Supporting Information

Novel Phenyldiazenyl Fibrate Analogues as PPAR α/γ/δ Pan-Agonists for the Amelioration of Metabolic Syndrome

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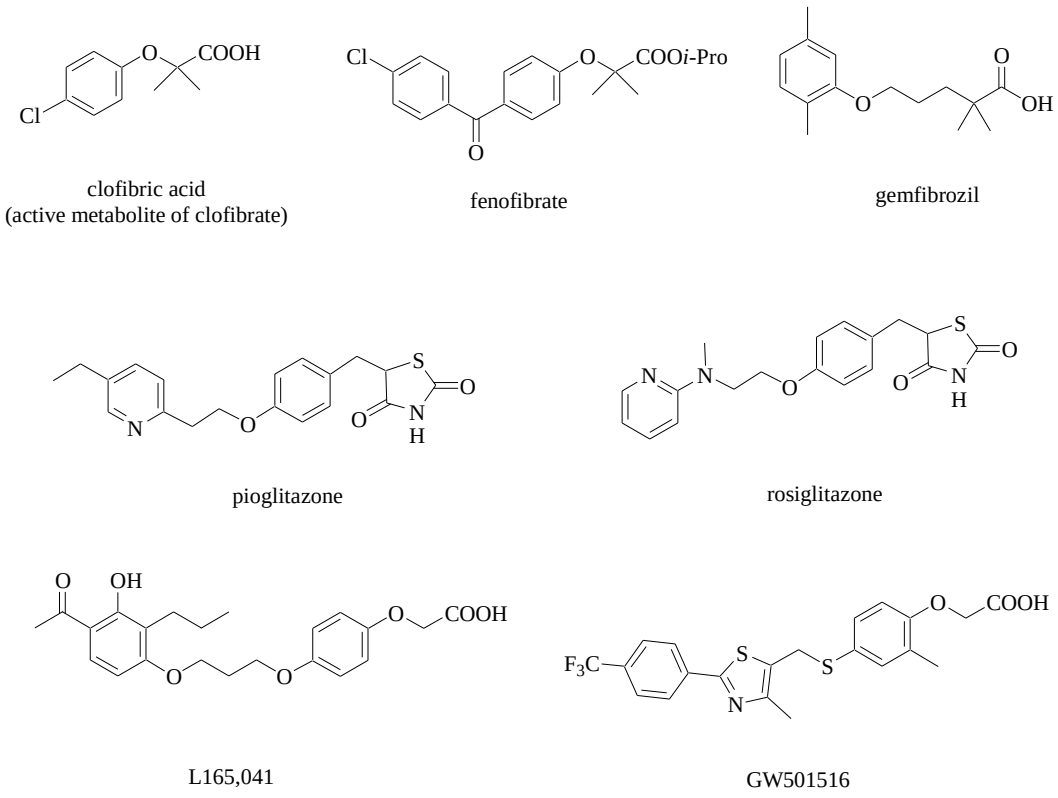


Figure S1. Chemical structures of representative fibrates, thiazolidinediones and phenoxyacetic acid derivatives.

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}; ^{1}H-NMR ($CDCl_3$) δ 6.94 (d, 2H, J=9.0 Hz, *CH*ar), 7.46 (d, 2H, J=8.7 Hz, *CH*ar), 7.82 (d, 2H, J=9.0 Hz, *CH*ar), 7.86 (d, 2H, J= 8.7 Hz, *CH*ar); ^{13}C-NMR ($CDCl_3$) δ 116.1 (*CH*ar), 124.1, 125.3, 129.5, 136.3 (*C*Har), 147.3 (*C*ar-N=N), 151.2 (N=N-*C*ar), 158.6 (*C*ar). Anal. calcd for $C_{12}H_9ClN_2O$: 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}; ^{1}H-NMR ($CDCl_3$) δ 6.94 (d, 2H, J=8.7 Hz *CH*ar), 7.62 (d, 2H, J=8.7 Hz *CH*ar), 7. 75 (d, 2H, J=9 Hz *CH*ar), 7.87 (d, 2H, J=9 Hz *CH*ar); ^{13}C-NMR ($CDCl_3$) δ 116.1, 124.3, 125.4, 132.5 (*C*Har), 147.3, (*C*ar), 151.6 (*C*ar-N=N), 152.5 (N=N-*C*ar), 158.6 (*C*ar). Anal. calcd for $C_{12}H_9BrN_2O$: 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}; ^{1}H-NMR ($CDCl_3$) δ 6.93 (d, 2H, J=8.7 Hz, C*H*ar), 7.81 (d, 2H, J=8.2 Hz, C*H*ar), 7.87 (d, 2H, J=8.7 Hz, C*H*ar), 7.98 (d, 2H, J=8.2 Hz, C*H*ar); ^{13}C-NMR ($CDCl_3$) δ 115.7, 122.6 (*C*Har), 122.9, 123.1 (*C*F_3), 125.3 (*C*Har), 126.1, 126.2 (*C*ar), 137.9 (*CH*ar), 146.2 (*C*ar-N=N), 152.8 (*C*ar-N=N), 161.9 (*C*ar). Anal. calcd for $C_{13}H_9F_3N_2O$: C 58.65, H 3.41, N 10.52. Found C 58.60, H 3.43, N 10.50.

*4-[(E)-(4-Hydroxyphenyl)diazenyl]benzonitrile (**2e**)*

Yellow solid; 52% yield; m.p. 175-178°C; IR (KBr): 3322 (OH), 2236 (CN), 1502 (N=N) cm^{-1}; ^{1}H-NMR (CD_3OD) δ 6.93 (d, 2H, J=8.7 Hz, *CH*ar), 7.88 (d, 2H, J=6.6 Hz, *CH*ar), 7.92 (d, 2H, J=6.6 Hz, *CH*ar), 7.95 (d, 2H, J=8.7 Hz, *CH*ar); ^{13}C-NMR (CD_3OD) δ 112. 9 (*C*ar),

115.8 (*C*Har), 118.3 (*C*N), 122.9, 125.6, 133.3 (*C*Har), 146.2 (*C*ar-N=N), 155.2 (N=N-*C*ar), 162.3 (*C*ar). Anal. calcd for $C_{13}H_9N_3O$: 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}; ^{1}H-NMR ($CDCl_3$) δ 6.93 (d, 2H, J=9 Hz, C*H*ar), 7.87 (d, 2H, J=9 Hz, C*H*ar), 7.97 (d, 2H, J=9 Hz, C*H*ar), 8.36 (d, 2H, J=9 Hz, C*H*ar); ^{13}C-NMR ($CDCl_3$) δ 116.3, 123.4, 125.0, 126,1 (*C*Har), 143.7 (*C*ar), 146.8 (*C*ar-N=N), 153.7 (*C*ar-N=N), 156.2 (*C*ar). Anal. calcd for $C_{12}H_9N_3O_3$: 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}; ^{1}H-NMR (CD_3OD) δ 3.86 (s, 3H, OC*H*$_3$), 6.88 (d, 2H, J=9.9 Hz, C*H*ar), 7.03 (d, 2H, J=9.9 Hz, C*H*ar), 7.75 (d, 2H, J=9 Hz, C*H*ar), 7.81 (d, 2H, J=9 Hz, C*H*ar); ^{13}C-NMR (CD_3OD) δ 54.8 (O*C*H$_3$), 114.0, 115.9, 123.9, 124.4 (*C*Har), 145.9 (*C*ar-N=N), 147.1 (*C*ar-N=N), 161.8 (*C*ar). Anal. calcd for $C_{13}H_{12}N_2O_2$: 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 CH_2Cl_2 (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 CH_2Cl_2 (3 x 30 mL). The combined organic layers were washed with 1 N HCl, saturated sodium

bicarbonate solution, water, brine and dried with $NaSO_4$. 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 CH_2Cl_2 (3 x 30 mL). The combined organic layers were dried with $NaSO_4$. 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; ^{1}H NMR ($CDCl_3$) δ 2.96 (t, 2H, J = 6.6 Hz, OCH_2CH_2), 3.92 (t, 2H, J = 6.6 Hz, OCH_2CH_2), 7.38 (d, 2H, J = 8.7 Hz, C*H*ar), 7.50 (m, 3H, C*H*ar), 7.89 (m, 4H, C*H*ar); ^{13}C NMR ($CDCl_3$) δ 39.3 ($HOCH_2CH_2$), 63.7 ($HOCH_2CH_2$), 123.0, 123.3, 129.3, 130.0, 131.1 (*C*ar), 142.2 (*C*ar CH_2), 151.7 (*C*arN), 152.9 (*C*arN). Anal. calcd for $C_{14}H_{14}N_2O$: 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; ^{1}H NMR ($CDCl_3$) δ 2.96 (t, 2H, J = 6.6 Hz, OCH_2CH_2), 3.92 (t, 2H, J = 6.6 Hz, OCH_2CH_2), 7.38 (d, 2H, J = 8.7 Hz, C*H*ar), 7.43 (d, 2H, J = 8.7 Hz, C*H*ar), 7.86 (m, 4H, C*H*ar); ^{13}C NMR ($CDCl_3$) δ 39.3 ($HOCH_2CH_2$), 63.7 ($HOCH_2CH_2$), 123.4, 124.3, 129.6, 130.0 (*C*ar), 137.0

(*C*arCl), 142.6 (*C*arCH$_2$), 151.2 (*C*arN), 151.5 (*C*arN). Anal. calcd for $C_{14}H_{13}ClN_2O$: 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; ^{1}H NMR ($CDCl_3$) δ 2.96 (t, 2H, J = 6.6 Hz, OCH$_2$C*H*$_2$), 3.92 (t, 2H, J = 6.6 Hz, OC*H*$_2$CH$_2$), 7.38 (d, 2H, J = 8.7 Hz, C*H*ar), 7.64 (d, 2H, J = 8.7 Hz, C*H*ar), 7.79 (d, 2H, J = 8.7 Hz, C*H*ar), 7.84 (d, 2H, J = 8.7 Hz, C*H*ar); ^{13}C NMR ($CDCl_3$) δ 39.3 (HOCH$_2$*C*H$_2$), 63.6 (HO*C*H$_2$CH$_2$), 123.4, 124.5 (*C*ar), 125.5 (*C*arBr), 130.1, 132.55 (*C*ar), 142.6 (*C*arCH$_2$), 151.5 (*C*arN), 151.6 (*C*arN). Anal. calcd for $C_{14}H_{13}BrN_2O$: 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}; ^{1}H-NMR ($CDCl_3$) δ 2.97 (d, 2H, J=6.3 Hz, C*H*$_2$CH$_2$OH), 3.93 (q, 2H, J=6.3 Hz, CH$_2$C*H*$_2$OH), 7.40 (d, 2H, J=8.4 Hz, C*H*ar), 7.77 (d, 2H, J=9.06 Hz, C*H*ar), 7.91 (d, 2H, J=8.4 Hz, C*H*ar), 7.99 (d, 2H, J=9.06 Hz, C*H*ar); ^{13}C-NMR ($CDCl_3$) δ 39.3 (*C*H$_2$CH$_2$OH), 63.6 (CH$_2$*C*H$_2$OH), 115.5, 123.2, 123.6 (*C*Har), 126.5 (*C*F$_3$), 130.1 (*C*Har), 132.5, 143.3, 151.4, 154.7 (*C*ar). Anal. calcd for $C_{15}H_{13}F_3N_2O$: 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; ^{1}H-NMR ($CDCl_3$) δ 2.97 (t, 2H, J = 6.6 Hz, OCH$_2$C*H*$_2$), 3.94 (t, 2H, J = 6.6 Hz, OC*H*$_2$CH$_2$), 7.4 (d, 2H, J = 8.7 Hz, C*H*ar), 7.81 (d, 2H, J = 8.7 Hz, C*H*ar), 7.91 (d, 2H, J = 8.7 Hz, C*H*ar), 7.98 (d, 2H, J = 8.7 Hz, C*H*ar); ^{13}C-NMR ($CDCl_3$) δ 39.3 (*C*H$_2$CH$_2$OH), 63.5

(CH_2CH_2OH), 114.0 (*C*arCN), 118.8 (*C*N) 123.5, 123.6, 123.8, 130.2, 133.5 (*C*ar), 143.8 (*C*arCN), 151.4 (*C*arN), 154.8 (*C*arN). Anal. calcd for $C_{15}H_{13}N_3O$: 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}; ^{1}H-NMR ($CDCl_3$) δ 2.98 (t, 2H, J=6.6 Hz, C*H*$_2$CH$_2$OH), 3.94 (q, 2H, J=6.6 Hz, CH$_2$C*H*$_2$OH), 7.42 (d, 2H, J=8.2 Hz, C*H*ar), 7.93 (d, 2H, J=8.4 Hz, C*H*ar), 8.01 (d, 2H, J=9 Hz, C*H*ar), 8.37 (d, 2H, J=9 Hz, C*H*ar); ^{13}C-NMR ($CDCl_3$) δ 39.4 (*C*H$_2$CH$_2$OH), 63.6 (CH_2CH_2OH), 100.4, 123.6, 123.9, 125.0 (*C*Har), 130.2, 132.8, 144.0, 151.4 (*C*ar). Anal. calcd for $C_{14}H_{13}N_3O_3$: 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 K_2CO_3 (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 Na_2SO_4 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}; ^{1}H NMR ($CDCl_3$) δ 1.28 (t, 3H, J = 7.2 Hz, CH$_2$C*H*$_3$), 1.57 (s, 6H, C(C*H*$_3$)$_2$), 2.91 (t, 2H, J = 7.8 Hz, OCH$_2$C*H*$_2$), 3.47 (t, 2H, J = 7.8 Hz, OC*H*$_2$CH$_2$), 4.34 (q, 2H, J = 7.2 Hz, C*H*$_2$CH$_3$), 6.84 (d, 2H, J = 8.5 Hz, C*H*ar),

6.91 (d, 2H, J = 8.5 Hz, C*H*ar); ^{13}C NMR ($CDCl_3$) δ 14.3 (*C*H_3CH_2), 24.9 (C(*C*H_3)$_2$), 39.0 (OCH_2*C*H_2), 61.1 (CH_3*C*H_2), 63.0 (O*C*H_2CH_2), 78.8 (*C*(CH_3)$_2$), 120.1, 128.5, (*C*Har), 132.6, 154.0 (*C*ar), 172.1 (*C*=O). Anal. calcd for $C_{14}H_{20}O_4$: 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 Na_2SO_4 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}; ^{1}H-NMR ($CDCl_3$): δ 1.27 (t, 3H, J = 6.9 Hz, CH_2C*H*$_3$), 1.52 (s, 6H, C(C*H*$_3$)$_2$), 4.23 (q, 2H, J = 6.9 Hz, C*H*$_2$$CH_3$), 6.73 (d, 2H, J = 6.9 Hz, C*H*ar), 6.91 (d, 2H, J = 6.9 Hz, C*H*ar); ^{13}C-NMR ($CDCl_3$): δ 14.3 (CH_2*C*H_3), 24.9 (C(*C*H_3)$_2$), 61.1 (*C*H_2CH_3), 78.8 (*C*(CH_3)$_2$), 120.1, 128.5 (*C*Har), 132.6, 154.0 (Car), 172.1 (C=O). Anal. calcd for $C_{12}H_{16}O_4$: 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}H-NMR ($CDCl_3$) δ 1.25 (t, 3H, J= 7.2 Hz, CH_2CH_3), 1.58 (s, 6H, $C(CH_3)_2$), 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}C-NMR ($CDCl_3$) δ 14.3 (CH_2CH_3), 25.0 ($C(CH_3)_2$), 37.2 (OCH_2CH_2), 61.1 (CH_2CH_3), 68.8 (OCH_2CH_2), 79.9 ($C(CH_3)_2$), 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 ($CarOC(CH_3)_2$), 154.5 ($CarOCH_2CH_2$), 174.6 (COOEt). Anal. calcd for $C_{26}H_{27}ClN_2O_4$: 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}H-NMR ($CDCl_3$) δ 1.27 (t, 3H, J= 7.2 Hz, CH_2CH_3), 1.52 (s, 6H, $C(CH_3)_2$), 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,

C*H*ar), 7.87 (d, 2H, J = 9 Hz, C*H*ar); ^{13}C-NMR ($CDCl_3$) δ 14.4 (CH_2CH_3), 25.5 ($C(CH_3)_2$), 36.0 (OCH_2CH_2), 61.6 (CH_2CH_3), 68.9 (OCH_2CH_2), 80.9 ($C(CH_3)_2$), 115.2, 121.8, 122.4, 123.3, 124.3 (*C*ar), 125.5 (*C*arBr), 129.5, 130.0 (*C*ar), 142.9 (*C*arCH$_2$), 149.3 (*C*arN), 151.2 (*C*arN), 151.5 (*C*arOC(CH$_3$)$_2$), 154.5 (*C*arOCH$_2$CH$_2$), 174.6 (*C*OOEt). Anal. calcd for $C_{26}H_{27}BrN_2O_4$: 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]diazenyl}phenoxy)ethyl]phenoxy}propanoate (**9d**)*

Strawberry oil; 46% yield; IR (KBr): 1736 (C=O), 1604 (N=N) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.25 (t, 3H, J=7.2 Hz, CH_2CH_3), 1.52 (s, 6H, $C(CH_3)_2$), 3.15 (t, 2H, J=6.6 Hz, OCH_2CH_2), 4.20 (t, 2H, J=6.6 Hz, OCH_2CH_2), 4.22 (q, 2H, J = 6.9, Hz, OCH_2CH_3), 6.80 (d, 2H, J=8.1 Hz, C*H*ar), 7.44 (d, 2H, J=8.4 Hz, C*H*ar), 7.77 (d, 4H, J=8.7 Hz, C*H*ar), 7.90 (d, 2H, J=8.4 Hz, C*H*ar), 7.98 (d, 2H, J=8.4 Hz, C*H*ar); ^{13}C-NMR ($CDCl_3$) δ 14.4 (OCH_2CH_3), 25.5 ($C(CH_3)_2$), 35.0 (OCH_2CH_2), 61.6 (OCH_2CH_3), 68.7 (OCH_2CH_2), 79.9 ($C(CH_3)_2$), 114.0, 115.1, 118.8, 120.8, 121.8 (*C*Har), 123.7-123.5 (*C*ar-CF$_3$), 130.15 (*C*Har), 133.4, 143.6, 149.3, 151.4, 154.8 (*C*ar), 174.5 (*C*OOEt). Anal. calcd for $C_{27}H_{27}F_3N_2O_4$: 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)diazenyl]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}; ^{1}H-NMR ($CDCl_3$) δ 1.25 (t, 3H, CH_2CH_3), 1.55 (s, 6H, $C(CH_3)_2$), 3.07 (t, 2H, OCH_2CH_2), 4.20-4.27 (m, 4H, OCH_2CH_3; OCH_2CH_2), 6.79 (d, 2H, J=8.4 Hz, *CH*ar), 7.01 (d, 2H, J=7.8 Hz, *CH*ar), 7.16 (d, 2H, J=7.5 Hz, *CH*ar), 7.59 (d, 2H, J=7.8 Hz, *CH*ar), 7.79 (d, 2H, J=8.4 Hz, *CH*ar), 7.93 (d, 2H, J=7.5 Hz, *CH*ar); ^{13}C-NMR ($CDCl_3$) δ 14.3 (CH_2CH_3),

25.6 (C(*C*H$_3$)$_2$), 35.0 (OCH$_2$*C*H$_2$), 61.7 (O*C*H$_2$CH$_3$), 69.4 (O*C*H$_2$CH$_2$), 79.3 (*C*(CH$_3$)$_2$), 115.2, 119.5, 123.3, 125.7, 129.9 (*C*Har), 131.5 (*C*ar-CN), 133.4 (*C*Har), 133.5 (*C*ar-CH$_2$CH$_2$O), 147.0 (*C*ar-N=N), 154.4 (*C*ar-N=N), 155.0 (*C*ar-O-C), 162.6 (*C*ar-OCH$_2$CH$_2$), 174.6 (*C*OOEt). Anal. calcd for $C_{27}H_{27}N_3O_4$: 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}; ^{1}H-NMR ($CDCl_3$) δ 1.25 (t, 3H, J=6.9 Hz, CH$_2$C*H*$_3$), 1.59 (s, 6H, C(C*H*$_3$)$_2$), 3.07 (t, 2H, J=6.9 Hz, OCH$_2$C*H*$_2$), 4.24 (m, 4H, C*H*$_2$CH$_3$–OC*H*$_2$CH$_2$), 6.80 (d, 2H, J=8.4 Hz, C*H*ar), 7.01 (d, 2H, J=8.7 Hz, C*H*ar), 7.16 (d, 2H, J=8.7 Hz, C*H*ar), 7.96 (t, 4H, J=8.7 Hz, C*H*ar), 8.36 (d, 2H, J=9 Hz, C*H*ar); ^{13}C-NMR ($CDCl_3$) δ 14.3 (OCH$_2$*C*H$_3$), 25.6 (C(*C*H$_3$)$_2$), 35.0 (OCH$_2$*C*H$_2$), 61.7 (O*C*H$_2$CH$_3$), 69.4 (O*C*H$_2$CH$_2$), 79.3 (*C*(CH$_3$)$_2$), 115.2, 119.5, 123.3, 125.0, 125.8 (*C*ar-NO$_2$), 129.9, 131.5, 147.1 (*C*ar), 148.4 (*C*Har), 154.4 (*C*ar), 156.2 (*C*Har), 174.6 (*C*OOEt). Anal. calcd for $C_{26}H_{27}N_3O_6$: C 65.40, H 5.70, N 8.80. Found C 65.43, H 5.67, 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}; ^{1}H-NMR ($CDCl_3$) δ 1.24 (t, 3H, J=7.2 Hz, CH$_2$C*H*$_3$), 1.58 (s, 6H, C(C*H*$_3$)$_2$), 3.06 (t, 2H, J=6.9, OCH$_2$C*H*$_2$), 3.86 (s, 3H, OC*H*$_3$), 4.17-4.27 (m, 4H, J=7.2 Hz, J=6.9 Hz, C*H*$_2$CH$_3$–OC*H*$_2$CH$_2$), 6.80 (d, 2H, J=8.5, C*H*ar), 6.97 (d, 2H, J=4.9 Hz, C*H*ar), 7.00 (d, 2H, J=4.9 Hz, C*H*ar), 7.16 (t, 2H, J=8.5 Hz, C*H*ar), 7.84 (d, 2H, J=5.2 Hz, C*H*ar), 7.87 (d, 2H, J=5.2 Hz, C*H*ar); ^{13}C-NMR ($CDCl_3$) δ 14.3 (OCH$_2$*C*H$_3$), 25.6 (C(*C*H$_3$)$_2$), 35.1 (OCH$_2$*C*H$_2$), 55.8

(O*C*H_3), 61.7 (O*C*H_2CH$_3$), 69.3 (O*C*H_2CH$_2$), 79.3 (*C*(CH$_3$)$_2$), 114.4, 114.9, 119.5, 124.6, 129.9 (CHar), 131.8, 147.3, 154.3, 161.0, 161.8 (*C*ar) 174.6 (*C*OOEt). Anal. calcd for $C_{27}H_{30}N_2O_5$: 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}; ^{1}H-NMR (CDCl$_3$) δ 1.27 (t, J= 3H, 7.2 Hz, OCH$_2$C*H*$_3$), 1.53 (s, 6H, C(C*H*$_3$)$_2$), 3.15 (t, 2H, J = 6.9 Hz, OCH$_2$C*H*$_2$), 4.16 (t, 2H, J = 6.9 Hz, OC*H*$_2$CH$_2$), 4.21 (q, 2H, J = 7.2, Hz, OC*H*$_2$CH$_3$), 6.75-6.85 (m, 3H, C*H*ar), 6.90 (d, 1H, J = 9.3 Hz, C*H*ar), 7.42 (d, 1H, J = 9.3 Hz, C*H*ar), 7.47-7.55 (m, 4H, C*H*ar), 7.86-7.92 (m, 4H, C*H*ar); ^{13}C-NMR (CDCl$_3$) δ 14.4 (OCH$_2$*C*H$_3$), 25.5 (C(*C*H$_3$)$_2$), 36.0 (OCH$_2$*C*H$_2$), 61.6 (O*C*H$_2$CH$_3$), 68.9 (O*C*H$_2$CH$_2$), 80.0 (*C*(CH$_3$)$_2$), 115.2, 121.8, 123.0, 123.3, 129.3, 130.0, 131.1 (*C*Har), 142.0 (*C*arCH$_2$), 149.3 (*C*arN), 151.6 (*C*arN), 151.9 (*C*arOC(CH$_3$)$_2$), 154.5 (*C*arOCH$_2$CH$_2$), 174.6 (*C*OOEt). Anal. calcd for $C_{26}H_{28}N_2O_4$: 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}; ^{1}H-NMR (CDCl$_3$) δ 1.27 (t, J= 3H, 7.2 Hz, OCH$_2$C*H*$_3$), 1.56 (s, 6H, C(C*H*$_3$)$_2$), 3.15 (t, 2H, J = 6.9 Hz, OCH$_2$C*H*$_2$), 4.16 (t, 2H, J = 6.9 Hz, OC*H*$_2$CH$_2$), 4.22 (q, 2H, J = 7.3 Hz, OC*H*$_2$CH$_3$), 6.81 (d, 2H, J = 9 Hz, C*H*ar), 6.91 (d, 2H, J = 9 Hz, C*H*ar), 7.43 (d, 2H, J = 8.4 Hz, C*H*ar), 7.48 (d, 2H, J = 8.4 Hz, C*H*ar), 7.85 (d, 2H, J = 2.1 Hz, C*H*ar), 7.87 (d, 2H, J = 2.1 Hz, C*H*ar); ^{13}C-NMR (CDCl$_3$) δ 14.3 (OCH$_2$*C*H$_3$), 24.9 (C(*C*H$_3$)$_2$), 37.3 (OCH$_2$*C*H$_2$), 61.1 (O*C*H$_2$CH$_3$), 70.0 (O*C*H$_2$CH$_2$), 78.7 (*C*(CH$_3$)$_2$), 114.9, 122.9, 124.9, 126.0, 129.6, 132.6 (CHar), 133.1 (*C*ar),

145.9 (*C*arCl), 146.9, 152.0, 153.9, 166.2 (*C*ar), 170.5 (*C*OOEt). Anal. calcd for $C_{26}H_{27}ClN_2O_4$: 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}; ^{1}H-NMR ($CDCl_3$) δ 1.27 (t, 3H, J= 7.2 Hz, OCH_2CH_3), 1.52 (s, 6H, $C(CH_3)_2$), 3.15 (t, 2H, J = 6.7 Hz, OCH_2CH_2), 4.18 (t, 2H, J = 6.7 Hz, OCH_2CH_2), 4.23 (q, 2H, J = 7.2, Hz, OCH_2CH_3), 6.81 (d, 2H, J = 9.3 Hz, C*H*ar), 6.90 (d, 2H, J = 9.3 Hz, C*H*ar), 7.27 (d, 4H, J = 8.4 Hz, C*H*ar), 7.64 (d, 2H, J = 8.4 Hz, C*H*ar), 7.87 (d, 2H, J = 8.4 Hz, C*H*ar); ^{13}C-NMR ($CDCl_3$) δ 14.4 (OCH_2CH_3), 25.5 ($C(CH_3)_2$), 35.9 (OCH_2CH_2), 61.6 (OCH_2CH_3), 68.8 (OCH_2CH_2), 80.9 ($C(CH_3)_2$), 115.2, 121.8, 122.4, 123.3, 124.30(*C*ar), 125.5 (*C*arBr), 129.5, 130.0 (*C*ar), 142.9 (*C*arCH_2), 149.3, 151.2 (*C*arN), 151.5 (*C*ar$OC(CH_3)_2$), 154.5 (*C*arOCH_2CH_2), 174.6 (*C*OOEt). Anal. calcd for $C_{26}H_{27}BrN_2O_4$: 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}; ^{1}H-NMR ($CDCl_3$) δ 1.27 (t, 3H, J=7.2 Hz, CH_2CH_3), 1.53 (s, 6H, $C(CH_3)_2$), 3.16 (t, 2H, J=6.6 Hz, OCH_2CH_2), 4.18 (q, 4H, J=6.6 Hz, OCH_2CH_2), 4.23 (q, 2H, J = 7.2 Hz, CH_2CH_3), 6.80 (d, 2H, J=8.1 Hz, C*H*ar), 7.45 (d, 2H, J=8.4 Hz, C*H*ar), 7.77 (d, 2H, J=8.7 Hz, C*H*ar), 7.90 (d, 2H, J=8.4 Hz, C*H*ar), 7.98 (d, 2H, J=8.4 Hz, C*H*ar); ^{13}C-NMR ($CDCl_3$) δ 14.4 (OCH_2CH_3), 25.5 ($C(CH_3)_2$), 36.0 (OCH_2CH_2), 61.6 (OCH_2CH_3), 68.7 (OCH_2CH_2), 79.9 ($C(CH_3)_2$), 14.0, 115.1, 118.8, 120.8 (CF_3), 116.2, 121.8, 123.5, 123.7, 130.2, 133.4 (*C*Har), 121.3, 129.7, 133.3, (*C*ar-CF_3),

143.6, 149.3, 151.4, 154.5, 154.8 (*C*ar), 174.5 (*C*OOEt). Anal. calcd for $C_{27}H_{27}F_3N_2O_4$: 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}; ^{1}H-NMR ($CDCl_3$) δ 1.27 (t, 3H, J= 7.2 Hz, OCH_2CH_3) 1.52 (s, 6H, $C(CH_3)_2$), 3.16 (t, 2H, J = 6.6 Hz, OCH_2CH_2), 4.17 (t, 2H, J = 6.6 Hz, OCH_2CH_2), 4.23 (q, 2H, J = 7.2, Hz, OCH_2CH_3), 6.77 (d, 2H, J = 9.3 Hz, C*H*ar), 6.83 (d, 2H, J = 9.3 Hz, C*H*ar), 7.45 (d, 2H, J = 8.4 Hz, C*H*ar), 7.80 (d, 2H, J = 9 Hz, CH_{ar}), 7.90 (d, 2H, J = 8.4 Hz, C*H*ar), 7.97 (d, J = 9, 2H, C*H*ar); ^{13}C-NMR ($CDCl_3$) δ 14.4 (OCH_2CH_3), 25.5 ($C(CH_3)_2$), 36.0 (CarCH_2), 61.6 (OCH_2CH_3), 68.7 (CH_2CH_2O), 79.9 ($C(CH_3)_2$), 113.9 (*C*arCN), 115.1 (*C*ar), 118.8 (*C*N), 121.8, 123.5, 123.7, 130.2, 133.4 (*C*ar), 143.6 (*C*arCH_2), 149.4, 151.4 (*C*arN), 154.5 (*C*ar$OC(CH_3)_2$), 154.8 (*C*arOCH_2CH_2), 174.5 (*C*OOEt). Anal. calcd for $C_{27}H_{27}N_3O_4$: 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}; ^{1}H-NMR ($CDCl_3$) δ 1.27 (t, 3H, J=6.3 Hz, CH_2CH_3), 1.52 (s, 6H, $C(CH_3)_2$), 3.16 (t, 2H, J=6.9 Hz, OCH_2CH_2), 4.21 (q, 4H, J=6.3 Hz, CH_2CH_3–OCH_2CH_2), 6.80 (q, 4H, J=8.1 Hz, C*H*ar), 7.46 (d, 2H, J=8.4 Hz, C*H*ar), 7.92 (d, 2H, J=8.1 Hz, C*H*ar), 8.01 (d, 2H, J=8.1 Hz, C*H*ar), 8.37 (d, 2H, J=8.4 Hz, C*H*ar); ^{13}C-NMR ($CDCl_3$) δ 14.4 (OCH_2CH_3), 25.5 ($C(CH_3)_2$), 36.0 (OCH_2CH_2), 61.6 (OCH_2CH_3), 68.7 (OCH_2CH_2), 79.9 ($C(CH_3)_2$), 115.1 (*C*Har), 121.2 (*C*Har), 123.8 (*CH*ar), 124.9 (*CH*ar), 130.2 (*C*Har), 143.9

(*C*ar), 148.8 (*C*ar), 149.3 (*C*ar), 154.4(*C*ar), 156.0 (*C*ar), 174.6 (*C*OOEt). 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}; ^{1}H-NMR ($CDCl_3$) δ 1.59 (s, 6H, C(*C*H_3)$_2$), 3.09 (t, 2H, J=6.9 Hz, OCH$_2$*C*H$_2$), 4.23 (t, 2H, J=6.9 Hz, O*C*H$_2$CH$_2$), 6.91 (d, 2H, J=8.4 Hz, *CH*ar), 6.99 (d, 2H, J=9 Hz, *CH*ar), 7.22 (d, 2H, J=8.4 Hz, *CH*ar), 7.46 (d, 2H, J=8.7 Hz, *CH*ar), 7.82 (d, 2H, J=9 Hz, *CH*ar), 7.88 (d, 2H, J=8.7 Hz, *CH*ar); ^{13}C-NMR ($CDCl_3$) δ 25.2 (C(*C*H$_3$)$_2$), 35.1 (OCH$_2$*C*H$_2$), 69.2 (O*C*H$_2$CH$_2$), 80.0 (*C*(CH$_3$)$_2$), 115.0, 121.1, 124.0, 125.1, 129.5 (*C*Har), 130.1 (*C*arCl), 133.2 (*C*arCH$_2$CH$_2$O), 136.4 (*C*Har), 147.0 (*C*arN=N), 151.3 (*C*arN=N), 153.1 (*C*arOC), 161.7 (*C*arOCH$_2$CH$_2$), 177.5 (*C*OOH). 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}H-NMR (CD_3OD) δ 1.53 (s, 6H, C(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, *CH*ar), 7.05 (d, 2H, J=9.0 Hz, *CH*ar), 7.22 (d, 2H, J=8.1 Hz, *CH*ar), 7.67 (d, 2H, J=7.8 Hz, *CH*ar), 7.77 (d, 2H, J=7.8 Hz, *CH*ar), 7.88 (d, 2H, J=9.0 Hz, *CH*ar); ^{13}C-NMR ($CDCl_3$) δ 25.1 (C(CH_3)$_2$), 35.2 (OCH_2CH_2), 69.1 (OCH_2CH_2), 83.8 (*C*(CH_3)$_2$), 115.0, 121.5, 124.3, 125.1, 130.2 (*C*Har), 130.6 (*C*arBr), 132.5 (*C*Har), 133.8 (*C*arCH_2CH_2O), 147.1, 152.9 (*C*arN=N), 161.7 (CarOC), 170.0 (*C*arOCH_2CH_2), 174.9 (*C*OOH). Anal. calcd for $C_{24}H_{23}BrN_2O_4$: 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**)*

*O*range solid; 50% yield; m.p. 103.6-106.5°C; IR (KBr): 3233 (OH), 1718 (C=O), 1606 (N=N) cm^{1}; ^{1}H-NMR ^{1}H-NMR ($CDCl_3$) δ 1.58 (s, 6H, C(CH_3)$_2$), 3.10 (t, 2H, J=6.9 Hz, OCH_2 CH_2), 4.24 (t, 2H, J=6.9 Hz, OCH_2 CH_2), 6.91 (d, 2H, J=8.4 Hz, C*H*ar), 7.01 (d, 2H, J=8.4 Hz, C*H*ar), 7.23 (t, 2H, J=8.4 Hz, C*H*ar), 7.74 (d, 2H, J=8.7 Hz, C*H*ar), 7.93 (m, 4H, J_1= 8.4 Hz, J_2=8.7 Hz, C*H*ar); ^{13}C-NMR ($CDCl_3$) δ 25.2 (C(CH_3)$_2$), 35.1 (OCH_2CH_2), 69.2 (OCH_2CH_2), 80.2 (*C*(CH_3)$_2$), 114.6, 120.0, 124.4, 133.4 (*C*F_3), 115.1, 121.3, 122.9, 125.4, 126.5, 130.2 (*C*Har), 147.0, 152.9, 154.8, 162.1 (*C*ar), 175.2 (*C*OOH). Anal. calcd for $C_{25}H_{23}F_3N_2O_4$: 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}; ^{1}H-NMR ($CDCl_3$) δ 1.58 (s, 6H, C(*CH*$_3$)$_2$), 3.10 (t, 2H, J=6.6 Hz, OCH$_2$*CH*$_2$), 4.25 (t, 2H, J=6.6 Hz, O*CH*$_2$CH$_2$), 6.92 (d, 2H, J=8.1 Hz, *CH*ar), 7.01 (d, 2H, J=8.7 Hz, *CH*ar), 7.17 (d, 2H, J=8.4 Hz, *CH*ar), 7.79 (d, 2H, J=8.4 Hz, *CH*ar), 7.93 (dd, 4H, J=8.1 Hz, *CH*ar); ^{13}C-NMR ($CDCl_3$) δ 25.2 (C(*C*H$_3$)$_2$), 35.1 (OCH$_2$*C*H$_2$), 69.3 (O*C*H$_2$CH$_2$), 80.1 (*C*(CH$_3$)$_2$), 115.1 (*C*Har), 118.4 (*C*arCN), 121.1, 123.3, 125.7, 130.1 (*C*Har), 133.1 (*C*arCH$_2$CH$_2$O), 133.4 (*C*Har), 147.1, 153.2 (*C*arN=N), 160.6 (*C*arOC), 162.5 (*C*arOCH$_2$CH$_2$), 177.1 (*C*OOH). Anal. calcd for $C_{25}H_{23}N_3O_4$: 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}; ^{1}H-NMR ($CDCl_3$) δ 1.59 (s, 6H, C(C*H*$_3$)$_2$), 3.09 (t, 2H, J=6.9 Hz, OCH$_2$C*H*$_2$), 4.24 (t, 2H, J=6.9 Hz, OC*H*$_2$CH$_2$), 6.91 (d, 2H, J=8.1 Hz, C*H*ar), 7.01 (d, 2H, J=8.7 Hz, C*H*ar), 7.23 (t, 2H, J=8.1 Hz, C*H*ar), 7.95 (t, 4H, J=8.1 Hz, C*H*ar), 8.35 (d, 2H, J=8.7 Hz, C*H*ar); ^{13}C-NMR ($CDCl_3$) δ 25.2 (C(*C*H$_3$)$_2$). 29.9 (*C*(CH$_3$)$_2$), 35.1 (OCH$_2$*C*H$_2$), 69.3 (O*C*H$_2$CH$_2$), 115.2, 121.2, 123.3, 124.9, 125.8, 130.1 (*C*Har), 133.2, 147.1, 148.4, 153.1, 156.2, 162.7 (*C*ar), 177.0 (*C*OOH). Anal. calcd for $C_{24}H_{23}N_3O_6$: 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}H-NMR ($CDCl_3$) δ 1.59 (s, 6H, C(C*H*$_3$)$_2$), 3.09 (t, 2H, J=6.9 Hz, OCH$_2$C*H*$_2$), 3.88 (s, 3H, OC*H*$_3$), 4.22 (t, 2H, J=6.9 Hz, OC*H*$_2$CH$_2$), 6.91 (d, 2H, J=8.4 Hz, C*H*ar), 6.95-7.01 (dd, 4H, J=7.2 Hz, C*H*ar), 7.22 (d, 2H, J=8.4 Hz, C*H*ar), 7.83-7.88 (dd, 4H, J=7.2 Hz, C*H*ar); ^{13}C-NMR ($CDCl_3$) δ 25.1 (C(*C*H$_3$)$_2$). 35.2 (OCH$_2$*C*H$_2$), 55.8 (O*C*H$_3$), 69.1 (O*C*H$_2$CH$_2$), 80.4 (*C*(CH$_3$)$_2$), 114.4, 114.9, 121.4, 124.6, 130.2 (*C*Har), 133.7, 147.3, 160.9, 166.4, 171.0 (*C*ar), 181.6 (*C*OOH). Anal. calcd for $C_{25}H_{26}N_2O_5$: 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}H-NMR ($CDCl_3$): δ 1.52 (s, 6H, C(C*H*$_3$)$_2$), 3.16 (t, 2H, J = 6.9 Hz, OCH$_2$C*H*$_2$), 4.19 (t, 2H, J = 6.9 Hz, OC*H*$_2$CH$_2$), 6.86-6.89 (m, 4H, C*H*ar), 7.42 (d, 1H, C*H*ar), 7.50 (d, 4H, J = 9.0 Hz, C*H*ar), 7.89 (t, 4H, J = 9.0 Hz, C*H*ar); ^{13}C-NMR ($CDCl_3$): δ 24.8 (C(*C*H$_3$)$_2$), 35.9 (OCH$_2$*C*H$_2$), 68.9 (O*C*H$_2$CH$_2$), 81.9 (*C*(CH$_3$)$_2$), 116.0, 122.4, 122.6, 125.8, 128.6, 129.8, 130.6 (*C*Har), 149.7, 1152.6, 143.1, 159.5, 151.5 (*C*ar), 175.2 (*C*OOH). Anal. calcd for $C_{24}H_{24}N_2O_4$: 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}H-NMR ($CDCl_3$): δ 1.52 (s, 6H, C(C*H*$_3$)$_2$), 3.16 (t, 2H, J = 6.9 Hz, OCH$_2$C*H*$_2$), 4.18 (t, 2H, J = 6.9 Hz, OC*H*$_2$CH$_2$), 6.81 (d, 2H, J = 9 Hz, C*H*ar), 6.91 (d, 2H, J = 9 Hz, C*H*ar), 7.43 (d, 2H, J = 8.7 Hz, C*H*ar), 7.48 (d, 2H, J = 8.7 Hz, C*H*ar), 7.85 (d, 2H, J = 2.1 Hz, C*H*ar), 7.88 (d, 2H, J = 2.1 Hz, C*H*ar); ^{13}C-NMR ($CDCl_3$): δ 24.9 (C(*C*H$_3$)$_2$), 35.9

(OCH$_2$*C*H$_2$), 68.9 (O*C*H$_2$CH$_2$), 80.9 (*C*(CH$_3$)$_2$), 115.3, 123.3, 123.5, 124.3, 129.5, 130.0 (*C*Har), 137.0 (*C*ar), 142.2 (*C*Cl), 147.2, 151.2, 151.5, 155.7 (*C*ar), 175.7 (*C*OOH). Anal. calcd for $C_{24}H_{23}ClN_2O_4$: 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}; ^{1}H-NMR (CDCl$_3$): δ 1.52 (s, 6H, C(C*H*$_3$)$_2$), 3.16 (t, 2H, J = 6.9 Hz, OCH$_2$C*H*$_2$), 4.18 (t, 2H, J = 6.9 Hz, OC*H*$_2$CH$_2$), 6.82 (d, 2H, J = 9 Hz, C*H*ar), 6.9 (d, 2H, J = 9 Hz, C*H*ar), 7.42 (d, 2H, J = 8.4 Hz, C*H*ar), 7.64 (d, 2H, J = 8.4 Hz, C*H*ar), 7.79 (d, 2H, J = 8.1, C*H*ar), 7.87 (d, 2H, J = 8.1 Hz, C*H*ar); ^{13}C-NMR (CDCl$_3$): δ 24.9 (C(*C*H$_3$)$_2$), 35.9 (OCH$_2$*C*H$_2$), 68.8 (O*C*H$_2$CH$_2$), 80.9 (*C*(CH$_3$)$_2$), 121.1, 122.4, 123.5, 124.5, 125.5. 130.0 (*C*Har), 132.5 (*C*arBr), 142.3, 151.5, 151.6, 155.7, 155.9 (*C*ar), 175.9 (*C*OOH). Anal. calcd for $C_{24}H_{23}BrN_2O_4$: 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}; ^{1}H-NMR (CD$_3$OD): δ 1.48 (s, 6H, C(C*H*$_3$)$_2$), 3.30 (t, 2H, J = 6.6 Hz, OCH$_2$C*H*$_2$), 4.21 (t, 2H, J = 6.6 Hz, OC*H*$_2$CH$_2$), 6.66 (d, 2H, J = 9.3 Hz, C*H*ar), 6.79 (d, 2H, J = 9.3 Hz, C*H*ar), 7.51 (d, 2H, J = 8.4 Hz, C*H*ar), 7.87 (d, 2H, J = 8.4 Hz, C*H*ar), 7.91 (d, 2H, J = 9.3, C*H*ar), 8.03 (d, 2H, J = 9.3 Hz, C*H*ar); ^{13}C-NMR (CD$_3$OD): δ 24.7 (C(*C*H$_3$)$_2$), 35.3 (OCH$_2$*C*H$_2$), 68.4 (O*C*H$_2$CH$_2$), 79.8 (*C*(CH$_3$)$_2$), 114.5, 121.0, 122.7, 122.8, 126.0, 129.7 (*C*Har), 126.1 (*C*F$_3$), 143.7 (*C*CF$_3$), 144.9, 147.6, 153.9, 154.6, 164.9 (*C*ar), 175.2 (*C*OOH). Anal. calcd for $C_{25}H_{23}F_3N_2O_4$: 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}; ^{1}H-NMR ($CDCl_3$): δ 1.52 (s, 6H, C(C*H*$_3$)$_2$), 3.18 (t, 2H, J = 6.6 Hz, OCH$_2$C*H*$_2$), 4.20 (t, 2H, J = 6.6 Hz, OC*H*$_2$CH$_2$), 6.82 (d, 2H, J = 9.3 Hz, C*H*ar), 6.91 (d, 2H, J = 9.3 Hz, C*H*ar), 7.46 (d, 2H, J = 8.7 Hz, C*H*ar), 7.81 (d, 2H, J = 9 Hz, C*H*ar), 7.91 (d, 2H, J = 8.7 Hz, C*H*ar), 7.97 (d, 2H, J = 9 Hz, C*H*ar); ^{13}C-NMR ($CDCl_3$): δ 24.9 (C(*C*H$_3$)$_2$), 35.9 (OCH$_2$*C*H$_2$), 68.7 (O*C*H$_2$CH$_2$), 80.8 (*C*(CH$_3$)$_2$), 114.0 (*C*N), 115.3 (*C*Har), 118.8 (*C*arCN), 123.4, 123.5, 123.7, 130.2, 133.5 (*C*Har), 143.5, 147.5, 151.4, 154.8, 155.5 (*C*ar), 176.3 (*C*OOH). Anal. calcd for $C_{25}H_{23}N_3O_4$: 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}; ^{1}H-NMR ($CDCl_3$): δ 1.52 (s, 6H, C(C*H*$_3$)$_2$), 3.18 (t, 2H, J = 6.6 Hz, OCH$_2$C*H*$_2$), 4.20 (t, 2H, J = 6.6 Hz, OC*H*$_2$CH$_2$), 6.82 (d, 2H, J = 9.3 Hz, C*H*ar), 6.9 (d, 2H, J = 9.3 Hz, C*H*ar), 7.46 (d, 2H, J = 8.4 Hz, C*H*ar), 7.9 (d, 2H, J = 8.4 Hz, C*H*ar), 8.02 (d, 2H, J = 9.3, C*H*ar), 8.4 (d, 2H, J = 9.3 Hz, C*H*ar); ^{13}C-NMR ($CDCl_3$): δ 25.0 (C(*C*H$_3$)$_2$), 36.0 (OCH$_2$*C*H$_2$), 68.7 (O*C*H$_2$CH$_2$), 80.8 (*C*(CH$_3$)$_2$), 115.3, 123.4, 123.6, 123.8, 125.0, 130.2 (*C*Har), 143.7, 147.5 (*C*ar), 148.8 (*C*arNO$_2$), 151.4, 155.5, 155.9 (*C*ar), 175.2 (*C*OOH). Anal. calcd for $C_{24}H_{23}N_3O_6$: 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, 10^5 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-PPARα-LBD, GAL4-PPARγ-LBD or GAL4-PPARβ/δ-LBD, 100 ng of reporter plasmid pGAL5TKpGL3, and 150 ng of control plasmid pCMVβgal 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 μM), reference compounds clofibric acid (100 μM), rosiglitazone (2 μM), and L165,041 (2 μM), 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 (VICTOR^3 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 5×10^5 cells/well in 2 mL of culture medium (DMEM with 10% FBS) per well and grown at 37 °C, 5% CO_2 for 24 h. Then, cells

were incubated for 48 h with a serum free medium containing the test compound **12a** (100 μM, 0.25% DMSO). Treatments with the commercial compounds L165,041 (2 μM, 0.1% DMSO), GW7647 (2 μM, 0.02% DMSO) or rosiglitazone (10 μM, 0.03% DMSO) were used as reference controls for activation of PPARβ/δ, PPARα or PPARγ, 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 described[3] 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'-CTGGAAGCACATGCCCACAATGAA-3' (RW) for GLUT1[5] 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 CO_2 inhalation (100% CO_2 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% CO_2 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 PPARα activator reference agent, the PPARγ agonist pioglitazone (1 μM), the PPARβ/δ agonist L165,041 (1 μM) and scalar concentrations of **12a** (0.1–10 μM). Tissue supernatants were collected and PGE_2 (ng/mg wet tissue) were measured by radioimmunoassay (RIA), as previously reported.[7] Briefly, specific anti-PGE_2 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 ^{3}H-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 ± 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 PPARα/**11a** (PDB ID: 4CI4), PPARγ/**11a** (PDB ID: 4CI5),[9] as well as PPARδ 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 PPARα, H449 and H323 into PPARγ, and H287 and H413 into PPARδ were set in their Nε 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 PPARα, PPARγ, and PPARδ 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 PPARα and PPARγ, and 6-(2-((N-isopropyl-[1,1'-biphenyl]-4-carboxamido)methyl)phenoxy)hexanoic acid for PPARδ 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 AClAP 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.

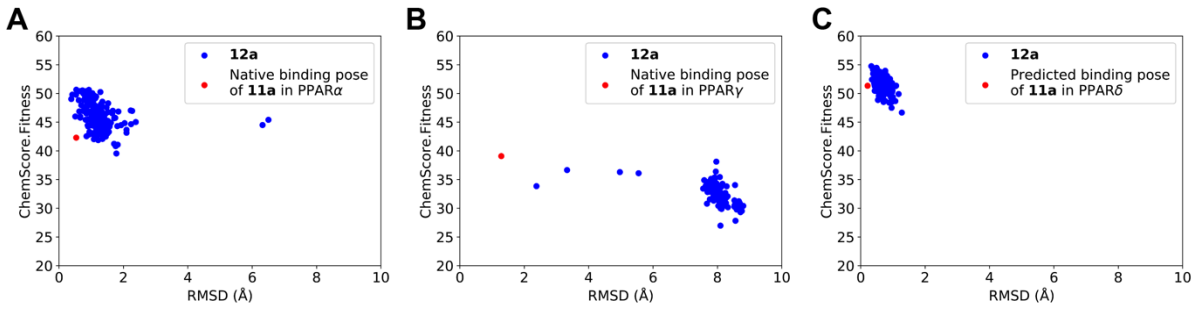


Figure S2. Docking of **12a** into the LBD of PPARα (A), PPARγ (B) and PPARδ (C) crystal structures. The RMSD between 10,000 binding poses of **12a** (shown in blue) and the pose closest to the native binding mode of **11a** (shown in red) was computed. The top 20 percent of poses by GOLD scores (ChemScore.Fitness)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

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

Subtype	Cluster N°	Cardinality	RMSD [Å]	ChemScore.Fitness
PPARα	1	28	1.27	50.65
PPARγ	1	105	8.01	36.39
PPARδ	1	50	0.33	54.75

[a]For 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.

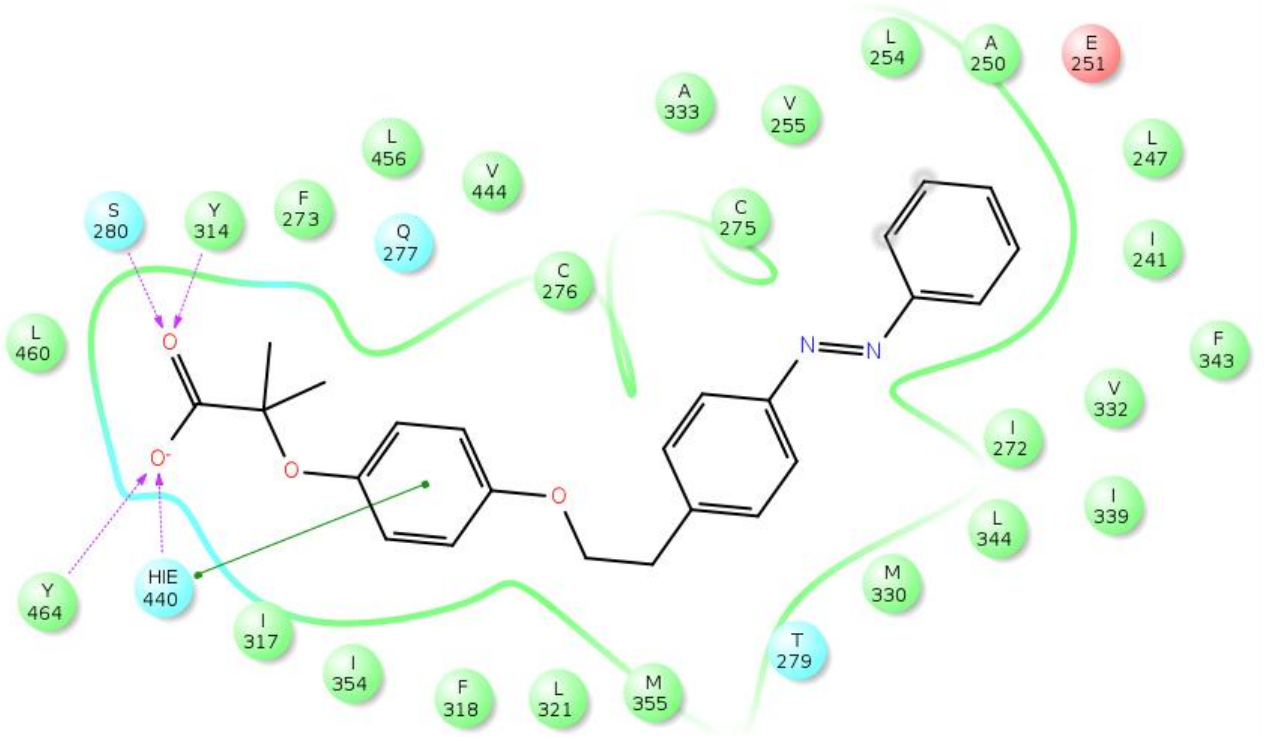


Figure S3. 2D ligand-interaction diagram of compound 12a into the PPARα binding pocket. Positively charged amino acids are represented with dark blue circles, negatively charged amino acids are represented with red circles, polar amino acids are represented with light blue circles and hydrophobic amino 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.

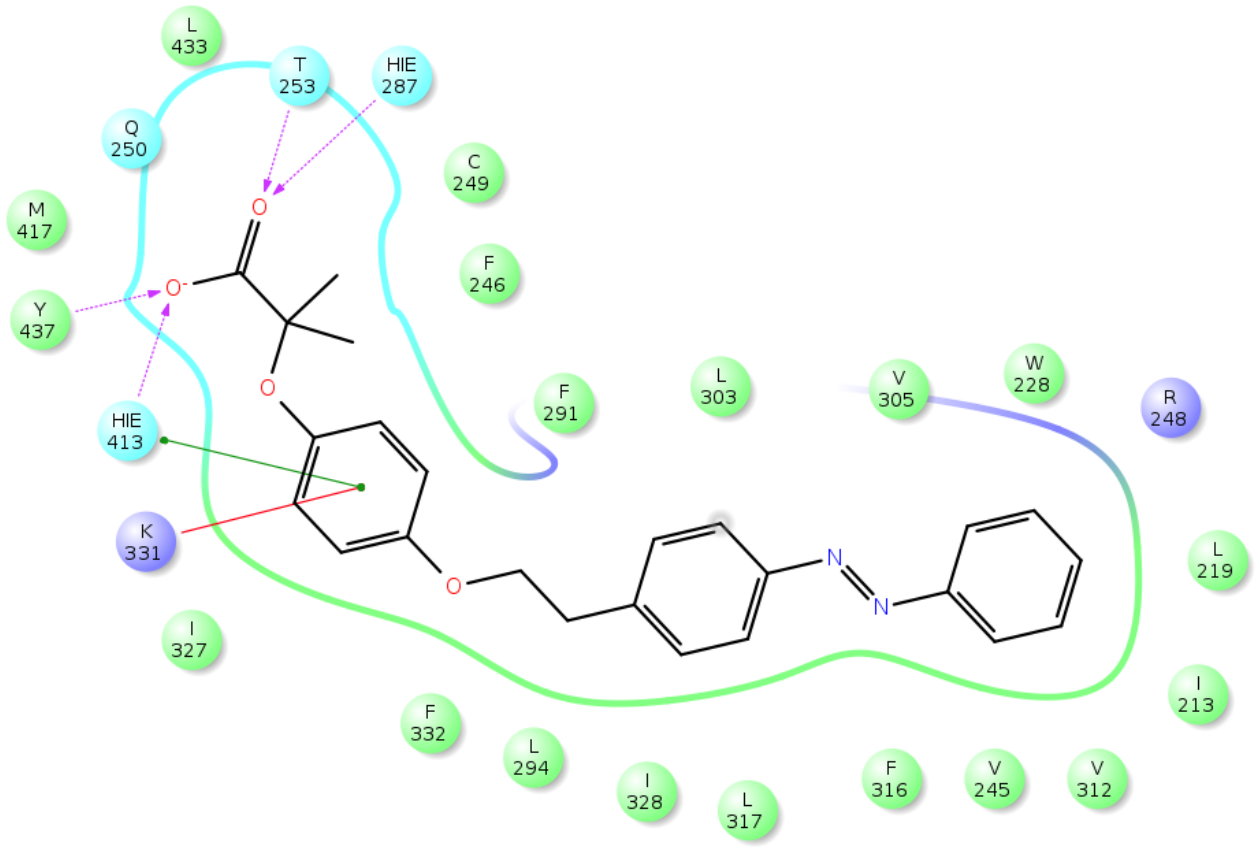


Figure S4. 2D ligand-interaction diagram of compound 12a into the PPARδ binding pocket. Positively charged amino acids are represented with dark blue circles, negatively charged amino acids are represented with red circles, polar amino acids are represented with light blue circles and hydrophobic amino 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.

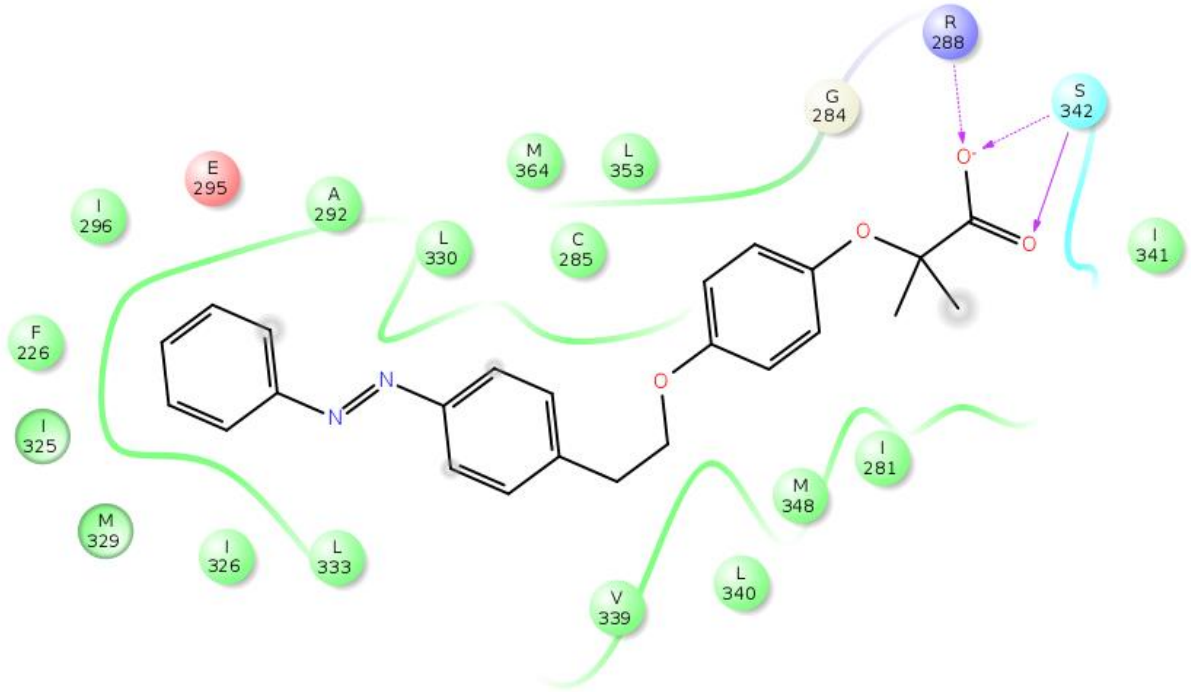
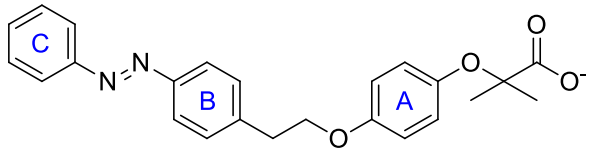


Figure S5. 2D ligand-interaction diagram of compound 12a into the PPARγ binding pocket. Positively charged amino acids are represented with dark blue circles, negatively charged amino acids are represented with red circles, polar amino acids are represented with light blue circles and hydrophobic amino 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 PPARα, PPARδ and PPARγ.



12a

PPAR subtype	Type of interaction	Ligand atoms	Residues involved	Distance (Å)
PPARα	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
PPARδ	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
PPARγ	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 PPARα potency of **11a** with respect to **12a**.

The increase of PPARα 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).

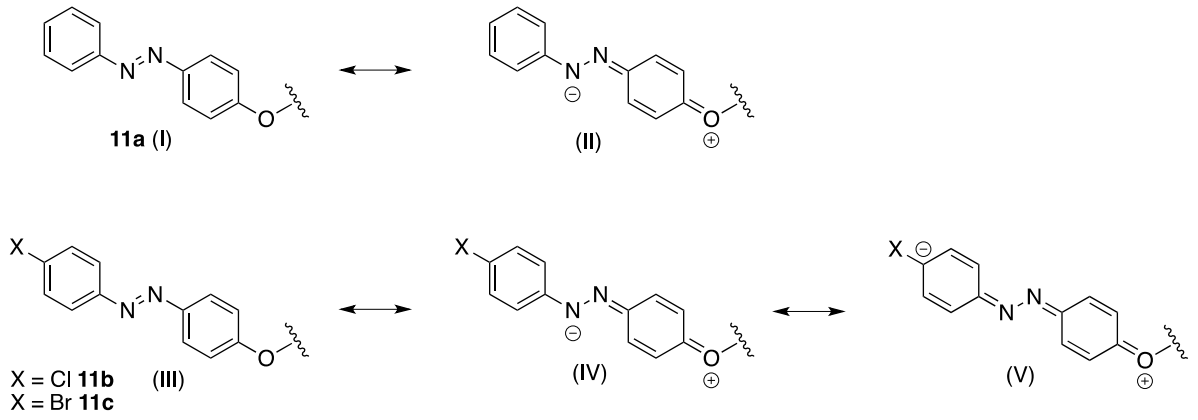


Figure S6. Resonance forms of derivatives **11a-c**.

Binding mode of 12a into PPARγ LBD

12a is situated at the entrance of the active site, between H3 and the β-sheet, a common site of PPARγ partial agonists, and does not interact directly with H12. Figure S7 shows the singular mode of binding of **12a** compared with that of the PPARγ-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 PPARγ might be ascribed to the peculiar binding mode, which is not able to replicate the spectrum of contacts of full agonists.[28]

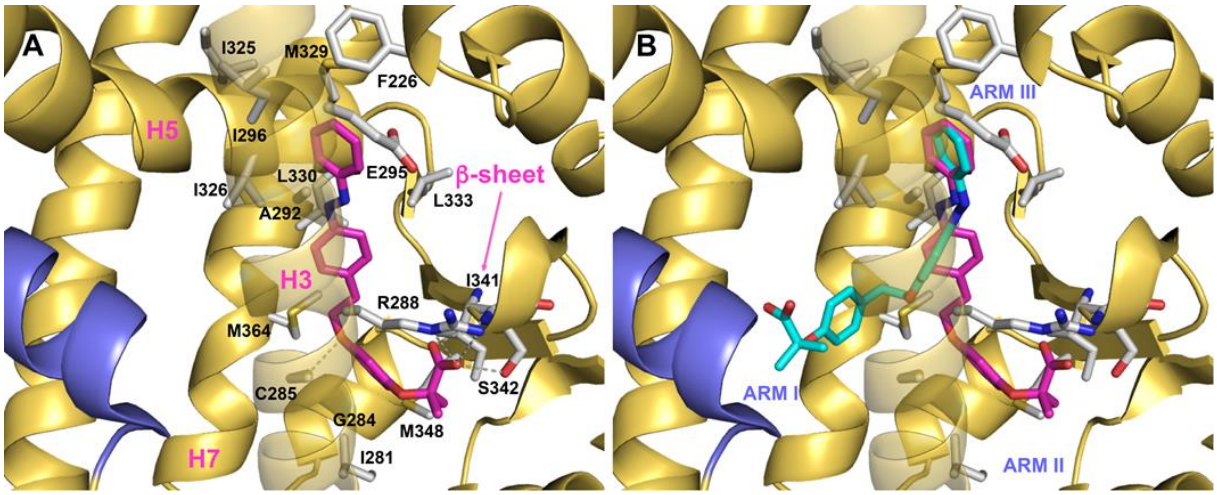
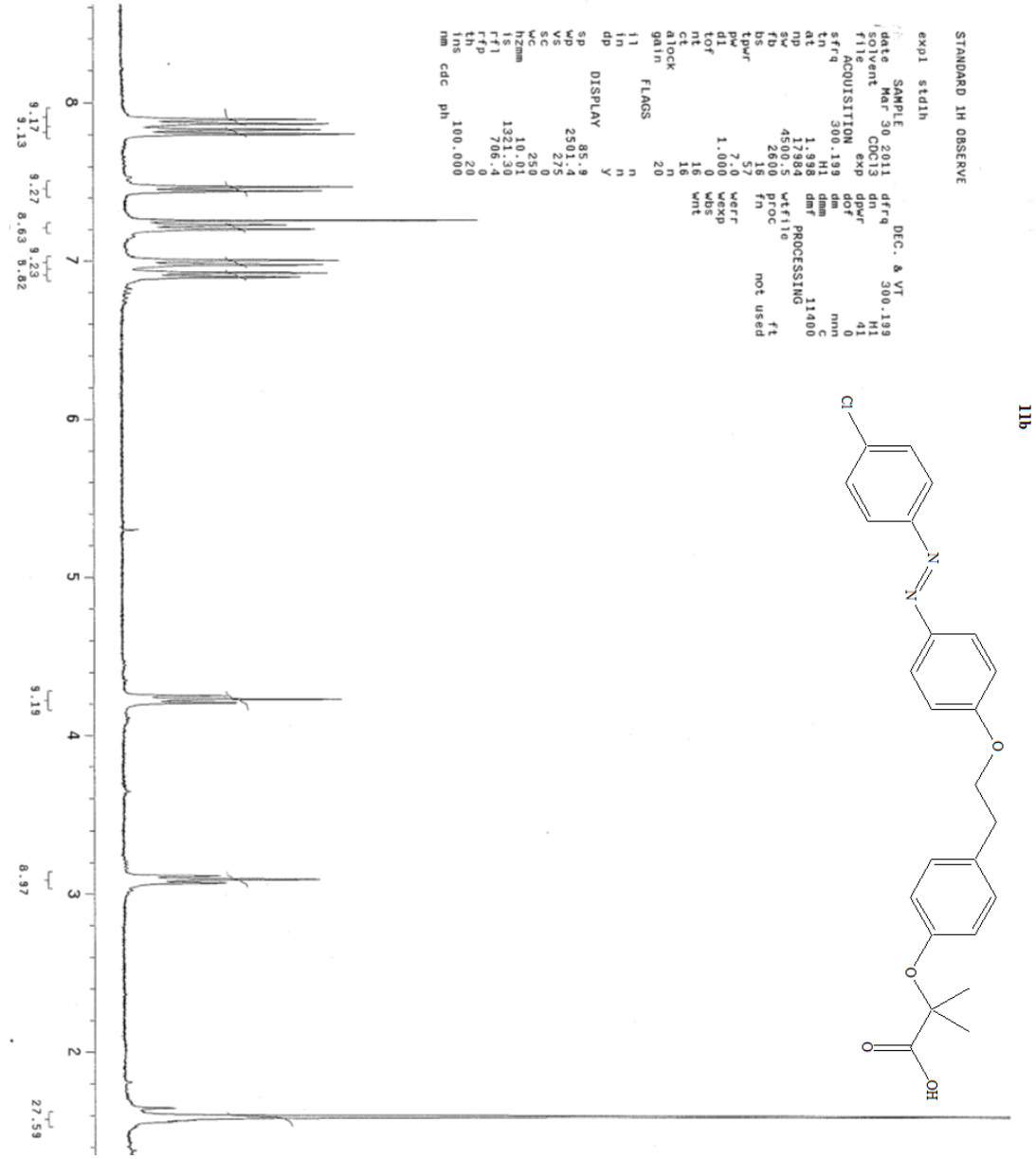


Figure S7. Binding mode of compound **12a** (magenta sticks) into the PPAR LBD (A) represented as a yellow ribbon model. Only amino acids located within 4.5 Å of the bound ligand are displayed (white sticks) and labeled. H-bonds discussed in the text are depicted as dashed grey lines. H12 is shown in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

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



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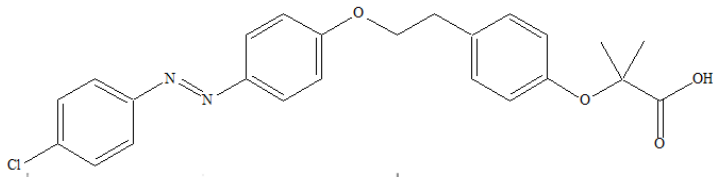


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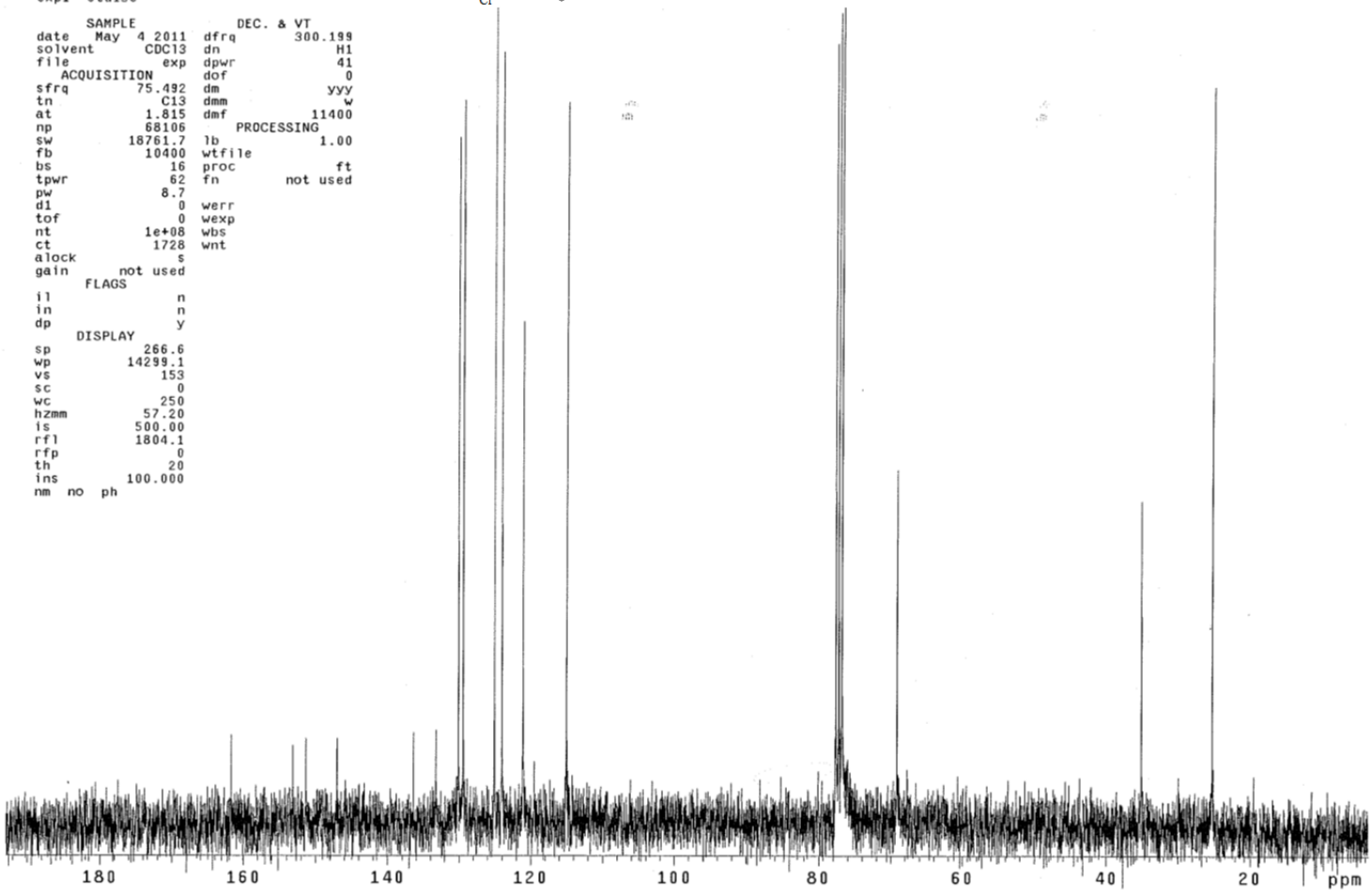

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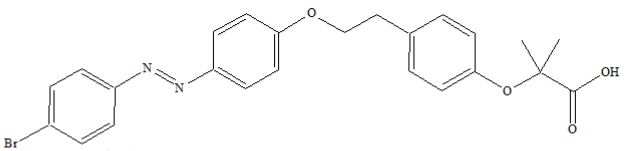
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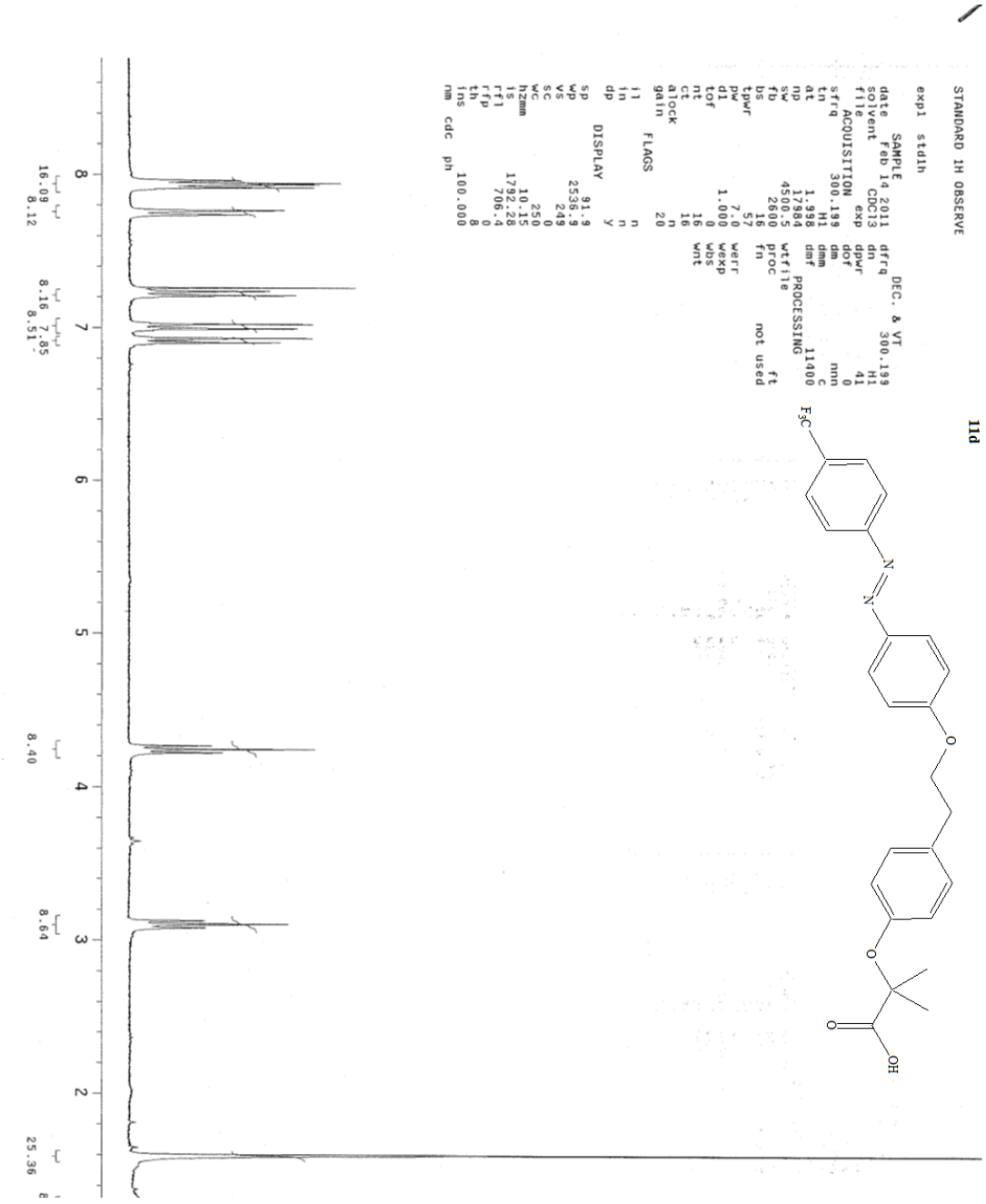
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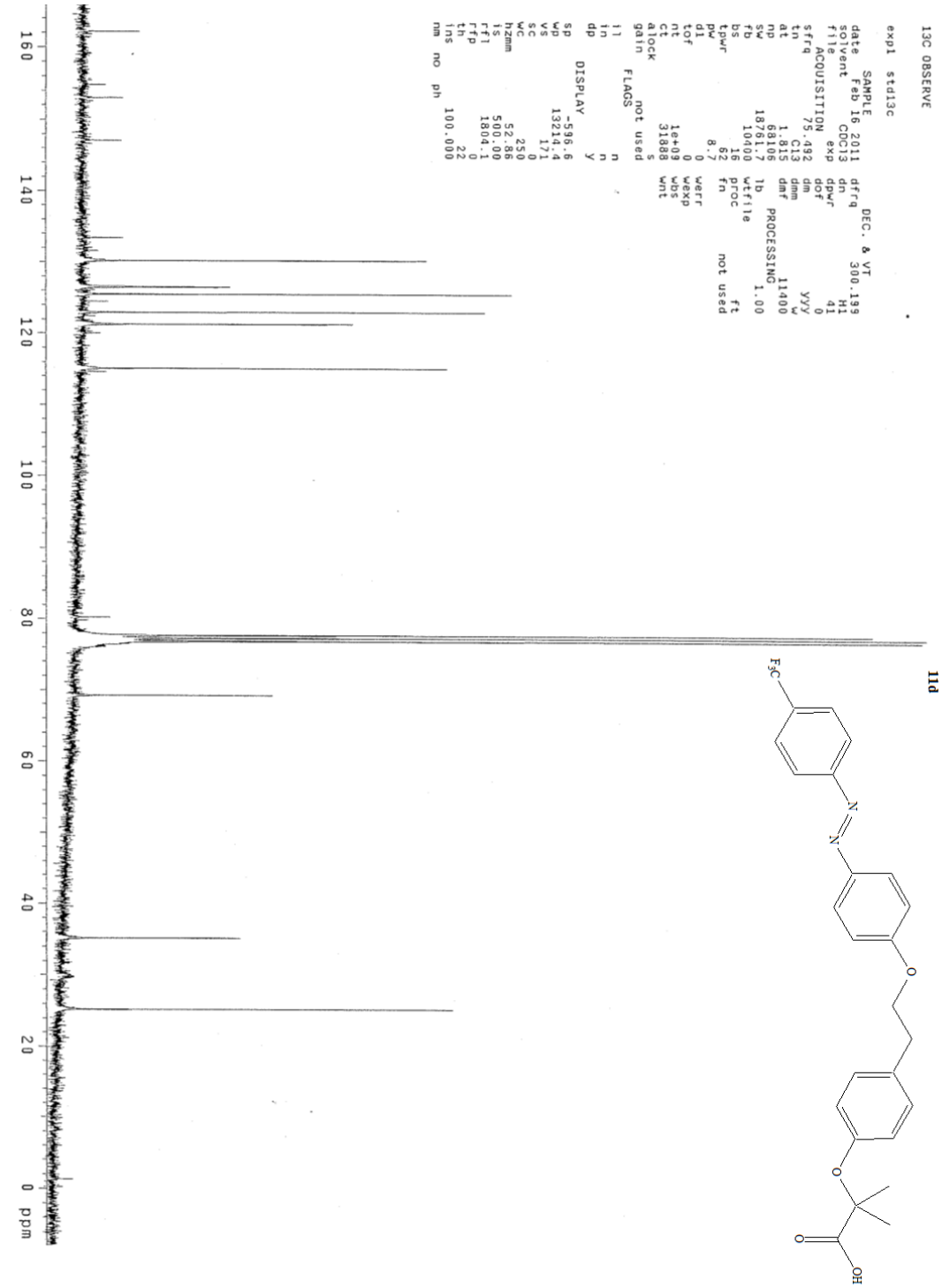
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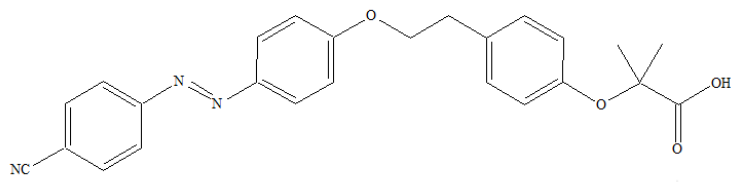

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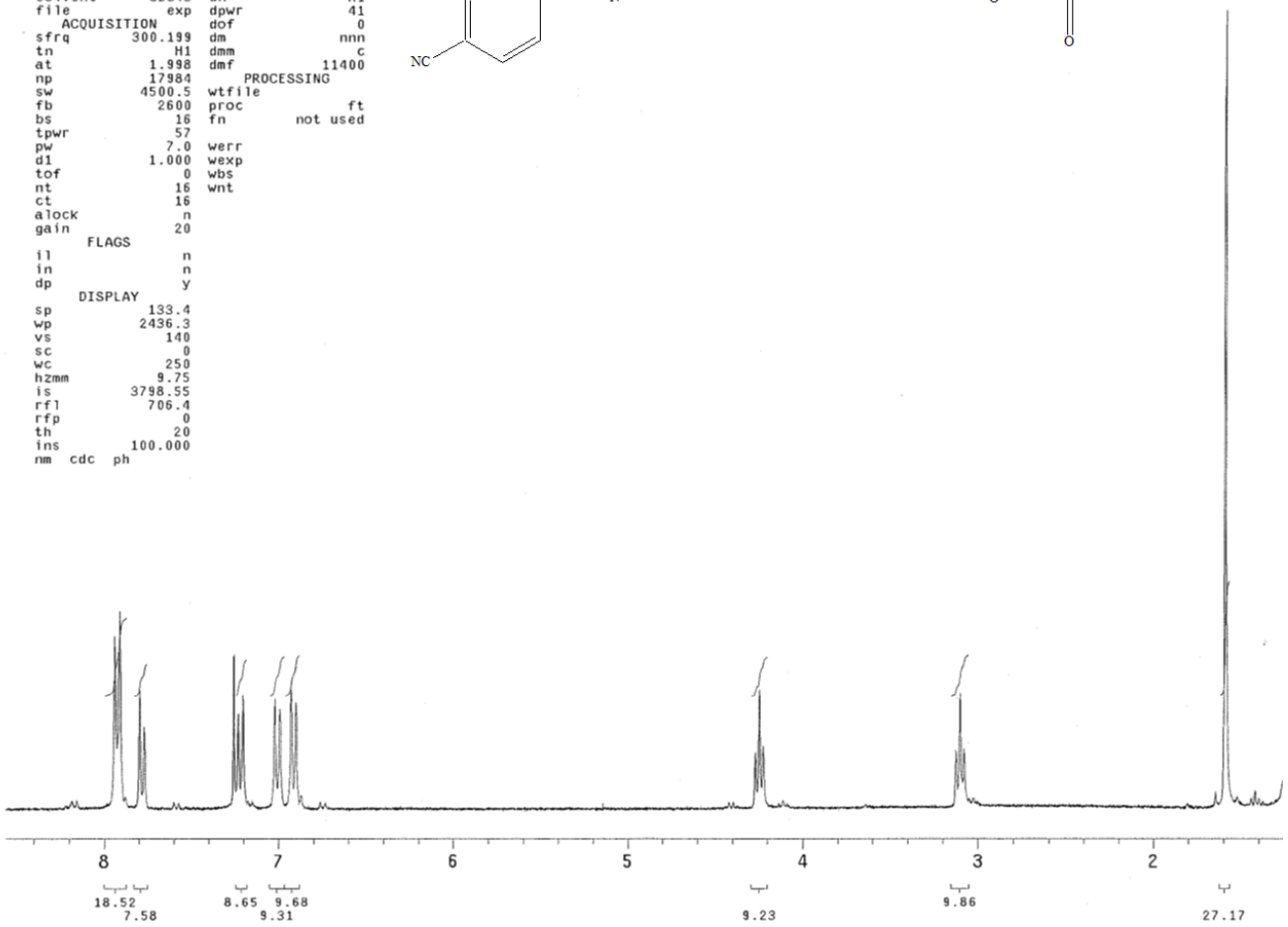

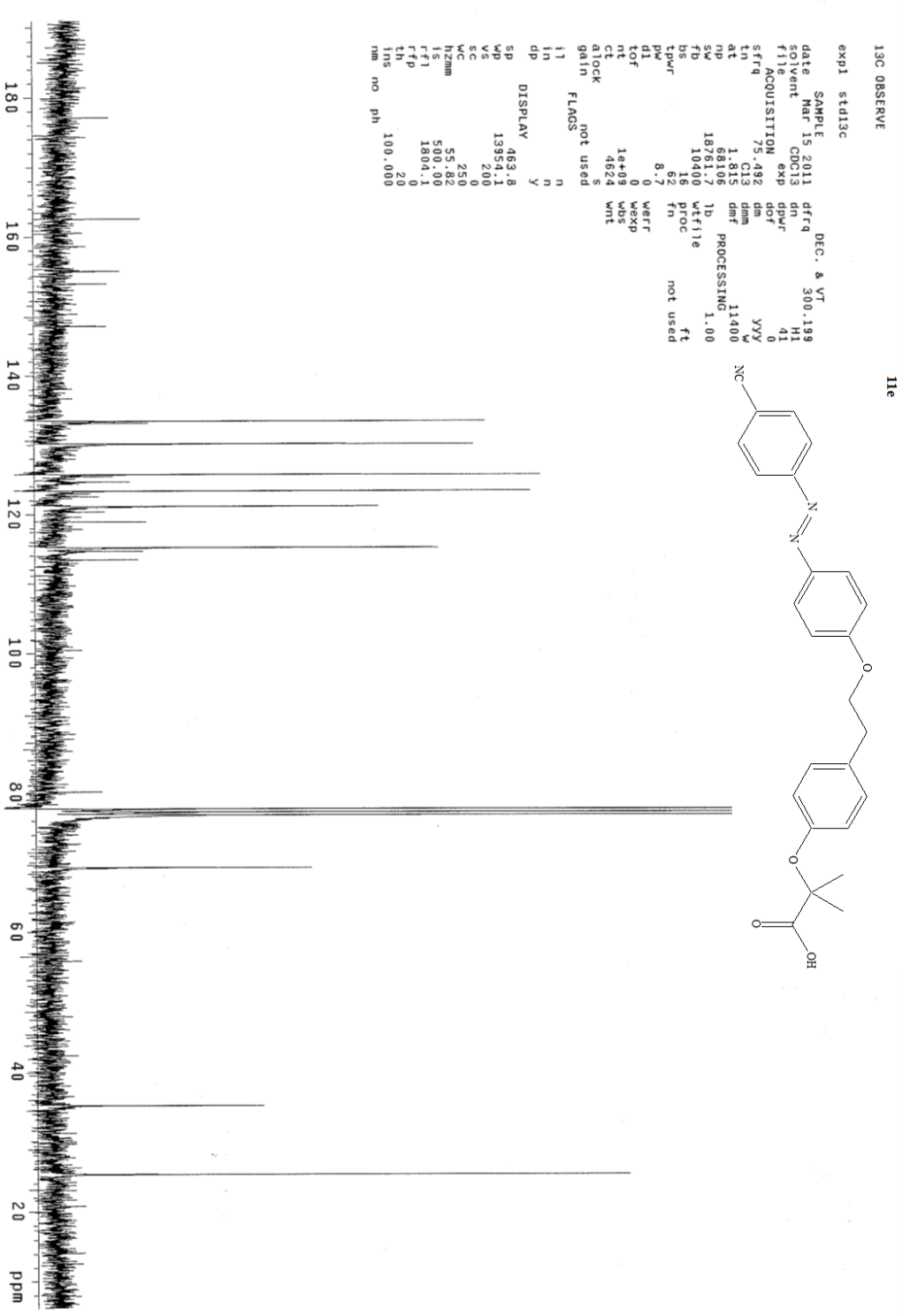
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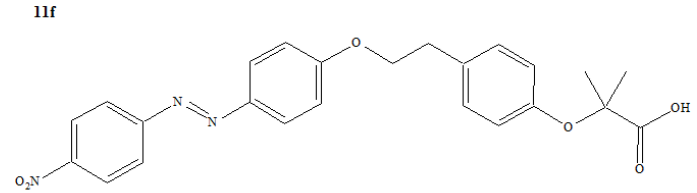


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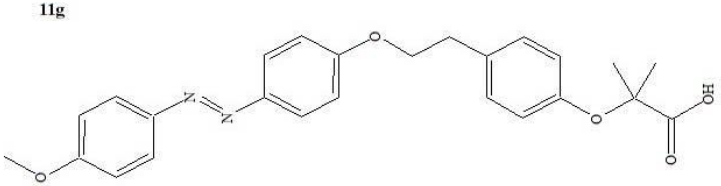

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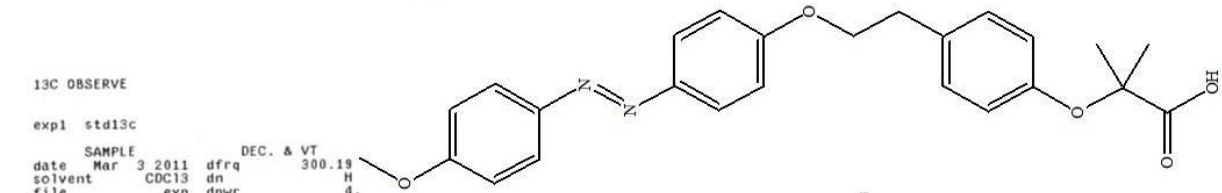


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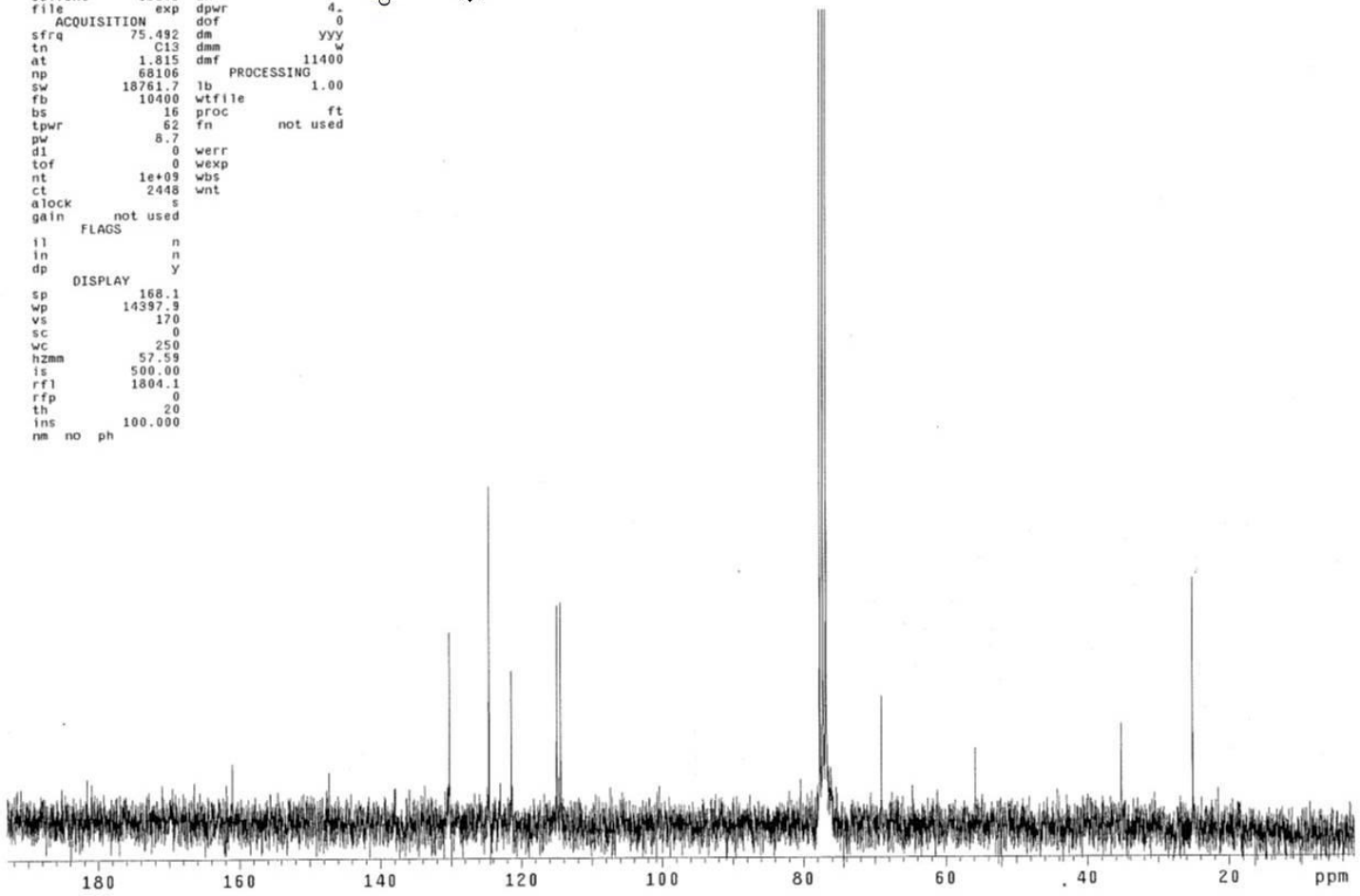

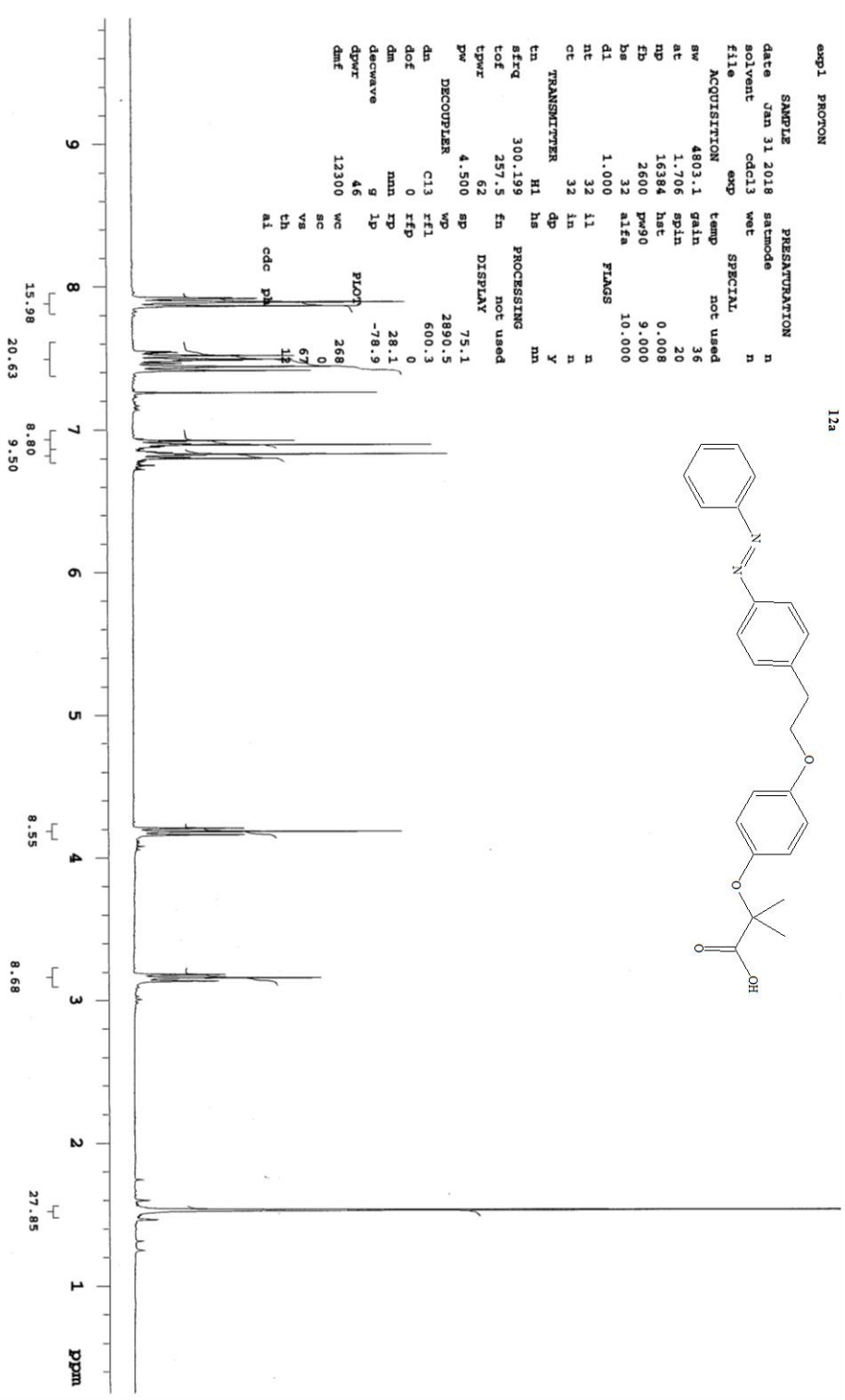
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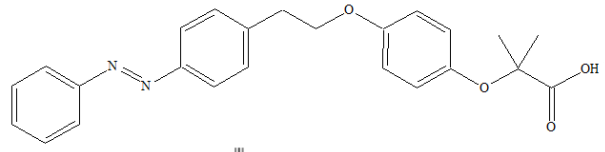
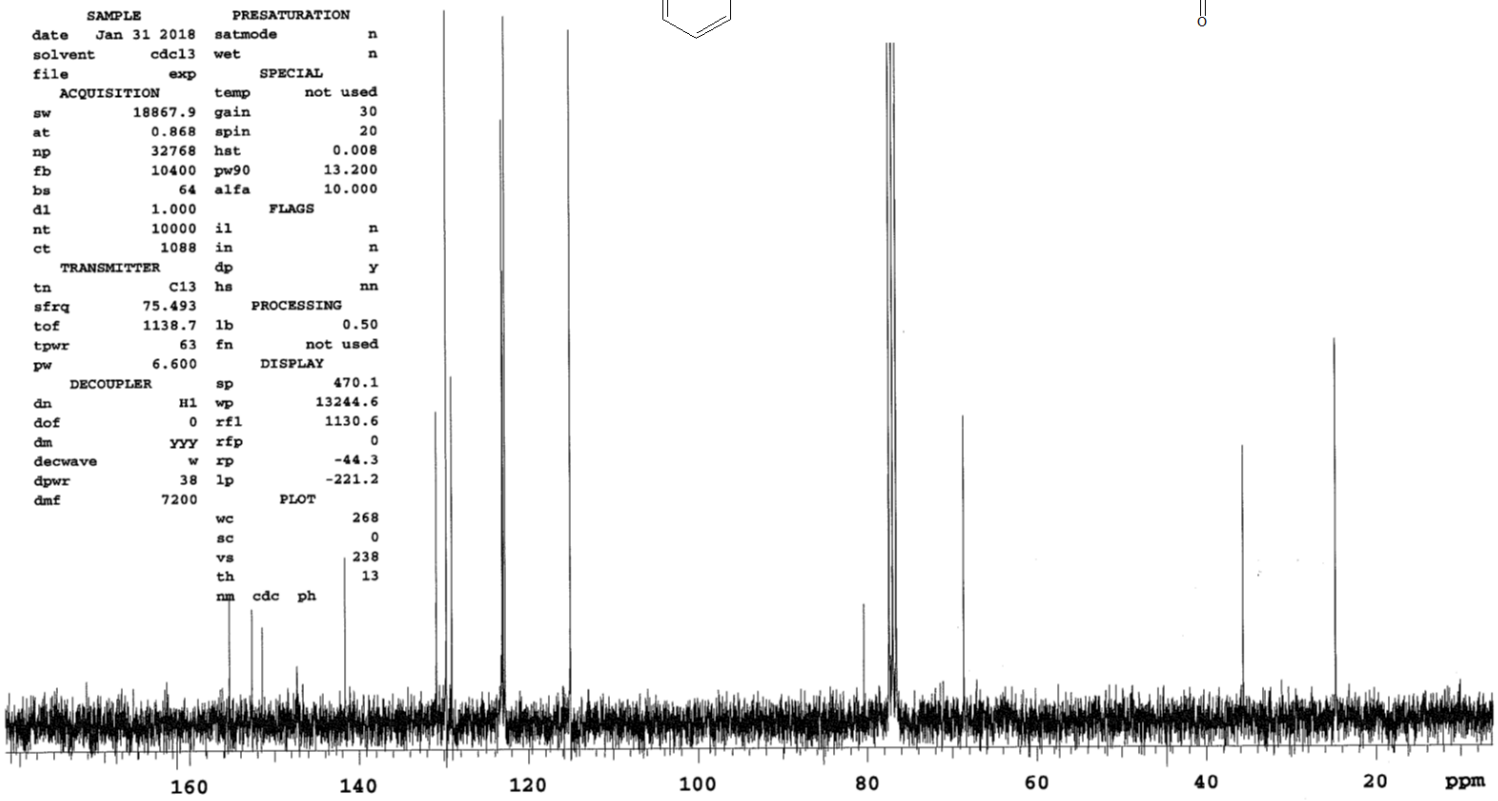
sw 4803.1
at 1.706
np 16384
fb 2600
bs 32
d1 1.000
nt 32
ct 32
TRANSMITTER
tn H1
sfrq 300.199
tof 257.5
tpwr 62
pw 4.500
DECOUPLER
dn C13
dof 0
dm nnn
decwave g
dpwr 46
dmf 12300
PRESATURATION
satmode n
wet n
SPECIAL
temp not used
gain 36
spin 20
hst 0.008
pw90 9.000
alfa 10.000
FLAGS
il n
in n
dp y
hs nn
PROCESSING
fn not used
DISPLAY
sp 75.1
wp 2890.5
rf1 600.3
rfp 0
rp 28.1
lp -78.9
PLOT
wc 268
sc 0
vs 67
th 12
ai cdc ph
12a
OH
9
8
7
6
5
4
3
2
1
ppm
15.98
20.63
8.80
9.50
8.55
8.68
27.85

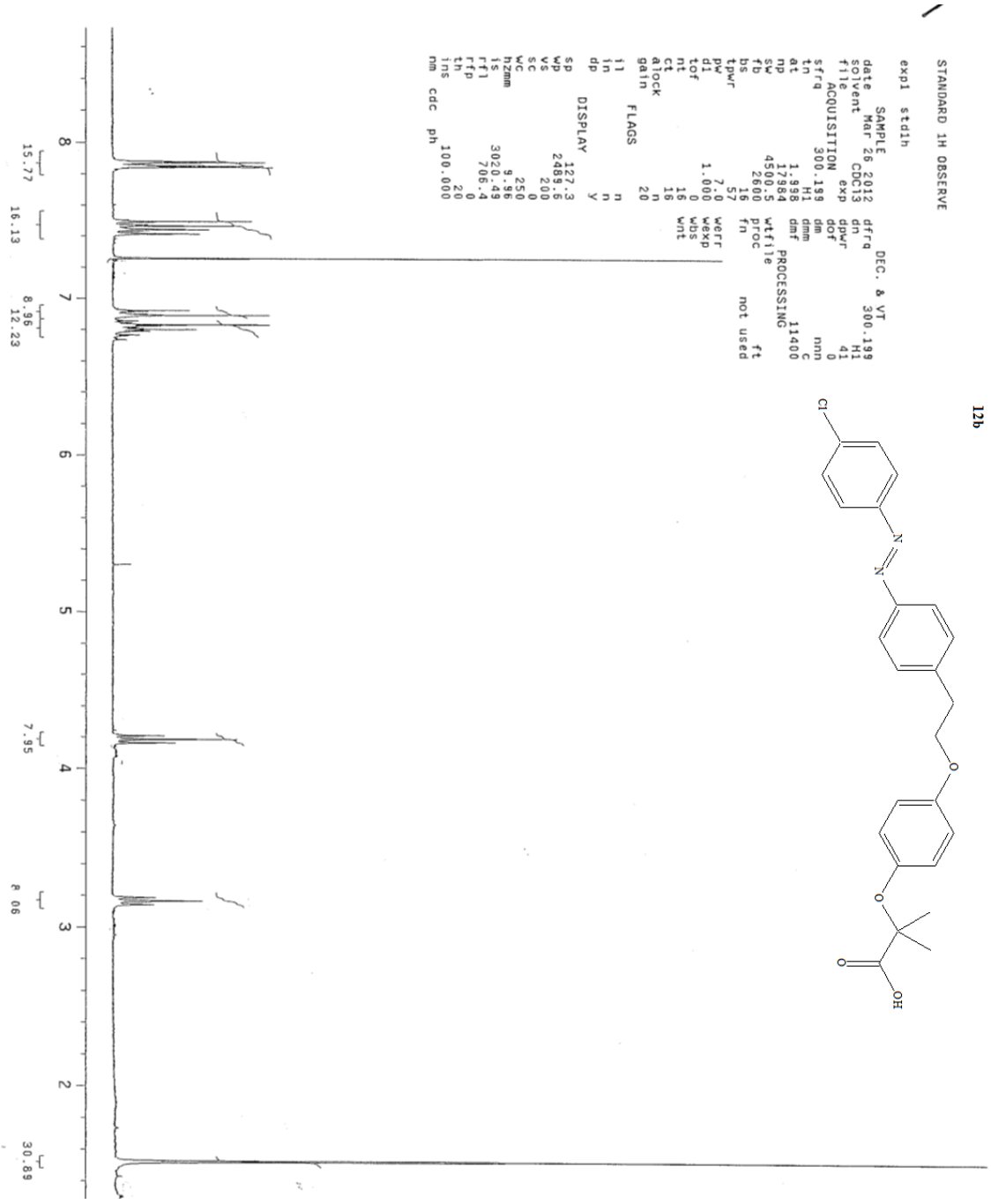
12a



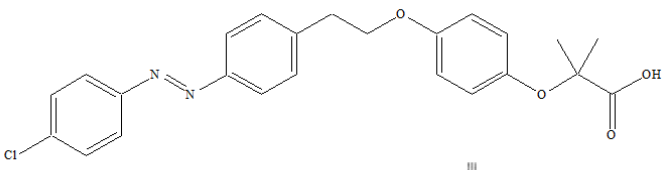
exp1 CARBON

SAMPLE		PRESATURATION	
date	Jan 31 2018	satmode	n
solvent	cdcl3	wet	n
file	exp	SPECIAL	
ACQUISITION		temp	not used
sw	18867.9	gain	30
at	0.868	spin	20
np	32768	hst	0.008
fb	10400	pw90	13.200
bs	64	alfa	10.000
d1	1.000	FLAGS	
nt	10000	il	n
ct	1088	in	n
TRANSMITTER		dp	y
tn	C13	hs	nn
sfrq	75.493	PROCESSING	
tof	1138.7	lb	0.50
tpwr	63	fn	not used
pw	6.600	DISPLAY	
DECOUPLER		sp	470.1
dn	H1	wp	13244.6
dof	0	rf1	1130.6
dm	yyy	rfp	0
decwave	w	rp	-44.3
dpwr	38	lp	-221.2
dmf	7200	PLOT	
		wc	268
		sc	0
		vs	238
		th	13
		nm	cdc ph

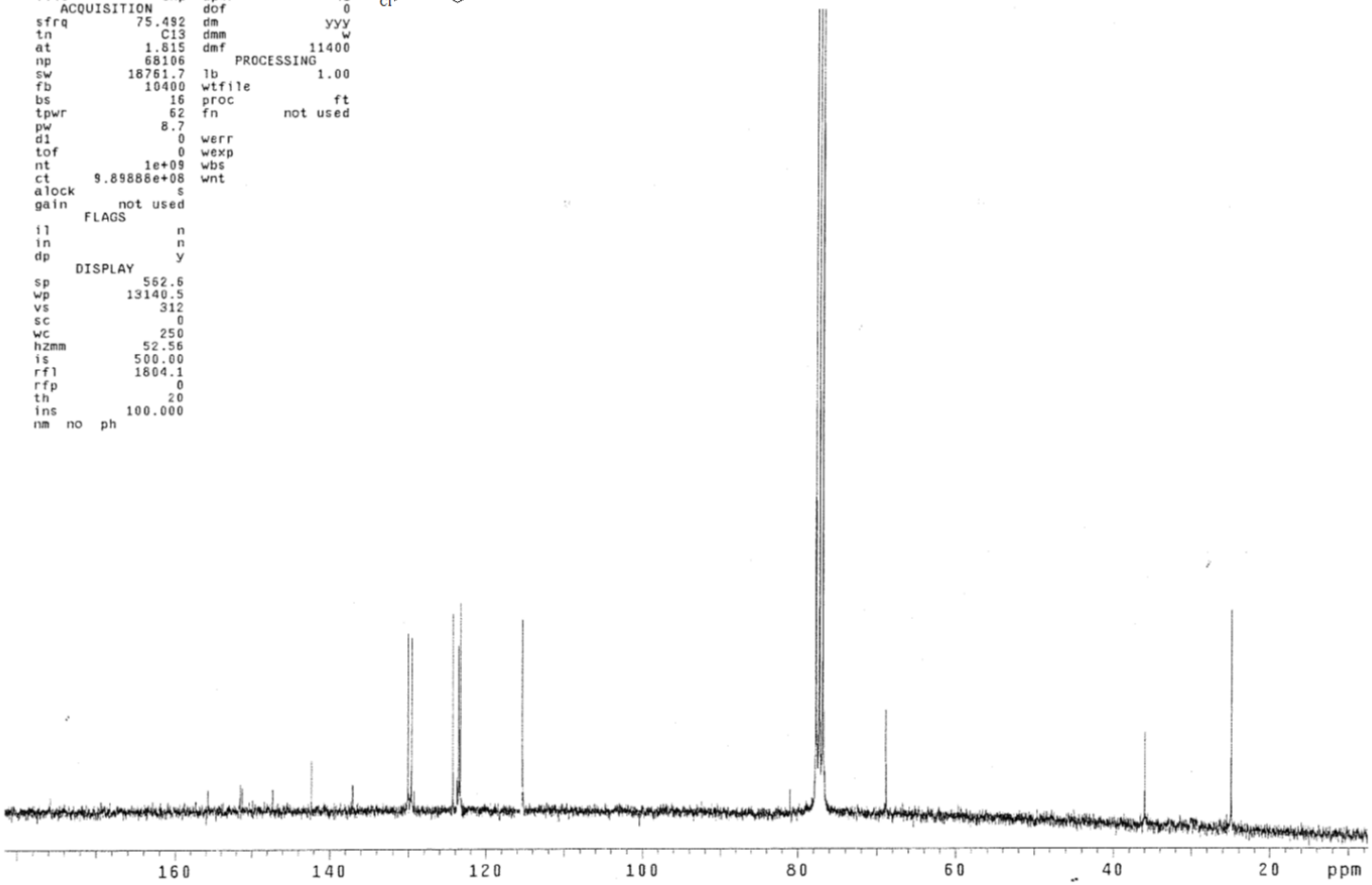


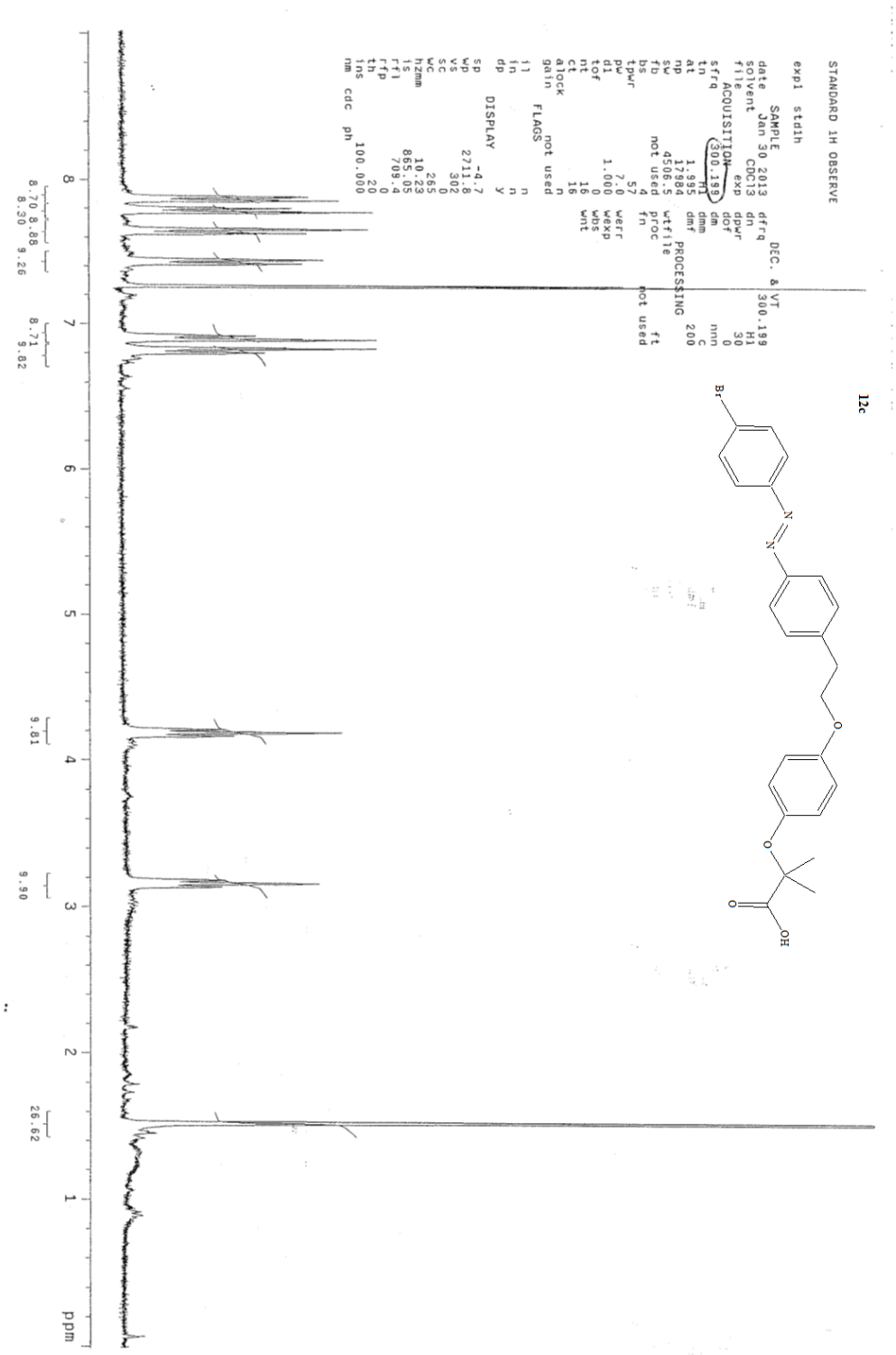

STANDARD 1H OBSERVE
12b
Cl
N
N
O
O
O
OH
8.96
12.23
7.95
8.06
30.89
15.77
16.13

12b

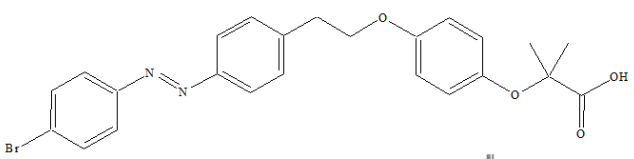



```
13C OBSERVE

exp1  std13c

      SAMPLE                DEC. & VT
date    May 29 2012  dfrq        300.199
solvent       CDCl3  dn             H1
file            exp  dpwr             41
   ACQUISITION       dof               0
sfrq         75.492  dm              yyy
tn              C13  dmm               w
at            1.815  dmf           11400
np            68106     PROCESSING
sw         18761.7   lb             1.00
fb           10400  wtfile
bs              16  proc              ft
tpwr            62  fn         not used
pw             8.7
d1               0  werr
tof              0  wexp
nt            1e+09  wbs
ct     9.89888e+08  wnt
alock            s
gain      not used
      FLAGS
il                n
in                n
dp                y
     DISPLAY
sp           562.6
wp         13140.5
vs             312
sc               0
wc             250
hzmm         52.56
is          500.00
rfl        1804.1
rfp              0
th              20
ins         100.000
nm  no  ph
```

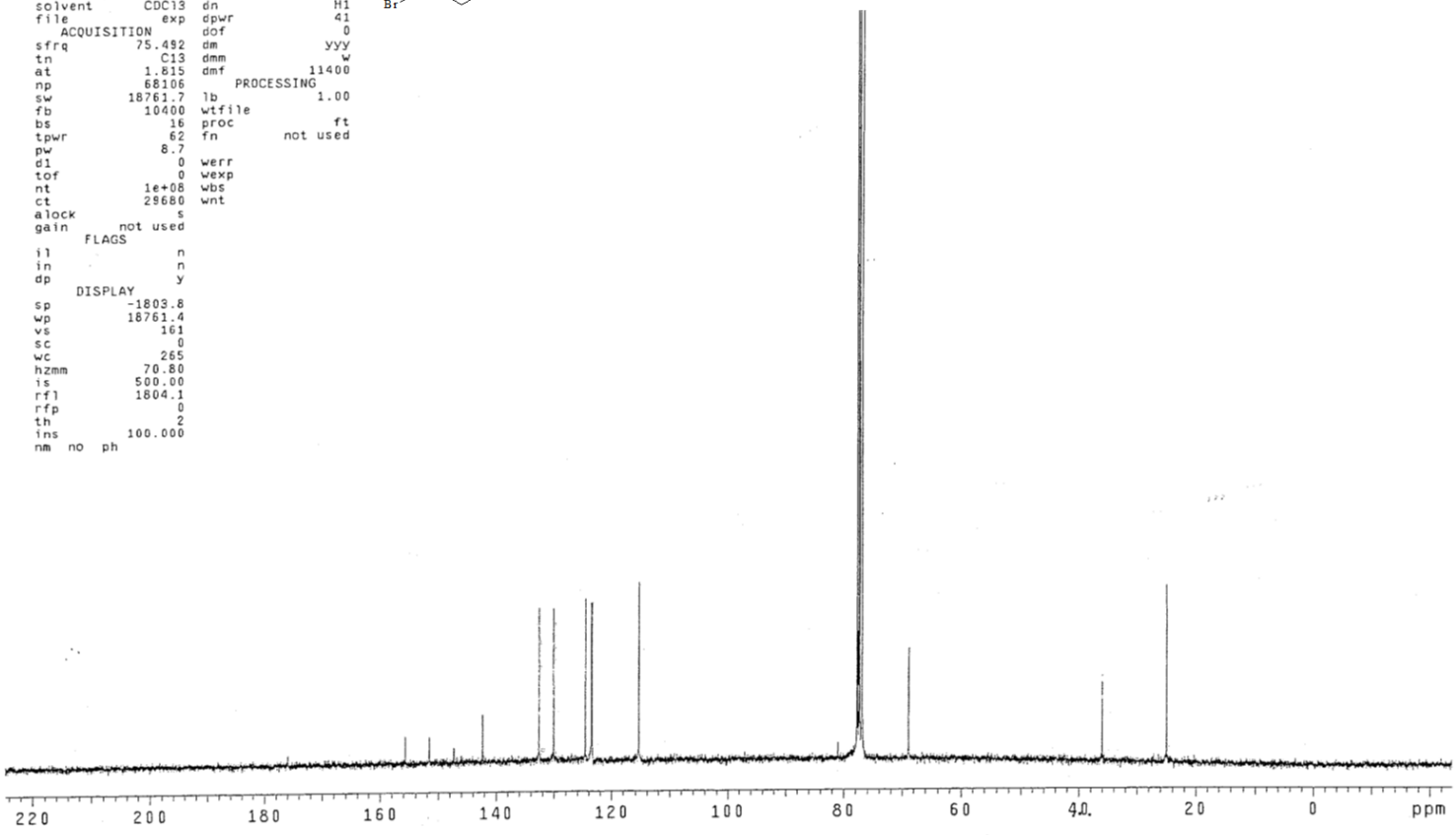


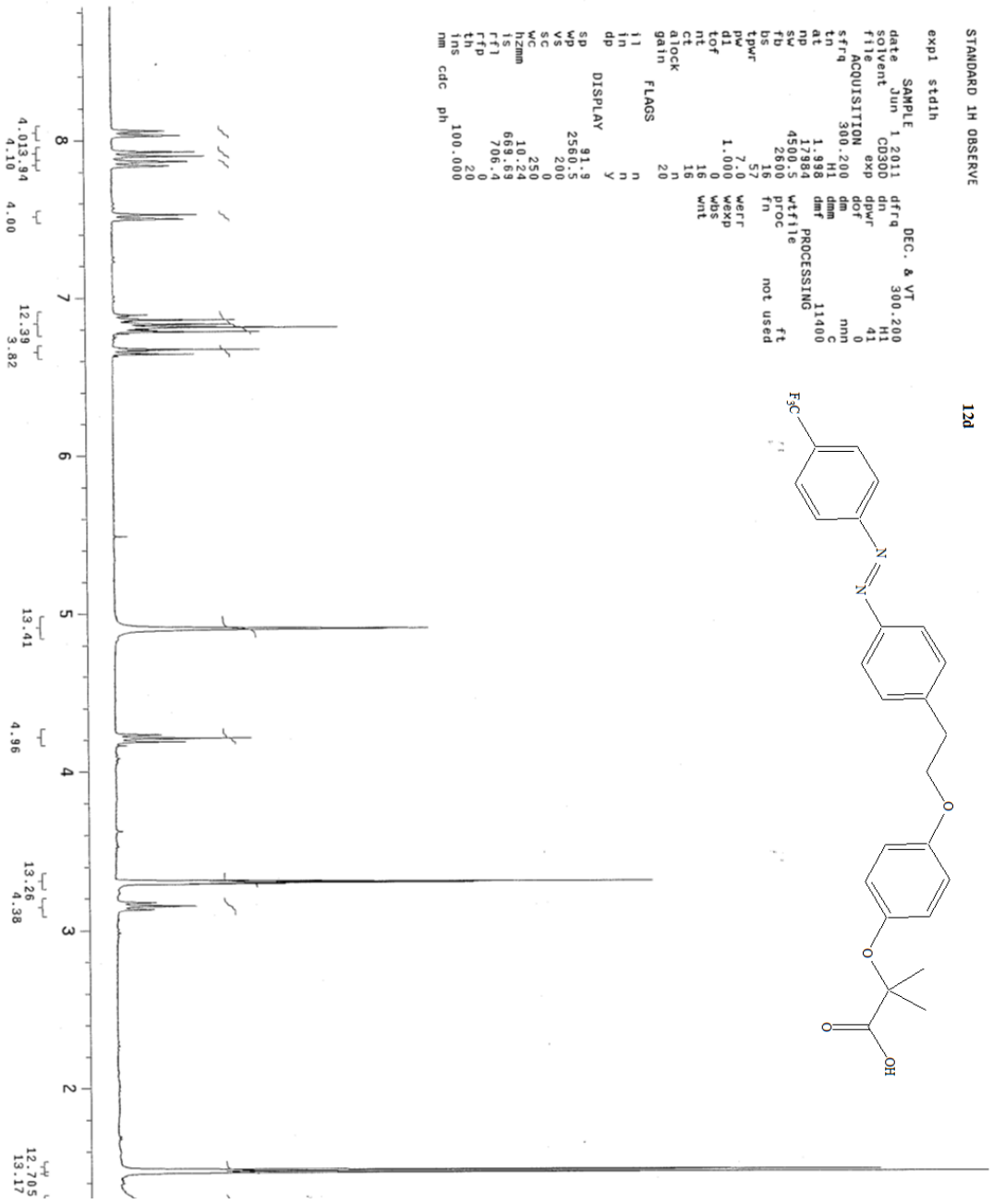

12c
Br
N
N
O
O
OH
O
STANDARD 1H OBSERVE
expl stdih
SAMPLE
date Jan 30 2013
solvent CDCl3
file
ACQUISITION
300.199 H1
sfrq
tn
at 1.995
np 17984
sw 4506.5
fb
bs
tpwr 57
pw 7.0
pl1 1.000
tof 0
nt 16
ct 16
alock n
gain n
FLAGS
il n
in n
dp y
DISPLAY
sp -4.7
wp 2711.8
vs 302
sc 0
wc 255
hzcm 10.23
is 865.05
rfl 709.4
rfp 0
th 20
ins
nm cdc ph 100.000
DEC. & VT
dfrq 300.199
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
PROCESSING
wtfile
proc
fn
not used ft
not used
wexp
wbs
wnt
8.70 8.88 8.71 9.82 9.81 9.90 26.62
8.30 9.26
ppm

12c

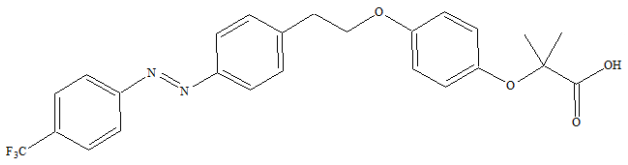



```
13C OBSERVE

exp1  std13c

      SAMPLE                DEC. & VT
date   Apr 22 2013   dfrq       300.199
solvent      CDCl3   dn              H1
file           exp   dpwr            41
   ACQUISITION       dof              0
sfrq         75.492  dm             yyy
tn               C13 dmm              w
at             1.815 dmf          11400
np             68106     PROCESSING
sw          18761.7  lb            1.00
fb             10400  wtfile
bs                16  proc            ft
tpwr              62  fn       not used
pw               8.7
d1                 0  werr
tof                0  wexp
nt             1e+08  wbs
ct             29680  wnt
alock              s
gain       not used
      FLAGS
il                 n
in                 n
dp                 y
     DISPLAY
sp          -1803.8
wp          18761.4
vs              161
sc                0
wc              265
hzmm          70.80
is           500.00
rfl         1804.1
rfp               0
th                2
ins         100.000
nm  no  ph
```




STANDARD 1H OBSERVE
12d
expl stdih
SAMPLE
date Jun 1 2011
solvent CD3OD
file
ACQUISITION
sfrq 300.200
tn H1
at 1.998
np 17984
sw 4500.5
fb 2600
bs 16
tpwr 57
pw 7.0
d1 1.000
tof 16
nt 16
ct 20
alock n
gain
FLAGS
il n
in n
dp y
DISPLAY
sp 91.9
wp 2560.5
vs 200
sc 0
wc 250
hzmm 10.24
is 669.69
rfl 706.4
rfp 0
th 20
ins 100.000
nm cdc ph
DEC. & VT
dfrq 300.200
dn H1
dpwr 41
dof 0
dm nnn
dmm c
dmf 11400
PROCESSING
wtfile
proc ft
fn not used
werr
wexp
wbs
wnt
F3C
N
N
O
O
O
OH
4.013.94
4.10
4.00
12.39
3.82
13.41
4.96
13.26
4.38
12.705
13.17
8
7
6
5
4
3
2

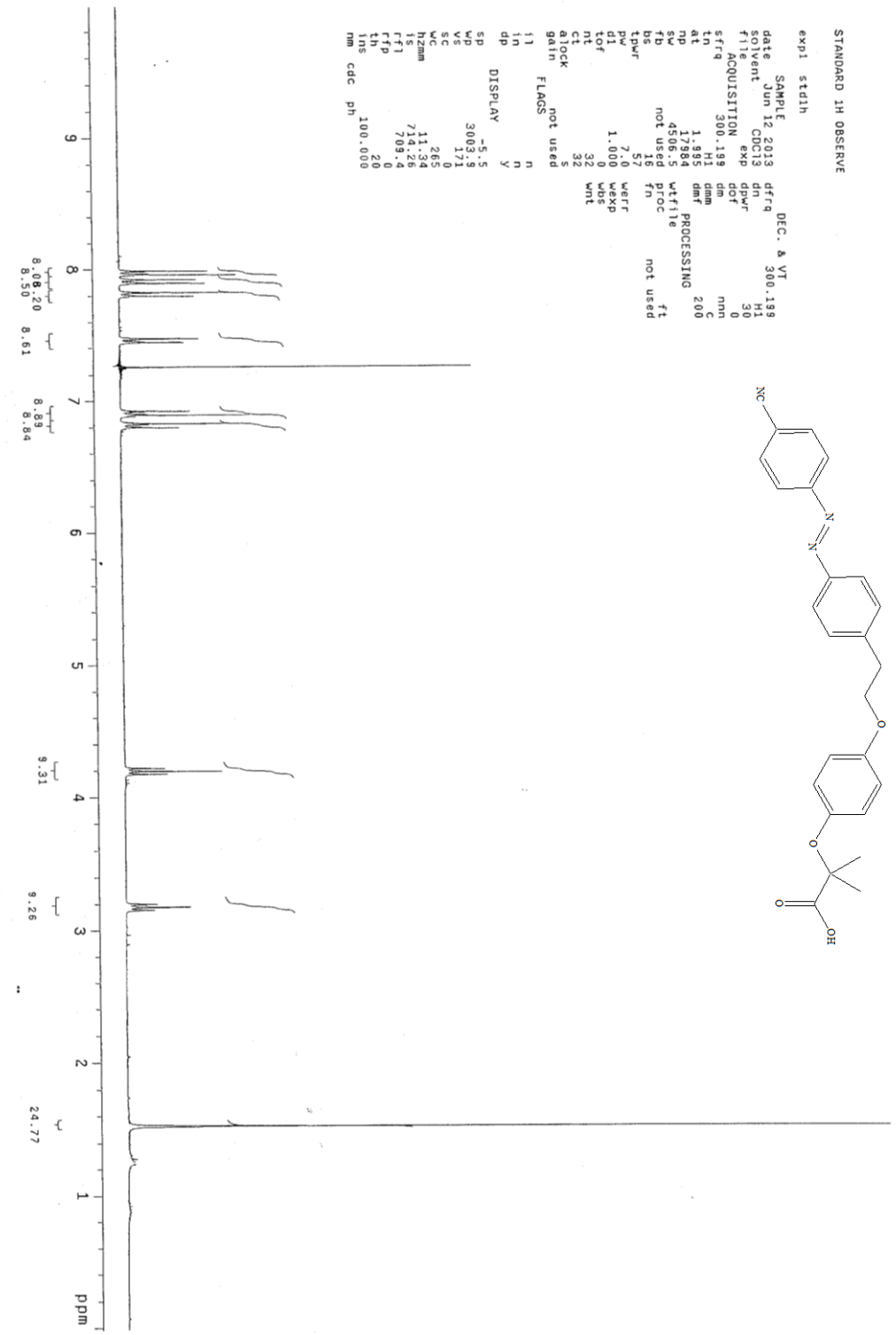
12d



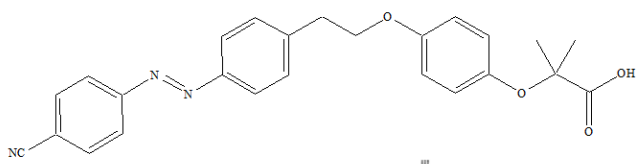
Gradient Shimming

exp1 CARBON

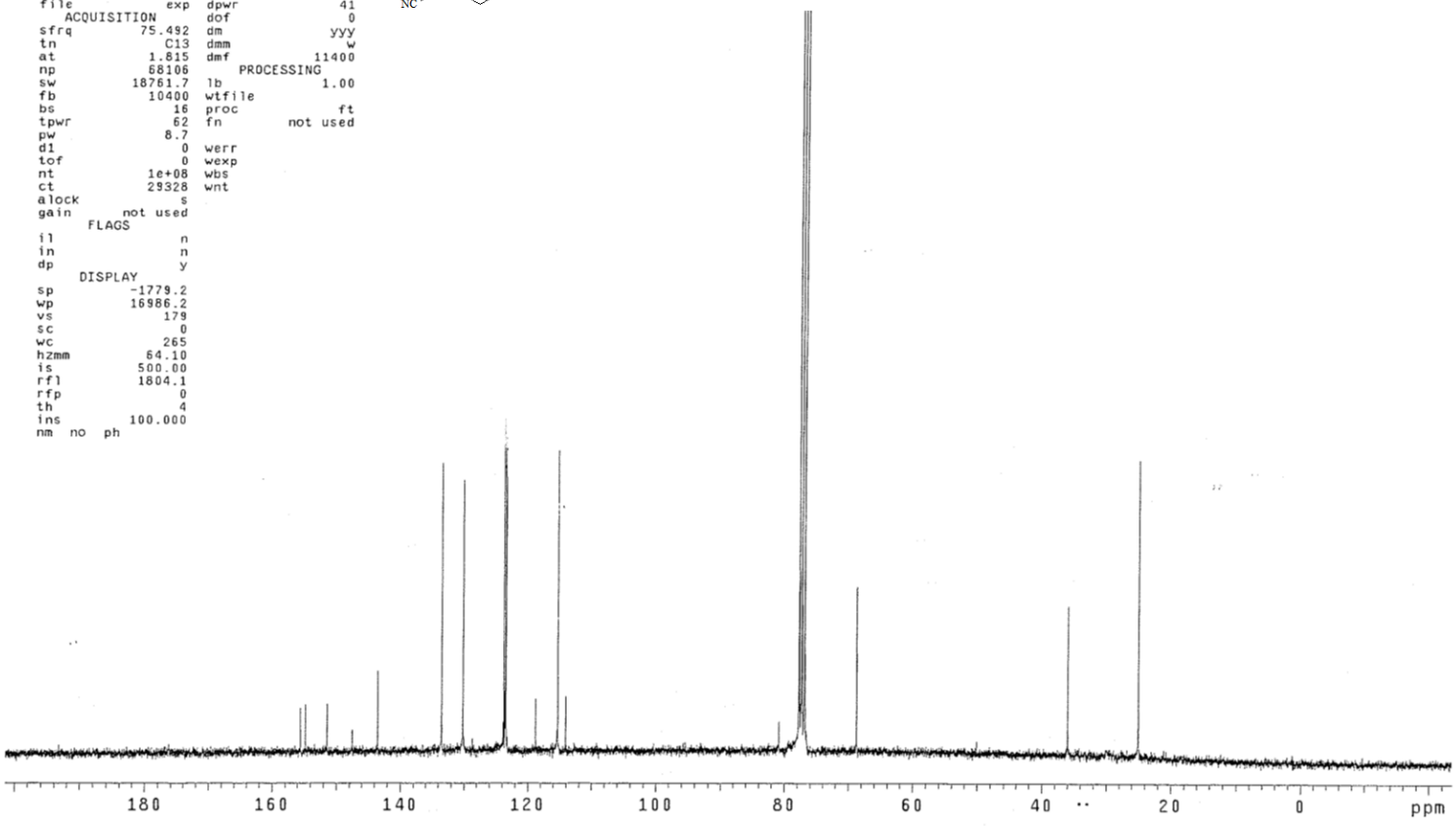
SAMPLE		PRESATURATION	
date	Feb 1 2018	satmode	n
solvent	cd3od	wet	n
file	/home/giampi/~	SPECIAL	
vnmrsys/data/GL605~		temp	not used
	_C13.fid	gain	30
ACQUISITION		spin	20
sw	18867.9	hst	0.008
at	0.868	pw90	13.200
np	32768	alfa	10.000
fb	10400	FLAGS	
bs	64	il	n
d1	1.000	in	n
nt	10000	dp	y
ct	10000	hs	nn
TRANSMITTER		PROCESSING	
tn	C13	lb	0.50
sfrq	75.493	fn	not used
tof	1138.7	DISPLAY	
tpwr	63	sp	138.5
pw	6.600	wp	13144.4
DECOUPLER		rfl	1130.6
dn	H1	rfp	0
dof	0	rp	14.7
dm	yyy	lp	-237.7
decwave	w	PLOT	
dpwr	38	wc	268
dmf	7200	sc	0
		vs	5141
		th	26
		ai cdc ph	


12e
NC
N
N
O
O
O
OH
STANDARD 1H OBSERVE
expl stdih
SAMPLE
date Jun 12 2013
solvent CDCl3
file
ACQUISITION
sfrq 300.199
tn H1
at 1.995
np 17984
sw 4506.5
fb not used
bs 16
tpwr 57
pw 7.0
d1 1.000
tof 0
nt 32
ct 32
alock s
gain not used
FLAGS
il n
in n
dp y
DISPLAY
sp -5.5
wp 3003.9
vs 171
sc 0
wc 265
hzmm 11.34
is 714.26
rfl 709.4
rfp 0
th 20
ins 100.000
nm cdc ph
DEC. & VT
dfrq 300.199
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
PROCESSING
wtfile
proc ft
fn not used
werr
wexp
wbs
wnt
8.20
8.50
8.61
8.89
8.84
9.31
9.26
24.77
ppm

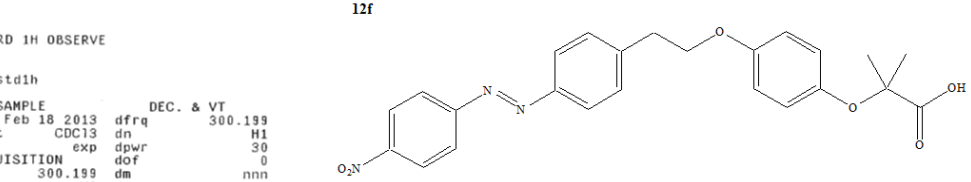
12e




```
13C OBSERVE

exp1  std13c

      SAMPLE              DEC. & VT
date    May 16 2013  dfrq        300.199
solvent       CDCl3  dn             H1
file            exp  dpwr            41
    ACQUISITION      dof             0
sfrq         75.492  dm            yyy
tn              C13  dmm             w
at            1.815  dmf         11400
np            68106      PROCESSING
sw          18761.7  lb            1.00
fb            10400  wtfile
bs               16  proc            ft
tpwr             62  fn         not used
pw              8.7
d1                0  werr
tof               0  wexp
nt             1e+08  wbs
ct             29328  wnt
alock             s
gain       not used
      FLAGS
il                n
in                n
dp                y
     DISPLAY
sp          -1779.2
wp          16986.2
vs              179
sc                0
wc              265
hzmm          64.10
is           500.00
rfl         1804.1
rfp               0
th                4
ins         100.000
nm  no  ph
```



12f



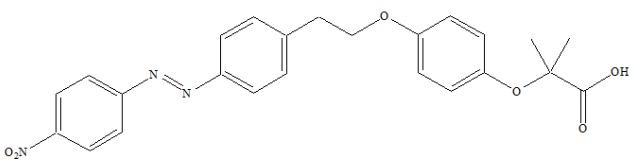
STANDARD 1H OBSERVE

exp1 std1h

SAMPLE		DEC. & VT	
date	Feb 18 2013	dfrq	300.199
solvent	CDCl3	dn	H1
file	exp	dpwr	30
ACQUISITION		dof	0
sfrq	300.199	dm	nnn
tn	H1	dmm	c
at	1.995	dmf	200
np	17984	PROCESSING	
sw	4506.5	wtfile	
fb	not used	proc	ft
bs	16	fn	not used
tpwr	57	werr	
pw	7.0	wexp	
d1	1.000	wbs	
tof	0	wnt	
nt	16		
ct	16		
alock	s		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
DISPLAY			
sp	107.8		
wp	2895.5		
vs	142		
sc	0		
wc	265		
hzmm	10.93		
is	2027.12		
rfl	709.4		
rfp	0		
th	20		
ins	100.000		

nm cdc ph

12f

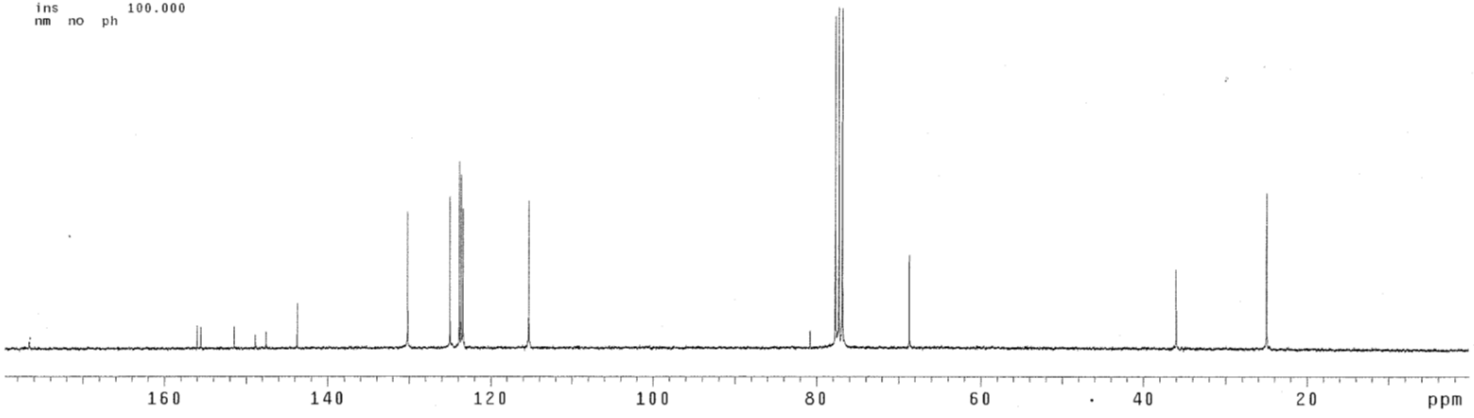


13C OBSERVE

exp1 std13c

SAMPLE		DEC. & VT	
date	Feb 18 2013	dfrq	300.199
solvent	CDCl3	dn	H1
file	exp	dpwr	41
ACQUISITION		dof	0
sfrq	75.492	dm	yyy
tn	C13	dmm	w
at	1.815	dmf	11400
np	68106	PROCESSING	
sw	18761.7	lb	1.00
fb	10400	wtfile	
bs	4	proc	ft
tpwr	62	fn	not used
pw	8.7		
d1	0	werr	
tof	0	wexp	
nt	1e+09	wbs	
ct	31600	wnt	
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
DISPLAY			
sp	20.4		
wp	13534.8		
vs	62		
sc	0		
wc	265		
hzmm	51.07		
is	500.00		
rfl	1804.1		
rfp	0		
th	20		
ins	100.000		

nm no ph



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