**Supporting Information**

# A Phosphorylated Prodrug for the Inhibition of Pin1

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## Experimental

### General

Unless otherwise indicated, all reactions were carried out under $N_2$ in flame-dried glassware. THF and $CH_2Cl_2$ were dried by passage through alumina. Anhydrous (99.8%) DMF was available commercially and used directly from SureSeal$^{TM}$ bottles. Dimethyl sulfoxide (DMSO) was anhydrous and dried with 4 Å molecular sieves. Triethylamine (TEA) was distilled from $CaH_2$, and oxalyl chloride $(COCl)_2$ was distilled before use each time. Diisopropylethylamine (DIEA) was distilled from $CaH_2$ under a $N_2$ atmosphere. Brine, $NaHCO_3$, and $NH_4Cl$ refer to saturated aqueous solutions unless otherwise noted. Flash chromatography was performed on 32-63 μm or 230-400 mesh, ASTM silica gel with reagent grade solvents. NMR spectra were obtained at rt in $CDCl_3$ unless otherwise noted. Proton (400 MHz) NMR spectra were obtained for compounds **4-10**, and carbon-13 (75 MHz) NMR spectra for compounds **4-10**. Proton (500 MHz) NMR spectra, and carbon-13 (125 MHz) NMR spectra were obtained for compounds **1** and **2**. Phosphorus (75 MHz) NMR spectra was obtained for compound **1**, **2**, **10**. Rotamer peaks are indicated by listing $^1$H chemical shifts separately; for $^{13}$C the minor rotamer peak is listed in parentheses. Coupling constant *J* values are given in Hz. Electrospray ionization (ESI-MS) was carried out on a triple quadrupole ThermoFinnigan TSQ MS. Human Pin1 recombinant protein was prepared as described.[1] Analytical reverse phase liquid chromatography (RP-HPLC) was performed on a RP C18, 250 × 4.4 mm, 5 μm column (Varian Solaris). Preparative HPLC was performed using on a RP C18, 250 × 21.4 mm, 5 μm (Varian Solaris). HPLC solvents were A: water, B: $CH_3CN$. UV detection was performed at 220 nm unless otherwise noted.

**Lactone 4.** Fmoc-Ser-Ψ[(*Z*)CH=C]-Pro-OH, **3** (40 mg, 0.096 mmol) and imidazole (33 mg, 0.48 mmol) were dissolved in DMF (2.0 mL), and TBSCl (29 mg, 0.19 mmol) was added. The mixture was stirred for 16 h, and $NH_4Cl$ (2 mL) was added. The mixture was stirred for an additional 50 min, and diluted with EtOAc (20 mL), washed with $NH_4Cl$ (2 × 10 mL), dried with $Na_2SO_4$, and concentrated. Chromatography on silica gel with 2% methanol in chloroform gave **4** (28 mg, 55%) as white powder. $^1$H NMR ($CDCl_3$) δ 7.77 (d, *J* = 8.0, 2H),

7.57 (d, *J* = 8.0, 2H), 7.40 (app. t, *J* = 7.5, 2H), 7.32 (app. t, *J* = 6.5, 2H), 5.50 (br s, 1H), 4.73 (d, *J* = 8.5, 1H), 4.52? (br s, 1H), 4.46 (d, *J* =6.0, 2H), 4.39 (t, *J* = 12, 1H) 4.22 (m, 2H), 3.92 (m, 1H), 2.37 (br s, 2H), 2.27 (two d, *J* = 7.1, 7.7, 1H), 1.97 (two d, *J* = 6.0, 7.1, 1H), 1.73 (two d, *J* = 6.0, 6.1, 1H), 1.60 (two d, *J* = 5.9, 7.8, 1H). $^{13}$C-NMR ($CDCl_3$) δ 173.2, 155.3, 143.6, 142.4, 141.3, 127.8, 127.0, 124.9, 120.0, 66.7, 66.3, 49.0, 47.1, 43.9, 35.1, 29.3, 24.4 ppm. HRMS calculated for $C_{24}H_{23}NO_4$ ($MH^+$) *m/z* = 390.1705, found *m/z* = 390.1715.

**Boc-Ser(TBS)-Ψ[(*Z*)CH=C]-Pro-OH 7.** Boc-Ser-Ψ[(*Z*)CH=C]-Pro-OH **6** (synthesized by the published method)[2] (111 mg, 0.388 mmol) and imidazole (136 mg, 2.00 mmol) were dissolved in DMF (2.0 mL), and TBSCl (151 mg, 1.00 mmol) was added with stirring. The reaction was stirred for 18 h at rt, then $NH_4Cl$ (5 mL) was added. The mixture was stirred for an additional 60 min, and then diluted with EtOAc (20 mL), washed with $NH_4Cl$ (2 × 10 mL), and brine (10 mL). The organic layer was dried over $MgSO_4$, and concentrated by rotary evaporation. Chromatography on silica gel with 30% EtOAc in hexane gave 150 mg (70%) of **7** as pale yellowish oil. $^1$H-NMR ($CDCl_3$) δ 11.10 (br s, 1H), 5.96 and 4.91 (br s, 1 H), 5.42 (d, *J* = 5.8, 1H), 4.23 (br s, 1H), 3.61 and 3.59 (d, *J* = 4.4, 1H), 3.55 and 3.48 (br s, 2H), 2.41 (m, 1H), 2.23 (m, 1H), 2.04 (br s, 1H), 1.90 (m, 1H), 1.80 (br s, 1H), 1.55 (m, 1H), 1.37 (s, 9H), 0.83 (s, 9H), - 0.01 (s, 6H). $^{13}$C-NMR ($CDCl_3$) δ 178.5, 155.4 (157.0), 144.1 (145.4), 122.2, 79.1 (79.9), 65.3, 51.8 (52.7), 45.8, 33.6, 31.1, 28.2, 25.7, 24.0, 18.2, -5.5 ppm. HRMS calculated for $C_{20}H_{38}NO_5Si$ ($MH^+$) *m/z* = 400.2519, found *m/z* = 400.2485.

**Boc-Ser(TBS)-Ψ[(*Z*)CH=C]-Pro-(2)-*N*-(3)-ethylaminoindole 8.** Boc-Ser(TBS)-Ψ[(*Z*)CH=C]-Pro-OH, **7** (150 mg, 0.376 mmol) was dissolved in DMF (20 mL), and cooled to 0 °C for 10 min. HOAt (101 mg, 0.751 mmol), HATU (287 mg, 0.751 mmol) and DMAP (10 mg, 0.075 mmol) were added. Then DIEA (260 μL, 1.50 mmol) was added to the stirred solution dropwise. Tryptamine (120 mg, 0.751 mmol) was added slowly. The mixture was stirred for 6 h, diluted with EtOAc (200 mL), washed with water (2 × 50 mL), and brine (20 mL). The aqueous layer was back-extracted with $CH_2Cl_2$ (2 × 75 mL). The organic layers were combined, dried with $Na_2SO_4$ and concentrated. Chromatography on silica gel with 2% MeOH in $CHCl_3$ gave 184 mg (90%) of **8** as colorless oil. $^1$H-NMR ($CDCl_3$), δ 8.82 (br s, 1H), 7.71 (br s, 1H), 7.65 (d, *J* = 7.6, 1H), 7.33 (d, *J* = 8.1, 1H), 7.13 (app t, *J* = 7.6, 1H), 7.05 (app t, *J* = 7.5, 1H), 6.97 (s, 1H), 5.32 (d, *J* = 8.8, 1H), 5.05 (s, 1H), 4.08 (m, 1H), 3.64 (m, 1H), 3.55 (m, 1H), 3.50 (two d, *J* = 6.2, 6.3, 2H), 3.35 (d, *J* = 7.0, 1H), 3.00 (app t, *J* = 7.7, 2H), 2.31 (m, 2H), 2.15 (m, 1H), 1.79 (m, 1H), 1.54 (m, 2H), 1.44 (s, 9H), 0.88 (s, 9H), 0.04 (s, 6H). $^{13}$C-NMR ($CDCl_3$) δ 171.9, 156.0, 144.3, 136.2, 127.5, 123.8, 121.9, 121.4, 118.7,

113.1, 111.1, 79.5, 64.9, 52.6, 47.5, 40.5, 38.5, 36.4, 32.8, 30.8, 28.3, 25.7, 25.0, 23.3, 18.2, -5.6. HRMS calculated for $C_{30}H_{48}N_3O_4$ ($MH^+$) *m/z* = 542.3414, found *m/z* = 542.3403.

**Boc-Ser-Ψ[(*Z*)CH=C]-Pro-(2)-*N*-(3) ethylaminoindole 9.** Boc-Ser(TBS)-Ψ[(*Z*)CH=C]-Pro-(2)-*N*-(3) ethylaminoindole, **8** (92 mg, 0.17 mmol) was dissolved in THF ( 2.5 mL), and cooled to 0 °C for 10 min. A solution of TBAF (117 mg, 0.342 mmol) in THF (2.5 mL) was added dropwise at 0 °C. The mixture was stirred at rt for 4 h. The reaction was quenched with $NH_4Cl$ (25 mL), and extracted with EtOAc (2 × 80 mL). The organic layer was washed with brine (20 mL), dried with $Na_2SO_4$ and concentrated. Chromatography on silica gel with 2% MeOH in $CHCl_3$ gave 85 mg (85%) of **9** as colorless oil. $^1$H-NMR ($CDCl_3$), δ 8.60 (br s, 1H), 7.73 (br s, 1H), 7.63 (d, *J* = 7.8, 1H), 7.33 (d, *J* = 8.1, 1H), 7.15 (app t, *J* = 7.5, 1H), 7.07 (app t, *J* = 7.4, 1H), 6.99 (s, 1H), 5.33 (s, 1H), 5.30 (d, *J* = 8.8, 1H), 4.04 (m, 1H), 3.87 (br s, 1H), 3.60 (m, 1H), 3.53 (m, 2H), 3.45 (m, 1H), 3.34 (d, *J* = 7.6, 1H), 3.01 (m, 2H), 2.29 (m, 1H), 2.17 (m, 2H), 1.80 (m, 1H), 1.52 (m, 2H), 1.41 (s, 9H). $^{13}$C-NMR ($CDCl_3$) δ 173.5, 156.5, 144.4, 136.1, 127.5, 123.4, 122.1, 121.7, 119.1, 118.8, 113.1, 111.1, 79.7, 64.7, 53.4, 47.4, 40.5, 33.4, 31.6, 28.3, 24.7, 23.8. HRMS calculated for $C_{24}H_{34}N_3O_4$ ($MH^+$) *m/z* = 428.2549, found *m/z* = 428.2553.

**Fmoc-Ser-Ψ[(*Z*)CH=C]-Pro-(2)-*N*-(3)-ethylaminoindole 5.** Boc-Ser-Ψ[(*Z*)CH=C]-Pro-(2)-*N*-(3)-ethylaminoindole **9** (62 mg, 0.14 mmol) was dissolved in $CH_2Cl_2$ (3 mL), and cooled to 0 °C for 10 min. TFA (1 mL) was added dropwise. The mixture was stirred at 0 °C for 10 min and cold bath was removed. The mixture was stirred at rt for an additional 45 min and the solvent was evaporated. $CH_2Cl_2$ was added and evaporated (3 × 20 mL). The remaining TFA was removed under high vacuum overnight. Without further purification, the crude product was dissolved in a mixture solution of 10% aq. $Na_2CO_3$ and $NaHCO_3$ (3:1, 2 mL), then cooled to 0 °C for 10 min. A solution of Fmoc-Cl (43 mg, 0.17 mmol) in dioxane (2 mL) was added dropwise. After stirring at 0 °C for 20 h, the mixture was diluted with water (20 mL) and extracted with EtOAc (2 × 25 mL). The aqueous layer was acidified with 1M HCl to pH 3-4 and extracted with EtOAc (3 × 30 mL) and $CH_2Cl_2$ (3 × 30 mL). The organic layers were combined, washed with brine (20 mL), dried with $Na_2SO_4$, and concentrated. Chromatography on silica gel with 10 % MeOH in $CHCl_3$ gave 63 mg (78 %) of **5** as a colorless oil. $^1$H-NMR ($CDCl_3$), δ 7.95 (br s, 1H), 7.77 (d, *J* = 7.5, 2H), 7.57 (d, *J* = 7.8, 2H), 7.53 (d, *J* = 6.2, 2H), 7.41 (app t, *J* = 7.5, 2H), 7.32 (app t, *J* = 6.8, 2H), 7.24 (s, 1H), 7.10 (app t, *J* = 7.5, 1H), 6.97 (app t, *J* = 7.4, 1H), 6.83 (br s, 1H), 5.23 (m, 2H), 4.37 (dd, *J* = 6.9, 10.5, 1H), 4.26 (d, *J* = 7.0, 9.3, 1H), 4.15 (app t, *J* = 6.6, 1H), 3.83 (m, 1H), 3.5-3.37 (m, 4H),

3.23 (d, $J$ = 6.8, 1H), 3.00 (m, 1H), 2.86 (m, 1H), 2.32 (m, 1H), 2.19 (m, 2H), 1.84 (m, 1H), 1.54 (m, 2H). $^{13}$C-NMR ($CDCl_3$) δ172.9, 156.7, 145.5, 143.9, 141.4, 136.2, 127.9, 127.3, 125.2, 125.1, 122.6, 122.2, 121.8, 120.2, 119.3, 118.9, 113.4, 111.2, 66.7, 64.6, 53.7, 47.9, 47.2, 40.7, 33.6, 31.7, 24.6, 24.0. HRMS calculated for $C_{34}H_{36}N_3O_4$ ($MH^+$) $m/z$ = 550.2706, found $m/z$ = 550.2711.

**Fmoc-Ser(PO(OH)$_2$)-Ψ[(*Z*)CH=C]-Pro-(2)-*N*-(3)-ethylaminoindole 1.** Fmoc-Ser-Ψ[(*Z*)CH=C]-Pro-(2)-*N*-(3)-ethylaminoindole, **5** (31 mg, 0.056 mmol) was dissolved in THF (3 mL). Tetrazole (36 mg, 0.22 mmol) and ($t$BuO)$_2$P(N-$i$Pr)$_2$ (40 μL, 0.11 mmol) were added. The mixture was stirred at rt for 20 h, then cooled to –40 °C for 10 min. *tert*-Butyl hydroperoxide (5 M in decane, 22 μL, 0.11 mmol) was added dropwise. The mixture was stirred at –40 °C for 40 min. The cold bath was removed and the reaction was stirred at rt for an additional 30 min. The mixture was cooled to 0 °C and 10% aq. $Na_2S_2O_3$ (3 mL) was added. After stirring for 10 min, the mixture was transferred to a separatory funnel using $Et_2O$ (3 × 30 mL). The combined organic layers were washed with 10 % aq. $Na_2S_2O_3$ (2 × 20 mL) and brine (20 mL), dried with $Na_2SO_4$, and concentrated to give 40 mg of the crude Fmoc-Ser(PO(O-$t$Bu)$_2$)-Ψ[(*Z*)CH=C]-Pro-(2)-*N*-(3)-ethylaminoindole, **10**, as a colorless oil. $^1$H-NMR ($CDCl_3$), δ 8.30 (s, 1H), 7.70 (t, $J$ = 10, 2H), 7.50-7.40 (m, 3H), 7.35 (m, 2H), 7.25 (m, 3H), 7.06 (app t, $J$ = 7.5, 1H), 6.92 (m, 2H), 5.67 (m, 1H), 5.30 (m, 1H), 4.27 (d, $J$ = 9.0, 1H), 4.10 (m, 2H), 3.90 (m, 2H), 3.50 (m, 3H), 3.35 (m, 1H), 2.95 (m, 2H), 2.22 (m, 3H), 1.90 (m, 1H), 1.45 (s, 9H). $^{31}$P-NMR ($CDCl_3$) δ –9.65 (s). ESI-MS gave the molecular ion $[M+H]^+$ $m/z$ = 742.3, $[M+Na]^+$ $m/z$ = 764.3. Without further purification (decomposes on silica gel), Fmoc-Ser(PO(O$t$Bu)$_2$)-Ψ[(*Z*)CH=C]-Pro-(2)-*N*-(3)-ethylaminoindole (40 mg, 0.054mmol) was dissolved in $CH_2Cl_2$ (4 mL), and cooled to 0 °C. TFA (1mL) was added to the reaction mixture slowly, followed by the addition of water (0.2 mL) as scavenger. After stirring at 0 °C for 10 min, the cold bath was removed and the reaction mixture was stirred at rt for an additional 30 min, and the solvent was evaporated. $CH_2Cl_2$ was added and evaporated (5 × 20 mL). The remaining TFA was removed under high vacuum overnight until constant weight was obtained. The crude product was purified by semipreparative $C_{18}$ HPLC at 15 mL/min, 10 % to 90 % B over 20 min. Purified **1** (12 mg, 70% yield) eluted at 19.6 min as a white solid. Purity was 98.8 % by analytical $C_{18}$ HPLC (2 mL/min, 10 % to 90 % B over 13 min, retention time 11.79 min). $^1$H-NMR ($CD_3OD$), δ 7.73 (dd, $J$ = 7.4, 5.1, 2H), 7.55 (app t, $J$ = 7.9, 2H), 7.39 (d, $J$ = 7.3, 1H), 7.32 (m, 3H), 7.21 (dd, $J$ = 7.0, 13.4, 2H), 7.03 (app t, $J$ = 7.3, 1H), 6.97 (s, 1H), 6.90 (app t, $J$ = 7.7, 1H), 5.43 (d, $J$ = 9.3, 1H), 4.40 (m, 1H),

4.24 (m, 2H), 4.08 (app t, $J$ = 6.6, 1H), 3.91 (m, 1H), 3.86 (m, 1H), 3.47 (m, 1H), 3.41 (app t, $J$ = 7.6, 2H), 2.91 (m, 2H), 2.41 (m, 1H), 2.30 (m, 1H), 1.99 (m, 2H), 1.72 (m, 1H), 1.57 (m, 1H). $^{13}$C-NMR ($CD_3OD$) δ 147.3, 145.4, 145.2, 142.5, 138.1, 128.8, 128.7, 128.1, 126.3, 126.2, 125.0, 123.4, 122.3, 120.8, 119.5, 119.4, 113.3, 112.2, 68.1, 67.9, 52.7, 41.7, 35.2, 35.1, 33.1, 30.8, 26.0, 25.5. $^{31}$P- NMR ($CD_3OD$) δ –1.16 (s). ESI-MS $[M+H]^+$ *m/z* = 630.3, $[M+Na]^+$ *m/z* = 652.2, $[M-H_3PO_4]^+$ *m/z* = 532.2. HRMS calcd for $C_{34}H_{37}N_3O_7P$ ($MH^+$) *m/z* = 630.2369, found *m/z* = 630.2340.

**Fmoc-Ser(PO(OPOM)$_2$)-Ψ[(*Z*)CH=C]-Pro-(2)-*N*-(3)-ethylaminoindole 2.** BisPOM phosphate was synthesized by a modification of the published procedure.[3] Oxalyl chloride (90 μL, 1.9 mmol) was added dropwise to $CH_2Cl_2$ (1.5 mL) at 0 °C. DMF (7 μL) was added in one portion. A solution of hydrogen bisPOM phosphate (62 mg, 0.19 mmol) in $CH_2Cl_2$ (1.5 mL) was added dropwise at 0 °C over 15 min. The reaction mixture was stirred at rt for 2 h. The solvent and $(COCl)_2$ were removed by rotary evaporation. The remaining oxalyl chloride was removed in vacuo until constant weight was obtained. The product was obtained as slightly yellowish oil (50 mg, 80%). Without further purification, the bisPOMphosphoryl chloride was used immediately in the next step. $^1$H-NMR ($CDCl_3$) δ 5.71 (m, 4H), 1.23 (s, 18H). $^{13}$C-NMR ($CDCl_3$) δ 83.52, 83.45, 31.0, 27.0. $^{31}$P-NMR ($CDCl_3$), δ 3.80 (s). Fmoc-Ser-Ψ[(*Z*)CH=C]-Pro-(2)-*N*-(3)-ethylaminoindole, **5** (21 mg, 0.038 mmol) was dissolved in THF (1.5 mL) and cooled to –40 °C. Pyridine (0.75 mL) was added, and the mixture was stirred at –40 °C for 20 min. DMAP (2.5 mg) was added, then a solution of bisPOM phosphoryl chloride (50 mg, 0.14 mmol) in THF (0.9 mL) was added to the reaction mixture dropwise via syringe at –40 °C. The mixture was stirred at –40 °C for 2 h. A second batch of bisPOM phosphoryl chloride solution, (20 mg, 0.058 mmol) in THF (0.5 mL) was added. The mixture was stirred for 30 min. The cold bath was removed and the reaction mixture was stirred at rt for 6 h. Water (2 mL) was added to quench the reaction and the reaction mixture was diluted with EtOAc (40 mL). The organic layer was washed with water (2 × 20 mL). The aqueous layer was back-extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine (20 mL), dried with $Na_2SO_4$, and concentrated. The crude product was purified using semipreparative $C_{18}$ HPLC (15 mL/min, 60% B for 5 min, then 60% to 95% B over 20 min). Purified **2** (3 mg, 20% yield) eluted at 23.0 min as a white solid. Purity > 95% by analytical $C_{18}$ HPLC (1.5 mL/min, 10% B for 5 min, then 10% to 90% B over 20 min, retention time 24.7 min). $^1$H-NMR ($CDCl_3$), δ 8.04 (s, 1H), 7.76 (d, $J$ = 7.1, 2H), 7.55 (m, 3H), 7.40 (m, 2H), 7.30 (m, 2H), 7.10 (app t, $J$ = 8.1, 1H), 6.97 (app t, $J$ = 7.4, 2H), 6.88 (s,

1H), 5.62 (m, 4H), 5.42(br s, 1H), 5.25 (m, 2H), 4.29 (d, $J$ = 7.0, 1H), 4.16 (app t, $J$ = 6.4, 1H), 4.11 (br s, 1H), 3.98 (m, 1H), 3.49 (m, 2H), 3.45-3.25 (m, 2H), 2.93 (m, 2H), 2.4-2.0 (m, 4H), 1.83 (m, 1H), 1.25 (s, 9H), 1.23 (s, 9H). $^{13}$C-NMR ($CDCl_3$) δ 177.2, 172.6, 156.1, 148.0, 144.0, 141.4, 136.3, 127.9, 127.3, 125.2, 122.2, 122.1, 120.3, 120.1, 119.4, 118.9, 113.4, 111.3, 83.0, 69.0, 67.0, 51.3, 48.0, 47.2, 40.4, 38.9, 33.6, 31.6, 27.0, 24.7, 23.9. $^{31}$P-NMR ($CDCl_3$) δ –3.93 (s). ESI-MS gave the molecular ion $[M+H]^+$ $m/z$ = 858.25; $[M+Na]^+$ $m/z$ = 880.37; $[M+K]^+$ $m/z$ = 896.47. HRMS calculated for $C_{46}H_{57}N_3O_{11}P$ ($MH^+$) $m/z$ = 858.3731, found $m/z$ = 858.3805.

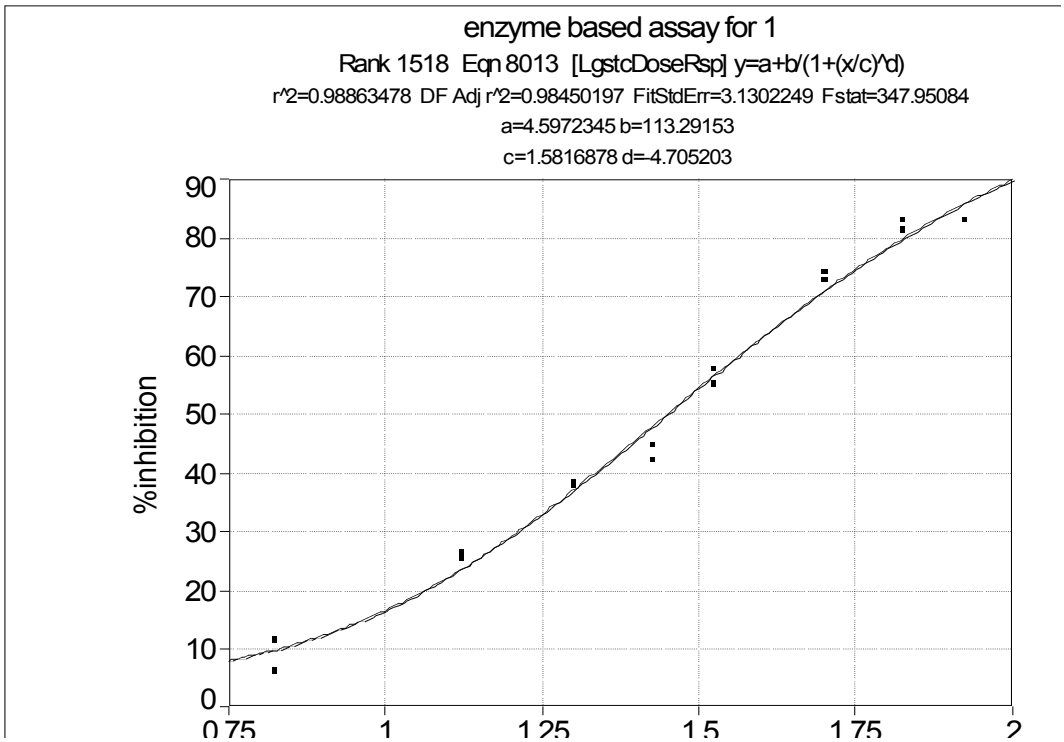
**Pin1 inhibition assay**

Pin1 inhibition assay of **1** was performed as published.[1] The inhibitor **1** (10 μL at concentrations of 800 μM, 1.6, 2.4, 3.2, 4.0, 6.0, 8.0, 10.0 mM in 1:2 DMSO: $H_2O$) was pre-equilibrated in the 1.0 mL quartz cuvette at 4 °C for 10 min. For each concentration, the assay was performed in duplicate, and all of the data were used for calculation of $IC_{50}$. The plot of % inhibition vs. log[I] (μM) produced a sigmoidal curve (SI Figure 1). The concentration of **1** for 50% inhibition of Pin1 activity ($IC_{50}$) was obtained by fitting all the experimental data to a dose response curve (95% confidence level) using equation (1) in TableCurve (version 3 for win32), where [I] is the inhibitor concentration (μM).

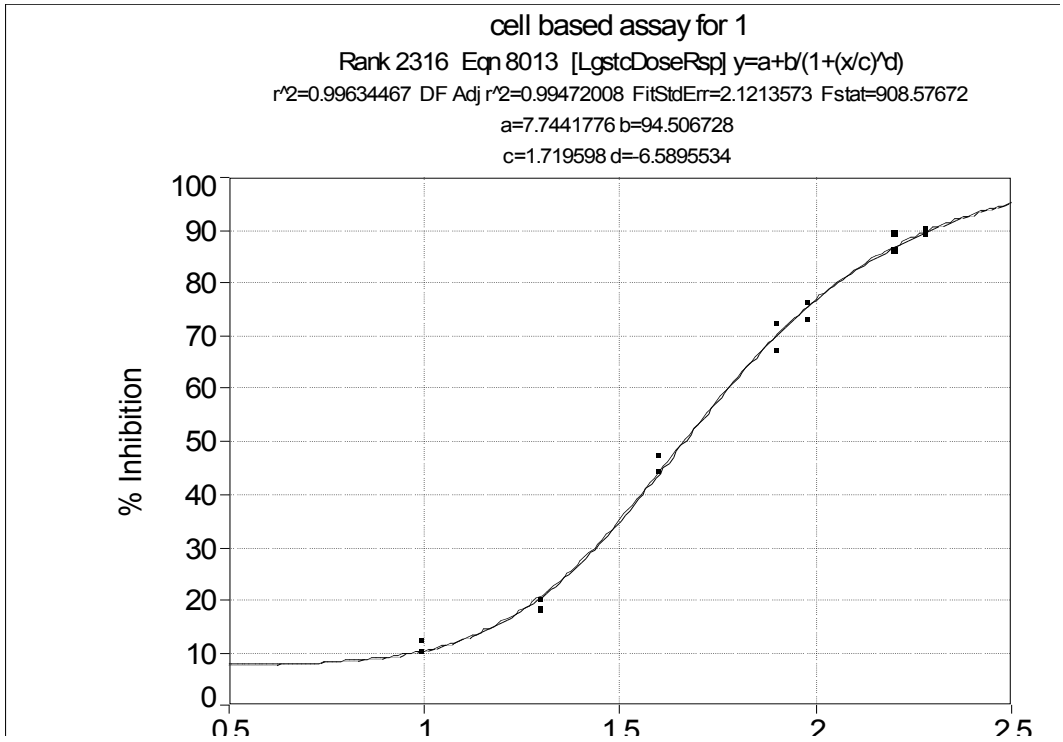
$$\%Inhibition = a + \frac{b}{\{1 + (\log[I]/c)^d\}} \qquad (1)$$

**A2780 cell based assay**

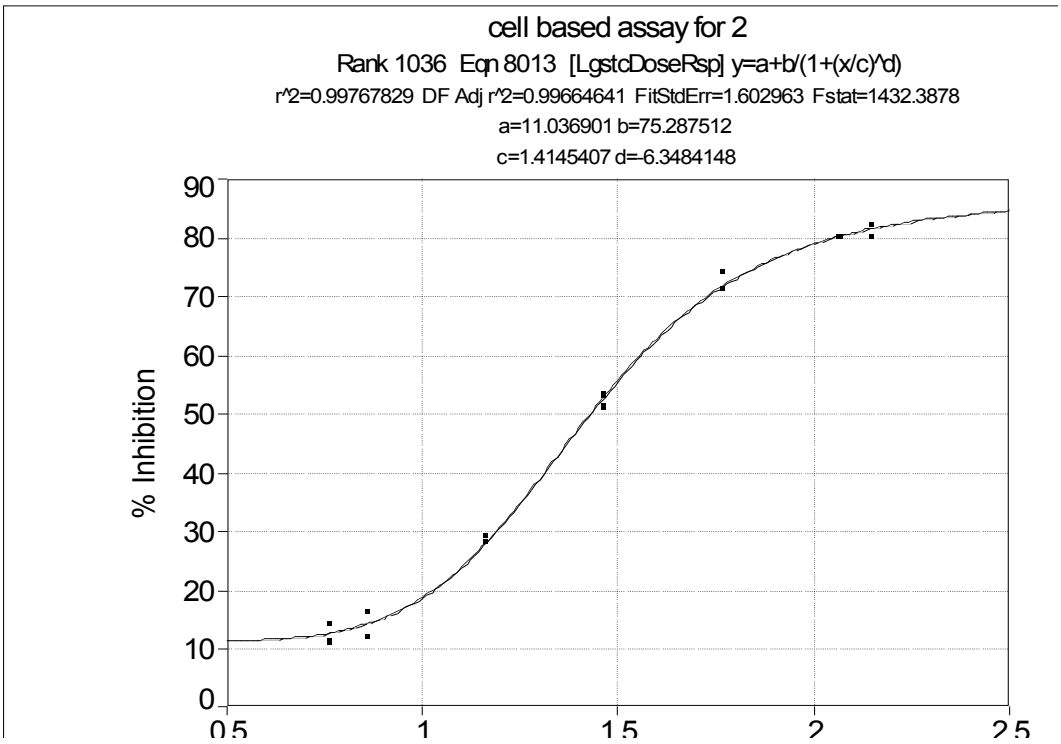
Antiproliferative activity towards the A2780 human ovarian cancer cell line was measured as published.[4, 5] The concentrations of **1** used were 190.8, 159.0, 95.4, 79.5, 39.7, 19.9, 9.9 μM (duplicates), and the concentrations of **2** were 140.0, 116.7, 58.3, 29.2, 14.6, 7.3, 5.8 μM (duplicates). The plot of % inhibition of proliferation activity against A2780 ovarian cancer cells vs. log[I] (μM) produced a sigmoidal curve for each inhibitor (SI Figures 2 and 3). The concentrations of **1** and **2** for 50% inhibition of Pin1 activity ($IC_{50}$) were obtained by fitting all the experimental data to a dose response curve (95% confidence level) using equation (1) in TableCurve (version 3 for win32). Where [I] is the inhibitor concentration (μM).



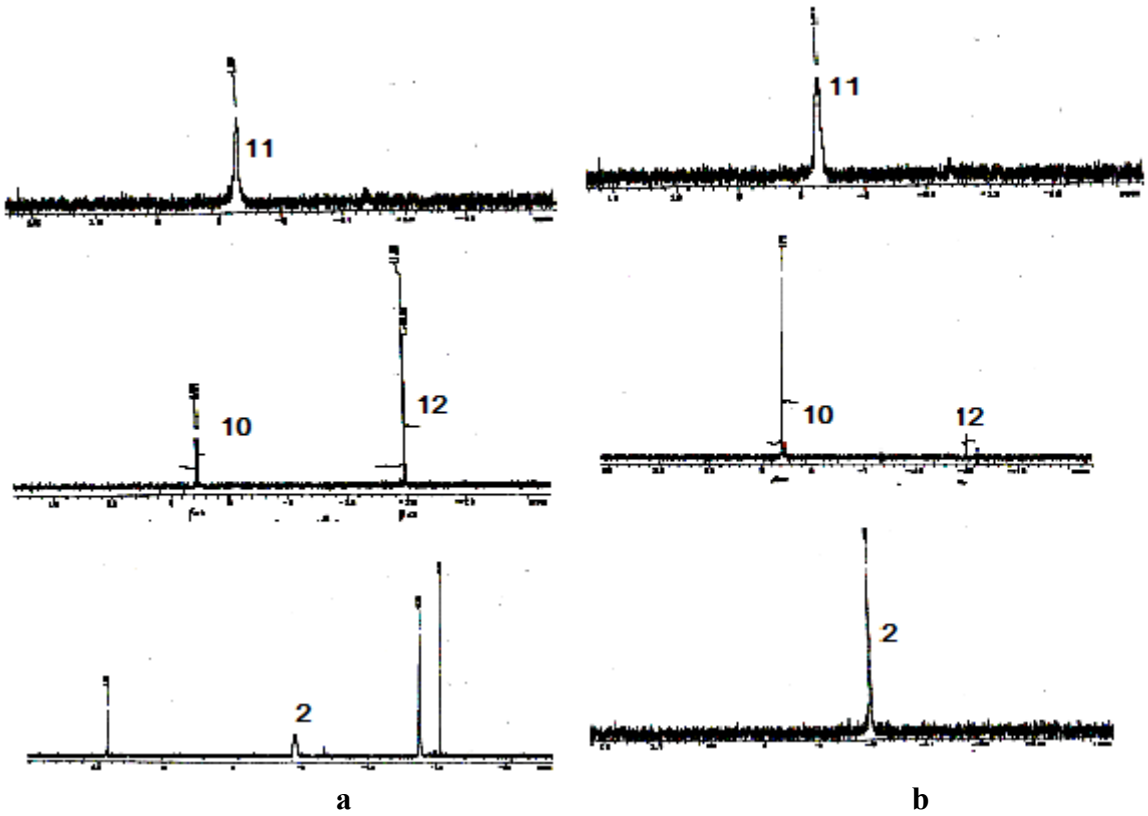

**SI Figure 1.** Dose response curve for inhibition of Pin1 by compound **1** ($IC_{50}$ = 28.3 ± 2.1 μM). In the equation, a = 4.60, b = 113.29, c = 1.58, and d = 4.71; $r^2$ = 0.989. $IC_{50}$ = 28.3 ± 2.1 μM.




**SI Figure 2.** Dose response curve for the inhibition of A2780 ovarian cancer cells proliferation activity of **1**. For **1**, from equation (1), a = 7.74, b = 94.51, c = 1.72, and d = 6.59; $r^2$ = 0.996. $IC_{50}$ = 46.2 ± 3.0 μM.




**SI Figure 3.** Dose response curve for the inhibition of A2780 ovarian cancer cells proliferation activity of **2.** For **2**, from the equation, a = 11.04, b = 75.29, c = 1.41, and d = 6.35; $r^2 = 0.998$. $IC_{50} = 26.9 \pm 1.5$ μM.



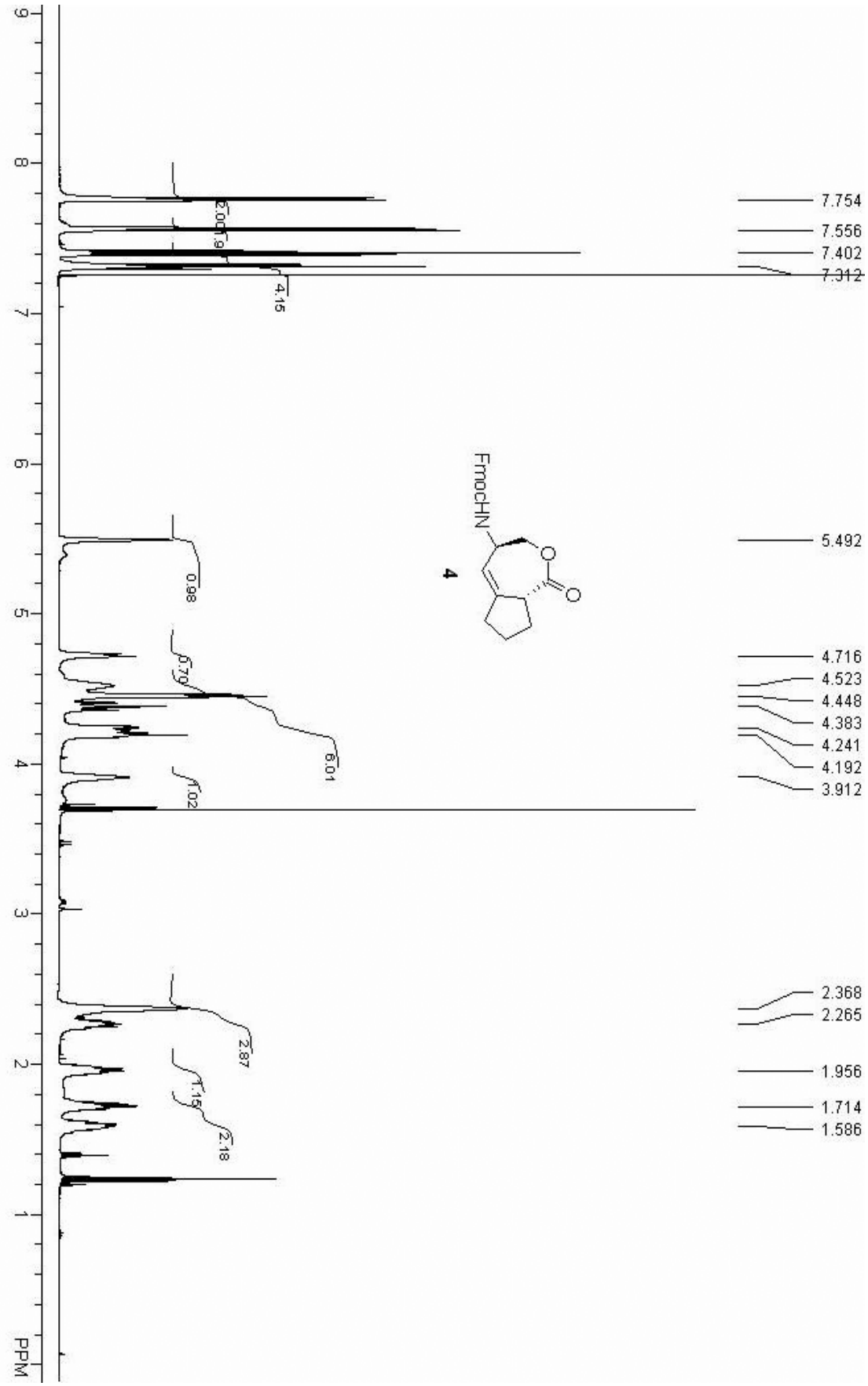

**SI Figure 4.** $^{31}$P-NMR study of the phosphorylation step. **a**: $(COCl)_2$ was added to **11**, followed by DMF; **b**: **11** was added very slowly to the mixture of $(COCl)_2$ and DMF. **10**: BisPOM phosphoryl chloride; **11**: bisPOM phosphate; **12**: $(POM)_4$pyrophosphate; **2**: Fmoc-Ser(PO(OPOM)$_2$)-Ψ[(*Z*)CH=C]-Pro-(2)-*N*-(3)-ethylaminoindole.

**SI Table 1.** Yields for the phosphorylation step using different bases.

| Base | Temperature | Yield |
|---|---|---|
| $Et_3N$ | -40 °C | < 20% |
| $Et_3N$ | rt | < 20% |
| DIEA | rt | < 20% |
| collidine | rt | < 20% |
| NMM | rt | < 20% |
| Pyridine (8 equivalents) | -40 °C | 20% |
| Pyridine (8 equivalents) | rt | 25% |
| Pyridine (large excess) | -40 °C | 22% |
| Pyridine (large excess) | rt | 30% |

## SI References


1. Wang, X. J.; Xu, B.; Mullins, A. B.; Neiler, F. K.; Etzkorn, F. A. *J. Am. Chem. Soc.* **2004,** *126*, 15533.
2. Wang, X. J.; Hart, S. A.; Xu, B.; Mason, M. D.; Goodell, J. R.; Etzkorn, F. A. *J. Org. Chem.* **2003,** *68*, 2343.
3. Hwang, Y.; Cole, P. A. *Org Lett* **2004,** *6*, 1555.
4. Abdel-Kader, M.; Berger, J. M.; Slebodnick, C.; Hoch, J.; Malone, S.; Wisse, J. H.; Werkhoven, M. C.; Mamber, S.; Kingston, D. G. *J. Nat. Prod.* **2002,** *65*, 11.
5. Kapustin, G.; Fejér, G.; Gronlund, J. L.; Seto, E.; McCafferty, D. G.; Etzkorn, F. A. *Org. Lett.* **2003,** *5*, 3053.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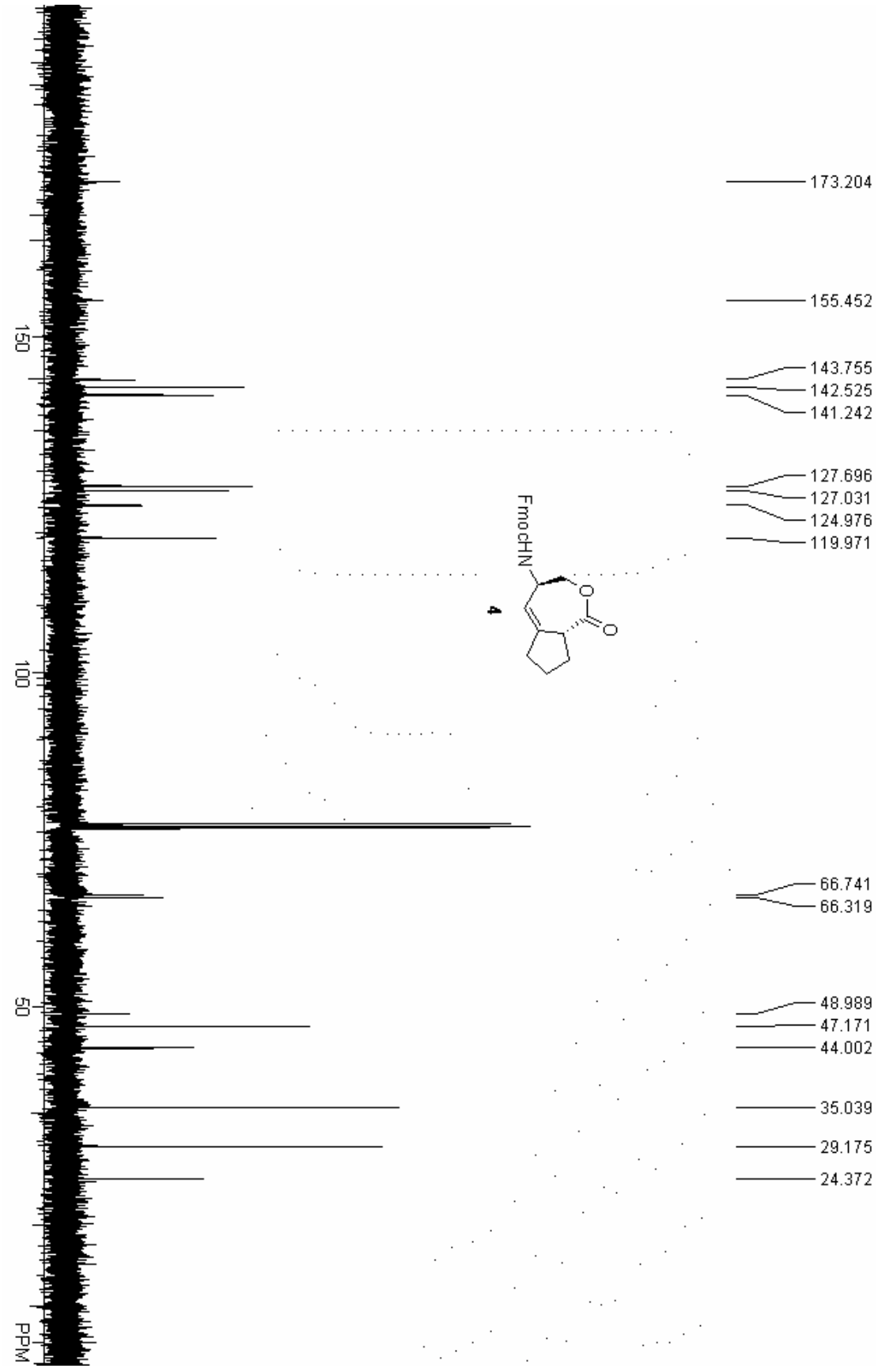
2.368
2.265
1.956
1.714
1.586
FmocHN
4
PPM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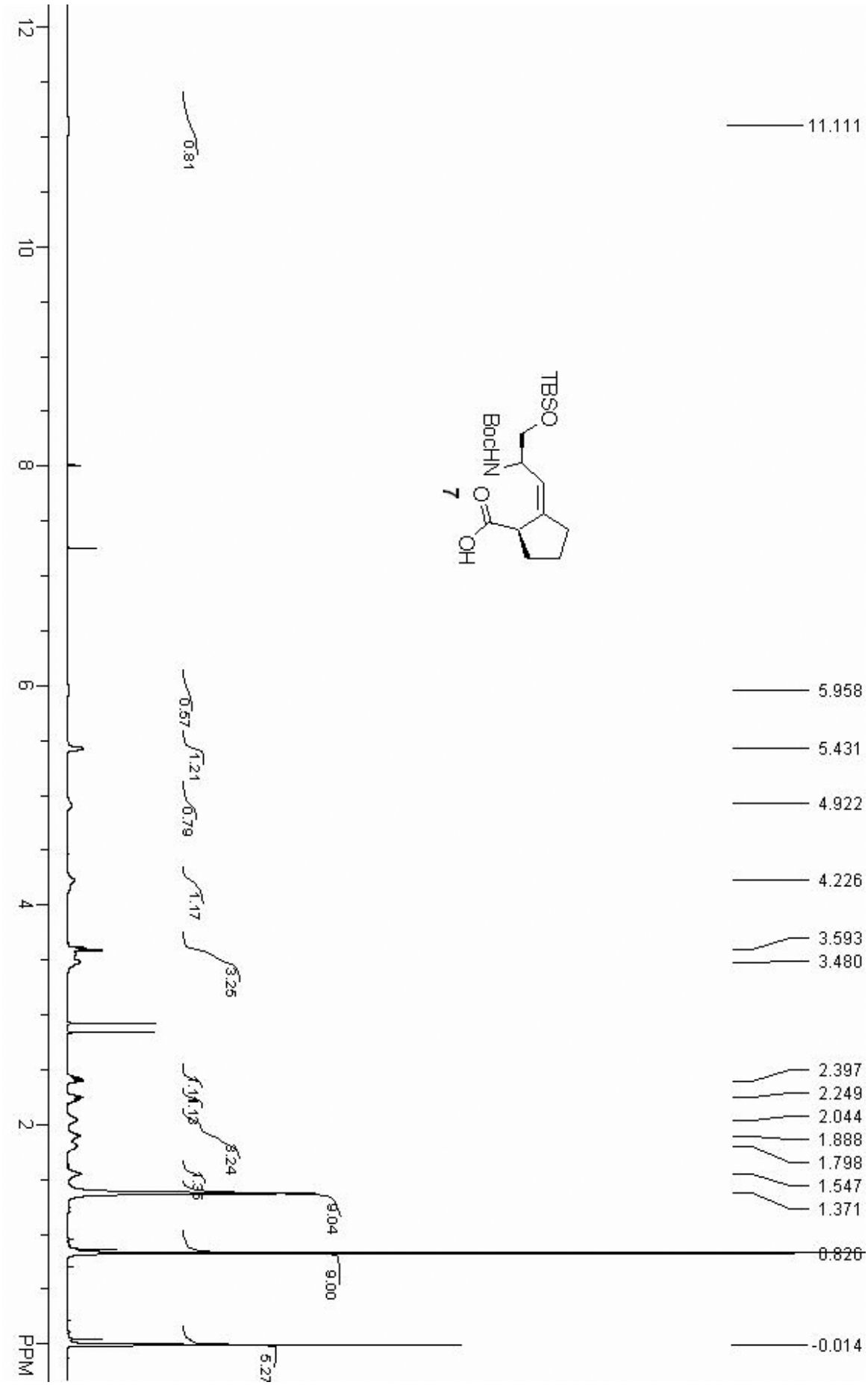
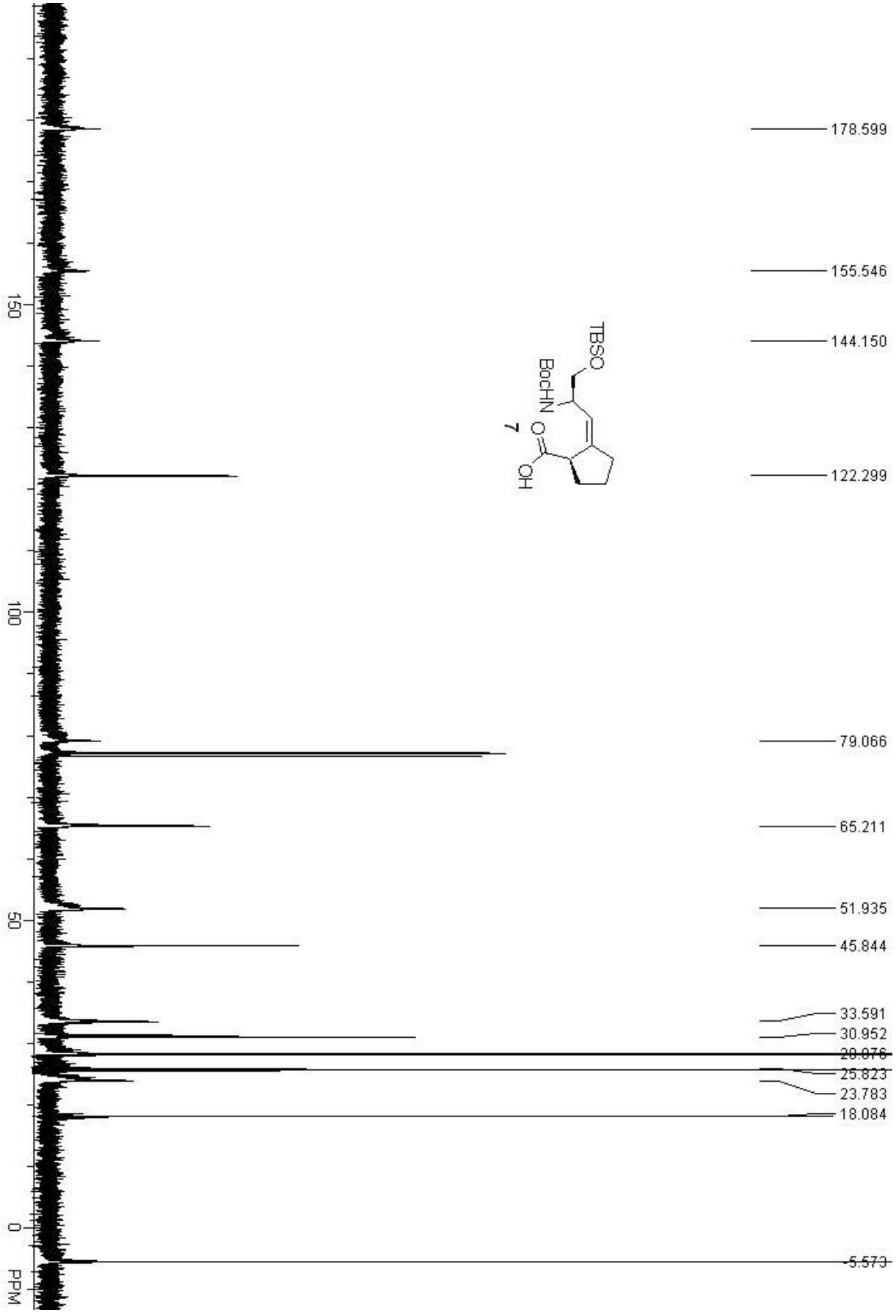
155.452
143.755
142.525
141.242
127.696
127.031
124.976
119.971
66.741
66.319
48.989
47.171
44.002
35.039
29.175
24.372
FmocHN
4
150
100
50
PPM


TBSO
BocHN
O
OH
7
11.111
5.958
5.431
4.922
4.226
3.593
3.480
2.397
2.249
2.044
1.888
1.798
1.547
1.371
0.826
-0.014
0.81
0.57
1.21
0.79
1.17
3.25
1.13
8.24
1.35
9.04
9.00
5.27
12
10
8
6
4
2
PPM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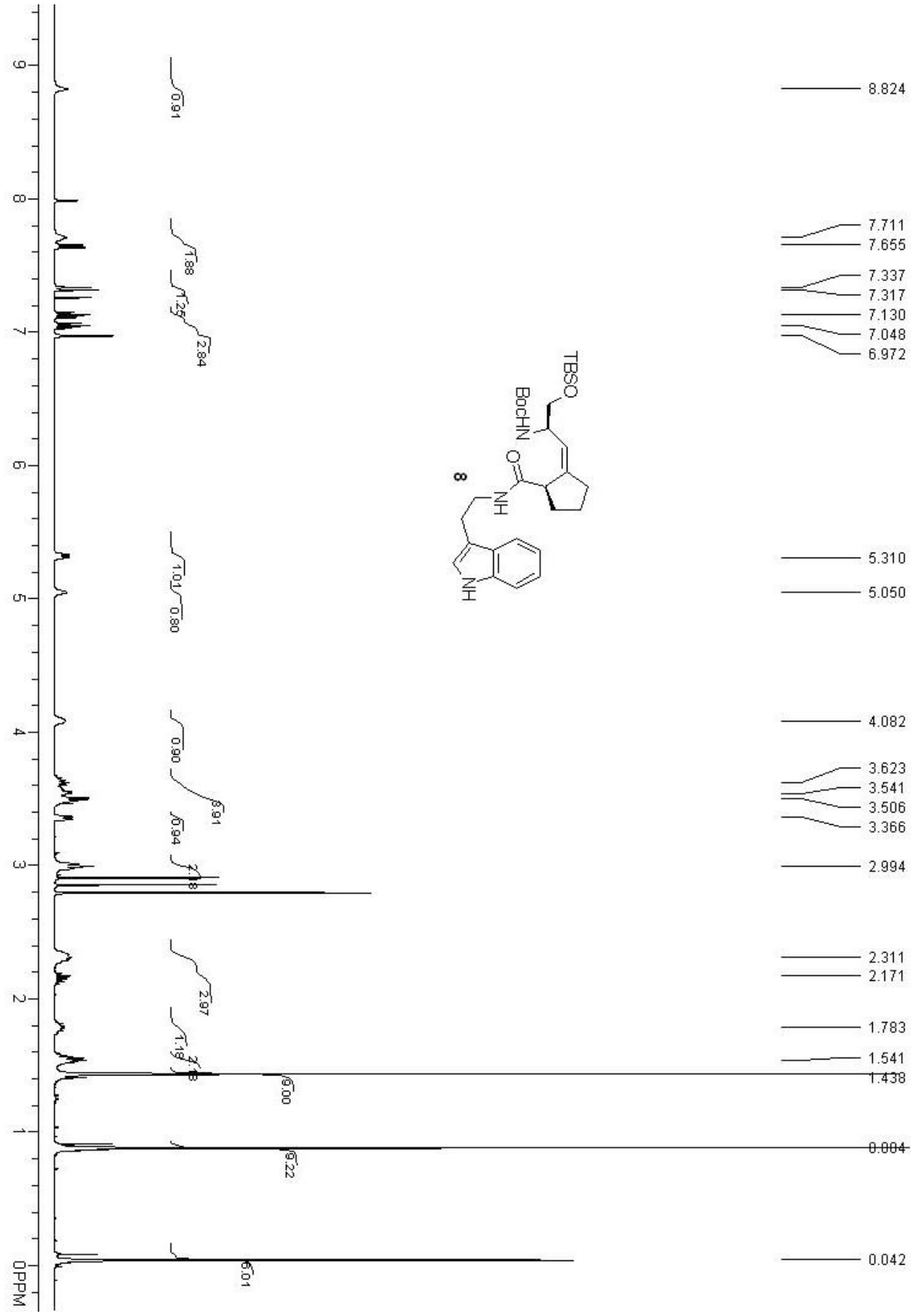
155.546
144.150
122.299
79.066
65.211
51.935
45.844
33.591
30.952
29.076
25.823
23.783
18.084
-5.573
150
100
50
0
PPM
TBSO
BocHN
O
OH
7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7.711
7.655
7.337
7.317
7.130
7.048
6.972
5.310
5.050
4.082
3.623
3.541
3.506
3.366
2.994
2.311
2.171
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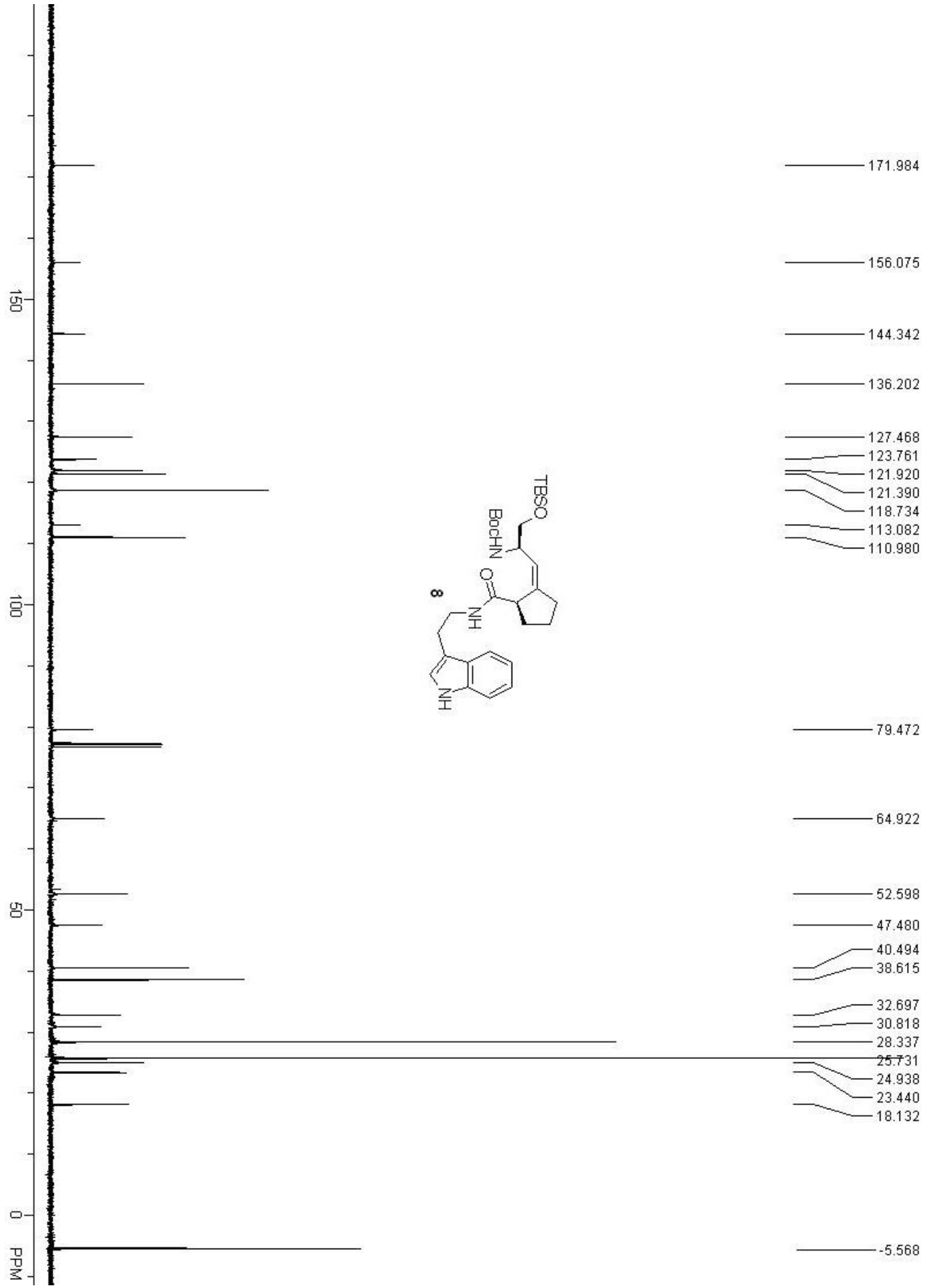
1.541
1.438
0.004
0.042
TBSO
BocHN
O
NH
NH
8
0PPM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127.468
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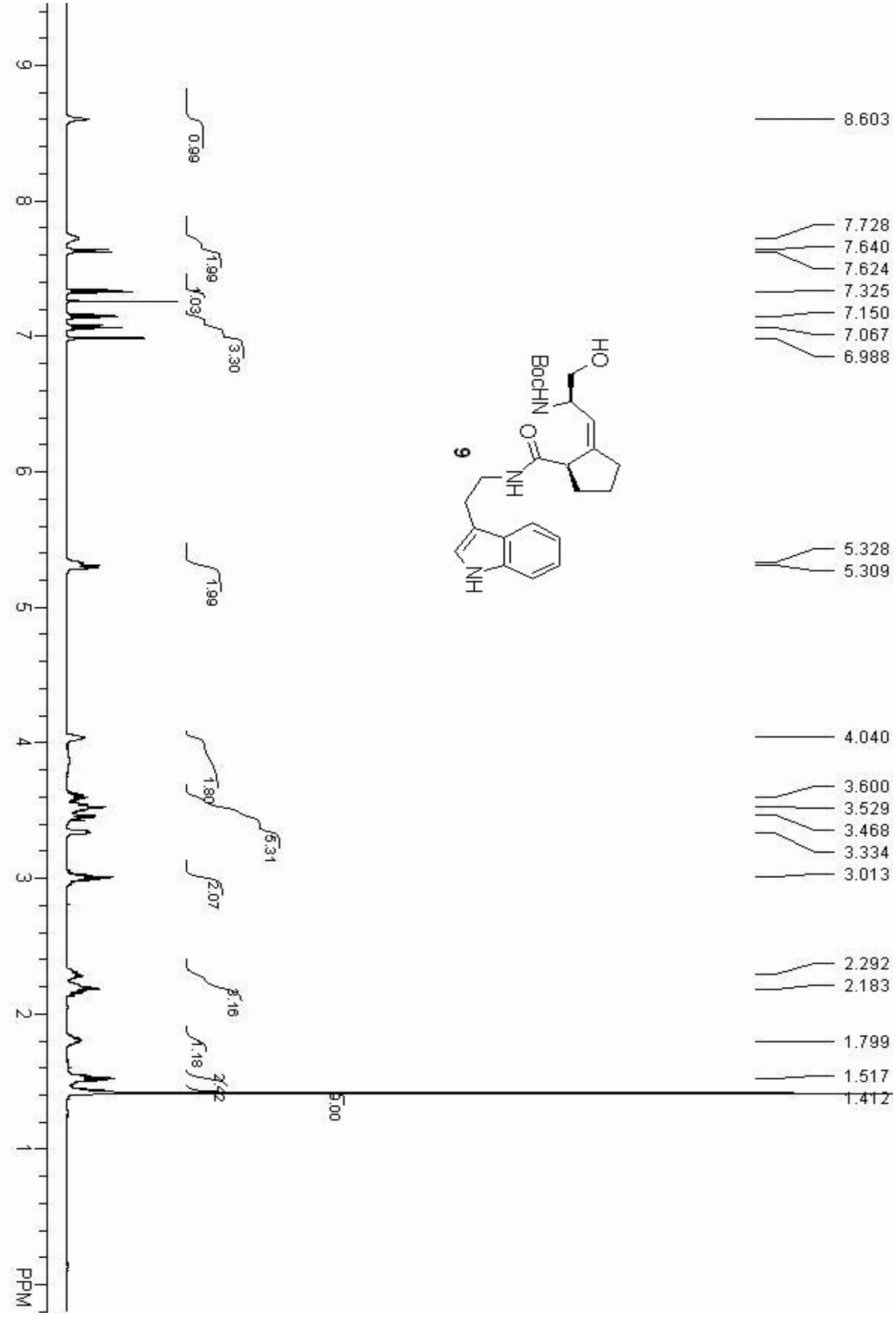
121.920
121.390
118.734
113.082
110.980
79.472
64.922
52.598
47.480
40.494
38.615
32.697
30.818
28.337
25.731
24.938
23.440
18.132
-5.568
150
100
50
0
PPM
TBSO
BocHN
O
NH
NH
8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3.468
3.334
3.013
2.292
2.183
1.799
1.517
1.412
0.99
1.99
1.03
3.30
1.99
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3.16
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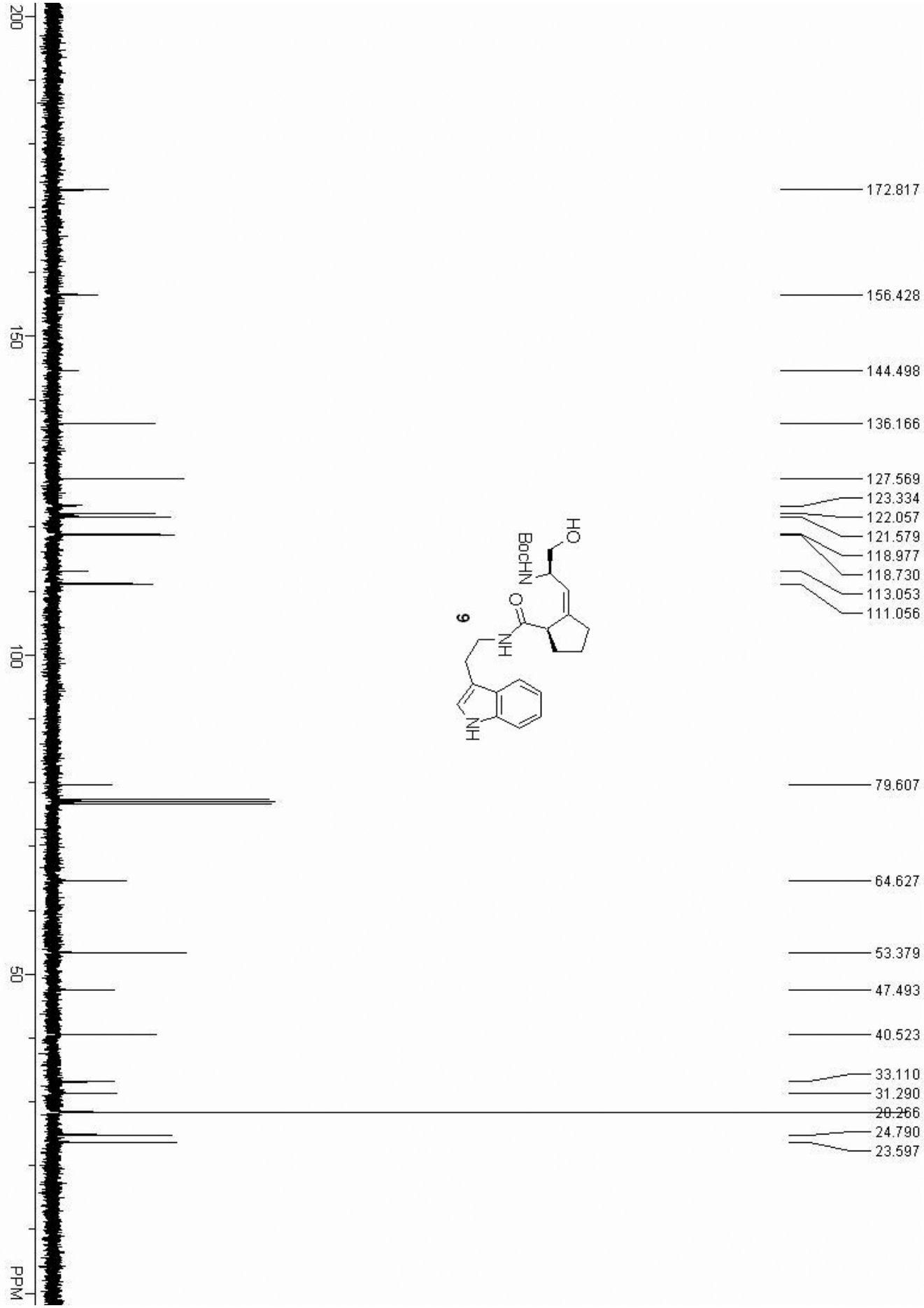
HO
BocHN
O
NH
NH
9
PPM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150
100
50
PPM
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113.053
111.056
79.607
64.627
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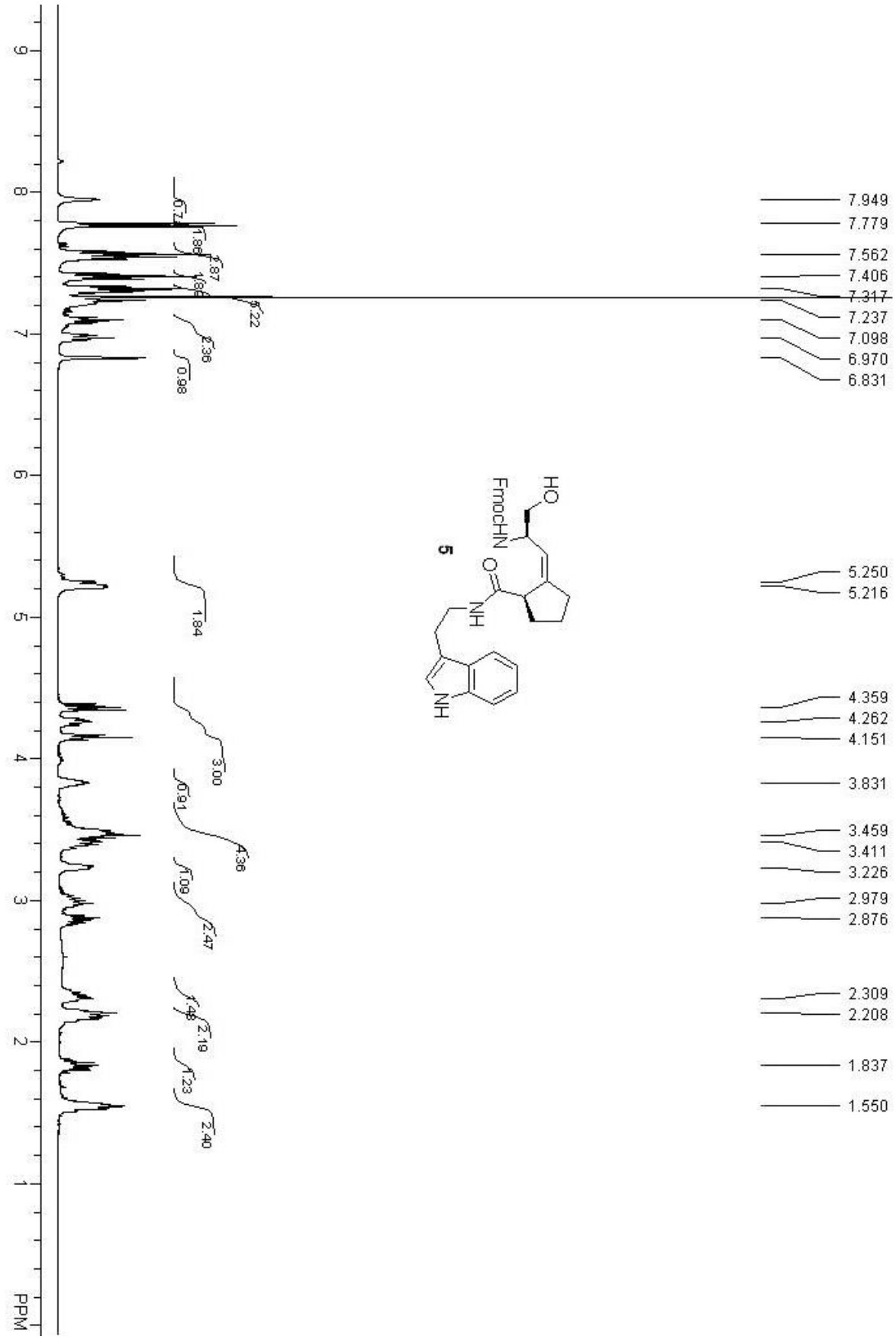
BocHN
O
NH
NH
9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5.250
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4.151
3.831
3.459
3.411
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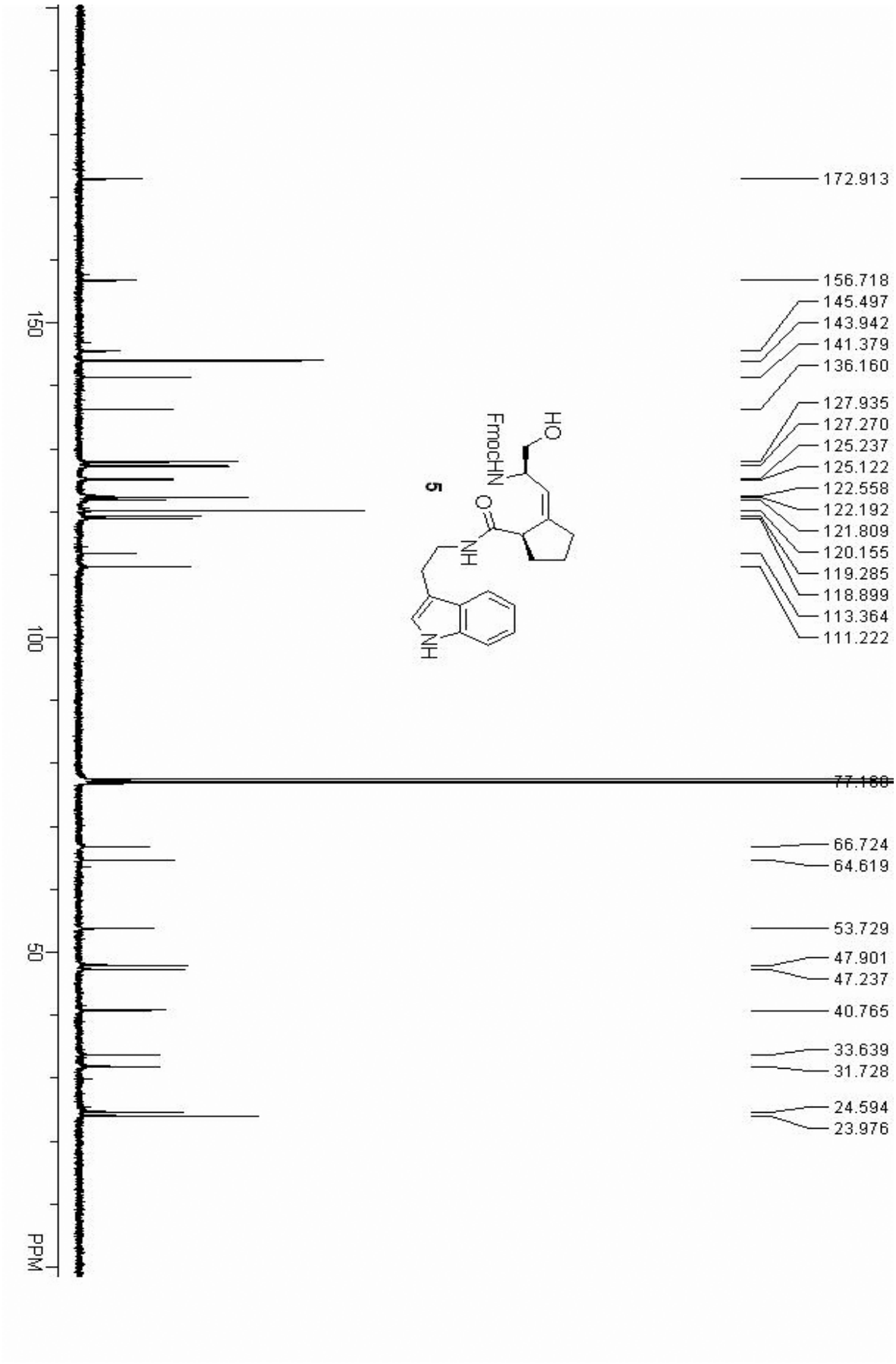
1.550
HO
FmocHN
O
NH
NH
5
PPM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122.192
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113.364
111.222
77.180
66.724
64.619
53.729
47.901
47.237
40.765
33.639
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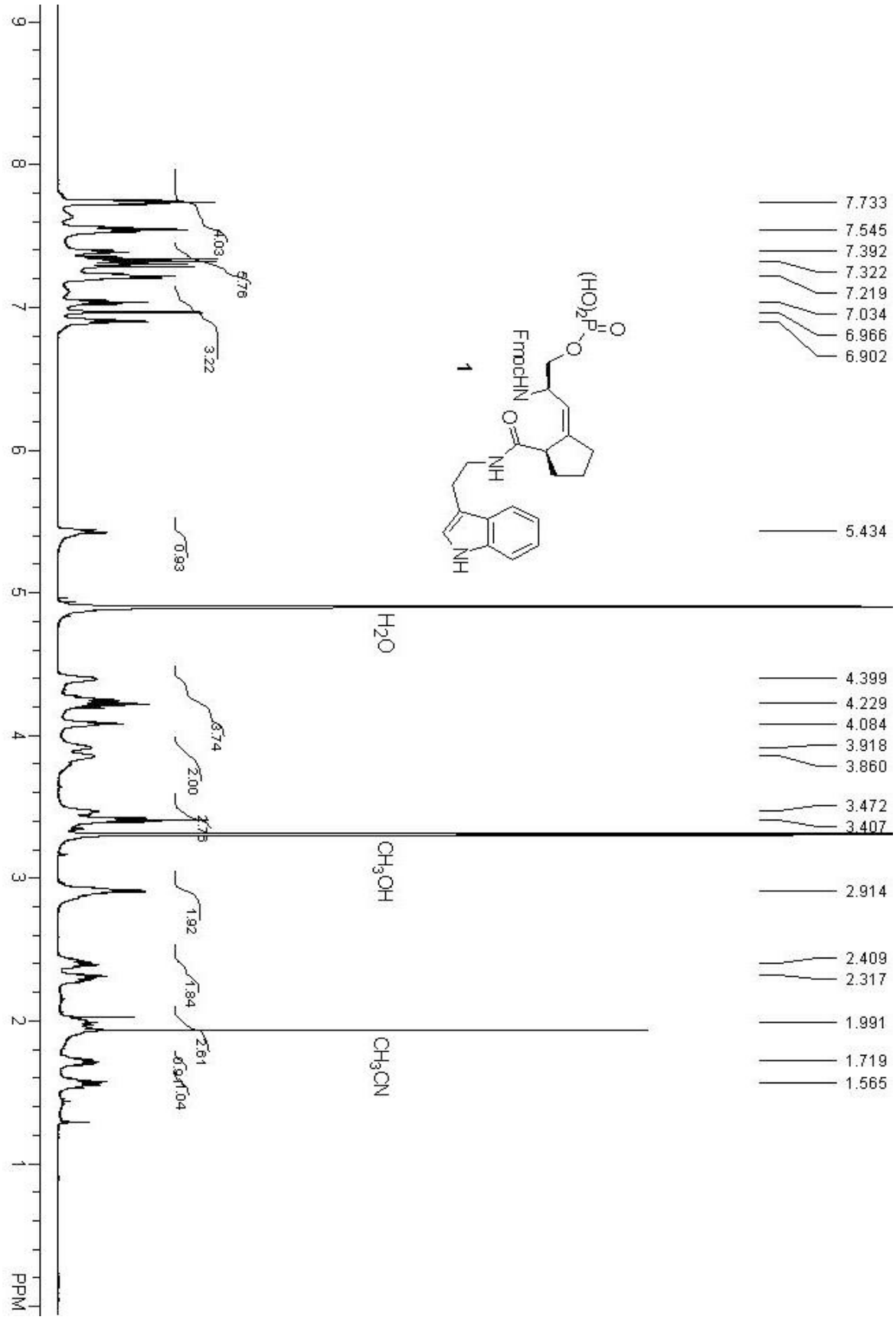
24.594
23.976
150
100
50
PPM
FmocHN
HO
O
NH
NH
5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4.229
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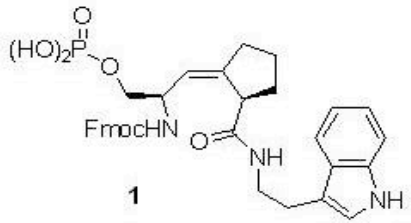
3.918
3.860
3.472
3.407
2.914
2.409
2.317
1.991
1.719
1.565
4.03
5.76
3.22
0.93
8.74
2.00
1.92
1.84
2.61
1.04
H2O
CH3OH
CH3CN
FmocHN
NH
NH
O
(HO)2P
O
O
1
PPM

13C OBSERVE

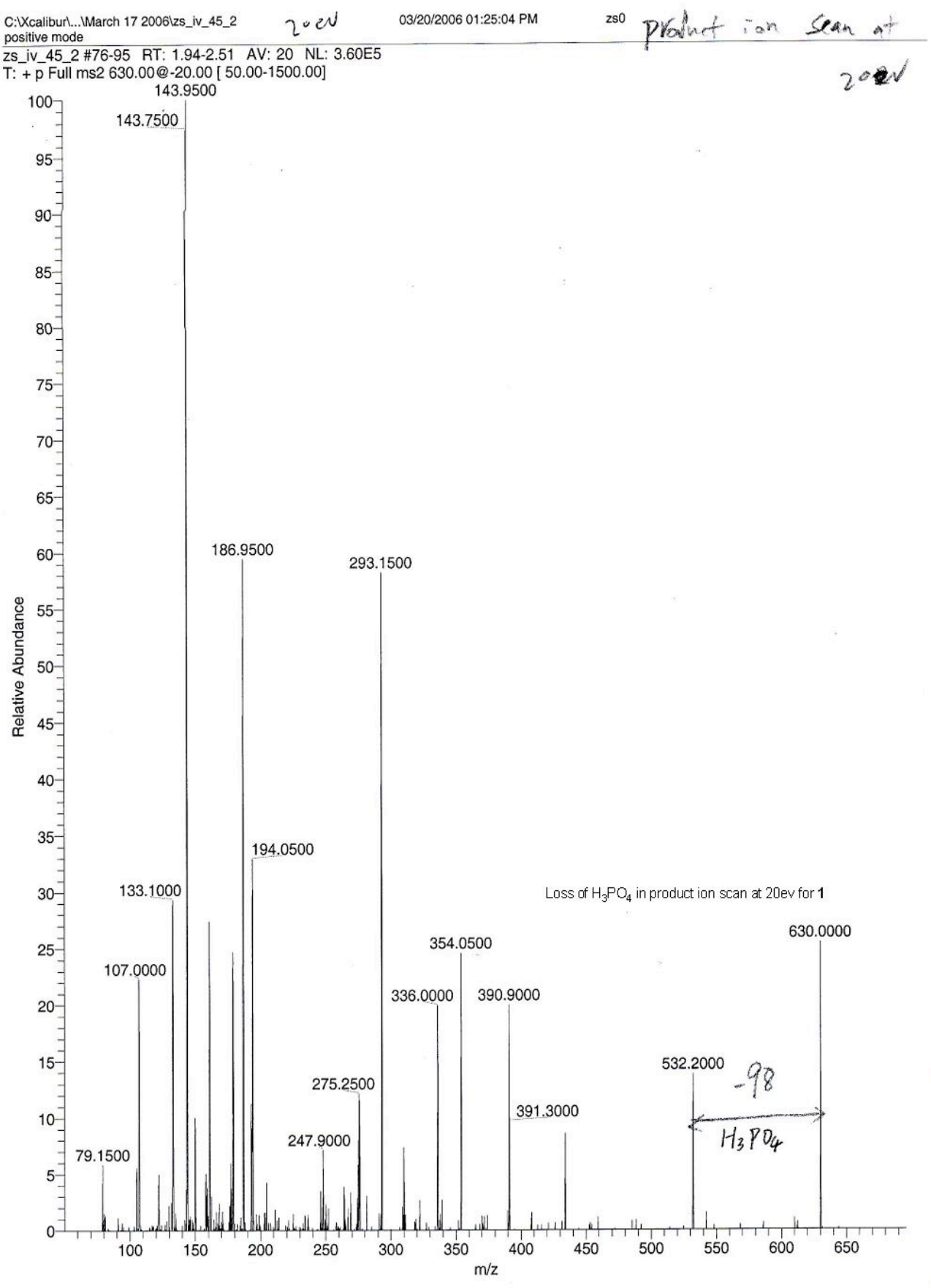
Pulse Sequence: s2pul
 Solvent: CD3OD
 Ambient temperature
UNITY-400 "unityultra"

 Relax. delay 1.000 sec
 Pulse 46.4 degrees
 Acq. time 1.199 sec
 Width 25000.0 Hz
 21696 repetitions
OBSERVE C13, 100.5675475 MHz
DECOUPLE H1, 399.9522799 MHz
 Power 40 dB
 continuously on
 WALTZ-16 modulated
DATA PROCESSING
 Line broadening 1.0 Hz
FT size 65536
Total time 613 hr, 56 min, 48 sec
Mar 20 2006
VA Tech Chemistry NMR Lab

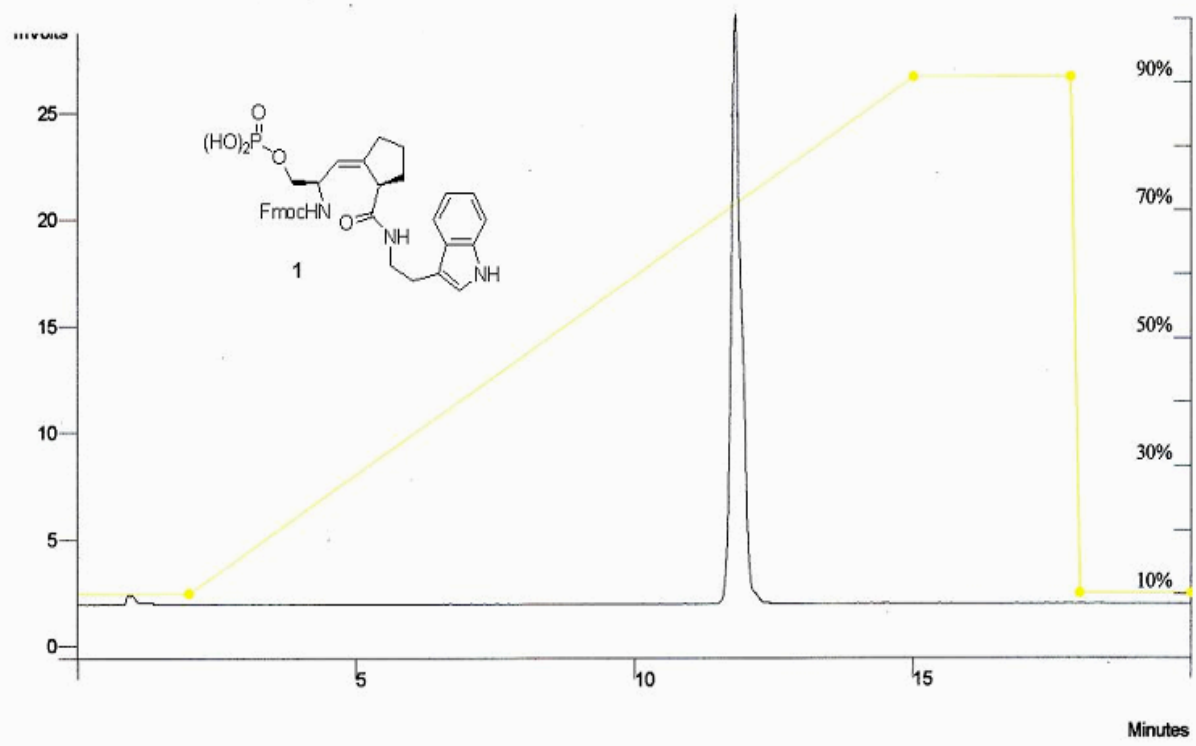



1

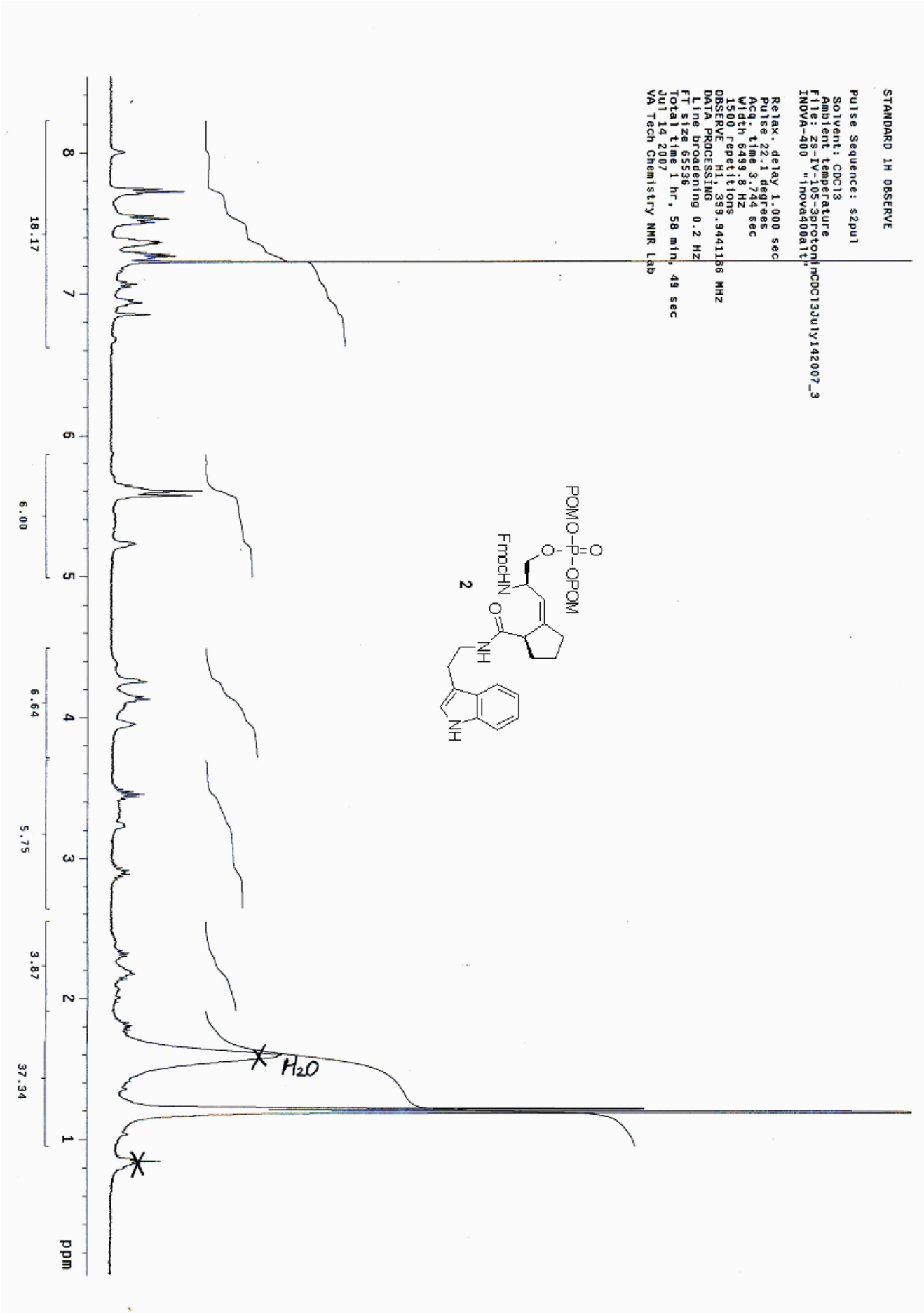
40
20
0
-20
-40
PPM
(HO)2P
O
FmocHN
NH
NH
1
31P-NMR
-1.164

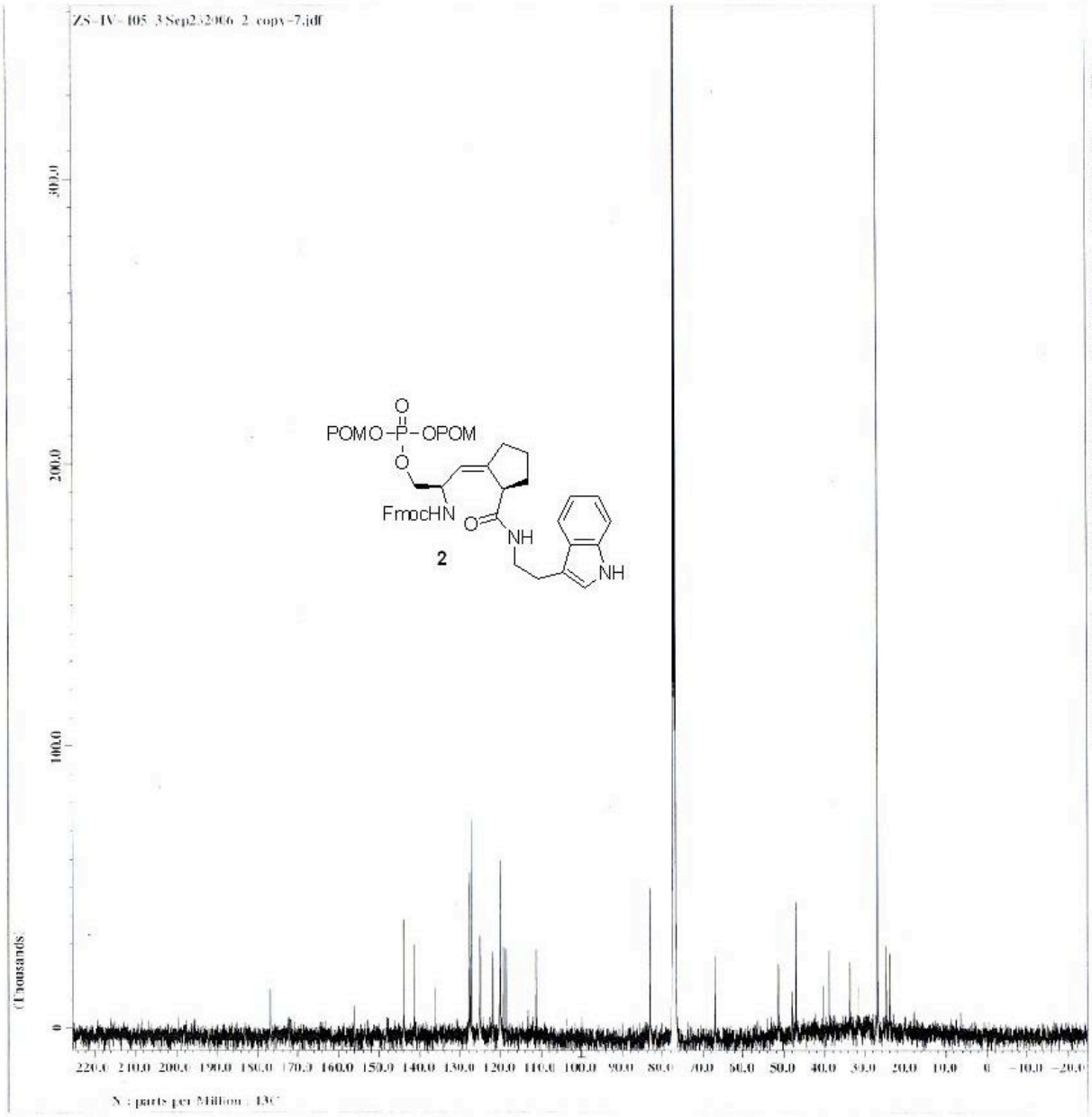

C:\Xcalibur\...\March 17 2006\zs_iv_45_2
positive mode
20eV
03/20/2006 01:25:04 PM
zs0
Product ion Scan at 20eV
zs_iv_45_2 #76-95 RT: 1.94-2.51 AV: 20 NL: 3.60E5
T: + p Full ms2 630.00@-20.00 [ 50.00-1500.00]
Relative Abundance
m/z
143.9500
143.7500
186.9500
293.1500
194.0500
133.1000
107.0000
79.1500
247.9000
275.2500
336.0000
354.0500
390.9000
391.3000
532.2000
630.0000
Loss of H3PO4 in product ion scan at 20ev for 1
-98
H3PO4

Data File: c:\star\guoyanxu\6-24-2007 5;57;33 pm
Sample ID: Manual Sample
Operator (Inj): Operator
Injection Date: 06/24/2007 05:57:33
Injection Method: c:\star\song zhao\analysis of
Peak Measurement:
Calculation Type: Percent




| Ret Time (min) | Peak Area (counts) | Result (area %) |
|---|---|---|
| 0.91 | 4722 | 1.2 |
| 11.79 | 235011 | 98.8 |
| | 239733 | 100 |


STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
File: zs-IV-105-3protonnCDCl3July142007_3
INOVA-400 "inova400a1t"
Relax. delay 1.000 sec
Pulse 22.1 degrees
Acq. time 3.744 sec
width 6499.8 Hz
1500 repetitions
OBSERVE H1, 399.9441186 MHz
DATA PROCESSING
Line broadening 0.2 Hz
FT size 65536
Total time 1 hr, 58 min, 49 sec
Jul 14 2007
VA Tech Chemistry NMR Lab
POMO-P-OPOM
FmocHN
2
NH
NH
H2O
18.17
6.00
6.64
5.75
3.87
37.34
8
7
6
5
4
3
2
1
ppm




JEOL

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|---|---|
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| Solvent | = CHLOROFORM-D |
| Creation_time | = 24-SEP-2006 22:01:18 |
| Revision_time | = 25-SEP-2006 09:55:41 |
| Current_time | = 25-SEP-2006 09:56:29 |
| Content | = Single Pulse with Bro |
| Data_format | = 1D COMPLEX |
| Dim_size | = 32768 |
| Dim_title | = 13C |
| Dim_units | = [ppm] |
| Dimensions | = X |
| Site | = Delta NMR |
| Spectrometer | = DELTA NMR |
| Field_strength | = 11.7473579[T] (500[MH |
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| X_domain | = 13C |
| X_freq | = 125.75529768[MHz] |
| X_offset | = 100[ppm] |
| X_points | = 32768 |
| X_prescans | = 4 |
| X_resolution | = 0.95967327[Hz] |
| X_sweep | = 31.44654080[kHz] |
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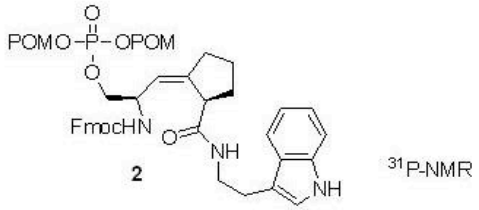
| Irr_freq | = 500.15991521[MHz] |
| Irr_offset | = 5[ppm] |
| Clipped | = FALSE |
| Mod_return | = 1 |
| Scans | = 61355 |
| Total_scans | = 61255 |
| X_90_width | = 11[us] |
| X_acq_time | = 1.0420224[s] |
| X_angle | = 45[deg] |
| X_pulse | = 5.5[us] |
| Initial_wait | = 1[s] |
| Phase_preset | = 3[us] |
| Recvr_gain | = 29 |
| Relaxation_delay | = 1[s] |
| Temp_get | = 22.8[dC] |
| Unblank_time | = 2[us] |

PHOSPHORUS OBSERVE
STANDARD PARAMETERS
PHOSPHATE REGION

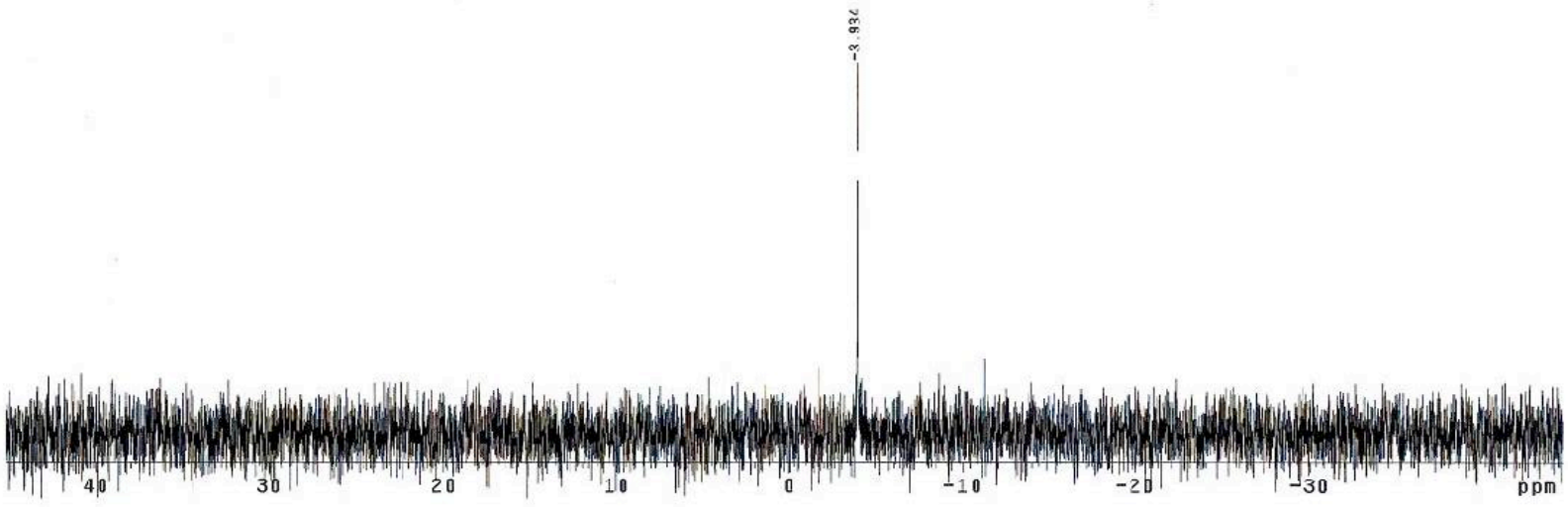
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
INOVA-400 "inova400alt"

Relax. delay 1.000 sec
Pulse 70.0 degrees
Acq. time 2.000 sec
Width 29986.3 Hz
512 repetitions
OBSERVE P31, 161.9003468 MHz
DECOUPLE H1, 399.9481183 MHz
Power 41 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 25 min, 40 sec
May 1 2006
VA Tech Chemistry NMR Lab




**2**

$^{31}$P-NMR



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Operator (Inj): Operator
Injection Date: 04/20/2006 08:26:17
Injection Method: c:\star\song zhao\10-90 for
Peak Measurement:
Calculation Type: Percent