Synthesis of Biphenyl Proteomimetics as Estrogen Receptor-alpha Coactivator Binding Inhibitors

Supporting Information

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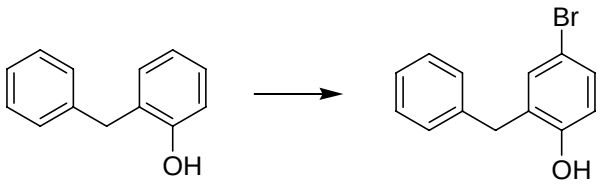
General

All commercially available reagents were purchased from Sigma-Aldrich and used without further purification. Solvents were distilled and reactions requiring inert conditions were performed under N_2 or argon. Column chromatography was performed using silica gel unless otherwise indicated. Flash chromatography was performed using the Argonaut FlashMaster Solo with an FC204 fraction collector. Thin layer chromatography was used to monitor reactions using Selecto Scientific 200 micron silica gel flexible TLC plates. ^{1}H was recorded on a Varian Unity-INOVA 500MHz spectrometer. ^{13}C NMR were recorded on a Varian 300MHz spectrometer. High resolution mass spectral data were obtained at the University of Massachusetts Mass Spectrometry Facility which is supported, in part, by the National Science Foundation.

Experimental Methods

Representative procedure for the synthesis of para-brominated 2-alkylphenols.

Preparation of 2-Benzyl-4-bromophenol (3e)



2-benzylphenol (4.61 g, 25 mmol) was dissolved in chloroform (200 mL). Tetra-*n*-butyl ammonium tribromide (14.47 g, 30 mmol) was added and the solution was stirred for 3 hours at room temperature. The solvent was removed by rotary evaporation and the crude product was partitioned between ether and water. The ether layer was washed sequentially with 1 N HCl (x2) and brine (x2). The organic layer was separated and dried over magnesium sulfate. Solvent was evaporated and the crude product was purified by chromatography on silica gel (Hexane/EtOAc, 80:20). The desired product (6.2 g, 95%) was isolated as a clear yellow oil. ^{1}H NMR (500 MHz, CDCl3): δ = 7.30 (t, J = 7.5 Hz, 2H), 7.25-7.20 (m, 5H), 6.66 (d, J = 7.0 Hz, 1H), 3.94 (s, 2H) ppm.

2-Isopropyl-4-bromophenol (3b)

Same procedure as **3e**. Product isolated in 88% yield as a yellow oil.
^{1}H NMR (500 MHz, CDCl3): δ = 7.35 (d, J = 2.5 Hz, 1H), 7.19 (dd, J = 8.5, 2.5 Hz, 1H), 6.67 (d, J = 9 Hz, 1H), 3.32 (m, J = 7.0 Hz, 1H), 1.27 (d, J = 8.0 Hz, 6H) ppm

2-sec-Butyl-4-bromophenol (3c)

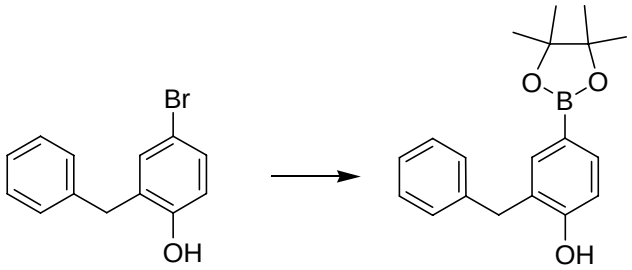
Same procedure as **3e**. Product isolated in essentially quantitative yield as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$-d): δ 7.24 (d, *J* = 2.5 Hz, 1 H), 7.16 (dd, *J* = 8 Hz, 2.5 Hz, 1H, 6.65 (d, *J* = 8 Hz, 1H), 4.82 (br s, 1H), 2.93 (m, 1H), 1.62 (m, 2H), 1.22 (d, *J* = 6.5 Hz, 3H), 0.877 (t, *J* = 7 Hz, 3H) ppm.

2-tert-Butyl-4-bromophenol (3d)

Same procedure as **3e**. Product isolated in essentially quantitative yield as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$-d): δ 7.36 (d, *J* = 2.5 Hz, 1H), 7.17 (dd, *J* = 8.5 Hz, 2.5 Hz, 1H), 6.57 (d, *J* = 8 Hz, 1H), 5.01 (br s, 1H), 1.40 (s, 9H) ppm.

Representative procedure for the synthesis of para-hydroxyphenylboronate esters

Preparation of 2-Benzyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (4e)



3e (0.79 g, 3.0 mmol), $PdCl_2$(dppf) (0.15 g, 6 mol %), and potassium acetate (0.59 g, 6.0 mmol) were added to the reaction vessel which was predried under vacuum and flushed with argon. Anhydrous dioxane (20 ml) was added via syringe. The reaction mixture was heated to 80 ºC and stirred for 30 minutes. Bis(pinacolato)diboron (0.84 g, 3.3 mmol), dissolved in 5 mL dioxane, was added and the reaction was kept under inert atmosphere, stirred, and heated at 80 ºC overnight. The crude mixture was filtered through activated carbon and Celite, and solvent was removed by rotary evaporation. The crude mixture was dissolved in ethyl acetate, washed with water (x2), brine (x2) and dried over magnesium sulfate. The product was purified by flash chromatography using silica gel (Hexane/EtOAc, 80:20) to afford 0.60 g (65 %) of the desired product as a white solid (m.p. 119-121ºC). ^{1}H NMR (500 MHz, CDCl3): δ = 7.70 (s, 1H), 7.65 (dd, *J* = 7.0, 1.5 Hz, 1H), 7.30 (t, *J* = 7.0 Hz, 2H), 7.25 (d, *J* = 8.0 Hz, 2H), 7.22 (t, *J* = 7.5 Hz, 1H), 6.81 (d, *J* = 7.0 Hz, 1H), 4.04 (s, 2H), 1.37 (s, 12H) ppm.

2-Isopropyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (4b)

Same procedure as **4e.** Product isolated in 95% yield as a white solid (m.p. 148-150 ºC). ^{1}H NMR (500 MHz, CDCl3): δ = 7.68 (s, 1H), 7.57 (dd, *J* = 8.0, 1.5 Hz, 1H), 6.76 (d, *J* = 8.0 Hz, 1H), 3.23 (m, *J* = 7.0 Hz, 1H), 1.37 (s, 12 H), 1.30 (d, *J* = 6.5 Hz, 6H) ppm.

2-sec-Butyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (4c)

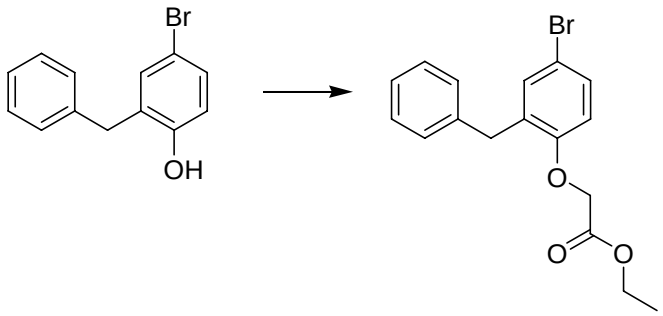
Same procedure as **4e.** Product isolated in 58% yield as a white solid (m.p. 114-117 ºC). ^{1}H NMR (500 MHz, $CDCl_3$-d): δ 7.61 (d, *J* = 1.5 Hz, 1H), 7.55 (dd, *J* = 7.5 Hz, 1.5 Hz, 1H), 6.74 (d, *J* = 7.5 Hz, 1H), 4.93 (br s, 1H), 2.95 (m, 1H), 1.67 (m, 2H), 1.34 (s, 12H), 1.27 (d, *J* = 6.5 Hz, 3H), 0.88 (t, *J* = 7 Hz, 3H) ppm.

2-tert-Butyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (4d)

Same procedure as **4e.** Product isolated in 61% yield as a white solid (m.p. 170-173 ºC). ^{1}H NMR (500 MHz, $CDCl_3$-d): δ 7.72 (d, *J* = 1.5 Hz, 1H), 7.55 (dd, *J* = 7.5 Hz, 1.5 Hz, 1H), 6.66 (d, *J* = 8 Hz, 1H), 4.96 (br s, 1H), 1.43 (s, 9H), 1.33 (s, 12H) ppm.

Representative procedure for the synthesis of ethyl 4-bromophenoxyacetates

Preparation of Ethyl 2-(2-benzyl-4-bromophenoxy)acetate (5e)



3e (2.10 g, 8 mmol) was dissolved in dry THF (75 mL). Sodium hydride (60 % in oil, 0.45 g, 11.2 mmol) was added and the solution was stirred at room temperature for 30 minutes. Ethyl bromoacetate (1.87 g, 11.2 mmol) was then added slowly and the solution was stirred for 2 hours at room temperature. The reaction mixture (milky-white suspension) was quenched with ethanol and concentrated. The concentrate was partitioned between ethyl acetate and water (x2). The combined organic layers were washed with brine (x2), dried, and concentrated. The mixture was purified by flash chromatography on silica gel (Hexane/EtOAc, 90:10) to afford 2.57 g (92%) of the desired product as a crystalline solid (m.p. 67-70 ºC). ^{1}H NMR (500 MHz, CDCl3): δ = 7.34-7.22 (m, 7H), 6.65 (d, *J* = 8.5 Hz, 1H), 4.62 (s, 2H), 4.30 (q, *J* = 6.5 Hz, 2H), 4.05 (s, 2H), 1.32 (t, *J* = 7.5 Hz, 3H) ppm.

Ethyl 2-(2-isopropyl-4-bromophenoxy)acetate (5b)

Same procedure as **5e**. Product was isolated in 78% yield as a yellow oil.
^{1}H NMR (500 MHz, CDCl3): δ = 7.35 (d, *J* = 2.5 Hz, 1H), 7.24 (dd, *J* = 8.5, 2.5 Hz, 1H), 6.61 (d, *J* = 8.5 Hz, 1H), 4.64 (s, 2H), 4.28 (q, *J* = 7.5 Hz, 2H), 3.41 (m, *J* = 7.0 Hz, 1H), 1.31 (t, *J* = 7.5 Hz, 3H), 1.26 (d, *J* = 6.5 Hz, 6H) ppm.

Ethyl 2-(2-sec-butyl-4-bromophenoxy)acetate (5c)

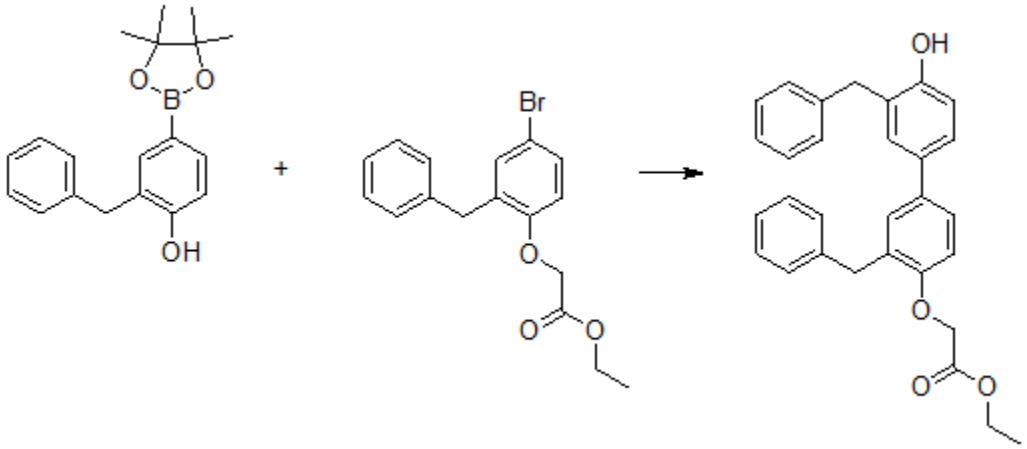
Same procedure as **5e**. Product was isolated in 92% yield as a pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$-d): δ 7.28 (d, *J* = 2.5 Hz, 1H), 7.22 (dd, *J* = 9 Hz, 2.5 Hz, 1H), 6.60 (d, *J* = 9 Hz, 1H), 4.60 (s, 2H), 4.26 (q, *J* = 7 Hz, 2H), 3.16 (m, 1H), 1.60 (m, 2H), 1.29 (t, *J* = 7.5 Hz, 3H), 1.21 (d, *J* = 7 Hz, 3H), 0.86 (t, *J* = 7.5 Hz, 3H) ppm.

Ethyl 2-(2-tert-butyl-4-bromophenoxy)acetate (5d)

Same procedure as **5e**. Product was isolated in 82% yield as a pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$-d): δ 7.41 (d, *J* = 2.5 Hz, 1H), 7.27 (dd, *J* = 9 Hz, 2.5 Hz, 1H), 6.62 (d, *J* = 8 Hz, 1H), 4.63 (s, 2H), 4.29 (q, *J* = 7 Hz, 2H), 1.42 (s, 9H), 1.32 (t, *J* = 7 Hz, 3H) ppm.

Representative procedure for the synthesis of biphenyl phenols.

Preparation of Ethyl 2-(1,1'-biphenyl-3,3'-dibenzyl-4'-ol-4-oxy) acetate (6e)



5e (0.210 g, 0.60 mmol), $PdCl_2(PPh_3)_2$ (0.026 g, 6 mol %), PPh_3 (0.010 g, 6 mol %), and 2M aqueous sodium carbonate (0.8 mL, 1.2 mmol) were added to the reaction vessel which was vacuumed and flushed with argon. Tetrahydrofuran and water (7 mL : 2 mL) were degassed with argon and added via syringe. The reaction was heated to reflux and stirred for 30 minutes. **4e** (0.224 g, 0.72 mmol), dissolved in 3 mL tetrahydrofuran, was then added and the reaction was stirred at reflux, overnight, under an inert atmosphere. Activated carbon was added to the crude solution and the resultant mixture was filtered through Celite and solvent was evaporated to dryness. The concentrate was dissolved in ethyl acetate, washed with water (x2) and brine (x2), dried over magnesium sulfate, and concentrated. The mixture was purified by flash chromatography on silica gel (Hexane/EtOAc, 80:20) to afford 0.165 g (61%) of the desired product as a white solid (m.p. 142-144 ºC). ^{1}H NMR (500 MHz, CDCl3): δ =

7.34-7.27 (m, 12H), 7.24 (t, *J* = 7.5 Hz, 1H) 7.20 (t, *J* = 6.5 Hz, 1H), 6.83 (d, *J* = 7.0 Hz, 1H), 6.81 (d, *J* = 8.5 Hz, 1H), 4.64 (s, 2H), 4.30 (q, *J* = 7.0 Hz, 2H), 4.11 (s, 2H), 4.05 (s, 2H), 1.33 (t, *J* = 7.5 Hz, 3H) ppm.

Ethyl 2-(1,1'-biphenyl-3,3'-diisopropyl-4'-ol-4-oxy) acetate (6b)

Same procedure as **6e.** Product was isolated in 49% yield as a white solid (m.p. 111-113 ºC). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.40 (d, *J* = 2 Hz, 1H), 7.36 (d, *J* = 2.5 Hz, 1H), 7.29 (dd, *J* = 8.0, 2.5 Hz, 1H), 7.25 (dd, *J* = 7.8, 2.3 Hz, 1H), 6.80 (d, *J* = 6.5 Hz, 1H), 6.77 (d, *J* = 7.0 Hz, 1H), 4.68 (s, 2H), 4.29 (q, *J* = 7.0 Hz, 2H), 3.46 (7let, *J* = 7.0 Hz, 1H), 3.26 (7let, *J* = 7.2 Hz, 1H), 1.32 (d, *J* = 6.5 Hz, 6H), 1.32 (t, *J* = 7.0 Hz, 3H), 1.30 (d, *J* = 8 Hz, 6H) ppm

Ethyl 2-(1,1'-biphenyl-3,3'-disec-butyl-4'-ol-4-oxy) acetate (6c)

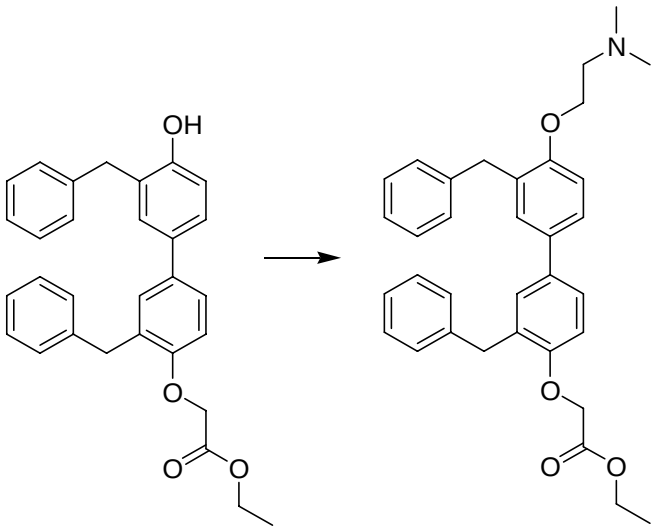
Same procedure as **6e.** Product was isolated in 27% yield as a white solid (m.p. 119-121 ºC). ^{1}H NMR (500 MHz, $CDCl_3$-d): δ 7.36 (d, *J* = 2 Hz, 1H), 7.32 (d, *J* = 2 Hz, 1H), 7.30 (dd, *J* = 8.5 Hz, 2.5 Hz, 1H), 6.82 (d, *J* = 7.5 Hz, 1H), 6.78 (d, *J* = 8 Hz, 1H), 4.73 (s, 1H), 4.67 (s, 2H), 4.29 (q, *J* = 7 Hz, 2H), 3.23 (m, 1H), 3.01 (m, 1H), 1.73 (m, 2H), 1.65 (m, 2H), 1.31 (m, 9H), 0.93 (t, *J* = 7.5 Hz, 3H), 0.91 (t, *J* = 7.5 Hz, 3H) ppm.

Ethyl 2-(1,1'-biphenyl-3,3'-ditert-butyl-4'-ol-4-oxy) acetate (6d)

Same procedure as **6e.** Product was isolated in 32% yield as a white solid (m.p. 174-177 ºC). ^{1}H NMR (500 MHz, $CDCl_3$-d): δ 7.48 (d, *J* = 2.5 Hz, 1H), 7.44 (d, *J* = 2.5 Hz, 1H), 7.32 (dd, *J* = 2 Hz, 8.5 Hz, 1 H), 7.25 (dd, *J* = 2 Hz, 8.5 Hz, 1H), 6.78 (d, *J* = 8 Hz, 1H), 6.73 (d, *J* = 7.5, 1H), 4.68 (s, 2H), 4.30 (q, *J* = 7 Hz, 2H), 1.48 (s, 9H), 1.46 (s, 9H), 1.33 (t, *J* = 7.5 Hz, 3H) ppm.

Representative procedure for the synthesis of the dimethylaminoethoxy ethyl ester

Preparation of Ethyl 2-(1,1'-biphenyl-3,3'-dibenzyl-4'-[2-(dimethylamino) ethoxy]-4-oxy) acetate (7e)



A mixture containing **6e** (81 mg, 0.18 mmol), 2-(dimethylamino)ethyl chloride hydrochloride (83 mg, 0.57 mmol), and potassium carbonate (132 mg, 0.95 mmol) in acetone (15 mL) was heated at reflux overnight. The reaction was monitored by thin layer chromatography using C18 plates. The reaction mixture was concentrated under reduced pressure. The resultant solid was then partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over magnesium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography on C18 gel (Hexane/EtOAc) to yield 55 mg (60%) of the desired product as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.31-7.16 (m, 14H), 6.87 (d, *J* = 9 Hz, 1H), 6.76 (d, *J* = 8.5 Hz, 1H), 4.61 (s, 2H), 4.26 (q, *J* = 7.5 Hz, 2H), 4.10 (t, *J* = 5.8 Hz, 2H), 4.08 (s, 2H), 4.00 (s, 2H), 2.76 (t, *J* = 5.5 Hz, 2H), 2.34 (s, 6H), 1.30 (t, *J* = 7.3 Hz, 3H) ppm; ^{13}C NMR (75.5 MHz , $CDCl_3$): δ = 169.2, 155.9, 155.1, 141.2, 141.0, 134.6, 133.6, 130.8, 130.1, 129.6, 129.4, 129.3, 129.2, 129.0 128.8, 128.7, 128.3, 126.3, 126.0, 125.8, 125.6, 112.2, 112.1, 111.9, 111.7, 66.5, 66.1, 61.5, 58.3, 46.0, 45.7, 36.9, 36.4, 14.1 ppm.

Ethyl 2-(1,1'-biphenyl-3,3'-diisopropyl-4'-[2-(dimethylamino) ethoxy]-4-oxy) acetate (7b)

Same procedure as **7e**. Product was isolated in 51% yield as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.40 (d, *J* = 2.5 Hz, 1H), 7.37 (d, *J* = 3.0 Hz, 1H), 7.30 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.29 (dd, *J* =8.5, 2.5 Hz, 1H), 6.89 (d, *J* = 8.5 Hz, 1H), 6.76 (d, *J* = 8.5 Hz, 1H), 4.66 (s, 2H), 4.27 (q, *J* = 8.5 Hz, 2H), 4.13 (t, *J* = 5.8 Hz, 2H), 3.45 (7let, *J* = 7 Hz, 1H), 3.37 (7let, *J* = 7 Hz, 1H), 2.81 (t, *J* = 5.8 Hz, 2H), 2.38 (s, 6H), 1.30 (t, *J* = 7.5 Hz, 3H), 1.29 (d, *J* = 7 Hz, 6H), 1.26 (d, *J* = 7 Hz, 6H) ppm; ^{13}C NMR (75.5 MHz , $CDCl_3$): δ = 169.4, 155.4, 154.5 ,137.9, 137.5, 135.3, 134.1, 125.5, 125.1 ,125.1, 125.0, 111.8, 111.8, 67.1, 66.1, 61.5, 58.7, 46.3, 27.4, 27.2, 23.0, 22.9, 14.4 ppm.

Ethyl 2-(1,1'-biphenyl-3,3'-disec-butyl-4'-[2-(dimethylamino) ethoxy]-4-oxy) acetate (7c)

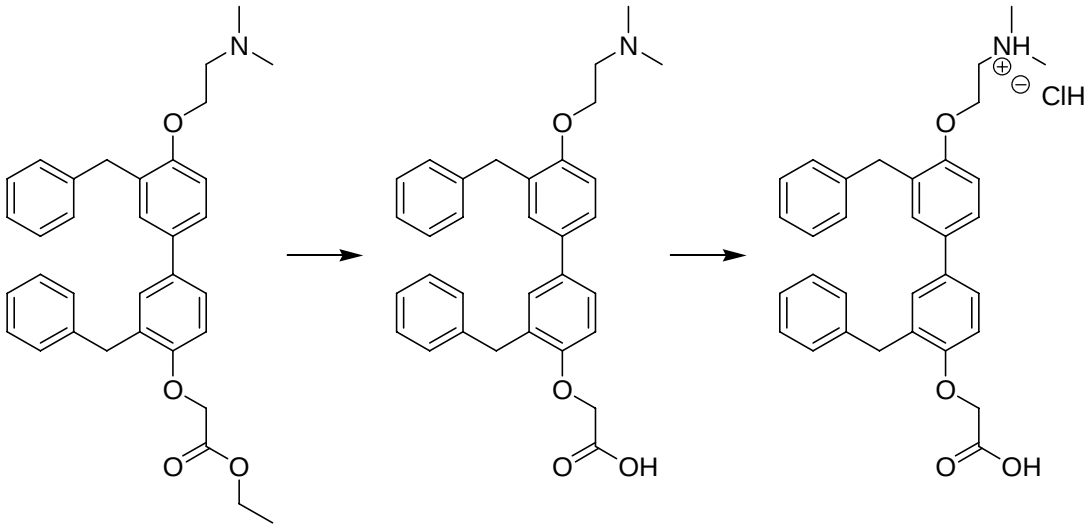
Same procedure as **7e.** Product was isolated in 50% yield as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$-d): δ 7.35 (d, *J* = 2 Hz, 1H), 7.30 (m, 3H), 6.89 (d, *J* = 8.5 Hz, 1H), 6.76 (d, *J* = 8 Hz, 1H), 4.65 (s, 2H), 4.27 (q, *J* = 7.5 Hz, 2H), 4.11 (t, *J* = 6 Hz, 2H), 3.22 (m, 1H), 3.14 (m, 1H), 2.78 (t, *J* = 6 Hz, 2H), 2.37 (s, 6H), 1.71 (m, 2H), 1.59 (m, 2H), 1.27 (m, 9H), 0.89 (t, *J* = 7.5Hz, 3 H), 0.88 (t, *J* = 7.5 Hz, 3H) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$-d): δ 169.42, 155.76, 154.80, 136.86, 136.53, 135.16, 133.96, 126.14, 125.74, 125.03, 124.95, 111.92, 111.85, 67.25, 66.13, 61.44, 58.68, 46.35, 34.28, 34.00, 30.18, 30.08, 20.67, 14.40, 12.54, 12.51 ppm.

Ethyl 2-(1,1'-biphenyl-3,3'-ditert-butyl -4'-[2-(dimethylamino) ethoxy]-4-oxy) acetate (7d)

Same procedure as **7e.** Product was isolated in 54% yield as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$-d): δ 7.48 (s, 1H), 7.45 (s, 1H), 7.33 (d, 1H), 7.31 (d, 1H), 6.93 (d, *J* = 8.5 Hz, 1H), 6.77 (d, *J* = 8 Hz, 1H), 4.67 (s, 2H), 4.29 (q, *J* = 7.5 Hz, 2H), 4.15 (t, *J* = 6.5, 2H), 2.84 (t, *J* = 6 Hz, 2H), 2.37 (s, 6H), 1.47 (s, 9H), 1.43 (s, 9H), 1.32 (t, *J* = 7 Hz, 3H) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$-d): δ 13.15, 28.75, 28.82, 33.92, 33.98, 45.11, 57.47, 60.24, 64.40, 65.62, 110.98, 111.45, 124.16, 124.27, 124.64, 124.95, 132.53, 133.59, 137.30, 137.64, 154.59, 155.78, 167.97 ppm.

Representative procedure for the synthesis of the carboxylic acid and the hydrochloride salt.

Preparation of 2-(1,1'-Biphenyl-3,3'-dibenzyl-4'-[2-(dimethylamino) ethoxy]-4-oxy) acetic acid (1e)



1 N aqueous sodium hydroxide (0.10 mL, 0.10 mmol) was added to a solution of **7e** (25 mg, 0.048 mmol) in methanol or ethanol (3 mL). The solution was heated to 40

°C for 2-4 hours. The reaction was monitored by thin layer chromatography using C18 plates. Upon completion, the solution was concentrated under reduced pressure. The residue was acidified with 1 N hydrochloric acid (25 mL) and extracted with ethyl acetate. The organic layer was then washed with water and brine, dried with magnesium sulfate, filtered and concentrated under reduced pressure yielding the carboxylic acid (21 mg, 89%)

The carboxylic acid of **7e** (21 mg, 0.042 mmol) was dissolved in ethyl acetate or dioxane (2 mL). To this solution was added 4 N hydrochloric acid in dioxane or ethyl acetate (3 mL). The reaction was allowed to stir at ambient temperature for 12-24 hours. The solvent was removed under reduced pressure. The resulting solid was rinsed with a minimal volume of ethyl acetate and hexanes, and the hydrochloride salt was collected by filtration (9 mg, 40%). ^{1}H NMR (500 MHz, CD_3OD): δ = 7.27 (s, 1H), 7.25 (s, 1H), 7.22-7.11 (m , 10H), 7.06 (s, 1H), 7.05 (s, 1H), 6.78 (d, *J* = 8.5 Hz, 1H), 6.74 (d, *J* = 9.0 Hz, 1H), 4.50 (s, 2H), 4.10 (t, *J* = 5.3 Hz, 2H), 4.04 (s, 2H), 3.94 (s, 2H), 3.34 (t, 2H), 2.72 (s, 6H) ppm

2-(1,1'-Biphenyl-3,3'-diisopropyl-4'-[2-(dimethylamino) ethoxy]-4-oxy) acetic acid (1b)

Same procedure as **1e**. Hydrolysis resulted in 100 % conversion. ^{1}H NMR (500 MHz, CD_3OD): δ = 7.37 (d, *J* = 1.5 Hz, 1H), 7.36 (d, *J* = 2 Hz, 1H), 7.22 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.17 (dd, *J* = 8.5, 2.5 Hz, 1H), 6.88 (d, *J* = 8.5 Hz, 1H), 6.82 (d, *J* = 8.0 Hz, 1H), 4.57 (s, 2H), 4.33 (t, *J* = 4.5 Hz, 2H), 3.61 (t, *J* = 4.5 Hz, 2H), 3.49 (7let, *J* = 6.8 Hz, 1H), 3.39 (7let, *J* = 6.8 Hz, 1H), 3.01 (s, 6H), 1.27 (d, *J* = 7.0 Hz, 6H), 1.24 (d, *J* = 7.5 Hz, 6H) ppm. Further conversion to the hydrochloride salt proceeded in 59 % yield.

2-(1,1'-Biphenyl-3,3'-disec-butyl-4'-[2-(dimethylamino) ethoxy]-4-oxy) acetic acid (1c)

Same procedure as **1e.** Hydrolysis resulted in 72% isolated yield. ^{1}H NMR (500 MHz, $CDCl_3$-d): δ 7.34 (m, 4H), 7.07 (d, *J* = 8.5 Hz, 1H), 6.90 (d, *J* = 8 Hz, 1H), 4.70 (s, 2H), 4.41 (br s, 2H), 3.68 (br s, 2H), 3.24 (m, 2H), 3.06 (s, 6H), 1.70 (m, 4H), 1.28 (d, *J* = 4.5 Hz, 2H), 1.27 (d, *J* = 4 Hz, 2H), 0.90 (t, *J* = 7.5 Hz, 3H), 0.89 (t, *J* = 7.5 Hz, 3H) ppm. Further conversion to the hydrochloride salt proceeded in 40% yield.

2-(1,1'-biphenyl-3,3'-ditert-butyl-4'-[2-(dimethylamino) ethoxy]-4-oxy) acetic acid (1d)

Same procedure as **1e.** Hydrolysis resulted in 89% isolated yield. ^{1}H NMR (500 MHz, $CDCl_3$-d): δ 7.37 (d, *J* = 1.5 Hz, 1H), 7.34 (d, *J* = 2.5 Hz, 1H), 7.26 (dd, *J* = 8.5 Hz, 2.5 Hz, 1H), 7.20 (dd, *J* = 8.5 Hz, 2 Hz, 1H), 6.98 (d, *J* = 8 Hz, 1 H), 6.79 (d, *J* = 8.5 Hz, 1H), 4.57 (s, 2H), 4.33 (br t, *J* = 4.5 Hz, 2H), 3.58 (br t, *J* = 5 Hz, 2H), 2.95 (s, 6H), 1.36 (s, 9H), 1.35 (s, 9H) ppm. Further conversion to the hydrochloride salt proceeded in 79% yield.

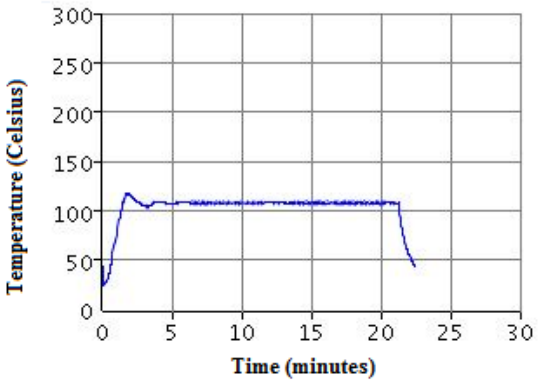
Synthesis of compound 1a.

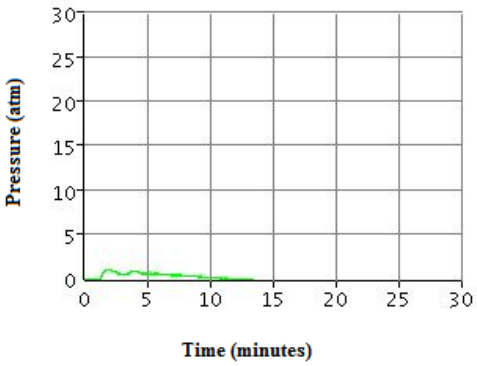
Preparation of Ethyl 2-(4-iodophenoxy)acetate (5a)

4-Iodophenol (1.1 g, 5 mmol) was dissolved in dry THF (45 mL). Sodium hydride, (60 % in oil, 0.28 g, 7 mmol) was added and the solution was stirred at room temperature for 30 minutes. Ethyl bromoacetate (1.2 g, 7 mmol) was then added slowly and the solution was stirred for 1.5 hours at room temperature. The reaction mixture was diluted with ethyl acetate and washed with water (x2). After drying over magnesium sulfate, the mixture was purified by column chromatography on silica gel (Hexane/EtOAc, 80:20) to afford 1.02 g (67%) of the desired product as a solid (m.p. 57-61 ºC). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.57 (d, *J* = 7 Hz, 2H), 6.69 (d, *J* = 6.5 Hz, 2H), 4.59 (s, 2H), 4.27 (q, *J* = 7Hz, 2 H), 1.30 (t, *J* = 6.5 Hz, 3H) ppm.

Preparation of Ethyl 2-(1,1'-biphenyl-4'-ol-4-oxy) acetate (6a)

2-(4-Iodo-phenyl-)-4-oxy) acetic acid (0.100g, 1equiv) and 4-hydroxyphenylboronic acid (0.0541g, 1.2 equiv) were combined with $PdCl_2(PPh_3)_2$ (0.0230g, 10 mol %), triphenyl phosphine (0.00858g, 10 mol %) and 2M sodium carbonate (0.0858g) in a sealed vial and dissolved in 4mL THF and 1mL H_2O. The reaction underwent microwave irradiation at 110^{o}C for 20 minutes using a Biotage Initiator (TM) 2.0. The crude reaction mixture was concentrated and purified by flash chromatography on silica gel(Hexane/EtOAc 90:10) to afford 0.030g (34%) of the desire product as a white solid (m.p. 131-133 ºC). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.45 (d, *J* = 7 Hz, 2H), 7.41 (d, *J* = 6.5 Hz, 2H), 6.95 (d, *J* = 6.5 Hz, 2H), 6.87 (d, *J* = 6.5 Hz, 2H), 4.97 (s, 1H), 4.66 (s, 2H), 4.29 (q, *J* = 6.5 Hz, 2H), 1.31 (t, *J* = 7.5 Hz, 3H) ppm





Preparation of Ethyl 2-(1,1'-biphenyl-4'-[2-(dimethylamino) ethoxy]-4-oxy) acetate (7a)

Same procedure as **7e.** Product was isolated in 74 % yield as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.47 (d, *J* = 7.0 Hz, 2H), 7.46 (d, *J* = 6.5 Hz, 2H), 6.97 (d, *J* = 6.0 Hz, 2H), 6.95 (d, *J* = 6.0 Hz, 2H), 4.65 (s, 2H), 4.29 (q, *J* = 7.5 Hz, 2H), 4.12 (t, *J* = 6.0 Hz, 2H), 2.79 (t, *J* = 5.5 Hz, 2H), 2.39 (s, 6H), 1.31 (t, *J* = 7.5 Hz, 3H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 207.2, 158.2, 157, 134.8, 134, 128.1, 128.0, 115.2, 115.1, 65.8, 61.7, 58.5, 46.0, 31.2, 14.3 ppm

Preparation of 2-(1,1'-Biphenyl-4'-[2-(dimethylamino) ethoxy]-4-oxy) acetic acid (1a)

1a was isolated as a sodium salt by stopping the procedure of **1e** after the initial hydrolysis step and recovering the salt from ethyl acetate.

Biological Methods

TR-FRET CBI Assay

This assay was performed as previously described.[1] Briefly, an N-terminally His-tagged ligand binding domain of ERα (residues 304-554; C381, 530S)[2] was expressed, purified and labeled with biotin-maleimide (Quanta BioDesign) through the one remaining reactive cysteine (Cys417) while bound to a nickel column. The SRC3 nuclear receptor domain (NRD) (residues 627-829) includes all three NR-boxes and was non-specifically labeled through four available cysteines using 5-iodoacetamido-fluorescein (Invitrogen).

The ER was subsequently tagged with a streptavidin-terbium complex (FRET donor, λ_{em} = 495 nm) and activated with a high concentration of agonist ligand estradiol to promote recruitment of fluorescein SRC3 (FRET acceptor, λ_{em} = 520 nm) and production of a high FRET signal. The biphenyl compounds were then serially diluted and added to each well of the assay mixture to test the ability of these compounds to disrupt the receptor/coactivator interaction and produce a subsequent decrease in FRET. The final concentrations of the reagents were as follows: ERα-417 (2 nM), streptavidin−terbium (0.5 nM), estradiol (1 μM), test compound (0−1 mM), SRC3-NRD (50 nM).

A 15-mer SRC1-Box II peptide and a published guanylhydrazone coactivator binding inhibitor compound[3] were used as positive controls in the TR-FRET and reporter gene assays, respectively. The compounds showed modest activity in this assay;

[1] Parent, A.A., Gunther, J.R., Carlson, K.E., Katzenellenbogen, J.A. *J. Med. Chem.* **2008**, *51*, 6512-6530.
[2] Tamrazi, A., Carlson, K.E., Daniels, J.R., Hurth, K.M., Katzenellenbogen, J.A. *Mol. Endocrinol.* **2002**, *16*, 2706-2719.
[3] Lafrate, A.L., Gunther, J.R., Carlson K.E., Katznellenbogen, J.A *Bioorganic& Medicinal Chemistry.* **2008**, 16, 10075-10084.

compound **1c** produced the most complete displacement of coactivator with a K_i of 33μM in the TR-FRET assay.

Radiometric Binding Assay

Performed as previously described.[4] Relative binding affinities (RBA) were determined using 2 nM [^{3}H]estradiol as tracer ([2,4,6,7-^{3}H]estra-1,3,5,(10)-triene-3,17β-diol, 89 Ci/mmol, Amersham/GE Healthcare Bio-Sciences Corporation, Piscataway, NJ) and purified full-length human ERα and ERβ receptors (PanVera/Invitrogen, Carlsbad, CA). Incubations were for 18–24 h at 0 °C. Hydroxyapaptite (Bio-Rad, Hercules, CA) was used to absorb the receptor–ligand complexes, and free ligand was washed away. The binding affinities are expressed as relative binding affinity (RBA) values with that of estradiol set to 100%. Estradiol binds to ERα with a K_d of 0.2 nM and to ERβ with a K_d of 0.5 nM.

Compound	ERα RBA	ERβ RBA
1a	<0.001	<0.001
1b	<0.001	<0.001
1c	~0.001	~0.001
1d	0.055 0.038 0.049 ± 0.016	0.018 0.017 0.018 ± 0.001
1e	<0.001	~0.001

The most promising CBI, compound **1c**, shows insignificant binding as a ligand and should not be considered a traditional antagonist.

Luciferase Reporter Gene Assay

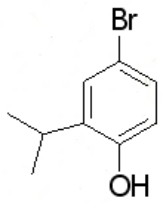
Human endometrial cancer (HEC-1) cells were maintained in culture as described and transfected in 24-well plates.[5] A mixture of HBSS (50 μL/well), Holo-transferrin (Sigma T1408) (20 μL/well), and lipofectin (Invitrogen #18292-011) (5 μL/well) were incubated at room temperature for 5 min. The DNA mixture was made by adding 200 ng of pCMVβ-galactosidase as internal control, 500 ng of the estrogen-responsive reporter gene plasmid 2ERE Luc, and 100 ng of full-length ERα expression vector with 75 μL HBSS per well and, after addition to the first mixture, allowed to incubate for 20 min at room temperature. The cell media were changed to Opti-MEM (350 μL/well) and 150 μL of the transfection mixture was added to each well. The cells were incubated at 37 °C in a 5% CO_2 containing incubator for 6 h. The medium was then replaced with fresh medium containing 5% charcoal–dextran-treated calf serum and the desired concentrations of

[4] Carlson, K.E., Choi, I., Gee, A., Katzenellenbogen, B.S., Katzenellenbogen, J.A. *Biochemistry*. **1997**, *36*, 14897-14905.

[5] J. Sun, M.J. Meyers, B.E. Fink, R. Rajendran, J.A. Katzenellenbogen and B.S. Katzenellenbogen, *Endocrinology* **140** (1999), p. 800.

compounds. Reporter gene activity was assayed at 24 h after compound addition. Luciferase activity, normalized for the internal control β-galactosidase activity, was assayed as previously described. In the initial screen, antagonist activity was determined at four concentrations, ranging from 20 to 0.6 μM, in the presence of 10^{-9} M estradiol (E_2).

3b



STANDARD PROTON PARAMETERS

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Ambient temperature
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Acq. time 2.048 sec
Width 8000.0 Hz
16 repetitions
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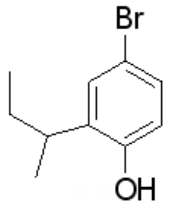
DATA PROCESSING
FT size 32768
Total time 0 min, 32 sec

AW-III-63-11

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
INOVA-500 "water500"

Pulse 45.0 degrees
Acq. time 2.048 sec
Width 8000.0 Hz
16 repetitions
OBSERVE H1, 499.7127366 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 32 sec

3c



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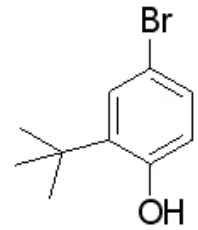
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
INOVA-500 "waters500"

Pulse 45.0 degrees
Acq. time 2.048 sec
Width 8000.0 Hz
16 repetitions
OBSERVE H1, 499.7127366 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 32 sec

3d



STANDARD PROTON PARAMETERS

PW-1-35

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Solvent: CDCl3
Ambient temperature
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INOVA-500 "waters500"

Pulse 45.0 degrees
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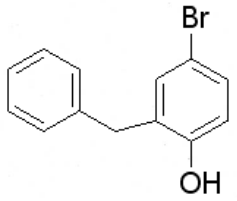
16 repetitions
OBSERVE H1, 499.7029798 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 32 sec

3e



STANDARD PROTON PARAMETERS

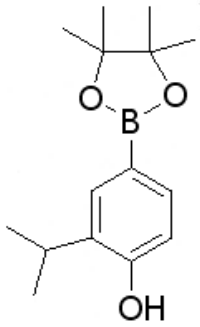
PW-1-71

Pulse Sequence: s2pul

Solvent: CDCl3
Ambient temperature
INOVA-500 "waters500"

Pulse 45.0 degrees
Acq. time 2.048 sec
Width 8000.0 Hz
16 repetitions
OBSERVE H1, 499.7029602 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 32 sec

4b

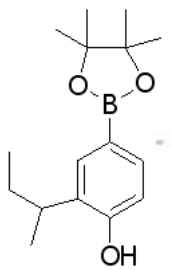


AW-III-64-14c

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
INOVA-500 "waters500"

Pulse 45.0 degrees
Acq. time 2.048 sec
Width 8000.0 Hz
16 repetitions
OBSERVE H1, 499.7127366 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 32 sec

4c



AW-III-69-28b

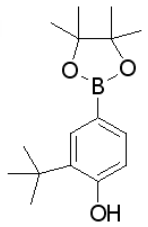
STANDARD PROTON PARAMETERS

Pulse Sequence: s2pul

Solvent: CDCl3
Ambient temperature
INOVA-500 "waters500"

Pulse 45.0 degrees
Acq. time 2.048 sec
Width 8000.0 Hz
16 repetitions
OBSERVE H1, 499.7127416 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 32 sec

4d



STANDARD PROTON PARAMETERS

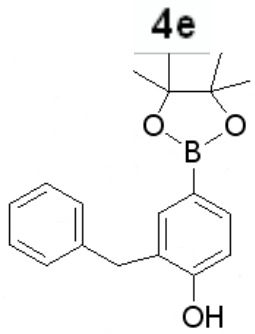
PW-1-75

Pulse Sequence: s2pul

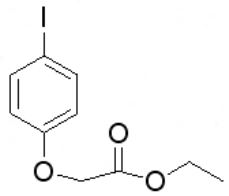
Solvent: CDCl3
Ambient temperature
INOVA-500 "waters500"

Pulse 45.0 degrees
Acq. time 2.048 sec
Width 8000.0 Hz
16 repetitions
OBSERVE H1, 499.7029602 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 32 sec

4e



5a



AW-III-81-53

STANDARD PROTON PARAMETERS

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
INOVA-500 "waters500"

Pulse 45.0 degrees
Acq. time 2.048 sec
Width 8000.0 Hz
16 repetitions
OBSERVE H1, 499.7029724 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 32 sec

STANDARD PROTON PARAMETERS

PW-1-44

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
INOVA-500 "waters500"

Pulse 45.0 degrees
Acq. time 2.048 sec
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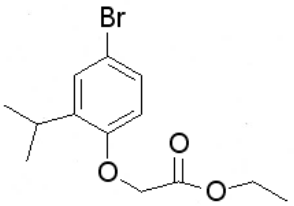
16 repetitions
OBSERVE H1, 499.7029602 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 32 sec

5b

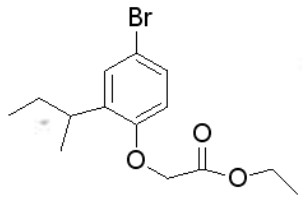


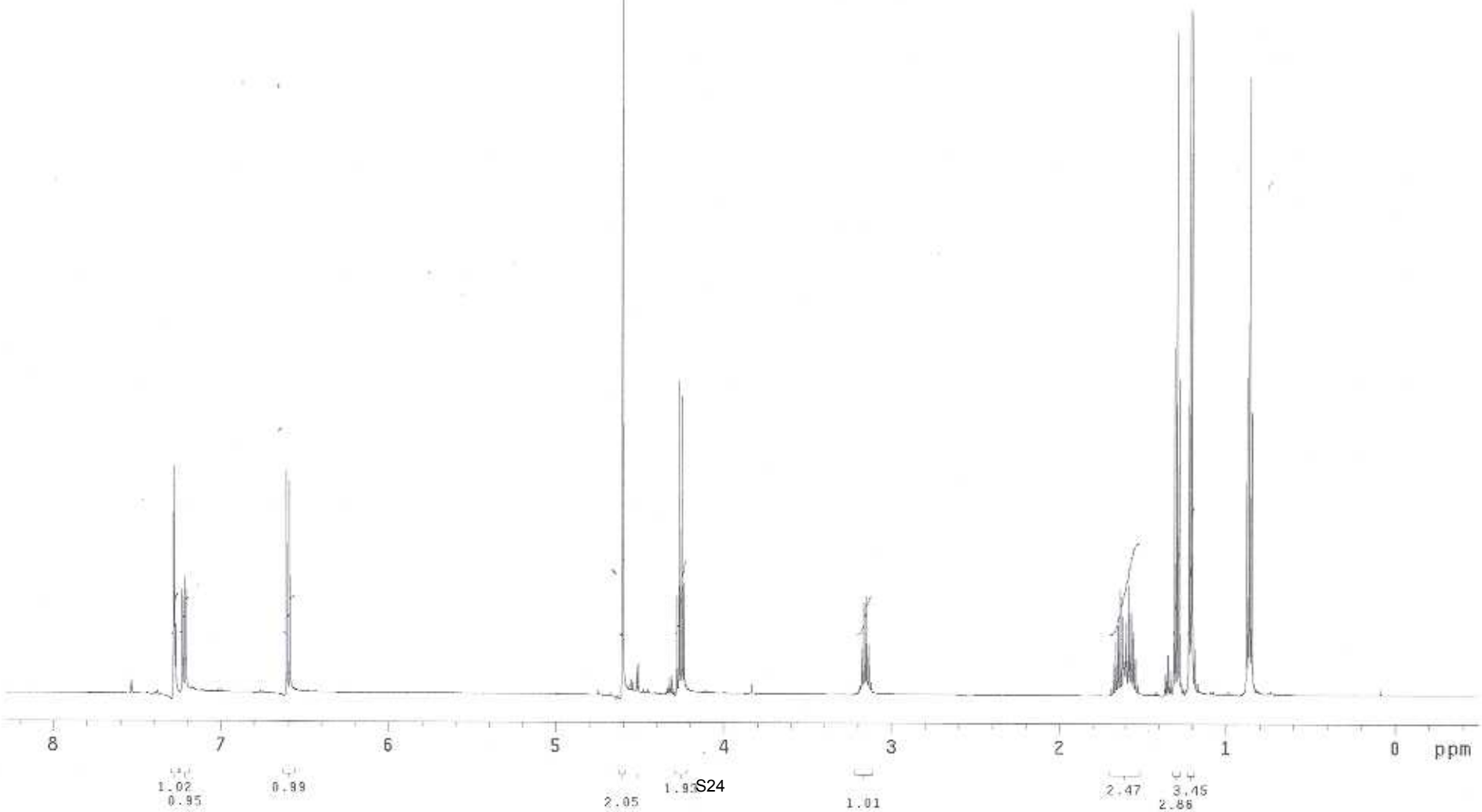
AW-III-89-71

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
INOVA-500 "waters500"

Pulse 45.0 degrees
Acq. time 2.048 sec
Width 8000.0 Hz
16 repetitions
OBSERVE H1, 499.7127366 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 32 sec

5c





AW-II-32-48a

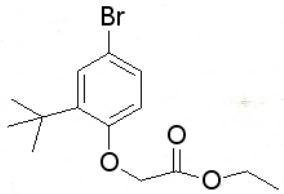
STANDARD PROTON PARAMETERS

Pulse Sequence: s2pul

Solvent: CDCl3
Ambient temperature
INOVA-500 "waters500"

Pulse 45.0 degrees
Acq. time 2.048 sec
Width 8000.0 Hz
16 repetitions
OBSERVE H1, 499.7029743 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 32 sec

5d



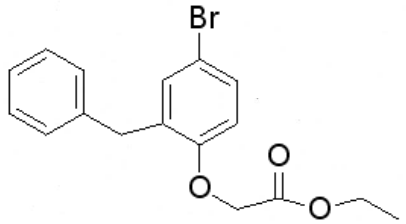
STANDARD PROTON PARAMETERS

PW-1-39

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
INOVA-500 "waters500"

Pulse 45.0 degrees
Acq. time 2.048 sec
Width 8000.0 Hz
16 repetitions
OBSERVE H1, 499.7029602 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 32 sec

5e



AW-III-84-62

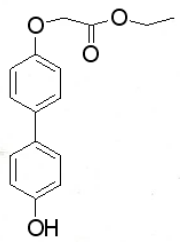
STANDARD PROTON PARAMETERS

Pulse Sequence: s2pul

Solvent: CDCl3
Ambient temperature
INOVA-500 "waters500"

Pulse 45.0 degrees
Acq. time 2.048 sec
Width 8000.0 Hz
16 repetitions
OBSERVE H1, 499.7029727 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 32 sec

6a



PW-1-74

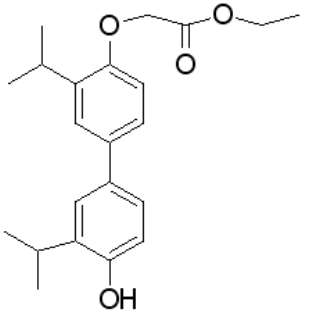
STANDARD PROTON PARAMETERS

Pulse Sequence: s2pul

Solvent: CDCl3
Ambient temperature
INOVA-500 "waters500"

Pulse 45.0 degrees
Acq. time 2.048 sec
Width 8000.0 Hz
16 repetitions
OBSERVE H1, 499.7127366 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 32 sec

6b



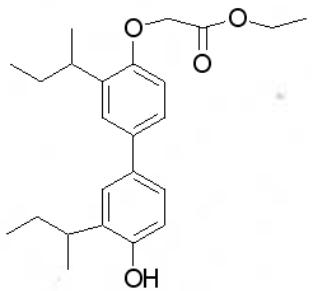
AW-III-78-43b

STANDARD PROTON PARAMETERS

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
INOVA-500 "waters500"

Pulse 45.0 degrees
Acq. time 2.048 sec
Width 8000.0 Hz
64 repetitions
OBSERVE H1, 499.7127317 MHz
DATA PROCESSING
FT size 32768
Total time 2 min, 11 sec

6c

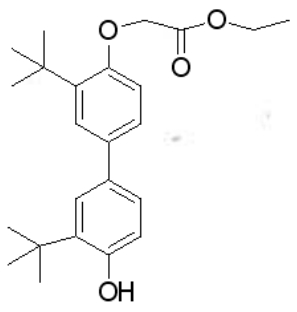


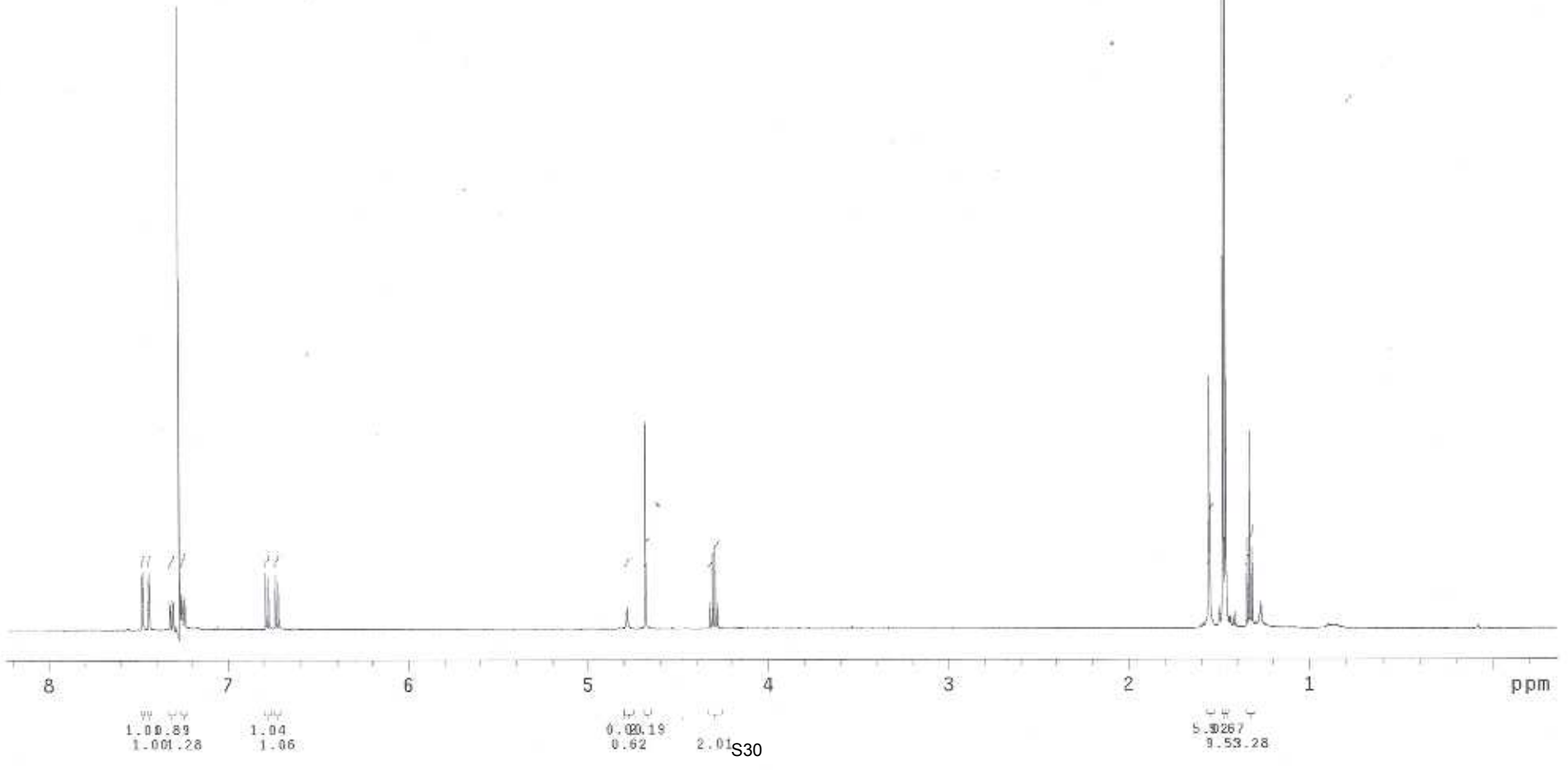
AW-III-73-34

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
INOVA-500 "waters500"

Pulse 45.0 degrees
Acq. time 2.048 sec
Width 8000.0 Hz
16 repetitions
OBSERVE H1, 499.7127366 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 32 sec

6d





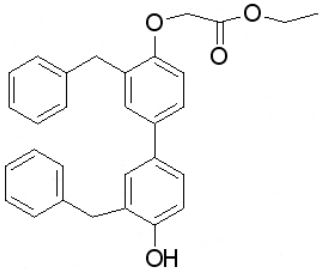
STANDARD PROTON PARAMETERS

PW-1-77

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
INOVA-500 "waters500"

Pulse 45.0 degrees
Acq. time 2.048 sec
Width 8000.0 Hz
16 repetitions
OBSERVE H1, 499.7029602 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 32 sec

6e



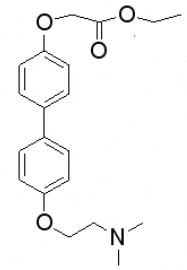
PW-I-82

STANDARD PROTON PARAMETERS

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
INOVA-500 "waters500"

Pulse 45.0 degrees
Acq. time 2.048 sec
Width 8000.0 Hz
16 repetitions
OBSERVE H1, 499.7029733 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 32 sec

7a

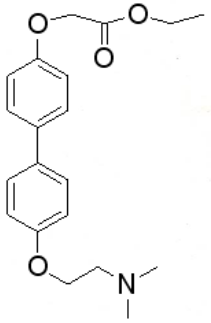


PW-1-82_C13

Weiser, Pat 8-20-09
Waters500:hanson

Pulse Sequence: s2pul

7a



PW-1-76
500 NMR

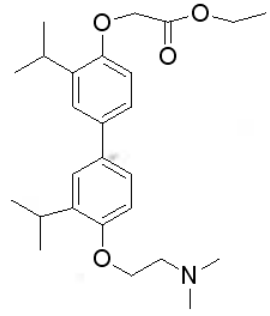
STANDARD PROTON PARAMETERS

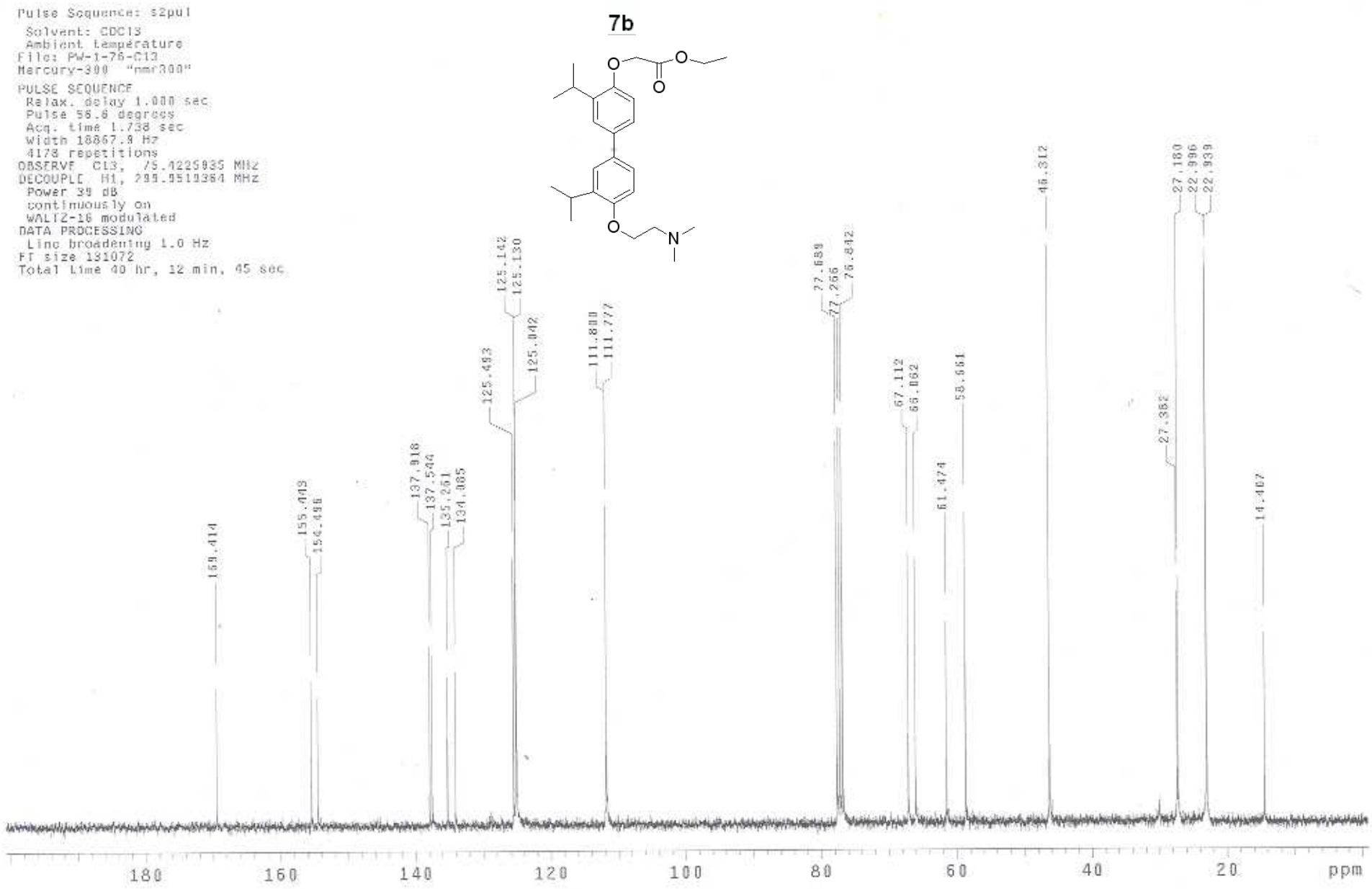
Pulse Sequence: s2pul

Solvent: CDCl3
Ambient temperature
INOVA-500 "waters500"

Pulse 45.0 degrees
Acq. time 2.048 sec
Width 8000.0 Hz
16 repetitions
OBSERVE H1, 499.7127455 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 32 sec

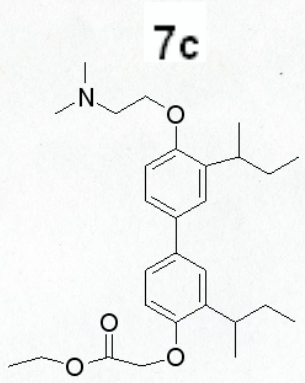
7b




Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
File: PW-1-76-C13
Mercury-300 "nmr300"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 56.6 degrees
Acq. time 1.738 sec
Width 18867.9 Hz
4178 repetitions
OBSERVE C13, 75.4225935 MHz
DECOUPLE H1, 299.9519364 MHz
Power 39 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 40 hr, 12 min, 45 sec
7b
O
O
O
O
N
159.414
155.043
154.496
137.918
137.544
135.261
134.985
125.483
125.142
125.130
125.042
111.880
111.777
77.689
77.266
76.842
67.112
66.862
61.474
58.661
46.312
27.382
27.180
22.996
22.939
14.467
180
160
140
120
100
80
60
40
20
ppm

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
INOVA-500 "waters500"

Pulse 45.0 degrees
Acq. time 2.048 sec
Width 8000.0 Hz
16 repetitions
OBSERVE H1, 499.7127440 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 32 sec



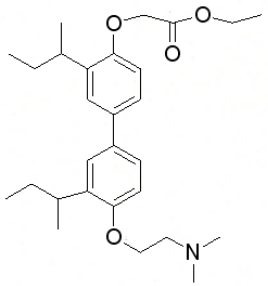
C13 AW-III-79-44a

sec-butyl product

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-300 "nmr300"

PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 56.6 degrees
Acq. time 1.738 sec
Width 18867.9 Hz
19524 repetitions
OBSERVE C13, 75.4225938 MHz
DECOUPLE H1, 299.9519364 MHz
Power 39 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 40 hr, 12 min, 45 sec

7c



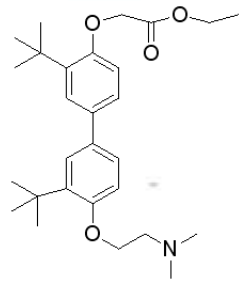
AW-111-77-41-post0A
500 NMR

STANDARD PROTON PARAMETERS

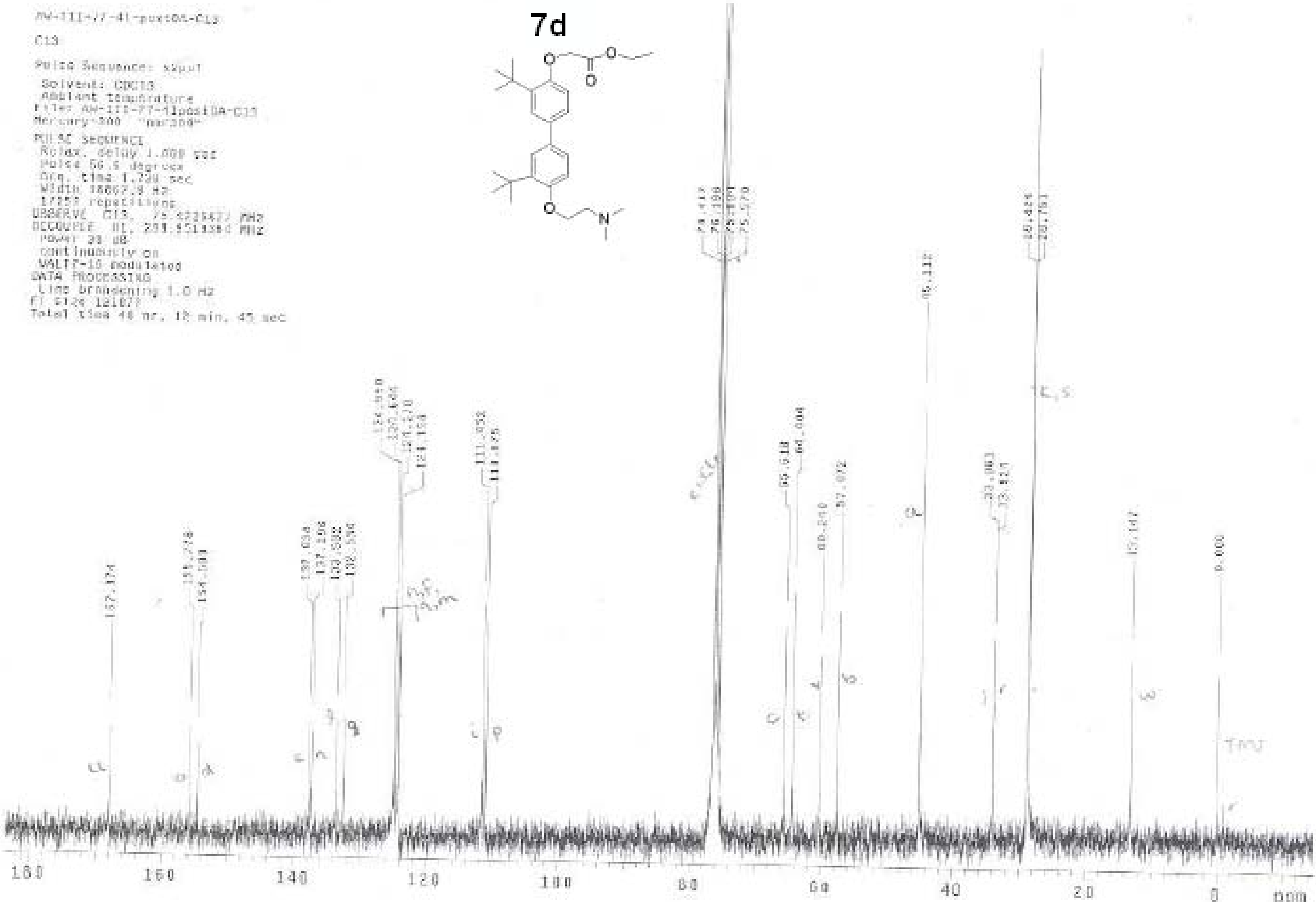
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
INOVA-500 "waters500"

Pulse 45.0 degrees
Acq. time 2.048 sec
Width 8000.0 Hz
2 repetitions
OBSERVE H1, 499.7127426 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 4 sec

7d



7d


C13
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-300
PULSE SEQUENCE
DATA PROCESSING
Line broadening 1.0 Hz
167.474
111.858
111.875
65.618
64.006
57.072
45.112
33.003
28.424
28.181
13.142
0.000
180
160
140
120
100
80
60
40
20
0
ppm

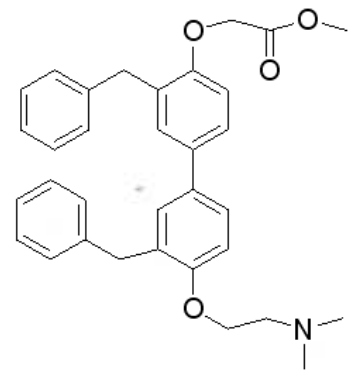
PW-1-69
500 NMR

STANDARD PROTON PARAMETERS

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
INOVA-500 "water500"

Pulse 45.0 degrees
Acq. time 2.048 sec
Width 8000.0 Hz
16 repetitions
OBSERVE H1, 499.7127421 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 32 sec

7e



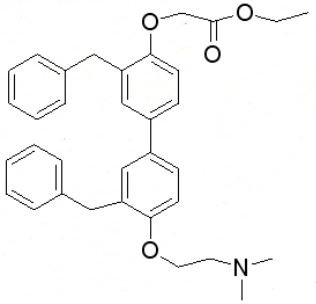
PW-1-78_C13_2

Pulse Sequence: s2pul

Solvent: CDCl3
Ambient temperature
Mercury-300 "nmr300"

PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 56.6 degrees
Acq. time 1.738 sec
Width 18867.9 Hz
21104 repetitions
OBSERVE C13, 75.4225938 MHz
DECOUPLE H1, 299.9519364 MHz
Power 39 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 31 hr, 39 min, 0 sec

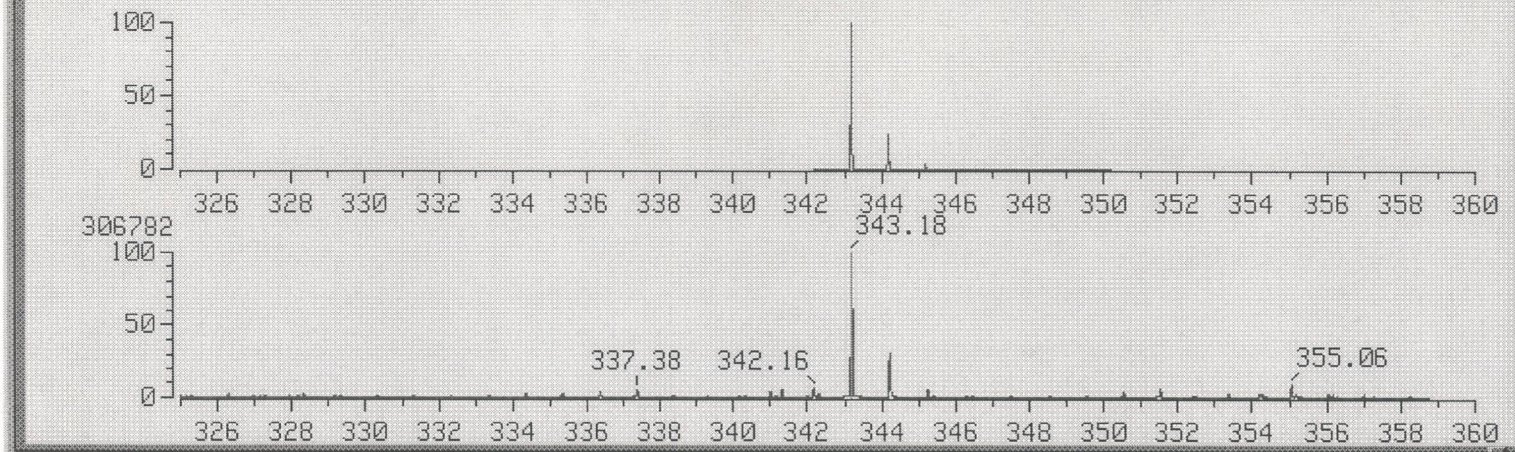
7e



Composition View

Edit Help

Spectrum #1 *1.00



Max Error (mmu) ≡	3	Max U.S. ≡	20.0
Min Error (mmu) ≡	3	Min U.S. ≡	-0.5
defect (ppm) ≡	3	Frg-CHK	Off
STD Error (mmu) ≡	3	STD-CHK	Off

Element	Max.	Min.	Isotope
C	40	0	1
H	50	0	1
O	5	0	1
N	2	0	1

Calculate Filter Cancel

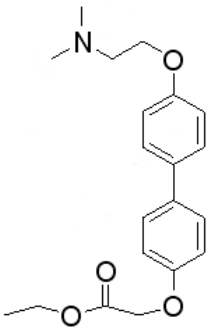
Edit Accept Delete Cancel

Format: Composition Formula | Type in | Print

Elements : C 40/0, H 50/0, O 5/0, N 2/0
Mass Tolerance : 3mmu
Unsaturation : -0.5 ~ 20.0

No.	m/z	Int%	Err-mmu	U.S.	Composition
1	343.1784	100.0	+0.0	9.0	$C_{20}H_{25}O_4N$
2			-2.7	13.5	$C_{23}H_{23}ON_2$

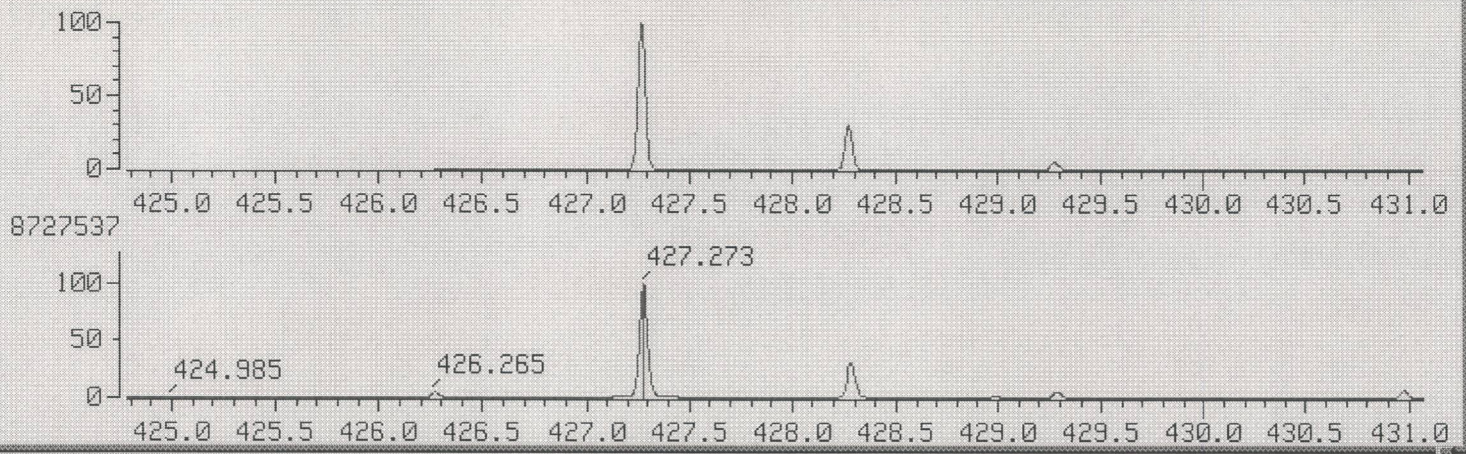
6a



Composition View

Edit Help

Spectrum #1 *0.79



Max Error (mmu) ≡	3	Max U.S. ≡	20.0
Min Error (mmu) ≡	3	Min U.S. ≡	−0.5
defect (ppm) ≡	3	Frg-CHK	Off
STD Error (mmu) ≡	3	STD-CHK	Off

Element	Max.	Min.	Isotope
C	40	0	1
H	50	0	1
O	5	0	1
N	2	0	1

Calculate Filter Cancel

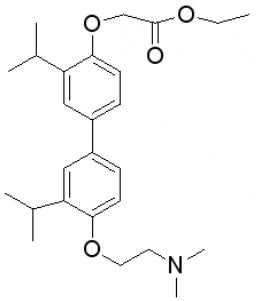
Edit Accept Delete Cancel

Format: Composition Formula | Type in | Print

Elements : C 40/0, H 50/0, O 5/0, N 2/0
Mass Tolerance : 3mmu
Unsaturation : −0.5 ~ 20.0

No.	m/z	Int%	Err-mmu	U.S.	Composition
1	427.2734	100.0	+1.2	9.0	$C_{26}H_{37}O_4N$
2			−1.5	13.5	$C_{29}H_{35}ON_2$

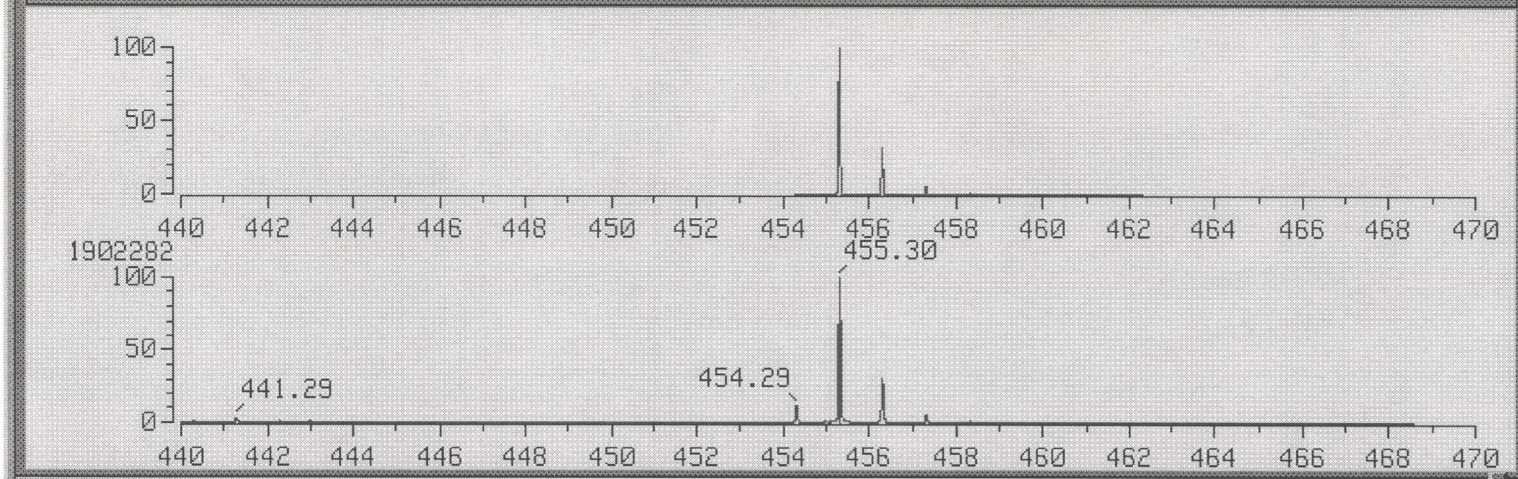
6b



Composition View

Edit Help

Spectrum #5 *1.00



Max Error (mmu) ≡	3	Max U.S. ≡	20.0
Min Error (mmu) ≡	3	Min U.S. ≡	-0.5
defect (ppm) ≡	3	Frg-CHK	Off
STD Error (mmu) ≡	3	STD-CHK	Off

Calculate Filter Cancel

Element	Max.	Min.	Isotope
C	40	0	1
H	50	0	1
O	5	0	1
N	2	0	1

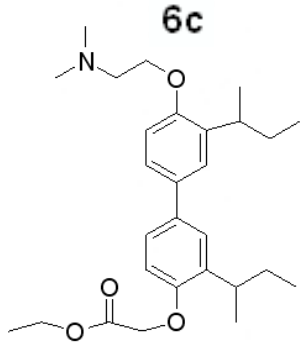
Edit Accept Delete Cancel

Format: Composition Formula | Type in | Print

Elements : C 40/0, H 50/0, O 5/0, N 2/0
Mass Tolerance : 3mmu
Unsaturation : -0.5 ~ 20.0

No.	m/z	Int%	Err-mmu	U.S.	Composition
1	455.3028	100.0	-0.8	9.0	$C_{28}H_{41}O_4N$

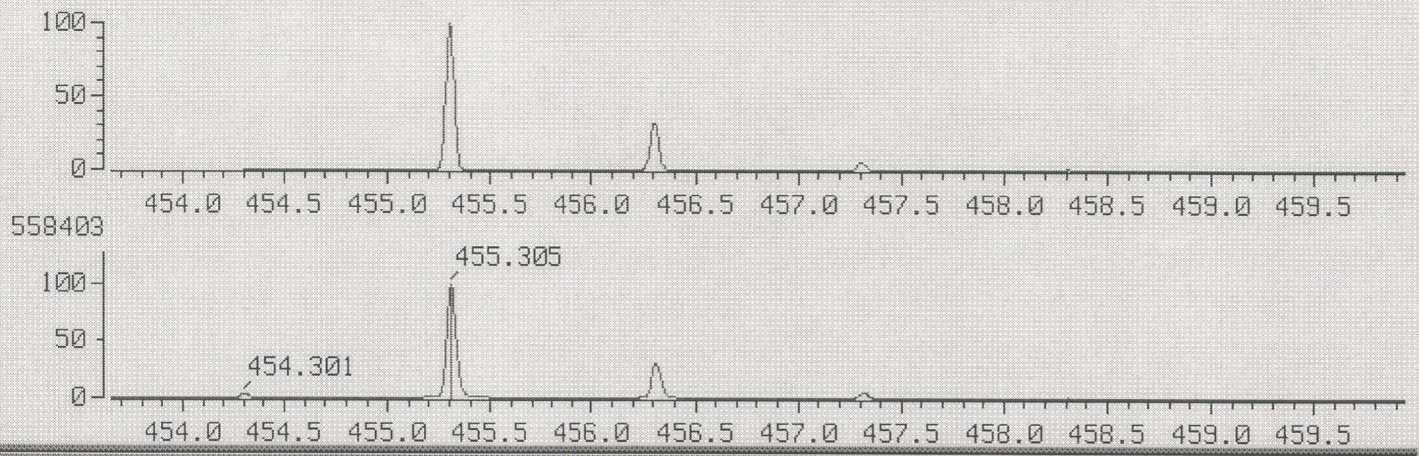
6c



Composition View

Edit Help

Spectrum #2 *0.79



Max Error (mmu) ≡	3	Max U.S. ≡	20.0
Min Error (mmu) ≡	3	Min U.S. ≡	-0.5
defect (ppm) ≡	3	Frg-CHK	Off
STD Error (mmu) ≡	3	STD-CHK	Off

Calculate Filter Cancel

Element	Max.	Min.	Isotope
C	40	0	1
H	50	0	1
O	5	0	1
N	2	0	1

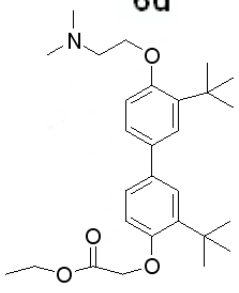
Edit Accept Delete Cancel

Format: Composition Formula | Type in | Print

Elements : C 40/0, H 50/0, O 5/0, N 2/0
Mass Tolerance : 3mmu
Unsaturation : -0.5 ~ 20.0

No.	m/z	Int%	Err-mmu	U.S.	Composition
1	455.3054	100.0	+1.8	9.0	$C_{28}H_{41}O_4N$
2			-0.8	13.5	$C_{31}H_{39}ON_2$

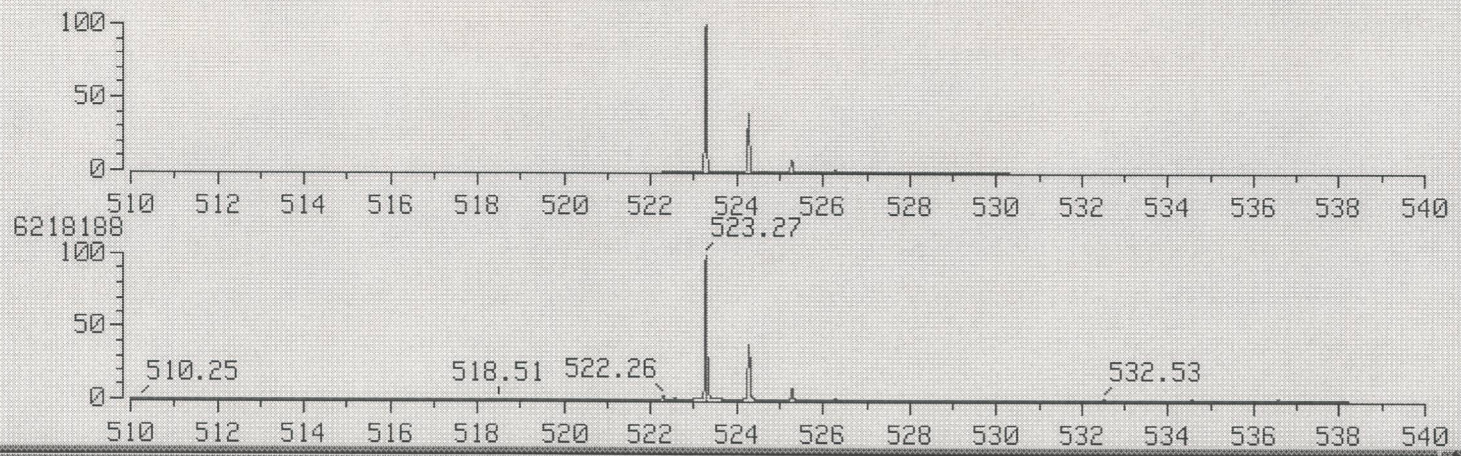
6d



Composition View

Edit Help

Spectrum #3 *1.00



Max Error (mmu) ≡	3	Max U.S. ≡	20.0
Min Error (mmu) ≡	3	Min U.S. ≡	−0.5
defect (ppm) ≡	3	Frg-CHK	Off
STD Error (mmu) ≡	3	STD-CHK	Off

Calculate Filter Cancel

Element	Max.	Min.	Isotope
C	40	0	1
H	50	0	1
O	5	0	1
N	2	0	1

Edit Accept Delete Cancel

Format Composition Formula Type in Print

Elements : C 40/0, H 50/0, O 5/0, N 2/0
Mass Tolerance : 3mmu
Unsaturation : −0.5 ~ 20.0

No.	m/z	Int%	Err-mmu	U.S.	Composition
1	523.2736	100.0	+1.4	17.0	C34H37O4N

6e