Supporting Information

Garcinoic Acid Is a Natural and Selective Agonist of Pregnane X Receptor

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General Methods

Synthetic Chemistry

Unless otherwise noted, chemicals were obtained from commercial suppliers and used without further purification. NMR spectra were recorded on a Bruker AC 400 MHz spectrometer in the indicated solvent. Chemical shifts are reported in parts per million (ppm) and are relative to $CDCl_3$ (7.26 ppm and 77.0 ppm) or to CD_3OD (3.31 ppm and 49.2 ppm). The abbreviations used are as follows: s, singlet; brs, broad singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; m, multiplet; brm, broad multiplet. Melting points were determined by the capillary method using a Buchi 535 instrument and they were not corrected. TLC was performed on aluminum backed silica plates (silica gel 60 F254). Flash chromatographic purifications were performed using Biotage Isolera™ Prime using the appropriate cartridge, eluent and gradient. Hydrogenations were performed with H-Cube apparatus (Thalesnano Nanotechnology Inc., Budapest, Hungary) using 10% Pd/C cartridge (s-cart, 30 × 4 mm i.d.). With the exception of compound **9**, all the synthesised compounds have been previously reported and their spectroscopic and analytical data were consistent with the literature.[1-3] Purity of synthesized compounds (> 95%) was assessed by HPLC-HRMS.

Analytical Chemistry

HPLC-MS

Samples were analyzed using Dionex UltiMate 3000 HPLC separations module combined with a HCT ultra ion trap (BRUKER) using an electrospray interface. The analytical column was a Waters Xselect CSH Phenyl-Hexyl 5 μm, 2.1 x 150 mm, protected by a guard column 2.1 x 4 mm. Compounds were analysed in elution gradient mode using 15 mM ammonium acetate buffer (pH = 8.0) as mobile phase A and MeCN/MeOH (75:25, v/v) as mobile phase B at a flow rate of 0.15 mL min-1. Sample injection volume was 5 μL. The chromatographic column was thermostated at 45 °C. The MS system was set with an electrospray ionization source (ESI) in the negative mode with optimized parameters. Chromatograms were acquired using the mass spectrometer in multiple reaction monitoring (MRM) mode. QuantAnalysis (Bruker Daltonic) software version 1.8 was used for data acquisition and processing.

GC-MS

The extracts were diluted with MeOH up to 10 μg mL^{-1} of final concentration. An aliquot (100 μL) was dried under nitrogen and resuspended in a solution (100 μL) of *N*,*O*-*bis*(trimethylsilyl)trifluoroacetamide (containing 1% of trimethylchlorosilane) and pyridine (1:1, v/v) at 70 °C for 30 min. The solution was dried under nitrogen and resuspended in toluene (100 μL). The same derivatization procedure was applied to the analytical standard of garcinoic acid (**1**) (declared purity ≥90% by LC/MS-ELSD). The GC-MS/MS analysis was carried out using an Agilent 7890A GC (Agilent technologies) coupled with a triple quadrupole Agilent 7000 MS and an autosampler 7693. An aliquot (5 μL) of the final extract in toluene was injected (vent modality: flow 50 mL min^{-1}, pressure 5 psi for 0.5 min and purge flow at 50 mL/min after 2 min from the injection). Method: 70 °C (0.5 min), 70 °C → 300 °C (1 °C min^{-1}), 300 °C → 325 °C (2 °C min^{-1}), 325 °C (5 min). Chromatographic separations were performed using a Rtx-5MS capillary column (RESTEK, 30 m x 0.25 mm x 0.25 μm) using the following conditions: 100 °C (3 min), 100 °C → 320 °C (20 °C min^{-1}), 320 °C (20 min). Temperatures of ion source, quadrupoles and interface were 280 °C, 150 °C and 300 °C, respectively, while He flow was 1 mL min^{-1}. MS method optimization was performed using Full Scan, Product Ion Scan and Multiple Reaction Monitoring modes. Data were analyzed using Agilent MassHunter workstation software and using NIST library. Acquisition time and acquisition window were optimized in order to obtain at least ten points of acquisition for the chromatographic pick. Quantification was performed by evaluating the transition of the most abundant ion while the transition of the less abundant ion was evaluated for the analyte identification. The MS/MS parameter and the selected transitions were 570.4> 249.1 and 570.4> 209.1, with a CV of 12 eV for both transitions. The linearity of the response was investigated by injecting the standard solution of garcinoic acid (**1**) at 0.5-100 μg mL^{-1}, which correspond to 1.17-234 μM.

Isolation and extraction of garcinoic acid (1) (Table S1)

Garcinia kola seeds were provided by Prof. Olatunde Farombi (Department of Biochemistry, University of Ibadan, Nigeria). After cleaning and decortication of the external part, seeds were grounded with a blade mill in dry ice at low temperature.

General procedure of extraction by maceration[3]

Finely ground seeds (100 g) were suspended in the appropriate solvent (MeOH or EtOH, 50 or 100 mL) and stirred at the desired temperature (25 °C, 40 °C or 60 °C) for 6.5 h. The resulting suspensions were filtered off under *vacuo* and the resulting solid residue was re-suspended in the

appropriate solvent (MeOH o EtOH, 50 or 100 mL) and stirred at the desired temperature (25 °C, 40 °C or 60 °C) for further 17.5 h. The suspension was filtered again under *vacuo* and the yield of extraction was determined by calibrated GC-MS.

General procedure for ultrasound extraction[4]

Finely ground seeds (100 g) were suspended in EtOH (100 mL) and sonicated at ambient temperature for 6.5 h. The resulting suspensions were filtered off under *vacuo* and analysed by calibrated GC-MS.

Table S1. Extraction of garcinoic acid (**1**) *G. kola* seeds.[a]

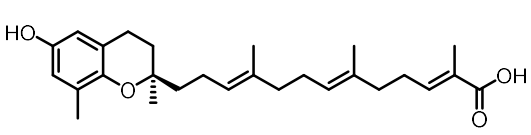
Entry	Temperature (°C)	Solvent	Method	Ratio seeds/solvent[b]	Time (h)	Amount of extract (g)[c]	Yield (%)[d]
1	25	EtOH	maceration	1:1	6.5	3.96	0.65
					17.5[e]	1.21	0.0044
2	25	EtOH	maceration	2:1	6.5	5.15	0.29
					17.5[e]	1.69	0.049
3	25	MeOH	maceration	1:1	6.5	2.39	1.64
					17.5[e]	0.70	0.013
4	25	EtOH	ultrasound	1:1	6.5	5.33	0.47
5	60	EtOH	maceration	1:1	6.5	8.09	0.20
					17.5[e]	0.94	0.0016
6	40	EtOH	maceration	1:1	6.5	4.70	0.87
					17.5[e]	1.01	0.027

[a]Extraction was performed on 100 g of finely ground *G. kola* seeds. [b]Ratio seeds/solvent is expressed in g/mL. [c]Crude extract. [d]Determined by calibrated GC-MS. [e]After 6.5 h, the suspension was filtered and the solid residue was re-suspended in the same solvent and stirred at the same temperature for further 17.5 h.

Optimised extraction protocol (Table S1, entry 3)

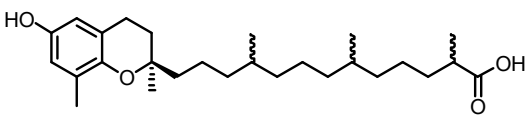
Finely ground seeds (1.2 kg) were suspended in MeOH (1.2 L) and stirred at 25 °C for 6.5 h. The resulting suspensions were filtered off under *vacuo* and the resulting solid residue was re-suspended in MeOH (1.2 L) and stirred at 25 °C for further 17.5 h. The suspension was filtered again under *vacuo* affording a crude brownish oil (56 g). The crude was purified by automated flash chromatography on silica (Eluent: CH_2Cl_2/MeOH, from 100:0 to 90:10, v/v) affording 7.4 g (yield 0.62% w/w) of pure garcinoic acid (**1**) as yellow-green oil and 4 g of impure product (purity estimation 50%).

(2*E*,6*E*,10*E*)-13-[(2*R*)-6-hydroxy-2,8-dimethyl-chroman-2-yl]-2,6,10-trimethyltrideca-2,6,10-trienoic acid (garcinoic acid, 1)[1]



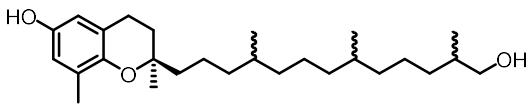
^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.27 (3H, s), 1.53-1.61 (1H, m), 1.63 (6H, m), 1.75-1.79 (2H, m), 1.84 (3H, s), 1.99-2.01 (2H, m), 2.06-2.13 (9H, m), 2.20 (3H, s), 2.29-2.30 (2H, m), 2.70 (2H, t, *J*= 6.49 Hz), 5.13-5.14 (2H, m), 6.41 (1H, d, *J*= 2.51 Hz), 6.50 (1H, d, *J*= 2.52 Hz), 6.88-6.92 (1H, m). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 12.0, 15.8, 15.9, 16.0, 22.1, 22.4, 24.0, 26.4, 27.5, 31.3, 38.0, 39.5, 42.0, 77.3, 112.6, 115.7, 121.2, 124.4, 125.1, 126.9, 127.3, 133.7, 134.8, 145.0, 145.9, 147.7, 173.3.

13-[(*R*)-6-hydroxy-2,8-dimethylchroman-2-yl)-2,6,10-trimethyltridecanoic acid (6)[2]



A solution of **1** (800 mg, 1.8753 mmol) in EtOAc (30 mL) was hydrogenated under continuous flow modality (Thalesnano Nanotechnology Inc.) using a 10% Pd/C cartdrige. The crude was concentrated under reduced pressure to give a green oil (800 mg, 1.8491 mmol, 99% yield as not separable mixture of isomers). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.85-1.79 (34H, m), 2.13 (3H, s), 2.45-2.50 (1H, m), 2.67 (2H, t, *J*= 6.04), 6.40 (1H, d, *J*= 2.19 Hz), 6.49 (1H, d, *J*= 2.20 Hz). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 16.1, 16.8, 16.9, 19.5, 19.6, 22.5, 24.2, 24.4, 24.6, 29.7, 31.27, 31.34, 32.6, 33.79, 33.84, 37.3, 39.4, 39.7, 39.8, 75.5, 112.6, 115.6, 121.3, 127.3, 145.9, 147.6, 182.8.

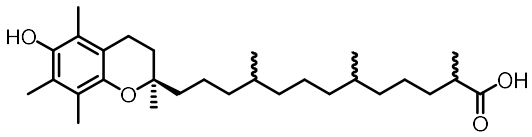
(2*R*)-2-(13-hydroxy-4,8,12-trimethyltridecyl)-2,8-dimethylchroman-6-ol (8)[3]



To a solution of **6** (100 mg, 0.2311 mmol) in dry THF (2 mL), $LiAlH_4$ (27 mg, 0.7115 mmol) was added portionwise at 0 °C. The resulting suspension was allowed to reach r.t. and stirred for 3 h. EtOAc (10 mL) was added followed by slow addition of H_2O (10 mL). The aqueous phase was extracted with EtOAc (2 x 10 mL) and the combined organic extracts were washed with H_2O (10 mL), brine (10 mL) and dried over anydrous Na_2SO_4. The crude wasa purified by automated flash chromatography on silica (Eluent: CH_2Cl_2/MeOH from 100:0 to 90:10, v/v) affording the desired derivative **8** (84 mg, 0.2006 mmol, 87% yield) as yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.85-1.79 (35H, m), 2.13 (3H, s), 2.68 (2H, t, *J*= 6.65 Hz), 3.42-3.46 (1H, m), 3.52-3.56 (1H, m), 6.39 (1H, d, *J*= 2.19 Hz), 6.49 (1H, d, *J*= 2.20 Hz). ^{13}C-NMR (100.6

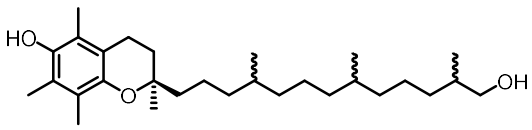
MHz, $CDCl_3$): δ 16.1, 16.6, 16.7, 19.7, 20.9, 21.0, 22.6, 24.3, 24.4, 24.5, 31.4, 31.5, 32.6, 32.8, 33.5, 35.7, 37.3, 37.4, 39.7, 68.5, 75.5, 112.7, 115.7, 121.3, 127.2, 145.8, 148.0.

13-[(*R*)-6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)]-2,6,10-trimethyltridecanoic acid (2)[2]



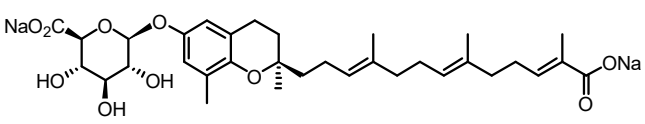
To a solution of **6** (100 mg, 0.2311 mmol) in dry Et_2O (4 mL), anhydrous $SnCl_2$ (350 mg, 1.8458 mmol), HCl 12 M (2 mL) and paraformaldehyde (51 mg, 1.6983 mmol) were sequentially added and the resulting suspension was stirred at 70 °C for 4 h. Then, H_2O (10 mL) was added and the mixture was extracted with Et_2O (3 x 10 mL). The combined organic extratcs were washed with brine (15 mL) and dried over anhydrous Na_2SO_4. The crude was purified by automated flash crhomatography (Eluent: Petroleum ether/EtOAc, from 100:0 to 70:30, v/v) affording the desired derivative **2** (98 mg, 0.2127 mmol, 92% yield) as pale yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.80-1.83 (34H, m), 2.12 (6H, s), 2.19 (3H, s), 2.47 (1H, m), 2.61 (2H, t, *J*= 6.69 Hz). ^{13}C-NMR (100.6 MHz, $CDCl_3$):δ 11.3, 11.8, 12.2, 16.8, 16.9, 19.5, 19.6, 20.7, 21.0, 23.8, 24.4, 24.6, 31.5, 32.6, 33.8, 36.8, 36.9, 37.3, 37.4, 39.3, 39.7, 74.5, 117.3, 118.5, 121.0, 122.6, 144.5, 145.5, 182.8.

(2*R*)-2-(13-hydroxy-4,8,12-trimethyltridecyl)-2,5,7,8-tetramethylchroman-6-ol (7)[2]



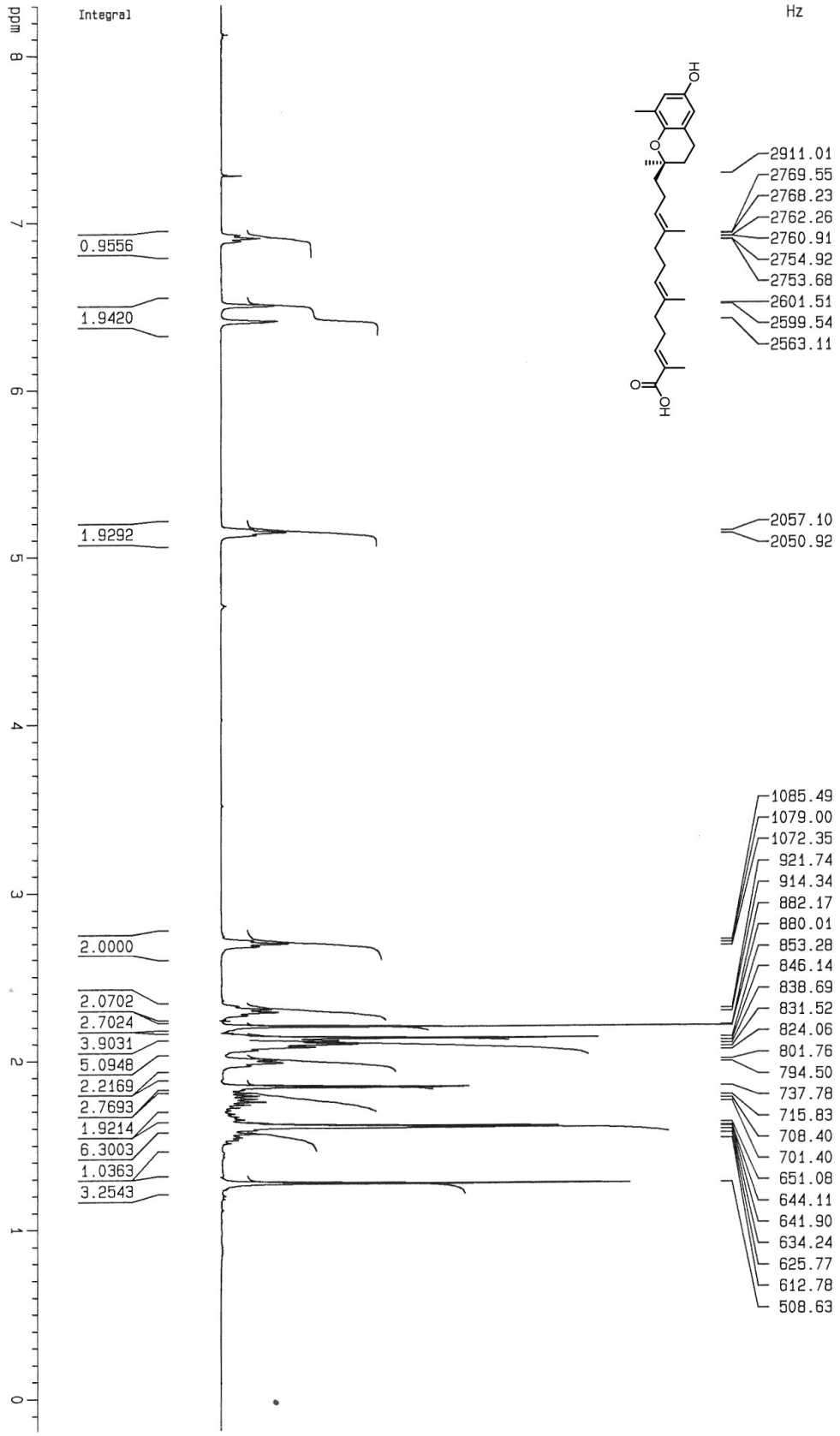
To a solution of **2** (100 mg, 0.2171 mmol) in dry THF (2 mL), $LiAlH_4$ (25 mg, 0.6513 mmol) was added portionwise at 0 °C. The resulting suspension was allowed to reach r.t. and stirred for 3 h. EtOAc (10 mL) was added followed by slow addition of H_2O (10 mL). The aqueous phase was extracted with EtOAc (2 x 10 mL) and the combined organic extracts were washed with H_2O (10 mL), brine (10 mL) and dried over anydrous Na_2SO_4. The crude wasa purified by automated flash chromatography on silica (Eluent: CH_2Cl_2/MeOH from 100:0 to 90:10, v/v) affording the desired derivative **7** (60 mg, 0.1343 mmol, 62% yield) as yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.83-1.83 (34H, m), 2.09 (6H, s), 2.15 (3H, s), 2.59 (2H, t, *J*= 6.72 Hz), 3.44-3.46 (2H, m), 4.10-4.12 (1H, m). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 11.3, 11.8, 12.3, 16.5, 16.6, 19.6, 19.7, 20.7, 21.0, 23.8, 24.4, 31.47, 31.53, 32.6, 32.7, 33.4, 35.7, 37.3, 39.67, 39.73, 68.27, 68.33, 74.5, 117.3, 119.0, 121.5, 122.5, 144.6, 145.4.

(2*S*,3*S*,4*S*,5*R*,6*S*)-6-[((*R*)-2-((3*E*,7*E*,11*E*)-12-carboxylato-4,8-dimethyltrideca-3,7,11-trien-1-yl)-2,8-dimethylchroman-6-yl)oxy]-3,4,5-trihydroxytetrahydro-2*H*-pyran-2-carboxylate, disodium salt (9)

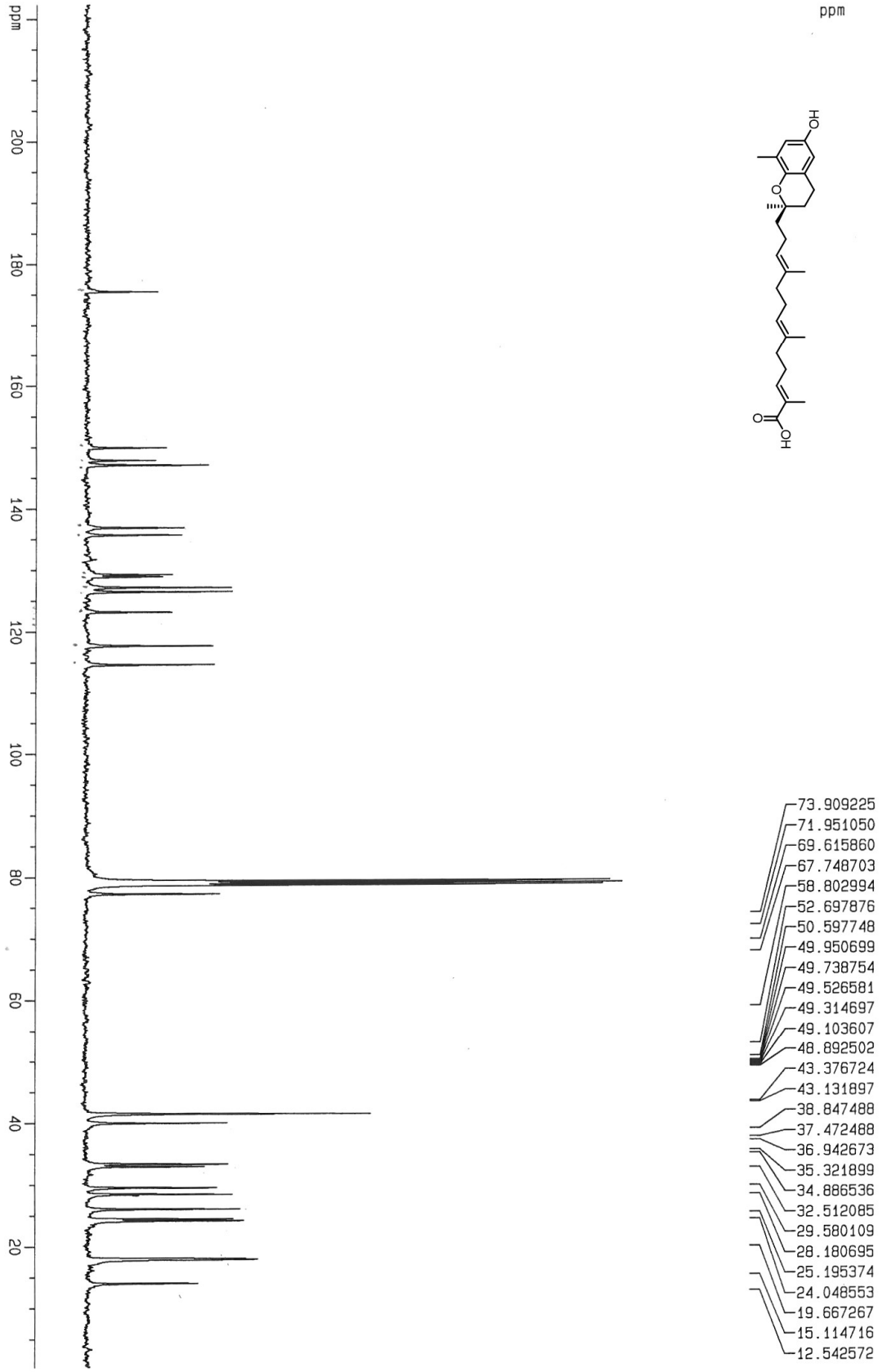


To a solution of garcinoic acid (**1**) (200 mg, 0.4688 mmol) in Et_2O (5 mL), a solution of diazomethane[5] in Et_2O (title: 2.3% w/v, 4 mL) was added dropwise at 0 °C and the resulting suspension was stirred at r.t. for 2 h. The solvent was removed *under vacuo* and the crude residue was dissolved in freshly distilled toluene (8 mL). Methyl 2,3,4-tri-*O*-acetyl-*a*-D-glucopyranosyluronate bromide (**13**, 484 mg, 1.2189 mmol), Fetizon reagent (2.085 g, loading 0.9 mmol g^{-1}) and molecular sieves (4 Å, 1.125 g) were sequentially added and the resulting suspension was stirred under argon atmosphere, under dark and at r.t. for 20 h.[6] The suspension was filtered on a pad of Celite and the filtrate was concentrated under reduced pressure. The crude was dissolved in MeOH (6 mL) and treated with Na_2CO_3 (508 mg, 4.800 mmol) at r.t. for 48 h. The solvent was removed under *vacuo* and the residue was dissolved with H_2O (10 mL) and washed with Et_2O (3 x 15 mL). The aquoeous phase was concentrated under reduced pressure and purified by reverse phase (C18) automated chromatography (Eluent: H_2O/MeOH from 100:0 to 0:100, v/v) affording the desired derivative **9** (50 mg, 0.077 mmol, 17% overall yield over three steps from **1**) as white solid (m.p.: 210 °C, dec.). ^{1}H-NMR (400 MHz, d^6-DMSO): δ 1.09-2.30 (30H, m), 2.59 (2H, m), 3.01-3.17 (5H, m), 3.79 (1H, m), 4.55 (1H, d, *J*= 3.68 Hz), 5.02-5.05 (4H, m), 6.14 (1H, d, *J*= 2.5 Hz), 6.53 (1H, s), 6.60 (1H, s).^{13}C-NMR (100.6 MHz, CD_3OD): δ 12.5, 14.5, 14.8, 15.0, 21.8, 22.1, 23.0, 26.3, 27.1, 31.2, 38.6, 39.3, 39.4, 72.2, 73.4, 75.1, 75.2, 76.4, 102.5, 115.2, 117.7, 120.8, 124.2, 124.3, 126.4, 133.4, 134.4, 134.6, 135.2, 147.3, 150.5, 175.3, 177.2. HRMS (ESI-) m/z $[M-H]^-$ calcd for $C_{33}H_{46}O_{10}$ 601.3013; found: 601.3017; Δ= 0.67 ppm; $[M-2H]^-$ calcd for $C_{33}H_{46}O_{10}$ 300.1468; found: 300.1472; Δ= 1.33 ppm.

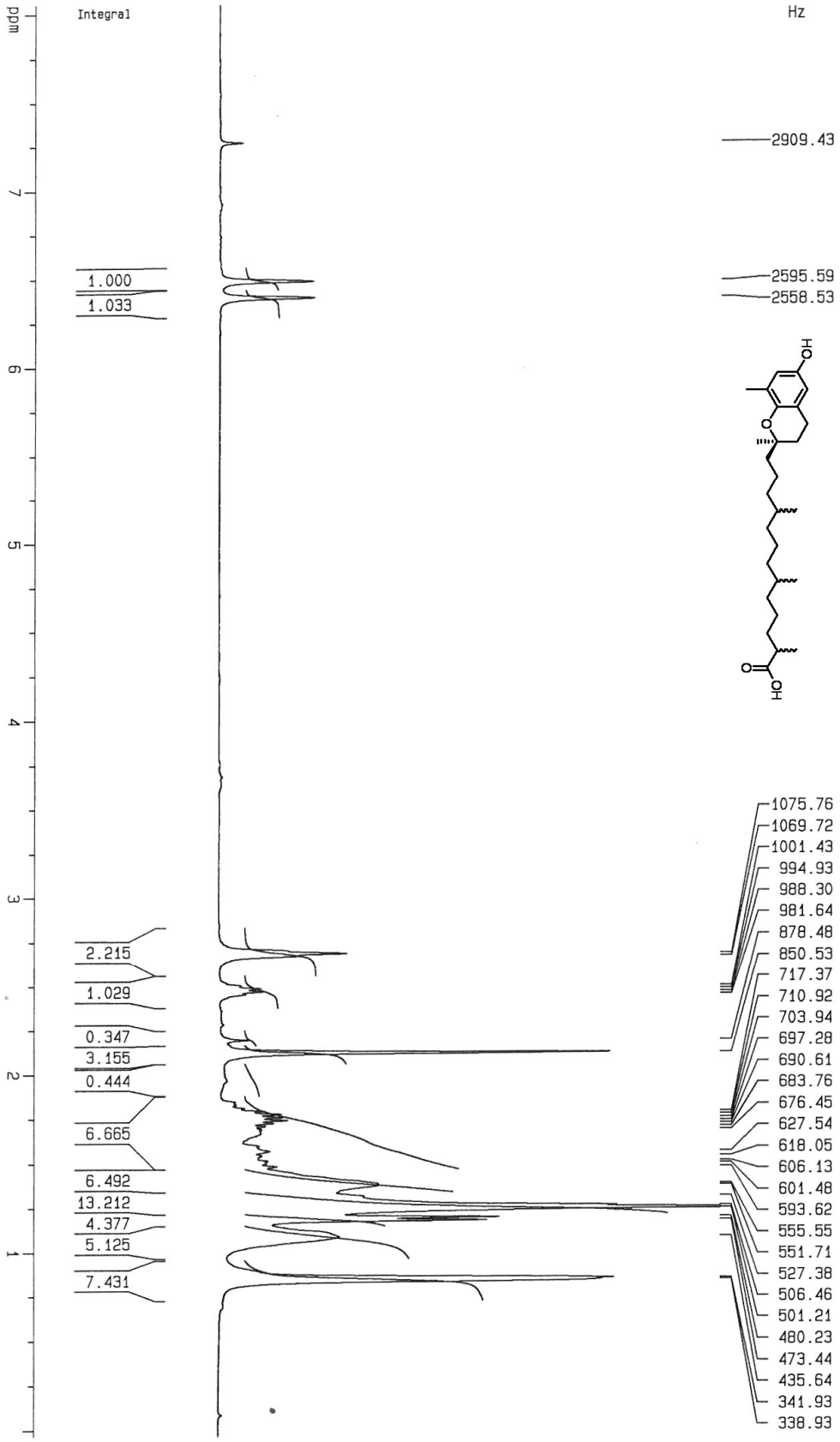
^{1}H-NMR ($CDCl_3$, 400 MHz) of (2*E*,6*E*,10*E*)-13-[(2*R*)-6-hydroxy-2,8-dimethyl-chroman-2-yl]-2,6,10-trimethyltrideca-2,6,10-trienoic acid (garcinoic acid, 1)



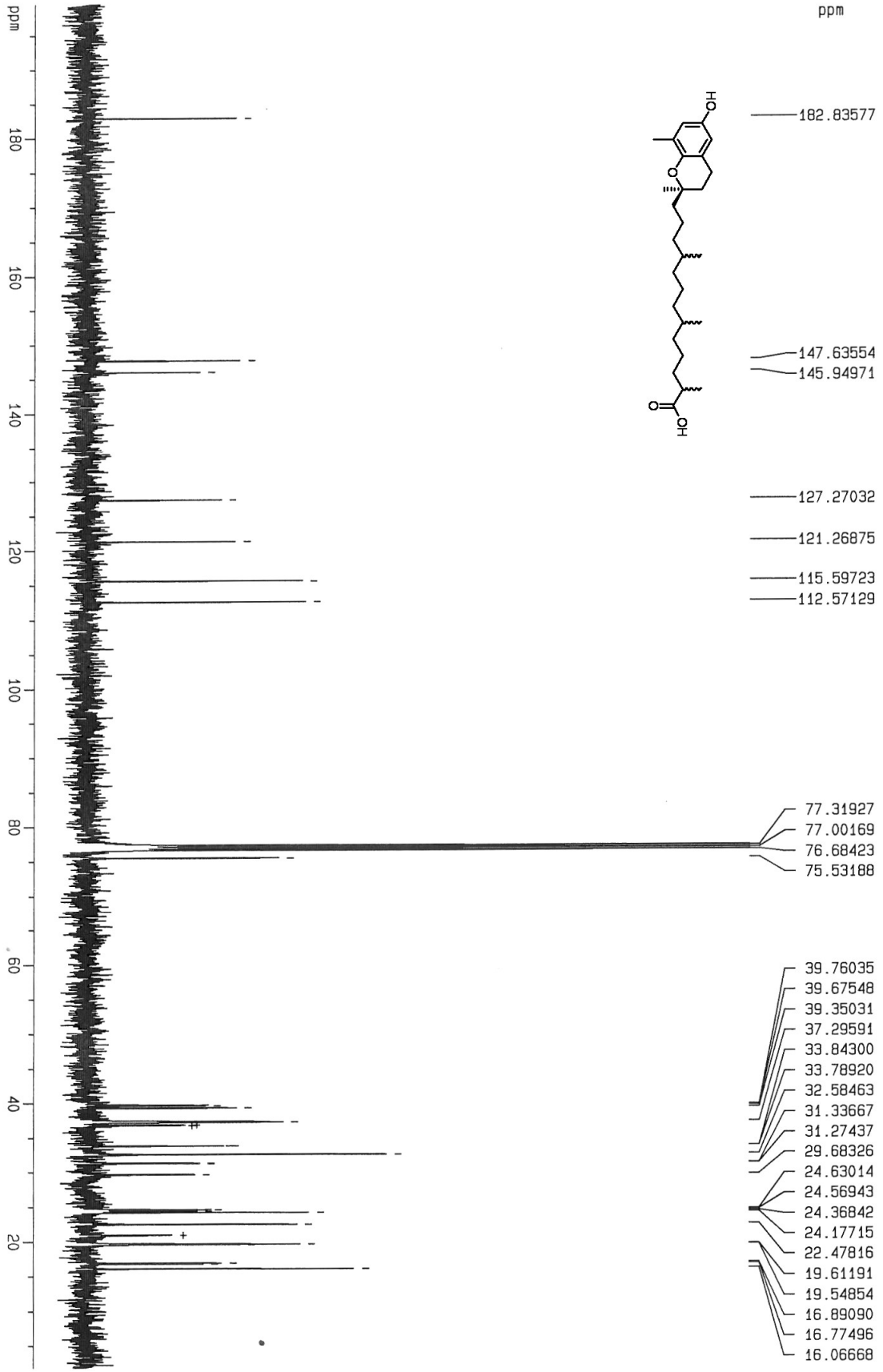
^{13}C-NMR ($CDCl_3$, 100.6 MHz) of (2*E*,6*E*,10*E*)-13-[(2*R*)-6-hydroxy-2,8-dimethyl-chroman-2-yl]-2,6,10-trimethyltrideca-2,6,10-trienoic acid (garcinoic acid, 1)



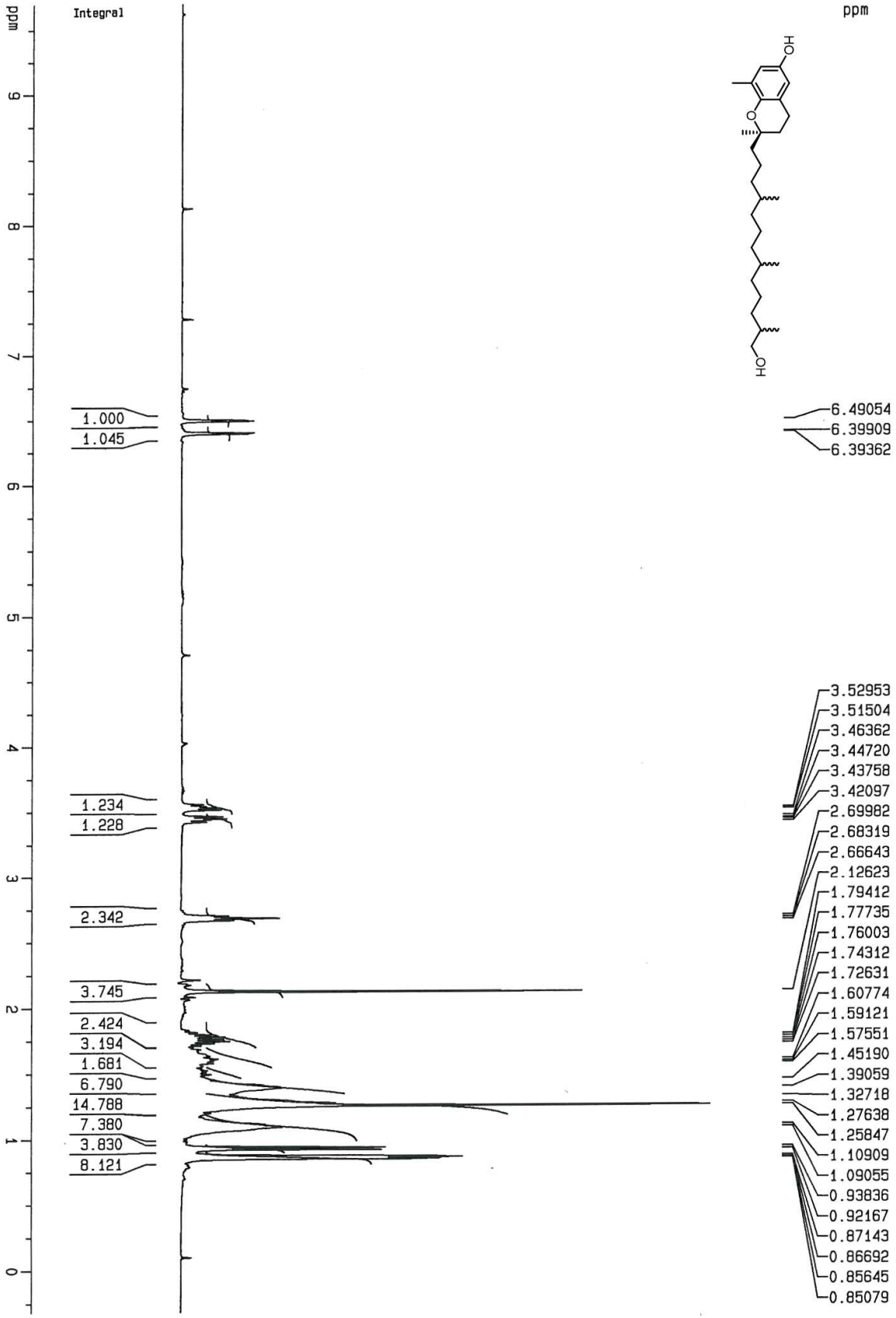
^{1}H-NMR ($CDCl_3$, 400 MHz) of 13-[(*R*)-6-hydroxy-2,8-dimethylchroman-2-yl)-2,6,10-trimethyltridecanoic acid (6)



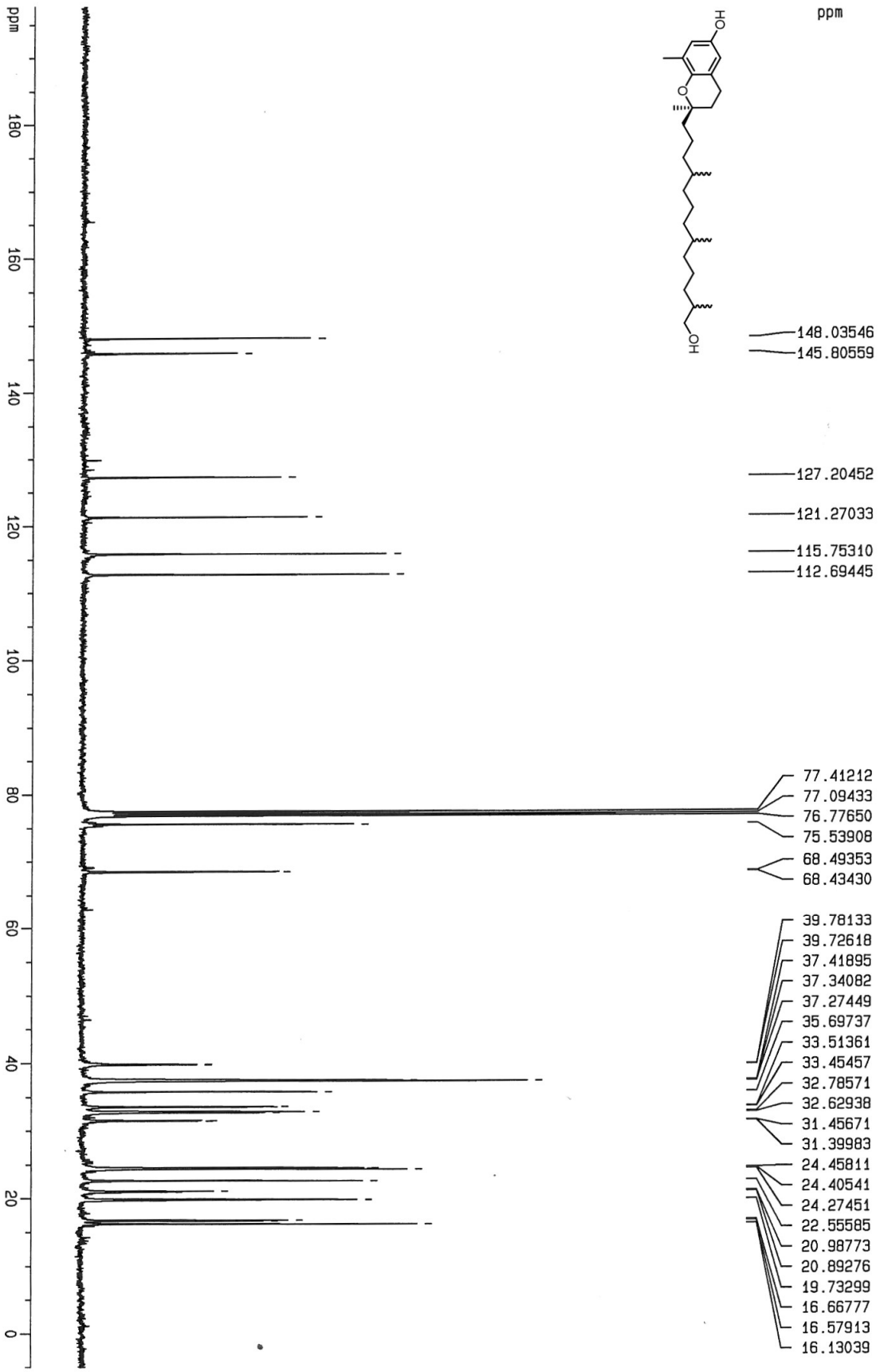
^{13}C-NMR ($CDCl_3$, 100.6 MHz) of 13-[(*R*)-6-hydroxy-2,8-dimethylchroman-2-yl)-2,6,10-trimethyltridecanoic acid (6)



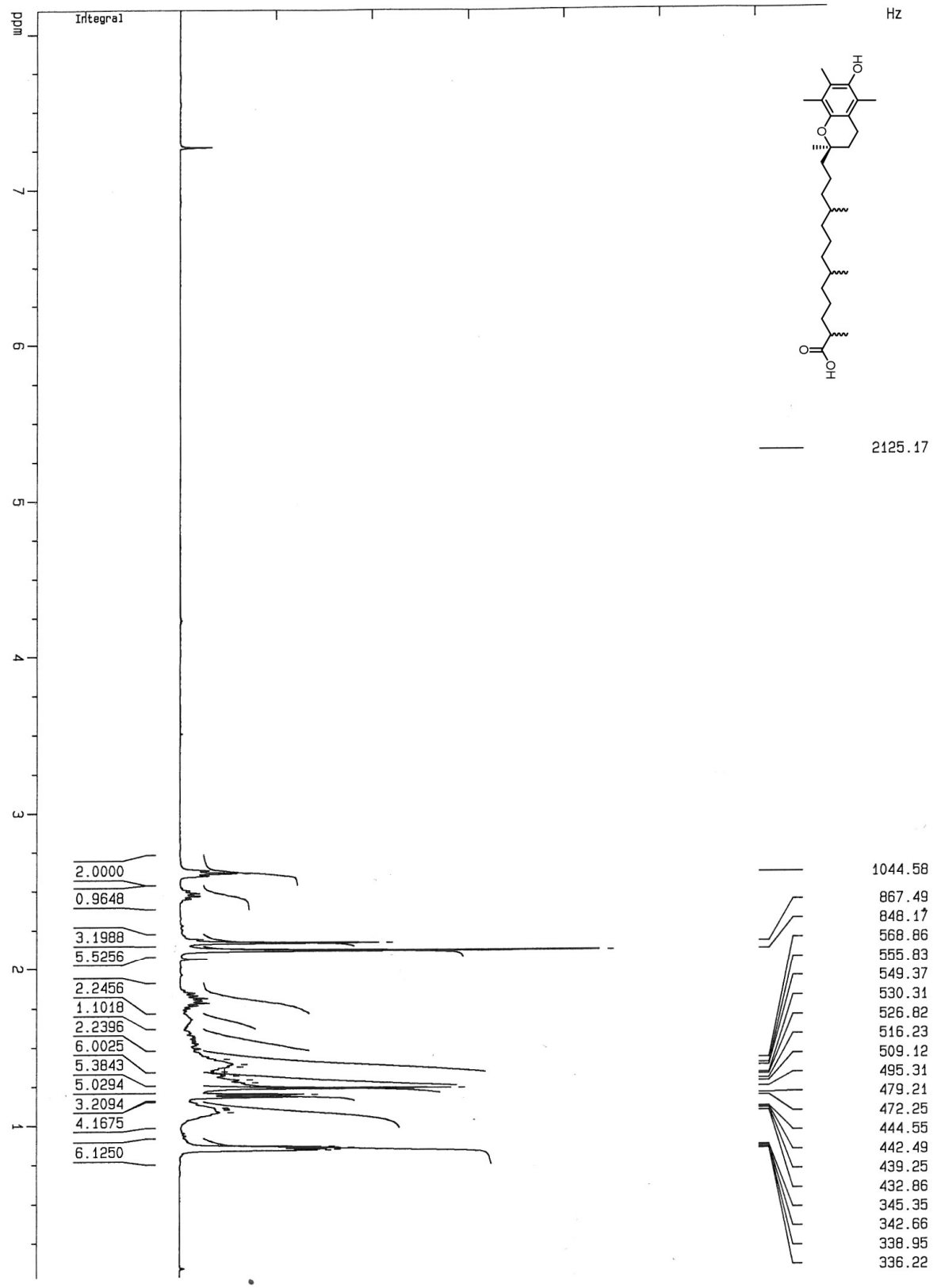
^{1}H-NMR ($CDCl_3$, 400 MHz) of (2*R*)-2-(13-hydroxy-4,8,12-trimethyltridecyl)-2,8-dimethylchroman-6-ol (8)



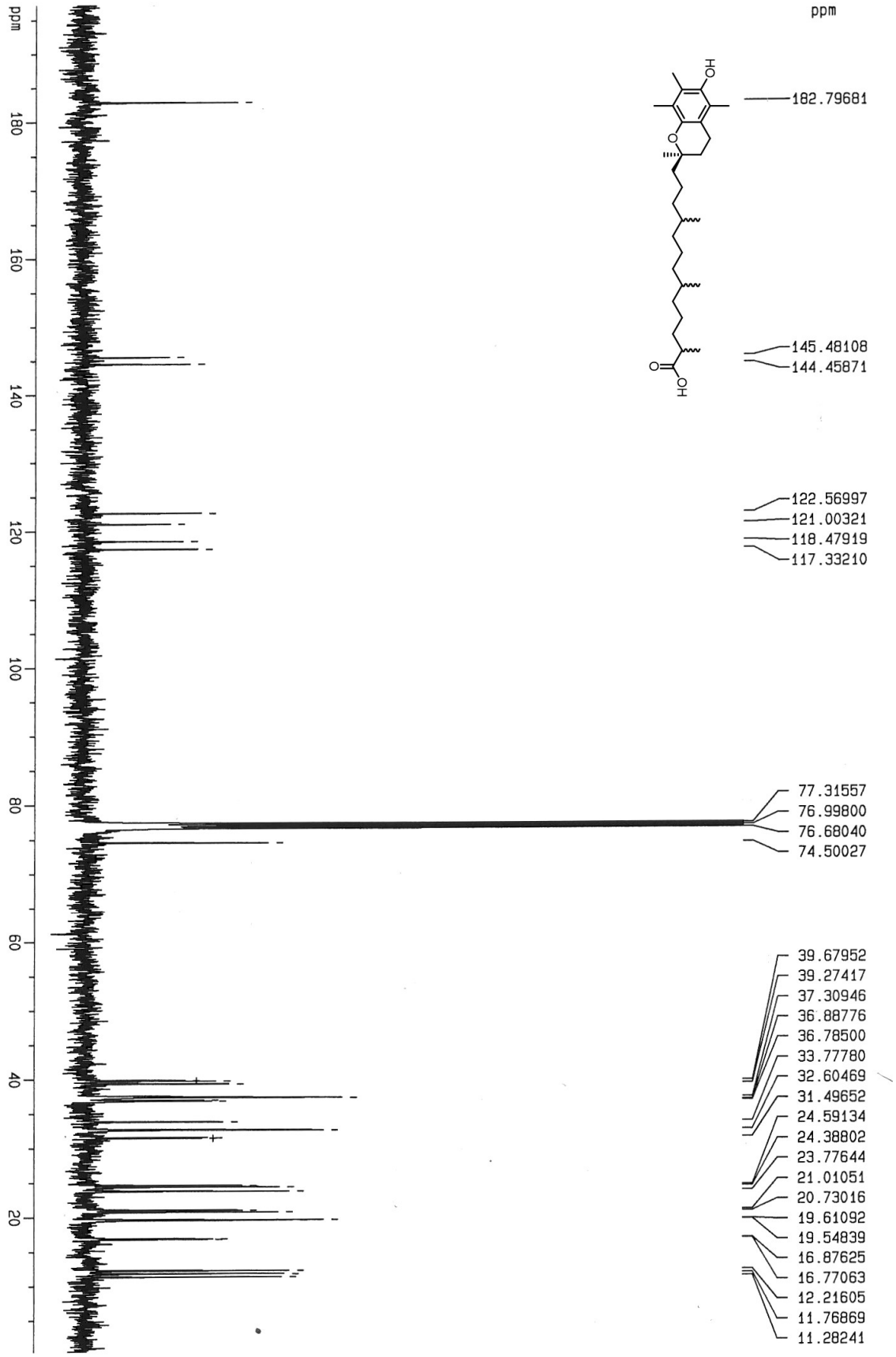
^{13}C-NMR ($CDCl_3$, 100.6 MHz) of (2*R*)-2-(13-hydroxy-4,8,12-trimethyltridecyl)-2,8-dimethylchroman-6-ol (8)



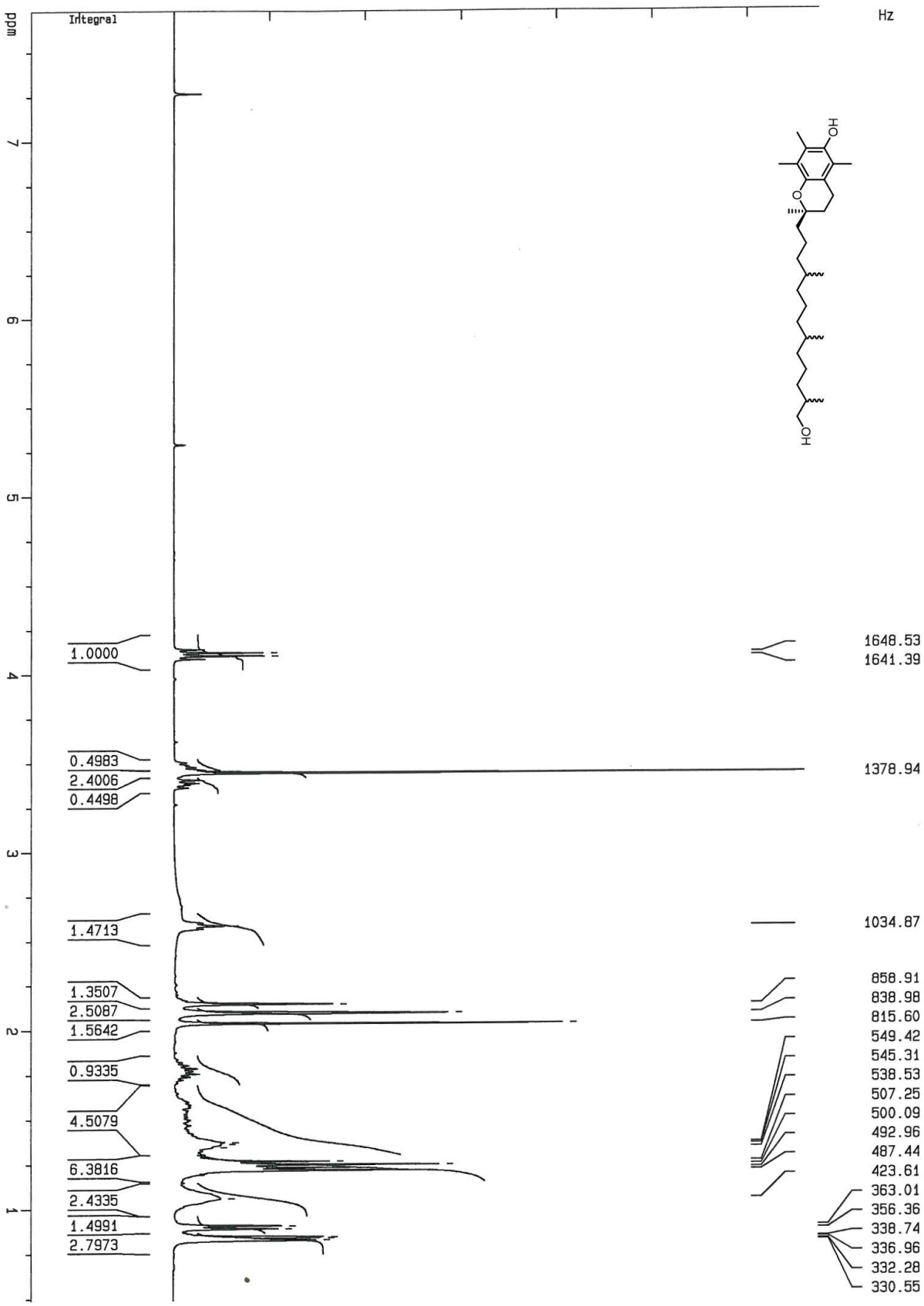
^{1}H-NMR ($CDCl_3$, 400 MHz) of 13-[(*R*)-6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)]-2,6,10-trimethyltridecanoic acid (2)



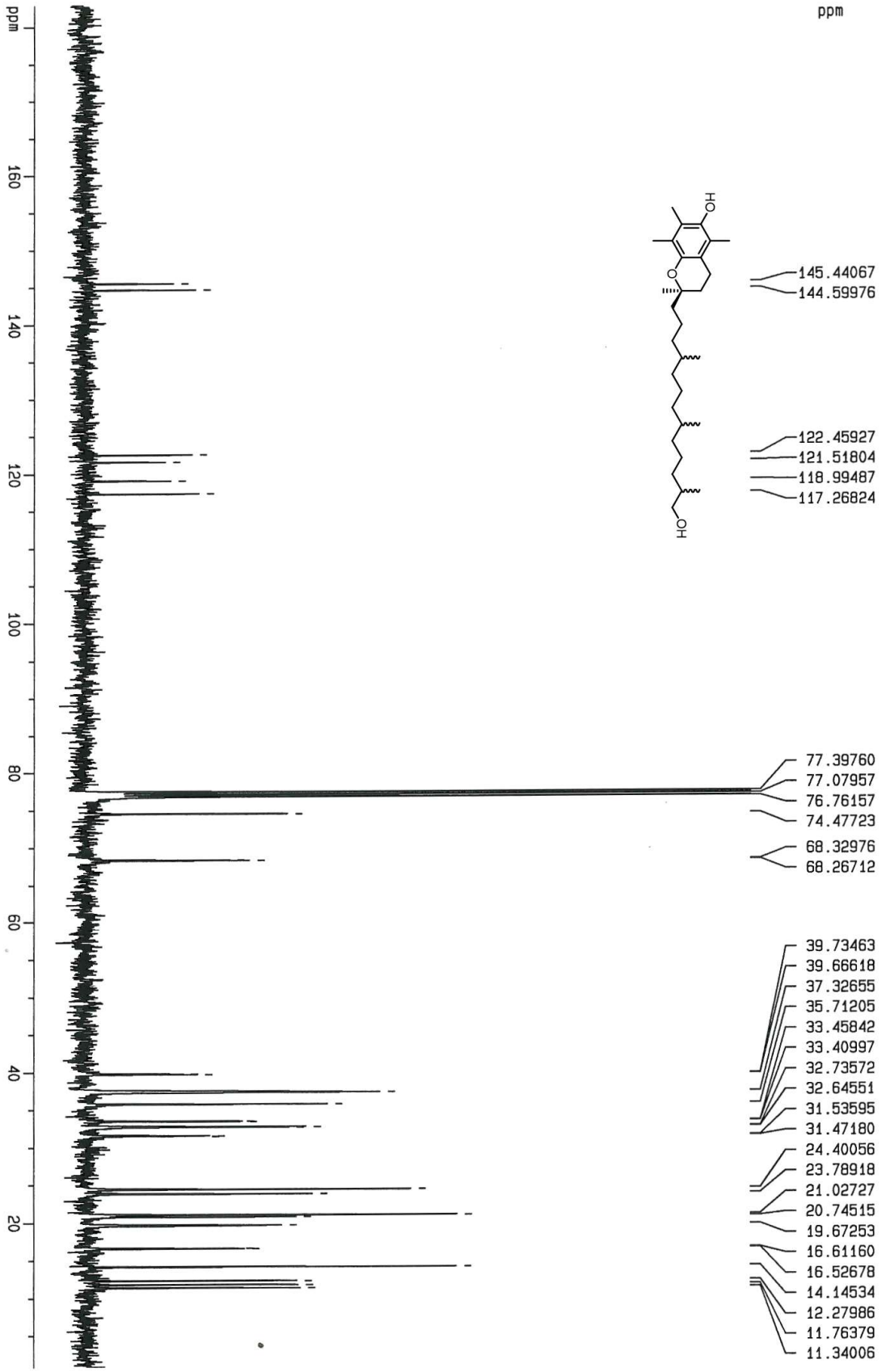
^{13}C-NMR ($CDCl_3$, 100.6 MHz) of 13-[(*R*)-6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)]-2,6,10-trimethyltridecanoic acid (2)



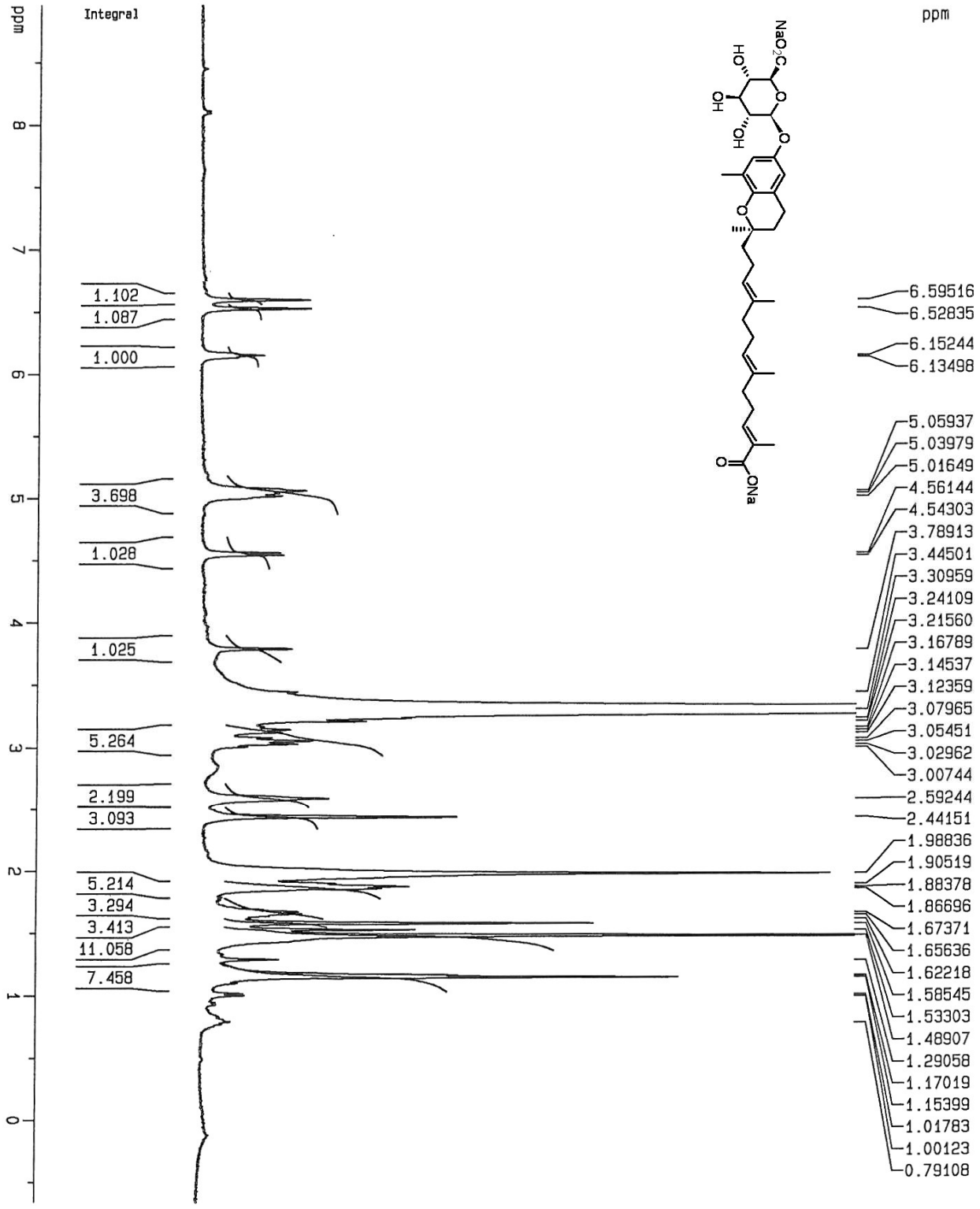
^{1}H-NMR ($CDCl_3$, 400 MHz) of (2*R*)-2-(13-hydroxy-4,8,12-trimethyltridecyl)-2,5,7,8-tetramethylchroman-6-ol (7)



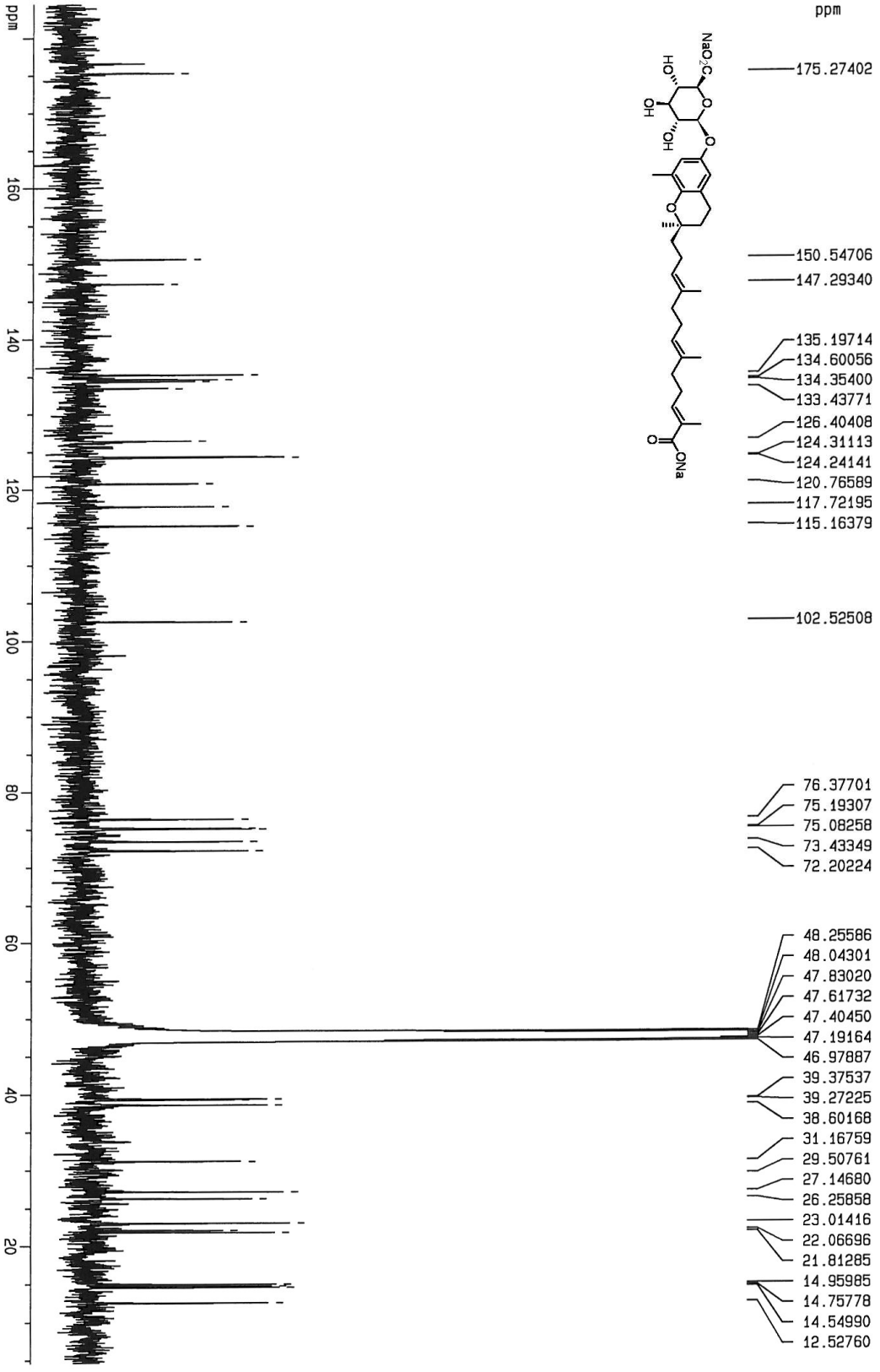
^{13}C-NMR ($CDCl_3$, 100.6 MHz) of (2*R*)-2-(13-hydroxy-4,8,12-trimethyltridecyl)-2,5,7,8-tetramethylchroman-6-ol (7)



^{1}H-NMR (d^6-DMSO, 400 MHz) of (2*S*,3*S*,4*S*,5*R*,6*S*)-6-[((*R*)-2-((3*E*,7*E*,11*E*)-12-carboxylato-4,8-dimethyltrideca-3,7,11-trien-1-yl)-2,8-dimethylchroman-6-yl)oxy]-3,4,5-trihydroxytetrahydro-2*H*-pyran-2-carboxylate, disodium salt (9)

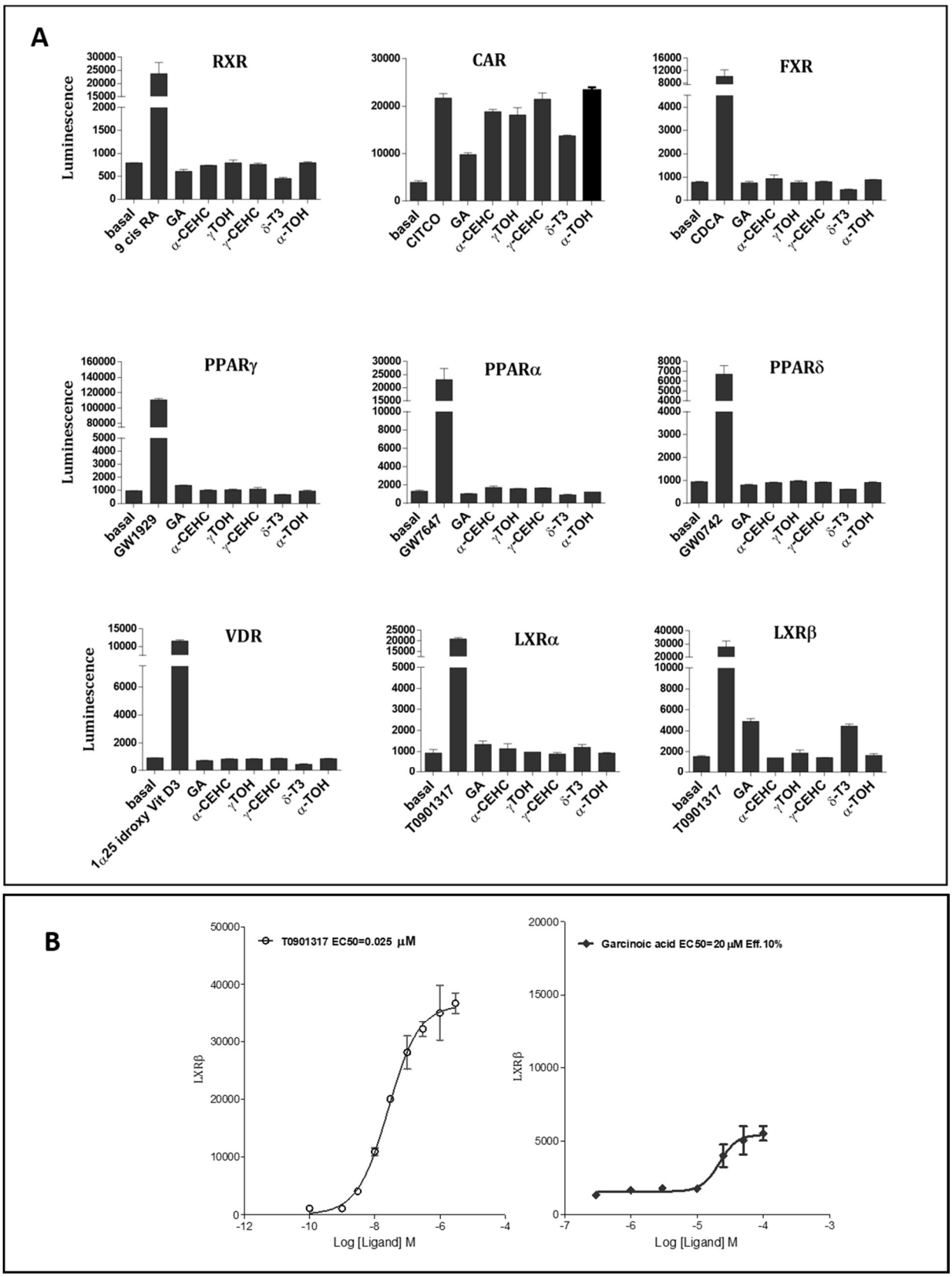


^{13}C-NMR (CD_3OD, 100.6 MHz) of (2*S*,3*S*,4*S*,5*R*,6*S*)-6-[((*R*)-2-((3*E*,7*E*,11*E*)-12-carboxylato-4,8-dimethyltrideca-3,7,11-trien-1-yl)-2,8-dimethylchroman-6-yl)oxy]-3,4,5-trihydroxytetrahydro-2*H*-pyran-2-carboxylate, disodium salt (9)



Additional biological data

Figure S1. Ligand binding activity of garcinoc acid and other vitamin E analogues on a series of nuclear receptors assessed by AlphaScreen test



(A) The test was carried out at 10 μM compound final concentration. Ligand binding to nuclear receptors was expressed as luminescence units. Pharmacological agonists were utilized at the specified concentrations as positive controls for each nuclear receptor. Vehicle was assessed to obtain a measure of background signal (basal evaluation). In the test, acceptor and donor beads support the GST-LBD of the receptor and the biotinylated coactivator, respectively. In presence of the ligand, the nuclear receptor LBD undergoes conformational changes, binds to the coactivator and the chemical energy is transferred from donor to acceptor beads in order to produce a signal, i.e. luminescence; in this case the compounds behaves as agonist, changing the conformation of the receptor to recruit the coactivator. Alternatively, when the signal induced by the agonist is reduced in presence of compounds, which make the receptor impossible to bind to coactivator, the competition can be read as signal reduction (the compound behaves as antagonist, blocking the ability of the receptor to bind the coactivator).

List of positive control used for AlphaScreen assay

Positive control	**Ligand**	**Concentration (μM)**
9-cis-Retinoic acid (9 cis RA)	RXR	0.4
6-(4-chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehydeO-(3,4-dichlorobenzyl)oxime (CITCO)	CAR	0.5
chenodeoxycholic acid (CDCA)	FXR	30
GW1929	PPARγ	0.03
GW7647	PPARα	0.015
GW0742	PPARδ	0.02
1alpha,25-dihydroxyvitamin D3 (1α25idroxy VitD3)	VDR	0.01
T0901317	LXRα	0.4
T0901317	LXRβ	0.05

(B) Dose-dependent activity of GA on LXRβ compared with the pharmacological inhibitor T0901317.

Legend: GA= garcinoic acid (**1**); α-CEHC= α-carboethylhydroxychroman (**10**); γ-TOH= γ-tocopherol (**4**); γ-CECH= γ-carboethylhydroxychroman (**11**); δ-T3= δ-tocotrienol (**5**); α-TOH= α-tocopherol (**3**).

Figure S2. Simple composite omit map contoured at 1 sigma level for the garcinoic acid (gray) ligand bound within the ligand binding pocket of human PXR.

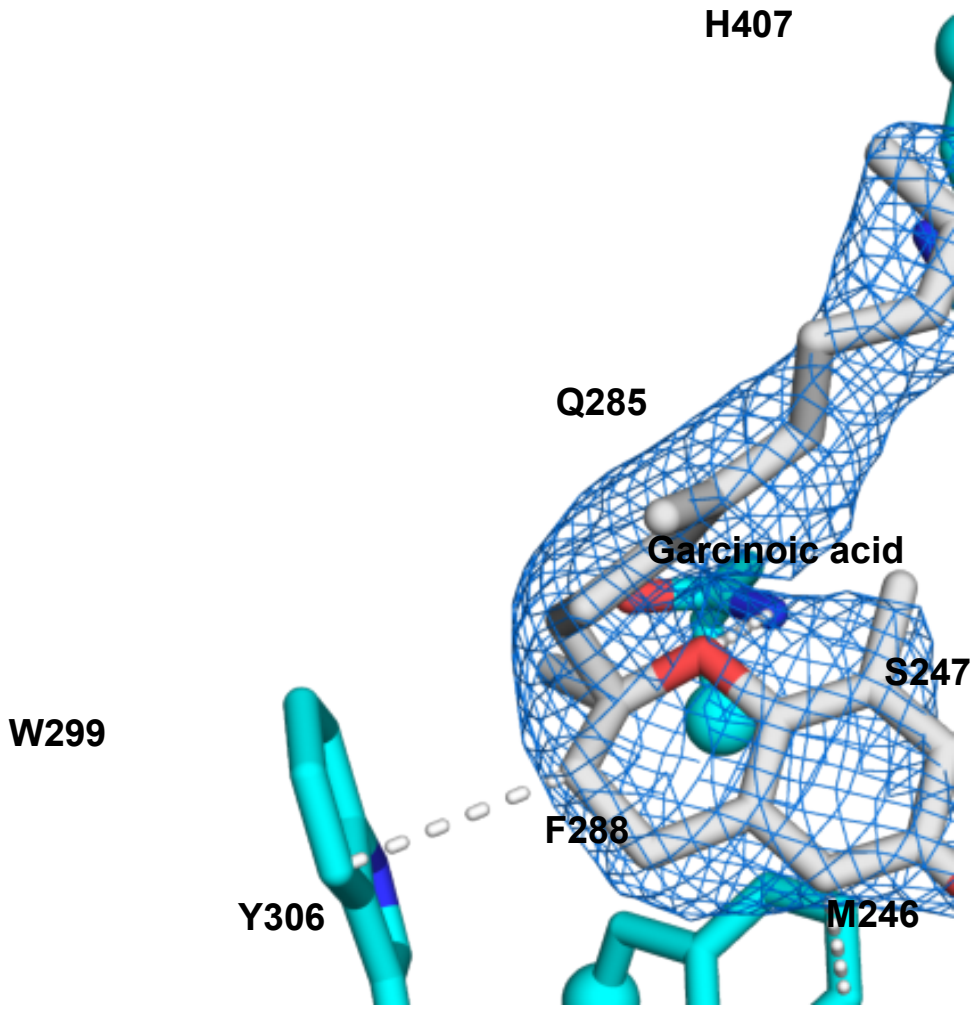
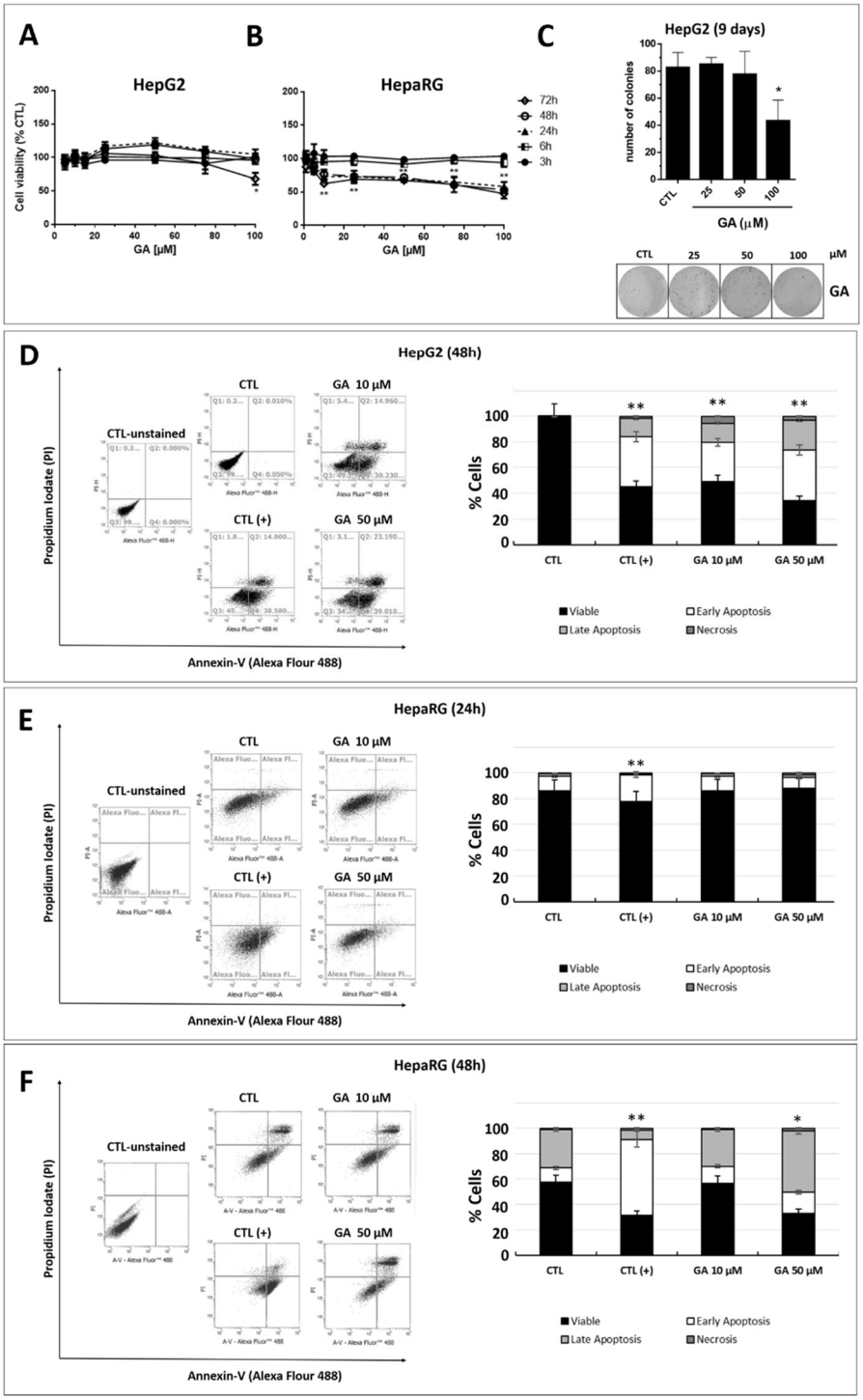


Table S2. Crystallographic data collection and refinement statistics.

Protein	Tethered PXR_LBD_INK_SRC1p
PDB code	6P2B
Resolution range	29.63-2.30 (2.38-2.30)
Space group	$P2_12_12_1$
Unit cell (a, b, c; α, β, γ)	85.0, 89.5, 107.5; 90.0, 90.0, 90.0
Total reflections	188079 (17950)
Unique reflections	36411 (3612)
Multiplicity	5.2 (5.0)
Completeness (%)	98.0 (99.1)
Mean I/sigma(I)	16.8 (3.8)
Wilson B-factor	46.4
R-merge	0.061 (0.512)
CC1/2	0.998 (0.901)
Reflections used in refinement	36409 (3613)
Reflections used for R-free	2000 (193)
R-work	0.186 (0.252)
R-free	0.227 (0.304)
Number of non-hydrogen atoms	4896
macromolecules	4700
ligands	74
solvent	122
Protein residues	569
RMS(bonds)	0.009
RMS(angles)	1.2
Ramachandran outliers (%)	0.72
Rotamer outliers (%)	4.03
Clashscore	9.86
Average B-factor	53.7
macromolecules	53.7
ligands	60.8
solvent	51.1

Statistics for the highest-resolution shell are shown in parentheses.

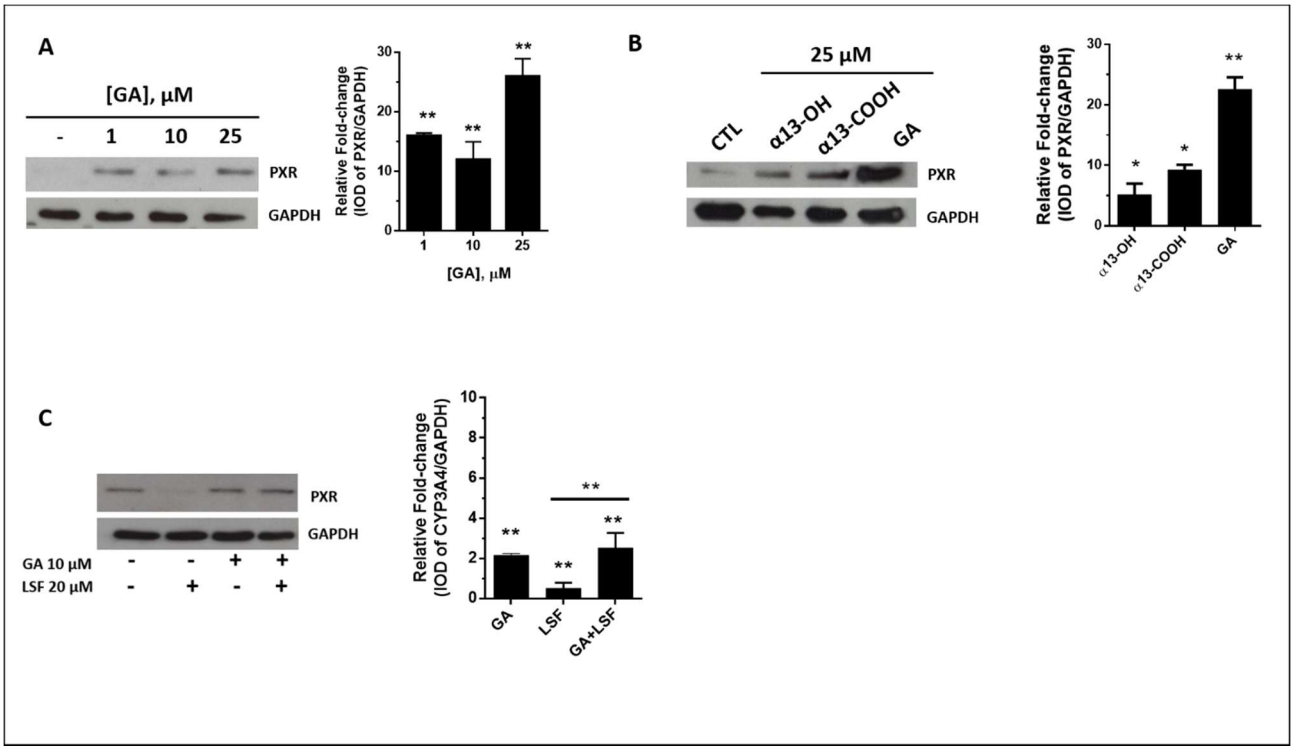
Figure S3. Cell viability, clonogenic activity and apoptotic cell death in human liver cell lines treated with garcinoic acid (GA, 1).



HepG2 and HepaRG cells (2×10^5 cells/well in 96-well microplate) were investigated to determine the concentration- and time-dependent effect of GA on cell viability by MTT test (A and B) was carried out with an in vitro toxicology assay kit (cat. Number M5655/M8910) by Sigma-Aldrich, MO, USA. Results were as

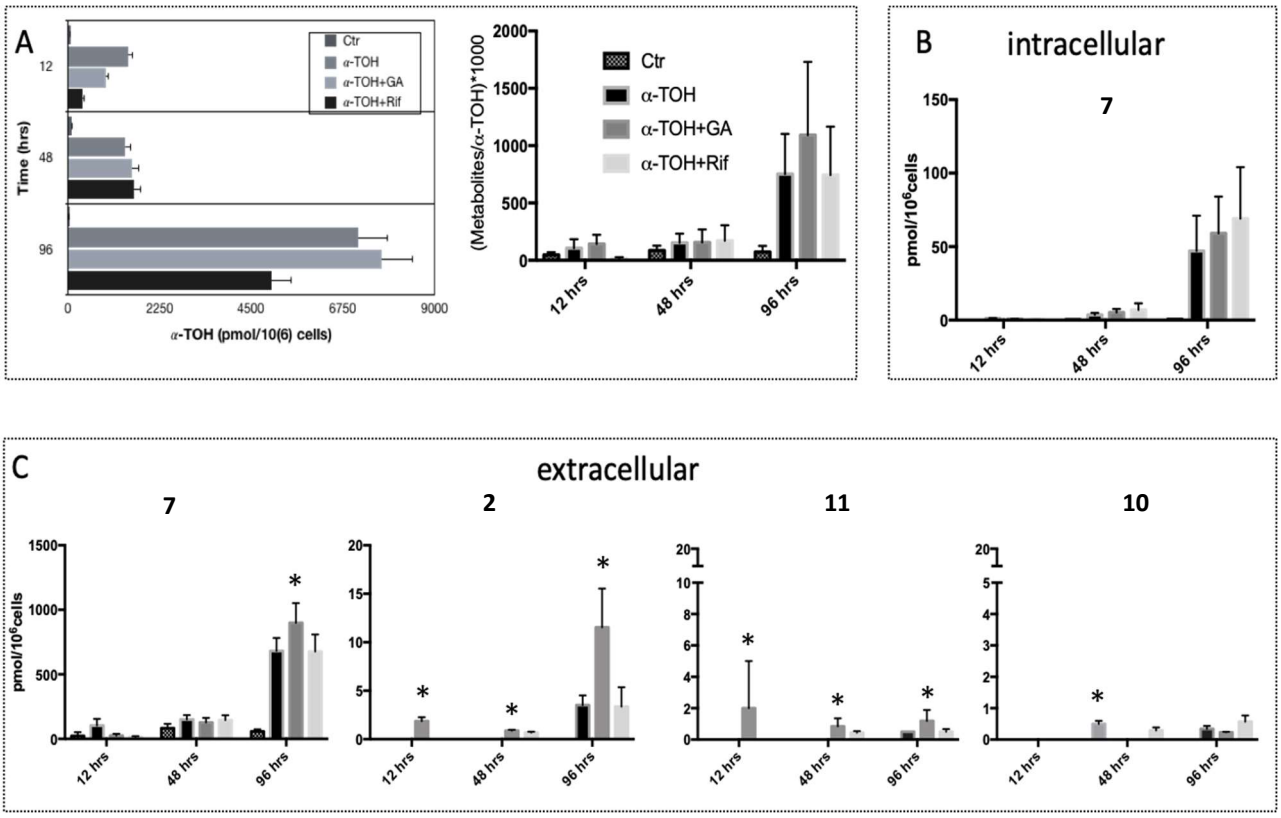
percentage of control cells. Colony forming activity (C) was investigated over 9 days in HepG2 cells plated in complete medium at 1 x10^2 cells/well in 24-well plates. The effect of GA treatment on apoptotic cell death was investigated by flow cytometry after 48h in HepG2 cells (2 × 10^5/well in 6-well plate) (D) and after 24h (E) and 48h (F) in HepaRG cells (3 × 10^5/well in 6-well plate). Flow cytometry was carried out on an Attune NxT Acustic Focusing Cytometer using the Annexin V (Alexa Fluor™ 488 conjugate, A13201) and propidium iodide (PI, Invitrogen™) staining procedure, according with the manufacturer's instructions (Thermo Fisher Scientific); staurosporine (50 µM) was utilized as a positive control for apoptotic cell death [CTL (+)]. Data were as means ± SD of three independent experiments. t-test CTL vs treated * $p<0.05$; ** $p<0.01$.

Figure S4. PXR protein expression in non-tumoral HepaRG cells treated with garcinoic acid (GA, 1).



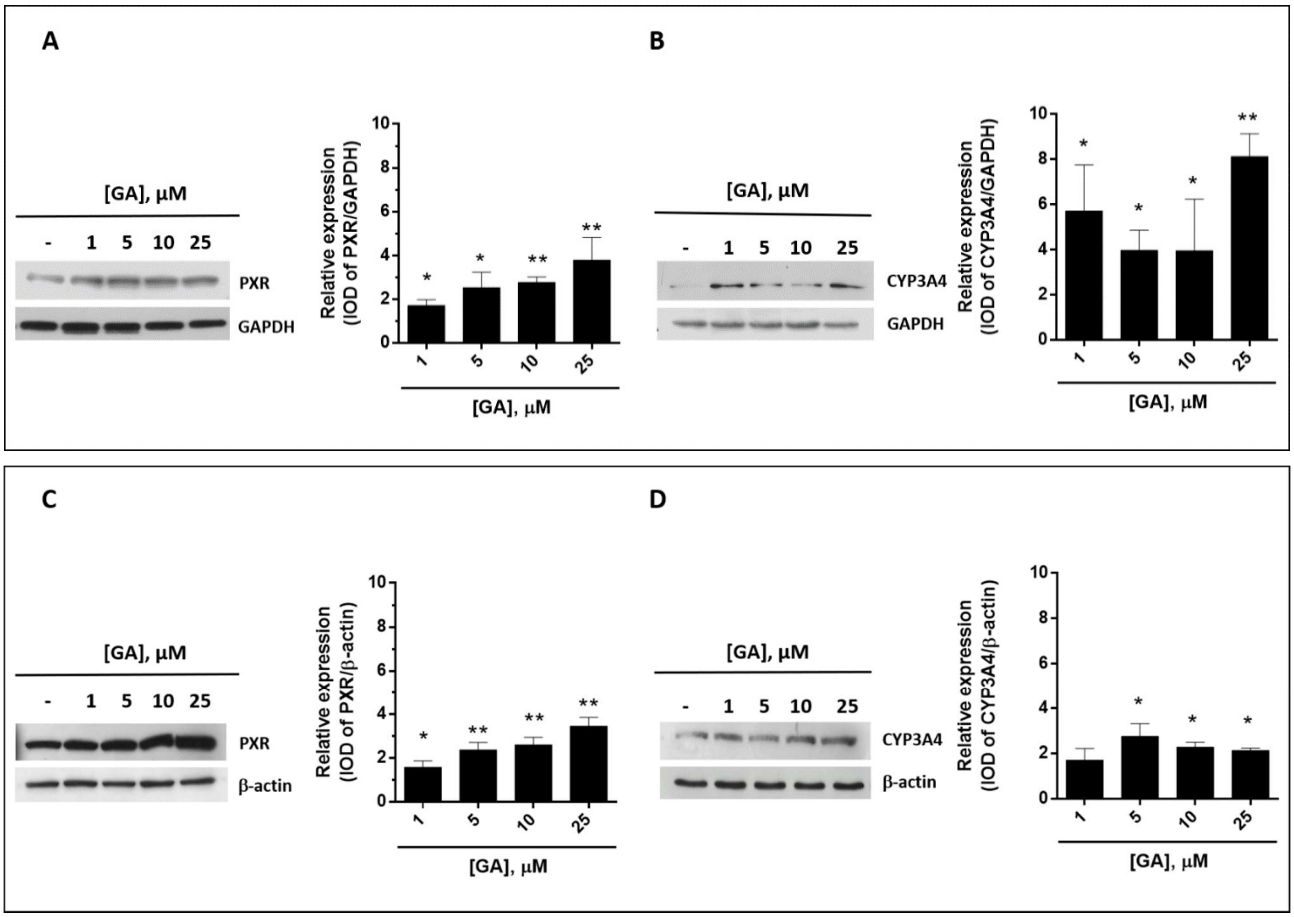
PXR protein was assessed by immunoblot (10 µg total protein loading) in HepaRG cells treated for 24 hrs with GA at concentrations between 1 and 25 µM (A). The effect of GA on PXR expression was compared with α-13-OH (**7**) and α-13-COOH (**2**) (25 µM final concentration) and immunoblot experiments were carried out on 20 µg of total proteins (B). GA agonist activity was investigated in cells pre-treatment for 24 hrs with the PXR antagonist LSF (C). The self-recruitment effect on PXR protein expression was investigated after 24 hrs of treatment with GA and was assessed on 10 µg of total proteins. t-test: control vs treatment, *$p<0.05$; **$p<0.01$. Legend: GA= garcinoic acid (**1**); α13-OH= (**7**); α13-COOH= (**2**).

Figure S5. Cellular uptake and transformation of α-tocopherol (α-TOH, 4) in HepG2 cells supplemented with RRR-α-tocopherol and co-treated with garcinoic acid (GA, 1) or Rifampicin.



HepG2 cells were treated with 100 μM RRR-α-tocopherol in the presence of 25 μM GA or 10 μM Rifampicin (Rif), and **3** (A, left panel), total metabolites (A, right panel), and **7** (B) were measures in total cell extract at the indicated time-points. Extracellular concentration of **2**, **7**, **10** and **11** were also measured by GC-(EI) MS analysis. t-test: α-TOH *vs* α-TOH+GA; *p<0.01. Legend: GA= garcinoic acid (**1**); α-TOH= α-tocopherol (**3**).

Figure S6. PXR (A, C) and CY3A4 (B, D) protein expression in human colorectal HT29 (A,B) and mucous-secreting HT29-MTX cells (C, D) treated with GA.



PXR and CYP3A4 protein was assessed by immunoblot (20 µg total protein loading) in HT29 cells (from ATCC) were cultured in RPMI (Invitrogen; Life Technologies) containing 10% FBS (*v*/*v*) and 1% (*v*/*v*) L-glutamine. Cultures were incubated at 37 C in a humidified 5% (*v*/*v*) CO_2 atmosphere and used between passages 10 to 20 and HT29-MTX cells (from Sigma-Aldrich) were cultured in DMEM supplemented with 2mM Glutamine, 1% Non essential amino acids and 10% FBS. Cells were treated for 24 hrs with GA at concentrations between 1 and 25 µM. t-test: control vs treatment, *$p<0.05$; **$p<0.01$.

References and notes

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5. Etheral solution of diazomethane was freshly prepared from Diazald® according to Moore, J. A.; Reed, D. E. *Organic Syntheses* **1973**, *5*, 351–354 by using a commercially available distillation equipment (https://wolfweb.unr.edu/homepage/wchalifoux/PDFs/diazomethane.pdf (accessed Feb 20, 2020).
6. Methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate bromide (**10**) and Fetizon reagent were prepared as previously reported (Mostarda, S.; Passeri, D.; Carotti, A.; Cerra, B.; Colliva, C.; Benicchi, T.; Macchiarulo, A.; Pellicciari, R.; Gioiello, A. Synthesis, physicochemical properties, and biological activity of bile acids 3-glucuronides: Novel insights into bile acid signalling and detoxification. *Eur. J. Med. Chem.* **2018**, *144*, 349–358).