

Supporting Information

Novel Phenyl diazenyl Fibrate Analogues as PPAR $\alpha/\gamma/\delta$ Pan-Agonists for the Amelioration of Metabolic Syndrome

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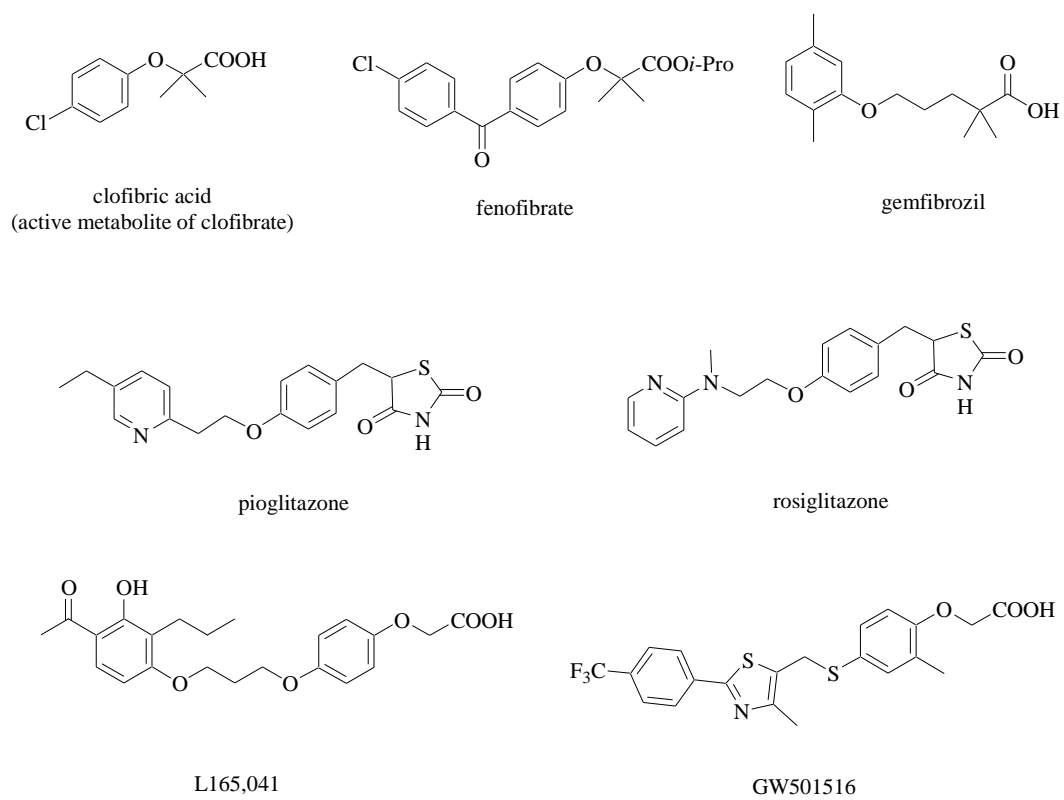


Figure S1. Chemical structures of representative fibrates, thiazolidinediones and phenoxyacetic acid derivatives.

Experimental Procedures

General information

Büchi B-540 apparatus was used to measure melting points and these values were uncorrected. Infrared spectra were recorded on a FT-IR 1600 Perkin–Elmer spectrometer. A Varian instrument was utilized to run NMR spectra at 300 MHz and chemical shifts (δ) are reported in ppm. Microanalyses were effected with an Eurovector Euro EA 3000 model analyser; the analytical results were not over 0.4% of the theoretical values. All commercial and cell culture reagents, medium and reference compounds were obtained from Sigma-Aldrich (Milan, Italy).

General procedure for the preparation of phenols 2a-g

p-Substituted aniline (0.5 g, 3.6 mmol) was dissolved in 6N hydrochloric acid (1.5 mL), then a solution of sodium nitrite (0.37 g, 5.3 mmol) in water (2 mL) was slowly added at 0–5 °C. The mixture was stirred for 1 h, filtered and the filtrate was then added dropwise to phenol (0.33 g, 3.5 mmol) in 4N sodium hydroxide (2.5 mL) at 0–5°C. The mixture was stirred for further 1 h and filtered to obtain an intense red precipitate that was washed several times with water. The residue was solubilized in 50 mL of ethanol/water (1:1,v/v), acidified with concentrated hydrochloric acid until a precipitate is formed. At last, the precipitate was filtrated and dried.

4-[(E)-Phenyldiazenyl]phenol (2a)

Characterization data are in agreement with those reported in the literature.¹

4-[(E)-(4-Chlorophenyl)diazenyl]phenol (2b)

Red solid, 56% yield; m.p. 149-151 °C; IR (KBr): 3229 (OH), 1585 (N=N) cm⁻¹; ¹H-NMR (CDCl₃) δ 6.94 (d, 2H, J=9.0 Hz, CHar), 7.46 (d, 2H, J=8.7 Hz, CHar), 7.82 (d, 2H, J=9.0 Hz, CHar), 7.86 (d, 2H, J= 8.7 Hz, CHar); ¹³C-NMR (CDCl₃) δ 116.1 (CHar), 124.1, 125.3, 129.5, 136.3 (CHar), 147.3 (Car-N=N), 151.2 (N=N-Car), 158.6 (Car). Anal. calcd for C₁₂H₉ClN₂O: C 61.95, H 3.90, N 12.04. Found C 61.93, H 3.90, N 12.07.

4-[(E)-(4-Bromophenyl)diazenyl]phenol (2c)

Red solid, 38% yield; m.p. 147-150 °C; IR (KBr): 3230 (OH), 1587 (N=N) cm⁻¹; ¹H-NMR (CDCl₃) δ 6.94 (d, 2H, J=8.7 Hz CHar), 7.62 (d, 2H, J=8.7 Hz CHar), 7.75 (d, 2H, J=9 Hz CHar), 7.87 (d, 2H, J=9 Hz CHar); ¹³C-NMR (CDCl₃) δ 116.1, 124.3, 125.4, 132.5 (CHar), 147.3, (Car), 151.6 (Car-N=N), 152.5 (N=N-Car), 158.6 (Car). Anal. calcd for C₁₂H₉BrN₂O: C 52.01, H 3.27 N 10.11. Found C 52.03, H 3.29 N 10.15.

4-[(E)-[4-(Trifluoromethyl)phenyl]diazenyl]phenol (2d)

Red solid, 53% yield; m.p. 149-151°C; IR (KBr): 3225 (OH), 1594 (N=N) cm⁻¹; ¹H-NMR (CDCl₃) δ 6.93 (d, 2H, J=8.7 Hz, CHar), 7.81 (d, 2H, J=8.2 Hz, CHar), 7.87 (d, 2H, J=8.7 Hz, CHar), 7.98 (d, 2H, J=8.2 Hz, CHar); ¹³C-NMR (CDCl₃) δ 115.7, 122.6 (CHar), 122.9, 123.1 (CF₃), 125.3 (CHar), 126.1, 126.2 (Car), 137.9 (CHar), 146.2 (Car-N=N), 152.8 (Car-N=N), 161.9 (Car). Anal. calcd for C₁₃H₉F₃N₂O: C 58.65, H 3.41, N 10.52. Found C 58.60, H 3.43, N 10.50.

4-[(E)-(4-Hydroxyphenyl)diazenyl]benzotrile (2e)

Yellow solid; 52% yield; m.p. 175-178°C; IR (KBr): 3322 (OH), 2236 (CN), 1502 (N=N) cm⁻¹; ¹H-NMR (CD₃OD) δ 6.93 (d, 2H, J=8.7 Hz, CHar), 7.88 (d, 2H, J=6.6 Hz, CHar), 7.92 (d, 2H, J=6.6 Hz, CHar), 7.95 (d, 2H, J=8.7 Hz, CHar); ¹³C-NMR (CD₃OD) δ 112.9 (Car),

115.8 (CHar), 118.3 (CN), 122.9, 125.6, 133.3 (CHar), 146.2 (Car-N=N), 155.2 (N=N-Car), 162.3 (Car). Anal. calcd for C₁₃H₉N₃O: C 69.95, H 4.06, N 18.82. Found C 69.97, H 4.05, N 18.80.

4-[(E)-(4-Nitrophenyl)diazenyl]phenol (2f)

Bronze solid; 44% yield; m.p. 209- 210°C; IR (KBr): 3402 (OH), 1583 (NO), 1508 (N=N), 1335 (NO) cm⁻¹; ¹H-NMR (CDCl₃) δ 6.93 (d, 2H, J=9 Hz, CHar), 7.87 (d, 2H, J=9 Hz, CHar), 7.97 (d, 2H, J=9 Hz, CHar), 8.36 (d, 2H, J=9 Hz, CHar); ¹³C-NMR (CDCl₃) δ 116.3, 123.4, 125.0, 126.1 (CHar), 143.7 (Car), 146.8 (Car-N=N), 153.7 (Car-N=N), 156.2 (Car). Anal. calcd for C₁₂H₉N₃O₃: C 59.26, H 3.73, N 17.28. Found C 59.24, H 3.75, N 17.30.

4-[(E)-(4-Methoxyphenyl)diazenyl]phenol (2g)

Brick red; 66% yield; m.p. 138-140°C; IR (KBr): 3418 (ν OH), 1593 (ν N=N), 1241 (ν C-O) cm⁻¹; ¹H-NMR (CD₃OD) δ 3.86 (s, 3H, OCH₃), 6.88 (d, 2H, J=9.9 Hz, CHar), 7.03 (d, 2H, J=9.9 Hz, CHar), 7.75 (d, 2H, J=9 Hz, CHar), 7.81 (d, 2H, J=9 Hz, CHar); ¹³C-NMR (CD₃OD) δ 54.8 (OCH₃), 114.0, 115.9, 123.9, 124.4 (CHar), 145.9 (Car-N=N), 147.1 (Car-N=N), 161.8 (Car). Anal. calcd for C₁₃H₁₂N₂O₂: C 68.41, H 5.30, N 12.27. Found 68.39, H 5.31, N 12.25.

General synthesis of alcohols 3a-f

To a solution of the *p*-substituted aniline (**1a-f**) (2.16 mmol) in CH₂Cl₂ (4 mL) was added Oxone® (4.3 mmol) dissolved in water (19 mL). The mixture was stirred under nitrogen at room temperature. Normally, when nitrosoarene was formed, the color of the solution turned to green. The two layers were separated and the aqueous phase was extracted with CH₂Cl₂ (3 x 30 mL). The combined organic layers were washed with 1 N HCl, saturated sodium

bicarbonate solution, water, brine and dried with NaSO₄. The solvent was removed under reduced pressure obtaining the corresponding nitrosoarene; it was labile and for this reason it was submitted to the next reaction without purification.

To the nitrosoarene (1.00 equiv) dissolved in acetic acid (15 mL) was added the 2-(4-aminophenyl)ethanol (1.00 equiv). The resulting mixture was stirred at room temperature for 24–48 h. To precipitate the product, a saturated sodium bicarbonate solution was added slowly. The mixture was extracted with CH₂Cl₂ (3 x 30 mL). The combined organic layers were dried with NaSO₄. After filtration, the solvent was evaporated and the residue was purified by column chromatography on silica gel (eluent cyclohexane/ethyl acetate 6:4) obtaining the desired products.

2-{4-[(E)-Phenyldiazenyl]phenyl}ethanol (3a)

Orange solid, 40% yield; m.p. 81-83 °C; IR (KBr) 3303 (OH), 1590 (N=N) cm⁻¹; ¹H NMR (CDCl₃) δ 2.96 (t, 2H, J = 6.6 Hz, OCH₂CH₂), 3.92 (t, 2H, J = 6.6 Hz, OCH₂CH₂), 7.38 (d, 2H, J = 8.7 Hz, CH_{ar}), 7.50 (m, 3H, CH_{ar}), 7.89 (m, 4H, CH_{ar}); ¹³C NMR (CDCl₃) δ 39.3 (HOCH₂CH₂), 63.7 (HOCH₂CH₂), 123.0, 123.3, 129.3, 130.0, 131.1 (C_{ar}), 142.2 (C_{ar} CH₂), 151.7 (C_{ar}N), 152.9 (C_{ar}N). Anal. calcd for C₁₄H₁₄N₂O: C 74.31, H 6.24, N 12.38. Found C 74.30, H 6.27, N 12.40.

2-{4-[(E)-(4-Chlorophenyl)diazenyl]phenyl}ethanol (3b)

Orange solid, 86% yield; m.p. 150-152 °C; IR (KBr) 3300 (OH), 1550 (N=N) cm⁻¹; ¹H NMR (CDCl₃) δ 2.96 (t, 2H, J = 6.6 Hz, OCH₂CH₂), 3.92 (t, 2H, J = 6.6 Hz, OCH₂CH₂), 7.38 (d, 2H, J = 8.7 Hz, CH_{ar}), 7.43 (d, 2H, J = 8.7 Hz, CH_{ar}), 7.86 (m, 4H, CH_{ar}); ¹³C NMR (CDCl₃) δ 39.3 (HOCH₂CH₂), 63.7 (HOCH₂CH₂), 123.4, 124.3, 129.6, 130.0 (C_{ar}), 137.0

(CarCl), 142.6 (CarCH₂), 151.2 (CarN), 151.5 (CarN). Anal. calcd for C₁₄H₁₃ClN₂O: C 64.49, H 5.03, N 10.74. Found C 64.53, H 5.00, N 10.75.

2-{4-[(E)-(4-Bromophenyl)diazenyl]phenyl}ethanol (3c)

Orange solid, 30% yield; m.p. 148.0-148.5 °C; IR (KBr) 3255 (OH), 1557 (N=N) cm⁻¹; ¹H NMR (CDCl₃) δ 2.96 (t, 2H, J = 6.6 Hz, OCH₂CH₂), 3.92 (t, 2H, J = 6.6 Hz, OCH₂CH₂), 7.38 (d, 2H, J = 8.7 Hz, CHar), 7.64 (d, 2H, J = 8.7 Hz, CHar), 7.79 (d, 2H, J = 8.7 Hz, CHar), 7.84 (d, 2H, J = 8.7 Hz, CHar); ¹³C NMR (CDCl₃) δ 39.3 (HOCH₂CH₂), 63.6 (HOCH₂CH₂), 123.4, 124.5 (Car), 125.5 (CarBr), 130.1, 132.55 (Car), 142.6 (CarCH₂), 151.5 (CarN), 151.6 (CarN). Anal. calcd for C₁₄H₁₃BrN₂O: C 55.10, H 4.29, N 9.18. Found C 55.08, H 4.33, N 9.20.

2-(4-{(E)-[4-(Trifluoromethyl)phenyl]diazenyl}phenyl)ethanol (3d)

Red solid; 49% yield; m.p. 111.5-112.8 °C; IR (KBr): 3377 (OH), 1604 (N=N) cm⁻¹; ¹H-NMR (CDCl₃) δ 2.97 (d, 2H, J=6.3 Hz, CH₂CH₂OH), 3.93 (q, 2H, J=6.3 Hz, CH₂CH₂OH), 7.40 (d, 2H, J=8.4 Hz, CHar), 7.77 (d, 2H, J=9.06 Hz, CHar), 7.91 (d, 2H, J=8.4 Hz, CHar), 7.99 (d, 2H, J=9.06 Hz, CHar); ¹³C-NMR (CDCl₃) δ 39.3 (CH₂CH₂OH), 63.6 (CH₂CH₂OH), 115.5, 123.2, 123.6 (CHar), 126.5 (CF₃), 130.1 (CHar), 132.5, 143.3, 151.4, 154.7 (Car). Anal. calcd for C₁₅H₁₃F₃N₂O: C 61.22, H 4.45, N 9.52. Found C 61.25, H 4.40 N 9.51.

4-{(E)-[4-(2-Hydroxyethyl)phenyl]diazenyl}benzonitrile (3e)

Orange solid; 51% yield; m.p. 159-160 °C; IR (KBr): 3488 (OH), 2236 (CN), 1599 (N=N) cm⁻¹; ¹H-NMR (CDCl₃) δ 2.97 (t, 2H, J = 6.6 Hz, OCH₂CH₂), 3.94 (t, 2H, J = 6.6 Hz, OCH₂CH₂), 7.4 (d, 2H, J = 8.7 Hz, CHar), 7.81 (d, 2H, J = 8.7 Hz, CHar), 7.91 (d, 2H, J = 8.7 Hz, CHar), 7.98 (d, 2H, J = 8.7 Hz, CHar); ¹³C-NMR (CDCl₃) δ 39.3 (CH₂CH₂OH), 63.5

(CH₂CH₂OH), 114.0 (CarCN), 118.8 (CN) 123.5, 123.6, 123.8, 130.2, 133.5 (Car), 143.8 (CarCN), 151.4 (CarN), 154.8 (CarN). Anal. calcd for C₁₅H₁₃N₃O: C 71.70, H 5.21, N 16.72. Found C 71.72, H 5.19, N 16.70.

2-[4-[(E)-(4-Nitrophenyl)diazenyl]phenyl]ethanol (3f)

Red solid; 42% yield; m.p. 122.4-124.6°C; IR (KBr): 3550 (OH), 1596 (N=N) 1508 (NO), 1335 (NO) cm⁻¹; ¹H-NMR (CDCl₃) δ 2.98 (t, 2H, J=6.6 Hz, CH₂CH₂OH), 3.94 (q, 2H, J=6.6 Hz, CH₂CH₂OH), 7.42 (d, 2H, J=8.2 Hz, CHar), 7.93 (d, 2H, J=8.4 Hz, CHar), 8.01 (d, 2H, J=9 Hz, CHar), 8.37 (d, 2H, J=9 Hz, CHar); ¹³C-NMR (CDCl₃) δ 39.4 (CH₂CH₂OH), 63.6 (CH₂CH₂OH), 100.4, 123.6, 123.9, 125.0 (CHar), 130.2, 132.8, 144.0, 151.4 (Car). Anal. calcd for C₁₄H₁₃N₃O₃: C 61.99, H 4.83, N 15.49. Found C 61.97, H 4.85, N 15.50.

Procedure for the preparation of ethyl 2-[4-(2-hydroxyethyl)phenoxy]-2-methylpropanoate (7)

Ethyl 2-bromo-2-methylpropanoate (**6**) (22.3 mmol, 3.3 mL) was added to a solution of 4-(2-hydroxyethyl)phenol (**4**) (7.2 mmol, 0.99 g) and dry K₂CO₃ (72.0 mmol, 9.96 g) in DMF (3 mL/mmol, 21.7 mL). The mixture was stirred for 4h at reflux. Water (25 mL) was used to quench the reaction and ethyl acetate (3 × 50 mL) to extract the mixture. The organic layer was washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (eluent cyclohexane/ethyl acetate 8:2).

Yellow oil, 80% yield, IR (KBr) 3420 (OH), 1713 (C=O) cm⁻¹; ¹H NMR (CDCl₃) δ 1.28 (t, 3H, J = 7.2 Hz, CH₂CH₃), 1.57 (s, 6H, C(CH₃)₂), 2.91 (t, 2H, J = 7.8 Hz, OCH₂CH₂), 3.47 (t, 2H, J = 7.8 Hz, OCH₂CH₂), 4.34 (q, 2H, J = 7.2 Hz, CH₂CH₃), 6.84 (d, 2H, J = 8.5 Hz, CHar),

6.91 (d, 2H, J = 8.5 Hz, *CH*_{ar}); ¹³C NMR (CDCl₃) δ 14.3 (CH₃CH₂), 24.9 (C(CH₃)₂), 39.0 (OCH₂CH₂), 61.1 (CH₃CH₂), 63.0 (OCH₂CH₂), 78.8 (C(CH₃)₂), 120.1, 128.5, (*CH*_{ar}), 132.6, 154.0 (*Car*), 172.1 (C=O). Anal. calcd for C₁₄H₂₀O₄: C 66.65, H 7.99. Found C 66.32, H 7.96.

Procedure for the preparation of ethyl 2-(4-hydroxyphenoxy)-2-methylpropanoate (8)

Hydroquinone (**5**) (0.2 g, 1.82 mmol) and ethyl 2-bromo isobutyrate (**6**) (0.13 mL, 0.91 mmol) were refluxed in ethanol (2 mL) with potassium hydroxide (0.5 g, 0.91 mmol) under nitrogen for 24 hours. A second portion of ethyl 2-bromo isobutyrate (0.13 mL) was added and the reflux was maintained for 48 hours. After ethanol removal, the residue was dissolved in ethyl acetate (15 mL), washed with water (15 mL) and brine (15 mL), dried over Na₂SO₄ and evaporated to dryness. The residue was purified by column chromatography on silica gel (eluent cyclohexane/ethyl acetate 8:2) affording pure title compound.

Brown solid, 37% yield, m.p. 85-86 °C; IR (KBr) 3427 (OH), 1719 (C=O) cm⁻¹; ¹H-NMR (CDCl₃): δ 1.27 (t, 3H, J = 6.9 Hz, CH₂CH₃), 1.52 (s, 6H, C(CH₃)₂), 4.23 (q, 2H, J = 6.9 Hz, CH₂CH₃), 6.73 (d, 2H, J = 6.9 Hz, *CH*_{ar}), 6.91 (d, 2H, J = 6.9 Hz, *CH*_{ar}); ¹³C-NMR (CDCl₃): δ 14.3 (CH₂CH₃), 24.9 (C(CH₃)₂), 61.1 (CH₂CH₃), 78.8 (C(CH₃)₂), 120.1, 128.5 (*CH*_{ar}), 132.6, 154.0 (*Car*), 172.1 (C=O). Anal. calcd for C₁₂H₁₆O₄: C 64.27, H 7.19. Found C 64.25, H 7.22.

General procedure for the preparation of esters 9a-g and 10a-f

A solution of diisopropyl azodicarboxylate (DIAD) (18.14 mmol, 2.86 mL) in dry THF (10 mL) was added dropwise to a stirred solution of alcohols **3a-f** or **7** (13.30 mmol), triphenylphosphine (4.76 g, 18.14 mmol), and appropriate phenols **2a-g** or **8** (18.14 mmol) in dry THF (40 mL) under nitrogen atmosphere. After stirring for 10-12 h at room temperature,

the solvent was evaporated under reduced pressure. The obtained crude product was purified by column chromatography on silica gel (eluent cyclohexane/ethyl acetate 8:2) to afford esters **9a-g** and **10a-f**.

Ethyl 2-methyl-2-[4-(2-[4-[(E)-phenyldiazenyl]phenoxy)ethyl]phenoxy]propanoate (9a)

Characterization data are in agreement with those reported in the literature.¹

Ethyl 2-[4-(2-[4-[(E)-(4-chlorophenyl)diazenyl]phenoxy)ethyl]phenoxy]-2-methylpropanoate (9b)

Orange oil; 58% yield; IR (KBr) 1734 (C=O), 1605 (N=N) cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3) δ 1.25 (t, 3H, $J = 7.2$ Hz, CH_2CH_3), 1.58 (s, 6H, $\text{C}(\text{CH}_3)_2$), 3.06 (t, 2H, $J = 6.6$ Hz, OCH_2CH_2), 4.21 (t, 2H, $J = 6.9$ Hz, OCH_2CH_2), 4.25 (q, 2H, $J = 7.2$, Hz, OCH_2CH_3), 6.81 (d, 2H, $J = 9.3$ Hz, CHar), 6.99 (d, 2H, $J = 9.3$ Hz, CHar), 7.16 (d, 2H, $J = 8.4$ Hz, CHar), 7.46 (d, 2H, $J = 8.4$ Hz, CHar), 7.82 (d, 2H, $J = 9$ Hz, CHar), 7.89 (d, 2H, $J = 9$ Hz, CHar); $^{13}\text{C-NMR}$ (CDCl_3) δ 14.3 (CH_2CH_3), 25.0 ($\text{C}(\text{CH}_3)_2$), 37.2 (OCH_2CH_2), 61.1 (CH_2CH_3), 68.8 (OCH_2CH_2), 79.9 ($\text{C}(\text{CH}_3)_2$), 115.2, 121.8, 123.3, 124.3, 129.5, 130.0 (CHar), 137.0 (CarCl), 142.9 (CarCH_2), 149.3 (CarN), 151.2 (CarN), 151.4 ($\text{CarOC}(\text{CH}_3)_2$), 154.5 ($\text{CarOCH}_2\text{CH}_2$), 174.6 (COOEt).
Anal. calcd for $\text{C}_{26}\text{H}_{27}\text{ClN}_2\text{O}_4$: C 66.88, H 5.83, N 6.00. Found C 66.90, H 5.85, N 6.02.

Ethyl 2-[4-(2-[4-[(E)-(4-bromophenyl)diazenyl]phenoxy)ethyl]phenoxy]-2-methylpropanoate (9c)

Orange oil; 32% yield; IR (KBr) 1738 (C=O), 1615 (N=N) cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3) δ 1.27 (t, 3H, $J = 7.2$ Hz, CH_2CH_3), 1.52 (s, 6H, $\text{C}(\text{CH}_3)_2$), 3.15 (t, 2H, $J = 6.7$ Hz, OCH_2CH_2), 4.18 (t, 2H, $J = 6.9$ Hz, OCH_2CH_2), 4.23 (q, 2H, $J = 6.9$, Hz, OCH_2CH_3), 6.81 (d, 2H, $J = 9.3$ Hz, CHar), 6.90 (d, 2H, $J = 9.3$ Hz, CHar), 7.27 (d, 4H, $J = 8.4$ Hz, CHar), 7.64 (d, 2H, $J = 8.4$ Hz,

CHar), 7.87 (d, 2H, *J* = 9 Hz, *CHar*); ¹³C-NMR (CDCl₃) δ 14.4 (CH₂CH₃), 25.5 (C(CH₃)₂), 36.0 (OCH₂CH₂), 61.6 (CH₂CH₃), 68.9 (OCH₂CH₂), 80.9 (C(CH₃)₂), 115.2, 121.8, 122.4, 123.3, 124.3 (*Car*), 125.5 (*CarBr*), 129.5, 130.0 (*Car*), 142.9 (*CarCH*₂), 149.3 (*CarN*), 151.2 (*CarN*), 151.5 (*CarOC*(CH₃)₂), 154.5 (*CarOCH*₂CH₂), 174.6 (COOEt). Anal. calcd for C₂₆H₂₇BrN₂O₄: C 61.06, H 5.32, N 5.48. Found C 61.09, H 5.30, N 5.50.

Ethyl

2-methyl-2-[4-[2-(4-{(E)-[4-

(trifluoromethyl)phenyl]diazanyl]phenoxy]ethyl]phenoxy}propanoate (9d)

Strawberry oil; 46% yield; IR (KBr): 1736 (C=O), 1604 (N=N) cm⁻¹; ¹H-NMR (CDCl₃) δ 1.25 (t, 3H, *J*=7.2 Hz, CH₂CH₃), 1.52 (s, 6H, C(CH₃)₂), 3.15 (t, 2H, *J*=6.6 Hz, OCH₂CH₂), 4.20 (t, 2H, *J*=6.6 Hz, OCH₂CH₂), 4.22 (q, 2H, *J* = 6.9, Hz, OCH₂CH₃), 6.80 (d, 2H, *J*=8.1 Hz, *CHar*), 7.44 (d, 2H, *J*=8.4 Hz, *CHar*), 7.77 (d, 4H, *J*=8.7 Hz, *CHar*), 7.90 (d, 2H, *J*=8.4 Hz, *CHar*), 7.98 (d, 2H, *J*=8.4 Hz, *CHar*); ¹³C-NMR (CDCl₃) δ 14.4 (OCH₂CH₃), 25.5 (C(CH₃)₂), 35.0 (OCH₂CH₂), 61.6 (OCH₂CH₃), 68.7 (OCH₂CH₂), 79.9 (C(CH₃)₂), 114.0, 115.1, 118.8, 120.8, 121.8 (*CHar*), 123.7-123.5 (*Car-CF*₃), 130.15 (*CHar*), 133.4, 143.6, 149.3, 151.4, 154.8 (*Car*), 174.5 (COOEt). Anal. calcd for C₂₇H₂₇F₃N₂O₄: C 67.79, H 5.44, N 5.60. Found C 67.81, H 5.45, N 5.58.

Ethyl 2-[4-(2-[4-{(E)-(4-cyanophenyl)diazanyl]phenoxy}ethyl]phenoxy]-2-methylpropanoate (9e)

Orange solid; 41% yield; m.p. 87.5-89.3 °C; IR (KBr): 2250 (CN), 1736 (C=O), 1601 (N=N) cm⁻¹; ¹H-NMR (CDCl₃) δ 1.25 (t, 3H, CH₂CH₃), 1.55 (s, 6H, C(CH₃)₂), 3.07 (t, 2H, OCH₂CH₂), 4.20-4.27 (m, 4H, OCH₂CH₃; OCH₂CH₂), 6.79 (d, 2H, *J*=8.4 Hz, *CHar*), 7.01 (d, 2H, *J*=7.8 Hz, *CHar*), 7.16 (d, 2H, *J*=7.5 Hz, *CHar*), 7.59 (d, 2H, *J*=7.8 Hz, *CHar*), 7.79 (d, 2H, *J*=8.4 Hz, *CHar*), 7.93 (d, 2H, *J*=7.5 Hz, *CHar*); ¹³C-NMR (CDCl₃) δ 14.3 (CH₂CH₃),

25.6 (C(CH₃)₂), 35.0 (OCH₂CH₂), 61.7 (OCH₂CH₃), 69.4 (OCH₂CH₂), 79.3 (C(CH₃)₂), 115.2, 119.5, 123.3, 125.7, 129.9 (CHar), 131.5 (Car-CN), 133.4 (CHar), 133.5 (Car-CH₂CH₂O), 147.0 (Car-N=N), 154.4 (Car-N=N), 155.0 (Car-O-C), 162.6 (Car-OCH₂CH₂), 174.6 (COOEt). Anal. calcd for C₂₇H₂₇N₃O₄: C 70.88, H 5.95, N 9.18. Found C 70.90, H 5.93, N 9.20.

Ethyl 2-methyl-2-[4-(2-[4-[(E)-(4-nitrophenyl)diazenyl]phenoxy]ethyl)phenoxy]propanoate (9f)

Red solid; 23% yield; m.p. 114.0-114.8 °C; IR (KBr): 1727 (C=O), 1601 (N=N) cm⁻¹; ¹H-NMR (CDCl₃) δ 1.25 (t, 3H, J=6.9 Hz, CH₂CH₃), 1.59 (s, 6H, C(CH₃)₂), 3.07 (t, 2H, J=6.9 Hz, OCH₂CH₂), 4.24 (m, 4H, CH₂CH₃-OCH₂CH₂), 6.80 (d, 2H, J=8.4 Hz, CHar), 7.01 (d, 2H, J=8.7 Hz, CHar), 7.16 (d, 2H, J=8.7 Hz, CHar), 7.96 (t, 4H, J=8.7 Hz, CHar), 8.36 (d, 2H, J=9 Hz, CHar); ¹³C-NMR (CDCl₃) δ 14.3 (OCH₂CH₃), 25.6 (C(CH₃)₂), 35.0 (OCH₂CH₂), 61.7 (OCH₂CH₃), 69.4 (OCH₂CH₂), 79.3 (C(CH₃)₂), 115.2, 119.5, 123.3, 125.0, 125.8 (Car-NO₂), 129.9, 131.5, 147.1 (Car), 148.4 (CHar), 154.4 (Car), 156.2 (CHar), 174.6 (COOEt). Anal. calcd for C₂₆H₂₇N₃O₆: C 65.40, H 5.70, N 8.80. Found C 65.43, H 5.67, N 8.82.

Ethyl 2-[4-(2-[4-[(E)-(4-methoxyphenyl)diazenyl]phenoxy]ethyl)phenoxy]-2-methylpropanoate (9g)

Orange solid; 37% yield; m.p. 50.8-51.7 °C; IR (KBr): 1731 (C=O), 1595 (N=N) cm⁻¹; ¹H-NMR (CDCl₃) δ 1.24 (t, 3H, J=7.2 Hz, CH₂CH₃), 1.58 (s, 6H, C(CH₃)₂), 3.06 (t, 2H, J=6.9, OCH₂CH₂), 3.86 (s, 3H, OCH₃), 4.17-4.27 (m, 4H, J=7.2 Hz, J=6.9 Hz, CH₂CH₃-OCH₂CH₂), 6.80 (d, 2H, J=8.5, CHar), 6.97 (d, 2H, J=4.9 Hz, CHar), 7.00 (d, 2H, J=4.9 Hz, CHar), 7.16 (t, 2H, J=8.5 Hz, CHar), 7.84 (d, 2H, J=5.2 Hz, CHar), 7.87 (d, 2H, J=5.2 Hz, CHar); ¹³C-NMR (CDCl₃) δ 14.3 (OCH₂CH₃), 25.6 (C(CH₃)₂), 35.1 (OCH₂CH₂), 55.8

(OCH₃), 61.7 (OCH₂CH₃), 69.3 (OCH₂CH₂), 79.3 (C(CH₃)₂), 114.4, 114.9, 119.5, 124.6, 129.9 (CHar), 131.8, 147.3, 154.3, 161.0, 161.8 (Car) 174.6 (COOEt). Anal. calcd for C₂₇H₃₀N₂O₅: C 70.11, H 6.54, N 6.06. Found C 70.13, H 6.55, N 6.03.

Ethyl 2-methyl-2-[4-(2-[4-[(E)-phenyldiazenyl]phenyl]ethoxy)phenoxy]propanoate (10a)

Orange oil; 73% yield; IR (KBr) 1733 (C=O), 1603 (N=N) cm⁻¹; ¹H-NMR (CDCl₃) δ 1.27 (t, J = 3H, 7.2 Hz, OCH₂CH₃), 1.53 (s, 6H, C(CH₃)₂), 3.15 (t, 2H, J = 6.9 Hz, OCH₂CH₂), 4.16 (t, 2H, J = 6.9 Hz, OCH₂CH₂), 4.21 (q, 2H, J = 7.2, Hz, OCH₂CH₃), 6.75-6.85 (m, 3H, CHar), 6.90 (d, 1H, J = 9.3 Hz, CHar), 7.42 (d, 1H, J = 9.3 Hz, CHar), 7.47-7.55 (m, 4H, CHar), 7.86-7.92 (m, 4H, CHar); ¹³C-NMR (CDCl₃) δ 14.4 (OCH₂CH₃), 25.5 (C(CH₃)₂), 36.0 (OCH₂CH₂), 61.6 (OCH₂CH₃), 68.9 (OCH₂CH₂), 80.0 (C(CH₃)₂), 115.2, 121.8, 123.0, 123.3, 129.3, 130.0, 131.1 (CHar), 142.0 (CarCH₂), 149.3 (CarN), 151.6 (CarN), 151.9 (CarOC(CH₃)₂), 154.5 (CarOCH₂CH₂), 174.6 (COOEt). Anal. calcd for C₂₆H₂₈ N₂O₄: C 72.20, H 6.53, N 6.48. Found C 72.18, H 6.55, N 6.47.

Ethyl 2-[4-(2-[4-[(E)-(4-chlorophenyl)diazenyl]phenyl]ethoxy)phenoxy]-2-methylpropanoate (10b)

Orange solid; 72% yield; m.p. 150.6-152.8 °C; IR (KBr): 1735 (C=O), 1605 (N=N) cm⁻¹; ¹H-NMR (CDCl₃) δ 1.27 (t, J = 3H, 7.2 Hz, OCH₂CH₃), 1.56 (s, 6H, C(CH₃)₂), 3.15 (t, 2H, J = 6.9 Hz, OCH₂CH₂), 4.16 (t, 2H, J = 6.9 Hz, OCH₂CH₂), 4.22 (q, 2H, J = 7.3 Hz, OCH₂CH₃), 6.81 (d, 2H, J = 9 Hz, CHar), 6.91 (d, 2H, J = 9 Hz, CHar), 7.43 (d, 2H, J = 8.4 Hz, CHar), 7.48 (d, 2H, J = 8.4 Hz, CHar), 7.85 (d, 2H, J = 2.1 Hz, CHar), 7.87 (d, 2H, J = 2.1 Hz, CHar); ¹³C-NMR (CDCl₃) δ 14.3 (OCH₂CH₃), 24.9 (C(CH₃)₂), 37.3 (OCH₂CH₂), 61.1 (OCH₂CH₃), 70.0 (OCH₂CH₂), 78.7 (C(CH₃)₂), 114.9, 122.9, 124.9, 126.0, 129.6, 132.6 (CHar), 133.1 (Car),

145.9 (CarCl), 146.9, 152.0, 153.9, 166.2 (Car), 170.5 (COOEt). Anal. calcd for C₂₆H₂₇ClN₂O₄: C 66.88, H 5.83, N 6.00. Found C 66.87, H 5.85, N 6.02.

Ethyl 2-[4-(2-[4-[(E)-(4-bromophenyl)diazenyl]phenyl]ethoxy)phenoxy]-2-methylpropanoate (10c)

Orange oil; 32% yield; IR (KBr): 1732 (C=O), 1602 (N=N) cm⁻¹; ¹H-NMR (CDCl₃) δ 1.27 (t, 3H, J= 7.2 Hz, OCH₂CH₃), 1.52 (s, 6H, C(CH₃)₂), 3.15 (t, 2H, J = 6.7 Hz, OCH₂CH₂), 4.18 (t, 2H, J = 6.7 Hz, OCH₂CH₂), 4.23 (q, 2H, J = 7.2, Hz, OCH₂CH₃), 6.81 (d, 2H, J = 9.3 Hz, CHar), 6.90 (d, 2H, J = 9.3 Hz, CHar), 7.27 (d, 4H, J = 8.4 Hz, CHar), 7.64 (d, 2H, J = 8.4 Hz, CHar), 7.87 (d, 2H, J = 8.4 Hz, CHar); ¹³C-NMR (CDCl₃) δ 14.4 (OCH₂CH₃), 25.5 (C(CH₃)₂), 35.9 (OCH₂CH₂), 61.6 (OCH₂CH₃), 68.8 (OCH₂CH₂), 80.9 (C(CH₃)₂), 115.2, 121.8, 122.4, 123.3, 124.30(Car), 125.5 (CarBr), 129.5, 130.0 (Car), 142.9 (CarCH₂), 149.3, 151.2 (CarN), 151.5 (CarOC(CH₃)₂), 154.5 (CarOCH₂CH₂), 174.6 (COOEt). Anal. calcd for C₂₆H₂₇BrN₂O₄: C 61.06, H 5.32, N 5.48. Found C 61.04, H 5.30, N 5.49.

Ethyl *2-methyl-2-[4-[2-(4-[(E)-[4-(trifluoromethyl)phenyl]diazenyl]phenyl]ethoxy]phenoxy]propanoate (10d)*

Strawberry oil; 46% yield; IR (KBr): 1736 (C=O), 1601 (N=N) cm⁻¹; ¹H-NMR (CDCl₃) δ 1.27 (t, 3H, J=7.2 Hz, CH₂CH₃), 1.53 (s, 6H, C(CH₃)₂), 3.16 (t, 2H, J=6.6 Hz, OCH₂CH₂), 4.18 (q, 4H, J=6.6 Hz, OCH₂CH₂), 4.23 (q, 2H, J = 7.2 Hz, CH₂CH₃), 6.80 (d, 2H, J=8.1 Hz, CHar), 7.45 (d, 2H, J=8.4 Hz, CHar), 7.77 (d, 2H, J=8.7 Hz, CHar), 7.90 (d, 2H, J=8.4 Hz, CHar), 7.98 (d, 2H, J=8.4 Hz, CHar); ¹³C-NMR (CDCl₃) δ 14.4 (OCH₂CH₃), 25.5 (C(CH₃)₂), 36.0 (OCH₂CH₂), 61.6 (OCH₂CH₃), 68.7 (OCH₂CH₂), 79.9 (C(CH₃)₂), 14.0, 115.1, 118.8, 120.8 (CF₃), 116.2, 121.8, 123.5, 123.7, 130.2, 133.4 (CHar), 121.3, 129.7, 133.3, (Car-CF₃),

143.6, 149.3, 151.4, 154.5, 154.8 (Car), 174.5 (COOEt). Anal. calcd for C₂₇H₂₇F₃N₂O₄: C 64.79, H 5.44, N 5.60. Found C 64.80, H 5.42, N 5.61.

Ethyl 2-[4-(2-{4-[(E)-(4-cyanophenyl)diazenyl]phenyl}ethoxy)phenoxy]-2-methylpropanoate (10e)

Red wax; 68% yield; m.p. 77.2-77.8 °C; IR (KBr): 2226 (CN), 1734 (C=O), 1603 (N=N) cm⁻¹; ¹H-NMR (CDCl₃) δ 1.27 (t, 3H, J= 7.2 Hz, OCH₂CH₃) 1.52 (s, 6H, C(CH₃)₂), 3.16 (t, 2H, J = 6.6 Hz, OCH₂CH₂), 4.17 (t, 2H, J = 6.6 Hz, OCH₂CH₂), 4.23 (q, 2H, J = 7.2, Hz, OCH₂CH₃), 6.77 (d, 2H, J = 9.3 Hz, CH_{ar}), 6.83 (d, 2H, J = 9.3 Hz, CH_{ar}), 7.45 (d, 2H, J = 8.4 Hz, CH_{ar}), 7.80 (d, 2H, J = 9 Hz, CH_{ar}), 7.90 (d, 2H, J = 8.4 Hz, CH_{ar}), 7.97 (d, J = 9, 2H, CH_{ar}); ¹³C-NMR (CDCl₃) δ 14.4 (OCH₂CH₃), 25.5 (C(CH₃)₂), 36.0 (CarCH₂), 61.6 (OCH₂CH₃), 68.7 (CH₂CH₂O), 79.9 (C(CH₃)₂), 113.9 (CarCN), 115.1 (Car), 118.8 (CN), 121.8, 123.5, 123.7, 130.2, 133.4 (Car), 143.6 (CarCH₂), 149.4, 151.4 (CarN), 154.5 (CarOC(CH₃)₂), 154.8 (CarOCH₂CH₂), 174.5 (COOEt). Anal. calcd for C₂₇H₂₇N₃O₄: C 70.88, H 5.95, N 9.18. Found C 70.90, H 5.93, N 9.17.

Ethyl 2-methyl-2-[4-(2-{4-[(E)-(4-nitrophenyl)diazenyl]phenyl}ethoxy)phenoxy]propanoate (10f)

Strawberry solid; 33% yield; m.p. 109-110.5 °C; IR (KBr): 1735 (C=O), 1605 (N=N), 1505 (NO), 1341 (NO) cm⁻¹; ¹H-NMR (CDCl₃) δ 1.27 (t, 3H, J=6.3 Hz, CH₂CH₃), 1.52 (s, 6H, C(CH₃)₂), 3.16 (t, 2H, J=6.9 Hz, OCH₂CH₂), 4.21 (q, 4H, J=6.3 Hz, CH₂CH₃-OCH₂CH₂), 6.80 (q, 4H, J=8.1 Hz, CH_{ar}), 7.46 (d, 2H, J=8.4 Hz, CH_{ar}), 7.92 (d, 2H, J=8.1 Hz, CH_{ar}), 8.01 (d, 2H, J=8.1 Hz, CH_{ar}), 8.37 (d, 2H, J=8.4 Hz, CH_{ar}); ¹³C-NMR (CDCl₃) δ 14.4 (OCH₂CH₃), 25.5 (C(CH₃)₂), 36.0 (OCH₂CH₂), 61.6 (OCH₂CH₃), 68.7 (OCH₂CH₂), 79.9 (C(CH₃)₂), 115.1 (CH_{ar}), 121.2 (CH_{ar}), 123.8 (CH_{ar}), 124.9 (CH_{ar}), 130.2 (CH_{ar}), 143.9

(Car), 148.8 (Car), 149.3 (Car), 154.4(Car), 156.0 (Car), 174.6 (COOEt). Anal. calcd for $C_{26}H_{27}N_3O_6$: C 65.40, H 5.70, N 8.80. Found C 65.43, H 5.72, N 8.79.

General procedure for the preparation of acids 11a-g and 12a-f

To esters **9a-g** or **10a-f** (3.0 mmol) in EtOH (20 mL), a solution of 1N NaOH (3.9 mmol) was added and the mixture was stirred at r.t. for 15-24 h. After solvent removal, the residue was poured into water (20 mL) and acidified with conc HCl at 0 °C. The aqueous layer was extracted with dichloromethane (3 × 20 mL) and the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude of the reaction was purified by crystallization or by column chromatography on silica gel (eluent cyclohexane/ethyl acetate 1:1 or dichloromethane/methanol 9:1) giving acids **11a-g** or **12a-f** in good yields.

2-Methyl-2-[4-(2-{4-[(E)-phenyldiazenyl]phenoxy}ethyl)phenoxy]propanoic acid (11a)

Characterization data are in agreement with those reported in the literature.¹

2-[4-(2-{4-[(E)-(4-Chlorophenyl)diazenyl]phenoxy}ethyl)phenoxy]-2-methylpropanoic acid (11b)

Orange solid; 91% yield; m.p. 143.4-145.7 °C; IR (KBr): 3238 (OH), 1719 (C=O), 1603 (N=N) cm^{-1} ; 1H -NMR ($CDCl_3$) δ 1.59 (s, 6H, $C(CH_3)_2$), 3.09 (t, 2H, $J=6.9$ Hz, OCH_2CH_2), 4.23 (t, 2H, $J=6.9$ Hz, OCH_2CH_2), 6.91 (d, 2H, $J=8.4$ Hz, $CHar$), 6.99 (d, 2H, $J=9$ Hz, $CHar$), 7.22 (d, 2H, $J=8.4$ Hz, $CHar$), 7.46 (d, 2H, $J=8.7$ Hz, $CHar$), 7.82 (d, 2H, $J=9$ Hz, $CHar$), 7.88 (d, 2H, $J=8.7$ Hz, $CHar$); ^{13}C -NMR ($CDCl_3$) δ 25.2 ($C(CH_3)_2$), 35.1 (OCH_2CH_2), 69.2 (OCH_2CH_2), 80.0 ($C(CH_3)_2$), 115.0, 121.1, 124.0, 125.1, 129.5 ($CHar$), 130.1 (CarCl), 133.2 (Car CH_2CH_2O), 136.4 ($CHar$), 147.0 (CarN=N), 151.3 (CarN=N), 153.1 (CarOC), 161.7 (Car OCH_2CH_2), 177.5 (COOH). Anal. calcd for $C_{24}H_{23}ClN_2O_4$: C 65.68, H 5.28, N 6.38. Found C 65.70, H 5.25, N 6.39.

2-[4-(2-{4-[(E)-(4-Bromophenyl)diazenyl]phenoxy}ethyl)phenoxy]-2-methylpropanoic acid (11c)

Yellow solid; 44% yield; m.p. 159.7-160.6 °C; IR (KBr): 3434 (OH), 1712 (C=O), 1602 (N=N) cm^{-1} ; $^1\text{H-NMR}$ (CD_3OD) δ 1.53 (s, 6H, $\text{C}(\text{CH}_3)_2$), 3.05 (t, 2H, $J=6.9$ Hz, OCH_2CH_2), 4.25 (t, 2H, $J=6.9$ Hz, OCH_2CH_2), 6.85 (d, 2H, $J=8.1$ Hz, CHar), 7.05 (d, 2H, $J=9.0$ Hz, CHar), 7.22 (d, 2H, $J=8.1$ Hz, CHar), 7.67 (d, 2H, $J=7.8$ Hz, CHar), 7.77 (d, 2H, $J=7.8$ Hz, CHar), 7.88 (d, 2H, $J=9.0$ Hz, CHar); $^{13}\text{C-NMR}$ (CDCl_3) δ 25.1 ($\text{C}(\text{CH}_3)_2$), 35.2 (OCH_2CH_2), 69.1 (OCH_2CH_2), 83.8 ($\text{C}(\text{CH}_3)_2$), 115.0, 121.5, 124.3, 125.1, 130.2 (CHar), 130.6 (CarBr), 132.5 (CHar), 133.8 ($\text{CarCH}_2\text{CH}_2\text{O}$), 147.1, 152.9 (CarN=N), 161.7 (CarOC), 170.0 ($\text{CarOCH}_2\text{CH}_2$), 174.9 (COOH). Anal. calcd for $\text{C}_{24}\text{H}_{23}\text{BrN}_2\text{O}_4$: C 59.64, H 4.80, N 5.80. Found C 59.66, H 4.82, N 5.77.

2-Methyl-2-{4-[2-(4-{(E)-[4-(trifluoromethyl)phenyl]diazenyl]phenoxy}ethyl)phenoxy]propanoic acid (11d)

Orange solid; 50% yield; m.p. 103.6-106.5°C; IR (KBr): 3233 (OH), 1718 (C=O), 1606 (N=N) cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3) δ 1.58 (s, 6H, $\text{C}(\text{CH}_3)_2$), 3.10 (t, 2H, $J=6.9$ Hz, OCH_2CH_2), 4.24 (t, 2H, $J=6.9$ Hz, OCH_2CH_2), 6.91 (d, 2H, $J=8.4$ Hz, CHar), 7.01 (d, 2H, $J=8.4$ Hz, CHar), 7.23 (t, 2H, $J=8.4$ Hz, CHar), 7.74 (d, 2H, $J=8.7$ Hz, CHar), 7.93 (m, 4H, $J_1=8.4$ Hz, $J_2=8.7$ Hz, CHar); $^{13}\text{C-NMR}$ (CDCl_3) δ 25.2 ($\text{C}(\text{CH}_3)_2$), 35.1 (OCH_2CH_2), 69.2 (OCH_2CH_2), 80.2 ($\text{C}(\text{CH}_3)_2$), 114.6, 120.0, 124.4, 133.4 (CF_3), 115.1, 121.3, 122.9, 125.4, 126.5, 130.2 (CHar), 147.0, 152.9, 154.8, 162.1 (Car), 175.2 (COOH). Anal. calcd for $\text{C}_{25}\text{H}_{23}\text{F}_3\text{N}_2\text{O}_4$: C 63.55, H 4.91, N 5.93. Found C 63.53, H 4.90, N 5.95.

2-[4-(2-{4-[(E)-(4-Cyanophenyl)diazenyl]phenoxy}ethyl)phenoxy]-2-methylpropanoic acid
(11e)

Orange solid; 43% yield; m.p. 130.2-132.8 °C; IR (KBr): 3142 (OH), 2227 (CN), 1715 (C=O), 1607 (N=N) cm⁻¹; ¹H-NMR (CDCl₃) δ 1.58 (s, 6H, C(CH₃)₂), 3.10 (t, 2H, J=6.6 Hz, OCH₂CH₂), 4.25 (t, 2H, J=6.6 Hz, OCH₂CH₂), 6.92 (d, 2H, J=8.1 Hz, CHar), 7.01 (d, 2H, J=8.7 Hz, CHar), 7.17 (d, 2H, J=8.4 Hz, CHar), 7.79 (d, 2H, J=8.4 Hz, CHar), 7.93 (dd, 4H, J=8.1 Hz, CHar); ¹³C-NMR (CDCl₃) δ 25.2 (C(CH₃)₂), 35.1 (OCH₂CH₂), 69.3 (OCH₂CH₂), 80.1 (C(CH₃)₂), 115.1 (CHar), 118.4 (CarCN), 121.1, 123.3, 125.7, 130.1 (CHar), 133.1 (CarCH₂CH₂O), 133.4 (CHar), 147.1, 153.2 (CarN=N), 160.6 (CarOC), 162.5 (CarOCH₂CH₂), 177.1 (COOH). Anal. calcd for C₂₅H₂₃N₃O₄: C 69.92, H 5.40, N 9.78. Found C 69.90, H 5.44, N 9.76.

2-Methyl-2-[4-(2-{4-[(E)-(4-nitrophenyl)diazenyl]phenoxy}ethyl)phenoxy]propanoic acid
(11f)

Orange solid; 26% yield; m.p. 110.0-110.9 °C; IR (KBr): 3421 (OH), 1716 (C=O), 1602 (N=N) cm⁻¹; ¹H-NMR (CDCl₃) δ 1.59 (s, 6H, C(CH₃)₂), 3.09 (t, 2H, J=6.9 Hz, OCH₂CH₂), 4.24 (t, 2H, J=6.9 Hz, OCH₂CH₂), 6.91 (d, 2H, J=8.1 Hz, CHar), 7.01 (d, 2H, J=8.7 Hz, CHar), 7.23 (t, 2H, J=8.1 Hz, CHar), 7.95 (t, 4H, J=8.1 Hz, CHar), 8.35 (d, 2H, J=8.7 Hz, CHar); ¹³C-NMR (CDCl₃) δ 25.2 (C(CH₃)₂), 29.9 (C(CH₃)₂), 35.1 (OCH₂CH₂), 69.3 (OCH₂CH₂), 115.2, 121.2, 123.3, 124.9, 125.8, 130.1 (CHar), 133.2, 147.1, 148.4, 153.1, 156.2, 162.7 (Car), 177.0 (COOH). Anal. calcd for C₂₄H₂₃N₃O₆: C 64.13, H 5.16, N 9.35. Found C 64.10, H 5.18, N 9.34.

2-[4-(2-{4-[(E)-(4-Methoxyphenyl)diazenyl]phenoxy}ethyl)phenoxy]-2-methylpropanoic acid
(11g)

Yellow solid; 44% yield; m.p. 111.1-113.8 °C; IR (KBr): 3425 (OH), 1716 (C=O), 1597 (N=N) cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3) δ 1.59 (s, 6H, $\text{C}(\text{CH}_3)_2$), 3.09 (t, 2H, $J=6.9$ Hz, OCH_2CH_2), 3.88 (s, 3H, OCH_3), 4.22 (t, 2H, $J=6.9$ Hz, OCH_2CH_2), 6.91 (d, 2H, $J=8.4$ Hz, CHar), 6.95-7.01 (dd, 4H, $J=7.2$ Hz, CHar), 7.22 (d, 2H, $J=8.4$ Hz, CHar), 7.83-7.88 (dd, 4H, $J=7.2$ Hz, CHar); $^{13}\text{C-NMR}$ (CDCl_3) δ 25.1 ($\text{C}(\text{CH}_3)_2$), 35.2 (OCH_2CH_2), 55.8 (OCH_3), 69.1 (OCH_2CH_2), 80.4 ($\text{C}(\text{CH}_3)_2$), 114.4, 114.9, 121.4, 124.6, 130.2 (CHar), 133.7, 147.3, 160.9, 166.4, 171.0 (Car), 181.6 (COOH). Anal. calcd for $\text{C}_{25}\text{H}_{26}\text{N}_2\text{O}_5$: C 69.11, H 6.03, N 6.45. Found C 69.09, H 6.05, N 6.46.

2-Methyl-2-[4-(2-{4-[(E)-phenyldiazenyl]phenyl}ethoxy)phenoxy]propanoic acid (12a)

Orange solid; 97% yield; m.p. 112-127 °C; IR (KBr): 3462 (OH), 1709 (C=O), 1605 (N=N) cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3): δ 1.52 (s, 6H, $\text{C}(\text{CH}_3)_2$), 3.16 (t, 2H, $J = 6.9$ Hz, OCH_2CH_2), 4.19 (t, 2H, $J = 6.9$ Hz, OCH_2CH_2), 6.86-6.89 (m, 4H, CHar), 7.42 (d, 1H, CHar), 7.50 (d, 4H, $J = 9.0$ Hz, CHar), 7.89 (t, 4H, $J = 9.0$ Hz, CHar); $^{13}\text{C-NMR}$ (CDCl_3): δ 24.8 ($\text{C}(\text{CH}_3)_2$), 35.9 (OCH_2CH_2), 68.9 (OCH_2CH_2), 81.9 ($\text{C}(\text{CH}_3)_2$), 116.0, 122.4, 122.6, 125.8, 128.6, 129.8, 130.6 (CHar), 149.7, 1152.6, 143.1, 159.5, 151.5 (Car), 175.2 (COOH). Anal. calcd for $\text{C}_{24}\text{H}_{24}\text{N}_2\text{O}_4$: C 71.27, H 5.98, N 6.93. Found C 71.29, H 5.96, N 6.92.

2-[4-(2-{4-[(E)-(4-Chlorophenyl)diazenyl]phenyl}ethoxy)phenoxy]-2-methylpropanoic acid (12b)

Orange solid; 72% yield; m.p. 150.6-152.8 °C; IR (KBr): 3462 (OH), 1707 (C=O), 1606 (N=N) cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3): δ 1.52 (s, 6H, $\text{C}(\text{CH}_3)_2$), 3.16 (t, 2H, $J = 6.9$ Hz, OCH_2CH_2), 4.18 (t, 2H, $J = 6.9$ Hz, OCH_2CH_2), 6.81 (d, 2H, $J = 9$ Hz, CHar), 6.91 (d, 2H, $J = 9$ Hz, CHar), 7.43 (d, 2H, $J = 8.7$ Hz, CHar), 7.48 (d, 2H, $J = 8.7$ Hz, CHar), 7.85 (d, 2H, $J = 2.1$ Hz, CHar), 7.88 (d, 2H, $J = 2.1$ Hz, CHar); $^{13}\text{C-NMR}$ (CDCl_3): δ 24.9 ($\text{C}(\text{CH}_3)_2$), 35.9

(OCH₂CH₂), 68.9 (OCH₂CH₂), 80.9 (C(CH₃)₂), 115.3, 123.3, 123.5, 124.3, 129.5, 130.0 (CHar), 137.0 (Car), 142.2 (CCl), 147.2, 151.2, 151.5, 155.7 (Car), 175.7 (COOH). Anal. calcd for C₂₄H₂₃ClN₂O₄: C 65.68, H 5.28, N 6.38. Found C 65.67, H 5.30, N 6.36.

2-[4-(2-{4-[(E)-(4-Bromophenyl)diazenyl]phenyl}ethoxy)phenoxy]-2-methylpropanoic acid (12c)

Orange solid; 79 % yield; m.p. 167.3-189.9 °C; IR (KBr): 3445 (OH), 1704 (C=O), 1605 (N=N) cm⁻¹; ¹H-NMR (CDCl₃): δ 1.52 (s, 6H, C(CH₃)₂), 3.16 (t, 2H, J = 6.9 Hz, OCH₂CH₂), 4.18 (t, 2H, J = 6.9 Hz, OCH₂CH₂), 6.82 (d, 2H, J = 9 Hz, CHar), 6.9 (d, 2H, J = 9 Hz, CHar), 7.42 (d, 2H, J = 8.4 Hz, CHar), 7.64 (d, 2H, J = 8.4 Hz, CHar), 7.79 (d, 2H, J = 8.1, CHar), 7.87 (d, 2H, J = 8.1 Hz, CHar); ¹³C-NMR (CDCl₃): δ 24.9 (C(CH₃)₂), 35.9 (OCH₂CH₂), 68.8 (OCH₂CH₂), 80.9 (C(CH₃)₂), 121.1, 122.4, 123.5, 124.5, 125.5, 130.0 (CHar), 132.5 (CarBr), 142.3, 151.5, 151.6, 155.7, 155.9 (Car), 175.9 (COOH). Anal. calcd for C₂₄H₂₃BrN₂O₄: C 59.64, H 4.80, N 5.80. Found C 59.62, H 4.81, N 5.78.

2-Methyl-2-[4-[2-(4-{(E)-[4-(trifluoromethyl)phenyl]diazenyl]phenyl}ethoxy)phenoxy]propanoic acid (12d)

Orange solid; 23 % yield; m.p. 103.4-105.5 °C; IR (KBr): 3233 (OH), 1720 (C=O), 1610 (N=N) cm⁻¹; ¹H-NMR (CD₃OD): δ 1.48 (s, 6H, C(CH₃)₂), 3.30 (t, 2H, J = 6.6 Hz, OCH₂CH₂), 4.21 (t, 2H, J = 6.6 Hz, OCH₂CH₂), 6.66 (d, 2H, J = 9.3 Hz, CHar), 6.79 (d, 2H, J = 9.3 Hz, CHar), 7.51 (d, 2H, J = 8.4 Hz, CHar), 7.87 (d, 2H, J = 8.4 Hz, CHar), 7.91 (d, 2H, J = 9.3, CHar), 8.03 (d, 2H, J = 9.3 Hz, CHar); ¹³C-NMR (CD₃OD): δ 24.7 (C(CH₃)₂), 35.3 (OCH₂CH₂), 68.4 (OCH₂CH₂), 79.8 (C(CH₃)₂), 114.5, 121.0, 122.7, 122.8, 126.0, 129.7 (CHar), 126.1 (CF₃), 143.7 (CCF₃), 144.9, 147.6, 153.9, 154.6, 164.9 (Car), 175.2 (COOH). Anal. calcd for C₂₅H₂₃F₃N₂O₄: C 63.55, H 4.91, N 5.93. Found C 63.58, H 4.89, N 5.95.

2-[4-(2-{4-[(E)-(4-Cyanophenyl)diazenyl]phenyl}ethoxy)phenoxy]-2-methylpropanoic acid
(12e)

Orange solid; 62 % yield; m.p. 162-165 °C; IR (KBr): 3193 (OH), 2246 (CN), 1742 (C=O), 1606 (N=N) cm⁻¹; ¹H-NMR (CDCl₃): δ 1.52 (s, 6H, C(CH₃)₂), 3.18 (t, 2H, J = 6.6 Hz, OCH₂CH₂), 4.20 (t, 2H, J = 6.6 Hz, OCH₂CH₂), 6.82 (d, 2H, J = 9.3 Hz, CHar), 6.91 (d, 2H, J = 9.3 Hz, CHar), 7.46 (d, 2H, J = 8.7 Hz, CHar), 7.81 (d, 2H, J = 9 Hz, CHar), 7.91 (d, 2H, J = 8.7 Hz, CHar), 7.97 (d, 2H, J = 9 Hz, CHar); ¹³C-NMR (CDCl₃): δ 24.9 (C(CH₃)₂), 35.9 (OCH₂CH₂), 68.7 (OCH₂CH₂), 80.8 (C(CH₃)₂), 114.0 (CN), 115.3 (CHar), 118.8 (CarCN), 123.4, 123.5, 123.7, 130.2, 133.5 (CHar), 143.5, 147.5, 151.4, 154.8, 155.5 (Car), 176.3 (COOH). Anal. calcd for C₂₅H₂₃N₃O₄: C 69.92, H 5.40, N 9.78. Found C 69.93, H 5.43, N 9.76.

2-Methyl-2-[4-(2-{4-[(E)-(4-nitrophenyl)diazenyl]phenyl}ethoxy)phenoxy]propanoic acid
(12f)

Red solid; 62 % yield; m.p. 148.8-150.3 °C; IR (KBr): 3463 (OH), 1709 (C=O), 1615 (N=N) cm⁻¹; ¹H-NMR (CDCl₃): δ 1.52 (s, 6H, C(CH₃)₂), 3.18 (t, 2H, J = 6.6 Hz, OCH₂CH₂), 4.20 (t, 2H, J = 6.6 Hz, OCH₂CH₂), 6.82 (d, 2H, J = 9.3 Hz, CHar), 6.9 (d, 2H, J = 9.3 Hz, CHar), 7.46 (d, 2H, J = 8.4 Hz, CHar), 7.9 (d, 2H, J = 8.4 Hz, CHar), 8.02 (d, 2H, J = 9.3, CHar), 8.4 (d, 2H, J = 9.3 Hz, CHar); ¹³C-NMR (CDCl₃): δ 25.0 (C(CH₃)₂), 36.0 (OCH₂CH₂), 68.7 (OCH₂CH₂), 80.8 (C(CH₃)₂), 115.3, 123.4, 123.6, 123.8, 125.0, 130.2 (CHar), 143.7, 147.5 (Car), 148.8 (CarNO₂), 151.4, 155.5, 155.9 (Car), 175.2 (COOH). Anal. calcd for C₂₄H₂₃N₃O₆: C 64.13, H 5.16, N 9.35. Found C 64.15, H 5.13, N 9.33.

Biological Methods

Reference compounds, the medium, and other cell culture reagents were purchased from Sigma-Aldrich (Milan, Italy) and Invitrogen (Carlsbad, CA).

Cell Culture and Transfections

Human hepatoblastoma cell line HepG2 was cultured at 37° C and in a humidified atmosphere of 5% CO₂ in growth medium composed of Minimum Essential Medium (MEM) containing 10% of heat-inactivated FBS, 1% penicillin G/streptomycin, 1% MEM non-essential amino acid, and 1% Glutamine. For transactivation assays, 10⁵ cells/well were seeded in a 24-well plate and cultured until confluency. Cells were transiently transfected with 30 ng of expression plasmids encoding the fusion protein GAL4-PPAR α -LBD, GAL4-PPAR γ -LBD or GAL4-PPAR β/δ -LBD, 100 ng of reporter plasmid pGAL5TKpGL3, and 150 ng of control plasmid pCMV β gal per well by CAPHOS®, a calcium-phosphate coprecipitation method, according to the manufacturer's guidelines. Four hours after transfection, medium was replaced with fresh serum-free medium supplemented with test compound (ranging from 100 nM to 100 μ M), reference compounds clofibric acid (100 μ M), rosiglitazone (2 μ M), and L165,041 (2 μ M), or DMSO 0.1%. After 20-22 h treatment, luciferase activity and β -galactosidase activity in cell extracts were determined by a Multilabel Plate Reader (VICTOR³ V, PerkinElmer) as previously described.² Luciferase activity were normalized to β -galactosidase activity to correct the transfection efficiencies. All transfection experiments were performed in triplicate and repeated at least twice.

Cell culture, RNA extraction and gene expression analysis

HepG2 cells were seeded in 6-well plates at a density of 5 × 10⁵ cells/well in 2 mL of culture medium (DMEM with 10% FBS) per well and grown at 37 °C, 5% CO₂ for 24 h. Then, cells

were incubated for 48 h with a serum free medium containing the test compound **12a** (100 μ M, 0.25% DMSO). Treatments with the commercial compounds L165,041 (2 μ M, 0.1% DMSO), GW7647 (2 μ M, 0.02% DMSO) or rosiglitazone (10 μ M, 0.03% DMSO) were used as reference controls for activation of PPAR β/δ , PPAR α or PPAR γ , respectively.

The effect of the treatments was evaluated on the expression of *PDK4*, *CPT1A* and *GLUT1* genes. The expression analyses were performed by real-time quantitative PCR (RTqPCR) as previously described³ with minor modifications. Briefly, after treatments, cells were washed with PBS and total RNA was isolated using the TRIzol reagent (Invitrogen, Carlsbad, CA), treated with Turbo DNA-Free kit (Life Technologies), quantified using the Nanodrop 2000 (Thermo Scientific) and retro-transcribed using the High Capacity cDNA Reverse Transcription kit (Life Technologies). RTqPCR assays were performed in 96-well optical reaction plates using the PowerUp SYBR Green Master Mix (Life Technologies) on the ABI 7900HT Real-Time PCR platform (Applied Biosystem) with the following primers: 5'-AGAGCCTGATGGATTTGGTG-3' (FW) and 5'-GCTTGGGTTTCCTGTCTGTG-3' (RW) for PDK4,⁴ 5'-TGCCATGGATCTGCTGTATATCC-3' (FW) and 5'-GCGTTGCCGGCTCTTG-3' (RW) for CPT1A;³ 5'-ATCGTGGCCATCTTTGGCTTTGTG-3' (FW) and 5'-CTGGAAGCACATGCCCAATGAA-3' (RW) for GLUT1⁵ and 5'-TGCCATCGCCAAGGAGTAG-3' (FW) and 5'-TGCACAGACGGTCACTCAAA-3' (RW) for cyclophilin (PPIB).⁶ RTqPCR was performed in duplicate plates, with each sample analyzed in triplicate wells for each plate. Quantitative normalization was performed using the expression of PPIB as internal control. Relative quantification was performed using the DDCT method using as a calibrator the cDNA obtained from cells cultured adding only the compound's vehicle (DMSO) in the medium.

Ex vivo studies

Male adult Sprague-Dawley rats (200-250 g) were housed in Plexiglas cages (40 cm × 25 cm × 15 cm), placed in climatized colony rooms (22 ± 1 °C; 60% humidity), on a 12 h/12 h light/dark cycle (light phase: 07:00–19:00 h). Rats were fed *ad libitum* a standard laboratory diet (chow; 3.20 kcal/g). Housing conditions and experimentation procedures were strictly in accordance with the EU Directive 63/2017/EU. Liver and cortex specimens were obtained as residual material from vehicle-treated rats randomized in our previous experiments approved by Italian Health Ministry (Italian Health Ministry authorization N. 880, delivered on 24th August 2015). Rats were sacrificed by CO₂ inhalation (100% CO₂ at a flow rate of 20% of the chamber volume per min), and both liver and cortex specimens were immediately collected and maintained in a humidified incubator with 5% CO₂ at 37°C for 4 h, in RPMI buffer with added bacterial LPS (10 µg/mL) (incubation period). During the incubation period, tissues were treated with WY14643 (1 µM), a widely used PPAR α activator reference agent, the PPAR γ agonist pioglitazone (1 µM), the PPAR β/δ agonist L165,041 (1 µM) and scalar concentrations of **12a** (0.1–10 µM). Tissue supernatants were collected and PGE₂ (ng/mg wet tissue) were measured by radioimmunoassay (RIA), as previously reported.⁷ Briefly, specific anti-PGE₂ were developed in the rabbit; the cross-reactivity against other prostanoids is <0.3%. One hundred microliters of prostaglandin standard or sample was incubated overnight at 4°C with the ³H-prostaglandin (3000 cpm/tube; NEN) and antibody (final dilution: 1:120000), in a volume of 1.5 mL of 0.025 M phosphate buffer. Free and antibody-bound prostaglandins were separated by the addition of 100 µL 5% bovine serum albumin and 100 µL 3% charcoal suspension, followed by centrifuging for 10 min at 4000 × g at 5°C and decanting off the supernatants into scintillation fluid (UltimaGold™, Perkin Elmer) for β emission counting. The detection limit of the assay method is 0.6 pg/mL.

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 5.01 for Windows (GraphPad Software, San Diego, CA). Means \pm SEM were determined for each experimental group and analyzed by one-way analysis of variance (ANOVA), followed by Newman-Keuls comparison multiple test. Statistical significance was set as $p < 0.05$. As regards the animals randomized for each experimental group, the number was calculated on the basis of the “Resource Equation” $N=(E+T)/T$ ($10 \leq E \leq 20$),⁸ according to the guidelines suggested by the “National Centre for the Replacement, Refinement and Reduction of Animals in Research” (NC3RS) and reported on the following web site: <https://www.nc3rs.org.uk/experimental-designstatistics>. In particular, N is the number of animals per treated group. E represents the degrees of freedom of the ANOVA). T is the number of treatments. Considering that E values should be between 10 and 20, the animal number N for ex vivo analysis was chosen in accordance to an E value of 20.

Computational Chemistry

Protein and Ligand Preparation

The crystal structures of PPAR α /**11a** (PDB ID: 4CI4), PPAR γ /**11a** (PDB ID: 4CI5),⁹ as well as PPAR δ in complex with the ligand 6-(2-((N-cyclopropyl-4-(furan-2-yl)benzamido)methyl)phenoxy)hexanoic acid (PDB entry: 5U3Q),¹⁰ recovered from Brookhaven Protein Database, were employed for the automated docking experiments.

The proteins were processed through the Protein Preparation Wizard in Maestro version 11.0 (Schrödinger, LLC, New York, NY, 2017). X-ray water molecules were removed, the appropriate bond orders as well as charges and atom types were assigned and the hydrogen atoms were added to the three protein structures. The H-bond network was optimized by exhaustive sampling of rotamers, tautomers and protonation states of titratable amino acids at neutral pH. Imidazole rings of H440 into PPAR α , H449 and H323 into PPAR γ , and H287 and H413 into PPAR δ were set in their N ϵ 2-H (N *tau*-H) tautomeric state. Finally, the protein structures were relaxed with by means of a restrained minimization using the Impref module with the OPLS2005 force field by imposing a 0.3 Å rmsd limit from the initial coordinates as the constraint.

The core structures of **11a** and **12a** were sketched using the Molecular Builder module in Maestro. The ligands were then preprocessed with LigPrep 3.3 (Schrödinger, LLC, New York, NY, 2017) and optimized by means of Macromodel 11.5 (Schrödinger, LLC, New York, NY, 2017), employing the MMFFs force field with 1000 steps of steepest descent; the resulting molecules were then submitted to 500 steps of truncated Newton conjugate gradient method. Partial atomic charges were assigned using the OPLS-AA force field.

Docking and Cluster Analysis

Docking of **11a** and **12a** to PPAR α , PPAR γ , and PPAR δ was performed with the genetic algorithm implemented in GOLD software (CCDC Software Limited: Cambridge, U.K.), for. The coordinates of the cocrystallized ligands **11a** for both PPAR α and PPAR γ , and 6-(2-((N-isopropyl-[1,1'-biphenyl]-4-carboxamido)methyl)phenoxy)hexanoic acid for PPAR δ were chosen as active-site origin. The active-site radius was set equal to 10 Å. For the purposes of this study, the number of GA runs was set to 1000 and the early termination option was switched off. The obtained docked poses were ranked according to the original ChemPLP scoring function and rescored with ChemScore.¹¹ Subsequently, the ACIAP program was used to perform a cluster analysis on the top 20 percent of poses.¹² Figure 2 in the manuscript shows the binding modes representative of the most significantly populated clusters on the basis of the Chaevenaut criterion contained in the script. Noteworthy, such poses correspond to the highest ranked poses according to ChemScore.

Figures in the manuscript were rendered with Pymol 2.0 (Schrödinger, LLC, New York, NY, 2017). All computations were performed on a E4 Computer Engineering E1080 workstation provided of a Intel Core i7-930 Quad-Core processor.

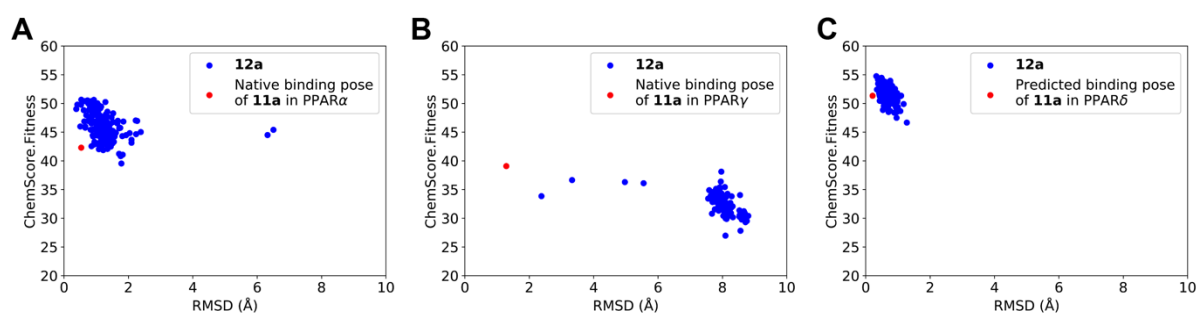


Figure S2. Docking of **12a** into the LBD of PPAR α (A), PPAR γ (B) and PPAR δ (C) crystal structures. The RMSD between 10,000 binding poses of **12a** (shown in blue) and the pose closest to the native binding mode of **11a** (shown in red) was computed. The top 20 percent of poses by GOLD scores (ChemScore.Fitness) are shown here.

Table S1. Docking and clustering outcomes for each of the investigated complexes^a

Subtype	Cluster N°	Cardinality	RMSD [Å]	ChemScore.Fitness
PPAR α	1	28	1.27	50.65
PPAR γ	1	105	8.01	36.39
PPAR δ	1	50	0.33	54.75

^aFor clarity, only the most relevant clusters are reported. The RMSD refers to the distance between the representative pose of each cluster and that closest to the experimental one. The representative structure is defined as the configuration closest to the geometric center of the cluster.

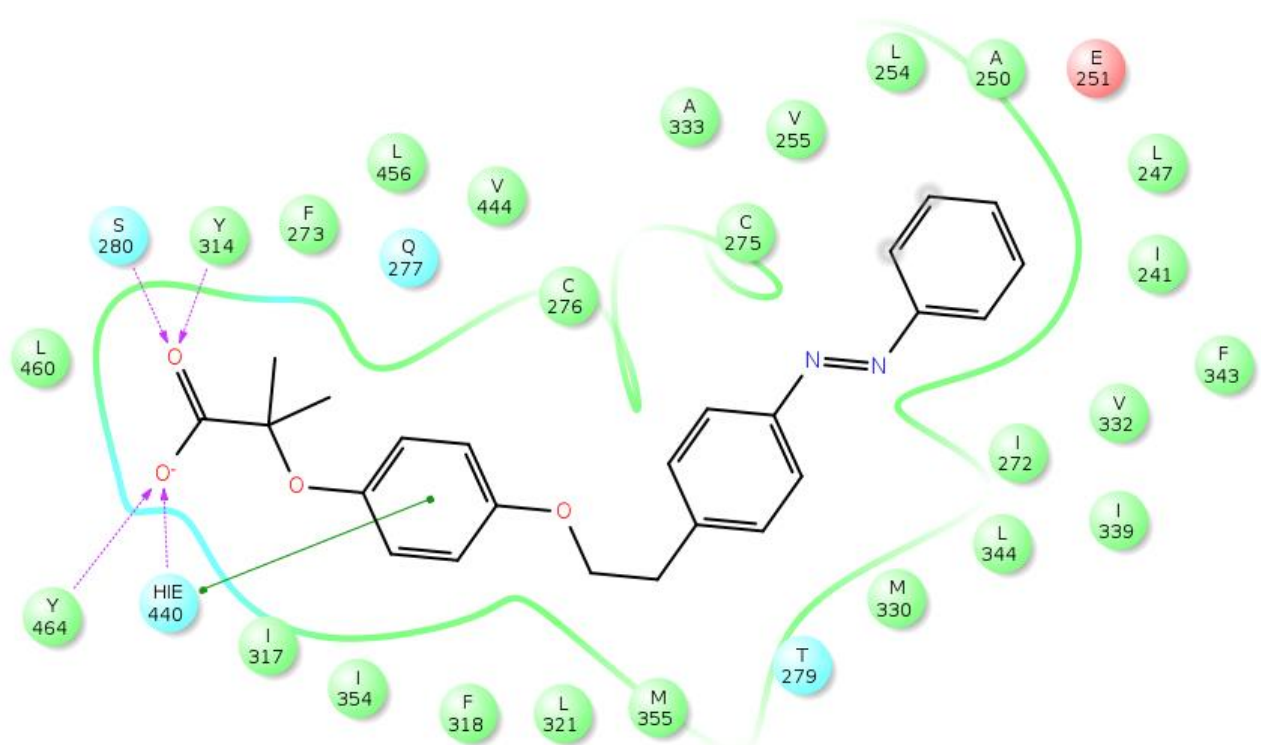


Figure S3. 2D ligand-interaction diagram of compound 12a into the PPAR α binding pocket. Positively charged amino acids are represented with dark blue circles, negatively charged amino acids are represented with red circles, polar amino acids are represented with light blue circles and hydrophobic amino acids are represented with green circles. H-bonds are depicted with purple arrows—dashed arrows for H-bonds involving amino acid side chain and regular arrows for H-bonds involving amino acid backbone. Straight green lines represent π -stacking interactions.

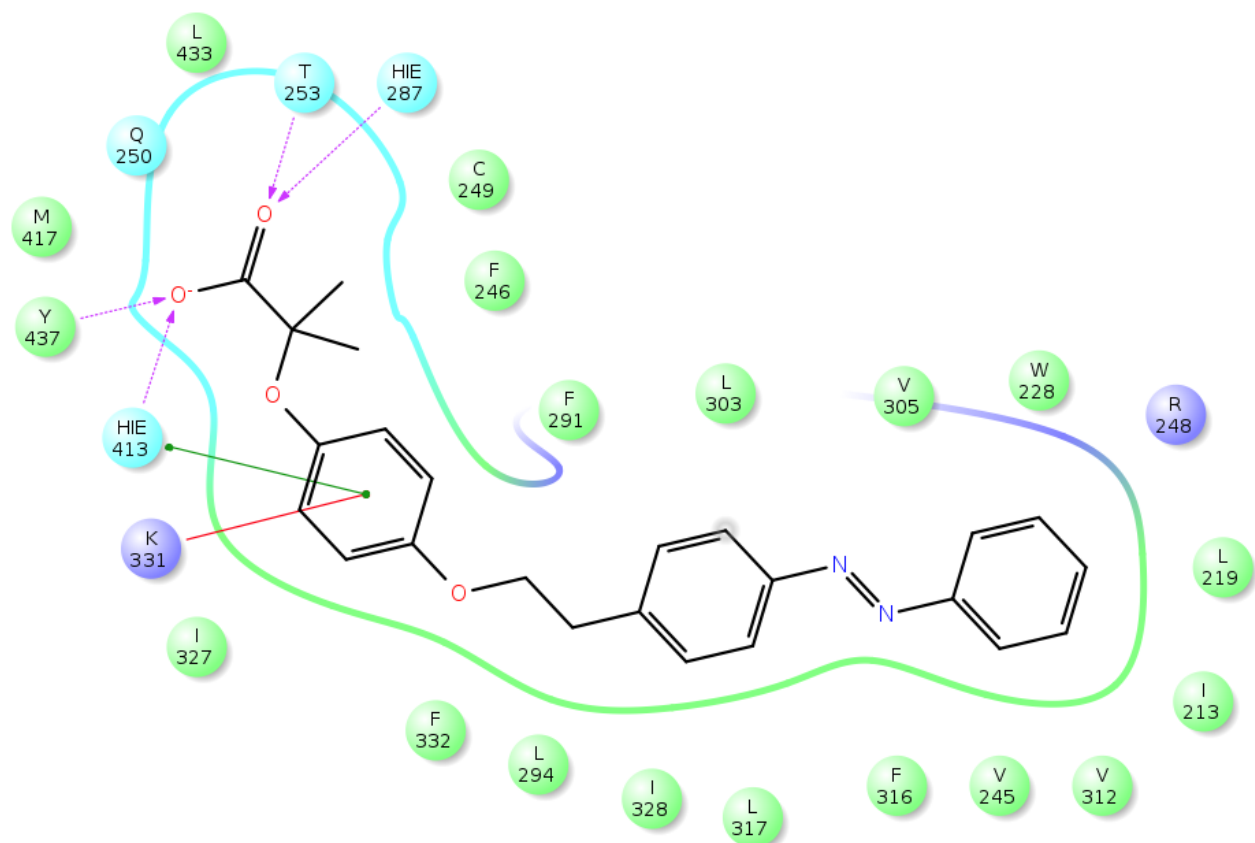


Figure S4. 2D ligand-interaction diagram of compound 12a into the PPAR δ binding pocket. Positively charged amino acids are represented with dark blue circles, negatively charged amino acids are represented with red circles, polar amino acids are represented with light blue circles and hydrophobic amino acids are represented with green circles. H-bonds are depicted with purple arrows—dashed arrows for H-bonds involving amino acid side chain and regular arrows for H-bonds involving amino acid backbone. Straight green lines represent π -stacking interactions, while straight red lines represent cation- π interactions.

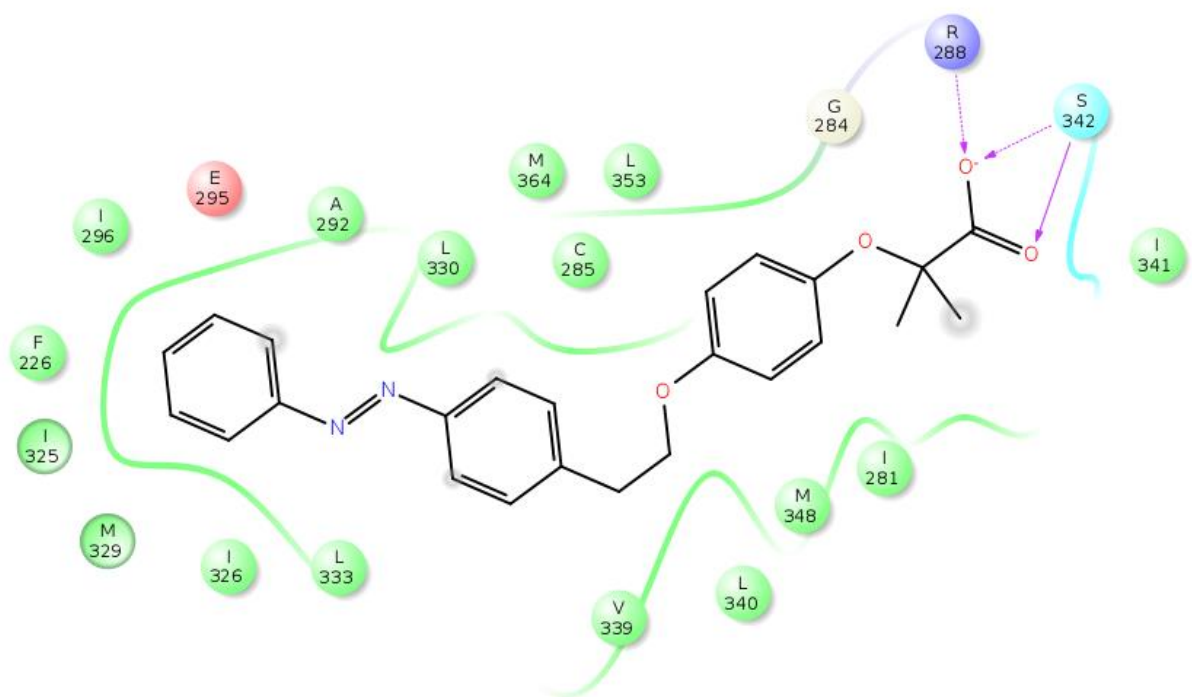
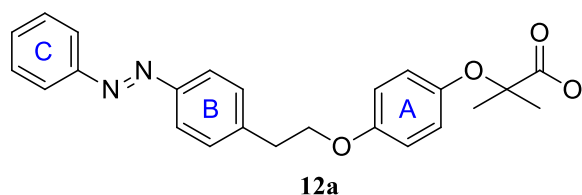


Figure S5. 2D ligand-interaction diagram of compound 12a into the PPAR γ binding pocket. Positively charged amino acids are represented with dark blue circles, negatively charged amino acids are represented with red circles, polar amino acids are represented with light blue circles and hydrophobic amino acids are represented with green circles. H-bonds are depicted with purple arrows—dashed arrows for H-bonds involving amino acid side chain and regular arrows for H-bonds involving amino acid backbone.

Table S2. Major interactions between 12a and PPAR α , PPAR δ and PPAR γ .



PPAR subtype	Type of interaction	Ligand atoms	Residues involved	Distance (Å)
PPAR α	H-bond	O ⁻	Y464 (OH)	2.5
		O ⁻	H440 (NE2)	2.6
		O	Y314 (OH)	3.1
		O	S280 (OG)	3.0
	π - π	Ring A (centroid)	H440 (centroid)	5.0
	Sulphur-aromatic	Ring C (closest atom)	C275 (SG)	3.7
		Ring B (closest atom)	C276 (SG)	3.6
Ring B (closest atom)		M355 (SD)	4.2	
Ring B (closest atom)		M330 (SD)	3.8	
PPAR δ	H-bond	O ⁻	Y347 (OH)	2.6
		O ⁻	H413 (NE2)	2.6
		O	H287 (NE2)	3.1
		O	T253 (OG1)	3.1
	π - π	Ring A (centroid)	H413 (centroid)	4.9
	Cation- π	Ring A (centroid)	K331 (NZ)	5.3
	Sulphur-aromatic	Ring B (closest atom)	C249 (SG)	3.6
PPAR γ	H-bond	O ⁻	S342 (OG)	2.9
		O ⁻	R288 (NE)	3.0
		O ⁻	R288 (NH ₂)	3.2
		O	S342 (N)	3.0
	Sulphur-aromatic	Ring A (closest atom)	C285 (SG)	3.0

Valence-bond (or resonance) approach to azobenzene system

It is expected that the oxygen directly linked to the azobenzene system in **11a** (structure I in Figure S6) generates a different electron distribution on its mesomeric quinonic form (structure II), accommodating the negative charge on the electronegative nitrogen atom of the azo group. This charge-separated resonance form decreases the electron donor π orbital behavior towards the electron acceptor S–H σ^* orbital, negatively affecting the above mentioned sulfur-aromatic interactions. This rationalizes the lower PPAR α potency of **11a** with respect to **12a**.

The increase of PPAR α potency of **11b** and **11c** (5.5- and 2.6-fold, respectively) compared to **11a** seems due to charge delocalization onto the distal phenyl ring of azobenzene as a consequence of the electron-withdrawing character of chlorine and bromine substituents (structure V).

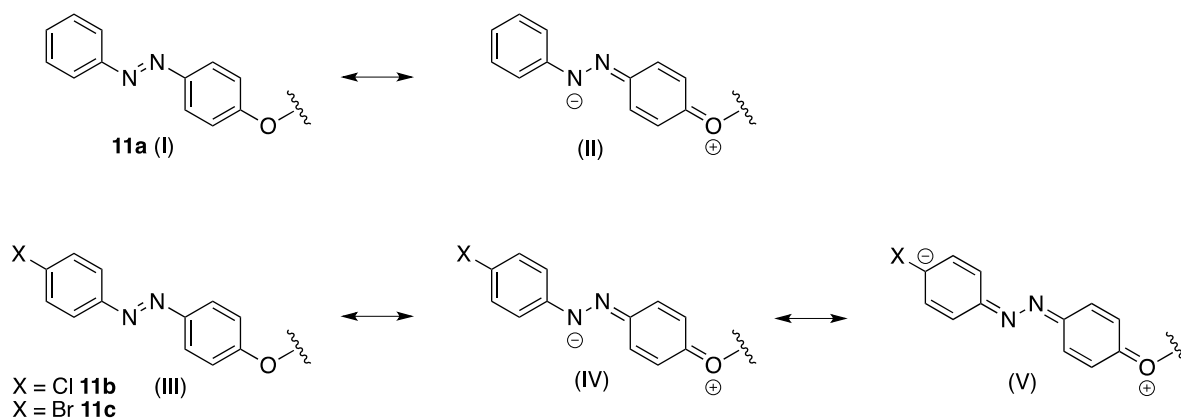


Figure S6. Resonance forms of derivatives **11a-c**.

Binding mode of 12a into PPAR γ LBD

12a is situated at the entrance of the active site, between H3 and the β -sheet, a common site of PPAR γ partial agonists, and does not interact directly with H12. Figure S7 shows the singular mode of binding of **12a** compared with that of the PPAR γ -bound crystal structure of **11a**.⁹ The clofibric acid group of the ligand makes H-bonds with the main chain amide nitrogen and the oxygen atom of S342 as well as with the guanidinium group of R288. Moreover, this group establishes several hydrophobic interactions with the β -sheet, in particular with I341 and M348. In addition, the phenoxy oxygen of **12a** accepts a H-bond from C285 on H3. The ring bearing the clofibric moiety forms numerous hydrophobic and van der Waals contacts, further increasing the stabilization of H3, while the azobenzene tail makes hydrophobic contacts at entrance region of binding site. Therefore, the low affinity and the attenuated transcriptional response of **12a** and its derivatives towards PPAR γ might be ascribed to the peculiar binding mode, which is not able to replicate the spectrum of contacts of full agonists.²⁸

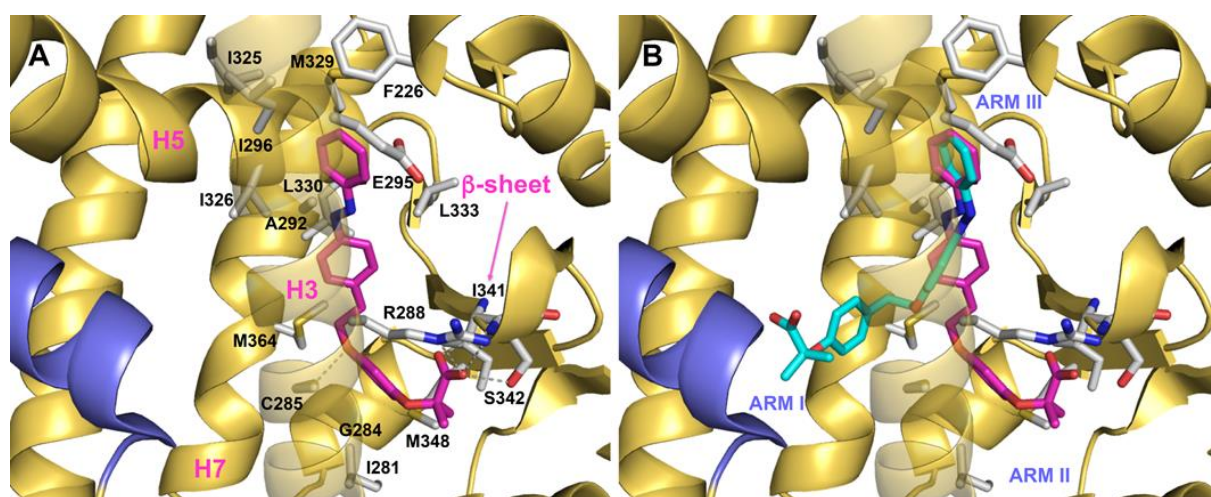
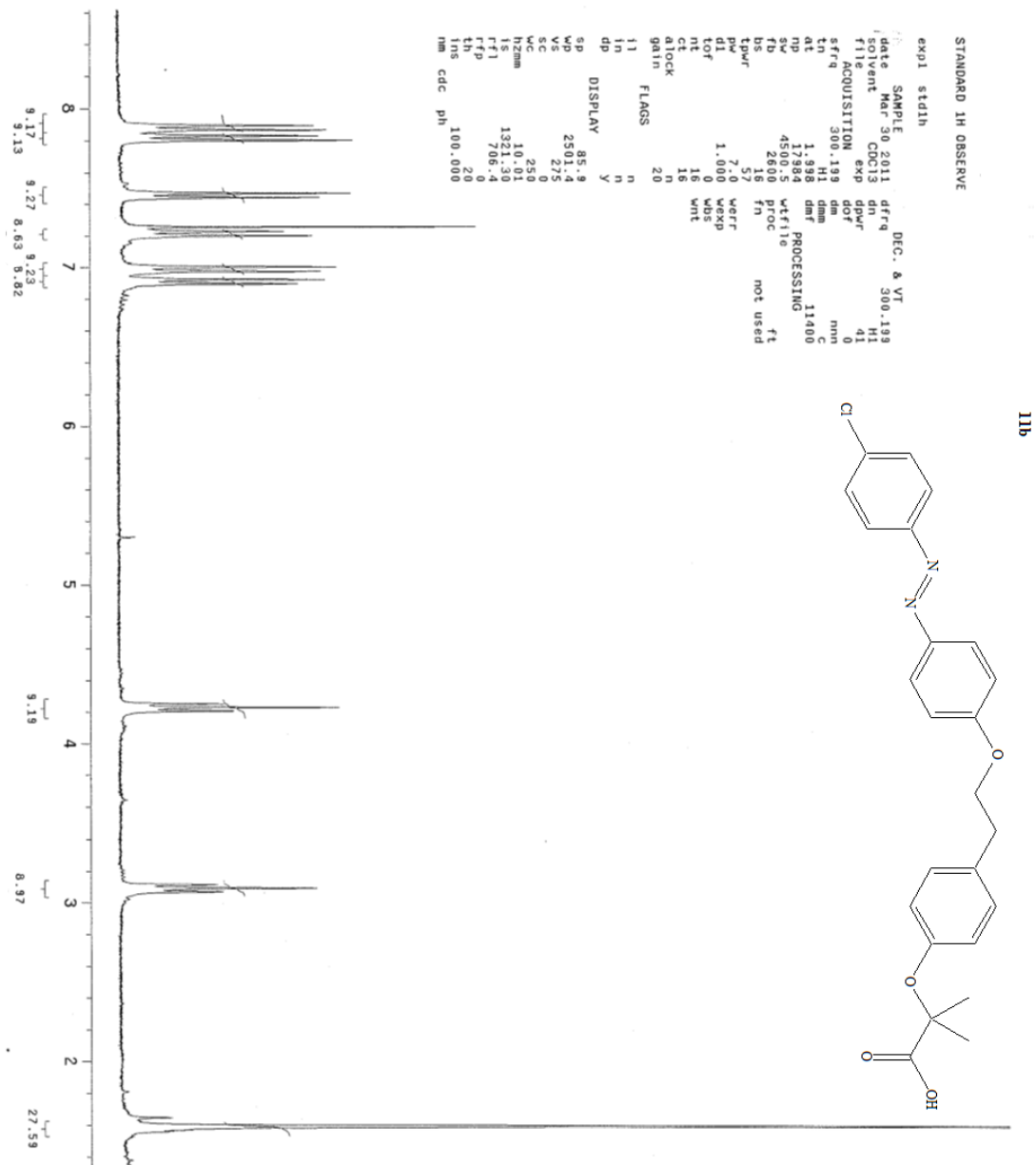
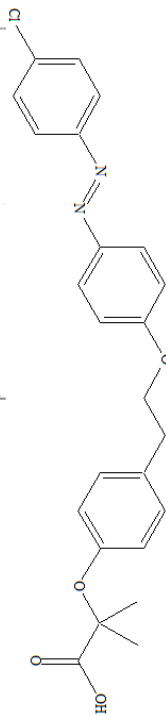


Figure S7. Binding mode of compound **12a** (magenta sticks) into the PPAR γ LBD (A) represented as a yellow ribbon model. Only amino acids located within 4.5 Å of the bound ligand are displayed (white sticks) and labeled. H-bonds discussed in the text are depicted as dashed grey lines. H12 is shown in slate. (B) $C\alpha$ superposition of the complex of PPAR γ with compounds **12a** and **11a** (cyan sticks, PDB entry: 4CI5).

Figure S8. ¹H and ¹³C spectra of final compounds 11b-g and 12a-f

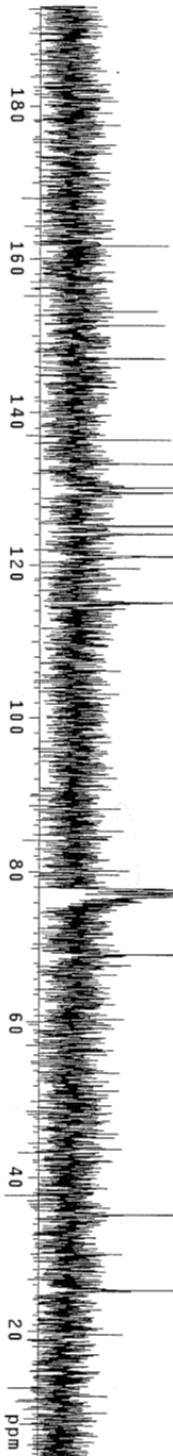


11b



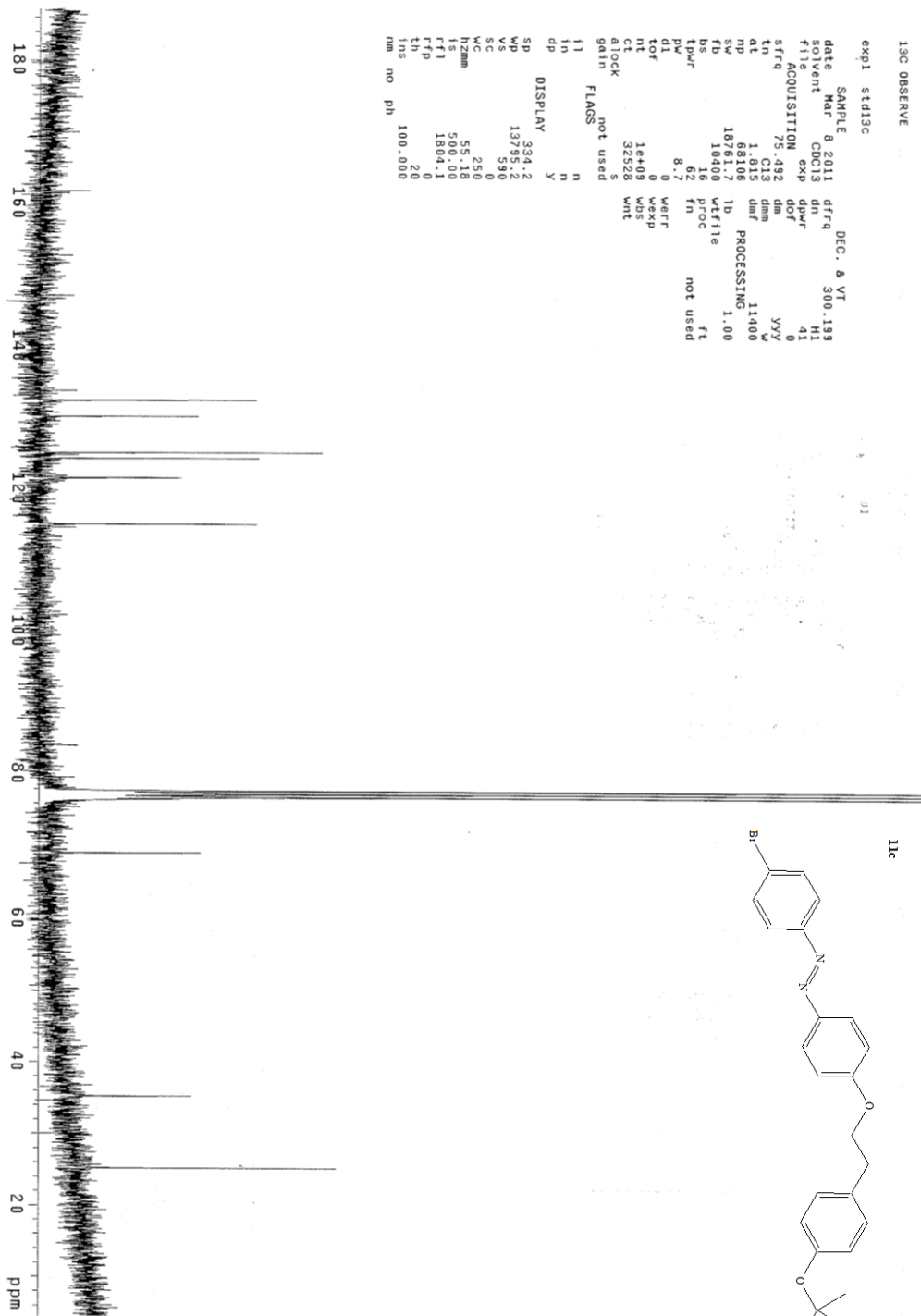
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f1 le ACQUISITION exp dpwr 41
sfreq 75.492 dmf YVW
tn C13 dmm W
at 1.815 dmf 11400
np 68106 PROCESSING 1.00
sw 18761.7 1b wtfile
fb 10400 proc
us 16 fn not used
dpwr 8.7
p 0 weff
dl 0 wexp
tof 1e+08 wbs
ct 1728 wnt
atlock not used
gain not used
ll n
in n
dp y
DISPLAY 286.6
wp 1429.1
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wc 250
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is 500.00
rf1 1804.1
rfp 20
th 20
rms 100.000
nm no ph



13C OBSERVE

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file exp dpar H1
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tn 1.615 dm W
np 68106 dm 11400
sw 18761.7 lb PROCESsing 1.00
fb 10400 wfile
bs 16 proc ft
lpar 62 fn not used
pw 8.7 wprt
nt 1e+09 wexp
ct 32528 wds
a1ock not used
gain 5 wnt
fl 11
in n
dp Y
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rfp 0
tms 20
nm no ph 100.000



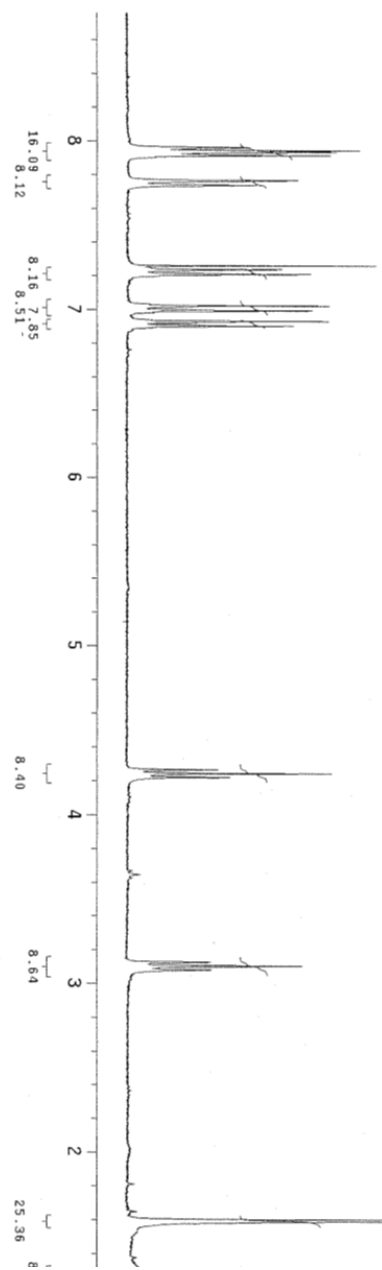
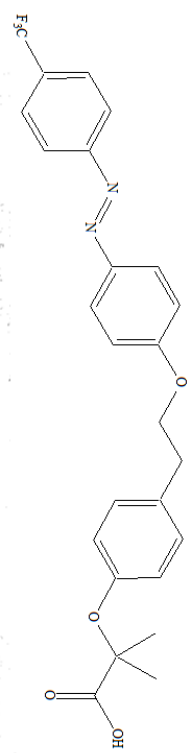
STANDARD 1H OBSERVE

11d

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sw 4506.3 wtf11e not used
bs 2016 tn
tpwr 57
pw 7.0 warr
d1 1.000 wexp
tof 0 wds
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stlck 16
gain 20
flags
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in n
dp y
DISPLAY 91.9
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nm cdc ph

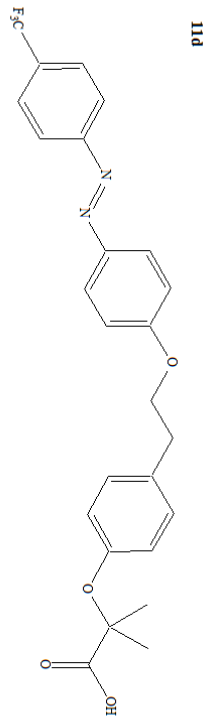
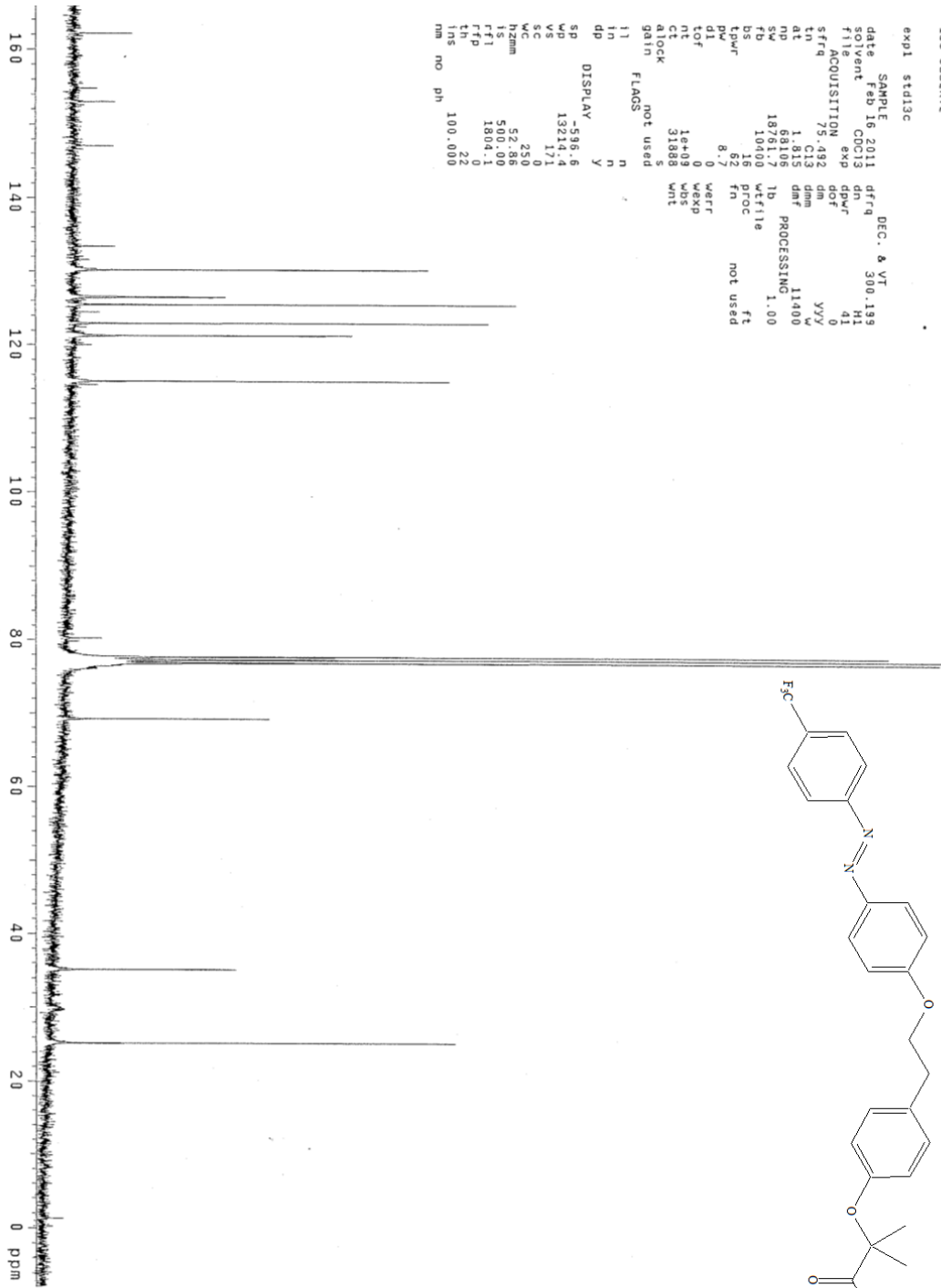
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13C OBSERVE

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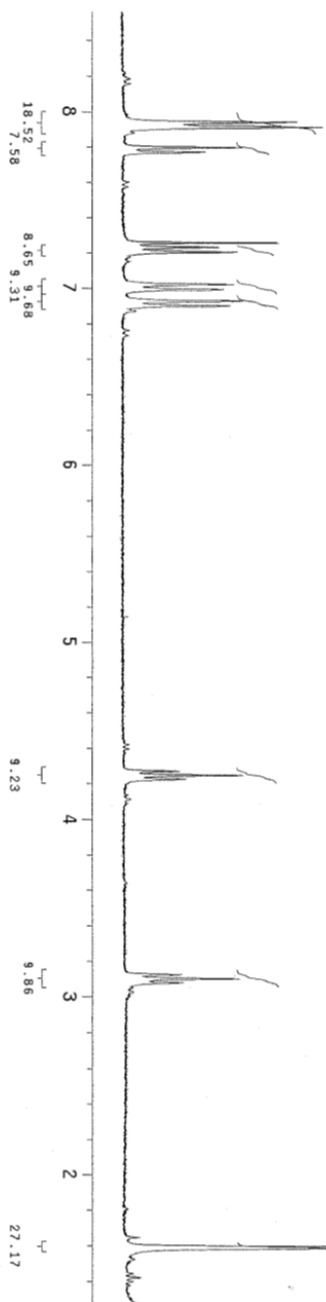
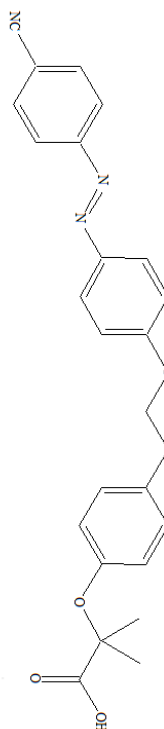
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gain not used
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ln n
in n
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STANDARD IN OBSERVE

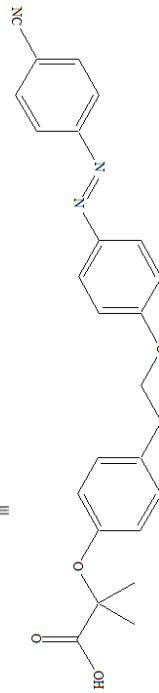
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sw 17984
sf 4500.5 wfile
fb 2600 proc
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tpwr 57
pw 7.0 werr
dt 1.000 wexp
of 16 wms
ct 16 wnt
atock n
gain 20
FLAGS
11 n
1n n
dn y
DISPLAY
sp 133.4
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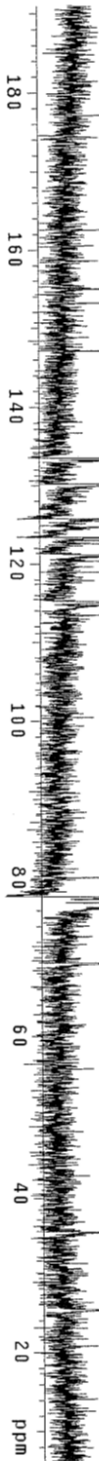


13C OBSERVE

11e



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IN 1.813 dnm 11400
AQ 68106 dmf PROCESSING 1.00
SW 18751.7 lb wifile
FD 10400 wifile ft
BS 16 PROC not used
TPWR 62 fn
PW 8.7
DI 0 WERT
DOF 10+09 WEXP
CT 4624 WNT
ATOCK S
gain not used
FLAGS
I1 n
I2 n
IN n
DP y
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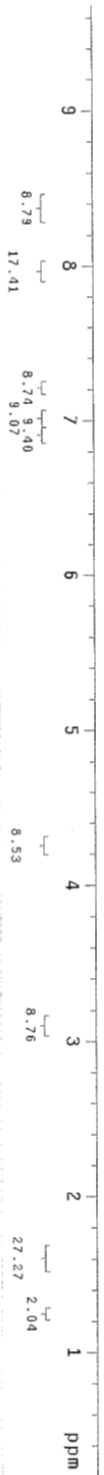
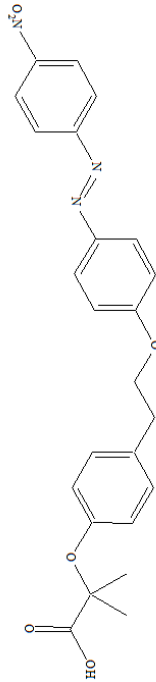


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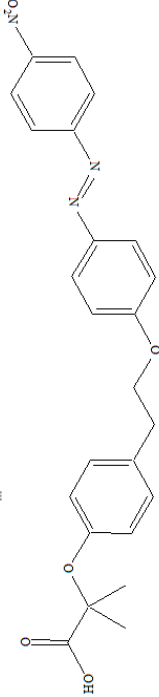
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pw 7.0
d1 1.000
tof 0
nt 16
ct 16
clock n
gain n
ll 1
in n
dp y
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nm cdc ph

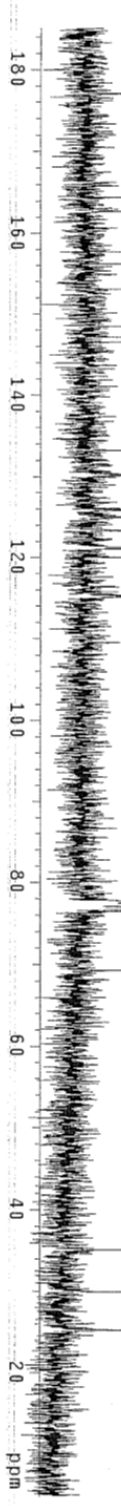
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300.199
dn H1
dppwr 41
dof 0
nmn C
11400
ft
not used

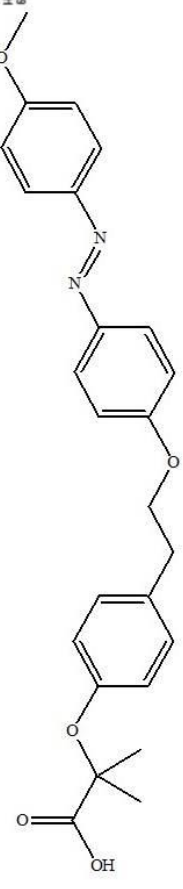


1H



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at 68106 dmf 11400
sw 18761.7 lb wtfile 1.00
fb 10400 proc ft
bs 16 not used
tpwr 82 fn
d1w 8.7 werr
d1v 0 wep
tof 1e+09 wbs
ct 4448 wnt
atlock gain not used
flags not used
11 n
1n n
dp y
DISPLAY
sp 365.4
wp 13508.9
vs 235
sc 0
wc 250
Hzmm 54.44
f1 500.44
rfi 1804.1
th 0
ins 38
nm no ph 100.000





13C OBSERVE

expt statisc

SAMPLE 3 2011 DEC. 8 VT

date Mar 3 2011 dfrq 300.19 H

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ACQUISITION

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an 1.813 dm

np 68106 dm 11400

sw 18761.7 lb PROCESSING 1.00

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bs 16 proc not used

tpwr 62 fn

pw 8.7 warr

nt 0 wexp

ct 1e+09 wds

gain 2448 wnt

atlock not used

flags

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dp n

DISPLAY

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fs 500.00

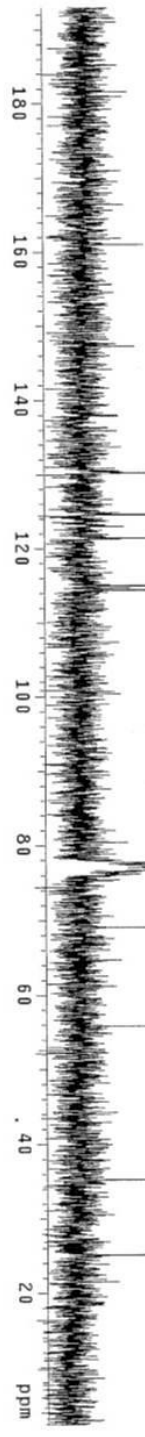
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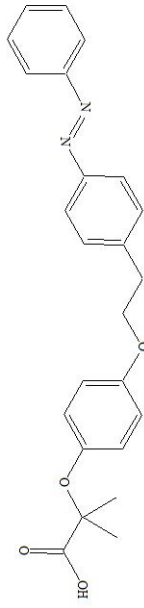
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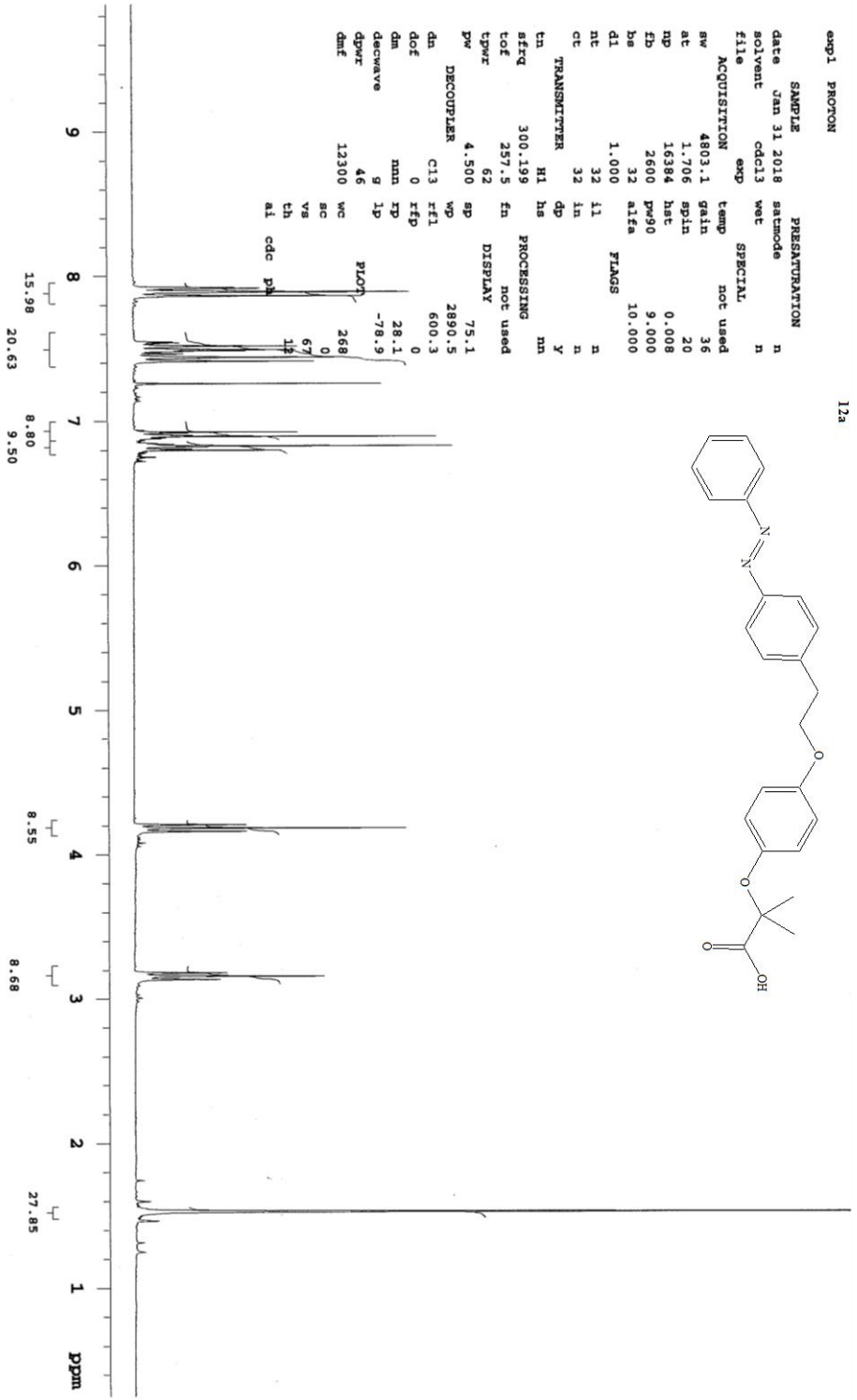
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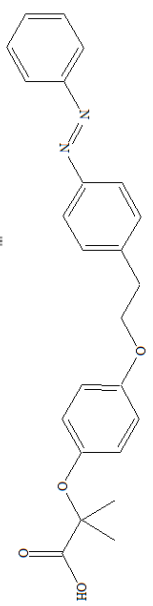
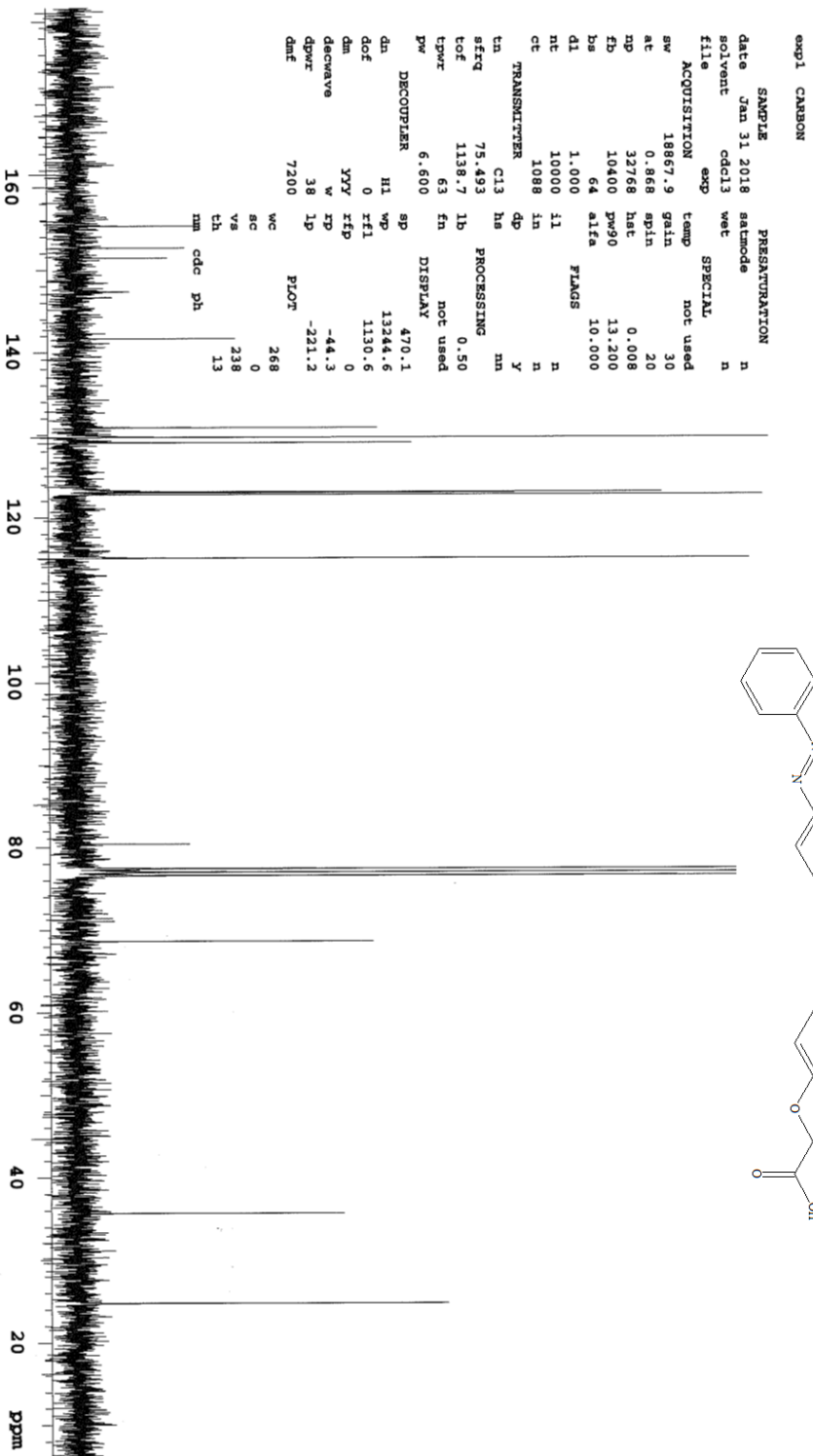
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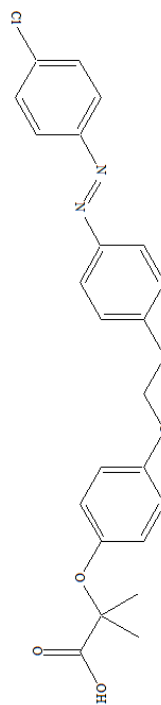
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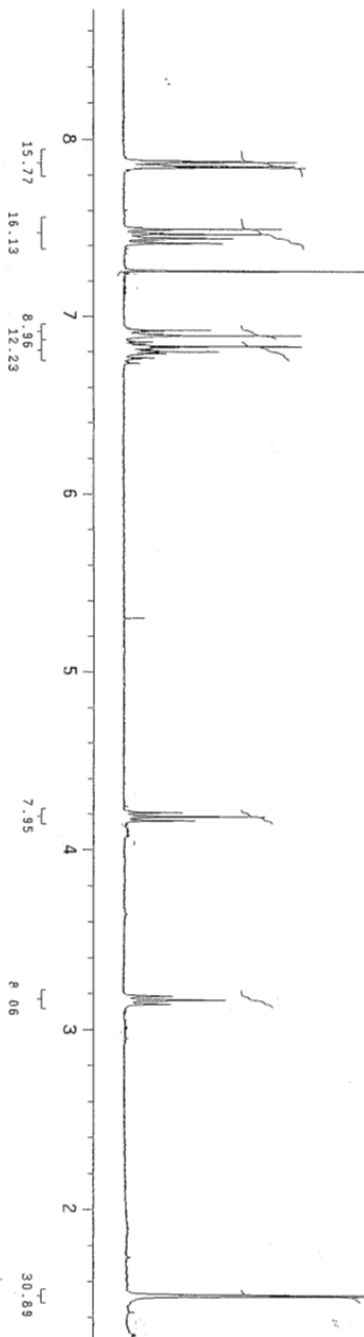


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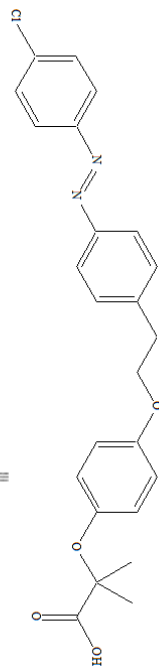
STANDARD 1H OBSERVE



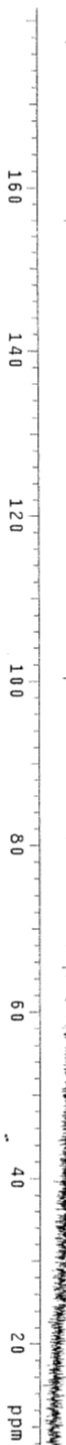
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tn H1 dmm nmh
gc 1.81 dmf C
nd 1.788 dmf PROCESSING 11400
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fb 2600 proc ft
bs 16 fn not used
tpwr 57
pw 7.0 weff
d1 1.000 wexp
tof 0 wds
nt 16 wnt
atlock 16
gain 20
FLAGS n
i1 n
in n
dp y
DISPLAY
SP 427.2
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tfr 0
tfr 0
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12b



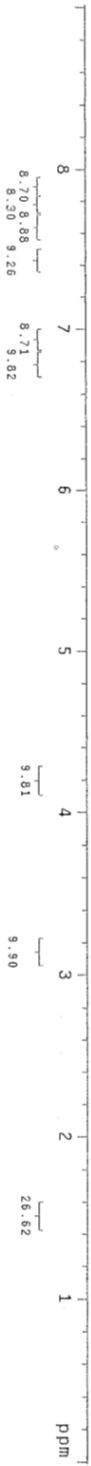
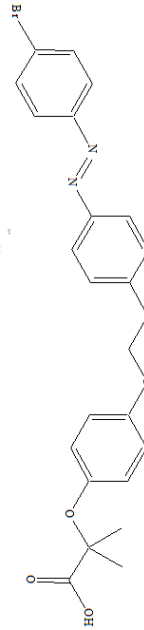
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d1 0 wexp
nt 1e+08 wps
ct 9.88888e+08 wnt
gain not used
gain FLAG not used
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in n
dp y
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vc 0
wc 250
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is 500.00
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f7 20
f8 20
f9 20
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nm no ph 100.000



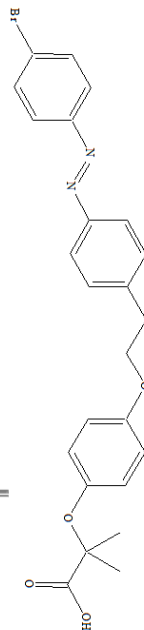
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12c

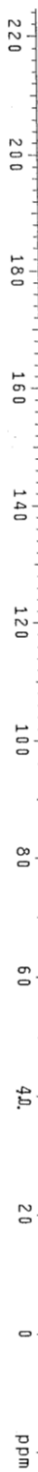
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sw 4508.5 not used ft
bs not used in not used
tpwr 57
pw 7.0 wefr
d1 1.000 wexp
tof 0 wns
ct 16 wnt
alock not used
gain not used
flags n
1) n
1n n
dp y
DISPLAY
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th 20
nm cdc ph 100.000



12c



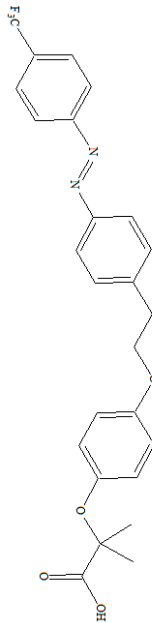
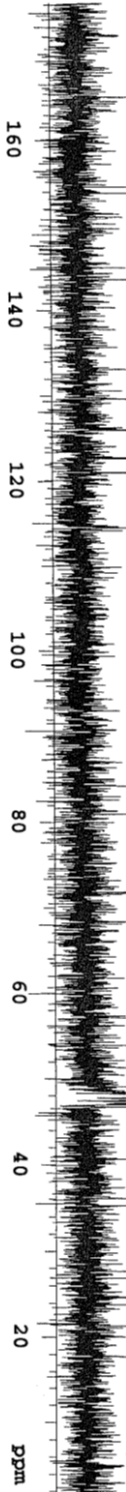
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dpvr 8.7 tn
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tof 1e+08 wds
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Gradient Shimming

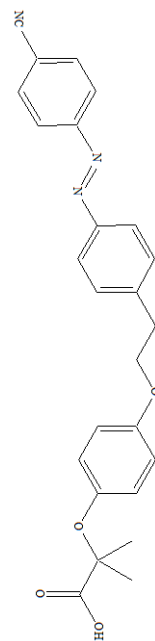
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fb 10400 FLAGS
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dl 1.000 in n
nt 10000 dp y
ct 10000 hs nm
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strq 75.493 fn not used
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pw 6.600 wp 13144.4
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dn H1 rfp 0
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dm yxy lp -237.7
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dmf 7200 vs 0
ai cdc ph 26
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124

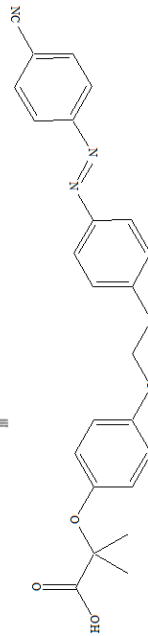
12e



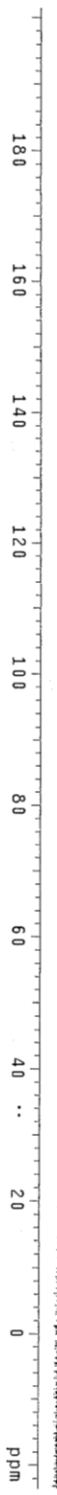
STANDARD 1H OBSERVE

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fb not used
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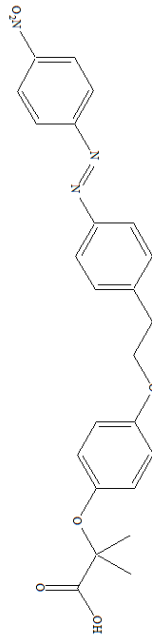


13C OBSERVE
expt1 std13c
SAMPLE DEC. & VT
date May 16 2013 dfrq 300.199
solvent CDCl3 dn H1
file ACQUISITION exp dppwr 41
75.432 dmf yyy
at 1.815 dmf 11400
nd 68106 1b PROCESSING 1.00
sw 18761.7 1b wtf1e
td 10400
dpwr 8.7 fn not used
d1 0 weff
tof 0 wexp
nt 14+08 wbs
clock 29328 wnt
gain not used
flags
11 n
1h n
dp DISPLAY y
sp -1779.2
wp 16986.2
vs 179
sc 0
hCmm 54.295
15 500.00
rf1 1804.1
rfp 0
th 4
ins no 100.000
ph

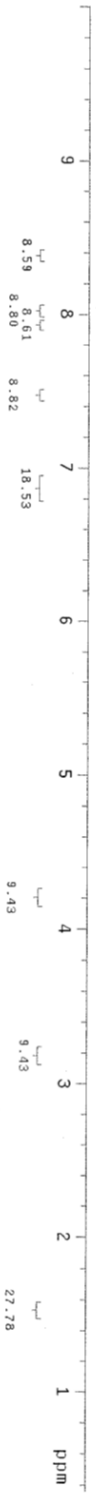


STANDARD 1H OBSERVE

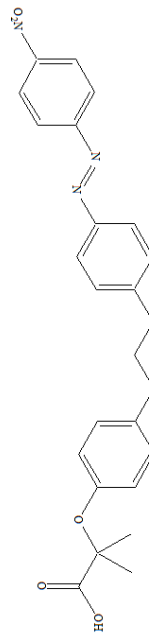
12f



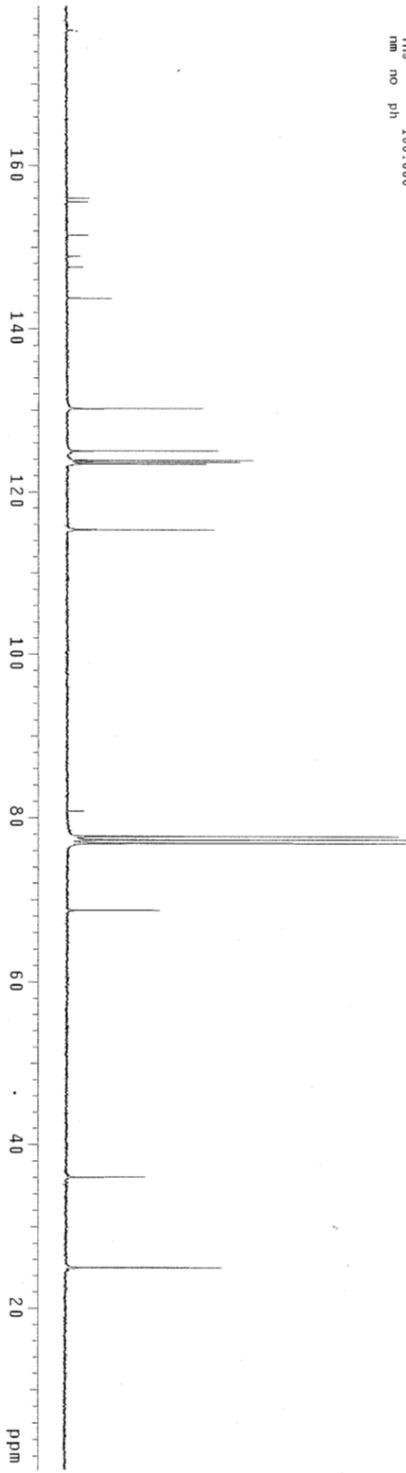
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expt stdin
SAMPLE
date 18.2013 DEC. 8 VT
solvent cdcl3 dn 300.199
file ACQUISITION exp dpwr 30
strf 300.199 dm dof 0
IN 1.938 dm nm
ND 1.7880 dm nm
SW 4508.5 wfile PROCESSING 200
FB not used wfile ft
BS not used fn
IS 57 not used
IPWR 7.0 werr
DPR 1.000 werr
TOF 0 werr
NT 16 werr
CT 16 werr
atlock not used
gain not used
II n
IN n
DP Y
SP DISPLAY 102.8
WC 2893.42
SC 1.0
WC 265
hznm 10.993
IS 1 2027.12
FID 7091.4
TH 20
INS nm
nm cdc ph 100.000
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13C OBSERVE
expt std13c
date SAMPLE DEC. 4 VT
Feb 18 2013 dfrq 300.199
solvent CDCl3 dm 4
11 ACQUISITION exp dof 4
75.492 dm yyy
C13 dm 11400
at 1.815 cmf PROCESSING
1828 1b 1.00
fb 10400 wfile
4 proc ft
bs tpwr 62 fn not used
pw 8.7 wgrf
dl 0 wtdp
nt 16+09 wds
ct 31600 wnt
atlock gain not used
flags
11 FLAOS n
in n
dp y
DISPLAY
sp 20.4
wp 13534.62
vc 0
wc 265
hzmm 51.07
fs 500.00
rfn 1004.4
th 20
nm no ph 100.000



References

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