Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Adamantane substituted aminocyclitols as pharmacological chaperones for Gaucher disease

Ana Trapero,[a] *Meritxell Egido-Gabás*[b] *and Amadeu Llebaria**[a]

[a]*Laboratory of Medicinal Chemistry, Department of Biomedicinal Chemistry, Institut de Química Avançada de Catalunya (IQAC–CSIC), Jordi Girona 18-26, 08034 Barcelona, Spain.*

[b]*Department of Biomedicinal Chemistry, Institut de Química Avançada de Catalunya (IQAC–CSIC), Jordi Girona 18-26, 08034 Barcelona, Spain.*

*Corresponding author: Fax: int +34-932045904. E-mail: amadeu.llebaria@cid.csic.es

Table of Contents

Chemistry: general methods

Reagents and solvents were purchased from commercial suppliers and were used without further purification.

The NMR experiments were carried out either on a Varian INOVA 500 spectrometer (500 MHz for ^{1}H) or a Varian MERCURY 400 spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C). Chemical shifts (δ) are reported in ppm relative to the singlet at 7.26 ppm of $CDCl_3$ for ^{1}H and in ppm relative to the center line of a triplet at 77.16 ppm of $CDCl_3$ for ^{13}C. IR spectra were measured as films and were recorded with a Thermo Nicolet Avatar 360 FT-IR Spectometer. High Resolution Mass Spectra (HRMS) were carried out at the IQAC Mass Spectroscopy Facility, using a UPLC-TOF equipment (Waters Corporation, Milford, MA). The purity of the compound was determined by HPLC on an Alliance 2695 system using a Kinetex C18 (4.6 mm x 50 mm, 2.6 μm) column under the following chromatography conditions: mobile phase A, H_2O with 0.2% HCO_2H; mobile phase B, CH_3CN with 0.2% HCO_2H; flow rate, 1.0 mL min^{-1}; injection volume, 25 μL; elution gradient, 0.0−2.9 min, 5−90% B; 2.9−3.4 min, 90% B; 3.4−4.6 min, 90−100% B; 4.6−6.0 min, 100−5% B; 6.0−10.0 min, 5% B. An evaporative light scattering detector (ELSD, model PL-ELS 1000, Polymer Laboratories) was used with the following parameters: a gas flow rate of 1.5 L min−1, a nebulizer temperature of 80 °C, and an evaporator temperature of 90 °C.

Synthesis and Compound Characterization

The following compounds were synthesized according to literature procedures:

N-Adamantanyl-4-hydroxybutanamide **3**,[1]

N-Adamantanyl-4-oxobutanamide **4**,[1]

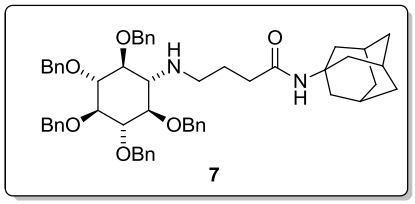
(1*s*,2*R*,3*S*,4*r*,5*R*,6*S*)-2,3,4,5,6-pentakis(benzyloxy)cyclohexanamine **5**,[2]

(1*S*,2*R*,3*S*,4*R*,5*R*,6*S*)-2-amino-3,4,5,6-tetrakis(benzyloxy)ciclohexanol **6**.[3]

General Procedure for Reductive Amination of aldehyde 4 with amines 5 and 6: Synthesis of 7 and 8.

A solution of the starting amine (0.12 mmol) in MeOH (5 mL) under an atmosphere of argon was treated successively with $NaBH_3CN$ (0.24 mmol), AcOH (7 μL) and aldehyde **4** (0.12 mmol). After stirring for 5 h at rt, the mixture was quenched by addition of few props of water and the solvents were removed under reduced pressure. The resulting residue was dissolved in EtOAc (20 ml) and washed with water (15 mL). The aqueous phase was extracted with EtOAc (2 x 20 mL). The combined organic layers were washed with brine and dried over MgSO4. Filtration and evaporation afforded crude compounds, which were purified as indicated below.

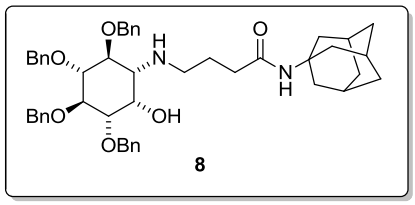
_N_-(Adamantanyl)-4-(((1*s*,2*R*,3*S*,4*r*,5*R*,6*S*)-2,3,4,5,6-pentakis(benzyloxy)cyclohexyl)amino)butanamide (7)



7

Obtained in 88% yield (84 mg, 0.10 mmol) from 75 mg (0.12 mmol) of amine **5**. The compound was purified by flash chromatography (2:1 to 1:2 hexane/EtOAc gradient). IR (film): υ = 3082, 3056, 3030, 2907, 2851, 1647, 1497, 1359, 1212, 1138, 1060, 1027, 734, 697 cm^{-1}; ^{1}H NMR (δ, 500 MHz, $CDCl_3$): 1.66-1.71 (m, 8H), 1.95-2.08 (m, 11H), 2.70 (t, 1H, *J* =9.7 Hz), 2.90 (m, 2H), 3.57-3.64 (m, 5H), 4.82-5.03 (m, 10H), 5.28 (br, 1H), 7.28-7.38 (m, 25H); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 29.4, 35.2, 36.4, 41.5, 49.7, 52.1, 62.1, 75.4, 75.8, 76.0, 82.9, 84.3, 127.8-128.7, 138.1, 138.2, 138.2, 172.0. ESIMS: *m*/*z* calcd for $C_{55}H_{65}N_2O_6$: 849.4843 $[M+H]^+$. Found: 849.4868.

_N_-(Adamantanyl)-4-(((1*R*,2*S*,3*R*,4*R*,5*S*,6*S*)-2,3,4,5-tetrakis(benzyloxy)-6-hydroxycyclohexyl)amino)butanamide (8)



8

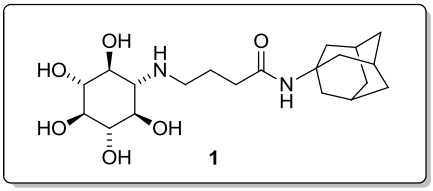
Obtained in 71% yield (70 mg, 0.07 mmol) from 50 mg (0.09 mmol) of amine **6**. The compound was purified on a Biotage *flash* chromatography (KP-Sil column, CH_2Cl_2:MeOH 15:0.6). $[\alpha]_D^{25}$ – 21.4 (*c* 1.0, $CHCl_3$); IR (film): υ = 3081, 3057, 3033, 2907, 2850, 1646, 1499, 1359, 1210, 1137, 1061, 1027, 735, 697 cm^{-1}; ^{1}H NMR (δ, 400 MHz, $CDCl_3$): 1.66-1.71 (m, 8H), 1.95-2.08 (m, 11H), 2.63 (dd, 1H, *J* =10.2, 2.2 Hz), 2.69-2.77 (m, 1H), 2.87-2.96 (m, 1H), 3.45 (dd, 1H, *J* = 9.6, 2.7 Hz), 3.52 (t, 1H, *J* = 9.3 Hz), 3.81 (t, 1H, *J* = 9.7 Hz), 3.97 (t, 1H, *J* = 9.5 Hz), 4.27 (t, 1H, *J* = 2.5 Hz), 4.68-4.99 (m, 8H), 5.36 (br, 1H), 7.21-7.37 (m, 20H); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 29.5, 35.4, 36.4, 41.6, 47.1, 52.4, 60.7, 65.0, 72.7, 75.5, 75.8, 76.0, 78.9, 80.7, 81.4, 84.5, 127.7-128.7, 138.1, 138.37, 138.41, 138.7, 172.5. ESIMS: *m*/*z* calcd for $C_{48}H_{59}N_2O_6$: 759.4373 $[M+H]^+$. Found: 759.4366.

Synthesis of aminocyclitols 1 and 2 by hydrogenolysis using Pd/C catalyst

General method: In a glass pressure flask, the benzylated amine **7** or **8** (0.1 mmol) was dissolved in a mixture of THF (3 mL) and concentrated HCl (4 drops). Pd/C (70 mg, 5–15% Pd on activated C, water-

wet) was then added. The flask was repeatedly filled and evacuated with hydrogen and vigorously stirred at room temperature for 24 h under H_2 (2 atm). After this period, the reaction mixture was filtered through a plug of Celite to separate the catalyst, and the filter was washed three times with MeOH. The filtrate and combined washings were concentrated to give the desired products.

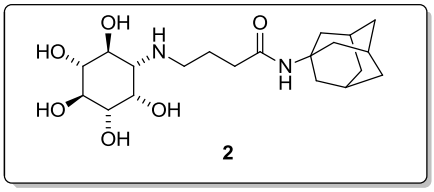
_N_-(Adamantanyl)-4-(((1*S*,2*R*,3*S*,4*R*,5*R*,6*S*)-2,3,4,5,6-pentahydroxycyclohexyl)amino)butanamide hydrochloride (1)



1

Obtained in 99% yield (42 mg, 0.10 mmol) as a white solid from 82 mg (0.10 mmol) of **7**. ^{1}H NMR (δ, 500 MHz, CD_3OD): 1.68-1.74 (m, 6H), 1.92-1.96 (m, 2H), 2.03-2.05 (m, 9H), 2.35 (t, 2H, *J* =6.6 Hz), 2.97 (t, 1H, *J* =10.8 Hz), 3.17-3.23 (m, 3H), 3.28 (t, 2H, *J* =9.2 Hz), 3.47-3.51 (m, 2H); ^{13}C NMR (δ, 100 MHz, CD_3OD): 23.3, 30.8, 35.0, 37.4, 42.2, 46.3, 53.1, 63.1, 70.2, 75.1, 76.6, 174.1. ESIMS: *m*/*z* calcd for $C_{20}H_{35}N_2O_6$: 399.2495 $[M+H]^+$. Found: 399.2503.

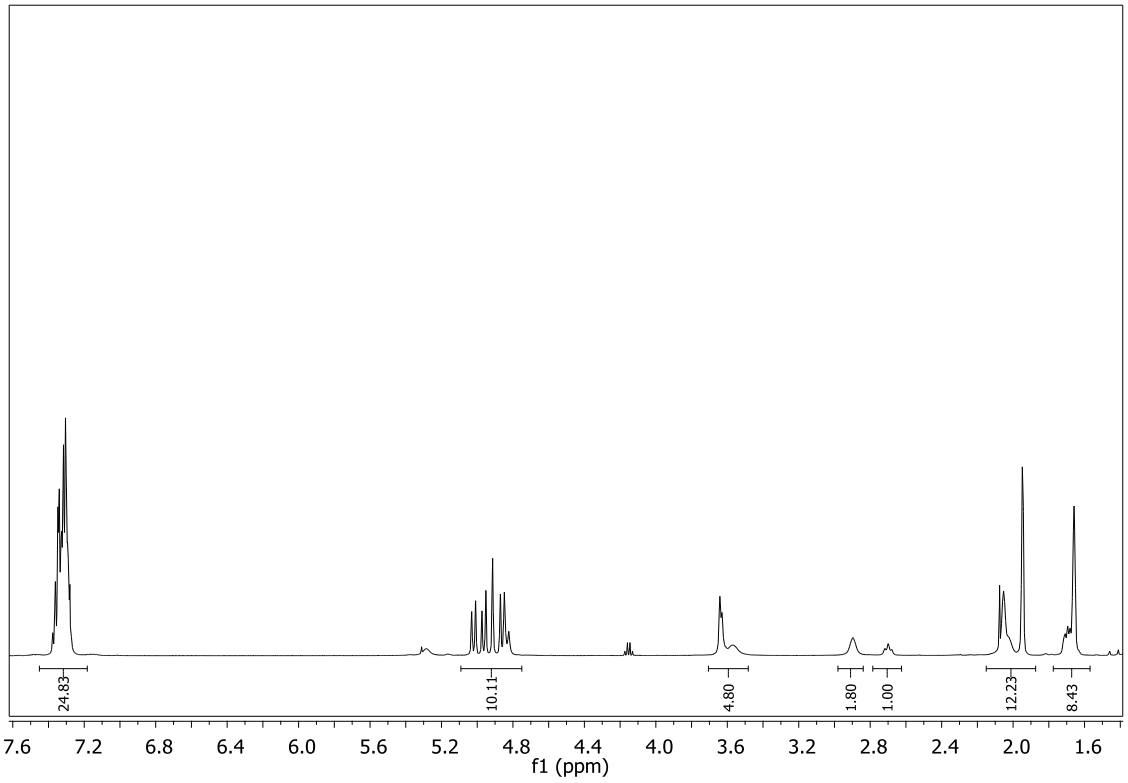
_N_-(Adamantanyl)-4-(((1*S*,2*S*,3*R*,4*S*,5*S*,6*S*)-2,3,4,5,6-pentahydroxycyclohexyl)amino)butanamide hydrochloride (2)



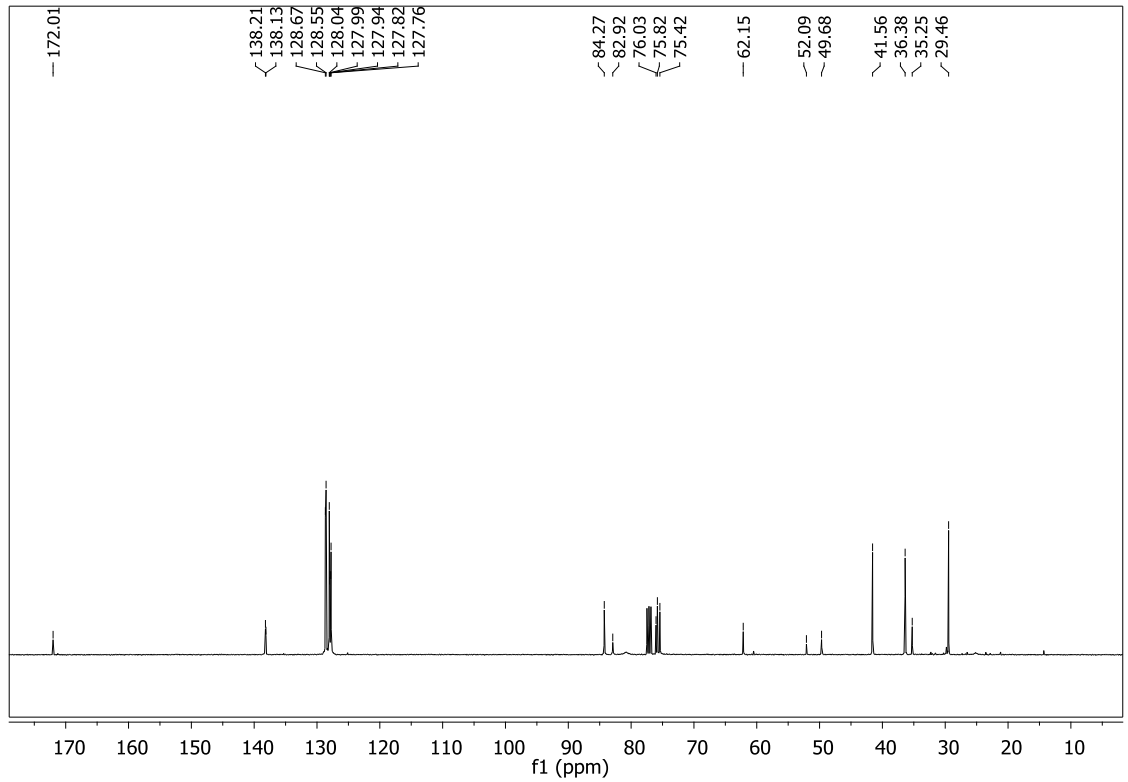
2

Obtained in 65% yield (15 mg, 0.03 mmol) as a white solid from 40 mg (0.05 mmol) of **8**. The compound was purified on a KP-C18-HS 12 g SNAP column using a Biotage Isolera One (H_2O/CH_3CN gradient). $[\alpha]_D^{25}$ – 18 (*c* 1.0, MeOH); ^{1}H NMR (δ, 400 MHz, CD_3OD): 1.68-1.74 (m, 6H), 1.92-1.96 (m, 2H), 2.03-2.05 (m, 9H), 2.34 (td, 2H, *J* =7.0, 2.2 Hz), 3.06-3.16 (m, 3H), 3.21 (t, 1H, *J* =9.3 Hz), 3.40 (dd, 1H, *J* =9.8, 2.8 Hz), 3.58 (t, 1H, *J* =9.5 Hz), 3.76 (dd, 1H, *J* =10.5, 9.3 Hz), 4.17 (t, 1H, *J* =2.7 Hz); ^{13}C NMR (δ, 100 MHz, CD_3OD): 22.9, 30.9, 35.0, 37.5, 42.3, 46.4, 53.0, 61.3, 68.1, 71.0, 73.5, 73.6, 76.9,174.0. ESIMS: *m*/*z* calcd for $C_{20}H_{35}N_2O_6$: 399.2495 $[M+H]^+$. Found: 399.2513.

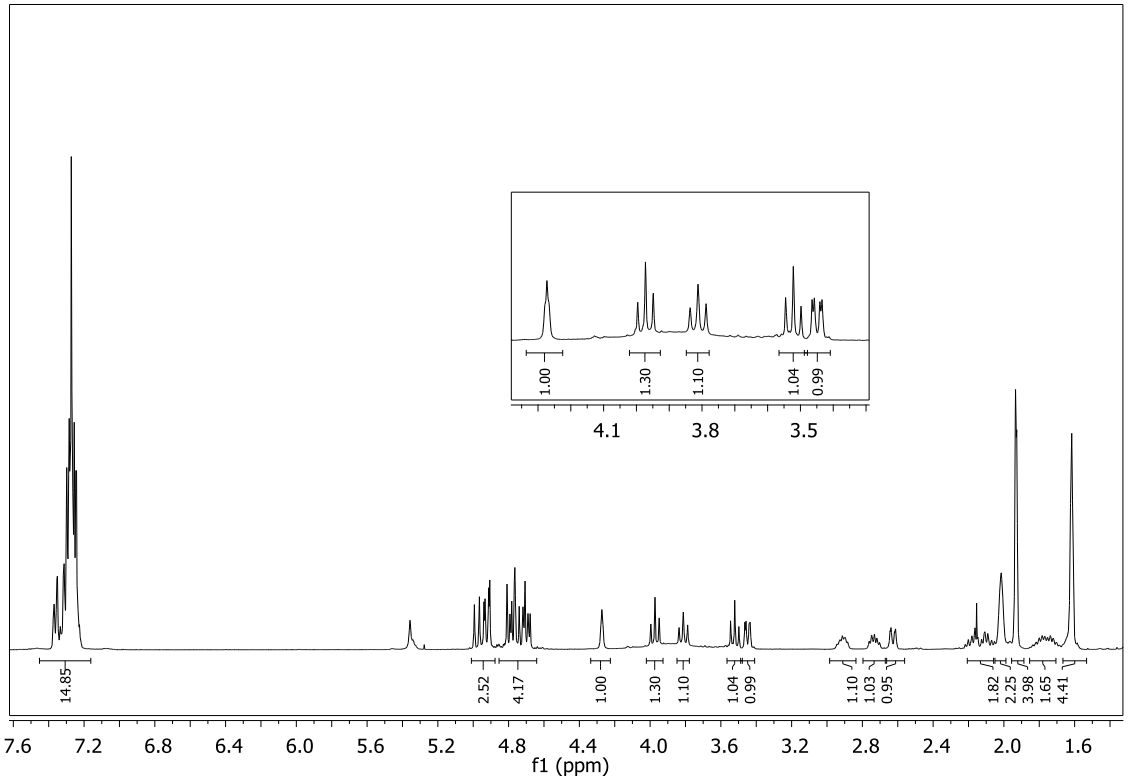
^{1}H NMR spectra (500 MHz, $CDCl_3$) of **7**



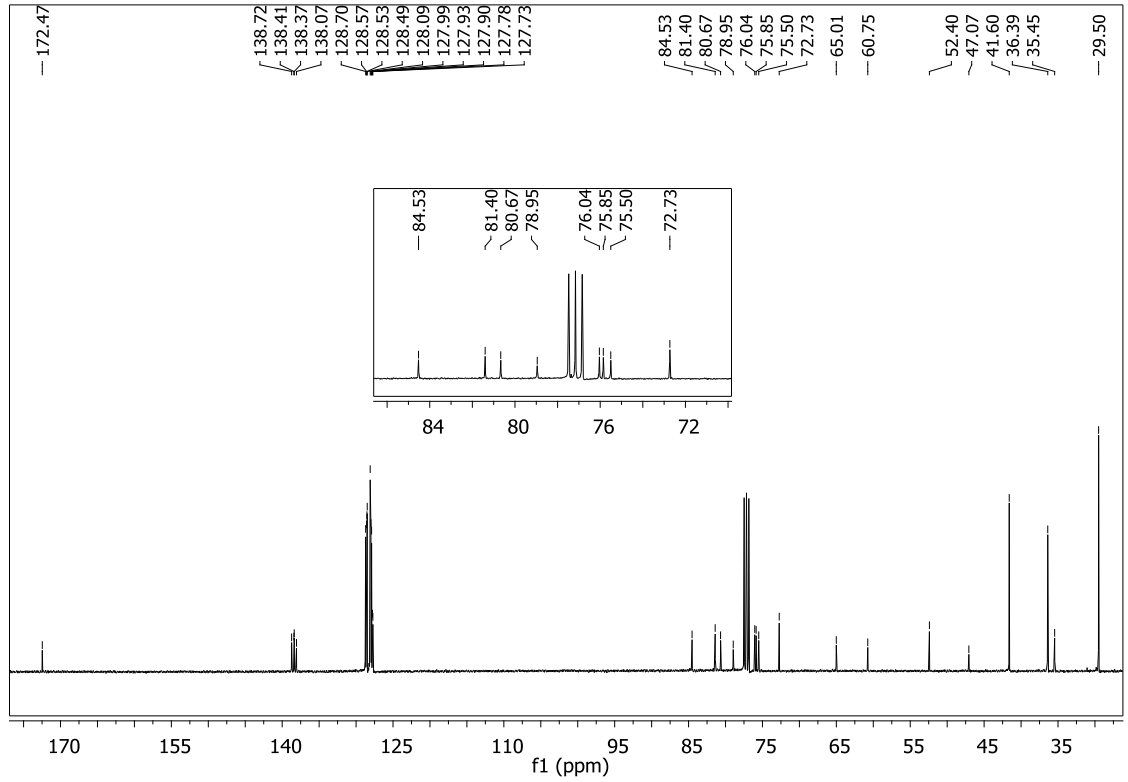
^{13}C NMR spectra (100 MHz, $CDCl_3$) of **7**



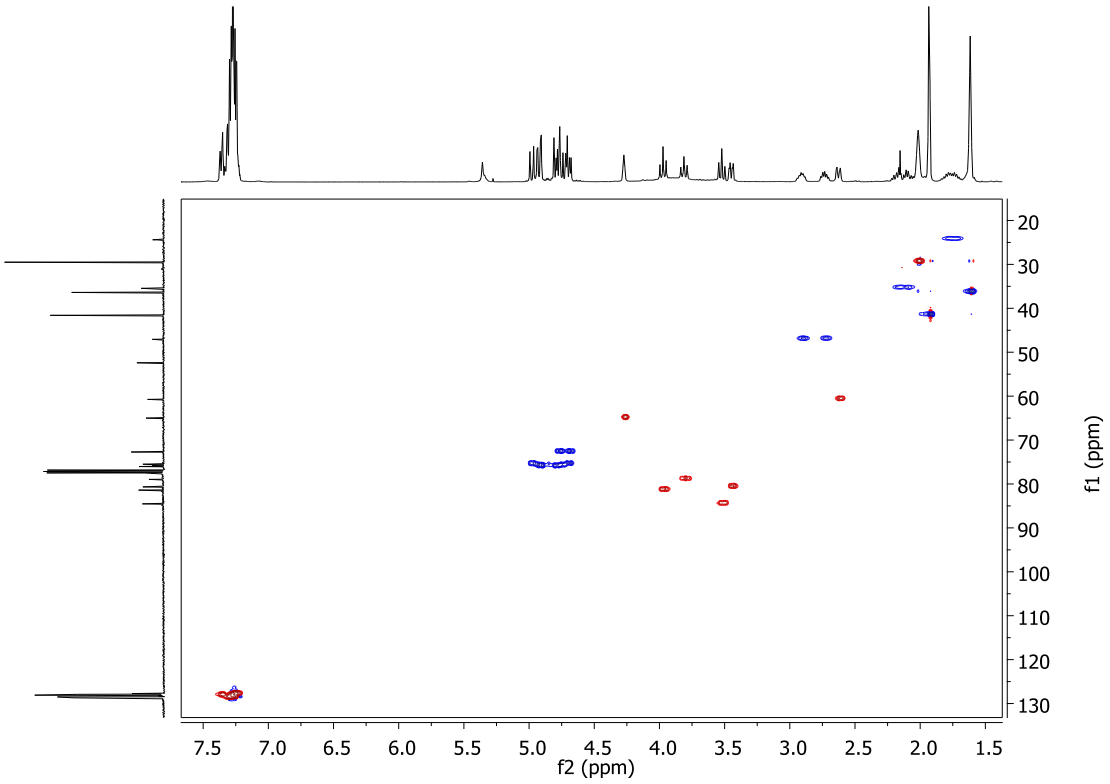
^{1}H NMR spectra (400 MHz, $CDCl_3$) of **8**



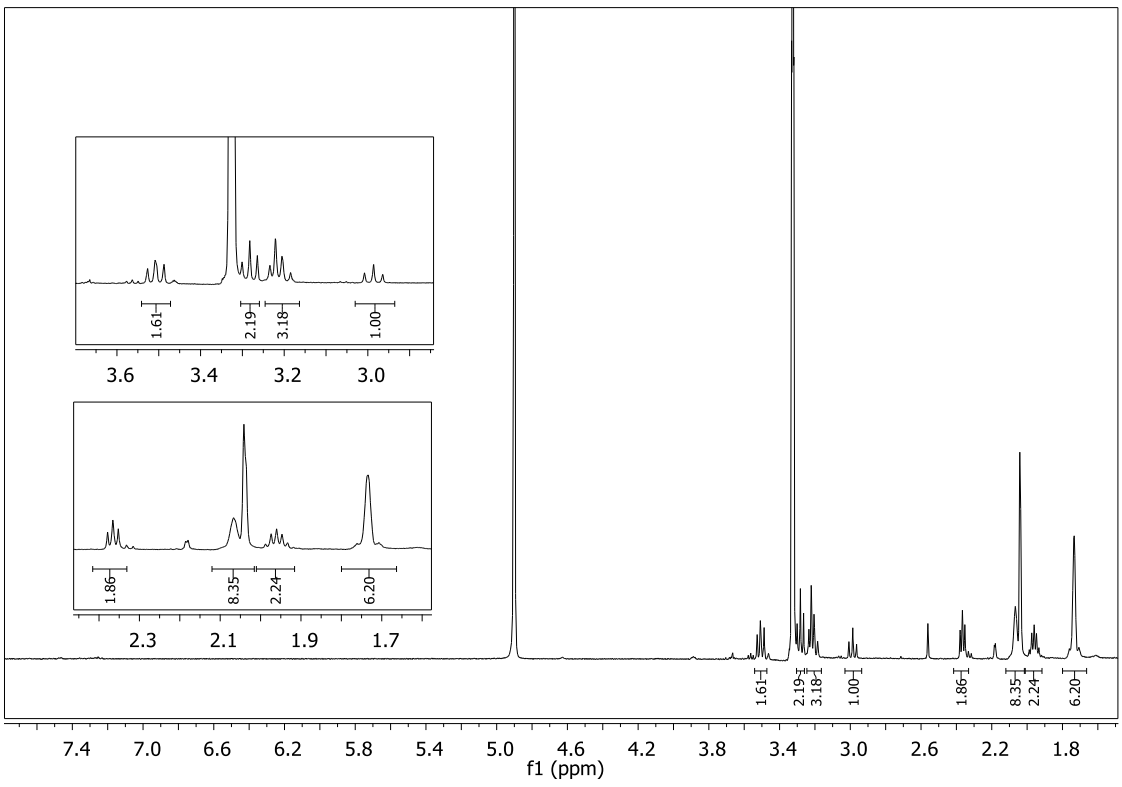
^{13}C NMR spectra (100 MHz, $CDCl_3$) of **8**



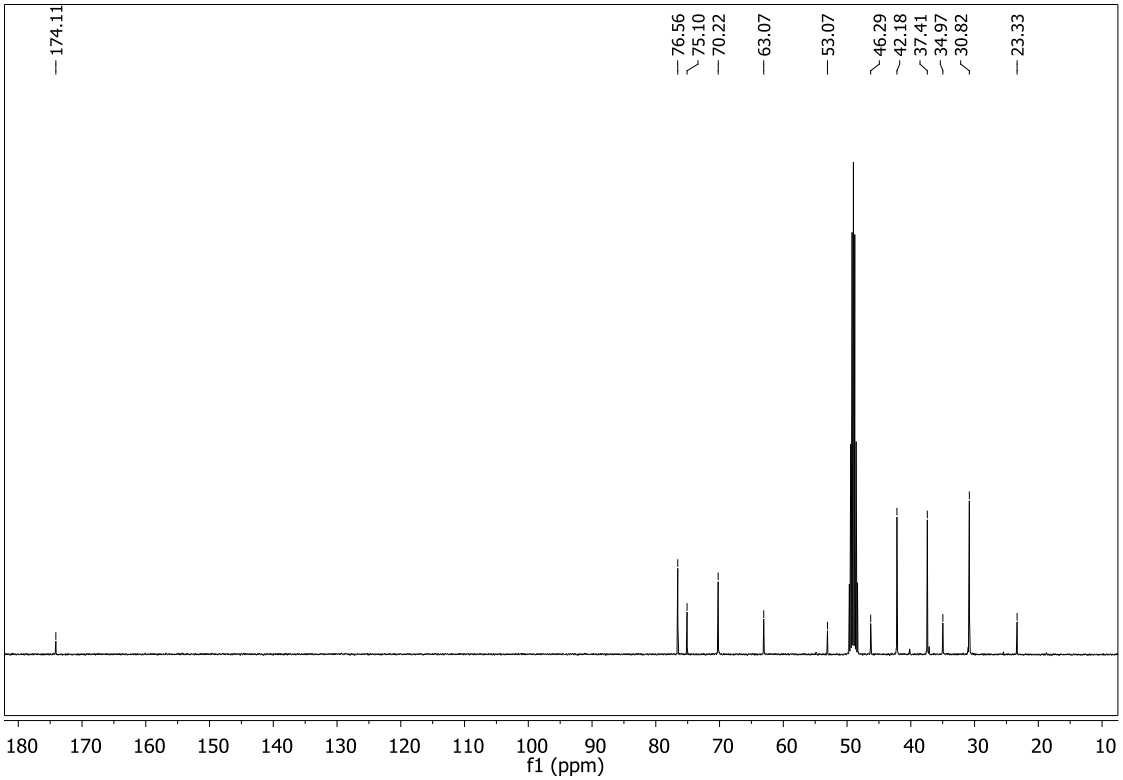
^{1}H-^{13}C gHSQC spectra (400 MHz, $CDCl_3$) of **8**



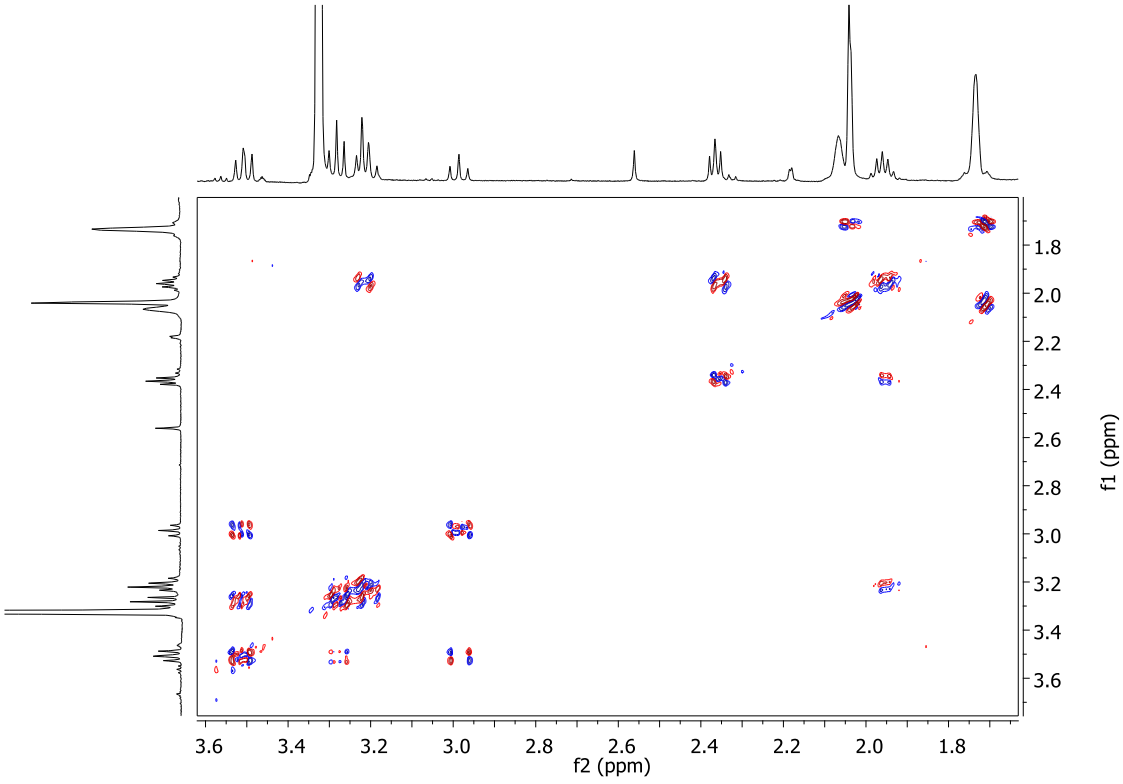
^{1}H NMR spectra (500 MHz, CD_3OD) of **1**



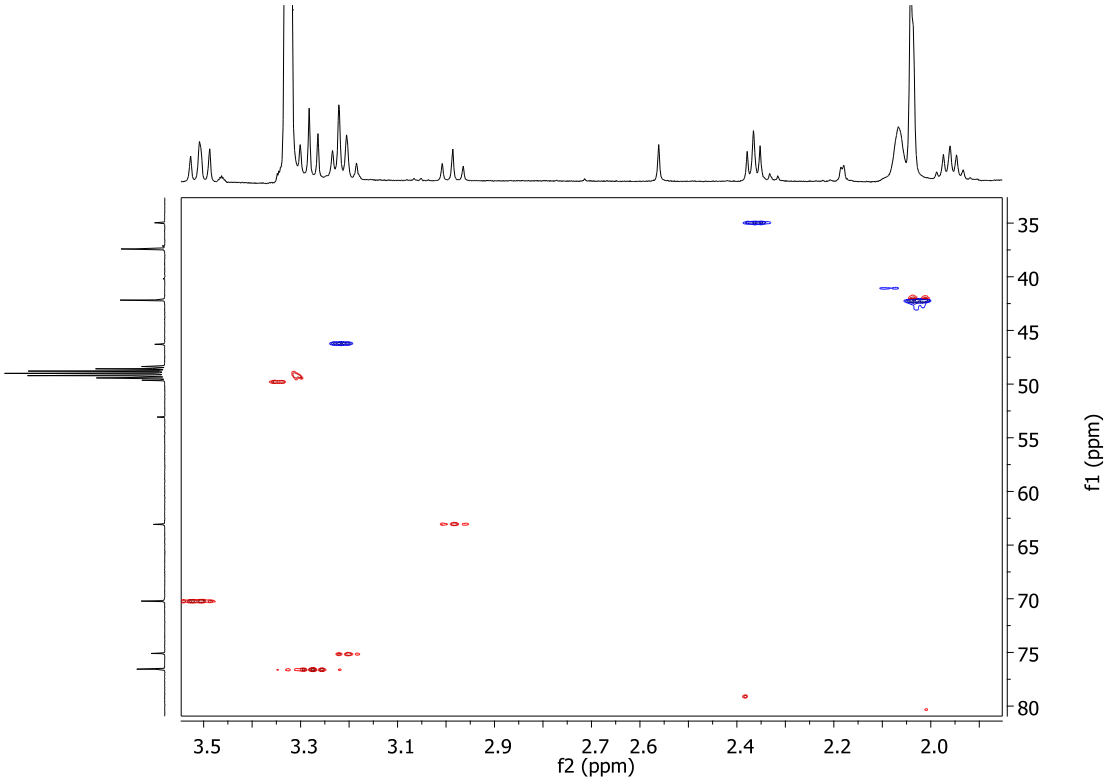
^{13}C NMR spectra (100 MHz, CD_3OD) of **1**



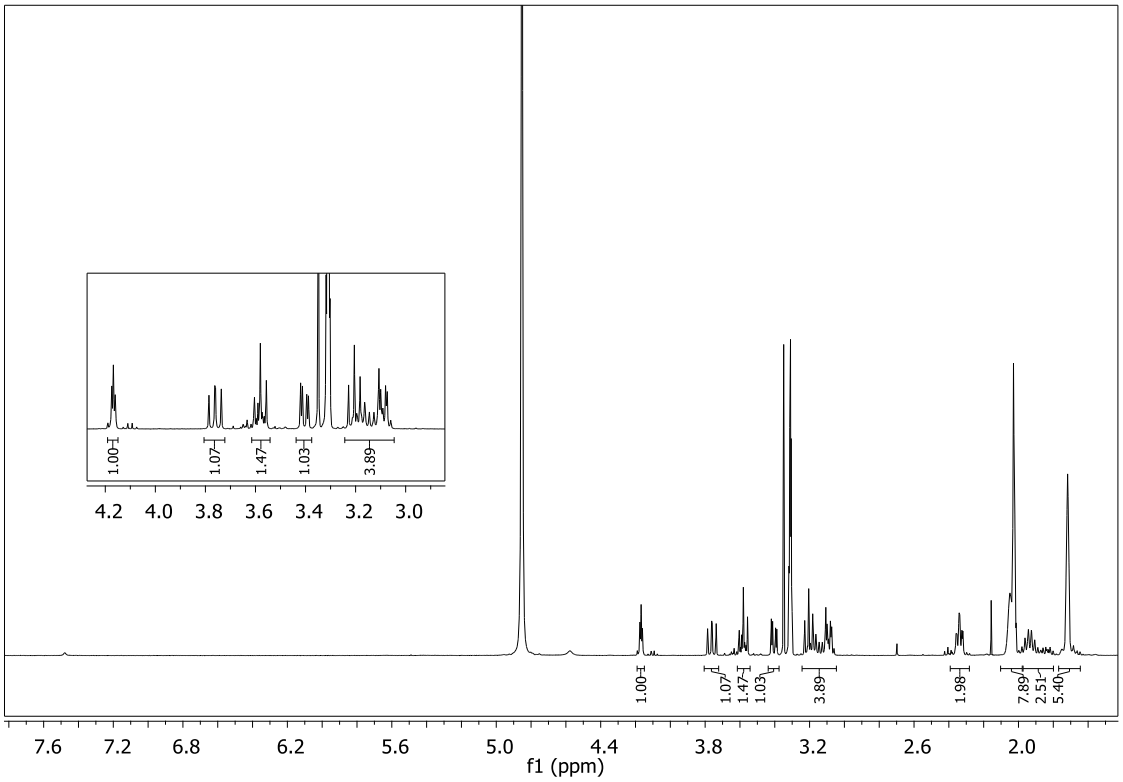
^{1}H-^{1}H gDQCOSY spectra (500 MHz, CD_3OD) of **1**



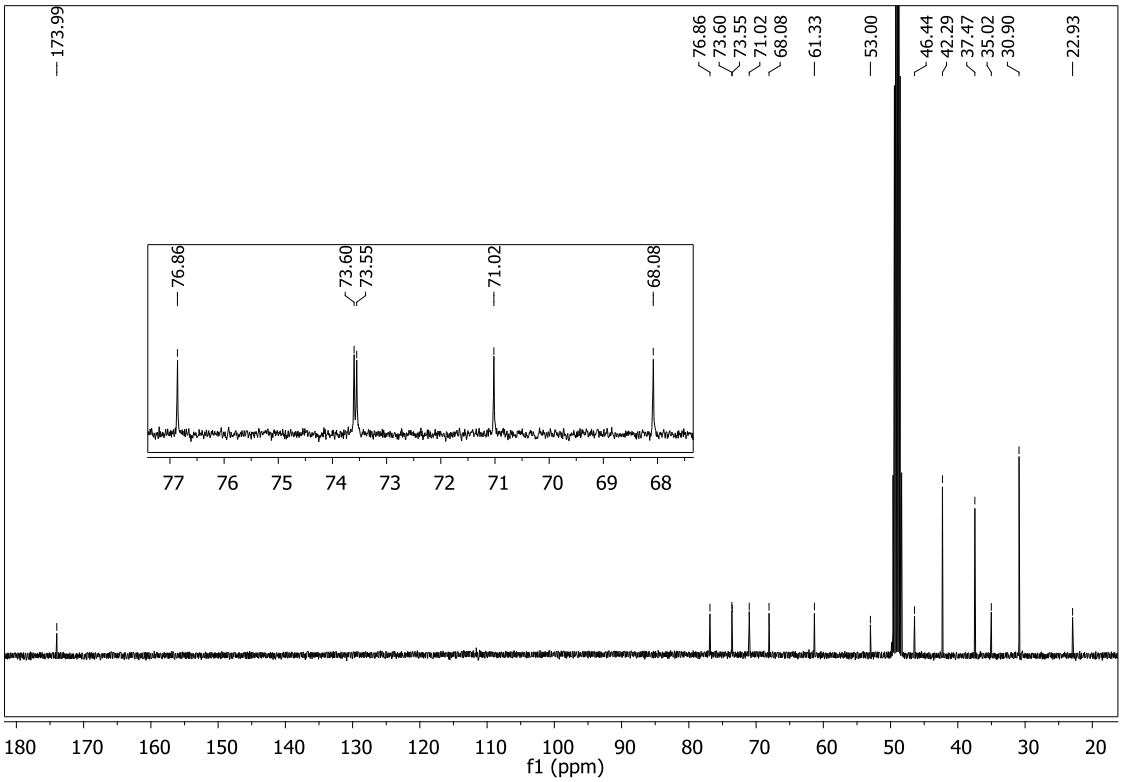
^{1}H-^{13}C gHSQC spectra (500 MHz, CD_3OD) of **1**



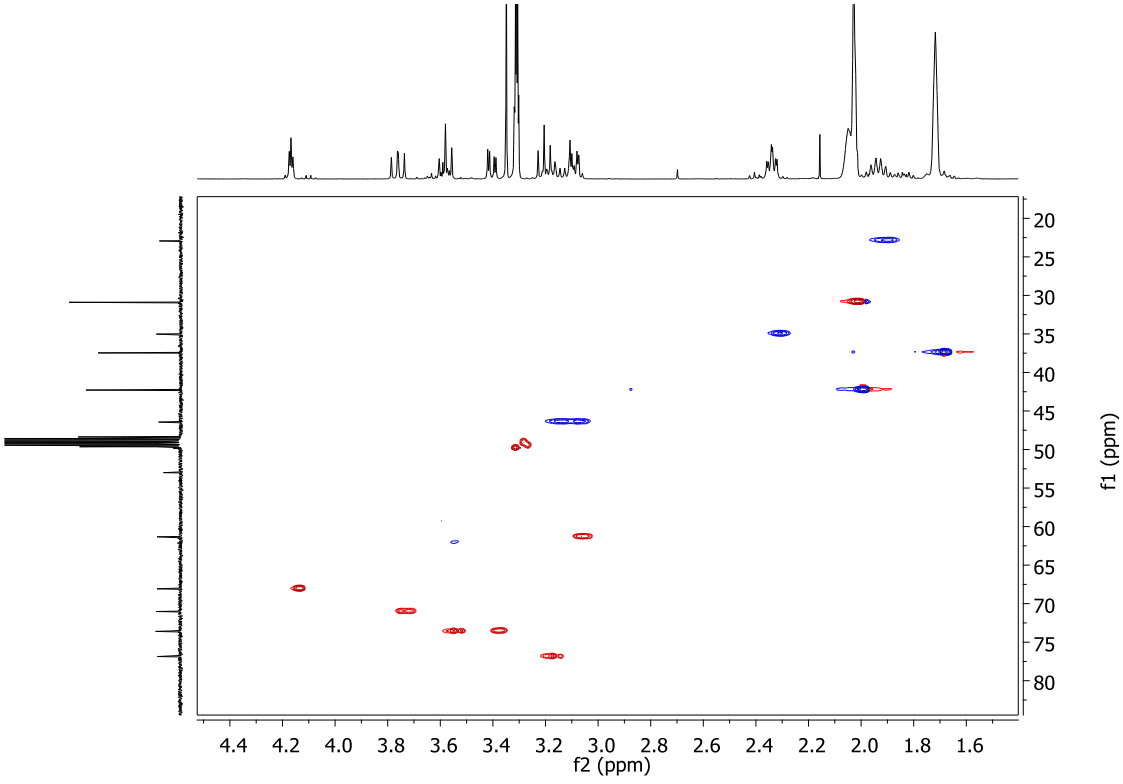
^{1}H NMR spectra (400 MHz, CD_3OD) of **2**



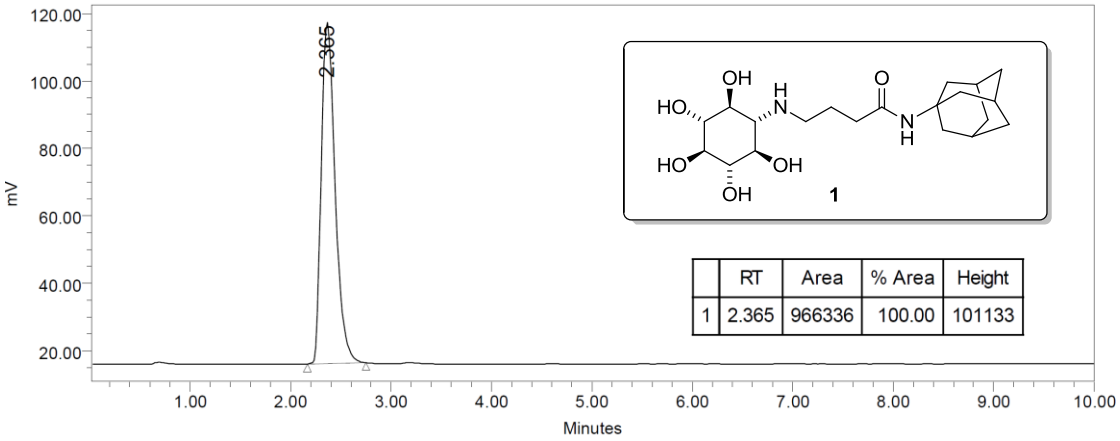
^{13}C NMR spectra (100 MHz, CD_3OD) of **2**



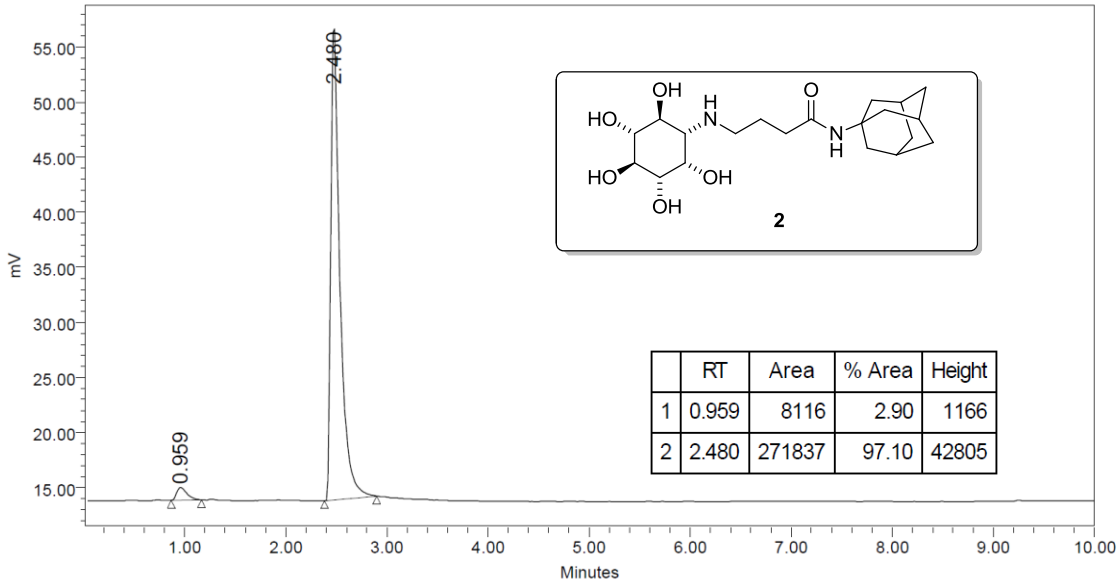
^{1}H-^{13}C gHSQC spectra (400 MHz, CD_3OD) of **2**



HPLC Chromatograms



	RT	Area	% Area	Height
1	2.365	966336	100.00	101133



	RT	Area	% Area	Height
1	0.959	8116	2.90	1166
2	2.480	271837	97.10	42805

Biological assays

Materials: The glycosidases α-glucosidase (from baker's yeast), *β*-glucosidase (from almond), *β*-galactosidase (from bovine liver) and α-galactosidase (from green coffee beans) that were used in the inhibition studies, as well as 4-methylumbelliferyl-*β*-D-glucoside and the corresponding *p*-nitrophenyl glycoside substrates, were purchased from Sigma. Imiglucerase (Cerezyme®; recombinant human *β*-glucocerebrosidase analogue) was kindly provided by Genzyme.

Cell Lines and Culture. Wild-type fibroblast and lymphoblasts derived from patients with Gaucher disease homozygous for N370S GCase (GM10873) or L444P GCase (GM08752) were obtained from Eucellbank and Coriell Cell Repositories, respectively. Fibroblast cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS, Invitrogen) and 1% penicillin-streptomycin (Invitrogen) at 37 °C in 5% CO_2. Culture medium was replaced every 3-4 days and all cells used in this study were between the 14th and 30th passages. Lymphoblast cell lines were cultured in RPMI-1640 medium (Gibco) supplemented with 15% FBS and 1% penicillin-streptomycin at 37 ºC in 5% CO_2. Culture medium was replaced every 2-3 days, and all cells used in this study were between the 5th and 16th passages. Total protein was determined using the Micro BCA protein assay kit according to the manufacture's instructions (Pierce, Thermo Scientific).

Recombinant GCase (Imiglucerase, Cerezyme®) inhibition assay.

Imiglucerase (Genzyme) activity was determined with 4-methylumbelliferyl-β-D-glucopyranoside as previously reported.[4] Briefly, enzyme solutions (25 *μ*L from a stock solution containing 0.1 mg mL^{-1}) in the presence of 0.25% (w/v) sodium taurocholate and 0.1% (v/v) Triton X-100 in McIlvaine buffer (100 mM sodium citrate and 200 mM sodium phosphate buffer, pH 5.2 or pH 7.0) were incubated at 37 ºC without (control) or with inhibitor at a final volume of 40 *μ*L for 30 min. After addition of 60 *μ*L 4-methylumbelliferyl-*β*-D-glucopyranoside (4 mM, McIlvaine buffer, pH 5.2 or pH 7.0), the samples were incubated at 37 ºC for 10 min. Enzymatic reactions were stopped by the addition of aliquots (150 *μ*L) of glycine/NaOH buffer (100 mM, pH 10.6). The amount of 4-methyumbelliferone formed was determined with a SpectraMax M5 fluorometer (Molecular Devices Corporation) at 355 nm (excitation) and 460 nm (emission). IC_{50} values were determined by plotting percent activity versus log [I], using at least five different inhibitor concentrations. The type of inhibition and K_i values for more active inhibitors were determined by Lineweaver−Burk or Dixon plots of assays performed with different concentrations of inhibitor and substrate.

Effects of compounds on the activity of other glycosidases

Table S1. Activity of Compounds against Commercial Glycosidases

Compound	α-glucosidase (baker's yeast)[a]	β-glucosidase (almonds)[a]	α-galactosidase (green coffee beans)[a]	β-galactosidase (bovine liver)[a]
1	0	0	0	0
2	0	1	0	24

[a] % Inhibition at 200 μM.

Inhibition assay against commercial glycosidases.

The enzyme assay methods were similar to those previously reported.[4]

Commercial enzyme solutions were prepared with the appropriate buffer and incubated in 96-well plates at 37 ºC without (control) or with inhibitor for 5 min. After addition of the corresponding substrate solution, incubations were prolonged for different time periods: 3 min for β-glucosidase (from almond) and α-glucosidase (from baker's yeast), 5 min for β-galactosidase, and 13 min for α-galactosidase and stopped by addition of Tris solution (50 μL, 1M) or Na_2CO_3 (180 μL, 1M), depending on the enzymatic inhibition assay. The amount of *p*-nitrophenol formed was determined at 405 nm with a SpectraMax M5 (Molecular Devices Corporation) spectrophotometer. For α-glucosidase (from baker yeast), the activity was determined with *p*-nitrophenyl-α-D-glucopyranoside (1 mM) in sodium phosphate buffer (100 mM, pH 7.2). For β-glucosidase (from almond), the activity was determined with *p*-nitrophenyl-β-D-glucopyranoside (1 mM) in sodium acetate buffer (100 mM, pH 5.0). β-galactosidase activity was determined with *p*-nitrophenyl-β-D-galactopyranoside (1 mM) in sodium phosphate buffer (100 mM, 0.1 mM $MgCl_2$, pH 7.2). α-galactosidase activity was determined with *p*-nitrophenyl-α-D-galactopyranoside (1 mM) in sodium phosphate buffer (100 mM, pH 6.8). The commercial glycosidase solutions were prepared as follows: α-glucosidase (from baker's yeast): (0.1 mg mL^{-1} buffer); β-glucosidase (from almond): (0.1 mg mL^{-1} buffer); α-galactosidase (from green coffee beans): 7.4 μL in buffer (1.99 mL); β-galactosidase from bovine liver (0.5 mg mL^{-1} buffer).

Cytotoxicity assay in wild-type human fibroblasts.

Wild-type fibroblasts were cultured in Dulbecco's modified Eagle's medium (DMEM; D5796; Sigma-aldrich) supplemented with 10% foetal bovine serum (FBS) and 1% penicillin-streptomycin at 37 °C in 5% CO_2/95% air. Cells used were between the 14th and 30th passage. At the time of the experiments, cells were seeded at a density of 25000 cells per well in 96-well plates. Media were renewed after 24 h and compounds were added to give final concentrations of 300-18 μM. All compounds were dissolved in DMSO and control experiments were performed with DMSO. Cells were incubated at 37 °C in 5% CO_2 for 24 h. Then, the media were replaced with 100 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution and the mixture was incubated for additional 3 h at 37 °C in 5% CO_2/95% air. The number of viable cells was quantified by the estimation of its dehydrogenase activity, which reduces MTT to water-insoluble formazan, which was dissolved in 100 μL of DMSO and measured at 570 nm with SpectraMax M5 (Molecular Devices Corporation) in 96-well format.

Table S2. Cytotoxicity of Compounds 1–2 in Wild-Type Human Fibroblasts.

Compound	Cytotoxicity CC_{50} (µM)[a]
1	>300
2	>300

[a] Wild-type fibroblasts were treated with different concentrations of compounds for 24 h, and the cytotoxicity was evaluated as described in the experimental procedures. The CC_{50} values were obtained by regression analysis of the dose-response curves obtained in a single experiment with triplicates.

GCase inhibition in intact human fibroblasts.

The intact cell GCase assay was performed as previously described.[5] Briefly, cells were plated into 24-well assay plates and incubated at 37 ºC under a 5% CO_2 atmosphere overnight. The media were then replaced with fresh media with or without a test compound and incubated at 37 °C in 5% CO_2 for 24 h. The enzyme activity assay was performed after removing media supplemented with the corresponding compound. The monolayers were washed with 100 μL of phosphate buffered saline (PBS) solution. Then, 80 μL of PBS and 80 μl of 200 mM acetate buffer (pH 4.0) were added to each well. The reaction was started by the addition of 100 μL of 5 mM 4-methylumbelliferyl-β-D-glucopyranoside (200 mM acetate buffer, pH 4.0) to each well, followed by incubation at 37 °C for 2 h. Enzymatic reactions were stopped by lysing the cells with 1.8 ml of glycine/NaOH buffer (100 mM, pH 10.6). Liberated 4-methylumbelliferone was measured (excitation 355 nm, emission 460 nm) with SpectraMax M5 fluorometer (Molecular Devices Corporation) in 24-well format. All determinations were performed in triplicate. Cells used were between the 14th and 30th passages.

Measurement of L444P or N370S GCase activity in lymphoblasts derived from patients with Gaucher disease.

The measurement of L444P or N370S GCase activity was performed as previously described.[6] Briefly, lymphoblasts were seeded at a density of $2x10^5$ cells per well in 1 mL of supplemented RPMI-1640 medium in 12-well plates. Cells were incubated in the absence or presence of various concentrations of compounds for 3 days before GCase activity was measured. After washing with PBS twice, the cell pellets were lysed in water by sonication.

All enzyme activation measurements were made using aliquots of homogenate (10 μL) and 6 mM of 4-methylumbelliferyl-β-D-glucopyranoside (50 μL) in 0.1 M citrate phosphate buffer, pH 5.2 containing 0.25% sodium taurocholate, 0.1% Triton X-100 and incubated at 37 ºC for 2 h. The enzyme reactions were stopped with 150 μL of glycine/NaOH buffer (200 mM, pH 10.6) and fluorescence was measured (excitation wavelength 355 nm, emission wavelength 460 nm) with SpectraMax M5 fluorometer (Molecular Devices Corporation) in 96-well format.

The non-specific GCase activity was evaluated by addition of conduritol B epoxide (500 μM) to control wells and was shown to account for about 2% of the total activity in control cells.

Supporting Information references

1 Z. Yu, A. R. Sawkar, L. J. Whalen, C.-H. Wong and J. W. Kelly, *J. Med. Chem.*, 2007, **50**, 94–100.

2 P. Serrano, A. Llebaria and A. Delgado, *J. Org. Chem.*, 2005, **70**, 7829–7840.

3 P. González-Bulnes, J. Casas, A. Delgado and A. Llebaria, *Carbohydr. Res.*, 2007, **342**, 1947–1952.

4 M. Egido-Gabás, P. Serrano, J. Casas, A. Llebaria and A. Delgado, *Org. Biomol. Chem.*, 2005, **3**, 1195–1201.

5 A. R. Sawkar, W.-C. Cheng, E. Beutler, C.-H. Wong, W. E. Balch and J. W. Kelly, *Proc. Natl. Acad. Sci. U.S.A.*, 2002, **99**, 15428–15433.

6 A. Trapero, I. Alfonso, T. D. Butters and A. Llebaria, *J. Am. Chem. Soc.*, 2011, **133**, 5474–5484.