 2.96 (s, 3H), 2.85 (s, 3H), 1.68 ~ 1.53 (m, 2H), 0.98 ~ 0.88 (m, 1H), 0.79 (d, J = 12.1 Hz, 3H), 0.77 (d, J = 12.0 Hz, 3H); ^{13}CNMR (75 MHz, $CDCl_3$), δ 180.86, 170.40, 142.35, 136.85, 133.62, 133.28, 130.04, 128.76, 127.16, 125.73, 121.87, 110.29, 68.90, 64.35, 63.60, 59.40, 38.66, 37.24, 36.24, 25.83, 23.42, 21.76; EI/MS, 460 (M^++1); HRMS $C_{24}H_{28}Cl_2N_3O_2$ ($[M+H]^+$) required 460.1559, found 460.1552. Anal. Calcd. For $C_{24}H_{27}Cl_2N_3O_2$: C, 62.61; H, 5.91; N, 9.13; found: C, 62.43; H, 6.25; N, 8.80.

(*2'R, 3S, 4'R, 5'R*) 6-Chloro-4'-(3-chloro-phenyl)-2-oxo-2'-(2,2-dimethylpropyl)-1,2-dihydro-spiro[indole-3,3'-pyrrolidine]-5'-carboxylic acid dimethylamide (1d). $[\alpha]^{25}_{D}$ 60.9 (c, 0.4 $CHCl_3$); ^{1}HNMR (300 MHz, $CDCl_3$), δ 9.36 (br, 1H), 7.35 ~ 6.97 (m, 4H), 6.90 (s, 1H), 6.69 (d, J = 8.10 Hz, 1H), 6.38 (d, J = 8.11 Hz, 1H), 4.54 (d, J = 47.41 Hz, 1H), 4.00 (d, J = 7.39 Hz, 1H), 3.50 (d, J = 9.41 Hz, 1H), 3.17 (br, 1H), 2.97 (s, 3H), 2.91 (s, 3H), 1.51 ~ 1.42 (m, 1H), 0.91 ~ 0.83 (m, 1H), 0.82 (s, 9H); ^{13}CNMR (75 MHz, $CDCl_3$), δ 181.37, 170.16, 142.63, 141.05, 134.33, 133.50, 129.78, 128.64, 127.52, 127.04, 126.54, 125.65, 121.65, 110.41, 68.13, 65.22, 64.41, 58.08, 43.10, 37.21, 36.19, 30.01, 29.79. EI/MS, 474 (M^++1); HRMS $C_{25}H_{30}Cl_2N_3O_2$ ($[M+H]^+$) required 474.1715, found 474.1713. Anal. Calcd. For $C_{25}H_{29}Cl_2N_3O_2$: C, 63.29; H, 6.16; N, 8.86; found: C, 62.99; H, 6.32; N, 8.63.

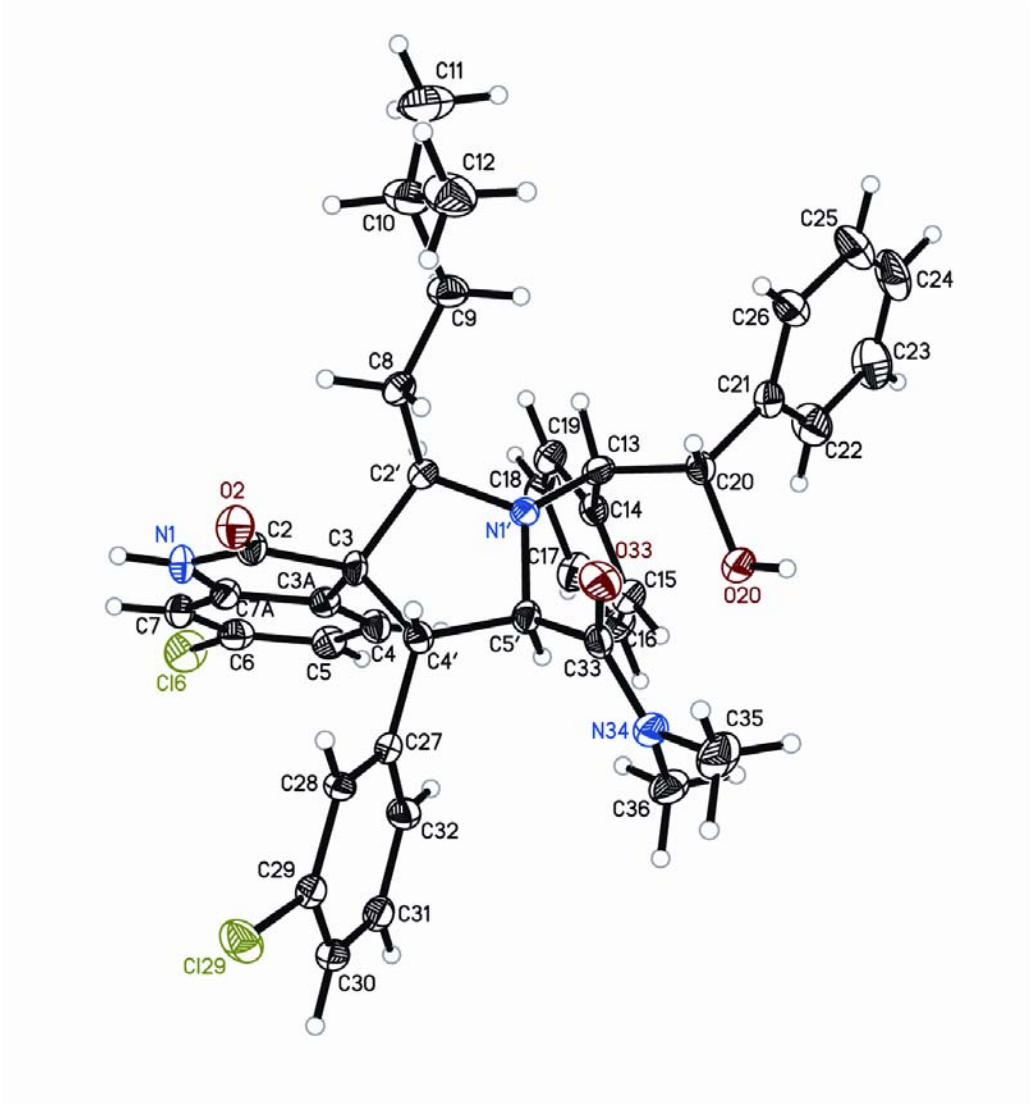
(*2'R, 3S, 4'R, 5'R*) 6-Chloro-4'-(3-chloro-phenyl)-2-oxo-2'-propyl-1,2-dihydro-spiro[indole-3,3'-pyrrolidine]-5'-carboxylic acid dimethylamide (1e). $[\alpha]^{25}_{D}$ 42.2 (c, 1.0 $CHCl_3$); ^{1}HNMR (300 MHz, $CDCl_3$), δ 9.39 (br, 1H), 7.16 ~ 7.05 (m, 4H), 6.87 (s, 1H), 6.74 (d, J = 7.98 Hz, 1H), 6.49 (d, J = 8.07 Hz, 1H), 4.62 (d, J = 7.82 Hz, 1H), 4.00 (d, J = 7.81 Hz, 1H), 3.51 (dd, J = 9.15, 9.27 Hz, 1H), 2.97 (s, 3H), 2.74 (s, 3H), 1.65 ~ 1.44 (m, 2H), 1.29 ~ 1.18 (m, 2H), 0.77 (t, J = 7.15 Hz, 3H), ^{13}CNMR (75 MHz, $CDCl_3$), δ 180.89, 170.27, 142.47, 140.30, 134.34, 133.59, 129.75, 128.67, 127.65, 127.21, 126.94, 125.73, 121.73, 110.34, 70.65, 63.85, 63.49, 59.66, 37.21, 36.21, 31.92, 20.77, 14.00; EI/MS, 446 (M^++1); HRMS $C_{23}H_{26}Cl_2N_3O_2$ ($[M+H]^+$) required 446.1402, found 446.1408. Anal. Calcd. For: $C_{23}H_{25}Cl_2N_3O_2$: C, 61.89; H, 5.65; N, 9.41; found: C, 61.48; H, 5.70; N, 9.11.

(*2'R, 3S, 4'R, 5'R*) 6-Chloro-4'-(3-chloro-phenyl)-2'-(3-methyl-butyl)-2-oxo-1,2-dihydro-spiro[indole-3,3'-pyrrolidine]-5'-carboxylic acid dimethylamide (1f). $[\alpha]^{25}_{D}$ 25.1(c, 0.5 $CHCl_3$); ^{1}HNMR (300 MHz, $CDCl_3$), δ 8.15 (br, 1H), 7.21 ~ 7.07 (m,4H), 6.81 (s, 1H), 6.76 (dd, J = 1.85, 8.06 Hz, 1H), 6.49 (d, d, J = 8.08 Hz, 1H), 4.69 (d, J = 7.90 Hz, 1H), 3.96 (d, J = 7.89 Hz, 1H), 3.53 (dd, J = 8.64, 9.19 Hz, 1H), 2.97 (s, 3H), 2.85 (s, 3H), 1.68 ~ 1.56 (m, 1H), 1.46 ~ 1.36 (m, 1H), 1.30 ~ 1.20 (m, 2H), 1.11 ~ 0.99 (m, 1H), 0.78 (d, J = 6.46 Hz, 3H), 0.75 (d, J = 6.48 Hz, 3H); ^{13}CNMR (75 MHz, $CDCl_3$), δ 180.00, 170.08, 141.83, 140.20, 134.45, 133.70, 129.82, 128.73, 127.78, 127.13, 126.93, 125.94, 121.98, 110.03, 70.69, 63.56, 63.33, 59.70, 40.43, 37.21, 36.51, 27.86, 27.33, 22.54, 22.15; EI/MS, 474 (M^++ 1); HRMS $C_{25}H_{30}Cl_2N_3O_2$ ($[M+H]^+$) required 474.1715, found 474.1714. Anal. Calcd. For $C_{25}H_{29}Cl_2N_3O_2$: C, 63.29; H, 6.16; N, 8.86; found: C, 62.82; H, 6.27; N, 8.74.

II. Structural Determination by X-Ray Analysis

The structure and the absolute configuration were determined for (*1''R, 2''S, 2'R, 3'R, 3S, 4'R*) 6-chloro-4'-(3-chloro-phenyl)-1'-(2-hydroxy-1,2-diphenyl-ethyl)-2'-(3-methyl-butyl)-2-oxo-1,2-dihydro-spiro[indole-3,3'-pyrrolidine]-5'-carboxylic acid dimethylamide, as shown in **Figure S1**.

Figure S1. X-ray structure of (*1''R, 2''S, 2'R, 3'R, 3S, 4'R*) 6-chloro-4'-(3-chloro-phenyl)-1'-(2-hydroxy-1,2-diphenyl-ethyl)-2'-(3-methyl-butyl)-2-oxo-1,2-dihydro-spiro[indole-3,3'-pyrrolidine]-5'-carboxylic acid dimethylamide.



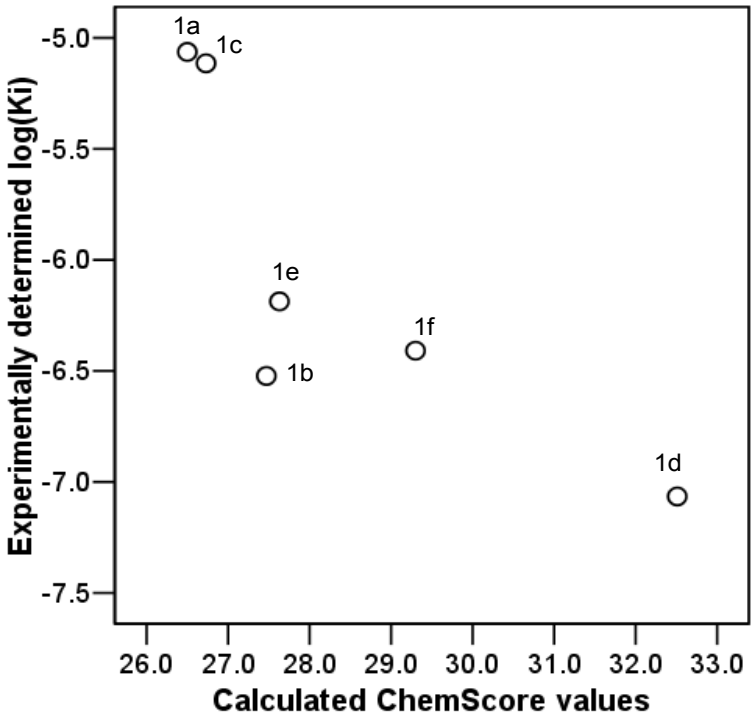
III. Molecular Docking

All docking studies were carried out using the GOLD program[3,4] (version 2.1) with the ChemScore fitness function. The structures of the designed compounds (**1a-1f**) were constructed using the SYBYL molecular modeling software[5] and were energy-minimized with the Tripos force field. The MDM2 structural coordinates were extracted from the crystal structure[6] of MDM2 complexed with a p53 transactivation domain peptide available from the Protein Data Bank (PDB code: 1YCR). Hydrogen atoms were added to the protein using SYBYL. The active site was defined to encompass all atoms within a 12 Å radius sphere, whose origin was located at the center of the ligand. The standard Genetic Algorithm protocol was selected for the docking. For each compound, 20 individual docking runs were conducted. The generated 20 solutions of each ligand were ranked according to their scores calculated by the ChemScore fitness function in the GOLD program. The best docking scores for compounds **1a-1f** as calculated by the ChemScore fitness function were provided in **Table S1**, together with the experimentally determined binding affinities for these compounds using a newly developed fluorescence polarization-based binding assay. The correlation between the ChemScore and log (K_i) is plotted in **Figure 2S**. The predicted binding model for compounds **1a** by GOLD is provided in **Figure 3S**.

Table S1. Docking scores of compounds **1a-1f** by the ChemScore function in the GOLD program together experimentally determined K_i values by our fluorescence polarization-based binding assay.

	Chemscore	Experimentally determined K_i value by FP-based Assay (μM)	Log(K_i)
1a	26.5	8.46	-5.07
1b	27.47	0.30	-6.52
1c	26.73	7.68	-5.11
1d	32.51	0.086	-7.07
1e	27.63	0.65	-6.19
1f	29.3	0.39	-6.41

Figure 2S. Correlation between the ChemScore values calculated by the GOLD program based upon the predicted binding models and the experimentally determined log (Ki) values for compounds **1a-1f.** The R^2 for the correlation is 0.66.



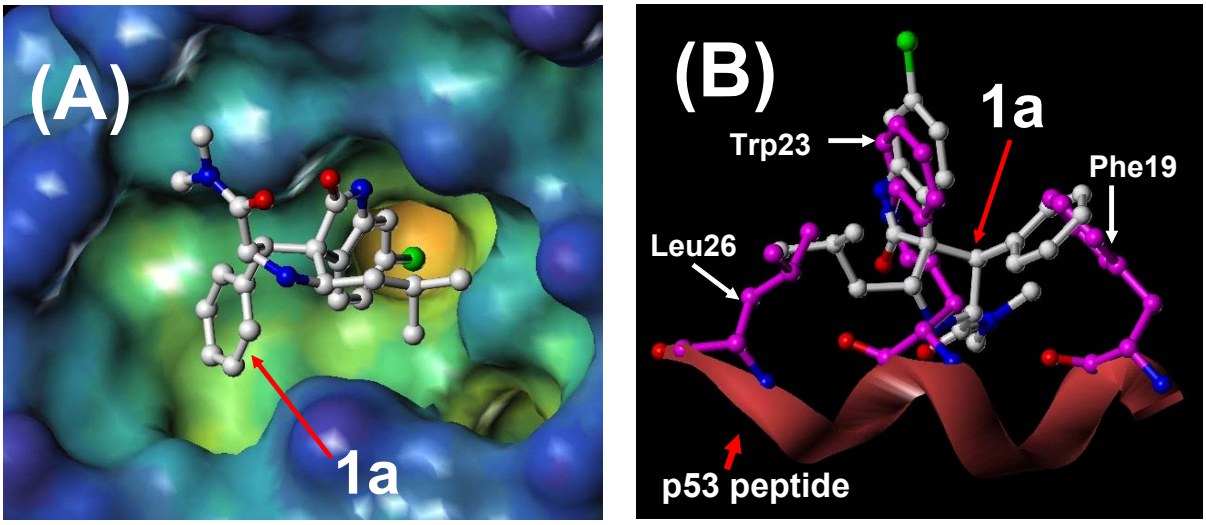


Figure 3S. (**A**). Predicted binding model of compound **1a** to MDM2 using the GOLD program. For **1a**, carbons are in white, nitrogens in blue, chloride in green and oxygens in red. MDM2 binding site is color-coded according to the cavity depth. Buried regions are coded in yellow and solvent exposed regions are coded in blue. (**B**). Superposition of compound **1a** to the **p53** peptide conformation in the crystal structure of p53 peptide in complex with MDM2. Three critical residues Phe19, Trp23 and Leu26 in p53 are colored in purple. For compound **1a**, the same colors are used to color-code atoms as in (**A**).

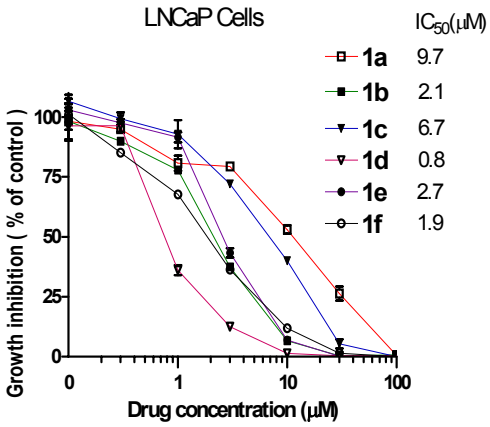
IV. Cell Growth Assay

The cellular growth inhibitory activities of compounds **1a-1f** were determined using two human prostate cancer LNCaP (wild type p53) and PC-3 (a deleted p53) cell lines, and normal human prostate epithelial cells (wild type p53). The p53 status of these cell lines has been previously determined.[7]

Cells were seeded in 96-well flat bottom cell culture plates at a density of $3\text{-}4\times10^3$ cells/well with compounds and incubated for 4 days. The rate of cell growth inhibition after treatment with increasing concentrations of the compounds was determined by WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt (Dojindo Molecular Technologies Inc., Gaithersburg, Maryland). WST-8 was added at a final concentration of 10% to each well, and then the plates were incubated at 37□ for 2-3 hrs. The absorbance of the samples was measured at 450 nm using a TECAN ULTRA Reader. Concentration of the compounds that inhibited cell growth by 50% (IC_{50}) was calculated by comparing absorbance in the untreated cells and the cells treated with the compounds. These compounds inhibited cell growth in a dose-dependent manner. The inhibitory curves for compounds **1a-1f** in LNCaP and PC-3 prostate cancer cells and in normal human prostate epithelial cells (PrEC) were provided in **Figure S4a**, **S4b** and **S4c**.

Figure S4. Inhibition of cell growth by MDM2 inhibitors in LNCaP (wild-type p53) and PC-3 (deleted p53) human prostate caner cell lines and in normal human prostate epithelial cells (PrEC) as determined by the WST cell growth assay.

Figure S4a.



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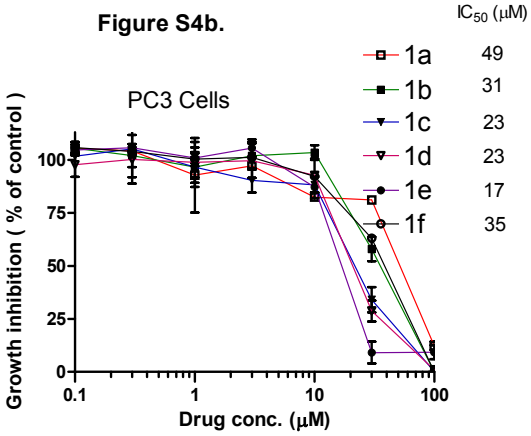
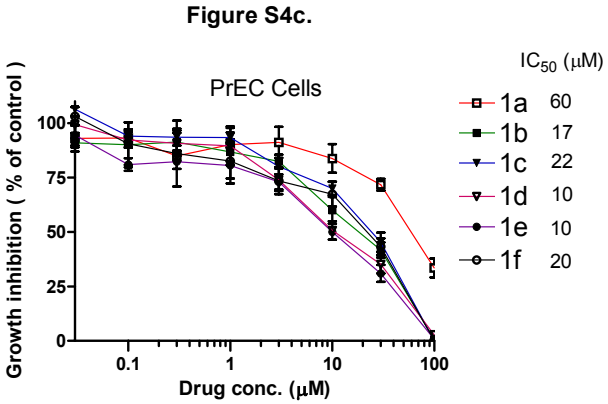


Figure S4c.



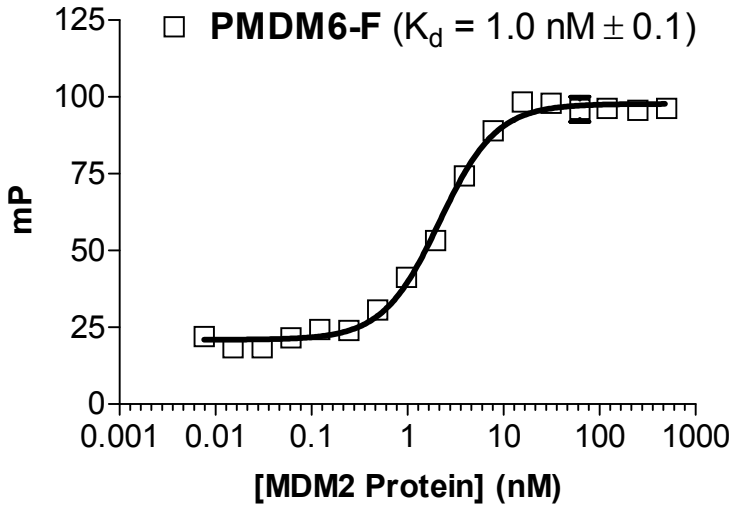
V. MDM2 Protein expression and purification

MDM2 (residues 1-118) was cloned into a pET28a expression vector with an n-terminal His_6-tag and transformed into *Escherichia coli* CD41 (DE3). Cultures were grown at 37°C in 2xYT medium containing 0.2% glycerol, and induced by 0.4mM IPTG at an OD_{600} of 0.6 at 18°C for 20 hours. Cells were lysed in 50mM Tris, pH7.5 buffer containing 500mM NaCl and 10% glycerol. MDM2 (1-118) was purified from the soluble fraction using Ni-NTA resin (QIAGEN), following the manufacturer's instruction, followed by a Source S column, using 30mM Tris (pH7.5) buffer containing a gradient from 50mM to 1M NaCl. Finally, MDM2 (1-118) was purified on a Superdex 75 column (Amersham Biosciences) in 30mM Tris pH7.5, 150mM NaCl and 10% glycerol. The protein was purified to >98% as judged SDS-PAGE.

VI. Fluorescence Polarization Competitive Binding Assay

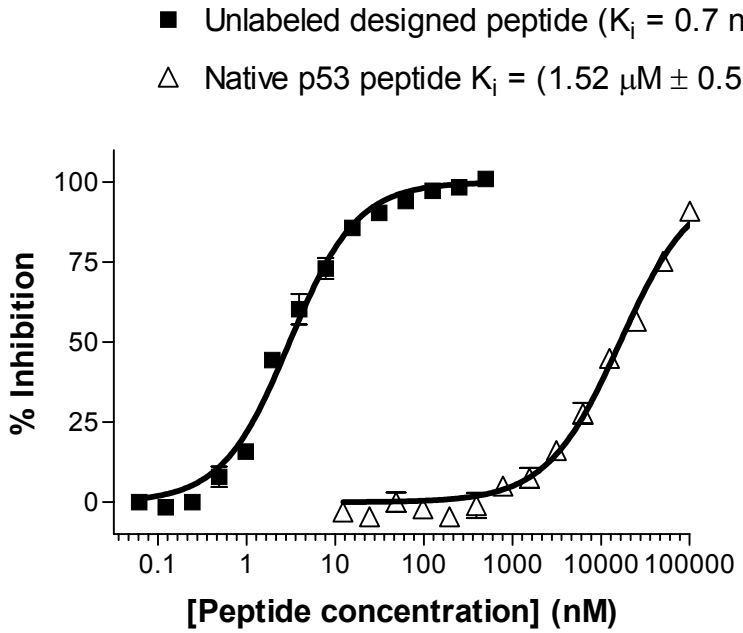
In order to determine quantitatively the binding affinities of designed compounds to MDM2 and to disrupt the interaction between MDM2 and p53, we have established a fluorescence polarization-based (FP-based) binding assay using a recombinant human MDM2 protein (residues 1-118) and a p53-based peptide labeled with a fluorescence tag. The design of a fluorescence probe was based upon a previously reported high-affinity peptide-based MDM2 inhibitor[9] (5-FAM-βAla-βAla-Phe-Met-Aib-pTyr-(6-Cl-*L*-Trp)-Glu-Ac3c-Leu-Asn-NH2), termed as **PMDM6-F**. The K_d value of **PMDM6-F** with the MDM2 protein was determined to be 1.0 nM $\pm$ 0.09 (**Figure S5**), consistent with its reported high-affinity determined using the ELISA method.[9] The specificity of the assay was confirmed by competitive displacement of **PMDM6-F** from MDM2 protein by its corresponding unlabeled peptide (termed PMDM6) without the fluorescence tag 5-FAM (**Figure S6**). As an additional control, we have synthesized and tested the natural p53 peptide (QETFSDLWKLLP-NH2), which has a K_i value of 1.52 μM in our binding assay, similar to the values reported in literature.[10]

Figure S5. Saturation curve of the fluorescently labeled peptide (**PMDM6-F**) to MDM2 protein



The dose-dependent binding experiments were carried out with serial dilutions of the tested compounds in DMSO. A 5 μl sample of the tested samples and preincubated MDM2 protein (0.010 μM) and **PMDM6-F** peptide (0.001 μM) in the assay buffer (100 mM potassium phosphate, pH 7.5; 100 μg/ml bovine gamma globulin; 0.02% sodium azide, purchased from Invitrogen™ Life Technology), were added in Dynex 96-well, black, round-bottom plates (Fisher Scientific) to produce a final volume of 125 μl. For each assay, the controls included the MDM2 protein and **PMDM6-F** (equivalent to 0% inhibition), only **PMDM6-F** peptide (equivalent to 100% inhibition). The polarization values were measured after 3 hrs of incubation using an ULTRA READER (Tecan U.S. Inc., Research Triangle Park, NC). The IC_{50} values, i.e. the inhibitor concentration at which 50% of bound peptide is displaced, were determined from a plot using nonlinear least-squares analysis. Curve fitting was performed using GRAPHPAD PRISM software (GraphPad Software, Inc., San Diego, CA).

Figure S6. Competitive binding curves of the unlabeled designed peptide (PMDM6) and a natural p53 peptide to MDM2 protein



To calculate the binding affinity constants (K_i) of inhibitors, we have used the following equation[11] developed for computing the K_i values in FP-based binding assays:

$$K_i = [I]_{50} / ([L]_{50} / K_d + [P]_0 / K_d + 1)$$

in which $[I]_{50}$ denotes the concentration of the free inhibitor at 50% inhibition, $[L]_{50}$ the concentration of the free labeled ligand at 50% inhibition, $[P]_0$ the concentration of the free protein at 0% inhibition, and K_d the dissociation constant of the protein-ligand complex. We developed a computational procedure to compute the accurate values of all of the parameters used in the equation.[11] A web-based computer program was developed for computing the K_i values for inhibitors in FP-based binding assays based upon this equation.[11]

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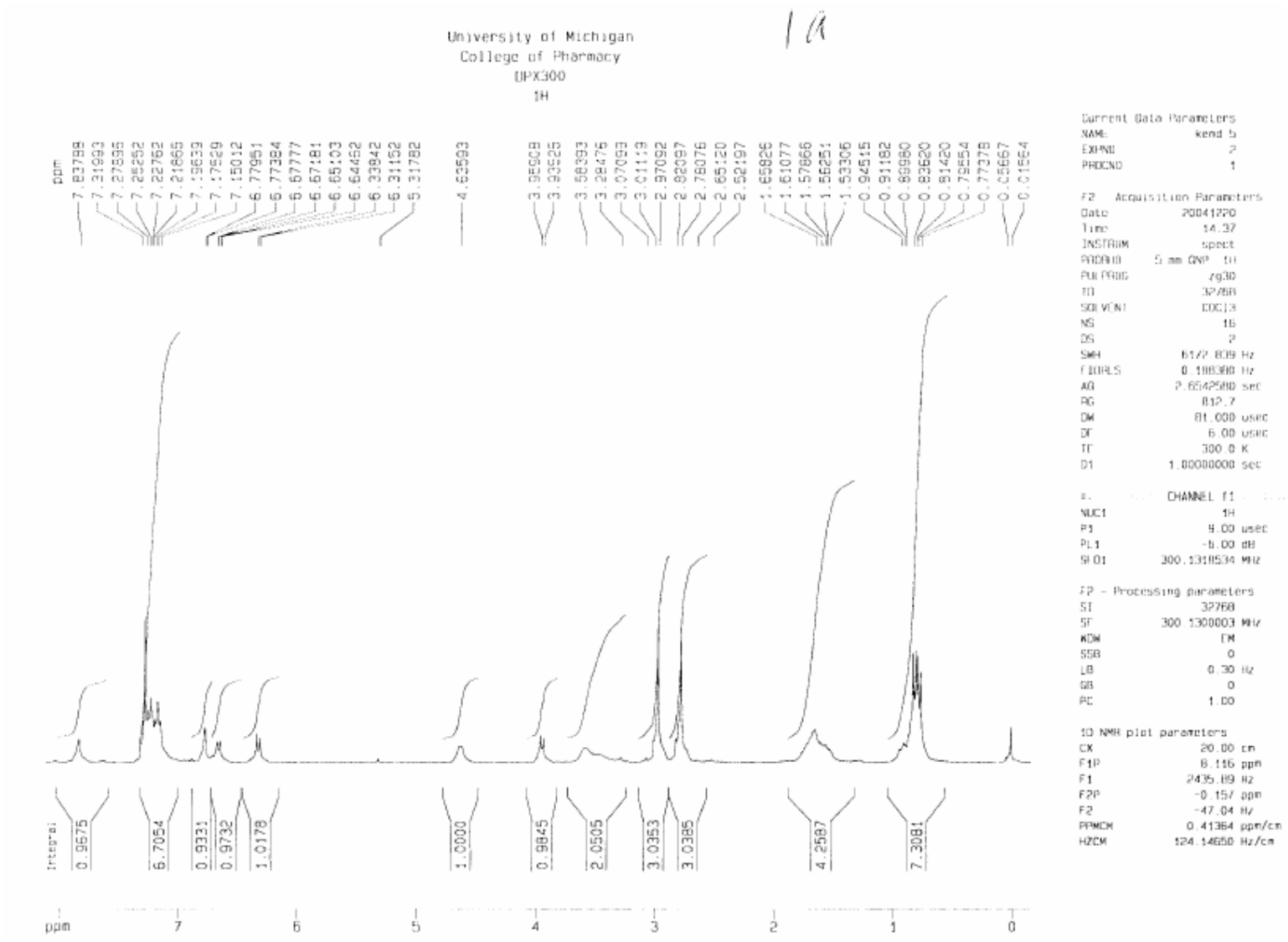
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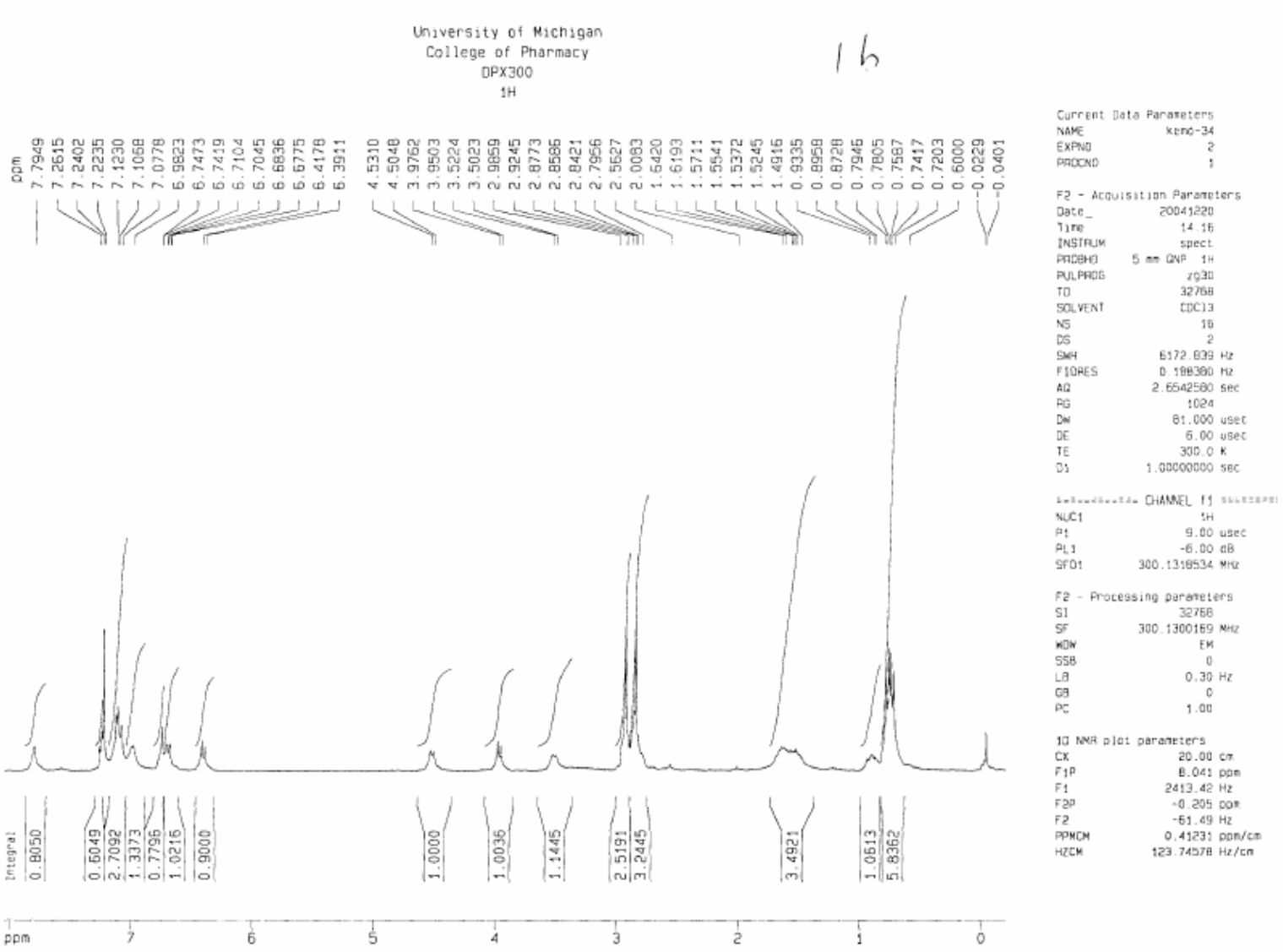
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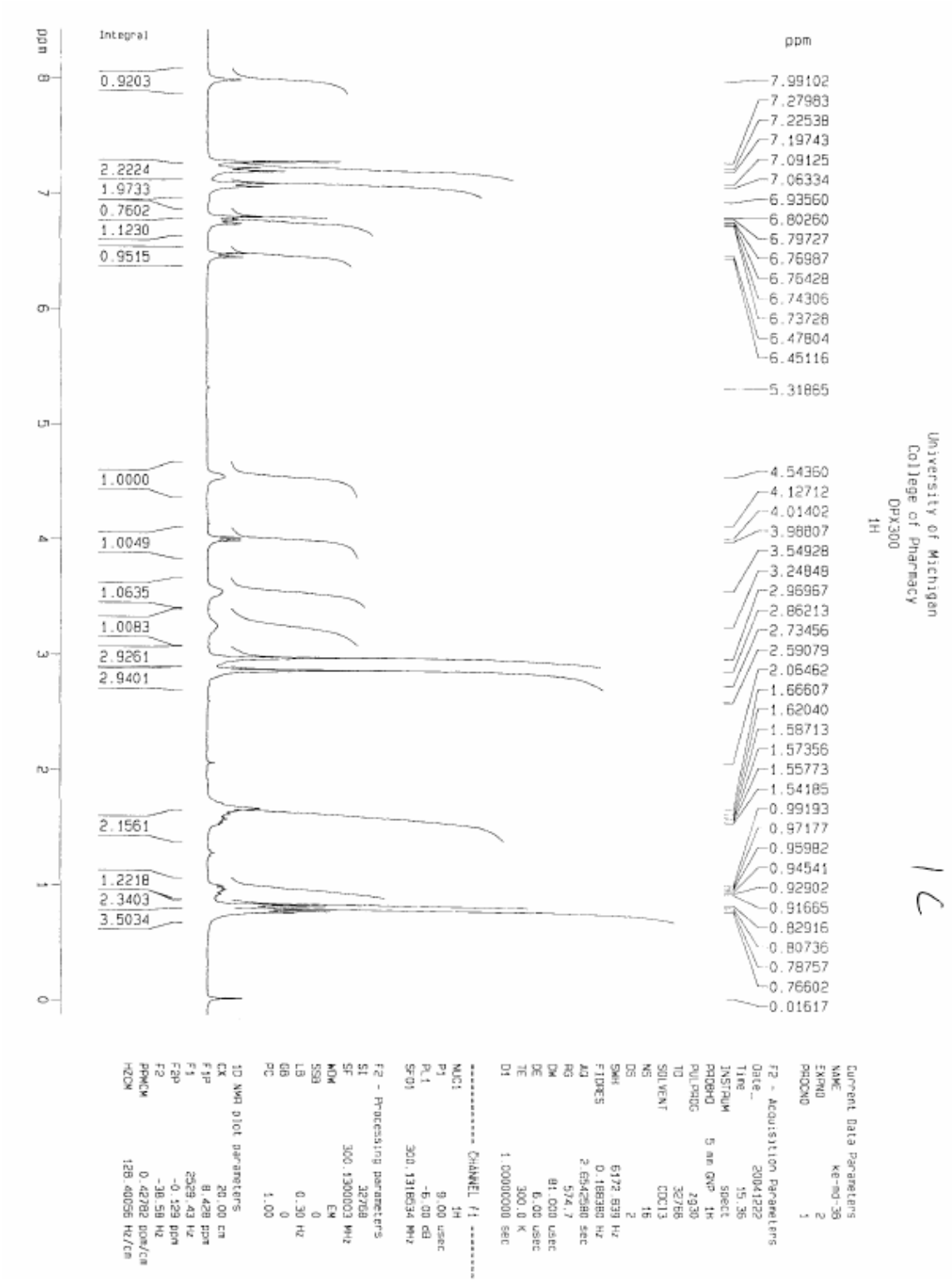
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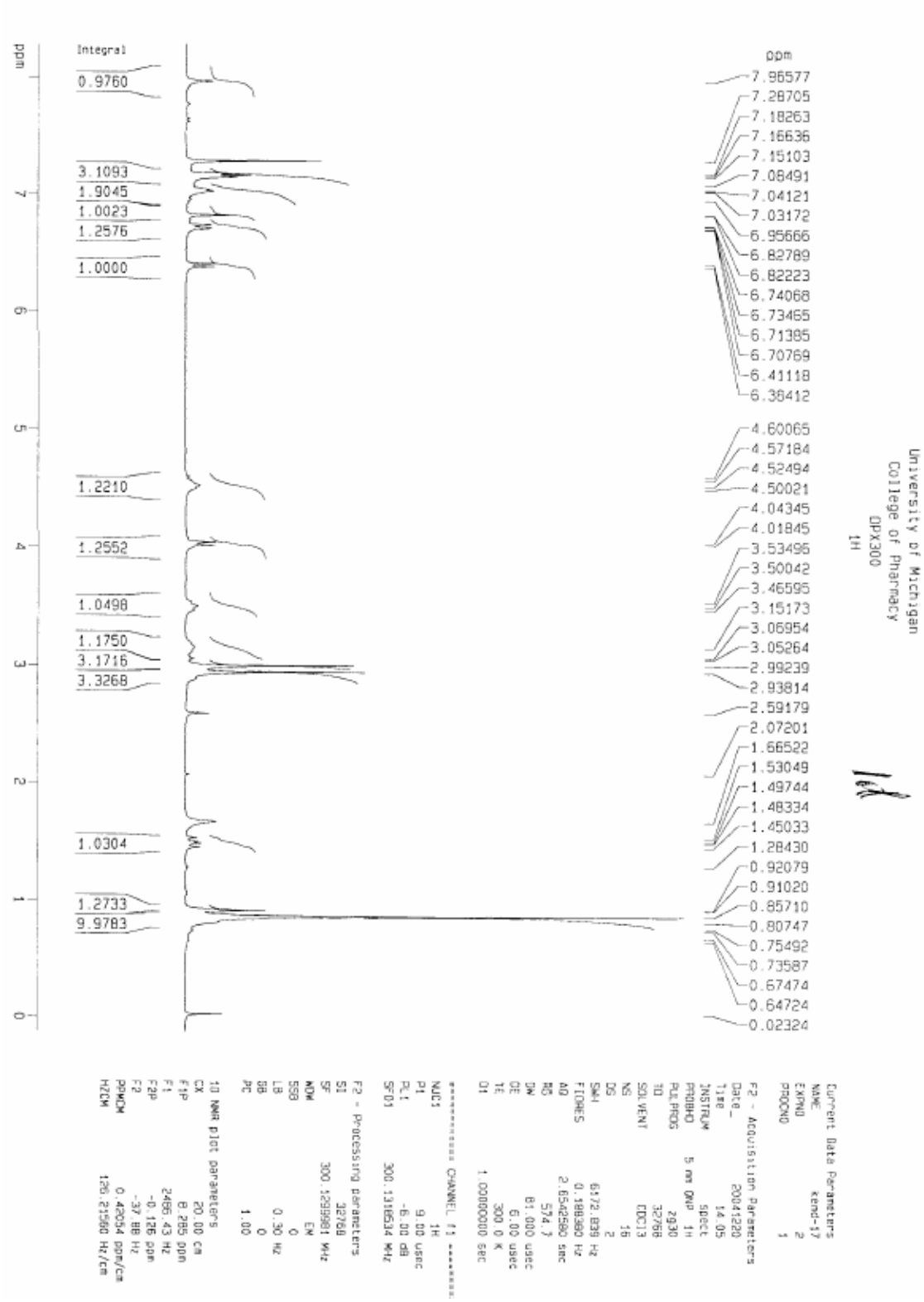
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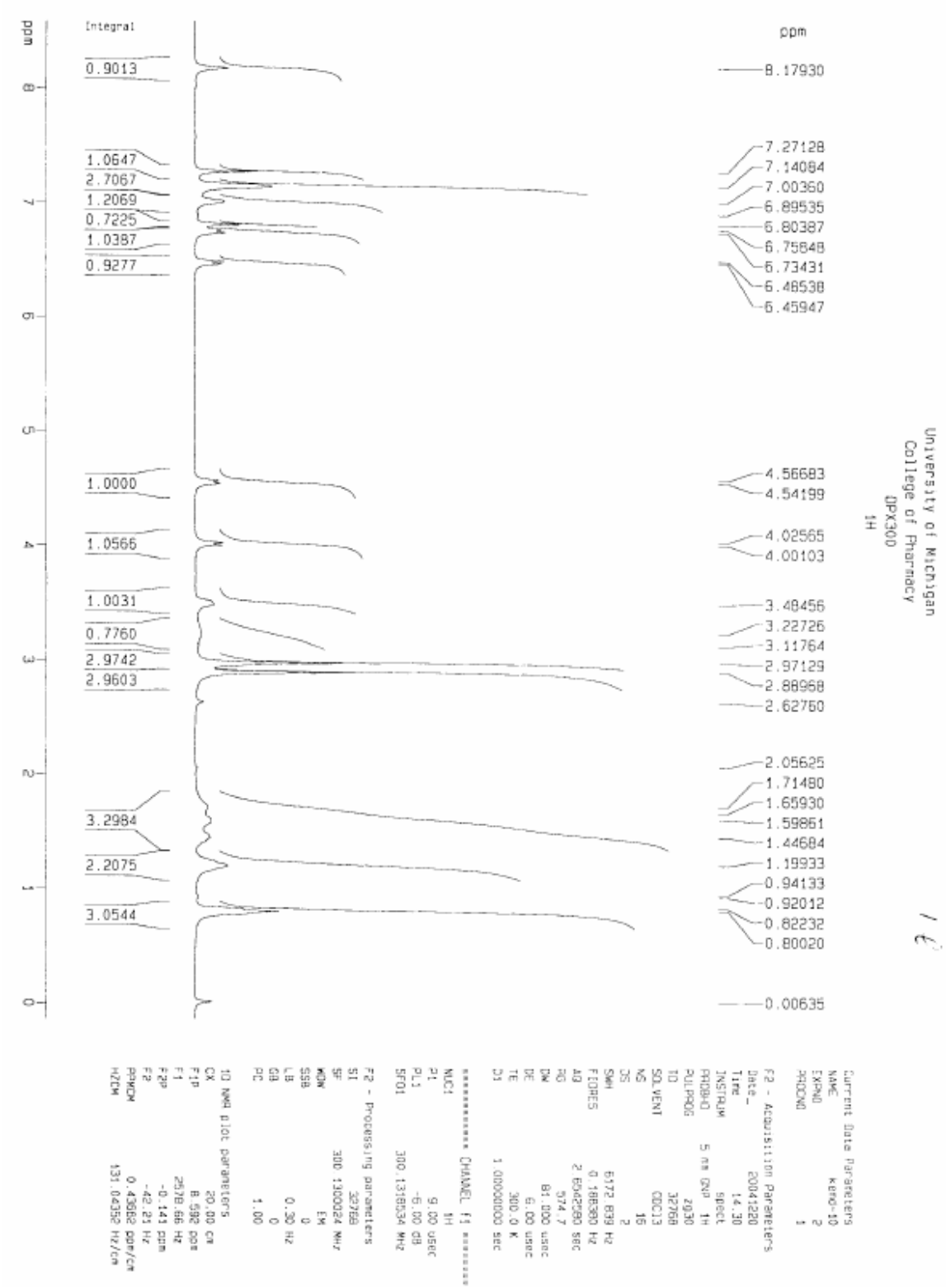
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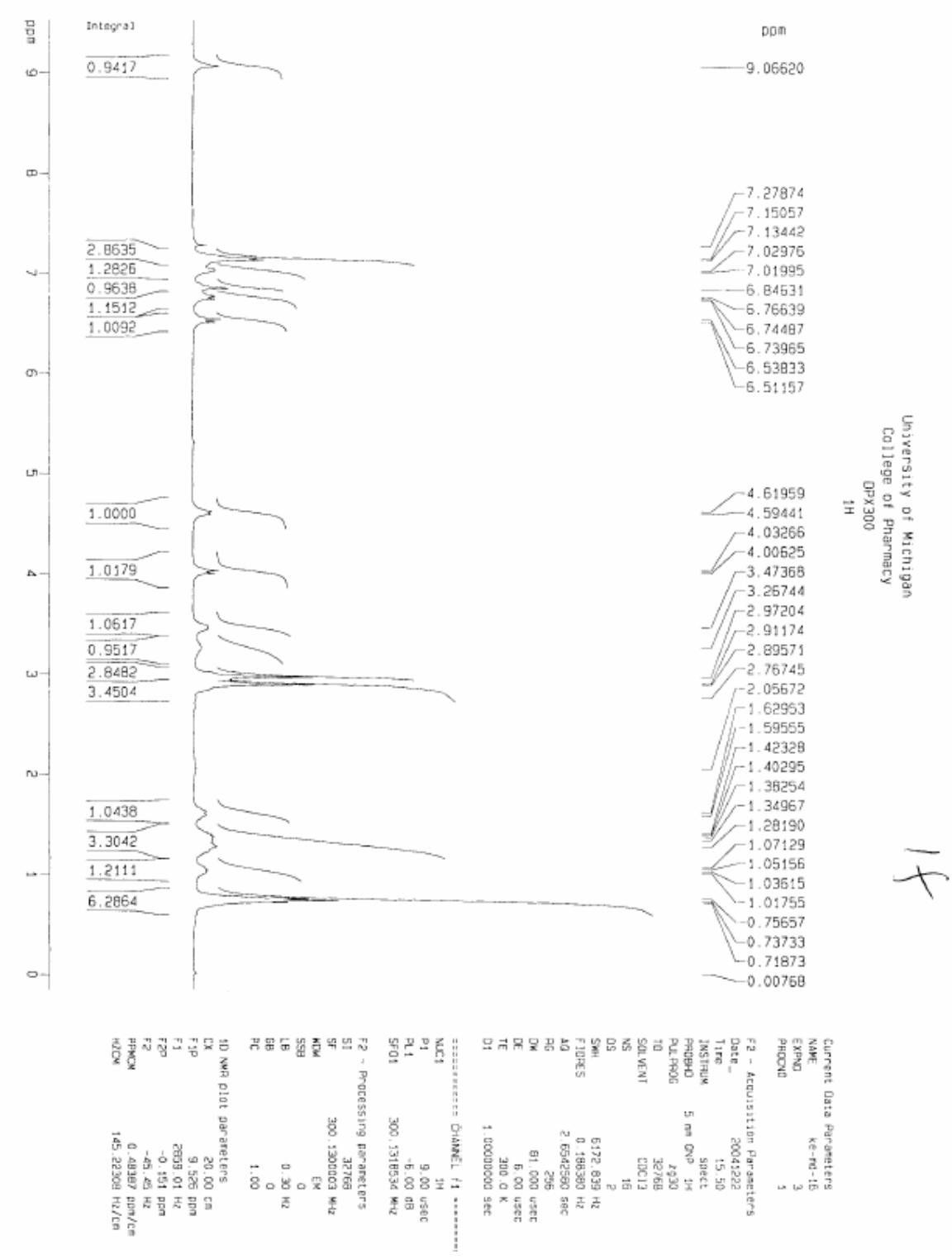
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1.0438
3.3042
1.2111
6.2864
Current Data Parameters
NAME ke-nd-16
EXPNO 3
PROCNO 1
F2 - Acquisition Parameters
Date_ 20041222
Time 15.50
INSTRUM spect
PROBHD 5 mm QNP 1H
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 16
DS 2
SWH 6172.839 Hz
FIDRES 0.188380 Hz
AQ 2.6542580 sec
RG 256
DW 81.000 usec
DE 6.00 usec
TE 300.0 K
D1 1.00000000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 9.00 usec
PL1 -6.00 dB
SFO1 300.1318534 MHz
F2 - Processing parameters
SI 32768
SF 300.1300003 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
1D NMR plot parameters
CX 20.00 cm
FIP 9.525 ppm
F1P 2859.01 Hz
F2P -0.151 ppm
F2 -45.45 Hz
PPMCM 0.48367 ppm/cm
HZCM 145.22369 Hz/cm