Design of Small Molecule Reagents that Enable Signal Amplification via an Autocatalytic, Base-Mediated Cascade Elimination Reaction

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Supporting Information

Table of Contents

General experimental procedures

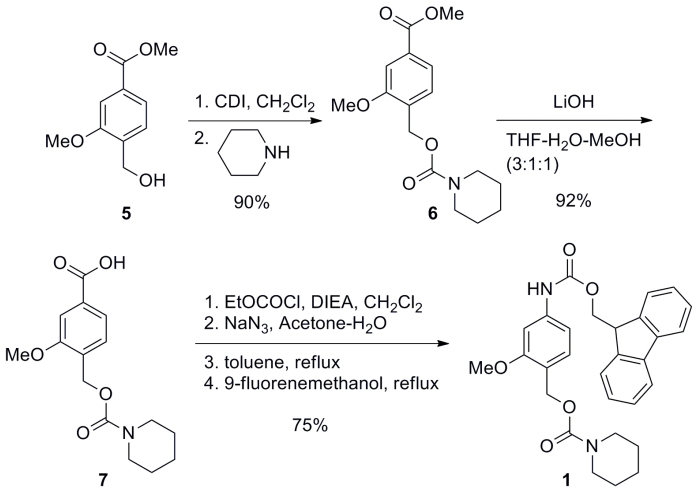
All reactions that required anhydrous conditions were performed in flame-dried glassware under a positive pressure of argon. Air- and moisture-sensitive liquids were transferred by syringe or stainless steel cannula. Organic solutions were concentrated by rotary evaporation (25–40 mmHg) at ambient temperature, unless otherwise noted. Methyl 4-(bromomethyl)-3-methoxybenzoate, 9-fluorenemethanol, piperidine, allyl alcohol, and all other reagents were purchased commercially and were used as received. Flash-column chromatography was performed as described by Still et al.,[1] employing silica gel (60-Å pore size, 32–63 μm, standard grade, Dynamic Adsobents). Thin layer chromatography was carried out on Dynamic Adsorbants silica gel TLC (20×20 cm w/h, F-254, 250 μm). Deionized water was purified using a Millipore-purification system (Barnstead EASYpure® II UV/UF).

Instrumentation

All UV-Visible data were obtained using a Beckman-Coulter DU®-800 spectrophotometer. Photographs were taken using a Nikon digital camera (D40). Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded using a Bruker DRX-400 (400 MHz) or AV-360 (360 MHz) at 25 °C. Proton chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to tetramethylsilane ($(CH_3)_4Si$, 0.00 ppm). Data are represented as follows:

Integration, chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and/or multiple resonances, br s = broad singlet, dd = doublet of doublet), and coupling constant (*J*) in hertz. Carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded using a Bruker DRX-400 (100 MHz). Carbon chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to the carbon resonance of the NMR solvent ($CDCl_3$, δ 77.16 ppm).

Synthesis of amplification reagent 1



2-Methoxy-4-(methoxycarbonyl)benzyl piperidine-1-carboxylate (6):

2-Methoxy-4-(hydroxymethyl)-3-methoxybenzoate **5**[2] (3.7 g, 19 mmol, 1 equiv) was dissolved in dry CH_2Cl_2 (100 mL) and the solution was cooled to 0 ^{o}C. Carbonyldiimidazole (3.75 g, 23.1 mmol, 1.2 equiv) was added slowly to the stirring solution, and, after 5 min at 0 ^{o}C, the reaction mixture was warmed to room temperature. The reaction mixture was stirred at room temperature for 45 min, after which piperidine (2.3 mL, 23 mmol, 1.2 equiv) was added. The solution was stirred at room temperature for 3 h, and then was diluted with CH_2Cl_2 (100 mL). The resulting solution was washed with aqueous hydrochloric acid (0.01 M, 100 mL). The aqueous layer was separated and was successively extracted using CH_2Cl_2 (2 × 100 mL). The combined organic layers were washed with water (1 × 100 mL), brine (2 × 100 mL), and dried over anhydrous sodium sulfate. The sodium sulfate was removed by filtration and the resulting solution was concentrated under reduced pressure. The resulting white solid was purified using column chromatography (elution with 30% ethyl acetate/hexanes) to obtain 2-methoxy-4-(methoxycarbonyl)benzyl piperidine-1-carboxylate **6** (5.19 g, 16.9 mmol, 90%). IR (cm^{-1}) 2936, 2854, 1695; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.64 (1H, d, J = 7.8), 7.52 (1H, s), 7.36 (1H, d, J = 7.8), 5.21 (2H, s), 3.91 (3H, s), 3.89 (3H, s), 3.48–3.46 (4H, m), 1.60–1.54 (6H, m); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 24.4, 25.7, 44.9, 52.2, 55.6, 61.9, 110.9, 121.9, 127.7, 130.6, 131.0, 155.2, 156.7, 166.9; MS (TOF MS ES+, *m/z*): 330.3 (11, M + Na^+), 179.0 (100, M – $C_6H_{11}O_2N$); HRMS (TOF MS ES+, *m/z*) Calculated for $C_{16}H_{22}NO_5$ (M + H^+): 308.1498; Found: 308.1494.

3-Methoxy-4-{[(piperidin-1-ylcarbonyl)oxy]methyl}benzoic acid (7):

2-Methoxy-4-(methoxycarbonyl)benzyl piperidine-1-carboxylate **6** (4.42 g, 14.4 mmol, 1 equiv) was dissolved in 68 mL of THF and 27 mL of methanol. Lithium hydroxide monohydrate (0.84 g, 20 mmol, 1.4 equiv) dissolved in 27 mL of water was added to the THF−MeOH solution. The resulting solution was stirred for 10 h at room temperature. The solution was acidified by slow addition of aqueous hydrochloric acid (1 M, 25 mL), and was extracted using ethyl acetate (5 × 100 mL). The combined organic layers were washed with brine (1 × 100 mL) and dried over anhydrous sodium sulfate. The sodium sulfate was removed by filtration and the resulting solution was concentrated under reduced pressure to obtain 3-methoxy-4-{[(piperidin-1-ylcarbonyl)oxy]methyl}benzoic acid **7** (3.88 g, 13.2 mmol, 92%). IR (cm^{-1}) 2937, 2859, 2822, 2668, 1684; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 12.24 (1H, br s), 7.73 (1H, d, J = 7.8), 7.58 (1H, s), 7.41 (1H, d, J = 7.8), 5.25 (2H, s), 3.90 (3H, s), 3.51–3.49 (4H, m), 1.59–1.57 (6H, m); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 24.3, 25.7, 45.0, 55.5, 62.1, 111.2, 122.6, 127.7, 130.0, 131.6, 155.4, 156.7, 171.1; MS (TOF MS ES–, *m/z*): 292.2 (100, M – H^+); HRMS (TOF MS ES–, *m/z*) Calculated for $C_{15}H_{18}NO_5$ (M – H^+): 292.1185; Found: 292.1201.

4-(((9H-fluoren-9-yl)methoxy)carbonylamino)-2-methoxybenzyl piperidine-1-carboxylate (1):

3-Methoxy-4-{[(piperidin-1-ylcarbonyl)oxy]methyl}benzoic acid **7** (3.65 g, 12.5 mmol, 1 equiv) dissolved in 100 mL of dry CH_2Cl_2 was cooled to 0 ^{o}C with stirring. DIEA (4.75 mL, 27.4 mmol, 2.2 equiv) was added to this cooled solution, followed by drop-wise addition of ethyl chloroformate (2.62 mL, 27.4 mmol, 2.2 equiv). The reaction mixture was stirred at 0 ^{o}C for 1 h, after which the solution was warmed to room temperature and concentrated under reduced pressure. The residue was dissolved in acetone (50 mL), and the resulting solution was cooled to 0 ^{o}C. A solution of sodium azide (4.0 g, 62 mmol, 5 equiv) in cold water (50 mL) (~10 ^{o}C) was added drop-wise to the acetone solution. The resulting suspension was stirred vigorously at 0 ^{o}C for 1 h. The solution was diluted with ethyl acetate (100 mL) and the aqueous layer was separated. The aqueous layer was extracted using ethyl acetate (3 × 100 mL), and the organic layers were combined. These organic extracts were washed with brine (1 × 50 mL) and dried over anhydrous sodium sulfate. The sodium sulfate was removed by filtration, and the resulting solution was concentrated under reduced pressure to provide a yellow gelatinous residue. The residue was dissolved in dry toluene (50 mL) and the resulting solution was heated to 100 ^{o}C for 1 h. The hot solution was cooled to room temperature, after which 9-fluorenemethanol (870 mg, 14.9 mmol, 1.2 equiv) was added. The solution was heated to reflux (112 ^{o}C) for 10 h, after which the solution was cooled to room temperature and the solvent was removed under reduced pressure with gentle heating (40 ^{o}C). The residue was purified by column chromatography (gradient elution with 20–30% ethyl acetate/hexanes) to obtain 4-(((9H-fluoren-9-yl)methoxy)carbonylamino)-2-methoxybenzyl piperidine-1-carboxylate **1** (4.7 g, 9.7 mmol, 78%). IR (cm^{-1}) 2856, 2838, 1725, 1673, 1615; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.76 (2H, d, J = 7.4), 7.60 (2H, d, J = 7.4), 7.39 (2H, t, J = 7.4), 7.30 (2H, t, J = 7.4), 7.24 (1H, s,), 7.21 (1H, d, J = 8.0), 7.07 (1H, s), 6.77 (1H, d, J = 8.0), 5.12 (1H, s), 4.50 (2H, d, J = 6.8), 4.25 (1H, t, J = 6.8), 3.78 (3H, s), 3.42 (4H, m), 1.55 (2H, m), 1.50 (4H, m); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 24.5, 25.8, 44.9, 47.2, 55.5, 62.2, 66.9, 101.7, 110.2, 120.1, 120.4, 125.1, 127.2, 127.9, 129.9, 139.1, 141.4, 143.8, 153.5, 155.7,

158.1; MS (TOF MS ES+, *m/z*): 509.1 (2, M + Na^+), 358.1 (100, M – $C_6H_{11}O_2N$); HRMS (TOF MS ES+, *m/z*) Calculated for $C_{29}H_{34}N_3O_5$ (M + NH_4^+): 504.2498; Found: 504.2509.

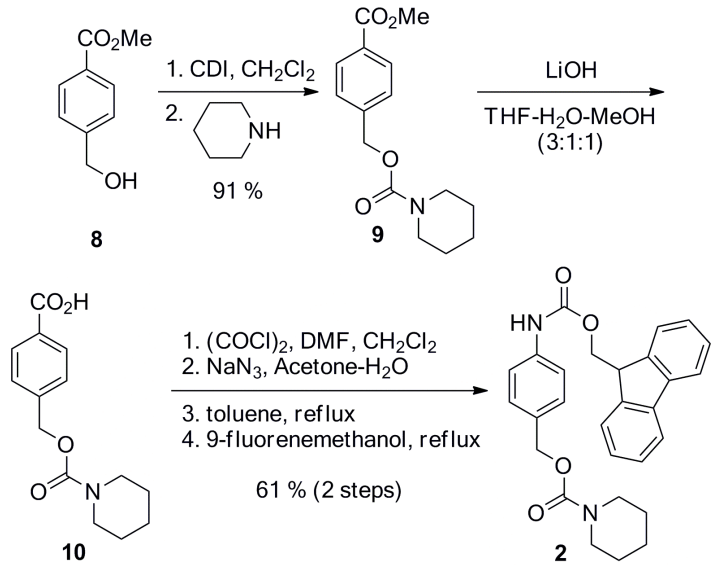
Synthesis of detection reagent 4

4-(Allyloxycarbonylamino)-2-methoxybenzyl piperidine-1-carboxylate (4):

3-Methoxy-4-{[(piperidin-1-ylcarbonyl)oxy]methyl}benzoic acid **7** (4.75 g, 16.2 mmol, 1 equiv) was dissolved in 150 mL of dry CH_2Cl_2 and cooled to 0 °C. DIEA (6.2 mL, 36 mmol, 2.2 equiv) was added to the solution followed by drop-wise addition of ethyl chloroformate (3.4 mL, 36 mmol, 2.2 equiv). The solution was stirred at 0 °C for 1 h, after which the reaction mixture was warmed to room temperature and all solvents were removed under reduced pressure. The resulting residue was dissolved in acetone (70 mL) and the solution was cooled to 0 °C. A solution of sodium azide (5.3 g, 81 mmol, 5 equiv) in cold water (70 mL) (~10 °C) was added drop-wise to the acetone solution. The resulting suspension was stirred vigorously at 0 °C for 2 h, after which the solution was diluted with ethyl acetate (200 mL) and the aqueous layer was separated. The aqueous layer was extracted using ethyl acetate (2 × 100 mL), and the combined organic extracts were washed with brine (2 × 100 mL) and dried over anhydrous sodium sulfate. The sodium sulfate was removed by filtration, and the resulting solution was concentrated under reduced pressure to obtain a yellow gelatinous residue. The residue was dissolved in dry toluene (100 mL) and the resulting solution was heated to 110 °C for 1 h. The reaction mixture was cooled to room temperature, allyl alcohol (3.3 mL, 49 mmol, 3 equiv) was added, and the solution was heated to reflux (112 °C) for 12 h. The solution was cooled to room temperature and the solvent was removed under reduced pressure with gentle heating (40 °C). The residue was purified by column chromatography (gradient elution with 30–40% ethyl acetate/hexanes) to obtain 4-(allyloxycarbonylamino)-2-methoxybenzyl piperidine-1-carboxylate **4** (3.67 g, 10.5 mmol, 65%). IR (cm^{-1}) 3260, 3204, 3074, 2933, 2850, 1726, 1668, 1610; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.43 (1H, s), 7.28 (1H, s), 7.21 (1H, d, J = 8.1), 6.82 (1H, d, J = 8.1), 5.94 (1H, m), 5.34 (1H, d, J = 17.2), 5.26 (1H, d, J = 15.7), 5.12 (2H, s), 4.65 (2H, d, J = 5.5), 3.79 (3H, s), 3.43 (4H, m), 1.57–1.55 (2H, m), 1.51 (4H, m); δ 24.4, 25.7, 44.9, 55.4, 62.2, 65.7, 101.6, 110.1, 118.1, 120.0, 129.8, 132.5, 139.4, 153.4, 155.7, 158.0; MS (TOF MS ES+, *m/z*): 371.1 (4, M + Na^+), 220.1 (100, M – $C_6H_{11}O_2N$); HRMS (TOF MS ES+, *m/z*) Calculated for $C_{18}H_{24}N_2O_5Na$ (M + Na^+): 371.1583; Found: 371.1576.

Synthesis of amplification reagent 2

Scheme S1. Synthesis of amplification reagent **2**.



4-(methoxycarbonyl)benzyl piperidine-1-carboxylate (9):

Methyl 4-(hydroxymethyl)benzoate **8** (0.66 g, 4 mmol, 1 equiv) was dissolved in dry CH_2Cl_2 (20 mL) and the solution was cooled to 0 ^oC. Carbonyldiimidazole (0.78 g, 4.8 mmol, 1.2 equiv) was added slowly to the stirring solution, and, after 5 min at 0 ^oC, the reaction mixture was warmed to room temperature. The reaction mixture was stirred at room temperature for 45 min, after which piperidine (0.47 mL, 4.8 mmol, 1.2 equiv) was added. The solution was stirred at room temperature for 12 h, and then was diluted with CH_2Cl_2 (20 mL). The resulting solution was washed with aqueous hydrochloric acid (0.01 M, 20 mL). The aqueous layer was separated and was successively extracted using CH_2Cl_2 (2 × 20 mL). The combined organic layers were washed with water (1 × 20 mL), brine (2 × 20 mL), and dried over anhydrous sodium sulfate. The sodium sulfate was removed by filtration and the resulting solution was concentrated under reduced pressure. The resulting white solid was purified using column chromatography (elution with 30% ethyl acetate/hexanes) to obtain 4-(methoxycarbonyl)benzyl piperidine-1-carboxylate **9** (1.0 g, 3.6 mmol, 91%). IR (cm^{-1}) 2940, 2856, 1723, 1701; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.00 (2H, d, J = 8.2), 7.37 (2H, d, J = 8.2), 5.14 (2H, s), 3.87 (3H, s), 3.44–3.40 (4H, m), 1.56–1.50 (6H, m); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 24.2, 25.6, 44.8, 52.0, 66.0, 127.2, 129.5, 129.7, 142.2, 154.9, 166.7; MS (TOF MS ES+, *m/z*): 278.1 (15, M + H^+), 149.0 (100, M – $C_6H_{11}O_2N$); HRMS (TOF MS ES+, *m/z*) Calculated for $C_{15}H_{20}NO_4$ (M + H^+): 278.1392; Found: 278.1391.

4-{[(piperidin-1-ylcarbonyl)oxy]methyl}benzoic acid (10):

4-(methoxycarbonyl)benzyl piperidine-1-carboxylate **9** (0.95 g, 3.4 mmol, 1 equiv) was dissolved in 18 mL of THF and 6 mL of methanol. Lithium hydroxide monohydrate (0.20 g, 4.8 mmol, 1.4 equiv) dissolved in 6 mL of water was added to the THF−MeOH solution. The resulting solution was stirred for 10 h at room temperature. The solution was acidified to a pH of 2 by slow addition of aqueous hydrochloric acid (1 M), and was extracted using

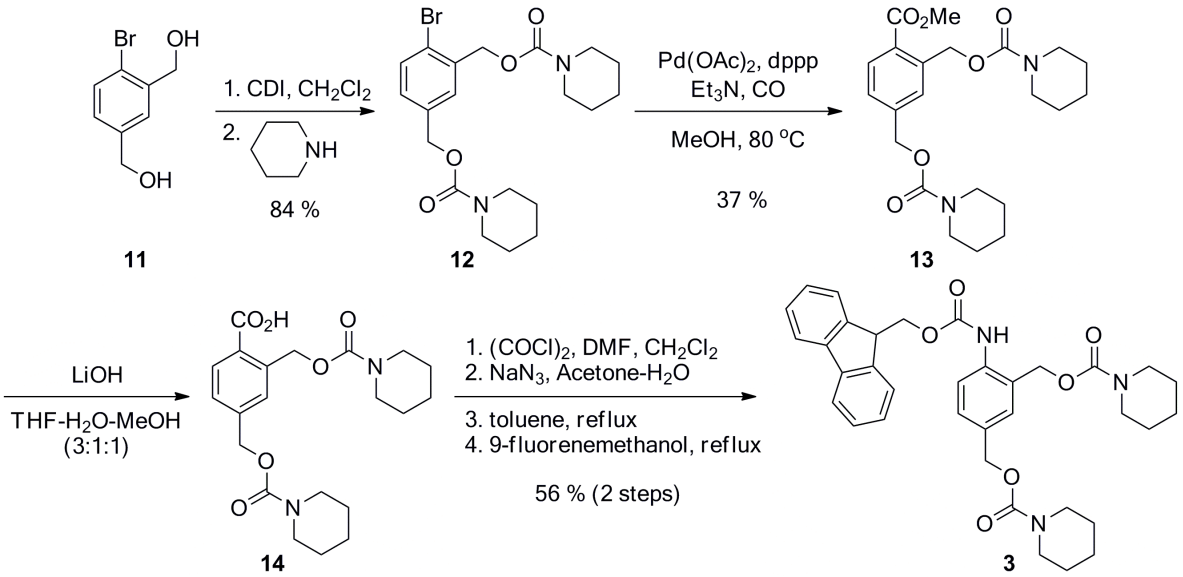
ethyl acetate (5 × 25 mL). The combined organic layers were washed with brine (1 × 50 mL) and dried over anhydrous sodium sulfate. The sodium sulfate was removed by filtration and the resulting solution was concentrated under reduced pressure to obtain 4-{[(piperidin-1-ylcarbonyl)oxy]methyl}benzoic acid **10**, which was used without further purification.

4-(((9H-fluoren-9-yl)methoxy)carbonylamino)benzyl piperidine-1-carboxylate (2):

4-{[(piperidin-1-ylcarbonyl)oxy]methyl}benzoic acid **10** (0.97 g, 3.7 mmol, 1 equiv) dissolved in 25 mL of dry CH_2Cl_2 and 1 mL of DMF was cooled to 0 ^oC with stirring. Oxalyl chloride (0.39 mL, 4.4 mmol, 1.2 equiv) was added to this cooled solution and the reaction mixture was stirred at 0 ^oC for 1 h, after which the solution was warmed to room temperature and concentrated under reduced pressure. The residue was dissolved in acetone (15 mL), and the resulting solution was cooled to 0 ^oC. A solution of sodium azide (0.72 g, 11.1 mmol, 3 equiv) in cold water (15 mL) (~10 ^oC) was added drop-wise to the acetone solution. The resulting suspension was stirred vigorously at 0 ^oC for 1 h. The solution was diluted with ethyl acetate (50 mL) and the aqueous layer was separated. The aqueous layer was extracted using ethyl acetate (3 × 30 mL), and the organic layers were combined. These organic extracts were washed with brine (1 × 50 mL) and dried over anhydrous sodium sulfate. The sodium sulfate was removed by filtration, and the resulting solution was concentrated under reduced pressure to provide a yellow gelatinous residue. The residue was dissolved in dry toluene (15 mL) and the resulting solution was heated to 100 ^oC for 1 h. The hot solution was cooled to room temperature, after which 9-fluorenemethanol (870 mg, 4.4 mmol, 1.3 equiv) was added. The solution was heated to reflux (112 ^oC) for 10 h, after which the solution was cooled to room temperature and the solvent was removed under reduced pressure. The residue was purified by column chromatography (gradient elution with 20–30% ethyl acetate/hexanes, then pure THF as eluent) to obtain 4-(((9H-fluoren-9-yl)methoxy)carbonylamino)benzyl piperidine-1-carboxylate **2** (1.03 g, 92.2 mmol, 61%). IR (cm^{-1}) 3292, 2937, 2855, 1732, 1676; ^{1}H-NMR (360 MHz, $CDCl_3$): 7.77 (2H, d, J = 7.5), 7.61 (2H, d, J = 7.5), 7.42–7.27 (8H, m), 6.72 (1H, s), 5.04 (1H, s), 4.54 (2H, d, J = 6.6), 4.27 (1H, t, J = 6.6), 3.42 (4H, m), 1.55 (6H, m); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 24.3, 25.7, 44.8, 47.1, 66.5, 66.8, 118.6, 120.0, 124.9, 127.1, 127.8, 128.9, 137.4, 141.3, 143.7, 155.3; MS (TOF MS ES+, *m/z*): 474.2 (40, M + NH_4^+), 328.1 (100, M – $C_6H_{11}O_2N$); HRMS (TOF MS ES+, *m/z*) Calculated for $C_{28}H_{32}N_3O_5$ (M + NH_4^+): 474.2393; Found: 474.2386.

Synthesis of amplification reagent 3

Scheme S2. Synthesis of amplification reagent **3**.



(4-bromo-1,3-phenylene)bis(methylene) dipiperidine-1-carboxylate (12):

(4-bromo-1,3-phenylene)dimethanol[3] **11** (1.66 g, 7.7 mmol, 1 equiv) was dissolved in dry THF (40 mL) and the solution was cooled to 0 °C. Carbonyldiimidazole (3.24 g, 20 mmol, 2.6 equiv) was added slowly to the stirring solution, and, after 15 min at 0 °C, the reaction mixture was warmed to room temperature. The reaction mixture was stirred at room temperature for 3 hours, after which piperidine (4.56 mL, 46 mmol, 6.0 equiv) was added. The solution was stirred at room temperature for 14 h, and then was diluted with ethyl acetate (40 mL). The resulting solution was successively washed with aqueous NH_4Cl (40 mL), aqueous hydrochloric acid (0.1 M, 2 × 20 mL) and brine. The combined organic layer was dried over anhydrous sodium sulfate. The sodium sulfate was removed by filtration and the resulting solution was concentrated under reduced pressure. The resulting white solid was purified using column chromatography (gradient elution with 20 – 60% ethyl acetate/hexanes) to obtain (4-bromo-1,3-phenylene)bis(methylene) dipiperidine-1-carboxylate **12** (2.85 g, 6.5 mmol, 84 %). IR (cm^{-1}) 2936, 2855, 1699; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.53 (1H, d, J = 8.1), 7.38 (1H, s), 7.17 (1H, d, J = 8.1), 5.19 (2H, s), 5.08 (2H, s), 3.47–3.42 (8H, m), 1.59–1.54 (12H, m); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 24.2, 24.2, 25.5, 44.7, 44.8, 65.9, 66.1, 122.2, 128.5, 132.6, 136.3, 136.5, 154.7, 154.8; MS (TOF MS ES+, *m/z*): 439.1 (100, M + H^+); HRMS (TOF MS ES+, *m/z*) Calculated for $C_{20}H_{28}N_2O_4Br$ (M + H^+): 439.1232; Found: 439.1232.

(4-(methoxycarbonyl)-1,3-phenylene)bis(methylene) dipiperidine-1-carboxylate (13)

An oven-dried 100-mL flask equipped with a stir bar was charged with (4-bromo-1,3-phenylene)bis(methylene) dipiperidine-1-carboxylate **12** (0.94 g, 2.1 mmol, 1equiv), $Pd(OAc)_2$ (38 mg, 0.17 mmol, 0.08 equiv), dppp (141 mg, 0.34 mmol, 0.16 equiv), triethylamine (0.45 mL, 3.2 mmol, 1.5 equiv), and methanol (4.5 mL). The resulting

brown solution was stirred at room temperature and CO gas was bubbled through it for 10 minutes. The reaction mixture was then stirred at 80 ^{o}C under an atmosphere of CO for 72 hours. The reaction mixture was cooled to room temperature and diluted with CH_2Cl_2. The reaction mixture was filtered through a bed of celite and the filtrate was concentrated under reduced pressure. The residue obtained was purified using column chromatography (gradient elution with 20 – 40% ethyl acetate/hexanes) to obtain (4-(methoxycarbonyl)-1,3-phenylene)bis(methylene) dipiperidine-1-carboxylate **13** (325 mg, 0.78 mmol, 37 %). IR (cm^{-1}) 2937, 2855, 1700, 1684; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.95 (1H, d, J = 7.9), 7.38 (1H, s), 7.34 (1H, d, J = 7.9), 5.52 (2H, s), 5.17 (2H, s), 3.89 (3H, s), 3.46 (8H, m), 1.59–1.55 (12H, m); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 24.4, 24.5, 25.7, 30.2, 45.0, 52.3, 65.3, 66.3, 105.7, 126.3, 126.7, 128.1, 131.2, 139.4, 141.6, 155.2, 167.3; MS (TOF MS ES+, *m/z*): 419.2 (90, M + H^+), 290.1 (100, M – $C_6H_{11}O_2N$); HRMS (TOF MS ES+, *m/z*) Calculated for $C_{22}H_{31}N_2O_6$ (M + H^+): 419.2182; Found: 419.2178.

2,4-bis((piperidine-1-carbonyloxy)methyl)benzoic acid (14):

(4-(methoxycarbonyl)-1,3-phenylene)bis(methylene) dipiperidine-1-carboxylate **13** (60 mg, 0.14 mmol, 1 equiv) was dissolved in 0.5 mL of THF and 0.5 mL of methanol. Lithium hydroxide monohydrate (6.5 mg, 0.15 mmol, 1.1 equiv) dissolved in 0.2 mL of water was added to the THF−MeOH solution. The resulting solution was stirred for 12 h at room temperature. An additional amount of lithium hydroxide monohydrate (6.6 mg, 0.16 mmol, 1.1 equiv) dissolved in 0.4 mL of water was added to the reaction mixture and the reaction mixture was stirred at room temperature for another 10 hours. The solution was then acidified to a pH of 2 by slow addition of aqueous hydrochloric acid (0.3 mL, 1 M), and was extracted using ethyl acetate (5 × 5 mL). The combined organic layers were washed with brine (2 × 5 mL) and dried over anhydrous sodium sulfate. The sodium sulfate was removed by filtration and the resulting solution was concentrated under reduced pressure to obtain 2,4-bis((piperidine-1-carbonyloxy)methyl)benzoic acid **14**, which was used without further purification.

(4-(((9H-fluoren-9-yl)methoxy)carbonylamino)-1,3-phenylene)bis(methylene) dipiperidine-1-carboxylate (3):

2,4-bis((piperidine-1-carbonyloxy)methyl)benzoic acid **14** (56 mg, 0.14 mmol, 1 equiv), dissolved in 1 mL of dry CH_2Cl_2 and 10 µL DMF, was cooled to 0 ^{o}C with stirring. Oxalyl chloride (36 µL, 0.43 mmol, 3.0 equiv) was added to this cooled solution and the reaction mixture was stirred at 0 ^{o}C for 1 h, after which the solution was warmed to room temperature and concentrated under reduced pressure. The residue was dissolved in acetone (0.5 mL), and the resulting solution was cooled to 0 ^{o}C. A solution of sodium azide (46 mg, 0.72 mmol, 5 equiv) in cold water (0.5 mL) (~10 ^{o}C) was added drop-wise to the acetone solution. The resulting suspension was stirred vigorously at 0 ^{o}C for 1 h. The solution was diluted with ethyl acetate (8 mL) and the aqueous layer was separated. The aqueous layer was extracted using ethyl acetate (3 × 5 mL), and the organic layers were combined. These organic extracts were washed with brine (2 × 5 mL) and dried over anhydrous sodium sulfate. The sodium sulfate was removed by filtration, and the resulting solution was concentrated under reduced pressure to provide a yellow gelatinous residue. The residue was dissolved in dry toluene (1 mL) and the resulting solution was heated to 100 ^{o}C for 1 h. The hot solution was cooled to room temperature, after which 9-fluorenemethanol (28 mg, 0.14 mmol, 1 equiv) was added. The solution was heated to reflux (112 ^{o}C) for 5 h, after which the solution was cooled to room

temperature and the solvent was removed under reduced pressure. The residue was purified by column chromatography (gradient elution with 20–30% ethyl acetate/hexanes) to obtain (4-(((9H-fluoren-9-yl)methoxy)carbonylamino)-1,3-phenylene)bis(methylene) dipiperidine-1-carboxylate **3** (47 mg, 0.08 mmol, 56 %). IR (cm^{-1}) 3256, 2937, 2855, 1734, 1697, 1672, 1605; ^{1}H-NMR (360 MHz, $CDCl_3$): 7.77 (2H, d, J = 7.5), 7.61 (2H, d, J = 7.6), 7.42–7.27 (8H, m), 6.72 (1H, s), 5.04 (1H, s), 4.54 (2H, d, J = 6.6), 4.27 (1H, t, J = 6.5), 3.42 (4H, m), 1.55 (6H, m); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 24.3, 25.7, 44.8, 47.1, 66.5, 66.8, 118.6, 120.0, 124.9, 127.1, 127.8, 128.9, 137.4, 141.3, 143.7, 155.3; MS (TOF MS ES+, *m/z*): 615.3 (100, M + Na^+); HRMS (TOF MS ES+, *m/z*) Calculated for $C_{35}H_{43}N_4O_6$ (M + NH_4^+): 615.3183; Found: 615.3179.

LCMS analysis of the reaction of 1 with piperidine

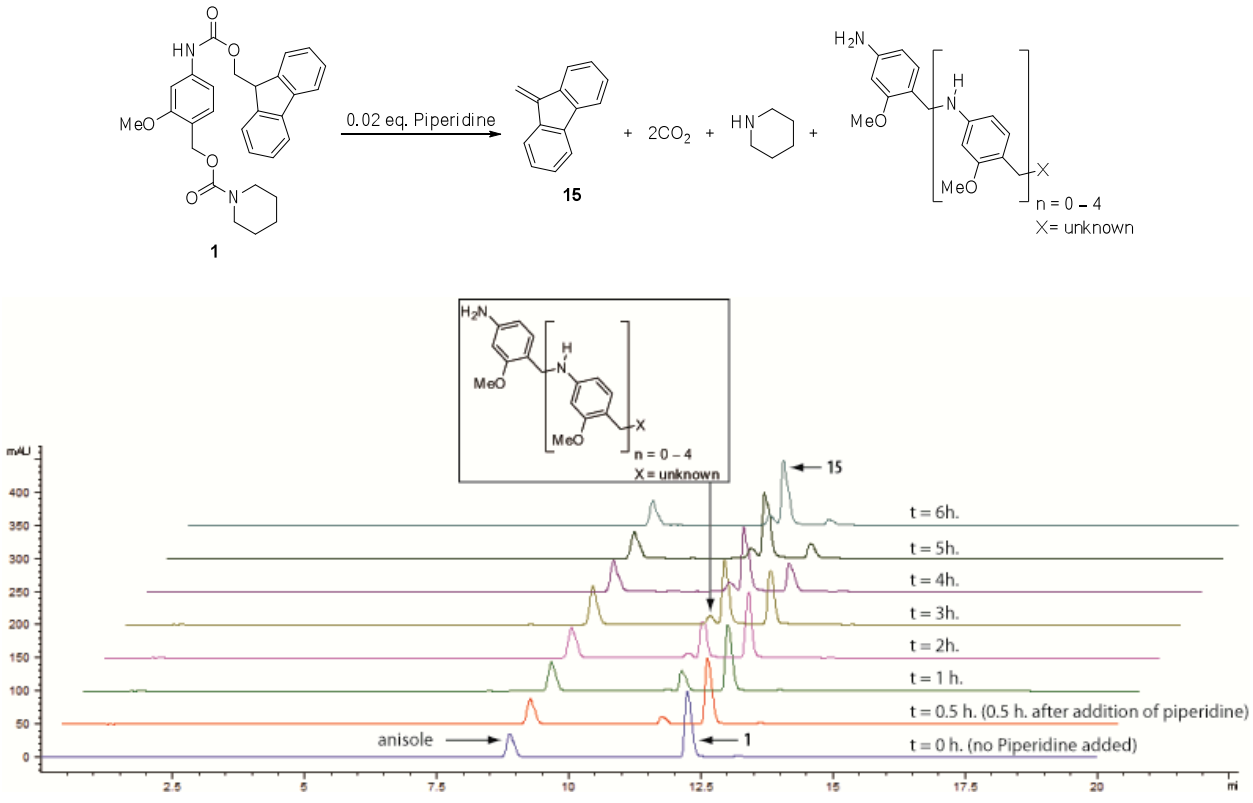


Figure S1: Stacked LC-MS traces (at 254 nm) obtained from the following reaction.

Procedure: A piperidine solution in water (7 µL, 40 mM, 0.02 equiv) was added to a solution of **1** in DMSO (350 µL, 40 mM) containing 61 mM anisole as the internal standard. At regular intervals, an aliquot (10 µL) of the reaction mixture was diluted with acetonitrile (500 µL) and the resulting solution was injected into an analytical reversed-phase HPLC coupled to a mass spectrometer. The LC-MS data were obtained on an Agilent Technologies 1200 series analytical reversed-phase HPLC coupled to an Agilent Technologies 6120 quadrupole mass spectrometer. The column used was a BETASIL Phenyl-Hexyl column (150 mm × 2.1 mm, 5 µm particle size). The mobile phase used was a mixture of 5 mM ammonium formate in H_2O (A) and 5 mM ammonium formate in CH_3CN (B). The column was equilibrated with 9:1 A-B at a flow rate of 0.5 mL/min. The solvent gradient was as follows:

Time (minutes)	A (%)	B (%)
0	90	10
3	75	25
6	50	50
9	75	25
12	90	10
15	90	10
17	10	90
20	10	90

A portion of the HPLC stream was automatically injected into the mass spectrometer. The mass spectrometer (ES) settings were as follows: gas temperature of 350 °C, drying gas flow of 10–13 L/min, nebulizer pressure of 40–60 psig, and a voltage of 3000 V.

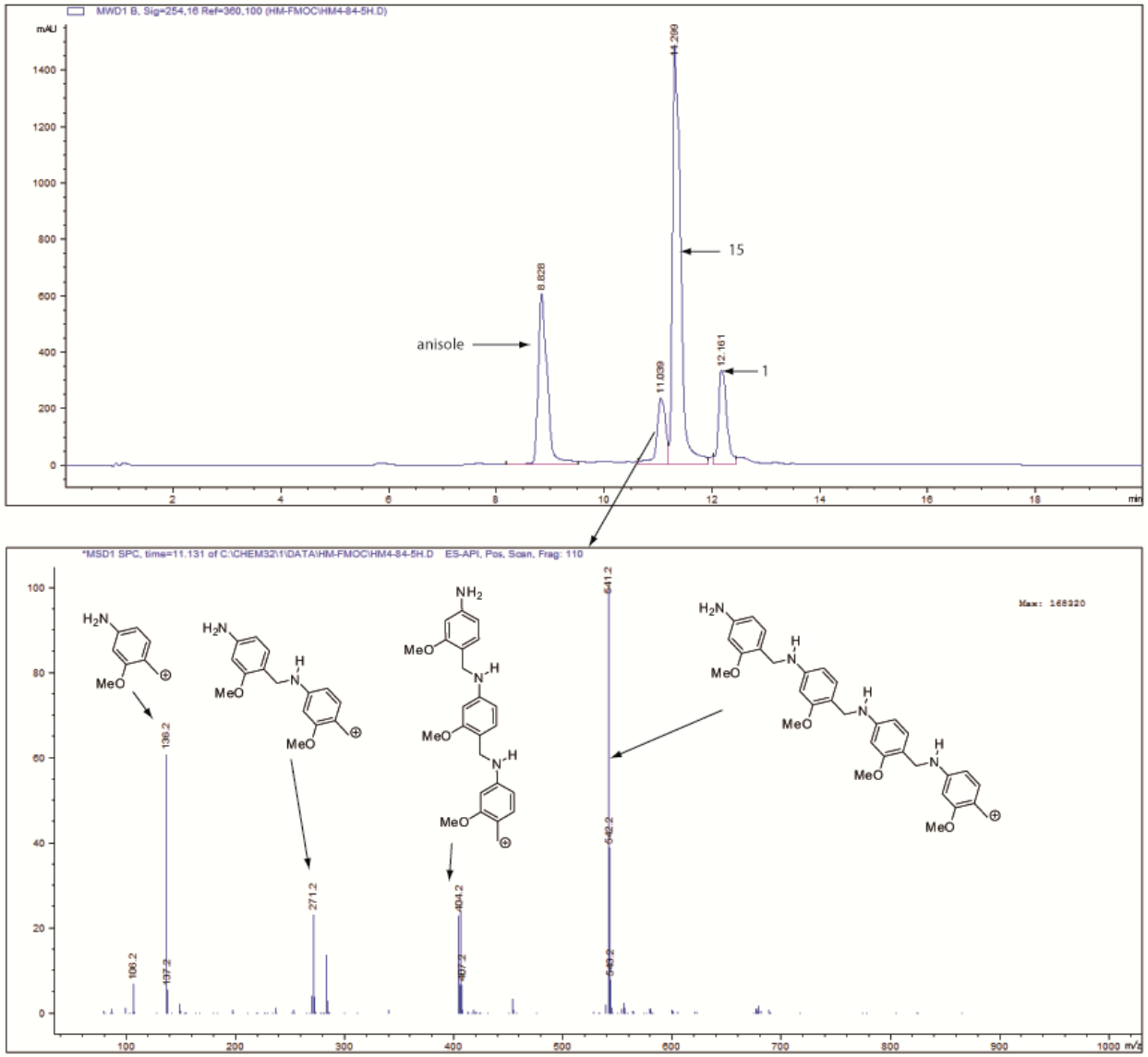


Figure S2: The LC-MS trace at 254 nm corresponding to the reaction at 6 h as described in Figure S1.

Stability of reagent 1 in the solid state

Procedure: 4-(((9H-fluoren-9-yl)methoxy)carbonylamino)-2-methoxybenzyl piperidine-1-carboxylate **1** (20 mg solid) was stored in a vial under air at 20 °C. At intervals the sample was dissolved in about 0.5 mL $CDCl_3$ and ^{1}H-NMR spectrum was obtained. The solution was transferred back into the vial using a small amount of CH_2Cl_2. The solvents were removed under reduced pressure. The vial was flushed with air and capped. No change was observed by ^{1}H-NMR for 14 days.

Quantification of dibenzofulvene when 1, 2, or 3 are exposed to substoichiometric quantities of piperidine

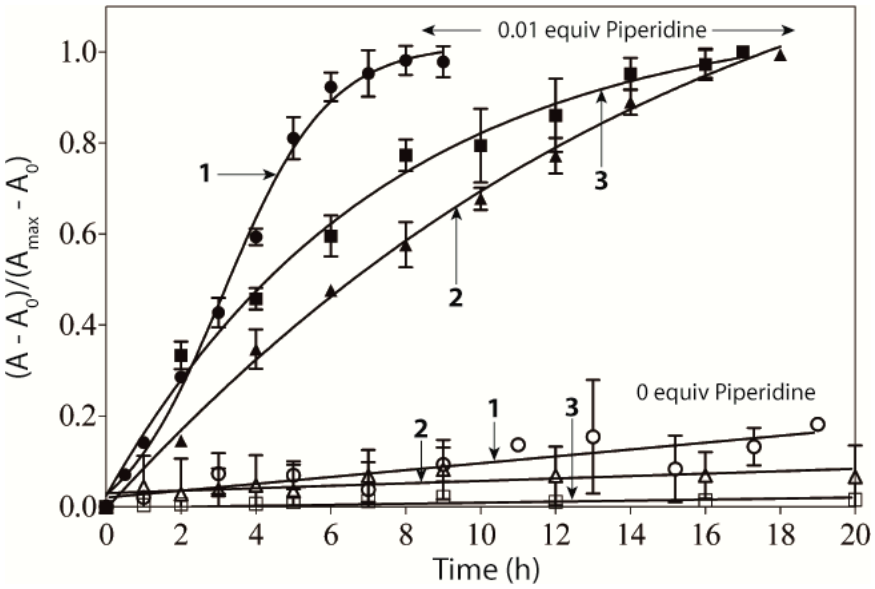


Figure S3. Quantification of dibenzofulvene when **1**, **2**, or **3** is exposed to substoichiometric quantities of piperidine. The graph provides the normalized absorbance of dibenzofulvene at 305 nm during the course of the signal amplification reaction for **1** (circular data points), **2** (triangular data points) or **3** (square data points). The experiments were conducted in triplicate, and the error bars reflect the standard deviations from the average values. The concentration of the amplification reagents was 40 mM in 45:5:1 DMSO–THF–H_2O, and the experiments were conducted at 18 °C.

General experimental procedure corresponding to Figures 3 and S3: A piperidine solution in water (2 μL, 2 M–2 mM, 1–0.001 equivalents relative to amplification reagent **1**, **2** or **3**) was added to a solution of the amplification reagent (**1**, **2** or **3**) in 9:1 DMSO−THF (100 μL, 40 mM). At regular intervals, an aliquot (1 μL) of the reaction mixture was diluted with THF (600 μL) and the absorbance of the sample was measured at 305 nm. The absorbance values were normalized using the formula $A_{normal} = (A - A_0)/(A_{max} - A_0)$ where A_{normal} = normalized absorbance, A = absorbance at time t, A_0 = absorbance for a negative control, A_{max} = maximum absorbance obtained for a particular assay.

Tables of data corresponding to Figure 3:

Table S1: Normalized absorbance data for 1.0 equivalent of added piperidine to **1**.

Time (h)	Absorbance at 305 nm
0.00	0.0000
0.03	0.4231
0.06	0.7560
0.07	0.7884
0.09	0.8585
0.13	0.8669
0.16	0.9100
0.23	0.9485
0.20	0.9330
0.25	0.9342
0.32	1.0000
0.38	0.9621
0.45	0.9137

Table S2: Normalized absorbance data for 0.1 equivalents of added piperidine to **1**.

	Absorbance at 305 nm		
Time (h)	Trial 1	Trial 2	Trial 3
0.01	0.0000	0.0000	0.0000
0.33	0.4124	0.4138	0.3765
0.66	0.5957	0.5597	0.6082
1.0	0.7283	0.7672	0.7462
1.3	0.8456	0.8379	0.8292
1.7	0.8506	0.9030	0.9091
2.0	0.9449	0.9815	0.9919
2.3	0.8981	0.9995	0.9496
2.7	0.9038	0.9639	0.9502
3.2	1.0000	1.0000	1.0000

Table S3: Normalized absorbance data for 0.01 equivalents of added piperidine to **1**.

	Absorbance at 305 nm		
Time (h)	Trial 1	Trial 2	Trial 3
0.01	0.0000	0.0000	0.0000
0.50	0.0697	0.0736	0.0692
1.0	0.1326	0.1375	0.1532
2.0	0.2718	0.2898	0.2943
3.0	0.4292	0.3933	0.4586
4.0	0.6109	0.5748	0.5946
5.0	0.7620	0.8160	0.8542
6.0	0.9500	0.8885	0.9313
7.0	0.9787	0.8940	0.9857
8.0	1.0000	1.0000	0.9447
9.0	0.9962	0.9393	1.0000

Table S4: Normalized absorbance data for 0.001 equivalents of added piperidine to **1**.

	Absorbance at 305 nm		
Time (h)	Trial 1	Trial 2	Trial 3
0.01	0.0000	0.0000	0.0000
2.0	0.0074	0.0611	0.0338
4.0	0.0345	0.0494	0.0354
6.0	0.0686	0.0868	0.0692
8.0	0.1611	0.2180	0.1787
10	0.3675	0.4009	0.4102
12	0.6855	0.7183	0.7531
13	0.8102	0.8305	0.8390
14	0.9590	0.9545	0.9243
15	0.9666	0.9560	0.9540
16	0.9738	0.9307	1.0000
18	1.0000	1.0000	0.9957

Table S5: Normalized absorbance data for 0.0 equivalents of added piperidine to **1**.

	Absorbance at 305 nm		
Time (h)	Trial 1	Trial 2	Trial 3
0.01	0.0000	0.0000	0.0000
1.0	0.0131	0.0227	0.0264
3.0	0.0583	0.0375	0.1233
5.0	0.1022	0.0419	0.0638
7.0	-0.0020	0.1070	0.0082
9.0	0.0565	0.0916	0.1311
11	0.1268	0.1463	0.1362
13	0.0410	0.2878	0.1330
15.2	0.0430	0.1678	0.0389
17.3	0.1184	0.1789	0.0999
19	0.1797	0.1850	0.1800

Tables of data corresponding to Figure S3:

Table S6: Normalized absorbance data for 0.01 equivalents of added piperidine to **2**.

	Absorbance at 305 nm		
Time (h)	Trial 1	Trial 2	Trial 3
0	0.0000	0.0000	0.0000
2	0.1531	0.1416	0.1418
4	0.3373	0.3939	0.3088
6	0.4748	0.4808	0.4719
8	0.5936	0.6146	0.5199
10	0.7017	0.6769	0.6533
12	0.7685	0.8124	0.7342
14	0.9166	0.8915	0.8607
16	1.0000	0.9403	0.9810
18	0.9818	1.0000	1.0000

Table S7: Normalized absorbance data for 0.0 equivalents of added piperidine to **2**.

	Absorbance at 305 nm		
Time (h)	Trial 1	Trial 2	Trial 3
0	0.0915	0.0948	0.1048
1	0.1017	0.0952	0.1014
2	0.1003	0.1008	0.1011
3	0.0995	0.0932	0.0279
4	0.0954	0.1006	0.1073
5	0.1027	0.1009	0.1061
7	0.1032	0.105	0.106
9	0.1073	0.104	0.1197
12	0.1023	0.1068	0.1023
16	0.1051	0.1048	0.1069
20	0.1036	0.1054	0.1098

Table S8: Normalized absorbance data for 0.01 equivalents of added piperidine to **3**.

	Absorbance at 305 nm		
Time (h)	Trial 1	Trial 2	Trial 3
0	0.0000	0.0000	0.0000
2	0.2999	0.3589	0.3398
4	0.4785	0.4318	0.4612
6	0.5455	0.6327	0.6080
8	0.7336	0.7995	0.7861
10	0.7476	0.7471	0.8874
12	0.7974	0.8342	0.9514
14	0.9726	0.9112	0.9722
16	0.9913	0.9325	0.9943
17	1.0000	1.0000	1.0000

Table S9: Normalized absorbance data for 0.0 equivalents of added piperidine to **3**.

	Absorbance at 305 nm		
Time (h)	Trial 1	Trial 2	Trial 3
0	0	0	0
1	0.1223	0.0021	0.0088
2	0.1172	-0.0275	-0.0052
3	0.0915	0.0126	0.0075
4	0.1234	0.0042	0.0134
5	0.0995	-0.0123	0.0165
7	0.1338	0.0382	0.0317
9	0.1575	0.0432	0.0446
12	0.1409	0.0199	0.0431
16	0.1278	0.0442	0.0346
20	0.1437	0.0139	0.0403

Demonstration that piperidine is amplified when 1 is exposed to substoichiometric piperidine

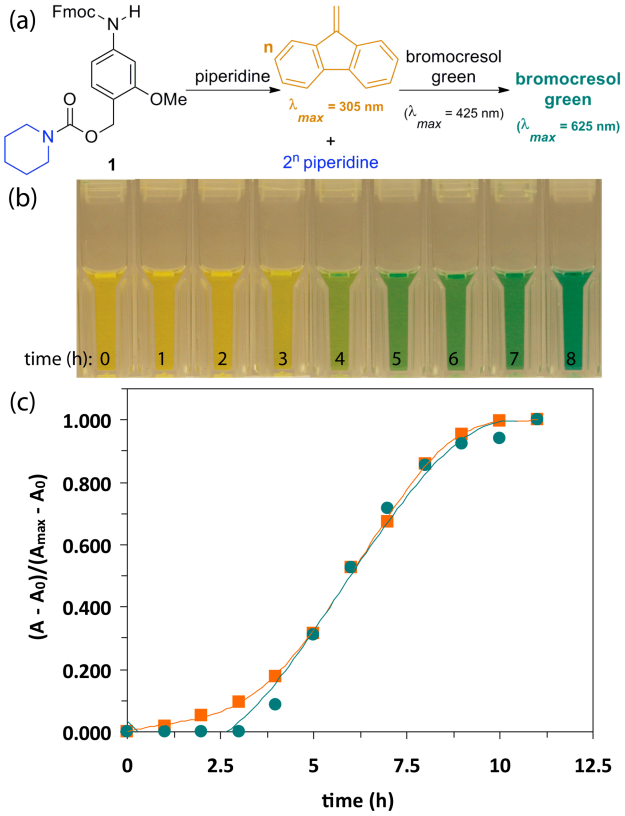


Figure S4. Demonstration that reagent **1** amplifies base autocatalytically. (a) The test reaction of reagent **1** when exposed to piperidine. (b) Photographs of the color produced when aliquots (1 μL) from the amplification reaction were added to 28.6 μM solutions of bromocresol green in isopropanol. (c) Normalized absorbance of bromocresol green (green circular data points) at 625 nm and dibenzofulvene (orange square data points) at 305 nm during the course of the signal amplification reaction.

Experimental procedure corresponding to Figure S4:

A stock dye solution (A) was prepared by dissolving bromocresol green (2 mg, 2.86 μmol) and aqueous sodium hydroxide (2 μL, 1 M) in isopropanol and by adjusting the volume of the solution to 100 mL using isopropanol. A piperidine solution in water (4 μL, 20 mM) was added to a solution of **1** in DMSO (200 μL, 40 mM). At regular intervals, an aliquot (1 μL) of the reaction mixture was added to a 1-mL aliquot of solution A and the absorbance at 625 nm was measured after an incubation time of 20 minutes. The progress of the reaction also was monitored using the method described for Figure S5. Briefly, an aliquot (1 μL) of the reaction mixture was diluted with THF (600 μL) and the absorbance was measured at 305 nm.

Tables of data corresponding to Figure S4:

Table S6: Normalized absorbance for the experiments described above.

Time(h)	Normalized absorbance at 305 nm $(A - A_0)/(A_{max} - A_0)$	Normalized absorbance at 625 nm $(A - A_0)/(A_{max} - A_0)$
0	0.000	0.000
1	0.019	0.001
2	0.052	0.001
3	0.095	0.001
4	0.177	0.086
5	0.314	0.312
6	0.528	0.525
7	0.672	0.715
8	0.859	0.856
9	0.954	0.922
10	0.996	0.939
11	1.000	1.000

Control experiments for the procedure described in Figure S4:

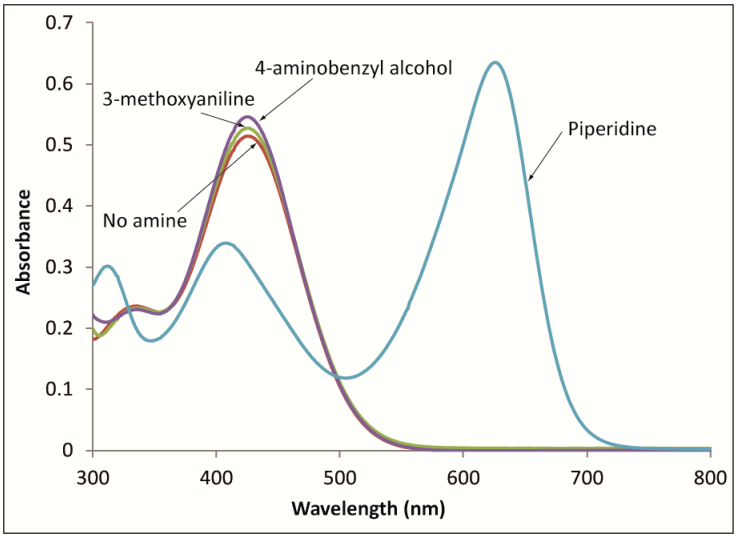


Figure S5: UV-Vis spectra of bromocresol green (1 mL, 28.6 μM, isopropanol) after addition of a solution of selected amines (1 μL, 50:1 DMSO−H_2O).

Detection of Pd using reagents 4 and 1

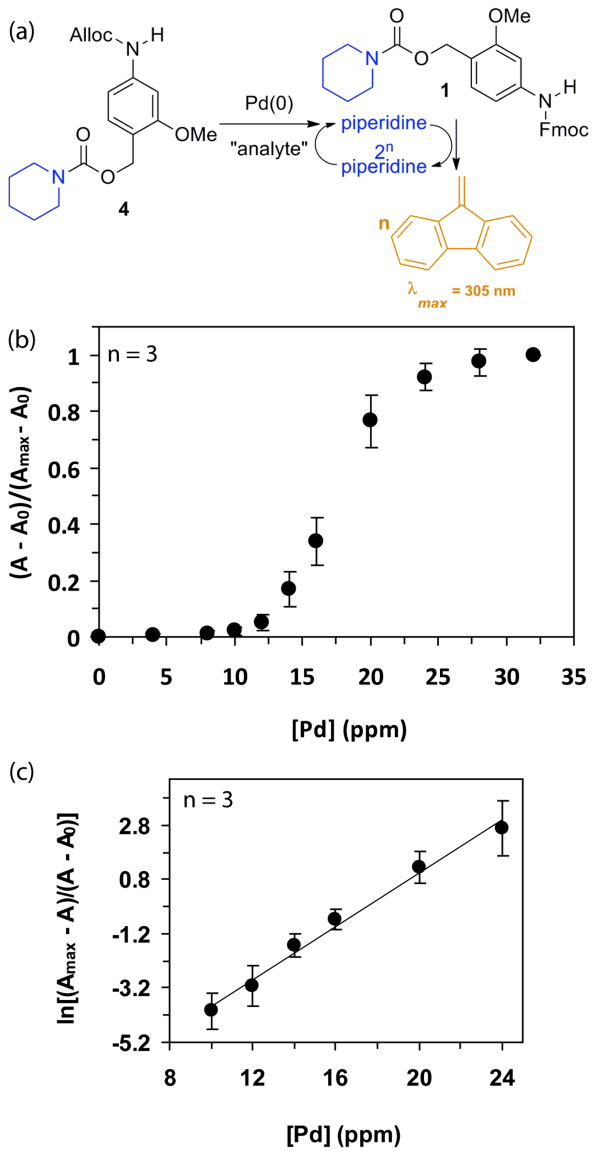


Figure S6: Detection of palladium using (a) a tandem reaction involving reaction of **4**, addition of **1**, and measurement of the absorbance of dibenzofulvene (305 nm) after 16 h of signal amplification. (b) Dose–response curve using normalized absorbance values. The experiments were repeated three times; the errors bars reflect the standard deviations from the average values. (c) Alternative plot that provides a linear calibration curve for quantitative measurements of Pd. The equation for the line is $y = 0.4905x - 8.7726$, and the R^2 value is 0.99.

Experimental procedure corresponding to Figure S6: A stock solution (Solution A) was prepared containing the detection reagent **4** in THF (0.2 M) and phenylsilane (438 mM). Tri-n-butylphosphine (10 μL) was added to a solution of $Pd(OAc)_2$ (1 mL, 0–32 ppm of Pd(II) in THF) and the resulting solution was mixed using a vortex mixer. After 5 minutes, an aliquot (100 μL) of this palladium solution was added to Solution A (100 μL) and the reaction mixture was agitated using a vortex mixture for 2 s. The solution was left undisturbed for 1 h. The reaction mixture

was then diluted with water (40 μL). An aliquot (50 μL) of this solution was combined with a solution of amplification reagent **1** in DMSO (56.8 μM, 360 μL). The amplification reaction was allowed to proceed for 16 h. An aliquot (1 μL) of the reaction mixture was diluted with THF (600 μL) and the absorbance of the solution was measured at 305 nm using THF as the blank sample. The absorbance data obtained were normalized using the formula $A_{normal} = (A - A_0)/(A_{max} - A_0)$ where A_{normal} = normalized absorbance, A = absorbance measurements obtained for a reaction, A_0 = absorbance values for a negative control containing no added Pd(II), and A_{max} = maximum absorbance obtained.

Tables of data corresponding to Figure S6:

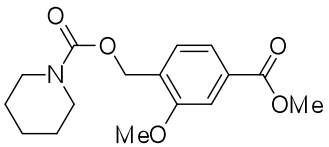
Table S7: Absorbance data obtained for the experiment described above.

	Normalized absorbance at 305 nm $(A - A_0)/(A_{max} - A_0)$		
[Pd(II)] in ppm	Trial 1	Trial 2	Trial 3
0	0.0000	0.0000	0.0000
4	0.0110	0.0086	-0.0017
8	0.0202	0.0134	0.0004
10	0.0133	0.0103	0.0359
12	0.0735	0.0533	0.0190
14	0.1367	0.1269	0.2404
16	0.2905	0.2849	0.4375
20	0.7122	0.7112	0.8737
24	0.8936	0.8923	0.9797
28	0.9156	1.0062	0.9979
32	1.0000	1.0000	1.0000

References

1. W. C. Still, M. Kahn,A. Mitra, *J. Org. Chem.* 1978, **43**, 2923.
2. F. A. Neugebauer, H. Fischer, H. Brunner, *Tetrahedron* 1981, **37**, 1391.
3. D. Suzuki, H. Urabe and F. Sato, *J. Am. Chem. Soc.*, 2001, **123**, 7925.

Copies of spectra



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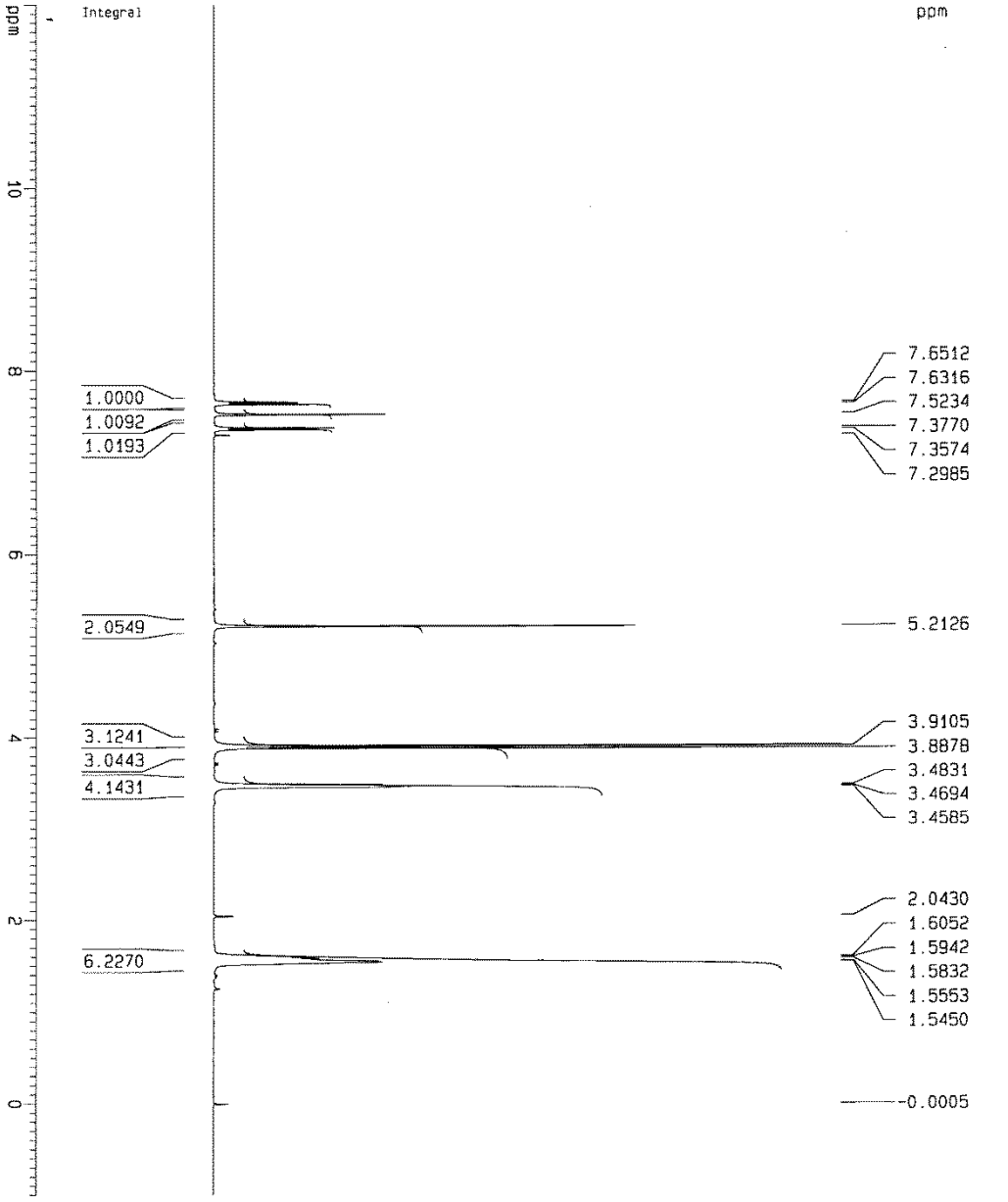


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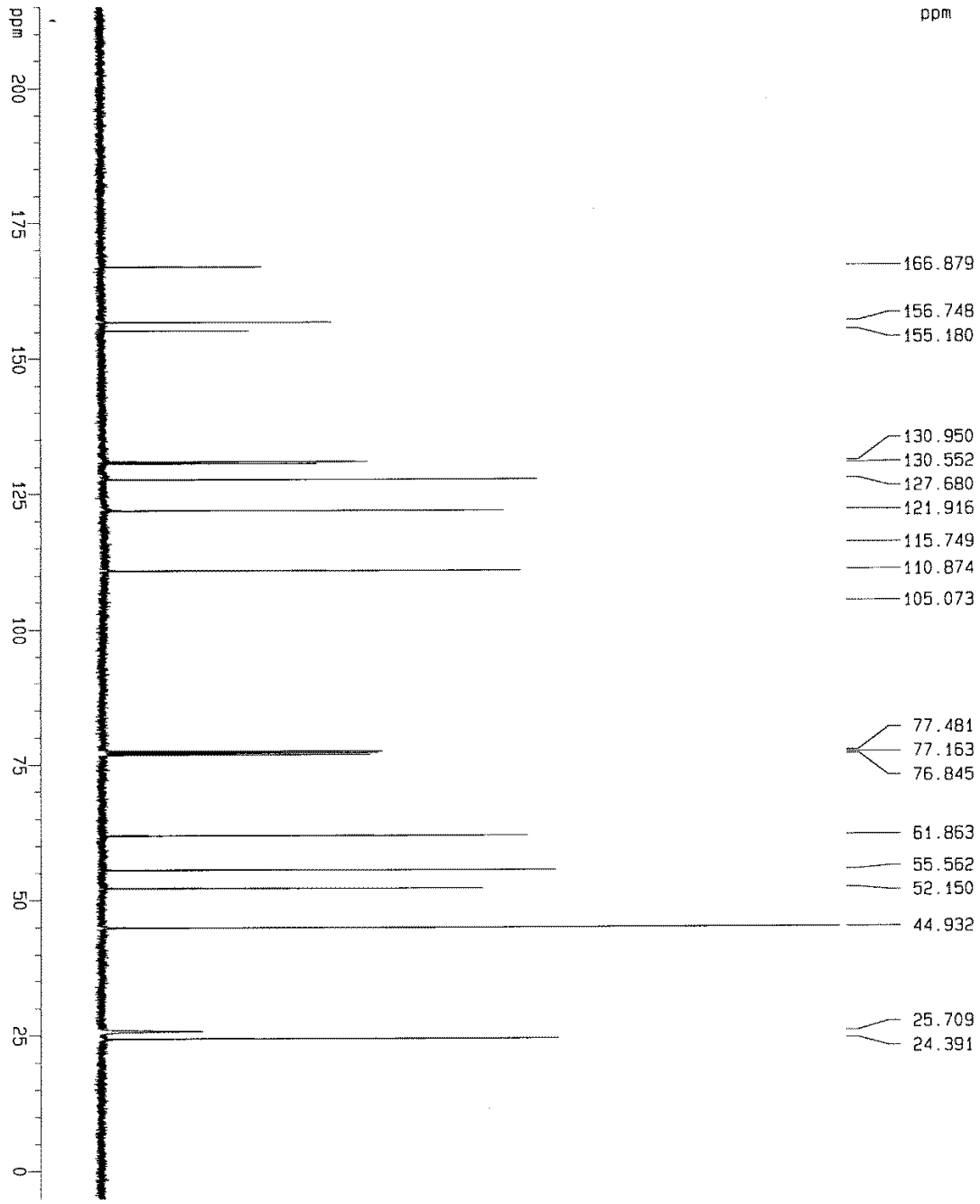


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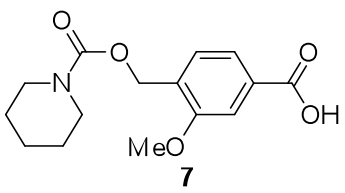

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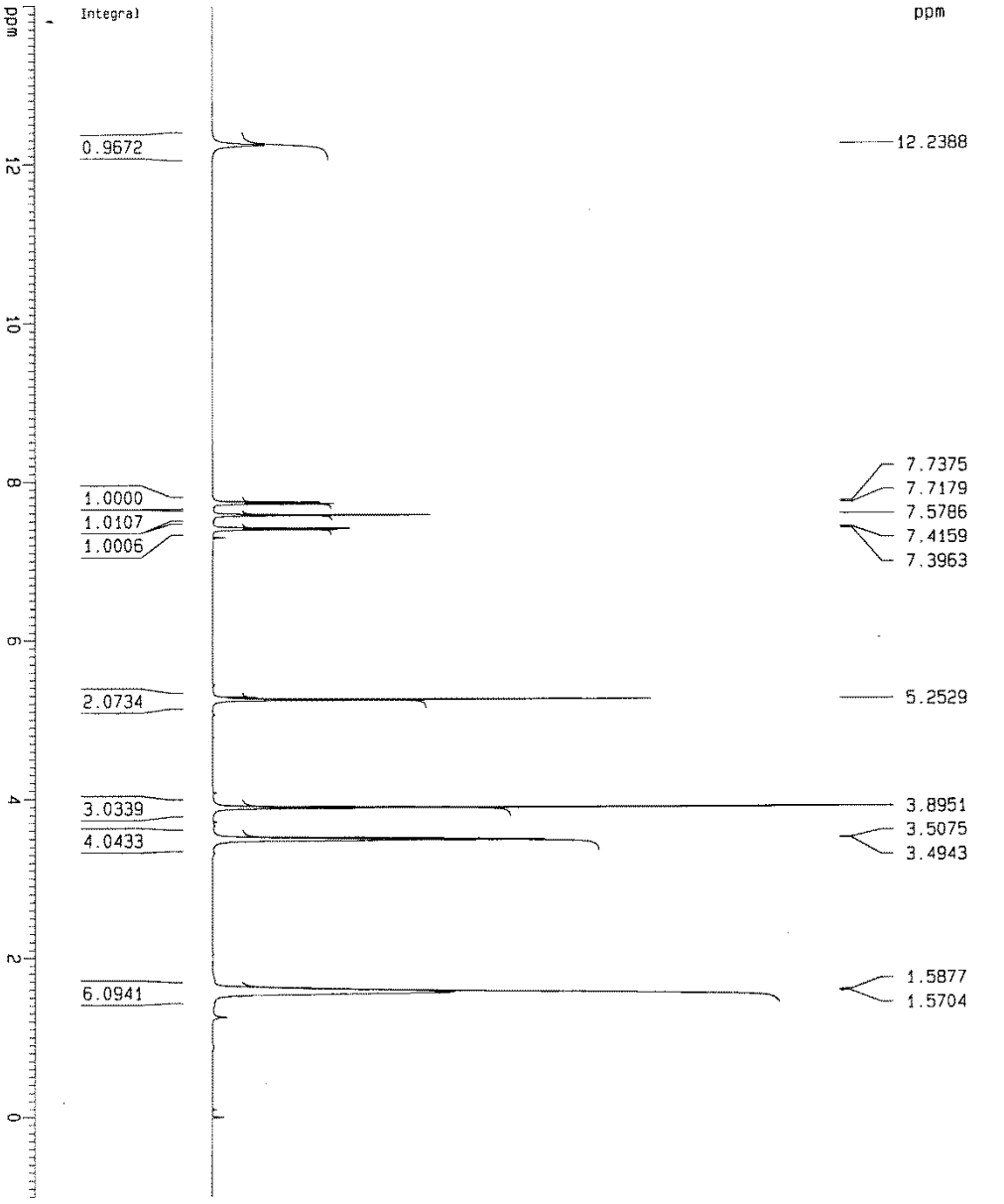


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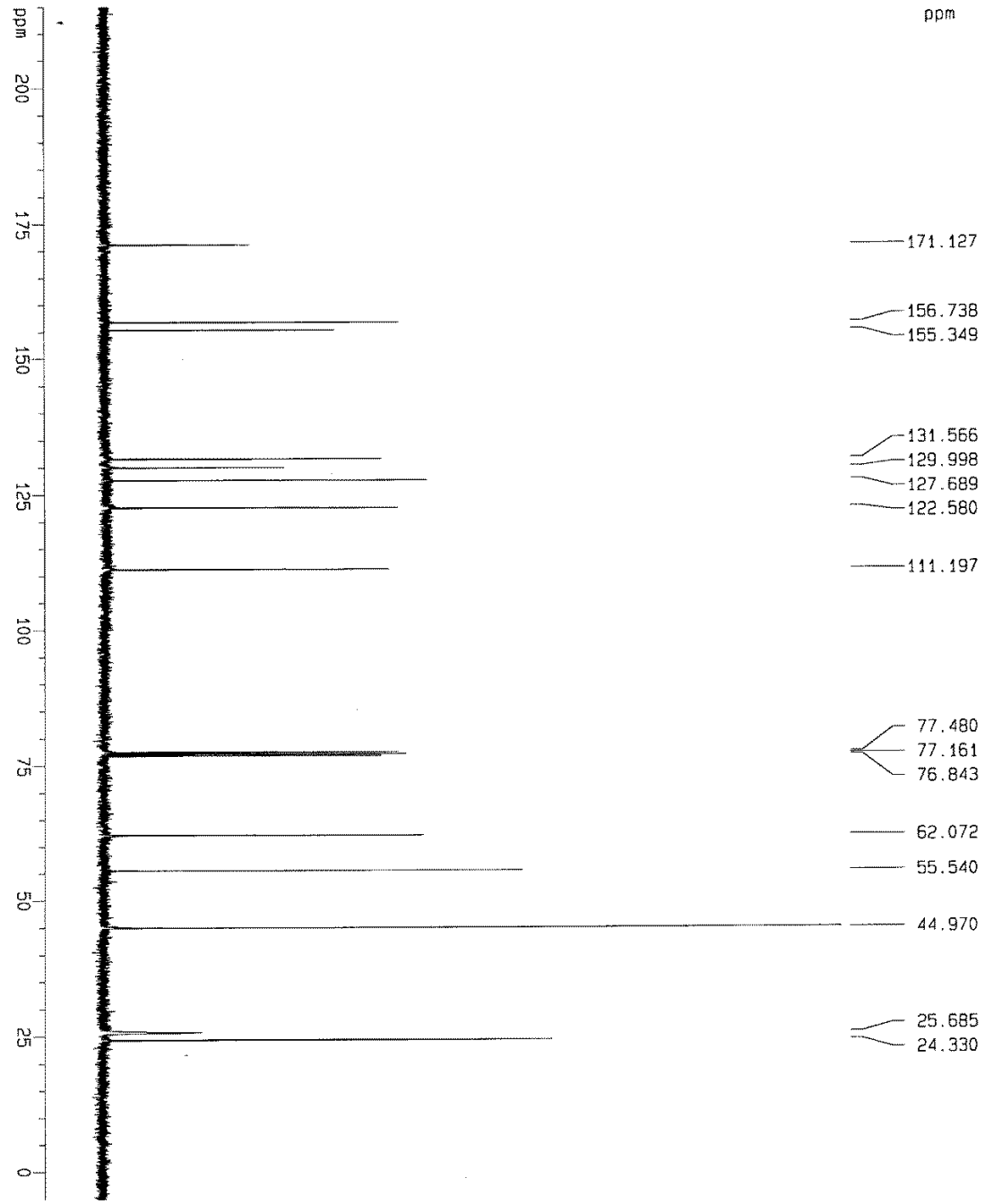


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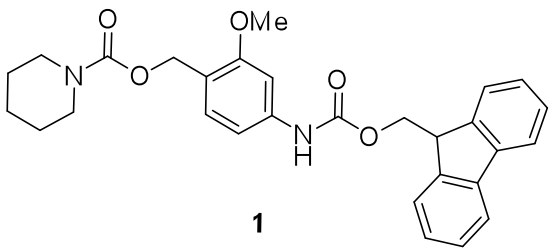

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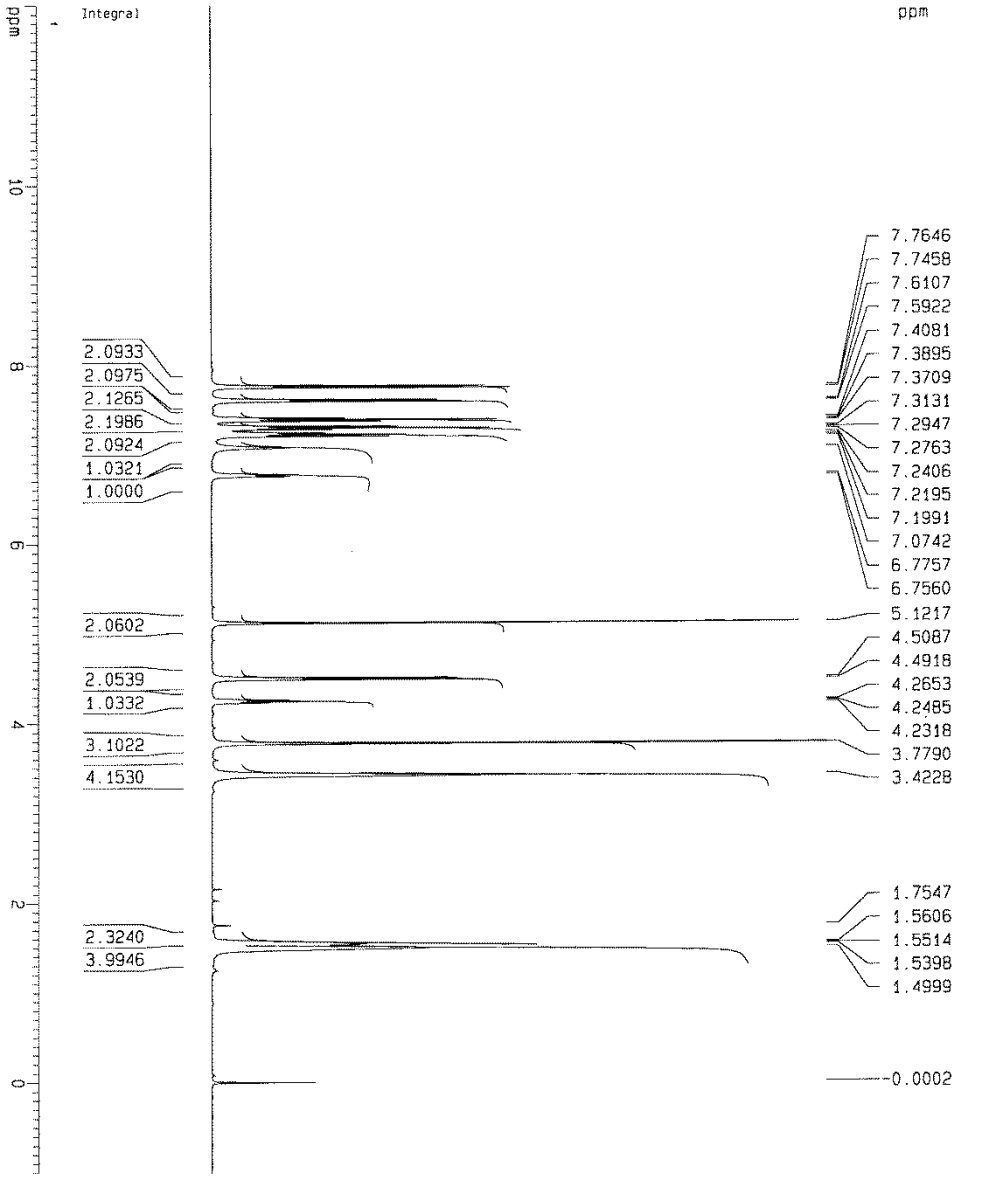


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F2 - Acquisition Parameters
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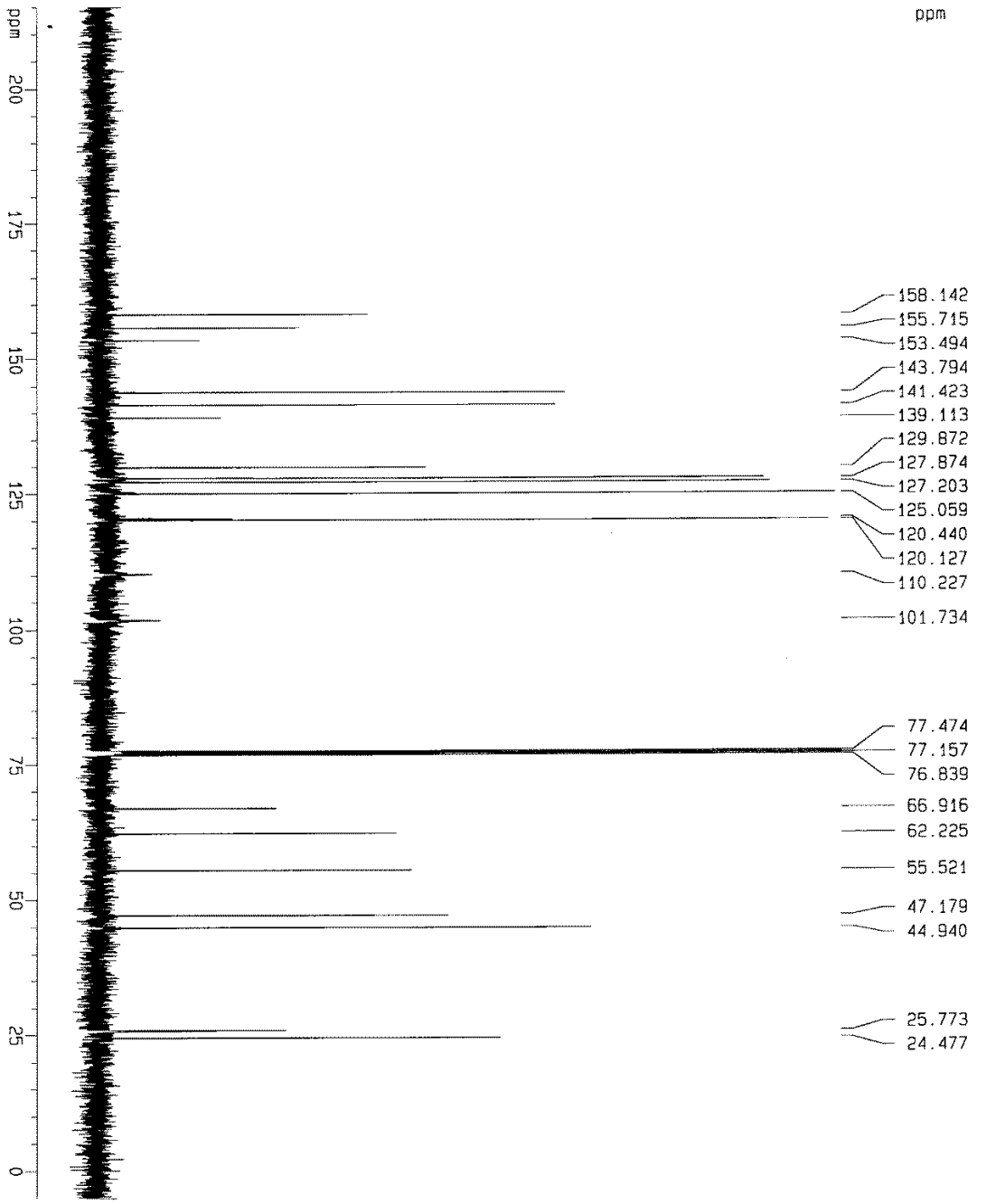
Time                          20.58
INSTRUM                       spect
PROBHD     5 mm BBI 1H-
PULPROG                        zg30
TD                            65536
SOLVENT                       CDCl3
NS                               16
DS                                2
SWH                       8278.146 Hz
FIDRES                    0.126314 Hz
AQ                        3.9584243 sec
RG                             90.5
DW                        60.400 usec
DE                         6.00 usec
TE                            300.0 K
D1                        1.00000000 sec

======== CHANNEL f1 ========
NUC1                            1H
P1                          6.45 usec
PL1                          0.00 dB
SFO1                  400.1324710 MHz

F2 - Processing parameters
SI                            32768
SF                    400.1300167 MHz
WDW                              no
SSB                               0
LB                         0.00 Hz
GB                                0
PC                             1.00

1D NMR plot parameters
CX                           20.00 cm
F1P                       12.000 ppm
F1                        4801.56 Hz
F2P                       -1.000 ppm
F2                        -400.13 Hz
PPMCM                  0.55000 ppm/cm
HZCM                   260.08450 Hz/cm
```




```
Current Data Parameters
NAME                  11-22-11mmmoc
EXPNO                            2
PROCNO                           1

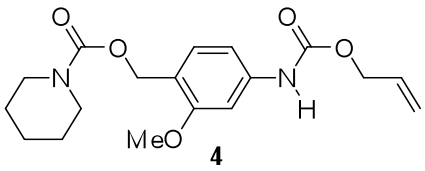
F2 - Acquisition Parameters
Date_                     20111122
Time                         21.33
INSTRUM                      spect
PROBHD     5 mm BBI 1H-
PULPROG                   zgpg30
TD                           65536
SOLVENT                      CDCl3
NS                             512
DS                               4
SWH                      25125.629 Hz
FIDRES                       0.383387 Hz
AQ                        1.3042164 sec
RG                            4096
DW                          19.900 usec
DE                           6.00 usec
TE                          300.0 K
D1                        2.00000000 sec
D11                       0.03000000 sec
D12                       0.00002000 sec

======== CHANNEL f1 ========
NUC1                           13C
P1                           16.35 usec
PL1                          -6.00 dB
SFO1                    100.6237959 MHz

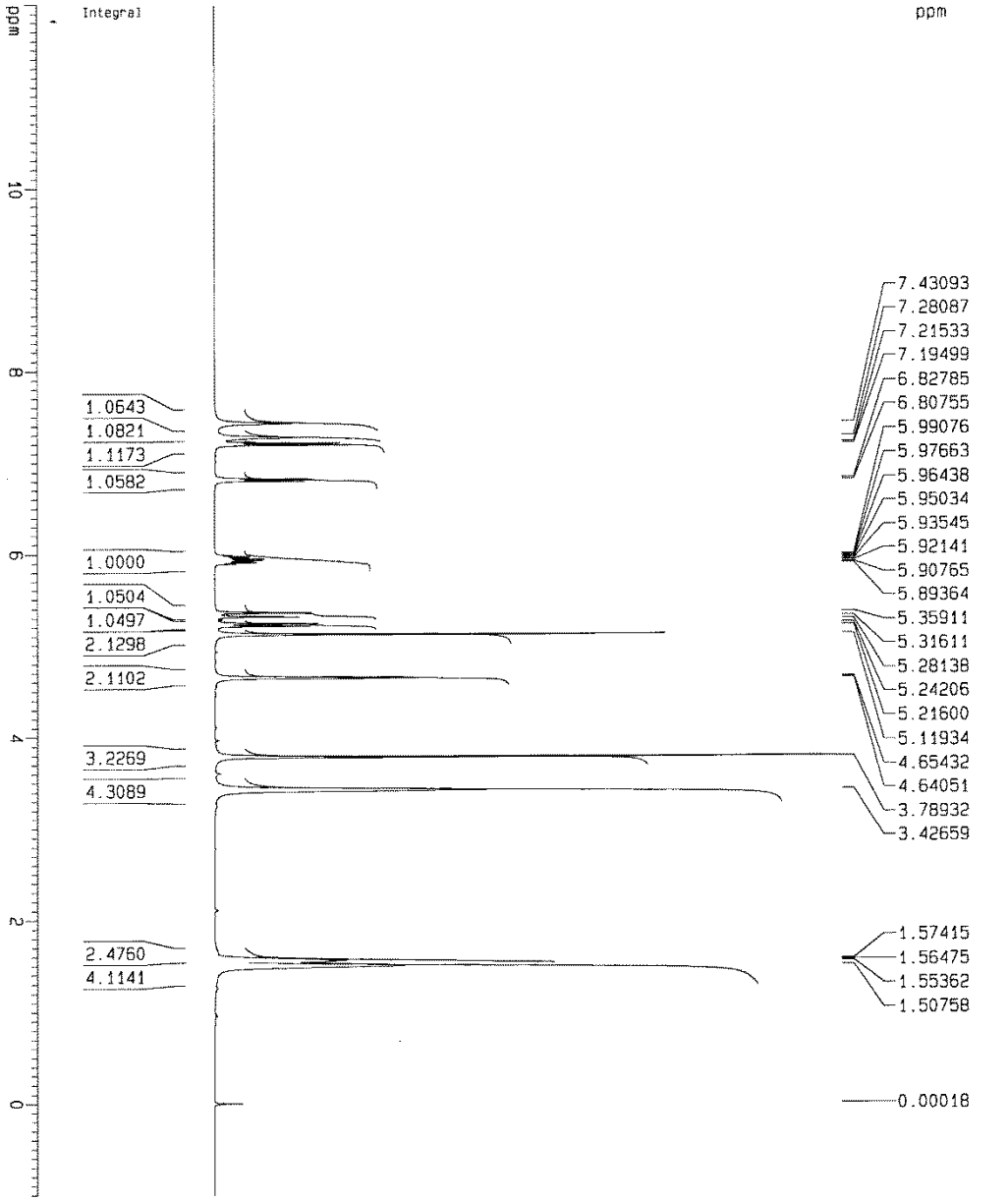
======== CHANNEL f2 ========
CPDPRG2                    waltz16
NUC2                           1H
PCPD2                       114.00 usec
PL2                          0.00 dB
PL12                        24.00 dB
PL13                        24.00 dB
SFO2                    400.1316005 MHz

F2 - Processing parameters
SI                            32768
SF                      100.6127631 MHz
WDW                              EM
SSB                               0
LB                            1.00 Hz
GB                                0
PC                             1.40

1D NMR plot parameters
CX                           20.00 cm
F1P                         215.000 ppm
F1                        21631.75 Hz
F2P                        -5.000 ppm
F2                        -503.06 Hz
PPMCM                      11.00000 ppm/cm
HZCM                    1105.74036 Hz/cm
```



4



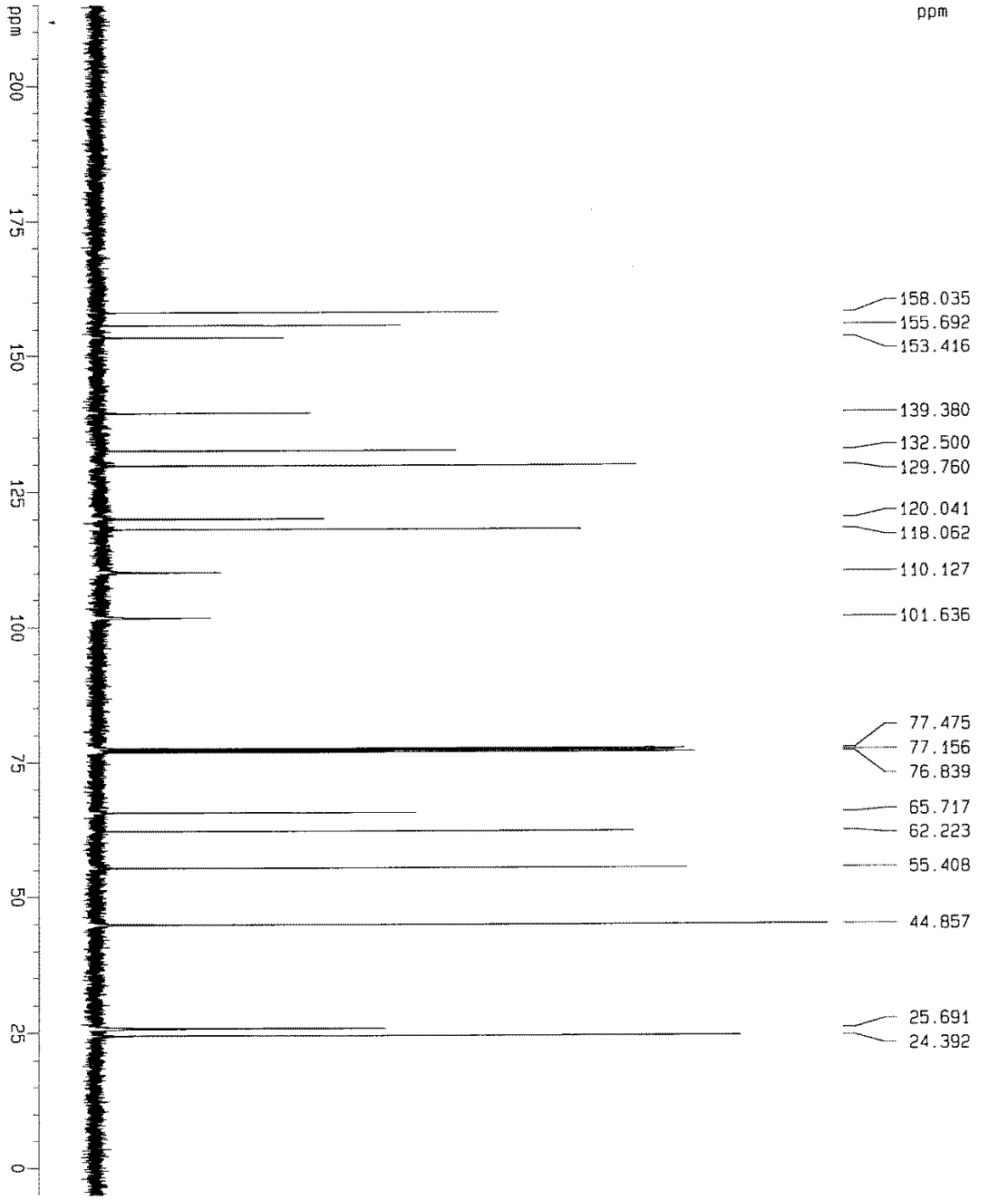

```
Current Data Parameters
NAME                  11-22-1fhmalio
EXPNO                             1
PROCNO                            1

F2 - Acquisition Parameters
Date_                      20111122
Time                          21.55
INSTRUM                       spect
PROBHD     5 mm BBI 1H-
PULPROG                       z930
TD                           65536
SOLVENT                      CDCl3
NS                              16
DS                               2
SWH                        8278.146 Hz
FIDRES                     0.126314 Hz
AQ                         3.9584243 sec
RG                              25.4
DW                         60.400 usec
DE                          6.00 usec
TE                           300.0 K
D1                         1.00000000 sec

======== CHANNEL f1 ========
NUC1                             1H
P1                             6.45 usec
PL1                            0.00 dB
SFO1                     400.1324710 MHz

F2 - Processing parameters
SI                           32768
SF                       400.1300000 MHz
WDW                              no
SSB                               0
LB                             0.00 Hz
GB                                0
PC                             1.00

1D NMR plot parameters
CX                            20.00 cm
F1P                        12.000 ppm
F1                         4801.56 Hz
F2P                        -1.000 ppm
F2                          -400.13 Hz
PPMCM                     0.65000 ppm/cm
HZCM                   260.08450 Hz/cm
```

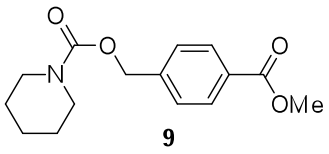



```
Current Data Parameters
NAME                  11-22-11hmalio
EXPNO                            2
PROCNO                           1

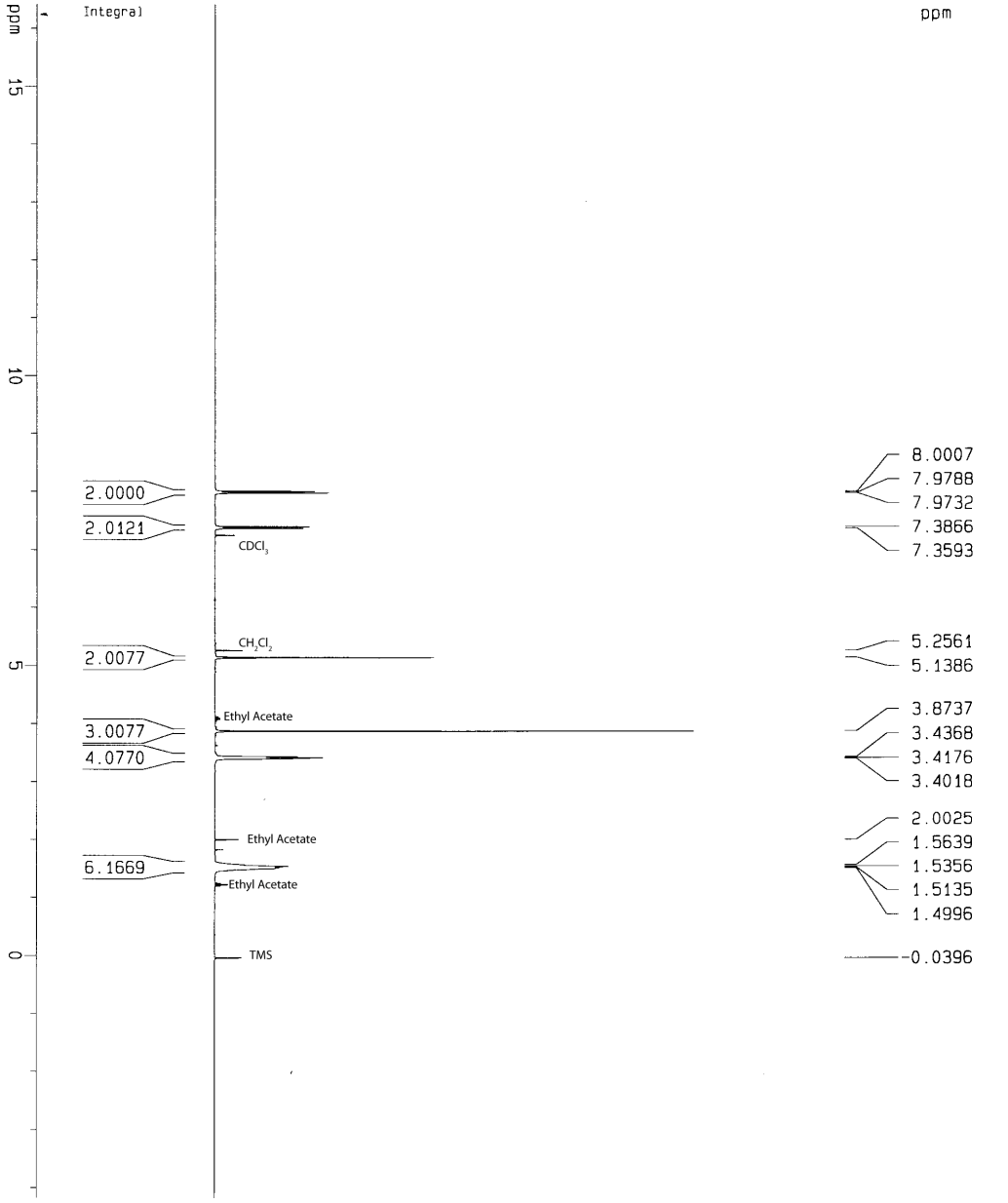
F2 - Acquisition Parameters
Date_                     20111122
Time                         22.32
INSTRUM                      spect
PROBHD     5 mm BBI 1H-
PULPROG                    zgpg30
TD                          65536
SOLVENT                     CDCl3
NS                            512
DS                              4
SWH                     25125.629 Hz
FIDRES                   0.383387 Hz
AQ                       1.3042164 sec
RG                          16384
DW                       19.900 usec
DE                        6.00 usec
TE                          300.0 K
D1                    2.00000000 sec
D11                   0.03000000 sec
D12                   0.00002000 sec
============== CHANNEL f1 ==========
NUC1                          13C
P1                         15.35 usec
PL1                        -6.00 dB
SFO1                 100.6237959 MHz
============== CHANNEL f2 ==========
CPDPRG2                    waltz16
NUC2                           1H
PCPD2                     114.00 usec
PL2                        0.00 dB
PL12                      24.00 dB
PL13                      24.00 dB
SFO2                 400.1316005 MHz

F2 - Processing parameters
SI                            32768
SF                   100.6127677 MHz
WDW                            EM
SSB                             0
LB                         1.00 Hz
GB                              0
PC                         1.40

1D NMR plot parameters
CX                        20.00 cm
F1P                      215.000 ppm
F1                     21631.75 Hz
F2P                       -5.000 ppm
F2                      -503.06 Hz
PPMCM                  11.00000 ppm/cm
HZCM                1106.74048 Hz/cm
```



9

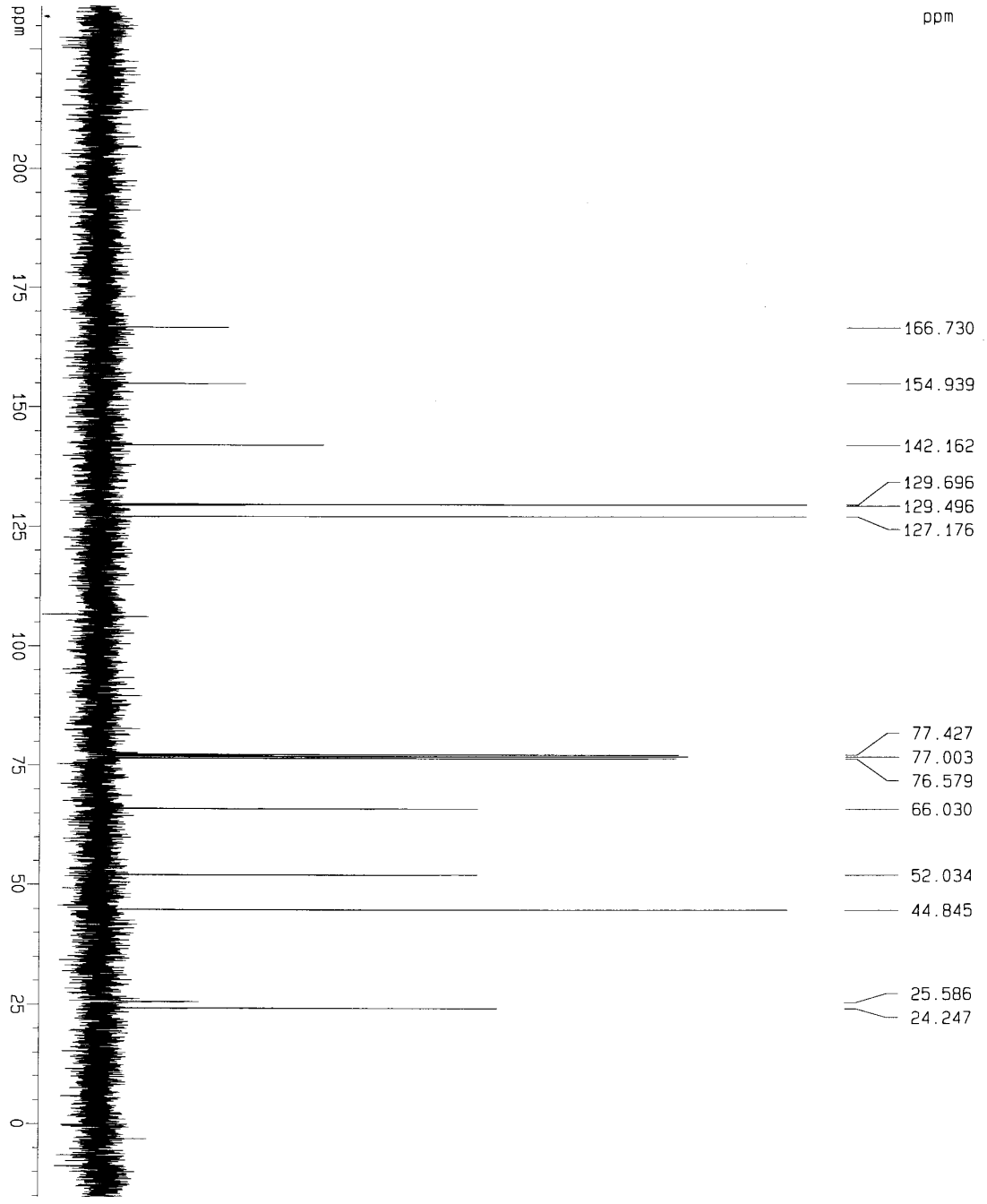



```
Current Data Parameters
NAME                  010512kms5-25
EXPNO                            1
PROCNO                           1

F2 - Acquisition Parameters
Date_                     20120105
Time                         17.05
INSTRUM                      spect
PROBHD     5 mm QNP 1H/1
PULPROG                      zg30
TD                          65536
SOLVENT                     CDCl3
NS                              16
DS                               2
SWH                      6172.839 Hz
FIDRES                   0.094190 Hz
AQ                       5.3084660 sec
RG                           143.7
DW                          81.000 usec
DE                           6.00 usec
TE                          300.0 K
D1                       1.00000000 sec
======== CHANNEL f1 ========
NUC1                           1H
P1                          12.10 usec
PL1                          0.00 dB
SFO1                 299.8718518 MHz

F2 - Processing parameters
SI                           32768
SF                   299.8700159 MHz
WDW                             no
SSB                              0
LB                            0.00 Hz
GB                               0
PC                            1.00

1D NMR plot parameters
CX                           20.00 cm
F1P                         16.415 ppm
F1                        4922.35 Hz
F2P                         -4.170 ppm
F2                        -1250.49 Hz
PPMCM                  1.02925 ppm/cm
HZCM                   308.64197 Hz/cm
```




```
Current Data Parameters
NAME                  010512kms5-25c
EXPNO                              1
PROCNO                             1

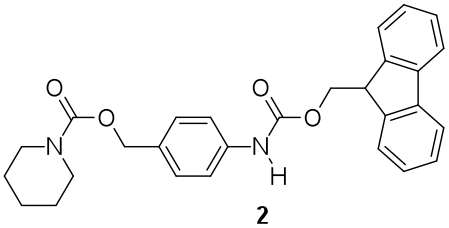
F2 - Acquisition Parameters
Date_                       20120105
Time                           17.19
INSTRUM                         spect
PROBHD     5 mm QNP 1H/1
PULPROG                      zgpg30
TD                            65536
SOLVENT                       CDCl3
NS                              300
DS                                4
SWH                       18796.992 Hz
FIDRES                     0.286819 Hz
AQ                         1.7433076 sec
RG                            14596.5
DW                           26.600 usec
DE                            6.00 usec
TE                           300.0 K
D1                        0.50000000 sec
D11                       0.03000000 sec
D12                       0.00002000 sec

======== CHANNEL f1 ========
NUC1                            13C
P1                            5.25 usec
PL1                          -6.00 dB
SFO1                  75.4106357 MHz

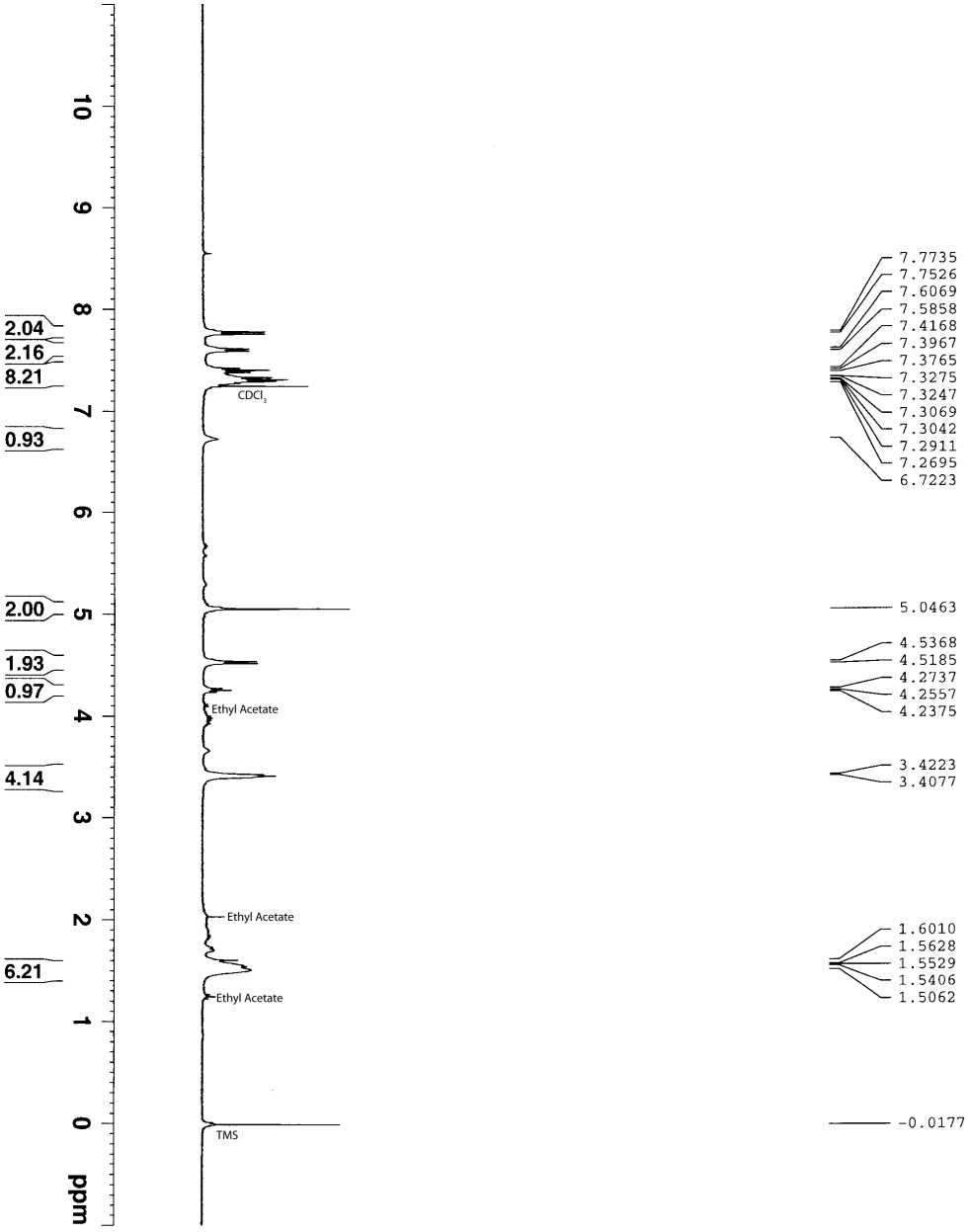
======== CHANNEL f2 ========
CPDPRG2                     waltz16
NUC2                            1H
PCPD2                       115.00 usec
PL2                           0.00 dB
PL12                         19.70 dB
PL13                         19.70 dB
SFO2                  299.8711995 MHz

F2 - Processing parameters
SI                             32768
SF                    75.4023786 MHz
WDW                               EM
SSB                                0
LB                              1.00 Hz
GB                                 0
PC                              1.40

1D NMR plot parameters
CX                            20.00 cm
F1P                          234.152 ppm
F1                         17655.64 Hz
F2P                          -15.137 ppm
F2                         -1141.35 Hz
PPMCM                      12.46446 ppm/cm
HZCM                    939.84967 Hz/cm
```



2

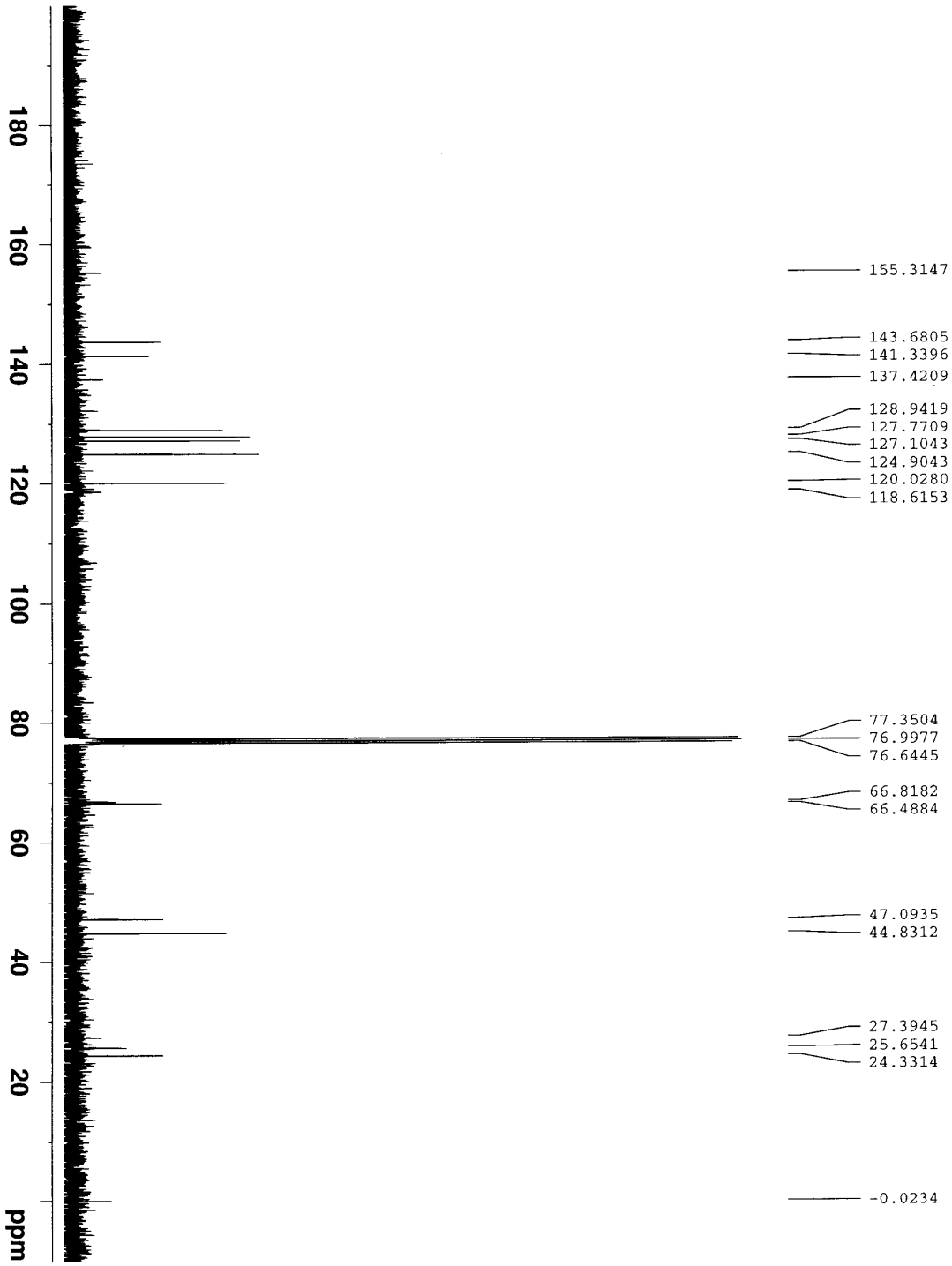


Current Data Parameters
NAME
EXPNO
PROCNO

F2 - Acquisition Parameters
Date_
Time
INSTRUM
PROBHD
PULPROG
TD
SOLVENT
NS
DS
SWH
FIDRES
AQ
RG
DW
DE
TE
D1
TD0

======== CHANNEL f1 ========
NUC1
P1
PL1
SFO1

F2 - Processing parameters
SI
SF
WDW
SSB
LB
GB
PC



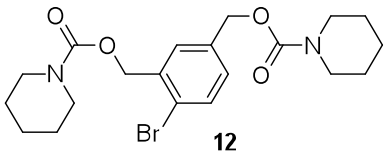

```
Current Data Parameters
NAME                  011112kms5-30c13
EXPNO                              1
PROCNO                             1

F2 - Acquisition Parameters
Date_                       20120111
Time                           21.49
INSTRUM                         spect
PROBHD     5 mm QNP 1H/15
PULPROG                     zgpg30
TD                               65536
SOLVENT                      CDCl3
NS                                 425
DS                                   4
SWH                          21645.021 Hz
FIDRES                        0.330277 Hz
AQ                            1.5139316 sec
RG                                13004
DW                            23.100 usec
DE                             6.00 usec
TE                            297.4 K
D1                            0.50000000 sec
D11                           0.03000000 sec
DELTA                         0.40000001 sec
TD0                                   1

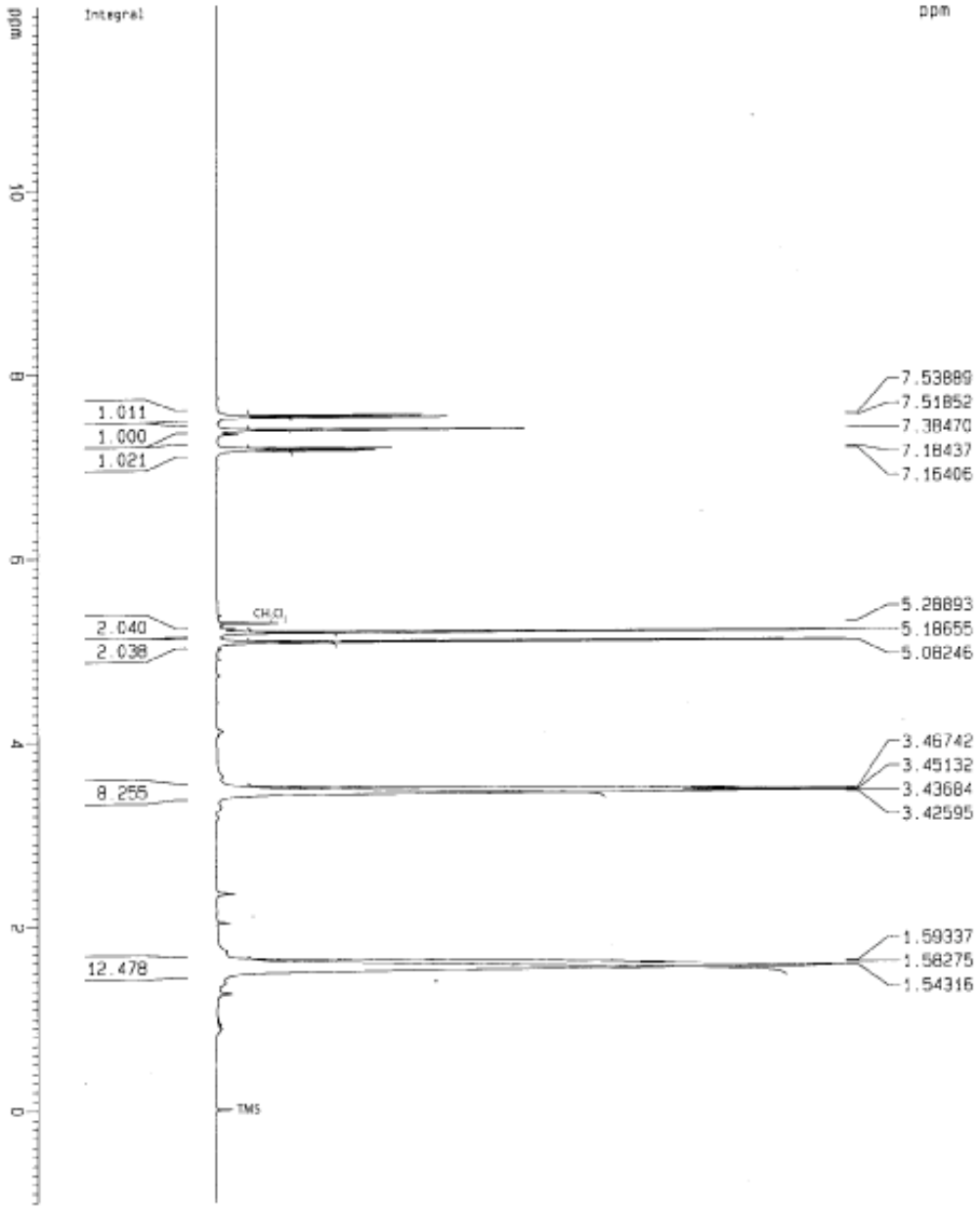
========== CHANNEL f1 ==========
NUC1                              13C
P1                              6.50 usec
PL1                            -3.00 dB
SFO1                    90.5638160 MHz

========== CHANNEL f2 ==========
CPDPRG2                       waltz16
NUC2                              1H
PCPD2                         110.00 usec
PL2                            -1.00 dB
PL12                           15.00 dB
PL13                           16.10 dB
SFO2                    360.1314405 MHz

F2 - Processing parameters
SI                                32768
SF                      90.5547658 MHz
WDW                                EM
SSB                                 0
LB                              1.00 Hz
GB                                  0
PC                              1.40
```



12



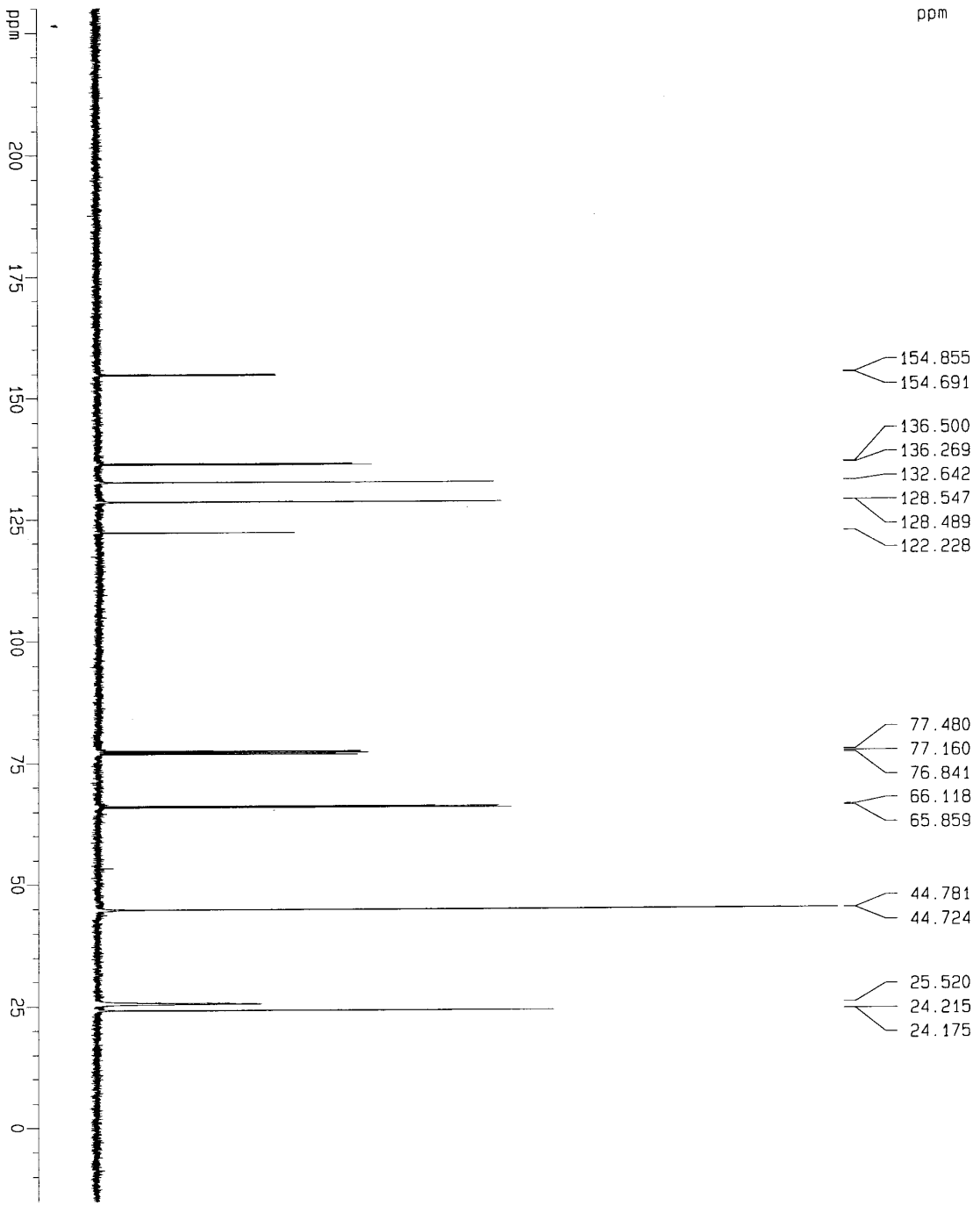
Current Data Parameters
NAME 1-10-12ma105a
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20120110
Time 21.09
INSTRUM spect
PROBHD 5 mm BBI 1H-
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8278.146 Hz
FIDRES 0.126314 Hz
AQ 3.9584243 sec
RG 14.3
DW 60.400 usec
DE 6.00 usec
TE 300.0 K
D1 1.00000000 sec

======== CHANNEL f1 ========
NUC1 1H
P1 6.45 usec
PL1 0.00 dB
SF01 400.1324710 MHz

F2 - Processing parameters
SI 32768
SF 400.1300763 MHz
WDW no
SSB 0
LB 0.00 Hz
GB 0
PC 1.00

1D NMR plot parameters
CX 20.00 cm
F1P 12.000 ppm
F1 4801.56 Hz
F2P -1.000 ppm
F2 -400.13 Hz
PPMCM 0.65000 ppm/cm
HZCM 260.08447 Hz/cm




```
Current Data Parameters
NAME                  1-10-12hm185a
EXPNO                            1
PROCNO                           1

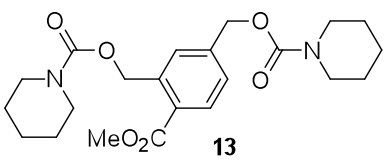
F2 - Acquisition Parameters
Date_                     20120110
Time                         21.42
INSTRUM                      spect
PROBHD     5 mm BBI 1H-
PULPROG                   zpg30
TD                        65536
SOLVENT                   CDCl3
NS                              512
DS                                4
SWH                       25125.629 Hz
FIDRES                    0.383387 Hz
AQ                        1.3042164 sec
RG                        16384
DW                        19.900 usec
DE                            6.00 usec
TE                            300.0 K
D1                        2.00000000 sec
d11                       0.03000000 sec
d12                       0.00002000 sec

============ CHANNEL f1 ============
NUC1                          13C
P1                          16.35 usec
PL1                         -6.00 dB
SF01                  100.6237959 MHz

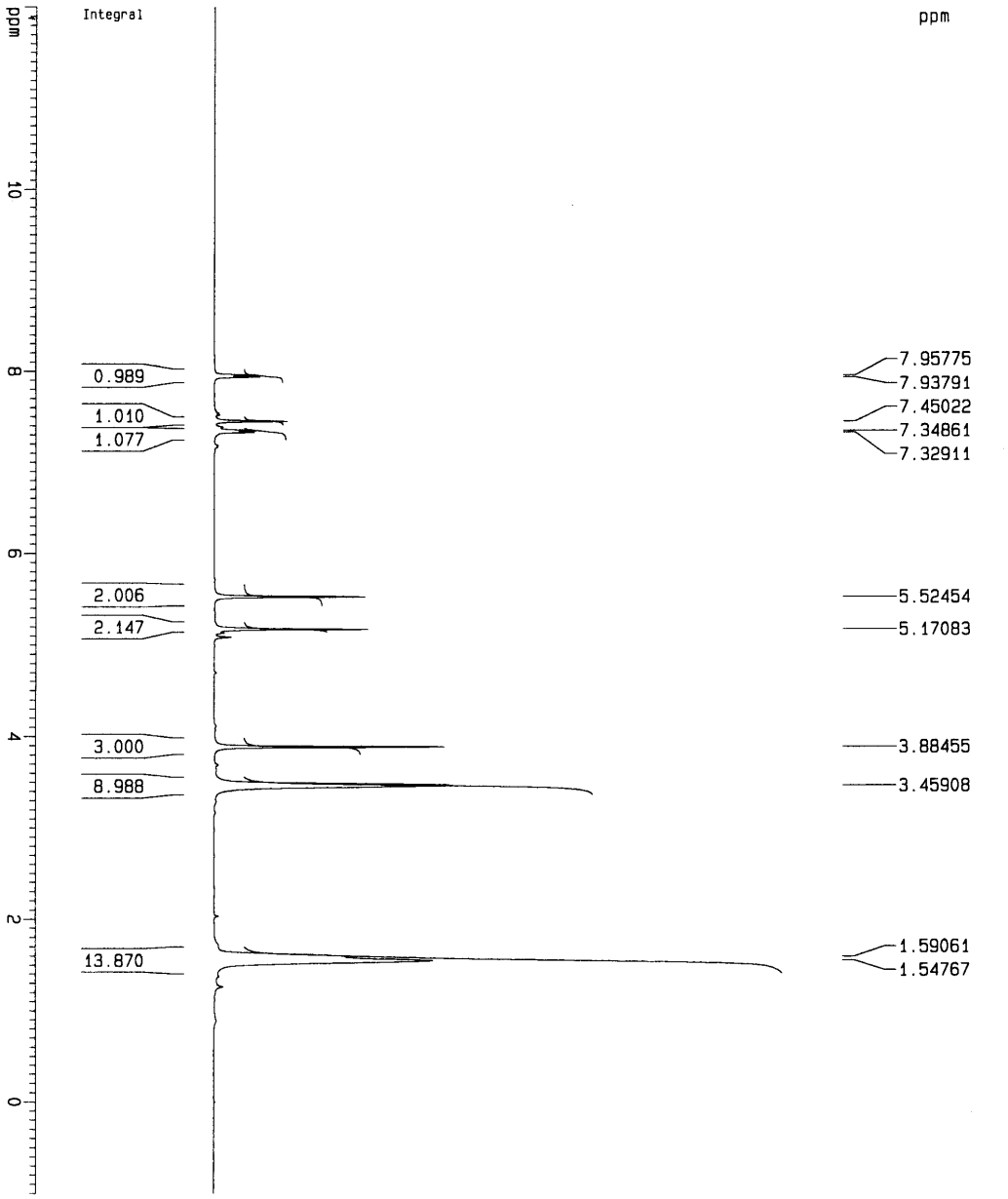
============ CHANNEL f2 ============
CPDPRG2                   waltz16
NUC2                          1H
PCPD2                      114.00 usec
PL2                          0.00 dB
PL12                        24.00 dB
PL13                        24.00 dB
SFO2                  400.1316005 MHz

F2 - Processing parameters
SI                            32768
SF                    100.6127807 MHz
WDW                              EM
SSB                               0
LB                             1.00 Hz
GB                                0
PC                             1.40

1D NMR plot parameters
CX                           20.00 cm
F1P                         230.000 ppm
F1                        23140.94 Hz
F2P                        -15.000 ppm
F2                        -1509.19 Hz
PPMCM                      12.25000 ppm/cm
HZCM                  1232.50647 Hz/cm
```



13



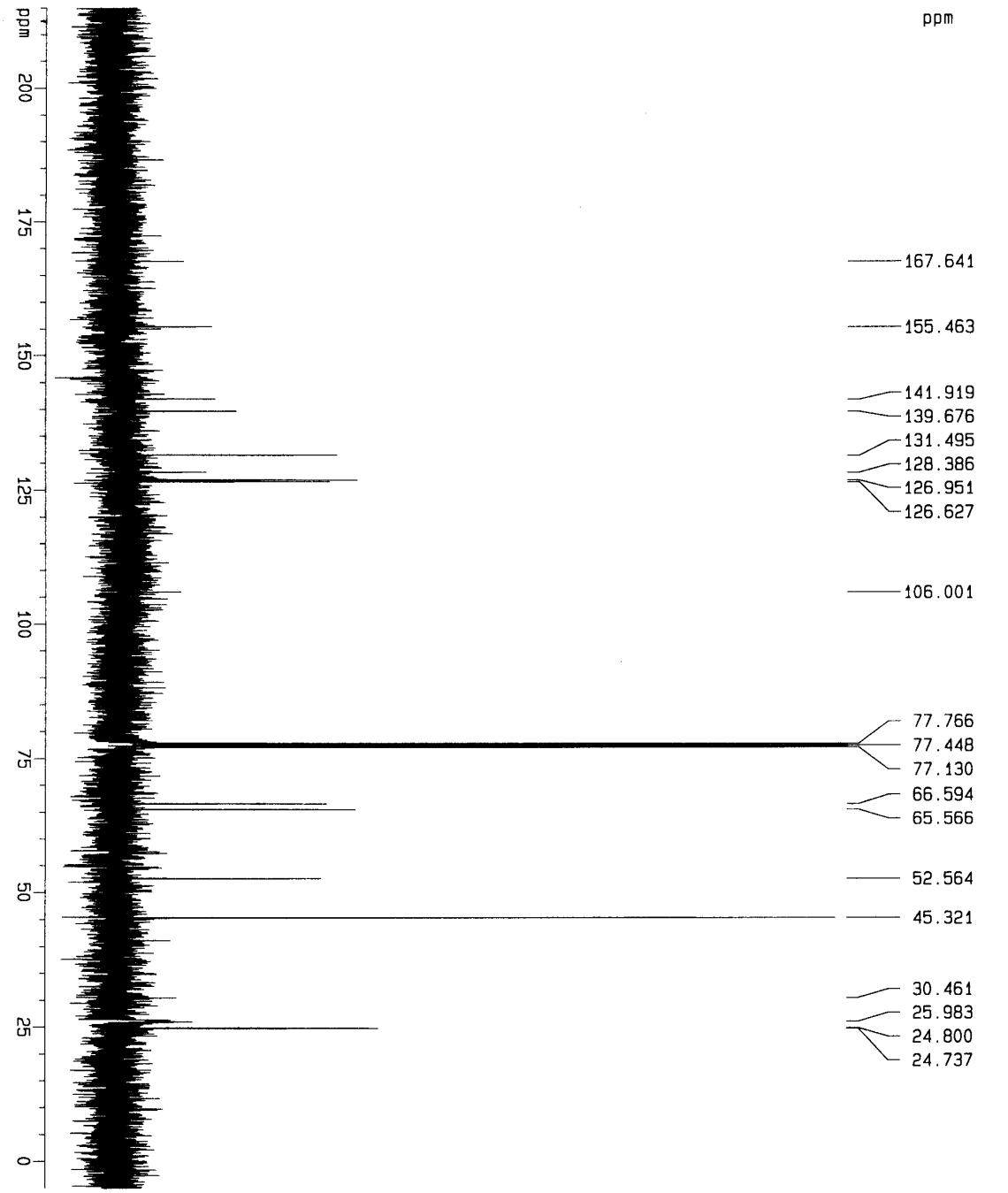

```
Current Data Parameters
NAME                  1-17-12hmester
EXPNO                             1
PROCNO                            1

F2 - Acquisition Parameters
Date_                      20120117
Time                           0.40
INSTRUM                       spect
PROBHD     5 mm BBI 1H-
PULPROG                       zg30
TD                           65536
SOLVENT                      CDCl3
NS                               8
DS                               2
SWH                     8278.146 Hz
FIDRES                  0.126314 Hz
AQ                      3.9584243 sec
RG                            10.1
DW                          60.400 usec
DE                           6.00 usec
TE                          300.0 K
D1                      1.00000000 sec

======== CHANNEL f1 ========
NUC1                           1H
P1                           6.45 usec
PL1                          0.00 dB
SFO1                400.1324710 MHz

F2 - Processing parameters
SI                           32768
SF                  400.1299639 MHz
WDW                             no
SSB                              0
LB                        0.00 Hz
GB                               0
PC                            1.00

1D NMR plot parameters
CX                          20.00 cm
F1P                        12.000 ppm
F1                       4801.56 Hz
F2P                       -1.000 ppm
F2                        -400.13 Hz
PPMCM                  0.65000 ppm/cm
HZCM                260.08447 Hz/cm
```




```
Current Data Parameters
NAME                  1-18-12hm13
EXPNO                           1
PROCNO                          1

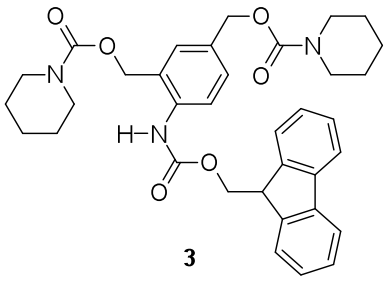
F2 - Acquisition Parameters
Date_                    20120118
Time                         10.24
INSTRUM                      spect
PROBHD     5 mm BBI 1H-
PULPROG                   zgpg30
TD                         65536
SOLVENT                    CDCl3
NS                           696
DS                             4
SWH                    25125.629 Hz
FIDRES                     0.383387 Hz
AQ                         1.3042164 sec
RG                           16384
DW                        19.900 usec
DE                         6.00 usec
TE                           300.0 K
D1                     0.50000000 sec
d11                    0.03000000 sec
d12                    0.00002000 sec

======== CHANNEL f1 ========
NUC1                          13C
P1                          16.35 usec
PL1                         -6.00 dB
SFO1                  100.6237959 MHz

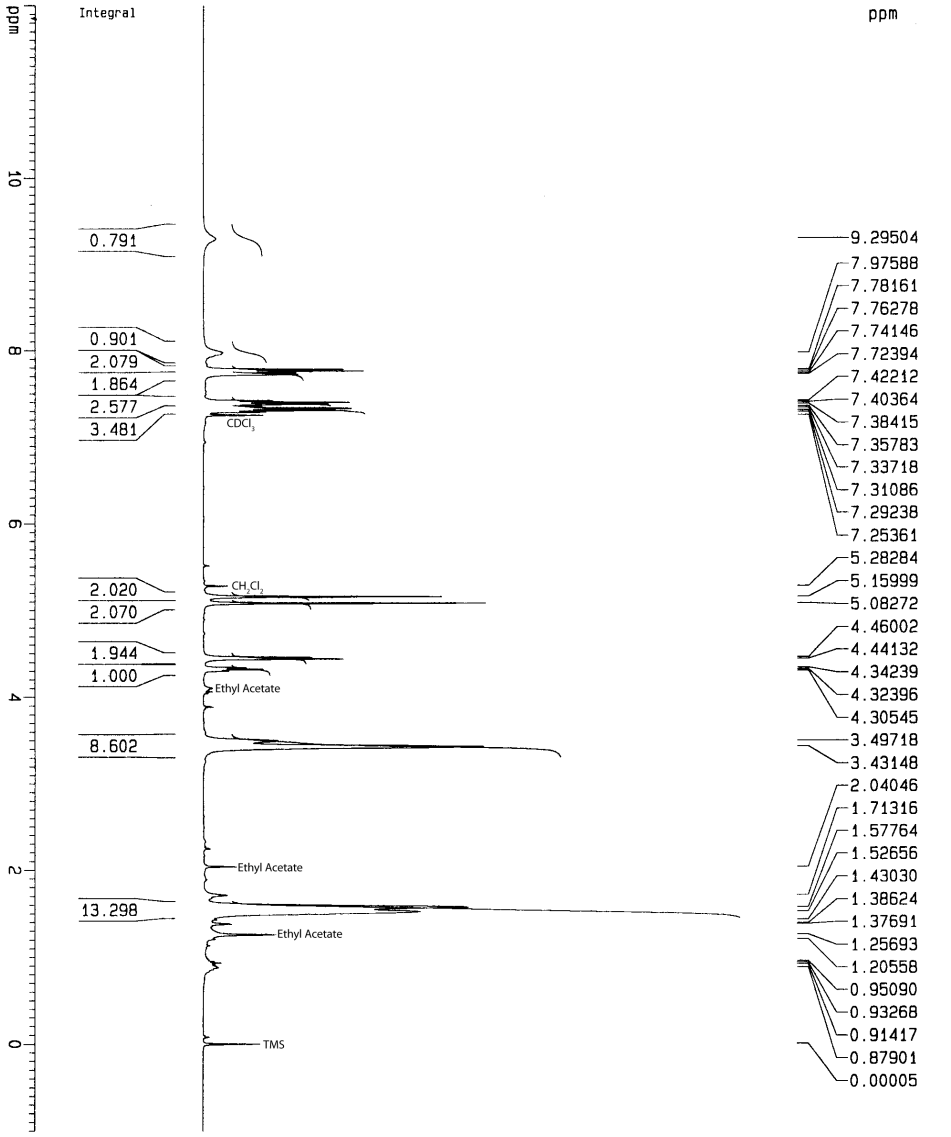
======== CHANNEL f2 ========
CPDPRG2                   waltz16
NUC2                           1H
PCPD2                     114.00 usec
PL2                         0.00 dB
PL12                       24.00 dB
PL13                       24.00 dB
SFO2                  400.1316005 MHz

F2 - Processing parameters
SI                            32768
SF                    100.6127290 MHz
WDW                              EM
SSB                               0
LB                             1.00 Hz
GB                                0
PC                             1.40

1D NMR plot parameters
CX                           20.00 cm
F1P                         215.000 ppm
F1                        21631.74 Hz
F2P                        -5.000 ppm
F2                         -503.06 Hz
PPMCM                      11.00000 ppm/cm
HZCM                  1106.73999 Hz/cm
```



3



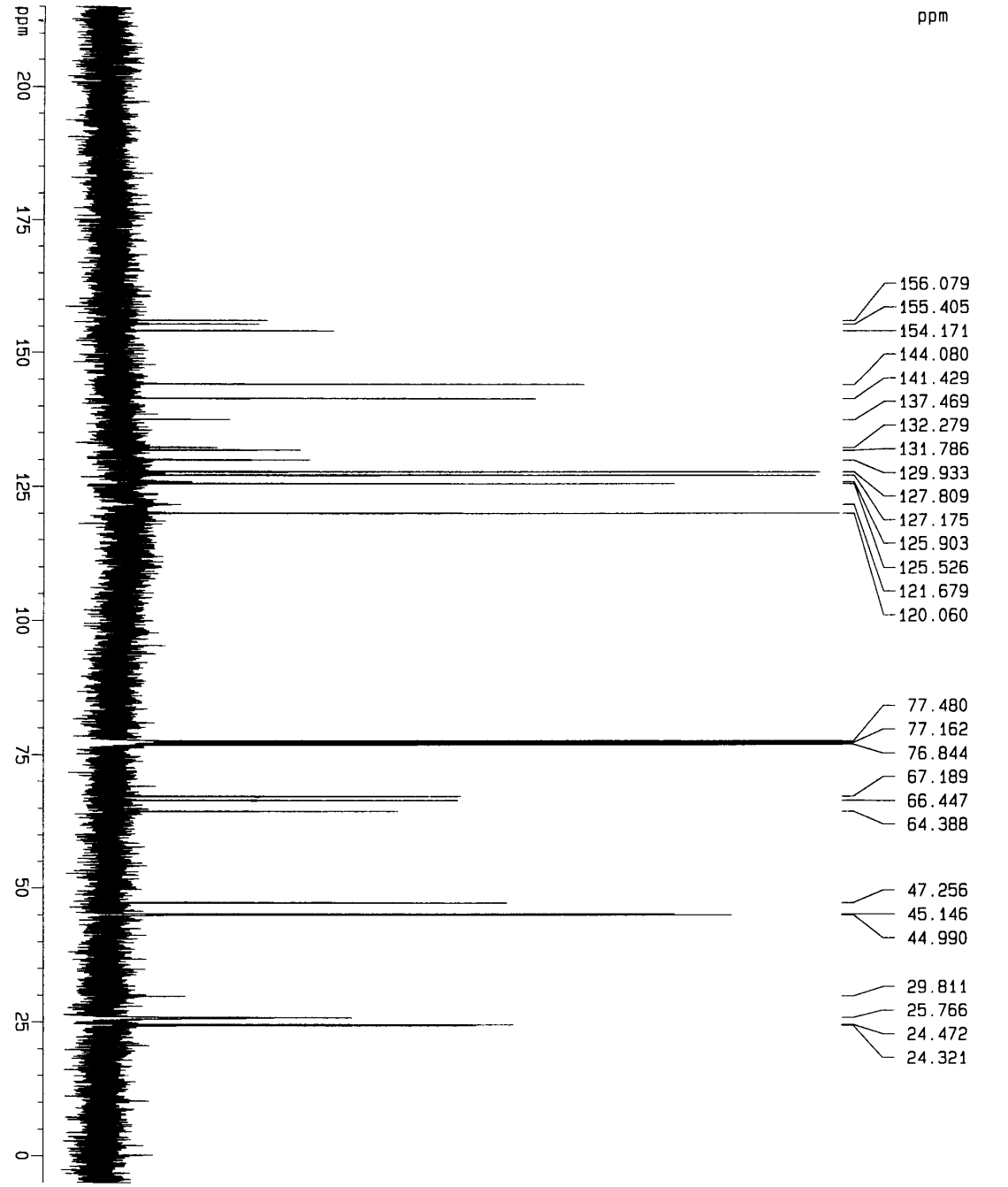

```
Current Data Parameters
NAME                  1-16-12hm199
EXPNO                            2
PROCNO                           1

F2 - Acquisition Parameters
Date_              20120116
Time                   23.23
INSTRUM                spect
PROBHD     5 mm BBI 1H-
PULPROG                zg30
TD                    65536
SOLVENT               CDCl3
NS                       16
DS                        2
SWH                827.146 Hz
FIDRES             0.126314 Hz
AQ                 3.9584243 sec
RG                      40.3
DW                 60.400 usec
DE                  6.00 usec
TE                  300.0 K
D1                 1.00000000 sec

======== CHANNEL f1 ========
NUC1                      1H
P1                     6.45 usec
PL1                    0.00 dB
SFO1           400.1324710 MHz

F2 - Processing parameters
SI                       32768
SF             400.1300114 MHz
WDW                         no
SSB                          0
LB                     0.00 Hz
GB                           0
PC                        1.00

1D NMR plot parameters
CX                    20.00 cm
F1P                 12.000 ppm
F1                 4801.56 Hz
F2P                -1.000 ppm
F2                 -400.13 Hz
PPMCM              0.65000 ppm/cm
HZCM               260.08450 Hz/cm
```




```
Current Data Parameters
NAME                  1-16-12hm199
EXPNO                            3
PROCNO                           1

F2 - Acquisition Parameters
Date_                     20120117
Time                          0.24
INSTRUM                       spect
PROBHD     5 mm BBI 1H-
PULPROG                    zgpg30
TD                          65536
SOLVENT                     CDCl3
NS                           1024
DS                              4
SWH                     25125.629 Hz
FIDRES                      0.383387 Hz
AQ                        1.3042164 sec
RG                          16384
DW                         19.900 usec
DE                          6.00 usec
TE                         300.0 K
D1                      2.00000000 sec
d11                     0.03000000 sec
d12                     0.00002000 sec

====== CHANNEL f1 ======
NUC1                          13C
P1                          16.35 usec
PL1                         -6.00 dB
SFO1                  100.6237959 MHz

====== CHANNEL f2 ======
CPDPRG2                     waltz16
NUC2                            1H
PCPD2                      114.00 usec
PL2                         0.00 dB
PL12                       24.00 dB
PL13                       24.00 dB
SFO2                  400.1316005 MHz

F2 - Processing parameters
SI                            32768
SF                    100.6127593 MHz
WDW                              EM
SSB                               0
LB                             1.00 Hz
GB                                0
PC                             1.40

1D NMR plot parameters
CX                            20.00 cm
F1P                          215.000 ppm
F1                         21631.75 Hz
F2P                         -5.000 ppm
F2                         -503.06 Hz
PPMCM                      11.00000 ppm/cm
HZCM                   1106.74036 Hz/cm
```