A thermally-stable enzyme detection assay that amplifies signal autonomously in water without assistance from biological reagents

*Kimy Yeung, Kyle M. Schmid, and Scott T. Phillips**
Department of Chemistry,
The Pennsylvania State University,
University Park, PA 16802

*Corresponding author E-mail: sphillips@psu.edu

Supporting Information

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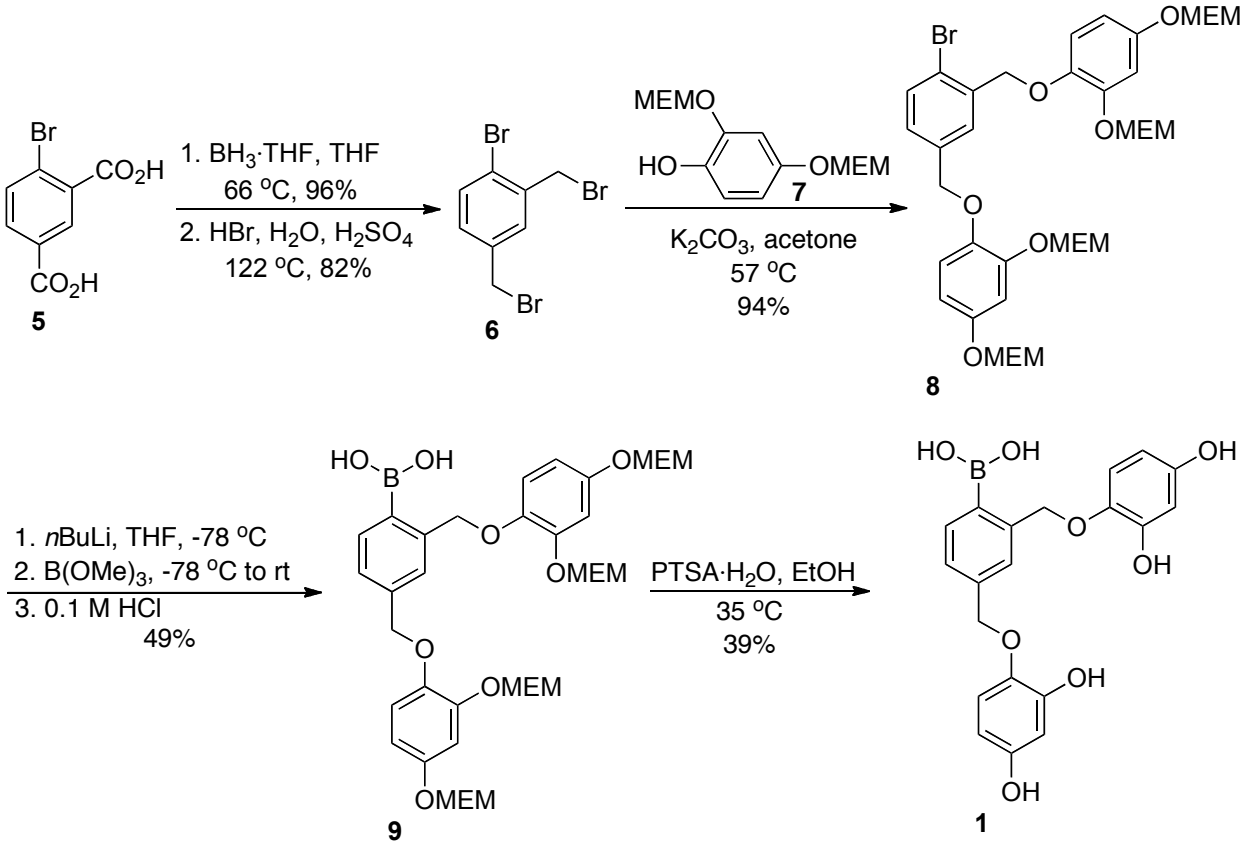
General experimental procedures

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. 4-Bromoisophthalic acid, 2,4-dihydroxybenzaldehyde, 2,4-diallyloxybenzaldehyde 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 Adsorbents). Thin layer chromatography was carried out on Dynamic Adsorbants silica gel TLC (20Å~20 cm w/h, F-254, 250 μm). Deionized water was purified using a millipore-purification system (Barnstead EASYpure® II UV/UF). Kinetics experiments were carried out in 1.5 mL plastic cuvettes.

Instrumentation

Photographs were acquired using a Nikon digital camera (D40, D3100) and analyzed using Adobe®Photoshop®. Proton nuclear magnetic resonance (^{1}H-NMR) spectra and carbon nuclear magnetic resonance spectra (^{13}C-NMR) were recorded using a Bruker DRX-400 (400 MHz), Bruker CDPX-300 (300 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 chloroform ($CDCl_3$, 7.26 ppm), methanol (CD_3OD, 3.31 ppm), acetonitrile (CD_3CN, 1.94 ppm), or acetone ($(CD_3)_2CO$, 2.05 ppm). Data are represented as follows: 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), integration, and coupling constant (*J*) in Hertz. Carbon chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to chloroform ($CDCl_3$, 77.16 ppm), methanol (CD_3OD, 49.0 ppm), acetonitrile (CD_3CN, 1.32 ppm), or acetone ($(CD_3)_2CO$, 29.84 ppm).

Synthesis of signal amplification reagent 1



1-Bromo-2,4-bis(bromomethyl)-benzene (6):

4-Bromoisophthalic acid **5** (3.12 g, 12.7 mmol, 1.0 equiv) was dissolved in dry tetrahydrofuran (32 mL), and the solution was cooled to 0 °C. Borane-tetrahydrofuran complex (1.0 M solution in tetrahydrofuran) was added dropwise slowly to the cooled solution, and the mixture was stirred at 0 °C for 15 min. The solution was warmed to room temperature and refluxed at 66 °C for 16.5 h. The reaction mixture was cooled to room temperature and quenched by slow addition of saturated ammonium chloride. The aqueous layer was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with saturated ammonium chloride (2 × 50 mL) and saturated sodium bicarbonate (2 × 50 mL). The organic layers were dried over sodium sulfate. The sodium sulfate was removed by filtration and the resulting solution was concentrated under reduced pressure, affording a white solid. The white solid was dried under reduced pressure and was without further purification.

The white solid (1.0 g, 4.6 mmol, 1.0 equiv) was dissolved in aqueous hydrobromic acid (48%) (5.1 mL, 46.1 mmol, 10.0 equiv). Concentrated sulfuric acid (123 µL, 2.3 mmol, 0.5 equiv) was added dropwise. The reaction mixture was stirred at 122 °C for 22 h. The solution was cooled to room temperature and diluted with ethyl acetate. The resulting mixture was washed with water (1 × 30 mL), saturated sodium bicarbonate (2 × 30 mL) and brine (1 × 50 mL). The organic layer was dried over sodium sulfate. The sodium sulfate was removed by filtration and the resulting solution was concentrated under reduced pressure. The residue was purified by column chromatography (gradient elution 0 – 5% ethyl acetate/hexanes) to afford compound **6** as a white solid (1.29 g, 3.76 mmol, 82%). ^{1}H-NMR (360 MHz, $CDCl_3$): δ 7.55 (d, 1 H, J = 8.17), 7.48 (d, 1 H, J = 2.27), 7.20 (dd, 1 H, J = 8.25, 2.3), 4.57 (s, 2 H), 4.42 (s, 2 H). This ^{1}H-NMR data matches previously published data for **6**.[2]

4,4'-(((4-Bromo-1,3-phenylene)bis(methylene))bis(oxy))bis(1,3-bis((2-methoxyethoxy)methoxy)benzene) (8):

An oven-dried two-necked round bottom flask equipped with a stir bar was charged with K_2CO_3 (237 mg, 1.72 mmol, 2.2 equiv), compound **6** (268 mg, 0.78 mmol, 1.0 equiv) and compound **7**[3] (495 mg, 1.64 mmol, 2.1 equiv) in acetone (8 mL). The reaction mixture was heated to reflux at 57 ºC for 24 h. 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 50 – 100% ethyl acetate/hexanes) to obtain compound **8** as a yellow oil (588 mg, 0.75 mmol, 96%). IR (cm^{-1}): 2882.3, 1503.6, 1082, 1000; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.60 (d, 1 H, *J* = 1.96), 7.53 (d, 1 H, *J* = 8.17), 7.25 (dd, 1 H, *J* = 8.16, 2.18), 6.91 (d 1 H, *J* = 2.8), 6.89 (d 1 H, *J* = 2.8), 6.81 (d, 1 H, *J* = 16.68), 6.78 (d, 1 H, *J* = 16.69), 6.65 (dd, 1 H, *J* = 8.85, 2.82), 6.62 (dd, 1 H, *J* = 8.80, 2.76), 5.27 (s, 2 H), 5.24 (s, 2 H), 5.17 (s, 2 H), 5.16 (s, 2 H), 5.09 (s, 2 H), 4.99 (s, 2 H), 3.86 – 3.77 (m, 8 H), 3.55 – 3.49 (8 H), 3.35 (s, 3 H), 3.34 (s, 3 H), 3.33 (s, 6 H); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 152.4, 152.2, 147.8, 147.8, 143.9, 137.0, 136.7, 132.5, 128.0, 127.7, 121.1, 116.0, 115.9, 109.5, 109.3, 107.3, 94.6, 94.6, 94.0, 71.5, 71.4,71.3, 71.2, 67.8, 67.8, 67.4, 58.8; MS (TOF MS ES+, *m/z*): 802.2 (+18, NH_4^+); HRMS (TOF MS ES+, *m/z*): calcd for $C_{36}H_{49}O_{14}Br$ (M + 1) 784, found 802.2659 (+18, NH_4^+).

(2,4-Bis((2,4-bis((2-methoxyethoxy)methoxy)phenoxy)methyl)phenyl)boronic acid (9):

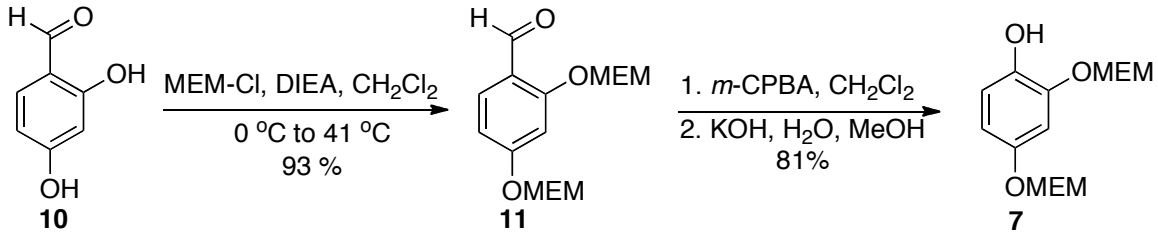
Compound **8** (500 mg, 0.64 mmol, 1.0 equiv) was dissolved in dry tetrahydrofuran (6.4 mL) and the solution was cooled to –78 °C. *n*-Butyl lithium (2.5 M solution in hexanes, 305 μL, 0.76 mmol, 1.2 equiv) was added slowly to the stirring solution, and the reaction mixture was stirred at –78 °C for 1 h. Trimethyl borate (357 μL, 3.2 mmol, 5.0 equiv) was added at –78 °C with continued stirring at –78 °C for 2.5 h. The solution was warmed to room temperature and was stirred at room temperature for 7 h. To this mixture was added 0.1 M HCl (6 mL) and the reaction mixture was stirred for 14 h. The solution was concentrated under reduced pressure. The residue was dissolved in ethyl acetate and the resulting solution was washed with deionized water (1 × 10 mL). The organic layer was collected and the aqueous layer was extracted with ethyl acetate (2 × 10 mL). The combined organic layers were dried over sodium sulfate. The sodium sulfate was removed by filtration and the resulting solution was concentrated under reduced pressure. The residue was purified by column chromatography (gradient elution with 90 – 100% ethyl acetate/hexanes to 8% methanol/ethyl acetate) to obtain compound **9** as a light yellow oil (236 mg, 0.31 mmol, 49%). IR (cm^{-1}): 3420.9, 2883.9, 1504.1, 1082.2, 1000; ^{1}H-NMR (300 MHz, $(CD_3)_2CO$): δ 7.80 (d, 1 H, *J* = 7.58), 7.61 (br s, 1 H), 7.43 (dd, 1 H, *J* = 7.6, 1.36), 7.39 (s, 2 H), 7.07 (d, 1 H, *J* = 8.87), 6.95 (d, 1 H, *J* = 8.89), 6.91 (dd, 2 H, *J* = 2.83, 0.92), 6.69 (dd, 1 H, *J* = 6.3, 2.83), 6.66 (dd, 1 H, *J* = 6.08, 2.63), 5.28 (s, 2 H), 5.25 (s, 2 H), 5.22 (s, 2 H), 5.18 (s, 2 H), 5.17 (s, 2 H), 5.04 (s, 2 H), 3.83 – 3.75 (m, 8 H), 3.52 – 3.46 (m, 8 H), 3.28 (s,

6 H), 3.26 (s, 3 H), 3.25 (s, 3 H); ^{13}C-NMR (300 MHz, $(CD_3)_2CO$): δ 153.3, 153.1, 148.9, 148.8, 142.5, 140.1, 135.8, 128.6, 127.2, 117.0, 116.7, 110.4, 110.3, 108.7, 108.0, 95.4, 95.3, 94.8, 73.3, 72.3, 72.3, 72.1, 68.6, 68.6, 68.3, 58.8; MS (TOF MS APCI-, *m/z*): 749.0, MS (TOF MS APCI+, *m/z*): 768.1 (+18, NH_4^+); HRMS (TOF MS ES+) Calculated for $C_{36}H_{55}BNO_{16}$ (M + NH_4^+) 768.3614; Found: 768.3635.

(2,4-Bis((2,4-dihydroxyphenoxy)methyl)phenyl)boronic acid (1):

Compound **9** (81 mg, 0.19 mmol, 1 equiv) was dissolved in ethanol (200 proof, 2 mL) and the solution was stirred at room temperature. *p*-Toluenesulfonic acid monohydrate (78 mg, 0.41 mmol, 2.2 equiv) was added to the stirred solution, and the reaction mixture was stirred at 35 °C for 24 h. The solution was cooled to room temperature and the solvent was removed under reduced pressure. The residue was first purified by column chromatography (90 –100% ethyl acetate/hexanes) to give a light brown oil residue. This residue was further purified using preparative HPLC to obtain **1** as an off-white solid (30 mg, 75 μmol, 39%). IR (cm^{-1}): 3356.2; ^{1}H-NMR (400 MHz, CD_3OD): δ 7.48 (s, 1 H), 7.41 (t, 1 H, *J* = 10.98), 7.38 (d, 1 H, *J* = 20.15), 6.79 (d, 1 H, *J* = 18.52), 6.77 (d, 1 H, *J* = 18.53), 6.34 (s, 2 H), 6.20 (d, 1 H, *J* = 10.12), 6.17 (d, 1 H, *J* = 9.72), 5.07 (s, 2 H), 5.05 (s, 2 H). ^{13}C-NMR (400 MHz, CD_3OD): 153.8, 153.6, 149.3, 149.2, 142.3, 141.3, 141.0, 139.7, 132.6, 127.6, 127.4, 117.4, 117.2, 106.7, 106.6, 104.7, 73.6, 73.1; MS (TOF MS ES+, *m/z*): 399.1; MS (TOF MS ES-, *m/z*): 397.0; HRMS (TOF MS ES-, *m/z*): calcd for $C_{20}H_{18}O_8B$ (M – 1) 397.1095, found 397.1105.

Synthesis of 2,4 –Dimethoxyethoxymethyloxyphenol (7)



2,4 –Dimethoxyethoxymethyloxybenzaldehyde (11):

2,4-Dihydroxybenzaldehyde **10** (2.0 g, 14.5 mmol, 1.0 equiv) was suspended in dry dichloromethane (29 mL), and the suspension was cooled to 0 ºC with stirring. *N*, *N*-

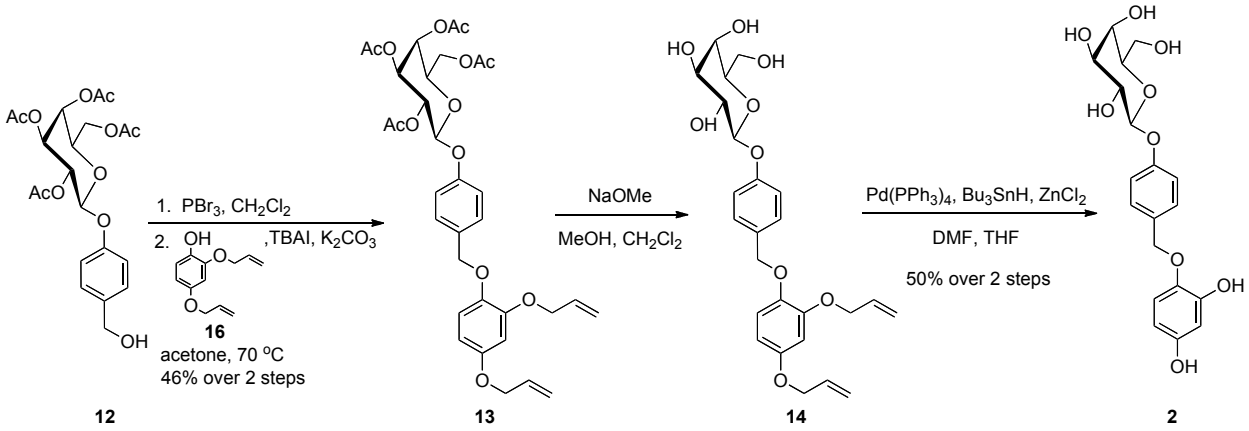
diisopropylethylamine (25 mL, 144.8 mmol, 10.0 equiv) was added to the cooled suspension and the solution was stirred for 10 min at 0 ºC. Methoxyethoxymethyl chloride (9.9 mL, 87 mmol, 6.0 equiv) was added slowly to the cooled solution. The reaction mixture was stirred at 41 ºC for 24 h. The solution was cooled to room temperature and the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate, and the resulting solution was washed with water (3 × 50 mL) and brine (2 × 50 mL). The organic layer was dried over sodium sulfate. The sodium sulfate was removed by filtration and the resulting solution was concentrated under reduced pressure. The residue was purified by column chromatography (gradient elution with 20 – 50% ethyl acetate/benzene) to obtain compound **11**[3] as a yellow oil (4.22 g, 13.4 mmol, 93%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 10.18 (s, 1 H), 7.65 (d, 2 H, J = 8.7), 6.75 (d, 1 H, J = 2.18), 6.64 (dd, 1 H, J = 8.69, 1.85), 5.24 (s, 2 H), 5.18 (s, 2 H), 3.74 – 3.71 (m, 2 H), 3.70 – 3.67 (m, 2 H), 3.45 – 3.40 (m, 4 H), 3.23 (s, 3 H), 3.22 (s, 3 H). This ^{1}H-NMR data matches previously published data for **11**.[3]

2,4 –Dimethoxyethoxymethyloxyphenol (7):

Compound **11** (1.84 g, 5.5 mmol, 1.0 equiv) was dissolved in dry dichloromethane (15 mL), and the solution was stirred at room temperature. meta-Chloroperoxybenzoic acid (1.51 g, 8.74 mmol, 1.5 equiv) was added to the stirring solution. The reaction mixture was stirred at room temperature for 1.5 h. The solution was diluted with dichloromethane and the resulting solution was washed with 1:1 solution of saturated sodium thiosulfate and saturated sodium bicarbonate (30 mL). The organic layer was removed, and the aqueous layer was extracted using dichloromethane (3 × 20 mL). The combined organic layers were washed with saturated sodium bicarbonate (2 × 30 mL) and then dried over sodium sulfate. The sodium sulfate was removed by filtration, and the resulting solution was concentrated under reduced pressure. The residue was dissolved in dry methanol (15 mL). A solution of potassium hydroxide (66 mg, 1.17 mmol, 0.2 equiv) in water (375 µL) was added dropwise to the solution. The reaction mixture was stirred at room temperature for 3 h, after which the solvent was removed under reduced pressure. The residue was dissolved in water and the solution was carefully neutralized with 1 M HCl. The solution was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were dried over sodium sulfate. The sodium sulfate was removed by filtration and the resulting solution was concentrated under reduced pressure. The residue was purified by column

chromatography (gradient elution with 25 – 50% ethyl acetate/benzene) to obtain compound **7**[3] as a light yellow oil (1.43 g, 4.73 mmol, 81%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.82 (d, 1 H, J = 6.27), 6.79 (s, 1 H), 6.66 (dd, 1 H, J = 8.66, 2.92), 6.11 (br s, 1 H), 5.21 (s, 2 H), 5.14 (s, 2 H), 3.85 – 3.82 (m, 2 H), 3.80 – 3.77 (m, 2 H), 3.58 – 3.52 (m, 4 H), 3.38 (s, 3 H), 3.35 (s, 3 H). This ^{1}H-NMR data matches previously published data for **7**.[3]

Synthesis of activity-based detection reagent 2



(2R,3S,4S,5R,6S)-2-(Acetoxymethyl)-6-(4-((2,4-bis(allyloxy)phenoxy)methyl)phenoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (13):

Compound **12**[4] (0.57 g, 1.2 mmol, 1.0 equiv) was dissolved in dry dichloromethane (10 mL) and cooled to 0 °C. Phosphorous tribromide (1.0 M solution in dichloromethane, 0.62 mL, 0.62 mmol, 0.5 equiv) was added dropwise and the solution was stirred at 0 °C for 1 h. The reaction mixture was diluted with dichloromethane (50 mL), washed with saturated sodium bicarbonate (1 × 50 mL) and washed with brine (1 × 50 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated by rotary evaporation and the residue was dried under reduce pressure, yielding a white solid. The white solid was dried under reduced pressure and used without further purification.

The white solid, compound **16** (0.31 g, 1.5 mmol, 1.2 equiv), potassium carbonate (0.51 g, 3.7 mmol, 3.0 equiv), and tetrabutylammonium iodide (93 mg, 0.25 mmol, 0.2 equiv) were suspended in acetone (2.5 mL). The suspension was heated in a sealed vial to 70 °C for 24 h.

The reaction mixture was filtered and the solvent was removed by rotary evaporation. The residue was dissolved in ethyl acetate (25 mL) and washed with saturated sodium chloride solution (1 × 25 mL). The organic layer was dried over sodium sulfate, filtered, and the solvent was removed by rotary evaporation. The residue was purified by flash column chromatography (gradient elution with 30 – 50% ethyl acetate/hexanes) to afford compound **13** (0.35 g, 0.55 mmol, 46%) as a white solid. IR (cm^{-1}): 2868.1, 1754.4 (b), 1753.5 (b); ^{1}H-NMR (360 MHz, $CDCl_3$): δ 7.35 (d, 2 H, *J* = 8.7), 6.97 (d, 2 H, *J* = 8.6), 6.79 (d, 1 H, *J* = 8.7), 6.53 (d, 1 H, *J* = 2.8), 6.36, (dd, 1 H, *J* = 8.7, 2.8), 6.08–5.96 (m, 2 H), 5.46–5.34 (m, 4 H), 5.27–5.23 (m, 2 H), 5.10 (dd, 1 H, *J* = 10.4, 3.4), 5.02 (d, 1 H, *J* = 8.0), 4.98 (s, 2 H), 4.56–4.54 (m, 2 H), 4.44–4.42 (m, 2 H), 4.23–4.11 (m, 2 H), 4.05 (t, 1 H, *J* = 7.0), 2.16 (s, 3 H), 2.04 (s, 3 H), 2.03 (s, 3 H), 1.99 (s, 3 H); ^{13}C-NMR (360 MHz, $CDCl_3$): δ 170.3, 170.2, 170.1, 169.4, 156.5, 153.9, 150.0, 142.7, 133.4, 133.2, 132.6, 129.0, 117.6, 117.6, 117.5, 116.8, 116.7, 105.0, 103.0, 99.7, 71.9, 71.0, 70.8, 69.7, 69.2, 68.6, 66.8, 61.3, 20.6; MS (TOF MS AP+, *m/z*): 660.2 (M + NH_4^+); HRMS (TOF MS ES+) Calculated for $C_{33}H_{42}NO_{13}$ (M + NH4+): 660.2656; Found: 660.2660.

(2S,3R,4S,5R,6R)-2-(4-((2,4-Bis(allyloxy)phenoxy)methyl)phenoxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol (14):

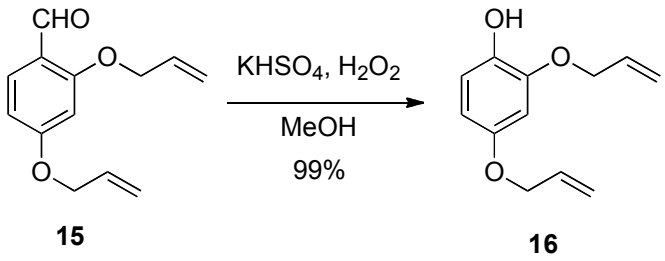
Sodium methoxide (1.2 mg, 22 μmol, 0.04 equiv) was dissolved in dry methanol (100 μL) and was added to a stirring solution of compound **13** (0.35 g, 0.55 mmol, 1.0 equiv) in a mixture of dry dichloromethane (1 mL) and dry methanol (4 mL). The reaction mixture was stirred at 23 °C for 2 h and an additional portion of sodium methoxide (1.2 mg, 22 μmol, 0.04 equiv, in 100 μL of methanol) was added. After 1 h, the solvent was removed by rotary evaporation to afford compound **14** as a white solid. Compound **14** was dried under reduced pressure and was used without further purification.

(2S,3R,4S,5R,6R)-2-(4-((2,4-Dihydroxyphenoxy)methyl)phenoxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol (2):

Compound **14** (0.14 g, 0.30 mmol, 1.0 equiv), tetrakis-(triphenylphosphine)palladium(0) (34 mg, 0.03 mmol, 0.1 equiv), and zinc(II) chloride (0.11 g, 0.8 mmol, 2.7 equiv) were dissolved in a mixture of dry tetrahydrofuran (4 mL) and dry *N,N*-dimethylformamide (1 mL). Tributyltin hydride (0.32 mL, 1.2 mmol, 4 equiv) was added slowly and the reaction mixture was stirred for

15 min. The suspension was then concentrated by rotary evaporation and dried under reduced pressure. The residue was dissolved in acetonitrile (20 mL), filtered through celite (eluting with 1:1 acetonitrile:methanol, 30 mL), and concentrated by rotary evaporation. The residue was purified by preparative HPLC, affording compound **2** (60 mg, 0.15 mmol, 50% over 2 steps) as an off white solid. IR (cm^{-1}): 3325.8, 2922.2, 1608.3, 1510.4; ^{1}H-NMR (360 MHz, CD_3OD): δ 7.36 (d, 2 H, J = 8.6), 7.10 (d, 2 H, J = 8.7), 6.71 (d, 1 H, J = 8.7), 6.31 (d, 1 H, J = 4.4), 6.16, (dd, 1 H, J = 8.6, 2.8), 4.94 (s, 2 H), 4.84 (s, 1 H), 3.89 (d, 1 H, J = 3.2), 3.81–3.67 (m, 4 H), 3.59 (dd, 1 H, J = 9.72, 3.4); ^{13}C-NMR (360 MHz, CD_3OD): δ 158.9, 153.6, 149.3, 141.1, 132.9, 130.4, 117.7, 117.6, 106.6, 104.6, 102.9, 76.9, 74.8, 73.1, 72.3, 70.2, 62.4; MS (TOF MS AP+, m/z): 412.1 ($M + NH_4^+$); HRMS (TOF MS ES-) Calculated for $C_{19}H_{21}O_9$ ($M - H^+$): 393.1186; Found: 393.1180.

Synthesis of 2,4-diallyloxyphenol (16)



15 **16**

2,4-Diallyloxyphenol (16):

2,4-Diallyloxybenzaldehyde **15** (0.87 g, 4.0 mmol, 1.0 equiv) and potassium hydrogen sulfate (81 mg, 5.0 mmol, 1.25 equiv) were dissolved in methanol (8 mL). Hydrogen peroxide solution (35% in water, 0.49 mL, 5 mmol, 1.25 equiv) was added dropwise and the reaction mixture was stirred at 23 °C for 1.5 h. A 1:1 solution of saturated sodium thiosulfate–saturated sodium bicarbonate (1 mL) was added dropwise to the reaction mixture. The mixture was diluted with ethyl acetate (60 mL) and washed with saturated sodium bicarbonate (50 mL) and brine (50 mL). The organic layer was dried over sodium sulfate, filtered and the solvent was removed by rotary evaporation. The residue was dried under reduce pressure, affording compound **16** (0.81 g, 3.96 mmol, 99%) as a white solid. IR (cm^{-1}): 3334.9, 2862.4, 1508.4; ^{1}H-NMR (360 MHz, $CDCl_3$): δ 6.82 (d, 1 H, J = 8.68), 6.51 (d, 1 H, J = 2.74), 6.41 (dd, 1 H, J = 8.68, 2.77), 6.08–5.97 (m, 2 H),

5.41–5.39 (m, 1 H), 5.36–5.35 (m, 1 H), 5.28–5.24 (m, 2 H), 4.55–4.53 (m, 2 H), 4.45–4.43 (m, 2 H); ^{13}C-NMR (360 MHz, $CDCl_3$): δ 152.3, 145.9, 140.1, 133.5, 132.6, 118.3, 117.5, 114.2, 105.9, 101.5, 69.7, 69.5; MS (TOF MS AP+, *m*/*z*) 207.1 ($M + H^+$).

HPLC studies showing consumption of reagent 1 when exposed to H_2O_2

General experimental procedure corresponding to Figure 2a:

A solution of reagent **1** in dry acetonitrile was added to phosphate buffer (0.1 M, pH 7.3). Hydrogen peroxide in buffered water was added to the solution and solution was vortexed for 60 s, and the resulting solution was injected into an analytical reversed-phase HPLC coupled to a mass spectrometer. The column used was an Agilent Eclipse XDB-C-18 column (4.6 × 150 mm, 5 μm). The mobile phase used was a mixture of 0.5 mM ammonium formate in H_2O (A) and CH_3CN (B). The column was equilibrated with 9:1 A-B at a flow rate of 0.4 mL/min. The solvent gradient was as follows:

Time (min)	A (%)	B (%)
0	90	10
18	10	90
23	10	90
25	90	10

For the assay that contained water treated with catalase, the buffered water was prepared as follows: 1.3 mg of catalase (3691 units/mg) was added to phosphate buffer (10 mL, 0.1 M, pH 7.3) and the treated water was stirred gently for 15 min, after which the catalase was removed by filter centrifugation. The pre-treated buffered water was used without further purification. For the assay that contained water with catalase present, the buffered water was prepared using the same procedure, but the catalase was not removed, and the buffered solution was used without further purification.

Tables of HPLC data for Figure 2a:

Table S1. HPLC data for consumption of reagent **1** with 0.5 equiv H_2O_2.

Time (min)	Area of Reagent 1	% Conversion
0	944.9	0
28	178.9	81.1
56	115.7	87.8
84	67.8	92.8
112	41.3	95.6
140	23.8	97.5

Table S2. HPLC data for consumption of reagent **1** with 0.25 equiv H_2O_2.

Time (min)	Area of Reagent 1	% Conversion
0	862.2	0
28	258.6	70.0
56	170	80.3
84	130.3	84.9
112	92	89.3
140	60.2	93.0
168	38.3	95.6
196	25.8	97.0
224	16.6	98.1
252	11.3	98.7
280	6	99.3

Table S3. HPLC data for consumption of reagent **1** with 0.1 equiv H_2O_2.

Time (min)	Area of Reagent 1	% Conversion
0	840.9	0
28	417.2	50.4
56	325.8	61.3
84	250.2	70.2
112	192.9	77.1
140	147.5	82.5
168	106.7	87.3
196	71.1	91.5
224	47.1	94.4
252	31.4	96.3
280	17.9	97.9

Table S4. HPLC data for consumption of reagent **1** with regular buffered water and no H_2O_2.

Time (min)	Area of Reagent 1	% Conversion
5	1000.7	0
33	637.5	36.3
61	510.7	49.0
89	384.8	61.5
117	325.6	67.5
145	268.3	73.2
173	213.9	78.6
201	170.4	83.0
229	134.5	86.6
257	95	90.5
285	66.9	93.3
313	44.7	95.5
341	29.8	97.0
369	18.9	98.1

Table S5. HPLC data for consumption of reagent **1** with catalase-treated buffered water and no H_2O_2.

Time (min)	Area of Reagent 1	% Conversion
5	1195.2	0
33	912.6	23.6
61	764.7	36.0
89	635.6	46.8
117	514.4	57.0
145	432	63.9
173	335.6	71.9
201	243.6	79.6
229	183.4	84.7
257	128.7	89.2
285	90.6	92.4
313	58.4	95.1
341	37.5	96.9
369	23.5	98.0

Table S6. HPLC data for consumption of reagent **1** with buffered water containing catalase and no H_2O_2.

Time (min)	Area of Reagent 1	% Conversion
0	1194.8	0.0
56	1178.7	1.3
82	1137.2	4.8
110	1150.3	3.7
138	1143.8	4.3
166	1142.2	4.4
194	1140.9	4.5
222	1141.7	4.4
250	1135.4	5.0
278	1132.3	5.2
306	1129	5.5
334	1120.9	6.2
362	1126.4	5.7
390	1124	5.9
418	1115.4	6.6
446	1109.6	7.1
474	1112.3	6.9
502	1111.9	6.9
530	1101.9	7.8
558	1104	7.6
586	1107	7.3
614	1099.4	8.0
642	1095.8	8.3
670	1094.2	8.4
698	1082.8	9.4
726	1081.8	9.5
754	1082	9.4
782	1080.4	9.6
810	1078.1	9.8
838	1075	10.0
866	1071.9	10.3
894	1074.2	10.1

Photographic analysis of colorimetric output when reagent 1 is exposed to H_2O_2

General experimental procedure corresponding to Figure S1a:

A hydrogen peroxide solution in phosphate buffered water (pH 7.3; 0.25 mM–2.5 mM, 0.1–1 equivalents relative to amplification reagent **1**) was added to a solution of the amplification reagent **1** in buffered water containing 1.5% DMF (2.5 mM). The colorimetric change of the solution was monitored via photography and image processing software.

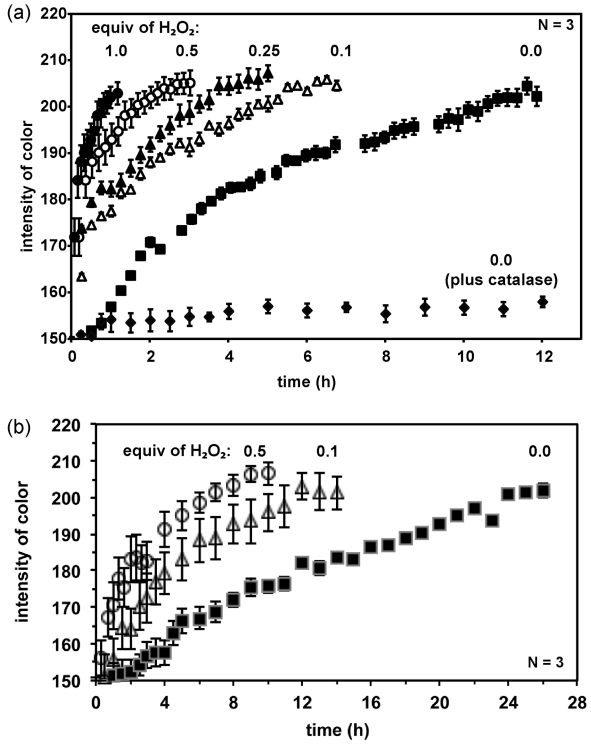


Figure S1. Amplified colorimetric response of **1** when exposed to hydrogen peroxide. (a) Intensity of color of the reaction mixture over time for experiments using 2.5 mM **1** and different initial quantities of hydrogen peroxide. The color of the solution was quantified from photographs using Adobe®Photoshop®. (b) An identical experiment as (a) with the exception that the amplification solution contained 0.5% Tween-20 (v/v), whereas the surfactant was not included in (a). The data points in both graphs are the average of three measurements, and the error bars represent the standard deviations from the averages.

Tables of Color Intensity for Figure S1a:

Table S7. Color intensity data for 1 equiv H_2O_2.

Time (min)	Trial 1	Trial 2	Trial 3
1	148.85	147.16	142.4
5	162.77	160.09	153.72
10	177.43	177.35	170.87
15	184.29	183.14	177.11
20	189.88	188.82	182.86
25	194.67	193.48	187.86
30	195.66	195.07	188.99
35	195.29	194.42	188.85
40	194.31	193.96	188.48
45	196.32	196.11	191.28
50	196.5	196.24	191.01
55	198.3	198.21	193.43
60	198.43	198.09	192.97
71	202.54	202.15	197.09
81	203.35	203.45	198.39
91	196.21	196.69	191.24

Table S8. Color intensity data for 0.5 equiv H_2O_2.

Time (min)	Trial 1	Trial 2	Trial 3
2	143.59	142.3	138.24
12	175.76	172.16	167.73
22	187.51	184.8	180.08
32	191.25	188.92	184.3
42	193.49	190.99	185.77
52	194.17	192.51	187.06
62	195.98	193.6	189.09
72	197.13	195.65	191.27
82	200.84	198.6	194.8
92	201.01	199.63	195.16
102	202.54	201.03	197.51
112	203.04	201.43	198.3
122	204.01	202.41	199.48
132	204.99	203.38	200.23
142	206.23	204.2	201.31
152	205.69	204.16	201.3
162	206.89	205.22	202.5
172	207.15	205.23	202.61
182	206.66	206.64	202.01

Table S9. Color intensity data for 0.25 equiv H_2O_2.

Time (min)	Trial 1	Trial 2	Trial 3
1	146.43	149.16	149.99
16	174.07	174.14	173.22
31	180.12	179.69	178.32
46	183.77	182.76	181.06
61	183.92	182.2	180.66
76	185.66	183.46	182.04
91	188.6	186.54	184.97
106	191.52	188.92	188.3
121	193.58	191.05	190.9
136	196.04	193.35	193.04
151	198.08	194.74	195.02
166	200.79	196.65	196.91
181	201.33	198.3	196.51
196	202.66	199.79	199.23
211	203.81	200.64	199.95
226	206.37	204.25	202.79
241	206.73	203.31	203.4
256	206.69	205.29	202.84
271	208.59	204.82	204.92
286	208.38	204.68	204.48
301	209.16	206.59	205.87

Table S10. Color intensity data for 0.1 equiv H_2O_2.

Time (min)	Trial 1	Trial 2	Trial 3
1	148.43	147.52	149.63
16	163.99	163.5	162.8
31	174.39	174.99	174.19
46	176.86	176.85	175.64
61	178.05	178.29	176.18
76	182.46	182.21	180.13
91	182.61	182.2	181.81
106	186.56	185.76	184.11
121	188.84	188.4	186.79
136	189.69	189.13	188.4
151	191.63	191.35	189.51
166	192.99	192.2	191.16
181	192.48	192.28	189.04
196	194.04	193.55	191.41
211	196.28	195.92	195.68
226	195.89	195.81	193.58
241	197.57	196.79	194.95
256	199.29	198.73	196.82
271	200.64	199.25	197.31
286	201.28	200.68	199.59
301	202.04	200.84	198.82
316	202.27	201.69	200.78
331	203.69	204.79	204.28
346	204.71	204.77	204.22
361	203.51	204.01	202.71
376	205.96	205.74	204.78
391	206.26	206.29	205
406	205.49	204.65	203.37

Table S11. Color intensity data for no H_2O_2.

Time (min)	Trial 1	Trial 2	Trial 3
1	144.46	144.24	144.24
16	148.77	147.97	147.3
31	152.5	151.3	151.48
46	153.84	153.28	152.92
61	157.12	156.95	156.57
76	160.9	160.79	159.38
91	164.42	163.53	162.91
106	168.24	168.34	167.01
121	171.73	170.95	169.65
136	169.45	169.55	168.82
169	173.21	173.82	172.94
184	176.2	176.42	174.64
199	178.67	178.78	176.69
214	179.94	180.17	178.72
229	181.65	182.36	179.86
244	183.31	183.01	181.34
259	182.94	183.31	181.8
274	184.03	184.26	181.84
289	185.54	186.08	183.5
314	186.47	186.68	184.2
329	189.11	189.07	187.01
344	188.85	188.68	187.47
359	190.3	190.15	188.04
374	191.39	190.32	188.38
389	191	190.32	188.79
404	193.07	192.43	189.99
449	193.66	192.38	190.09
464	193.85	193.03	190.33
479	194.29	193.82	192
494	196.43	195.08	193.01
509	197.07	195.42	193.4
524	197.2	196.1	193.81
562	197.64	196.71	194.31
577	199.47	197.38	195.5
592	144.46	144.24	144.24
607	148.77	147.97	147.3
622	152.5	151.3	151.48
637	153.84	153.28	152.92
652	157.12	156.95	156.57
577	160.9	160.79	159.38
592	199.54	197.27	194.73
607	200.84	199.56	197.56
622	201.14	198.78	197
637	202.08	200.98	198.85
652	203.3	201.88	200.16
667	203.56	202.31	199.97
682	203.65	202.2	199.85
697	205.9	205.02	202.35
712	204.08	202.53	199.95

Table S12. Color intensity data for catalase-treated water with no H_2O_2.

Time (min)	Trial 1	Trial 2	Trial 3
1	149.49	149.64	147.59
15	151.44	151.09	150.11
31	152.36	150.3	148.79
46	155.28	153.95	151.94
61	155.89	155.5	151.05
91	155.67	153.1	151.56
121	156.58	153.34	151.95
151	155.67	154.27	151.43
181	156.11	155.59	152.51
211	155.51	154.87	153.69
241	157.53	155.83	154.33
301	157.98	157.68	155.26
361	157.76	155.62	154.89
421	157.34	157.32	155.58
481	156.96	155.74	153.4
541	158.62	156.82	154.95
601	158.09	156.99	155.07
661	157.54	156.95	154.65
721	159.08	157.94	156.79

General experimental procedure corresponding to Figure S1b:

A hydrogen peroxide solution in phosphate buffered water (pH 7.3; 0.25 mM–1.25 mM, 0.1–0.5 equivalents relative to amplification reagent **1**) was added to a solution of the amplification reagent **1** in buffered water containing 1.5% DMF (2.5 mM) and 0.5% Tween-20 (v/v). The colorimetric change of the solution was monitored via photography and image processing software.

Tables of Color Intensity for Figure S1b:

Table S13. Color intensity data for 0.5 equiv H_2O_2 with 0.5% Tween-20.

Time (h)	Trial 1	Trial 2	Trial 3
0	138.82	142.04	151.78
0.33	152.04	154.64	161.79
0.67	163.35	165.08	173.09
1	166.08	167.45	177.97
1.33	173.65	175.42	184.23
1.67	171.56	172.82	181.52
2	178.29	180.91	190.2
2.33	179.28	180.44	190.64
2.67	177.52	180.88	187.52
3	178.81	180.74	188.83
4	187.1	189.76	196.58
5	191.35	194.41	199.12
6	196.03	198.16	201.37
7	198.98	201.1	204.08
8	200.59	202.93	206.34
9	203.92	206.63	208.23
10	203.58	206.34	209.72

Table S14. Color intensity data for 0.1 equiv H_2O_2 with 0.5% Tween-20.

Time (h)	Trial 1	Trial 2	Trial 3
0	134.78	142.23	147.16
0.5	144.2	153.25	155.63
1	149.34	156.51	160.86
1.5	157.67	166.36	168.86
2	158.23	164.26	169.25
2.5	162.78	172.75	174.92
3	164.88	174.79	177.86
3.5	170.25	178.17	182.33
4	173.07	180.71	184.24
5	176.69	184.28	187.94
6	181.51	190.65	192.47
7	182.16	190.69	193.41
8	186.95	193.43	197.53
9	186.72	195.3	198.54
10	190.25	197.28	200.22
11	191.33	199.07	202.72
12	199.27	202.99	206.76
13	196.18	202.36	206.07
14	196.5	201.72	205.43

Table S15. Color intensity data for no H_2O_2 with 0.5% Tween-20.

Time (h)	Trial 1	Trial 2	Trial 3
0	149.88	146.07	143.09
0.5	150.59	146.76	145.4
1	155.02	150.81	147.6
1.5	155.82	151.21	148.46
2	155.99	151.74	149.72
2.5	157.05	154.28	151.03
3	160.51	156.25	153.76
3.5	161.74	156.98	154.23
4	161.05	156.87	154.52
4.5	166.75	162.01	160.27
5	169.92	164.82	163.8
6	170.05	166.58	164.26
7	171.61	169.1	165.85
8	173.98	171.1	170.34
9	177.88	174.89	173.34
10	177.18	176.09	173.78
11	178.63	175.96	174.98
12	183.63	182.15	181.22
13	182.97	180.54	178.94
14	184	183.44	182.85
15	184.54	183.34	181.91
16	186.01	186.9	185.92
17	187.06	186.87	186.35
18	189.47	188.85	187.69
19	190.34	190.3	190.92
20	191.78	193.28	192.5
21	195.02	195.99	195.02
22	196.68	197.98	196.31
23	192.35	194.92	193.18
24	199.57	202.4	200.85
25	200.25	202.73	201.46
26	200.11	203.69	201.74

Calibration curve for quantifying hydrogen peroxide using 1

General experimental procedure corresponding to Figure S2:

A hydrogen peroxide solution in phosphate buffered water (pH 7.3; 0.01 mM–2.5 mM, 0.004–1 equivalents relative to amplification reagent **1**) was added to a solution of the amplification reagent **1** in buffered water containing 1.5% DMF (2.5 mM). After 30 min, the colorimetric change of the solution was monitored via photography and image processing software.

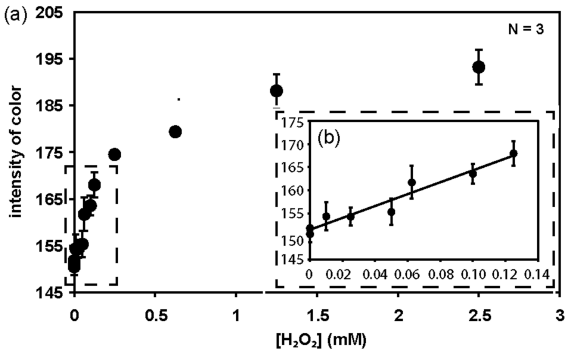


Figure S2. Calibration curve for a 30 min assay in which (a) 2.5 mM **1** in 0.1 M phosphate buffer containing 1.5% DMF (pH 7.3) was exposed to various concentrations of hydrogen peroxide at 23 °C. The intensity of color for each experiment was obtained using image analysis of photographs. The data points represent the averages of three measurements. (b) Expanded view of the dotted region of (a). The limit of detection was calculated as 3 × (*sd*/*s*) where *sd* is the standard deviation at 0.01 mM and *s* is the slope of the calibration curve.

Table S16. Color intensity data for the calibration curve (corresponding to Figure S2).

Conc. of H_2O_2 (mM)	Trial 1	Trial 2	Trial 3
0	152.5	151.3	151.48
0.01	156.19	155.95	150.79
0.025	156.54	153.2	153.11
0.05	158.42	154.59	152.89
0.0625	165.86	162.34	159.08
0.1	165.19	164.33	161.17
0.125	171.12	166.61	166.28
0.25	174.39	174.99	174.19
0.625	180.12	179.69	178.32
1.25	191.25	188.92	184.3
2.5	195.66	195.07	188.99

Detection of β-D-galactosidase using reagents 1 and 2

Experimental procedure corresponding to Figure 3:

For the assay containing both activity-based detection reagent **2** and signal amplification reagent **1** (denoted as ●):

A 5 μL solution of reagent **2** in DMF was mixed with 25 μL of Tween-20 in phosphate buffer (0.1 M, pH 7.3). To the solution was added 7.5 μL solution of reagent **1** in DMF and 437.5 μL phosphate buffer, respectively. 25 μL solution of β-D-galactosidase in phosphate buffer was subsequently added. The colorimetric change of the solution was monitored via photography and image processing software. Overall, the assay contained 2.5 mM of reagents **1** and **2**, respectively, 194 nM of the β-D-galactosidase, 2.5% DMF, and 0.5% Tween-20 (v/v).

For the assay containing only activity-based detection reagent **2** (denoted as ▲):

A 5 μL solution of reagent **2** in DMF was mixed with 25 μL of Tween-20 in phosphate buffer (0.1 M, pH 7.3). To the solution was added 445 μL phosphate buffer, and 25 μL solution of β-D-galactosidase in phosphate buffer was subsequently added. The colorimetric change of the solution was monitored via photography and image processing software. Overall, the assay contained 2.5 mM of reagent **5**, 194 nM of the β-D-galactosidase, 1% DMF, and 0.5% Tween-20 (v/v).

For the assay containing only activity-based detection reagent **1** (denoted as ■):

A 7.5 μL solution of reagent **1** in DMF was mixed with 25 μL of Tween-20 in phosphate buffer (0.1 M, pH 7.3). To the solution was added 442.5 μL phosphate buffer, and 25 μL solution of β-D-galactosidase in phosphate buffer was subsequently added. The colorimetric change of the solution was monitored via photography and image processing software. Overall, the assay contained 2.5 mM of reagent **1**, 194 nM of the β-D-galactosidase, 1.5% DMF, and 0.5% Tween-20 (v/v).

For the assay containing only activity-based detection reagent **2** with no enzyme (denoted as ◆):

A 5 μL solution of reagent **2** in DMF was mixed with 25 μL of Tween-20 in phosphate buffer (0.1 M, pH 7.3) and 470 μL phosphate buffer. The colorimetric change of the solution was

monitored via photography and image processing software. Overall, the assay contained 2.5 mM of reagent **2**, 1% DMF, and 0.5% Tween-20 (v/v).

For all assays, the absolute intensities were calculated using the formula $I - I_0$, where I = intensity at time t, and I_0 = intensity at time 0 min.

Tables of Absolute Color Intensity for Figure 3:

Table S17: Absolute color intensity data for the one-pot assay containing reagents **1** and **2**, assay containing only **2**, and assay containing only **1**

Time (h)	1 + 2 ●	2 ▲	1 ■
0	0.0	0.0	0.0
0.5	-1.9	-0.7	-0.2
1	1.9	2.1	0.4
1.5	3.2	5.6	-0.3
2	5.5	6.0	-4.1
2.5	9.0	9.0	-1.3
3	12.3	13.1	-2.3
3.5	13.9	14.7	-4.0
4	19.9	18.5	-3.6
4.5	25.5	22.5	-3.2
5	28.9	24.8	-3.2
5.5	33.0	28.4	-2.0
6	34.8	27.9	-2.7
6.5	41.4	31.3	-0.1
7	49.0	35.7	-1.0
7.5	47.6	35.2	0.8
8	51.7	36.4	1.3
8.5	55.4	37.7	-3.1
9	61.5	40.2	-1.7
9.5	60.0	39.3	1.7
10	65.1	43.5	4.2
10.5	65.8	44.3	2.9
11	71.2	44.8	5.5
11.5	72.5	45.6	6.7
12	76.1	46.3	9.2

Table S18: Absolute color intensity data for activity-based detection reagent (**2**) with no enzyme

Time (h)	2 (no enzyme)
0	0
0.5	0.71
1	-0.16
1.5	1.52
2	0.77
2.5	1.17
3	3.33
3.5	3.46
4	3.44
4.5	4.62
5	2.7
5.5	3.58
6	3.25
7	1.3
8	0.12
9	-0.68
12	-0.57
14	-4.31
16	-1.99
18	-1.38
20	-1.98
22	1.02
24	0.1

Quantification of β-D-galactosidase using reagents 1 and 2

Experimental procedure corresponding to Figure 4:

A 5 μL solution of reagent **2** in DMF was mixed with 25 μL of Tween-20 in phosphate buffer (0.1 M, pH 7.3). To the solution was added 7.5 μL solution of reagent **1** in DMF and β-D-galactosidase solution in phosphate buffered water (12.5 nM – 250 nM) sequentially. After 10 h, the colorimetric change of the solution was monitored via photography and image processing software. Overall, the assay contained 2.5 mM of reagents **1** and **2**, respectively, 2.5% DMF, and 0.5% Tween-20 (v/v).

Table of Absolute Color Intensity for Figure 4:

Table S19. Color intensity data for the calibration curve (corresponding to Figure 4).

Conc. of enzyme (nM)	Trial 1	Trial 2	Trial 3
0	13.8	10.9	12.0
12.5	13.8	15.3	10.6
25	14.5	15.6	22.5
50	19.7	25.1	28.0
75	40.4	41.6	35.0
100	47.4	49.3	48.3
125	54.8	57.8	54.5
150	63.8	64.8	61.7
194	65.1	62.6	62.6
250	63.6	65.7	61.3

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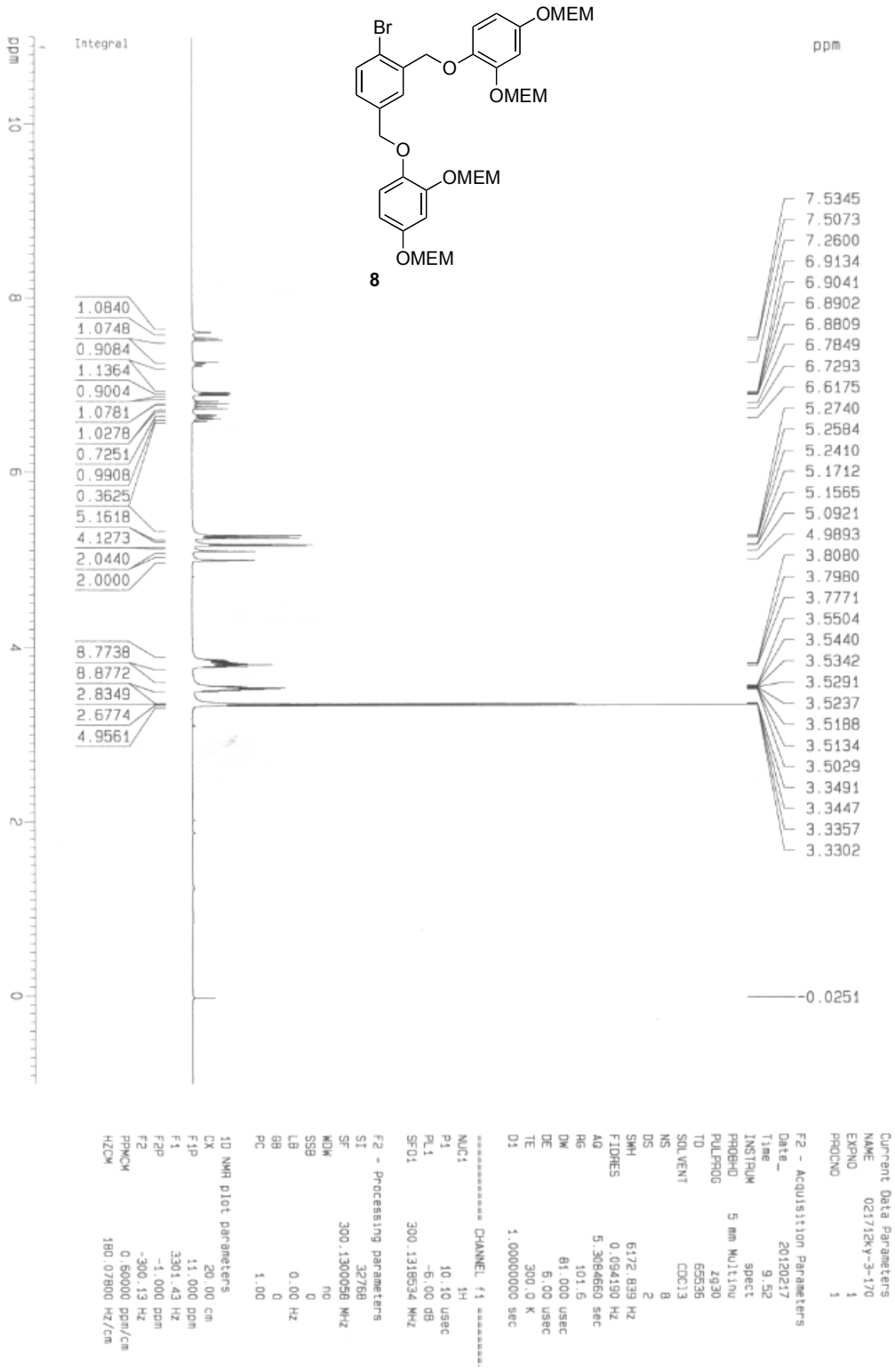
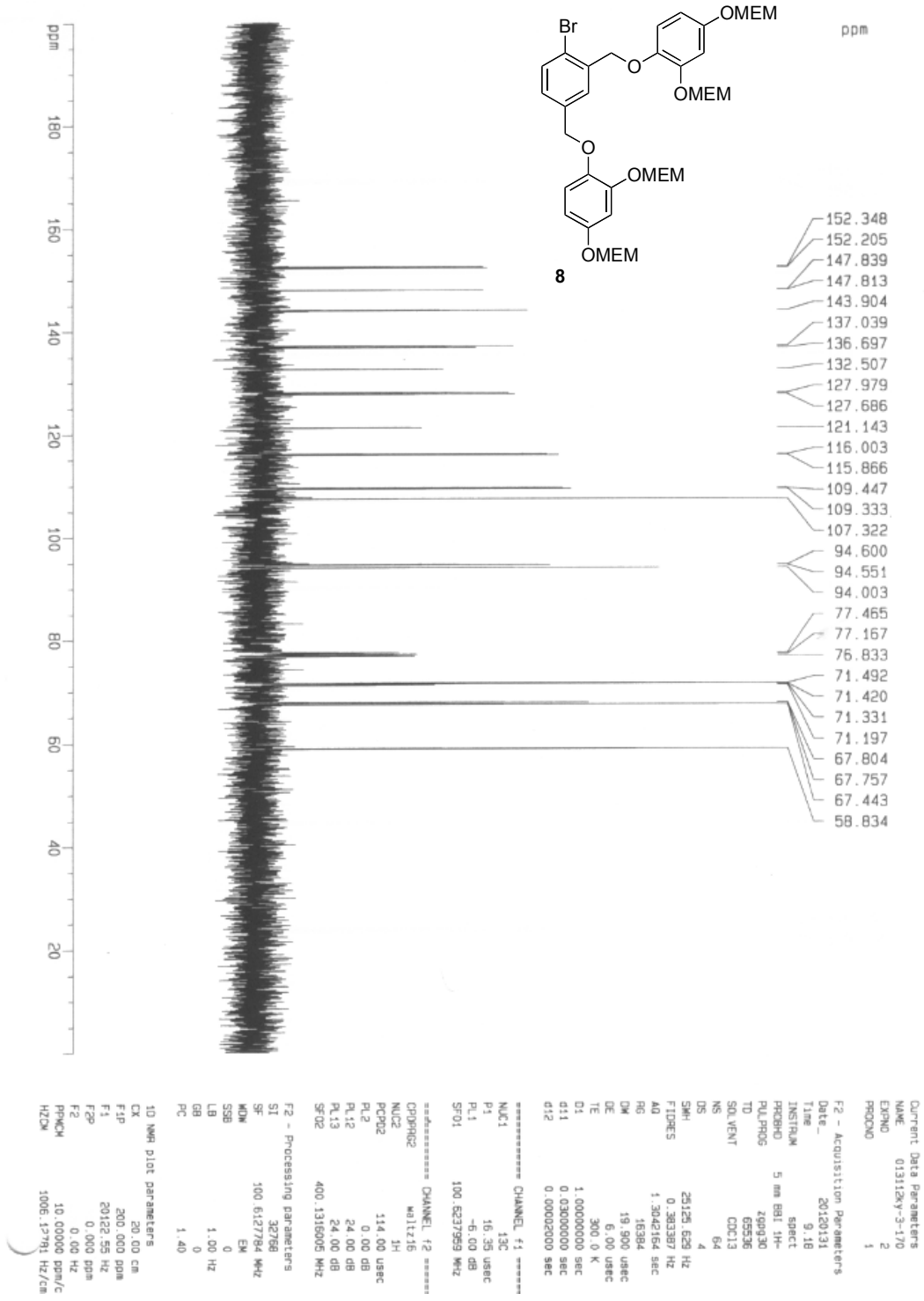
Copies of spectra

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DE 6.00 usec
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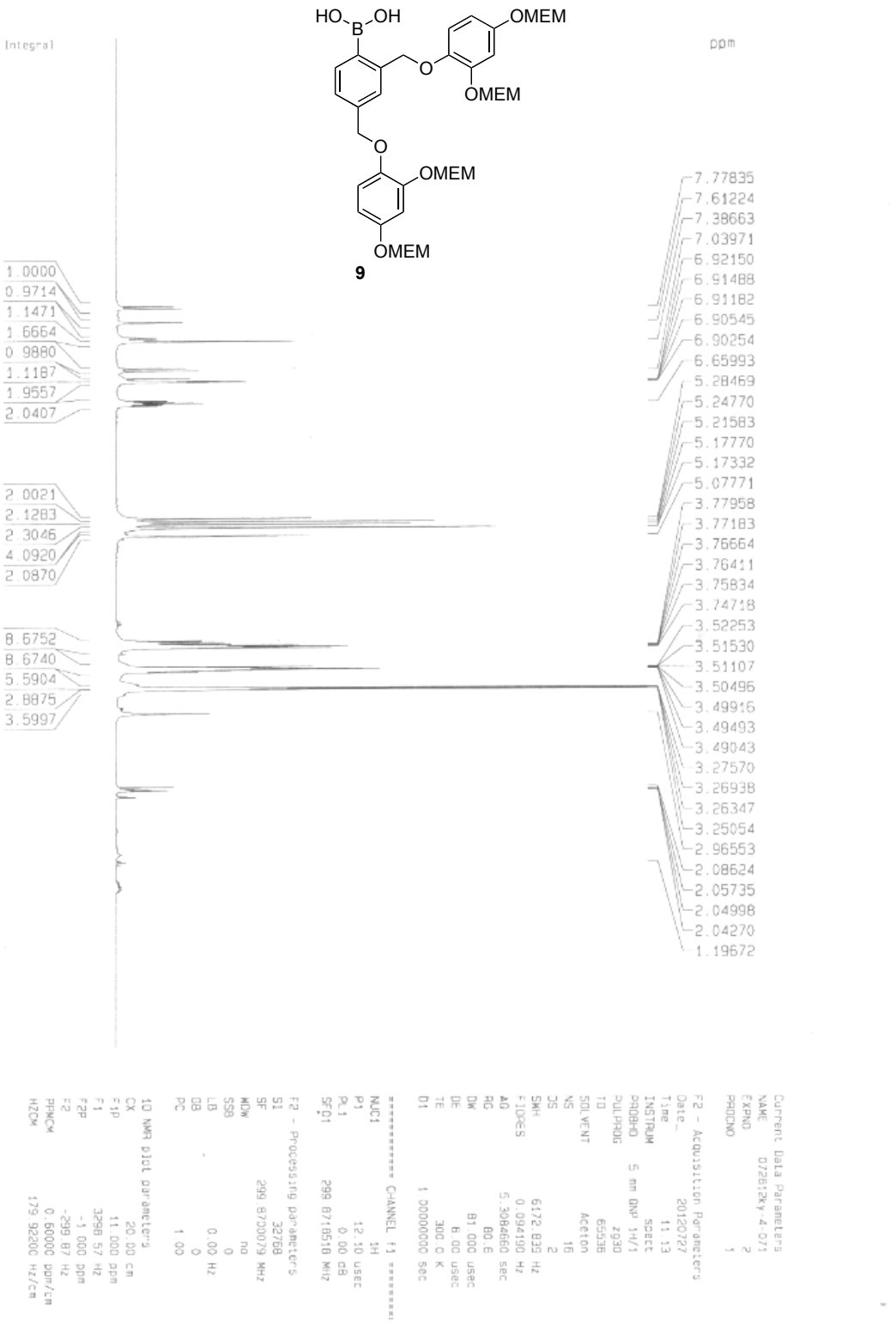
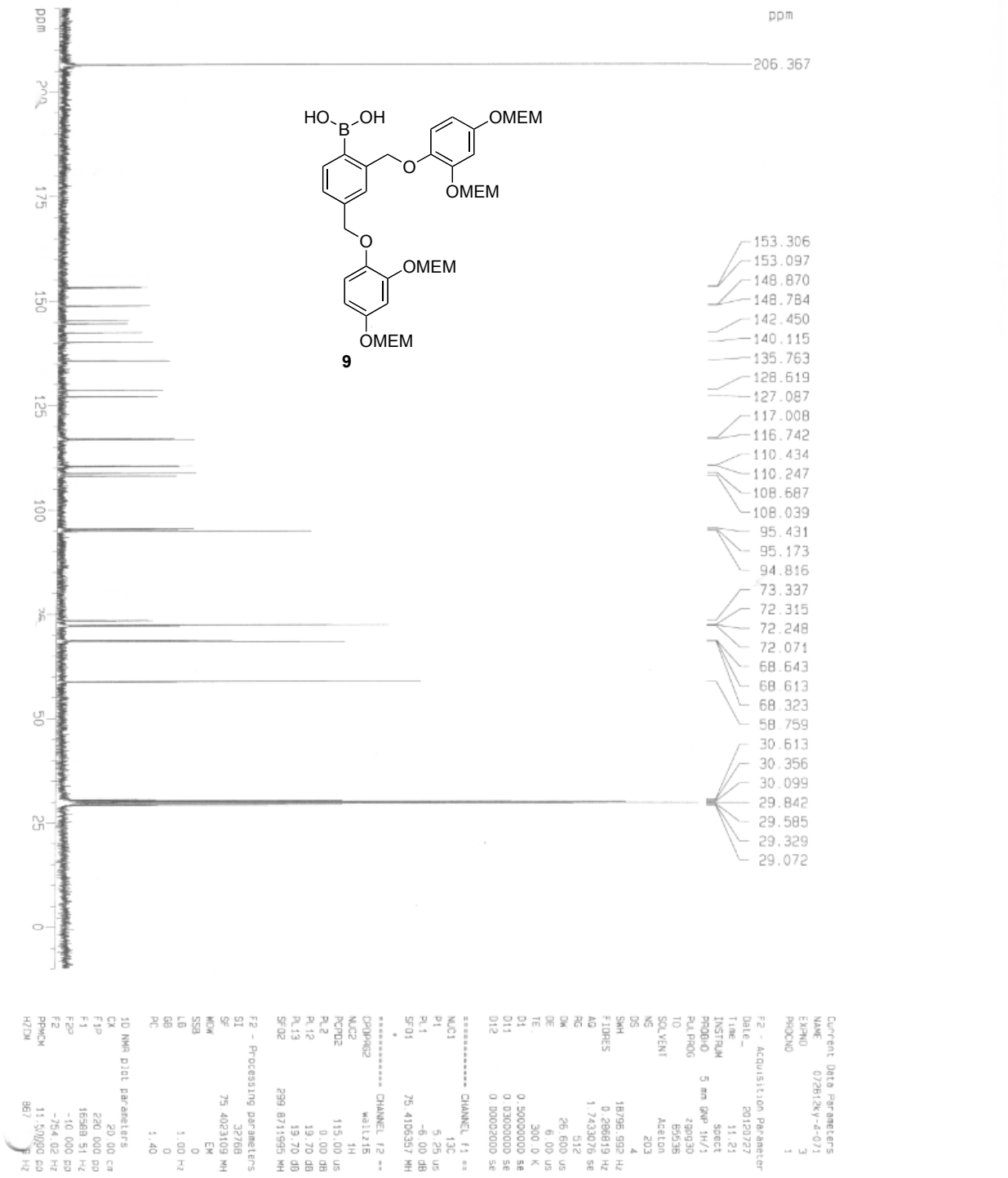
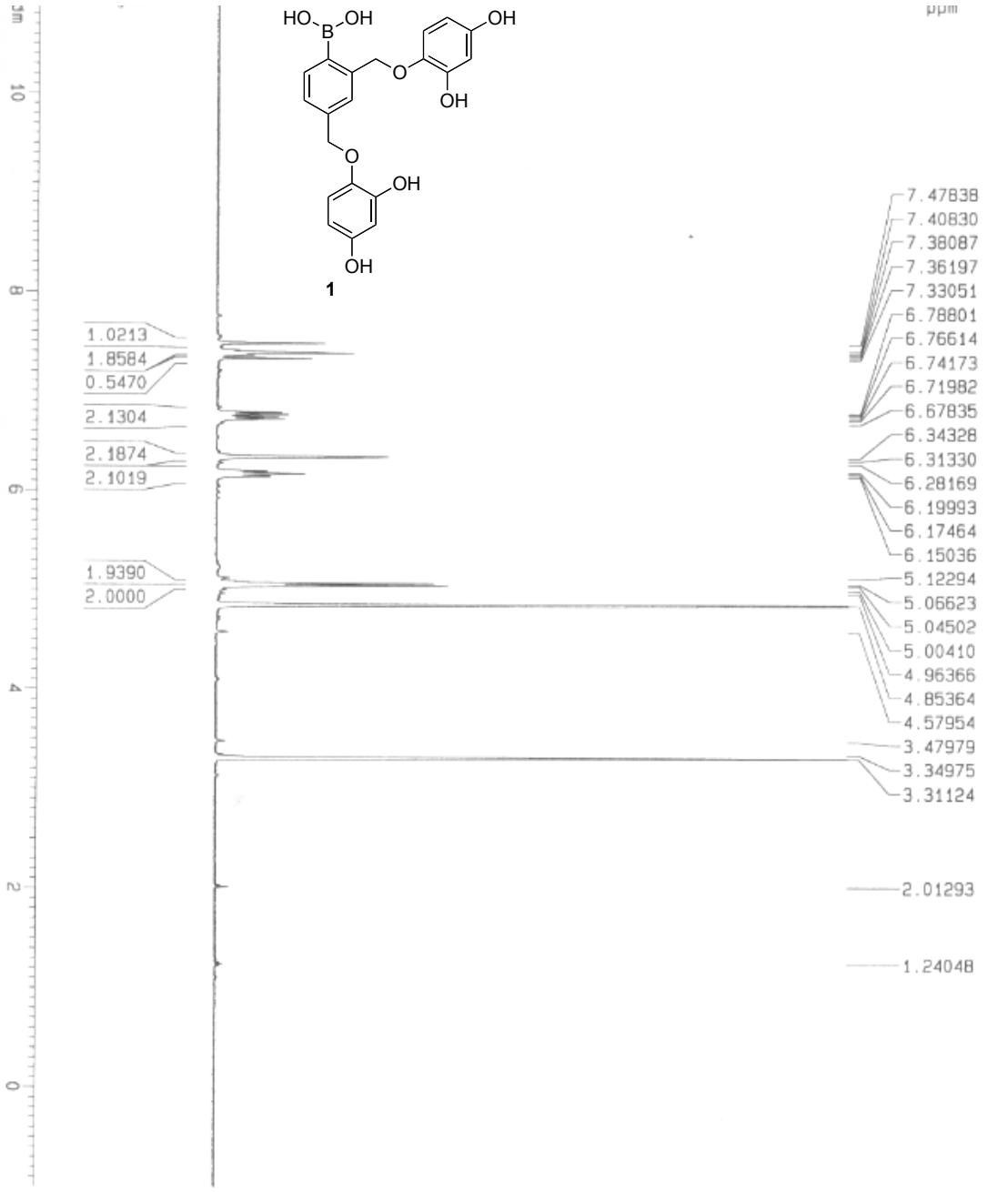
ppm
180
160
140
120
100
80
60
40
20


HO
B
OH
O
OMEM
OMEM
O
OMEM
OMEM
9
Integral
ppm
1.0000
0.9714
1.1471
1.6664
0.9880
1.1187
1.9557
2.0407
2.0021
2.1283
2.3046
4.0920
2.0870
8.6752
8.6740
5.5904
2.8875
3.5997
7.77835
7.61224
7.38663
7.03971
6.92150
6.91488
6.91182
6.90545
6.90254
6.65993
5.28469
5.24770
5.21583
5.17770
5.17332
5.07771
3.77958
3.77183
3.76664
3.76411
3.75834
3.74718
3.52253
3.51530
3.51107
3.50496
3.49916
3.49493
3.49043
3.27570
3.26938
3.26347
3.25054
2.96553
2.08624
2.05735
2.04998
2.04270
1.19672
Current Data Parameters
NAME 072612ky-4-071
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20120727
Time 11 13
INSTRUM spect
PROBHD 5 mm BNI 1H/1
PULPROG z930
TD 65536
SOLVENT Aceton
NS 16
DS 2
SWH 6172.835 Hz
FIDRES 0.094190 Hz
AQ 5.3084660 sec
RG 80.6
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 299.8715510 MHz
F2 - Processing parameters
SI 32768
SF 299.9700079 MHz
WDW no
SSB 0
LB 0.00 Hz
GB 0
PC 1.00
1D NMR plot parameters
CX 20.00 cm
F1P 11.000 ppm
F1 3298.57 Hz
F2P -1.000 ppm
F2 -299.87 Hz
PPMCM 0.60000 ppm/cm
HZCM 179.92200 Hz/cm


HO
B
OH
OMEM
O
OMEM
O
OMEM
OMEM
9
ppm
206.367
153.306
153.097
148.870
148.784
142.450
140.115
135.763
128.619
127.087
117.008
116.742
110.434
110.247
108.687
108.039
95.431
95.173
94.816
73.337
72.315
72.248
72.071
68.643
68.613
68.323
58.759
30.613
30.356
30.099
29.842
29.585
29.329
29.072
Current Data Parameters
NAME 072812ky-4-071
EXPNO 3
PROCNO 1
F2 - Acquisition Parameter
Date_ 20120727
Time 11.21
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG zgpg30
TD 65536
SOLVENT Aceton
NS 203
DS 4
SWH 18796.992 Hz
FIDRES 0.286819 Hz
AQ 1.7433076 sec
RG 512
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
SF01 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 75.4023109 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
CX 20.00 cm
F1P 220.000 ppm
F1 16568.51 Hz
F2P -10.000 ppm
F2 -754.02 Hz
PPMCM 11.50000 ppm/cm
HZCM 867.58 Hz



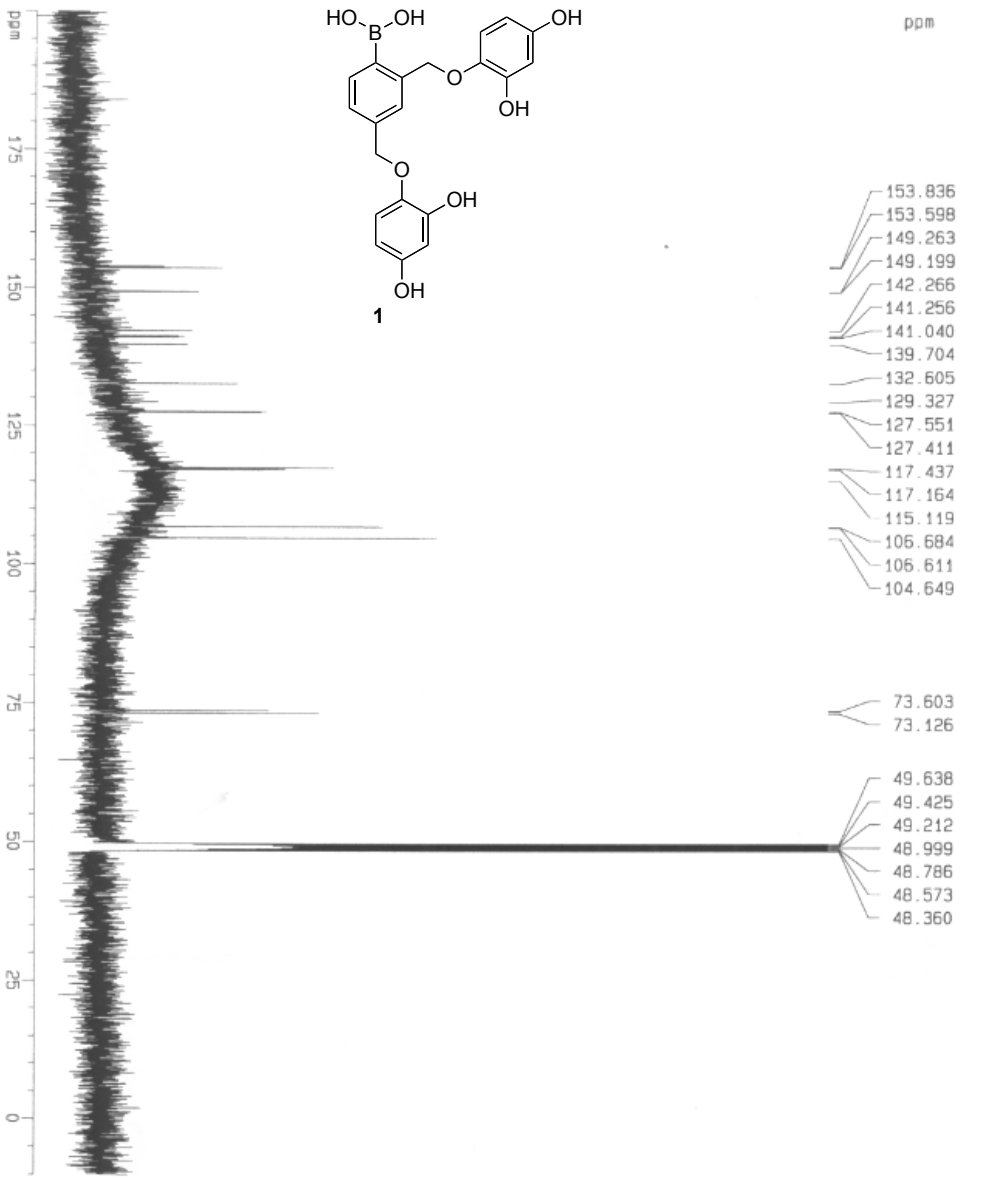
Current Data Parameters
NAME 071912ky-nb4
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20120719
Time 18.54
INSTRUM spect
PROBHD 5 mm BBI 1H-
PULPROG zg30
TD 65536
SOLVENT MeOH
NS 16
DS 2
SWH 8278.146 Hz
FIDRES 0.126314 Hz
AQ 3.956423 sec
RG 406.4
DW 60.400 usec
DE 6.00 usec
TE 300.0 K
D1 1.00000000 sec

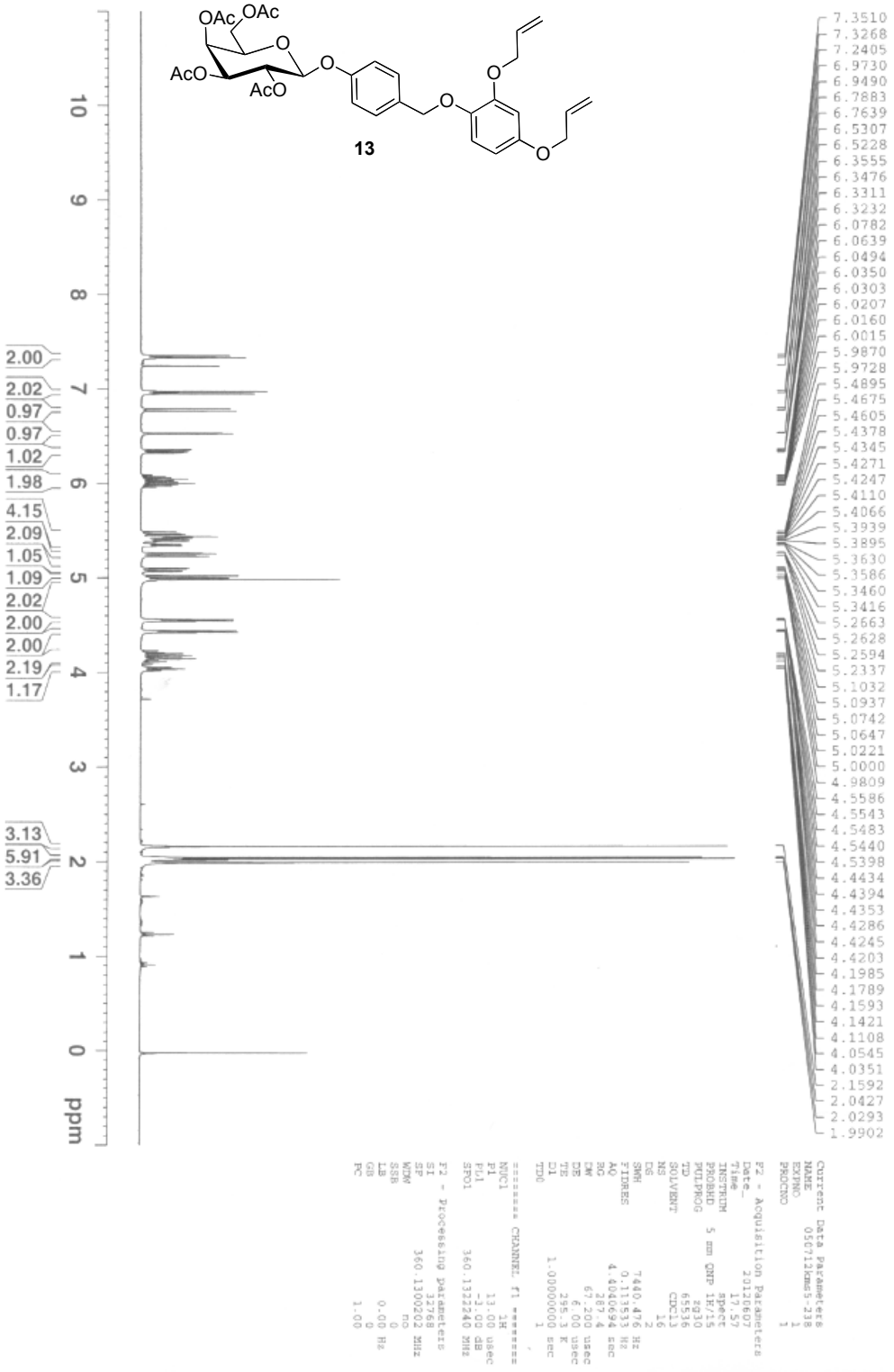
======== CHANNEL f1 ========
NUC1 1H
P1 6.45 usec
PL1 0.00 dB
SFO1 400.1324710 MHz

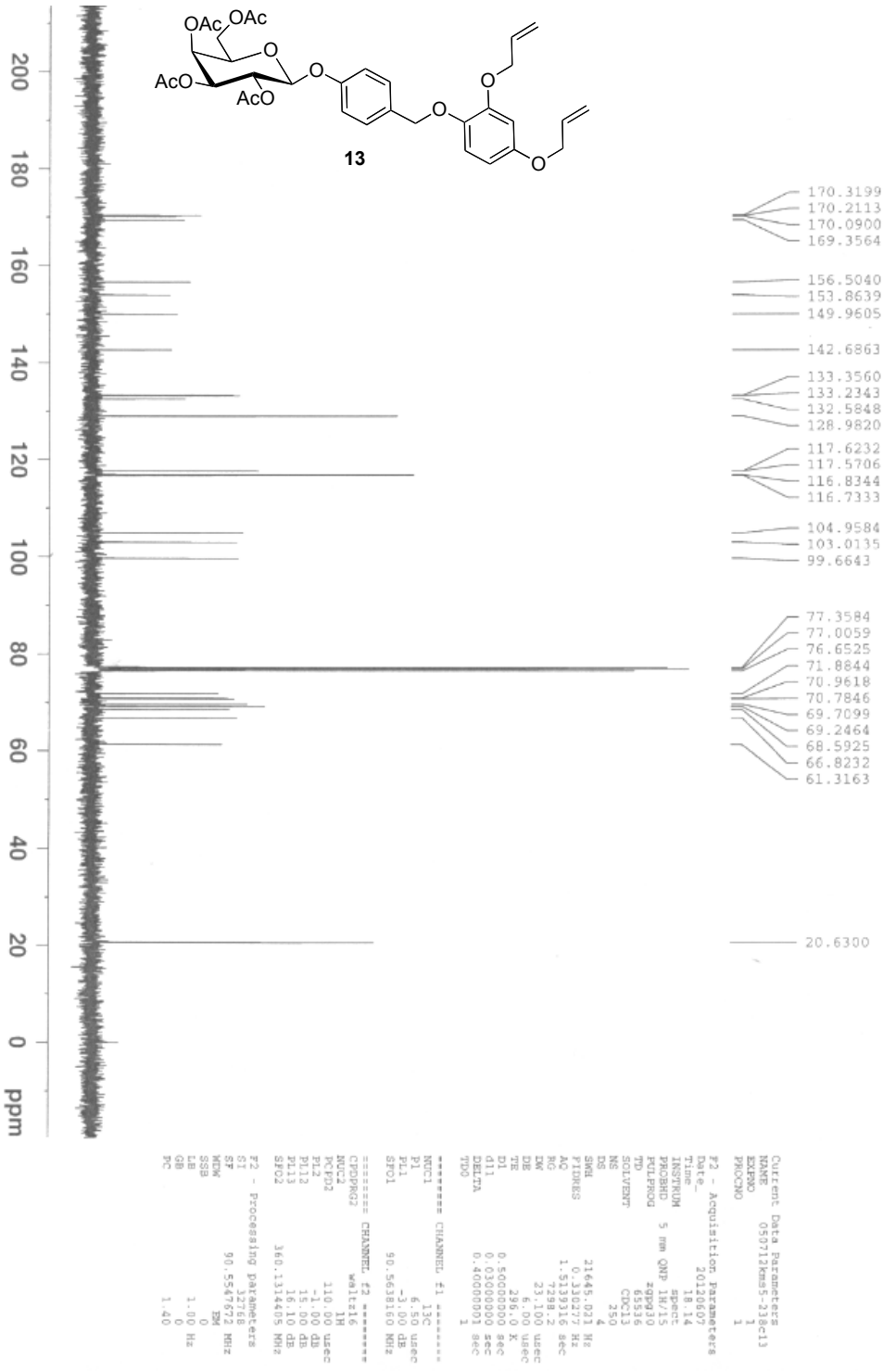
F2 - Processing parameters
SI 32768
SF 400.1300067 MHz
WDW no
SSB 0
LB 0.00 Hz
GB 0
PC 1.00

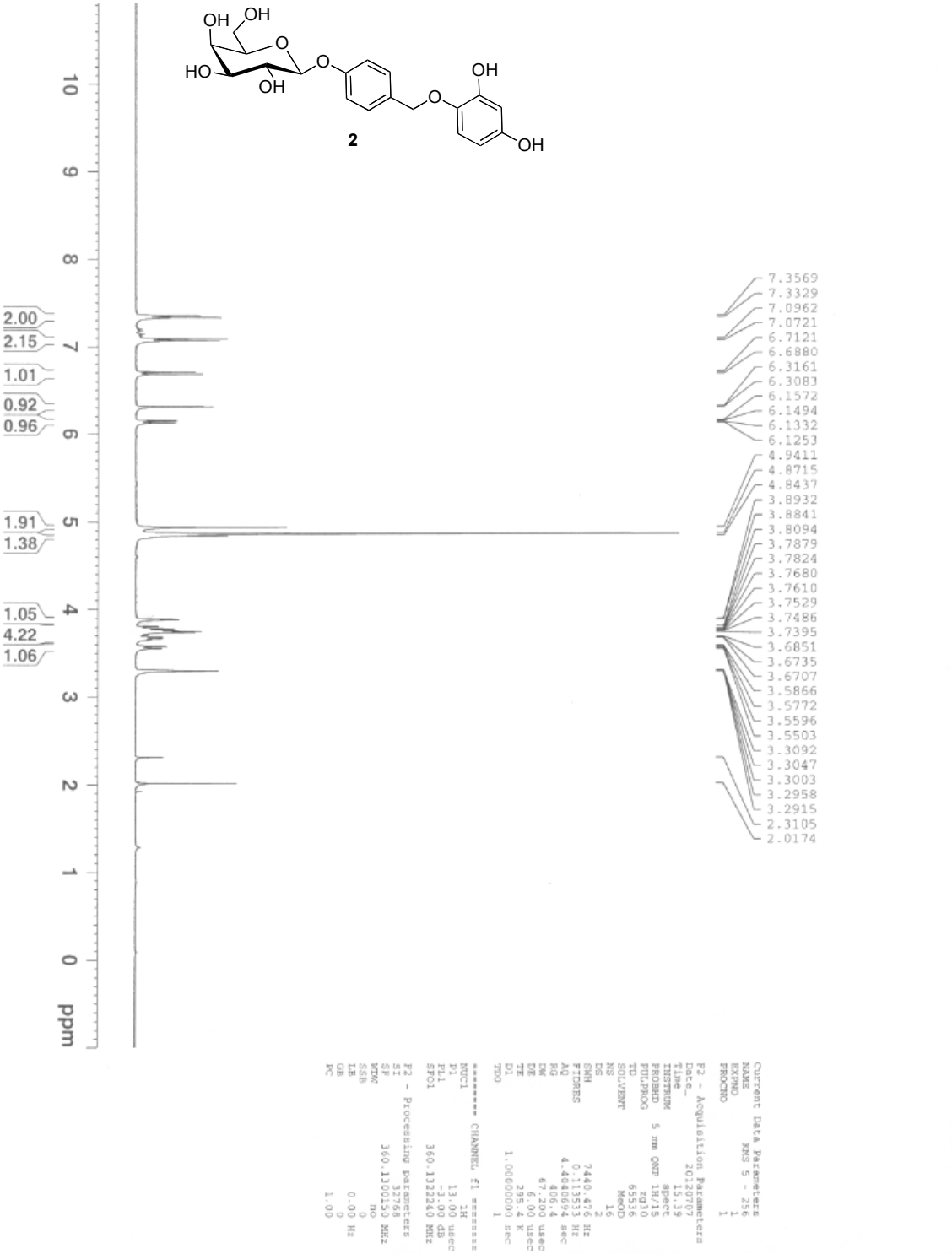
1D NMR plot parameters
CX 20.00 cm
F1P 11.000 ppm
F1 4401.43 Hz
F2P -1.000 ppm
F2 -400.13 Hz
PPMCM 0.60000 ppm/cm
HZCM 240.07800 Hz/cm

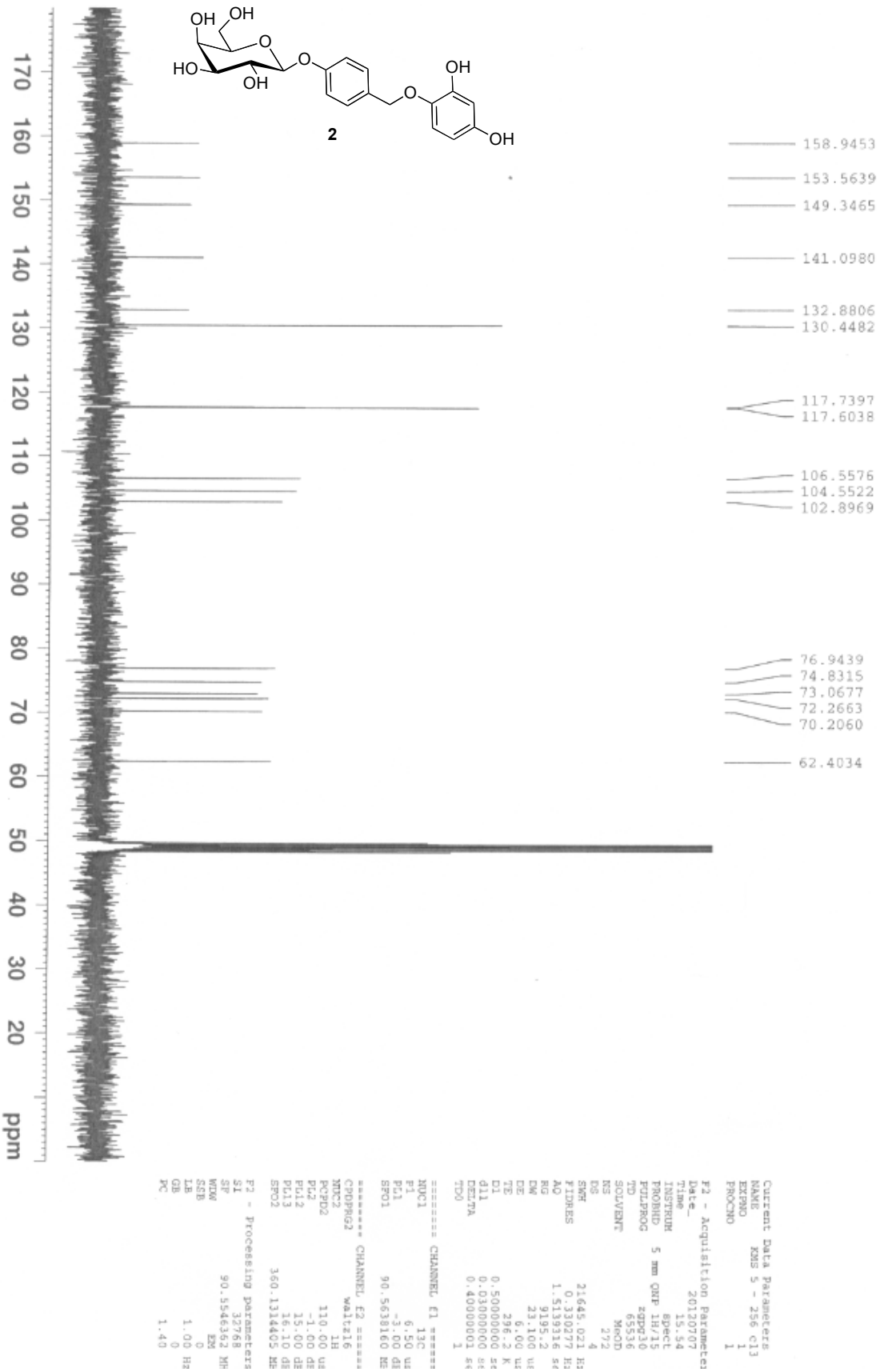


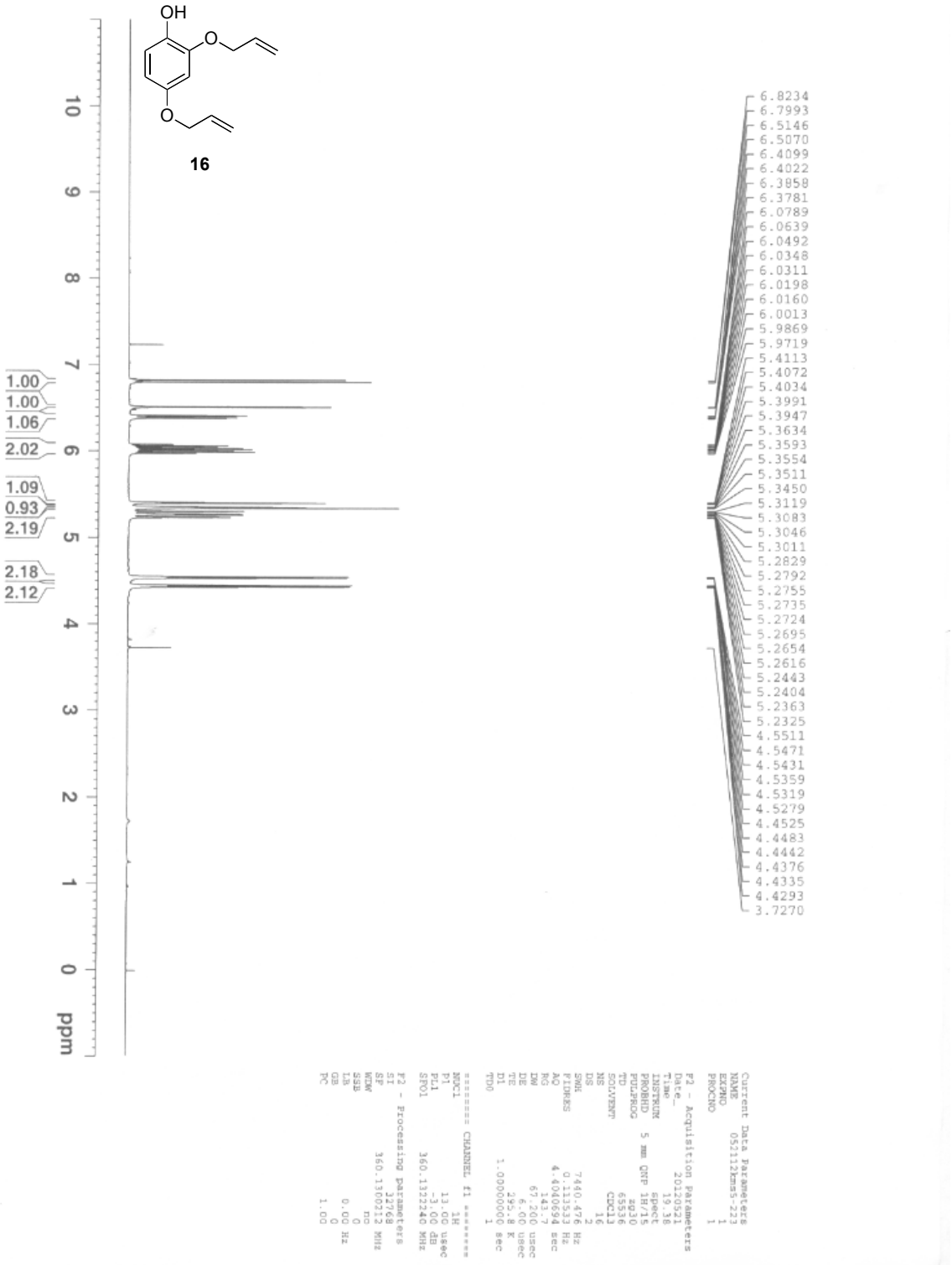
Current Data Parameters
NAME 071912ky-rb4
EXPNO 3
PROCNO 1
F2 - Acquisition Parameters
Date_ 20120719
Time 18.59
INSTRUM spect
PROBHD 5 mm BBI 1H-
PULPROG zgpg30
TD 65536
SOLVENT MeOH
NS 24000
DS 4
SWH 25125.629 Hz
FIDRES 0.383387 Hz
AQ 1.3042164 sec
RG 16384
DW 19.900 usec
DE 6.00 usec
TE 300.0 K
D1 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 100.6126274 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
CX 20.00 cm
F1P 200.000 ppm
F1 20122.53 Hz
F2P -10.000 ppm
F2 -1006.13 Hz
PPMCM 10.50000 ppm/cm
HZCM 1056.43262 Hz/cm

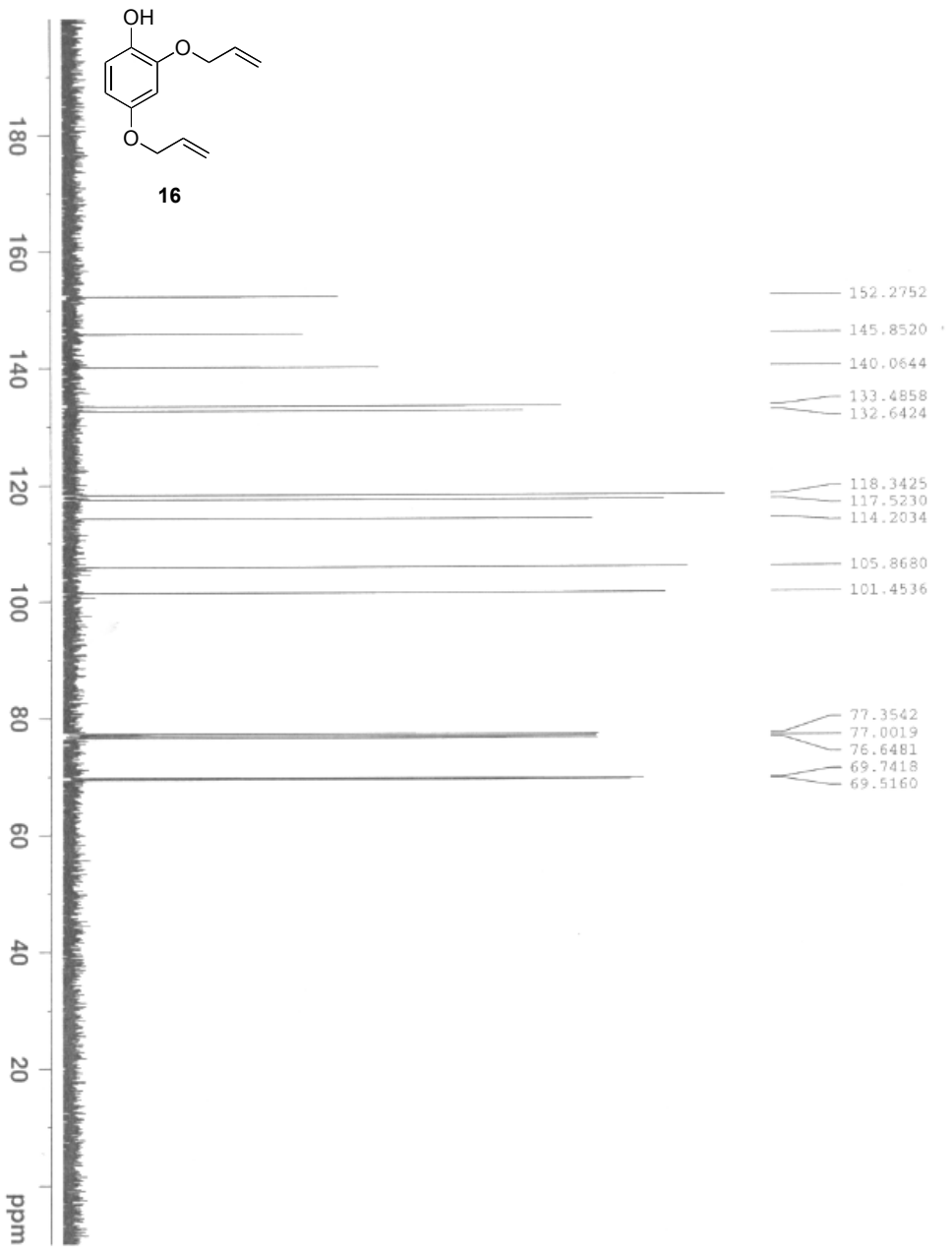

OAc
OAc
AcO
AcO
O
O
O
O
O
13
2.00
2.02
0.97
0.97
1.02
1.98
4.15
2.09
1.05
1.09
2.02
2.00
2.00
2.19
1.17
3.13
5.91
3.36
10
9
8
7
6
5
4
3
2
1
0
ppm
7.3510
7.3268
7.2405
6.9730
6.9490
6.7883
6.7639
6.5307
6.5228
6.3555
6.3476
6.3311
6.3232
6.0782
6.0639
6.0494
6.0350
6.0303
6.0207
6.0160
6.0015
5.9870
5.9728
5.4895
5.4675
5.4605
5.4378
5.4345
5.4271
5.4247
5.4110
5.4066
5.3939
5.3895
5.3630
5.3586
5.3460
5.3416
5.2663
5.2628
5.2594
5.2337
5.1032
5.0937
5.0742
5.0647
5.0221
5.0000
4.9809
4.5586
4.5543
4.5483
4.5440
4.5398
4.4434
4.4394
4.4353
4.4286
4.4245
4.4203
4.1985
4.1789
4.1593
4.1421
4.1108
4.0545
4.0351
2.1592
2.0427
2.0293
1.9902
Current Data Parameters
NAME 05t712kms5-238
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20120607
Time 17.57
INSTRUM spect
PROBHD 5 mm QNP 1H/15
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 7440.476 Hz
FIDRES 0.113533 Hz
AQ 4.4040694 sec
RG 287.4
DW 67.200 usec
DE 6.00 usec
TE 295.3 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 13.00 usec
PL1 -1.00 dB
SFO1 360.1322240 MHz
F2 - Processing parameters
SI 32768
SF 360.1300202 MHz
WDW no
SSB 0
LB 0.00 Hz
GB 0
PC 1.00


OAc
OAc
AcO
AcO
O
O
O
O
O
13
170.3199
170.2113
170.0900
169.3564
156.5040
153.8639
149.9605
142.6863
133.3560
133.2343
132.5848
128.9820
117.6232
117.5706
116.8344
116.7333
104.9584
103.0135
99.6643
77.3584
77.0059
76.6525
71.8844
70.9618
70.7846
69.7099
69.2464
68.5925
66.8232
61.3163
20.6300
200
180
160
140
120
100
80
60
40
20
0
ppm


OH
OH
O
HO
OH
O
O
OH
OH
2
2.00
2.15
1.01
0.92
0.96
1.91
1.38
1.05
4.22
1.06
ppm
7.3569
7.3329
7.0962
7.0721
6.7121
6.6880
6.3161
6.3083
6.1572
6.1494
6.1332
6.1253
4.9411
4.8715
4.8437
3.8932
3.8841
3.8094
3.7879
3.7824
3.7680
3.7610
3.7529
3.7486
3.7395
3.6851
3.6735
3.6707
3.5866
3.5772
3.5596
3.5503
3.3092
3.3047
3.3003
3.2958
3.2915
2.3105
2.0174


OH
OH
O
HO
OH
O
O
OH
OH
2
158.9453
153.5639
149.3465
141.0980
132.8806
130.4482
117.7397
117.6038
106.5576
104.5522
102.8969
76.9439
74.8315
73.0677
72.2663
70.2060
62.4034
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
ppm


OH
O
O
16
1.00
1.00
1.06
2.02
1.09
0.93
2.19
2.18
2.12
10
9
8
7
6
5
4
3
2
1
0
ppm
6.8234
6.7993
6.5146
6.5070
6.4099
6.4022
6.3858
6.3781
6.0789
6.0639
6.0492
6.0348
6.0311
6.0198
6.0160
6.0013
5.9869
5.9719
5.4113
5.4072
5.4034
5.3991
5.3947
5.3634
5.3593
5.3554
5.3511
5.3450
5.3119
5.3083
5.3046
5.3011
5.2829
5.2792
5.2755
5.2735
5.2724
5.2695
5.2654
5.2616
5.2443
5.2404
5.2363
5.2325
4.5511
4.5471
4.5431
4.5359
4.5319
4.5279
4.4525
4.4483
4.4442
4.4376
4.4335
4.4293
3.7270
Current Data Parameters
NAME 052112kms5-223
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20120521
Time 19.38
INSTRUM spect
PROBHD 5 mm QNP 1H/15
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 7440.476 Hz
FIDRES 0.113533 Hz
AQ 4.4040694 sec
RG 143.7
DW 67.200 usec
DE 6.00 usec
TE 295.8 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 13.00 usec
PL1 -3.00 dB
SFO1 360.1322240 MHz
F2 - Processing parameters
SI 32768
SF 360.1300212 MHz
WDW no
SSB 0
LB 0.00 Hz
GB 0
PC 1.00


OH
O
O
16
152.2752
145.8520
140.0644
133.4858
132.6424
118.3425
117.5230
114.2034
105.8680
101.4536
77.3542
77.0019
76.6481
69.7418
69.5160
180
160
140
120
100
80
60
40
20
ppm