Supporting Information

Fructose-6-phosphate Aldolase in Organic Synthesis: Preparation of D-Fagomine, *N*-Alkylated Derivatives and Preliminary Biological Assays

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Materials. α-D-Glucosidase from baker's yeast, β-D-glucosidase from almonds, α-D-glucosidase type V from rice, α-D-mannosidase from jack bean, α-D-rhamnosidase (naranginase) from *Penicillium decumbens* and β-D-galactosidase grade III: crude from Bovine Liver and the corresponding substrates *p*-nitrophenol–α-D-glucopyranoside, *p*-nitrophenyl-β-D-glucopyranoside, *p*-nitrophenol–α-D-mannopyranoside, *p*-nitrophenol–α-D-rhamnopyranoside and *p*-nitrophenol–α-D-galactopyranoside were purchased from Sigma. Butanal 99 %, hexanal 98%, octanal 99 %, nonanal, 95 % dodecanal 92 %, phenylacetaldehyde 90 %, palladium over charcoal (10 % Pd), and *N*-(benzyloxycarbonyl)succinimide (Cbz-OSu) were obtained from Aldrich. Dihydroxyacetone and aluminium oxide 90 active neutral were purchased from Merck. 3-Amino-1-propanol and Celite-545 (particle size 26 μm, mean pore diameter 17.000 nm, specific surface area BET method 2.19 $m^2 g^{-1}$) were obtained from Fluka. Deionized water was used for preparative HPLC and Milli-Q-grade water for analytical HPLC. All other solvents used were of analytical grade. Dodecanal and phenylacetaldehyde were purified prior to use by flash chromatography on neutral alumina and silica using CH_3Cl and CH_3Cl/Hexane 1:1 as eluents, respectively. Human blood from healthy volunteers was obtained from the Blood Bank of the Hospital Clinic (Barcelona, Spain).

Methods

NMR analysis: High field ^{1}H and ^{13}C nuclear magnetic resonance (NMR) analyses were carried out using an AVANCE 500 BRUKER spectrometer equipped with a high-sensitive CryoProbe for D_2O and CD_3OD solutions. Full characterization of the described compounds was performed using typical gradient-enhanced 2D experiments: COSY, NOESY, HSQC and HMBC, recorded under routine conditions.

When possible, NOE data was obtained from selective 1D NOESY versions using a single pulsed-field-gradient echo as a selective excitation method and a mixing time of 500 ms. When necessary, proton and NOESY experiments were recorded at different temperatures in order to study the different behavior of the exchange phenomena to avoid the presence of false NOE cross-peaks that difficult both structural and dynamic studies. Routine, ^{1}H (500 MHz) NMR spectra of compounds were recorded with a Varian Anova-500 spectrometer and ^{13}C (101 MHz) NMR Varian Mercury-400.

Specific rotations were measured with a Perkin Elmer Model 341 (Überlingen, Germany) polarimeter.

HPLC analyses: HPLC analyses were performed on a RP-HPLC cartridge, 250 x 4 mm filled with Lichrosphere® 100, RP-18, 5 μm from Merck (Darmstadt, Germany). Samples (50 μL) from the aldol reaction were withdrawn from the reaction medium, dissolved in MeOH to stop the reaction and analyzed by HPLC. The solvent system used was: solvent (A): H_2O 0.1 % (v/v) trifluoroacetic acid (TFA) and solvent (B): ACN/H_2O 4/1 0.095 % (v/v) TFA, gradient elution from 10 % to 70 % B in 30 min, flow rate 1 mL min^{-1}, detection 215 nm. Retention factor (*k*') for the aldol adduct **2** is given below.

Preparation of D-fructose-6-phosphate aldolase. D-Fructose-6-phosphate aldolase (FSA) was prepared as described by Schürmann et al.[1] and was purified as follows: after collecting the cells by centrifugation, the pellet was broken with a shot cell disrupter system (Constant Systems, Northants, UK) and centrifuged (17,000 x *g*) at 4°C during 30 min. The supernatant was taken up and heated to 75°C during 40 min. Then, the solution was centrifuged (50,000 x *g*) during 40 min and the supernatant lyophilized obtaining a brown powder with 1.7 U mg^{-1} powder. Unit (U) definition:

one U will synthesize 1 μmol of fructose-6-phosphate from D-glyceraldehyde-3-phosphate and dihydroxyacetone per minute at pH 8.5 (glycyl-glycine 50mM buffer) and 30°C.

(3*S*,4*R*)-6-[(Benzyloxycarbonyl)amino]-5,6-dideoxyhex-2-ulose (2). Dihydroxyacetone (2.1 g, 22.9 mmol) and FSA aldolase powder (2.09 g, 3445 U) were dissolved in boric/borate buffer 50 mM pH 7.0 (155 mL) and cooled down to 4°C. *N*-Cbz-aminoaldehyde **1**[2] (4.71 g, 22.9 mmol) dissolved in DMF (40 mL) at 4°C was added to this mixture. The reaction mixture was then placed in a reciprocal shaker (120 rpm) at 4°C. After 24 h, MeOH (200 mL) was added to the mixture to stop the reaction and centrifuged (1000 rpm) at 10 °C for 40 min. The supernatant was filtered on a 0.45 μm filter and purified by preparative HPLC as follows. The crude of **2** was loaded onto a preparative column (47 x 300 mm) filled with Bondapack C18 (Waters), 300 Å, 15-20 μm stationary phase. Products were eluted using ACN gradient from 0 % to 28 % ACN in 35 min**.** The flow rate was 100 mL min^{-1} and the products were detected at 254 nm. Analysis of the fractions was accomplished under isocratic conditions (33 % of solvent B) on the analytical HPLC. Pure fractions were pooled and lyophilized to give **2** (4.7 g, 69 %) as a white solid. HPLC retention factor *k*' 3.7; $[\alpha]_D^{22}$ +9.0 (*c* 1.0 in MeOH); ^{1}H NMR (500 MHz, D_2O, 22 °C): δ[ppm] = 7.47-7.35 (5H, Ar), 5.09 (s, 2H, 8-H), 4.54 (d, *J* = 19.4 Hz, 1H, 1-H), 4.43 (d, *J* = 19.4 Hz, 1H, 1-H), 4.28 (s, 1H, 3-H), 4.02 (dt, *J* = 6.8, 6.8, 2.2 Hz, 1H, 4-H), 3.2 (m, 2H, 6-H) and 1.75 (q, *J* = 6.8 Hz, 2H, 5-H); ^{13}C NMR (100 MHz, D_2O, 22 °C): δ[ppm] = 212.9 (C-2), 158.6 (C-7), 136.7 (Ar), 128.9 (Ar), 128.5 (Ar), 127.8 (Ar), 77.7 (C-3), 69.5 (C-1), 67.0 (C-8), 66.1(C-4), 37.2 (C-6), 32.5 (C-5).

D-Fagomine [(2*R*,3*R*,4*R*)-2-hydroxymethylpiperidine-3,4-diol] (3). Pd/C (100 mg) was added to a solution of the aldol adduct **2** (373 mg, 1.26 mmol) in H_2O/EtOH 9:1

(50 mL). The reaction mixture was shaken under hydrogen gas (50 psi) overnight at room temperature. After removal of the catalyst by filtration through neutralized and deactivated aluminum, the solvent was evaporated under reduced pressure and then lyophilized. D-Fagomine **3** (164 mg, 89 %) was afforded as a brown solid $[\alpha]_D^{20}$ + 20.4 (*c* 1.0 in H_2O). A fraction of the crude D-fagomine (24 mg) was purified by ion exchange chromatography on a FPLC system following a method described by Asano et al.[3] Bulk stationary phase CM-Sepharose CL-6B (Amersham Pharmacia) in NH_4^+ form was packed into a glass column (120x10 mm) to a final bed volume of 8 mL. The flow rate was 0.7 ml min^{-1}. The CM-Shepharose was equilibrated initially with H_2O. Then, an aqueous solution of the crude material at pH 7 was loaded onto the column. Minor coloured impurities were washed away with H_2O (2-3 column volumes). Then, D-fagomine and D-2,4-di-*epi*-fagomine were eluted in this order with 0.01 M NH_4OH. The fractions were analysed by NMR. Pure fractions were pooled and lyophilized affording D-fagomine (20 mg, 83 %) and D-2,4-di-*epi*-fagomine (<1 mg) as pale brown solids. ^{1}H NMR (500 MHz, D_2O, 22 °C) δ [ppm] = 3.86 (dd, *J* = 11.8, 3.0 Hz, 1H, 7-H), 3.66 (dd, *J* = 11.8, 6.5 Hz, 1H, 7-H), 3.56 (ddd, *J* = 11.5, 9.0, 5.0, 1H, 4-H), 3.21 (t, *J* = 9.5 Hz, 1H 3-H), 3.06 (ddd, *J* = 12.9, 4.4, 2.3 Hz, 1H, 6-H), 2.68 (dt, *J* = 12.9, 12.9, 2.7 Hz, 1H, 6-H), 2.61 (ddd, *J* = 9.7, 6.4, 3.0, 1H, 2-H), 2.01 (tdd, *J* = 13.0, 4.9, 2.5,2.5, 1H, 5-H), 1.53-1.43 (m, 1H, 5-H); ^{13}C NMR (101 MHz, D_2O, 22 °C) δ [ppm] = 72.9 (C-4), 72.7 (C-3), 61.1 (C-2), 60.9 (C-7), 42.6 (C-6), 32.1 (C-5).

D-2,4-Di-*epi*-fagomine [(2*S*,3*R*,4*S*)-2-hydroxymethylpiperidine-3,4-diol] ^{1}H NMR (500 MHz, D_2O, 22 °C) δ [ppm] = 3.91 (s, 1H, 3-H), 3.77-3.71 (m, 1H, 4-H), 3.68-3.60 (m, 2H, 7-H), 3.10-3.04 (m, 1H, 6-H), 2.74 (t, *J* = 6.8 Hz, 1H, 2-H), 2.64-2.57 (dt, *J* = 12.3, 12.3, 4.5 Hz, 1H, 6-H), 1.75-1.62 (m, 2H, 5-H); ^{13}C NMR (101 MHz,

D_2O, 22 °C) δ[ppm] = 69.61 (C-4), 67.48 (C-3), 61.42 (C-7), 59.19 (C-2), 42.81(C-6), 26.96 (C-5).

_N_-Butyl-D-fagomine [(2_R_,3_R_,4_R_)-_N_-butyl-2-hydroxymethylpiperidine-3,4-diol] (4a)

Strategy (a): from D-fagomine. Butanal (610 mg, 8.46 mmol) and **3** (249 mg, 1.69 mmol) were dissolved in EtOH/H_2O 20:7 (27 mL), and Pd/C (128 mg) was added. The mixture was shaken under H_2 (50 psi) overnight at room temperature. After removal of the catalyst by filtration through neutralized and deactivated alumina or Celite, the solvent was evaporated under reduced pressure. The brown oily residue obtained was purified by flash column chromatography on silica using MeOH/$CHCl_3$ from 0:1 to 4:1 as eluent. Pure fractions were pooled, the solvent evaporated to dryness and the residue was dissolved in H_2O and lyophilized, yielding **4a** as a white-brown solid (160 mg, 47 %). $[\alpha]_D^{20}$ = -24.5 (*c* 1.2 in MeOH); ^{1}H NMR (500 MHz, D_2O, 22 °C) δ[ppm] = 3.90 (dd, *J* = 12.7, 2.4, 1H, 7-H), 3.82 (dd, *J* 12.7, 2.9 Hz, 1H, 7-H), 3.45 (ddd, *J* = 11.5, 9.1, 5.1, 1H, 4-H,), 3.30 (t, *J* = 9.4, 1H, 3-H)**,** 2.90 (td, *J* = 12.2, 3.5, 3.5, 1H, 6-H), 2.73 (ddd, *J* = 13.3, 11.2, 5.4, 1H, 8-H), 2.50 (ddd, *J* = 13.3, 11.1, 5.2, 1H, 8-H), 2.36 (dt, *J* = 12.6, 12.6, 2.4, 1H, 6-H), 2.16 (td, *J* = 9.8, 2.6, 2.6 Hz, 1H, 2-H), 1.92 (tdd, *J*= 12.7, 5.0, 2.5, 2.5, 1H, 5-H), 1.55-1.35 (m, 3H, 5-H, 9-H), 1.30-1.21 (m, 2H, 10-H), 0.88 (t, *J* = 7.4, 3H, 11-H); ^{13}C NMR (101 MHz, D_2O, 22 °C) δ[ppm] = 73.2 (C-4), 72.0 (C-3), 65.8 (C-2), 58.1 (C-7), 52.2 (C-8), 49.1 (C-6), 30.5 (C-5), 25.6 (C-9), 20.4 (C-10), 13.3 (C-11).

*Strategy (b): one pot reaction from the aldol adduct **2**.* Butanal (54 mg, 0.74 mmol) and the aldol adduct **2** (150 mg, 0.51 mmol) were dissolved in EtOH/H_2O 7:3 (10 mL) and Pd/C (55 mg) was added. After treatment with H_2 (50 psi) overnight at room

temperature, the same procedure was followed as in the *strategy a* to furnish **4a** (52 mg, 51 %) as a solid.

***N*-Hexyl-D-fagomine [(2*R*,3*R*,4*R*)-*N*-hexyl-2-hydroxymethylpiperidine-3,4-diol] (4b).** The title compound was obtained by the previous described *strategy a* (140 mg, 40 %) as a solid. $[\alpha]_D^{20}$ -27.1 (*c* 1.3 in MeOH). ^{1}H NMR (500 MHz, D_2O, 22 °C) δ [ppm] = 3.94-3.84 (m, 2H, 7-H), 3.50-3.42 (m, 1H, 4-H), 3.38 (t, *J* = 9.2 Hz, 1H, 3-H), 2.97 (d, *J* = 11.7 Hz, 1H, 6-H), 2.87-2.77 (m, 1H, 8-H), 2.69-2.59 (m, 1H, 8-H), 2.46 (t, *J* = 12.0 Hz, 1H, 6-H), 2.24 (d, *J* = 9.4 Hz, 1H, 2-H), 1.97 (dd, *J* = 12.8, 2.4 Hz, 1H, 5-H), 1.58 (dq, *J* = 13.7, 13.7, 13.5, 3.2 Hz, 1H, 5-H), 1.54-1.45 (m, 2H, 9-H), 1.34-1.25 (br s, 6H, 10,11,12-H), 0.88 (t, *J* = 5.8 Hz, 3H, 13-H); ^{13}C NMR (101 MHz, D_2O, 22 °C) δ [ppm] = 72.9 (C-4), 71.5 (C-3), 65.8 (C-2), 57.4 (C-7), 52.7 (C-8), 49.5 (C-6), 31.6 (C-5), 30.4 (C-9), 27.1 (C-10), 23.3 (C-11), 22.5 (C-12), 13.8 (C-13).

***N*-Octyl-D-fagomine [(2*R*,3*R*,4*R*)-*N*-octyl-2-hydroxymethylpiperidine-3,4-diol] (4c).**

The title compound was obtained by the previous described *strategy a* (79 mg, 19 %) and *b* (153 mg, 54 %) as a solid. $[\alpha]_D^{20}$ -29.5 (*c* = 1.0 in MeOH). ^{1}H NMR (500 MHz, CD_3OD, 22 °C) δ [ppm] = 3.91-3.84 (m, 2H, 7-H), 3.38-3.31 (m, 2H, 3-H, 4-H), 2.94 (td, *J* = 11.8, 3.4, 3.4 Hz, 1H, 6-H), 2.87-2.78 (m, 1H, 8-H), 2.56 (ddd, *J* = 13.3, 10.2, 5.9 Hz, 1H, 8-H), 2.36 (dt, *J* = 12.2, 12.0, 1.5 Hz, 1H, 6-H), 2.13 (d, *J* = 8.5 Hz, 1H, 2-H), 1.92-1.85 (m, 1H, 5-H), 1.62-1.44 (m, 3H, 5-H, 9-H), 1.31 (b. s., 10H, 10, 11, 12, 13, 14-H), 0.90 (t, *J* = 6.7 Hz, 3H, 15-H); ^{13}C NMR (101 MHz, CD_3OD, 22 °C) δ [ppm] = 74.3 (C-4), 73.3 (C-3), 67.6 (C-2), 58.9 (C-7), 54.1 (C-8), 50.8 (C-6), 33.0 (C-5), 32.0 (C-9), 30.6 (C-10), 30.4 (C-11), 28.6 (C-12), 25.7 (C-13), 23.8 (C-14), 14.5 (C-15).

***N*-Nonyl-D-fagomine [(2*R*,3*R*,4*R*)-*N*-nonyl-2-hydroxymethylpiperidine-3,4-diol] (4d)**. The title compound was obtained by the previous described *strategy a* (96 mg, 22 %) and b (110 mg, 40 %) as a solid. $[\alpha]_D^{20}$ -25.1 (*c* = 1.2 in MeOH). ^{1}H NMR (500 MHz, CD_3OD, 22 °C) δ[ppm] = 3.91-3.84 (m, 1H, 7-H), 3.39-3.32 (m, 2H, 3-H, 4-H), 2.96 (d, *J* = 11.9 Hz, 1H, 6-H), 2.88-2.80 (m, 1H, 8-H), 2.63-2.54 (m, 1H, 8-H), 2.43-2.34 (m, 1H, 6-H), 2.16 (d, *J* = 7.6 Hz, 1H, 2-H), 1.93-1.86 (m, 1H, 5-H), 1.62-1.46 (m, 3H, 5-H, 9-H), 1.38-1.24 (br s, 12H, 10,11,12,13,14,15-H), 0.90 (t, *J* = 7.0, 3H, 16-H); ^{13}C NMR (101 MHz, CD_3OD, 22 °C) δ[ppm] = 74.5 (C-4), 73.5 (C-3), 67.6 (C-2), 59.2 (C-7), 54.1 (C-8), 50.9 (C-6), 33.1 (C-5), 32.2 (C-9), 30.8 (C-10), 30.7 (C-11), 30.5 (C-12), 28.7 (C-13), 25.3 (C-14), 23.8 (C-15), 14.5 (C-16).

***N*-Dodecyl-D-fagomine [(2*R*,3*R*,4*R*)-*N*-dodecyl-2-hydroxymethylpiperidine-3,4-diol] (4e).** The title compound was obtained by the previous described *strategy a* (289 mg, 57 %) as a solid $[\alpha]_D^{20}$ -11.1 (*c* = 1.0 in MeOH). ^{1}H NMR (500 MHz, CD_3OD, 22 °C) δ[ppm] = 4.10 (d, *J* = 12.4, 1H, 7-H), 3.90 (dd, *J* = 12.4, 2.7, 1H, 7-H) 3.62-3.50 (m, 2H, 3-H, 4-H), 3.42 (d, *J* = 12.1 Hz, 1H, 6-H), 3.30-3.20 (br s, 1H, 8-H), 3.16-2.99 (br s, 2H, 6-H, 8-H), 2.92 (br s, 1H, 2-H), 2.11 (dd, *J* = 14.1, 3.1, 1H, 5-H), 1.82-1.63 (br s, 3H, 5-H, 9-H), 1.41-1.30 (br s, 2H, 10-H), 1.30-1.20 (br s, 16H, 11,12,13,14,15,16,17,18-H), 0.90 (t, *J* = 6.94 Hz, 3H, 19-H); ^{13}C NMR (101 MHz, CD_3OD, 22 °C) δ[ppm] = 71.8 (C-4), 71.1 (C-3), 67.5 (C-2), 56.1 (C-7), 54.0 (C-8), 50.3 (C-6), 33.1 (C-5), 30.8 (C-9), 30.7 (C-10, C-11), 30.6 (C-12), 30.5 (C-13), 30.3 (C-14), 29.8 (C-15), 27.9 (C-16), 24.6 (C-17), 23.8 (C-18), 14.5 (C-19)

***N*-Phenylethyl-D-fagomine [(2*R*,3*R*,4*R*)-*N*-phenyethyl-2-hydroxymethylpiperidine-3,4-diol] (4f).** The title compound was obtained by the previous described *strategy a* (96 mg, 23 %) as a solid $[\alpha]_D^{20}$ -21.8 (*c* 1.1 in MeOH). ^{1}H NMR (500 MHz, CD_3OD, 22 °C) δ[ppm] = 7.29-7.13 (5H, Ar), 3.96-3.88 (m, 2H,

7-H), 3.41-3.31 (m, 2H, 4-H, 3-H), 3.05-2.96 (m, 2H, 6H, 8-H), 2.93-2.72 (m, 3H, 8-H, 9-H), 2.52 (dt, *J* = 12.3, 12.3, 2.4 Hz, 1H, 6-H), 2.26 (td, *J* = 9.0, 2.7, 2.7 Hz, 1H, 2-H), 1.95-1.88 (m, 1H, 5-H), 1.65-1.54 (m, 1H, 5-H); ^{13}C NMR (101 MHz, CD_3OD, 22 °C) δ[ppm] = 141.5 (Ar), 129.71-129.1 (Ar), 74.6 (C-4), 73.5 (C-3), 67.0 (C-2), 59.4 (C-7), 56.1 (C-8), 50.9 (C-6), 32.4 (C-5), 31.5 (C-9).

Antimicrobial activity

The antimicrobial activities were determined in vitro on the basis of minimum inhibitory concentration (MIC) values. The antibacterial test was performed as described[4] using Mueller-Hinton broth and the MICs values were determined after 24-48 h at 30 °C. The antifungal test was performed using Sabouraud liquid medium and the MICs values were determined after 48-72 h at 30 °C. Tables 1 and 2 show the MICs values of compound **4e** against bacteria and fungi, respectively. Compounds **3** and **4a-d** and **4f** were inactive at 256 mg L^{-1}.

Table 1 Minimum inhibitory concentrations (MICs) of *N*-dodecylfagomine **4e** against 15 bacteria

Gram-positive	MIC (mg L^{-1})	Gram-negative	MIC (mg L^{-1})
Bacillus cereus var. mycoides ATCC 11778	32	*Bordetella bronchiseptica* ATCC 4617	128
Bacillus subtilis ATCC 6633	32	*Enterobacter aerogenes* CECT 684	>256
Enterococcus hirae ATCC 10541	32	*Escherichia coli* ATCC 8739	128
Micrococcus luteus ATCC 9341	32	*Klebsiella pneumoniae* ATCC 4352	256
Mycobacterium phlei ATCC 41423	64	*Pseudomonas aeruginosa* ATCC 9027	256
Mycobacterium smegmatis ATCC 3017	128	*Salmonella typhimurium* ATCC 14028	256
Staphylococcus aureus ATCC 6538	64	*Serratia marcescens* ATCC 274	>256
Staphylococcus epidermidis ATCC 12228	64		

Table 2 Minimum inhibitory concentrations (MICs) of *N*-dodecylfagomine **4e** against 7 fungi.

Moulds	MIC (mg L^{-1})	Yeasts	MIC (mg L^{-1})
Aspergillus repens ATCC 28604	32	*Candida albicans* ATCC 10231	>256
Penicillium chrysogenum ATCC 9480	256	*Saccharomyces cerevisiae* ATCC 9763	256
Cladosporium cladosporoides ATCC 16022	64		
Trichophyton mentagrophytes ATCC 18748	128		

Penicillium funiculosum CECT 2914	128

Enzymatic inhibition assays. Commercial glycosidase solutions were prepared with the appropriate buffer and incubated in 96-well plates at 37ºC without (control) or with inhibitor (1 mM) during 3 min for α-glycosidase, α-mannosidase, and for α-rhamnosidase, and 5 min for β-galactosidase. After addition of the corresponding substrate solution, incubations were prolonged during different time periods: 10 min for α-glucosidase, 6 min for α-mannosidase, 5 min for α-rhamnosidase and 16 min for β-galactosidase and stopped by addition of 50 μL of 1 M Tris solution or 180 μL of 100 mM glycine buffer pH 10, depending on the enzymatic inhibition assay. The amount of *p*-nitrophenol formed was determined at 405 nm with UV/VIS Lector Spectramax Plus (Molecular Devices Corporation) spectrophotometer. α-D-Glucosidase from baker's yeast activity was determined with *p*-nitrophenyl-α-D-glucopyranoside (1 mM) in 100 mM sodium phosphate buffer (pH 7.2). β-Glucosidase activity was determined with *p*-nitrophenyl-β-D-glucopyranoside (1 mM) in 100 mM sodium acetate buffer (pH 5.0). α–Glucosidase from rice activity was determined with *p*-nitrophenyl-α-D-glucopyranoside (1 mM) in 50 mM sodium acetate buffer (pH 5.0). α-Mannosidase activity was determined with *p*-nitrophenyl-α-D-mannopyranoside (1 mM) in 50 mM sodium acetate buffer (pH 5.0). α-Rhamnosidase activity was determined with *p*-nitrophenyl-α-D-rhamnopyranoside (1 mM) in 50 mM sodium acetate buffer (pH 5.0). β-Galactosidase activity was determined with *p*-nitrophenyl-β-D-galactopyranoside (1 mM) in 100 mM sodium phosphate buffer, 0.1 mM $MgCl_2$ (pH 7.2). The commercial glycosidase solutions were prepared as follows: α-glycosidase from rice: 100 μL commercial suspension/5 mL buffer, α-mannosidase from jack bean: 25 μL commercial suspension/10 mL

buffer, naringinase from *Penicilium decumbens*: 0.3 mg/1 mL buffer; β-galactosidase from bovine liver: 0.1 mg /1 mL buffer.

Kinetics of Inhibition. The nature of the inhibition against enzymes and the K_i values were determined from the Lineweaver-Burk or Dixon plots.

Haemolysis assays. The haemolysis assays were performed as described previously.[5]

HPLC Chromatograms

HPLC Chromatogram of the FSA-catalyzed aldol addition of DHA to *N*-Cbz-3-aminopropanal in Glycylglycine 50 mM pH 7.0 buffer/DMF 4:, after 1 h of reaction

D-7000 HSM: Aldolasas Series: 1275 Report: original System: Sys 1

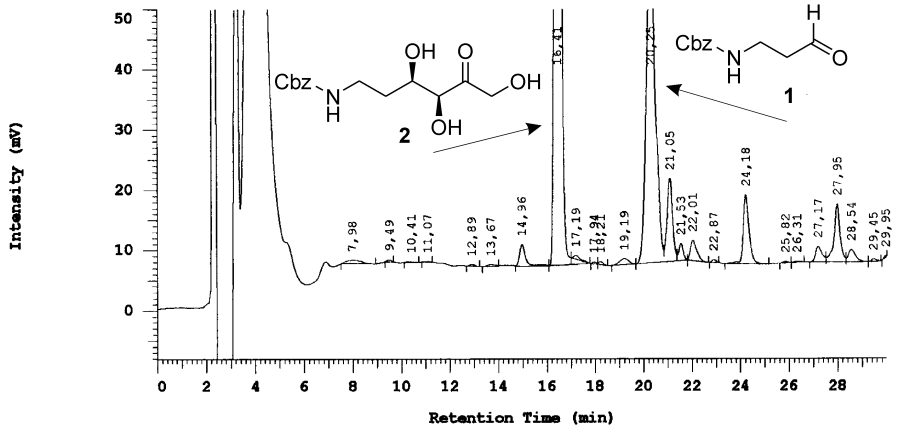
D-7000 HPLC System Manager Report

Analyzed: 29/09/05 17:59
Reported: 29/09/05 18:43
Processed: 29/09/05 18:43

Data Path: C:\WIN32APP\HSM\DATA\1275\
Processing Method: JC003

Application: Aldolasas
Sample Name: exp 908 1h

Series:1275
Vial Number: 3
Volume: 30,0 ul



No.	RT	Area	Conc 1	BC
1	7,98	24346	0,366	BB
2	9,49	4054	0,061	BB
3	10,41	2286	0,034	BB
4	11,07	1391	0,021	BB
5	12,89	4002	0,060	BB
6	13,67	8047	0,121	BV
7	14,96	63719	0,957	BB
8	16,41	4353287	65,406	BV
9	17,19	11238	0,169	TBB
10	17,94	3681	0,055	BV
11	18,21	6245	0,094	VB
12	19,19	25936	0,390	BB
13	20,25	1369740	20,580	BV

HPLC Chromatogram of the FSA-catalyzed aldol addition of DHA to *N*-Cbz-3-aminopropanal in Boric-Borate 50 mM pH 7.0 buffer/DMF 4:1, after 1 h of reaction.

D-7000 HSM: Aldolasas Series: 1275 Report: original System: Sys 1

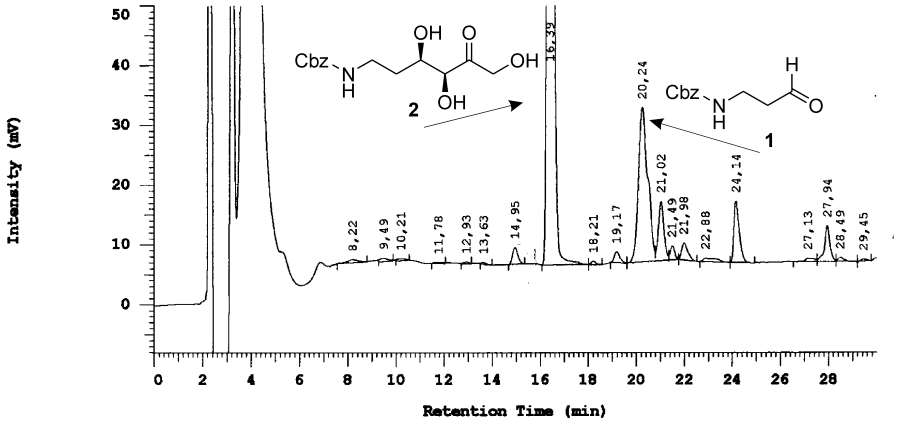
D-7000 HPLC System Manager Report

Analyzed: 29/09/05 18:45
Reported: 29/09/05 19:29
Processed: 29/09/05 19:29

Data Path: C:\WIN32APP\HSM\DATA\1275\
Processing Method: JC003

Application: Aldolasas
Sample Name: exp 909 1h

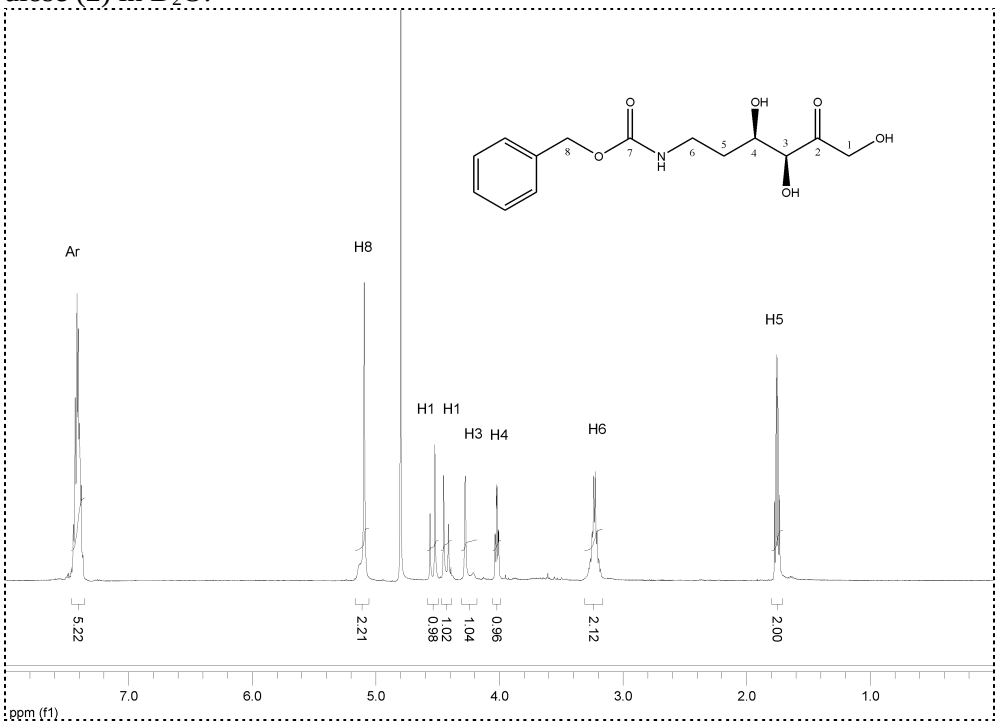
Series:1275
Vial Number: 4
Volume: 30,0 ul



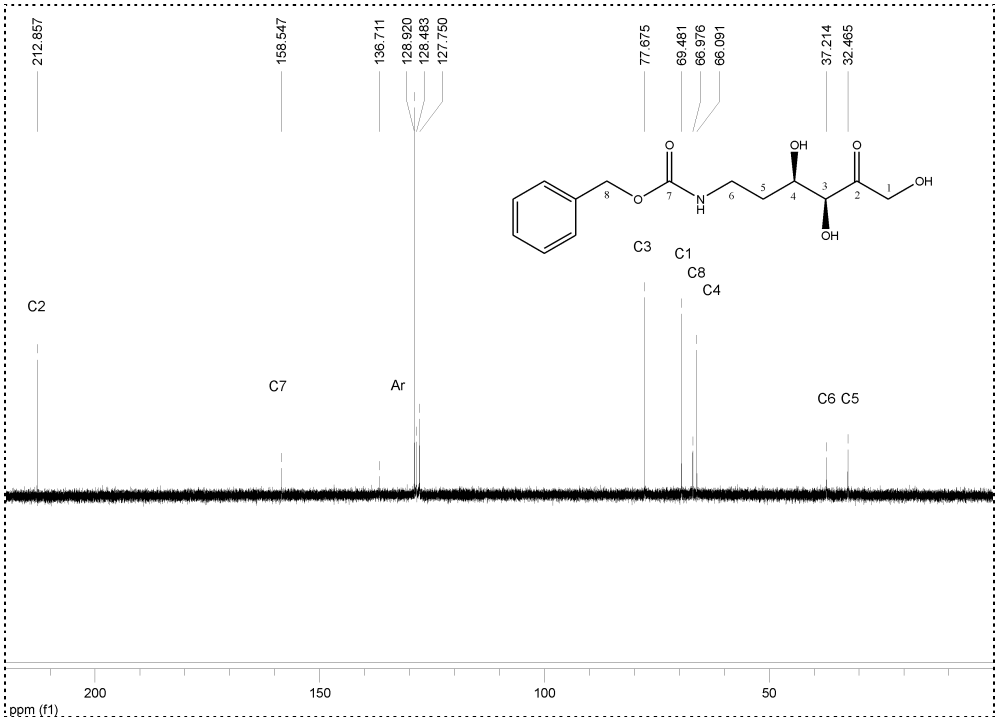
No.	RT	Area	Conc 1	BC
1	8,22	17482	0,316	BV
2	9,49	14418	0,260	VV
3	10,21	8511	0,154	VB
4	11,78	3442	0,062	BV
5	12,93	5157	0,093	BV
6	13,63	4349	0,079	VB
7	14,95	41718	0,754	BB
8	16,39	4174789	75,415	BB
9	18,21	6979	0,126	BB
10	19,17	29166	0,527	BB
11	20,24	689882	12,462	BV
12	21,02	156640	2,830	VV

NMR Spectra

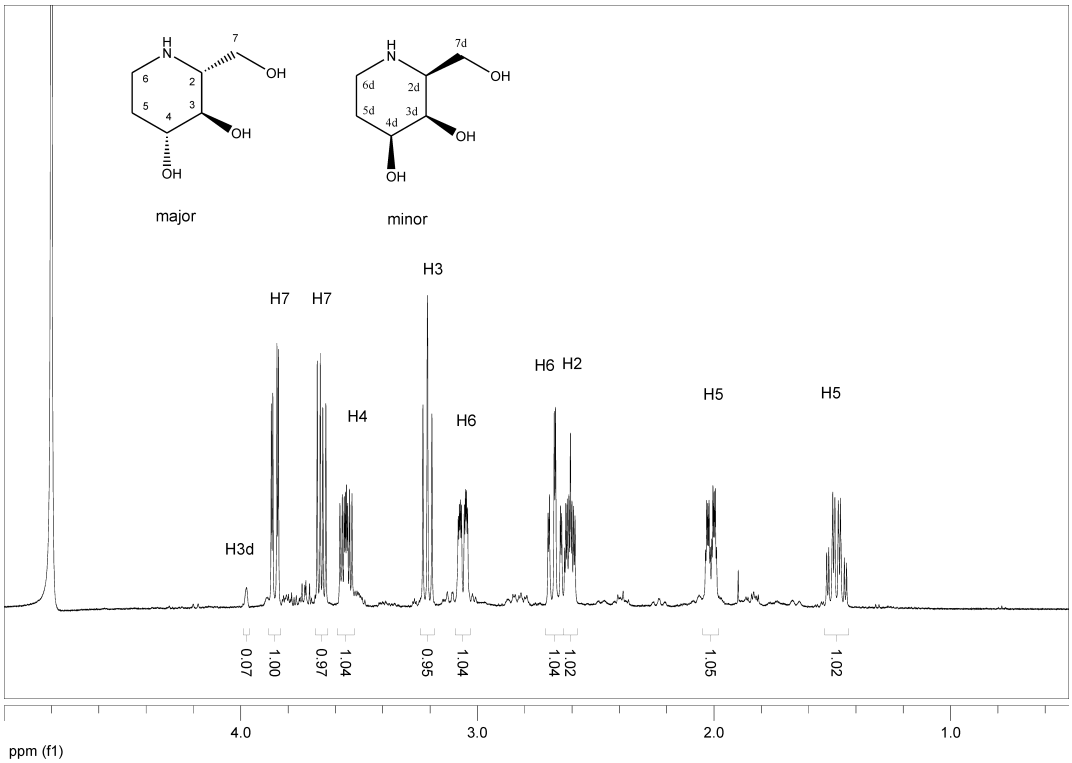
^{1}H-NMR spectrum of (3*S*,4*R*)-6-[(Benzyloxycarbonyl)amino]-5,6-dideoxyhex-2-ulose (2) in D_2O.



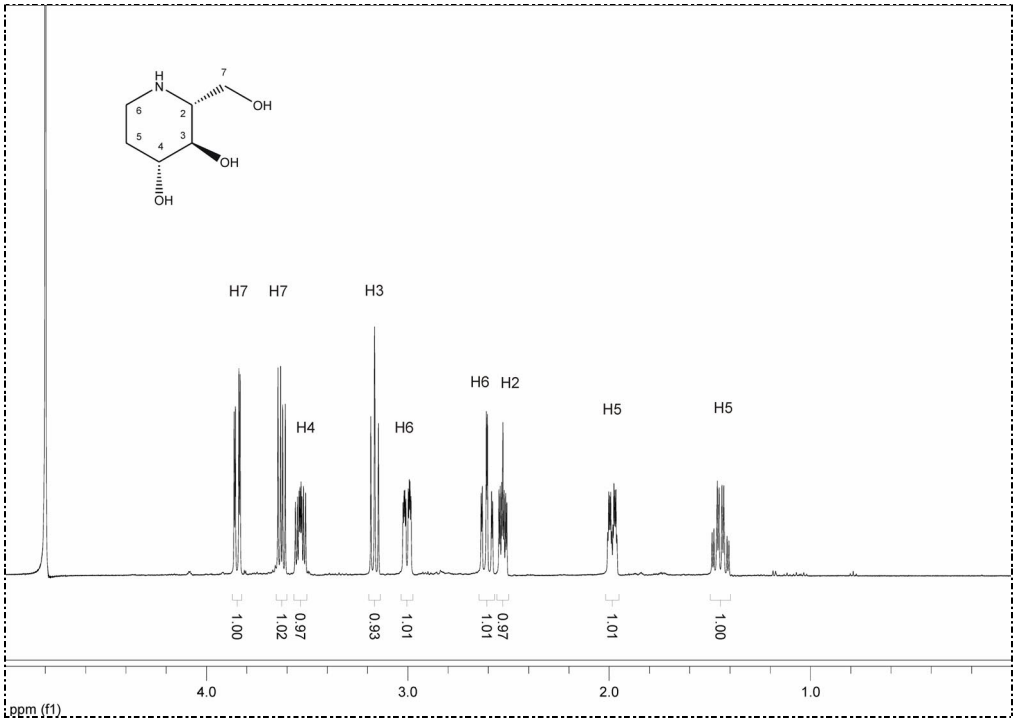
^{13}C-NMR spectrum of (3*S*,4*R*)-6-[(benzyloxycarbonyl)amino]-5,6-dideoxyhex-2-ulose (2) in D_2O.



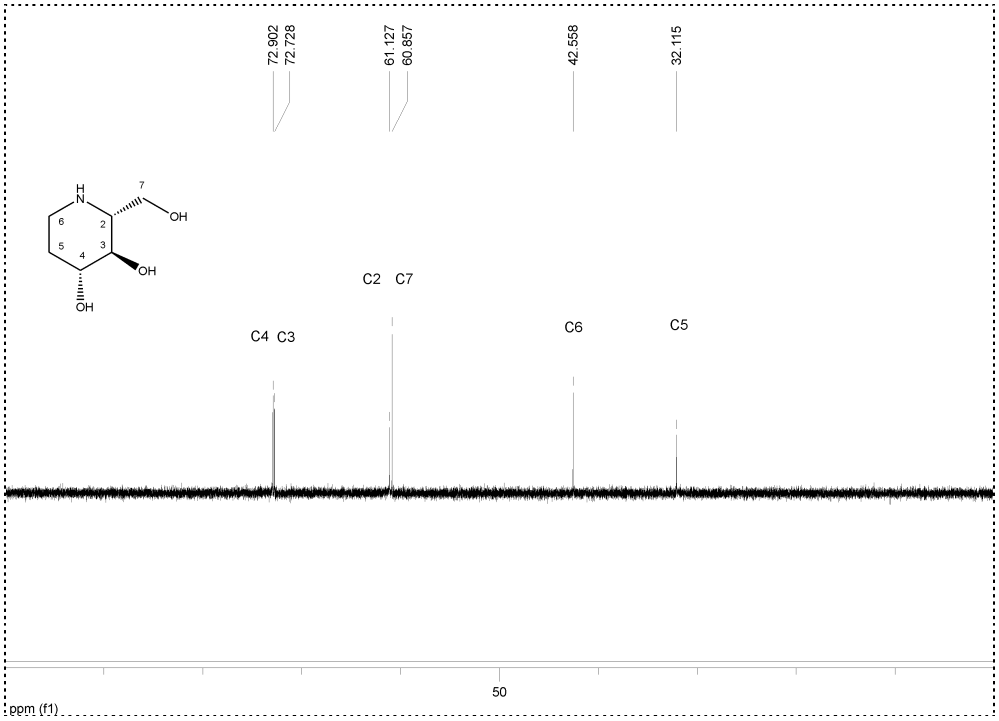
^{1}H-NMR spectrum of D-fagomine in D_2O before purification by cation exchange chromatography



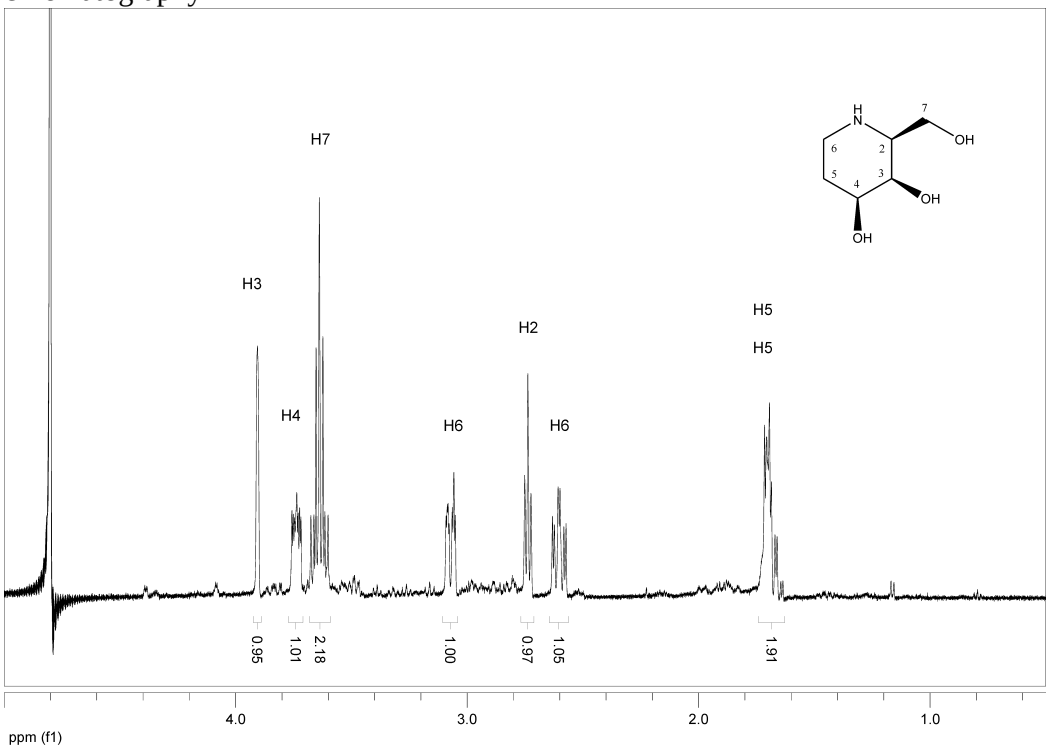
^{1}H-NMR spectrum of D-fagomine in D_2O purified by cation exchange chromatography:



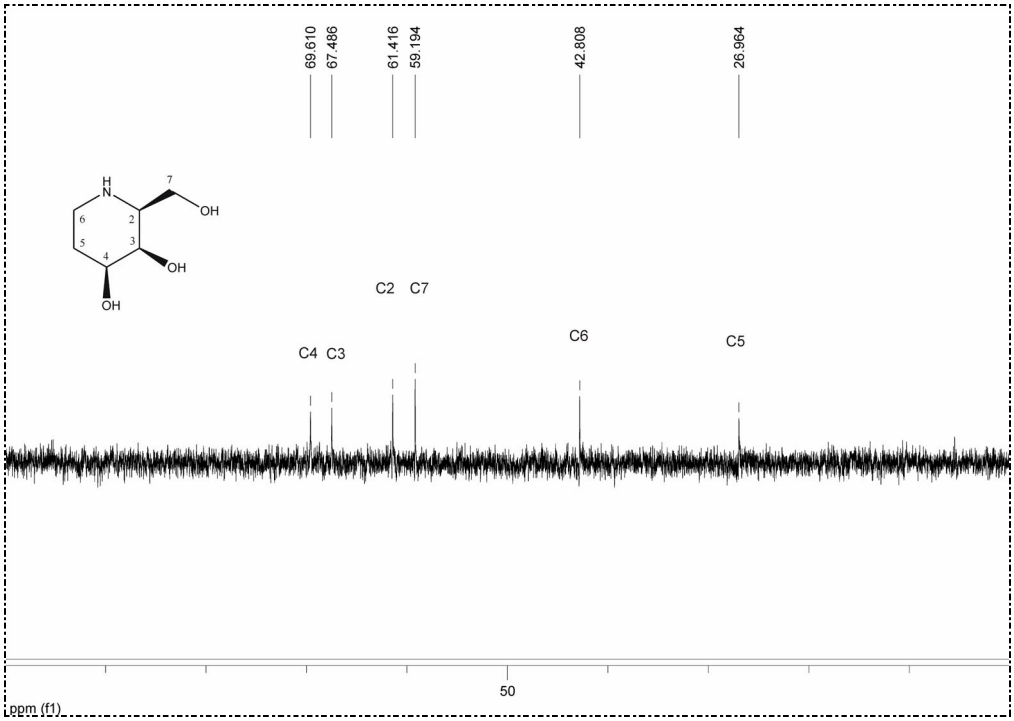
^{13}C-NMR spectrum of D-fagomine in D_2O purified by cation exchange chromatography



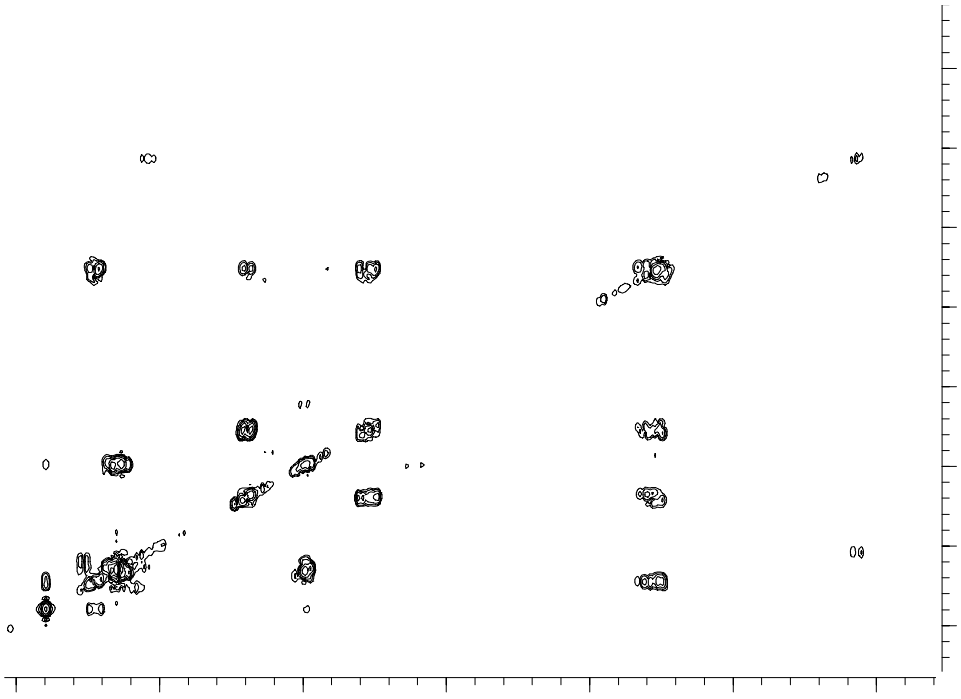
^{1}H-NMR spectrum of D-2,4-di-*epi*-fagomine in D_2O purified by cation exchange chromatography



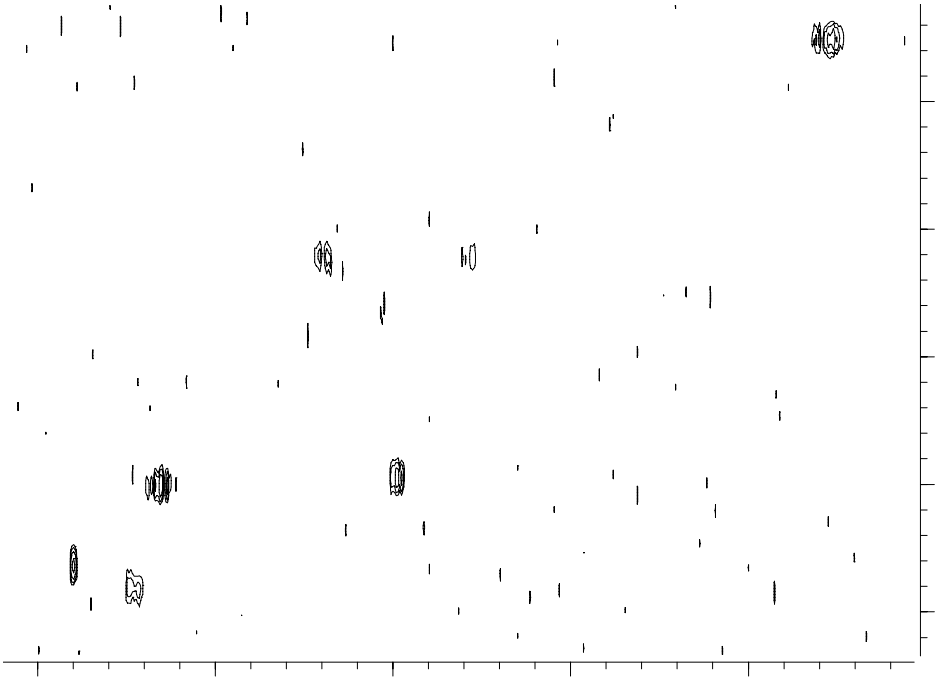
^{13}C-NMR spectrum of D-2,4-di-*epi*-fagomine in D_2O purified by cation exchange chromatography



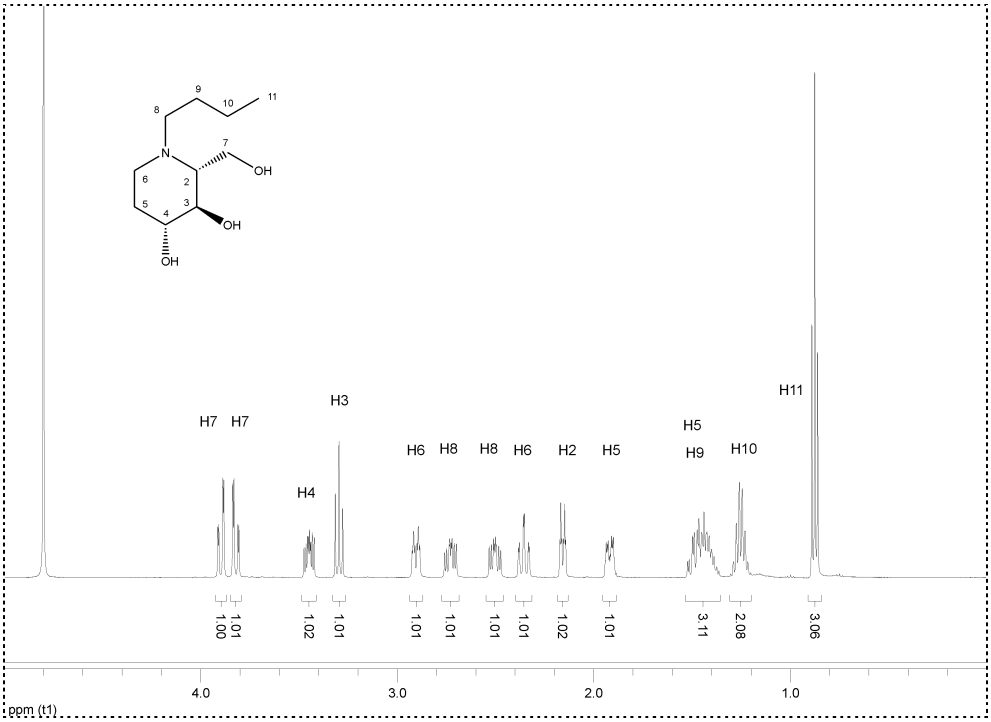
COSY spectrum of D-2,4-di-*epi*-fagomine in D_2O purified by cation exchange chromatography



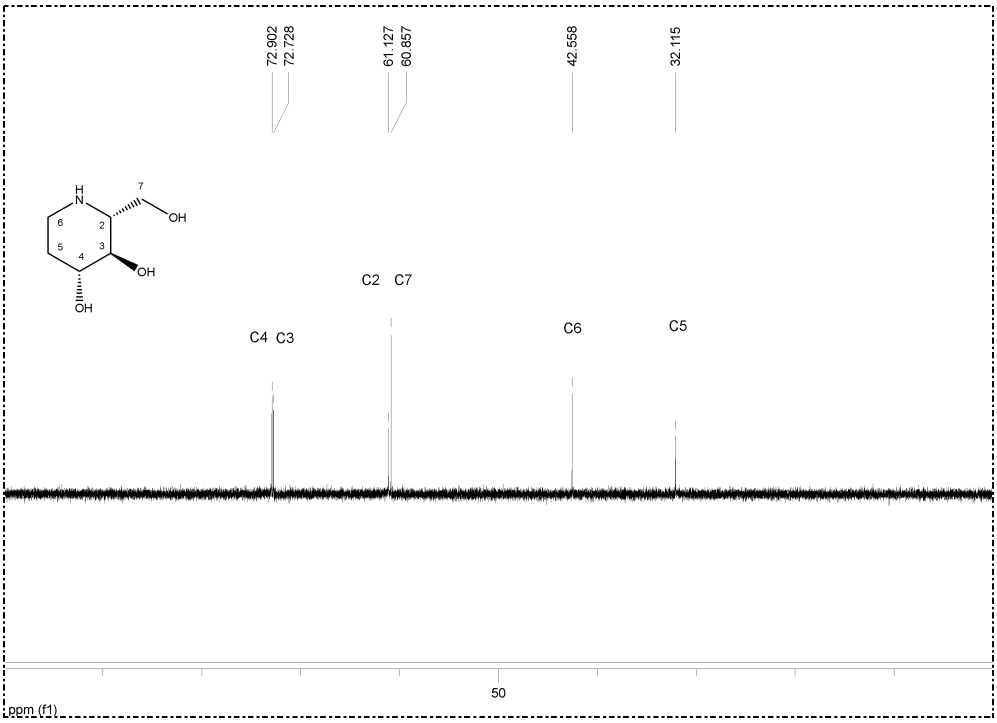
HSQC spectrum of D-2,4-di-*epi*-fagomine in D_2O purified by cation exchange chromatography



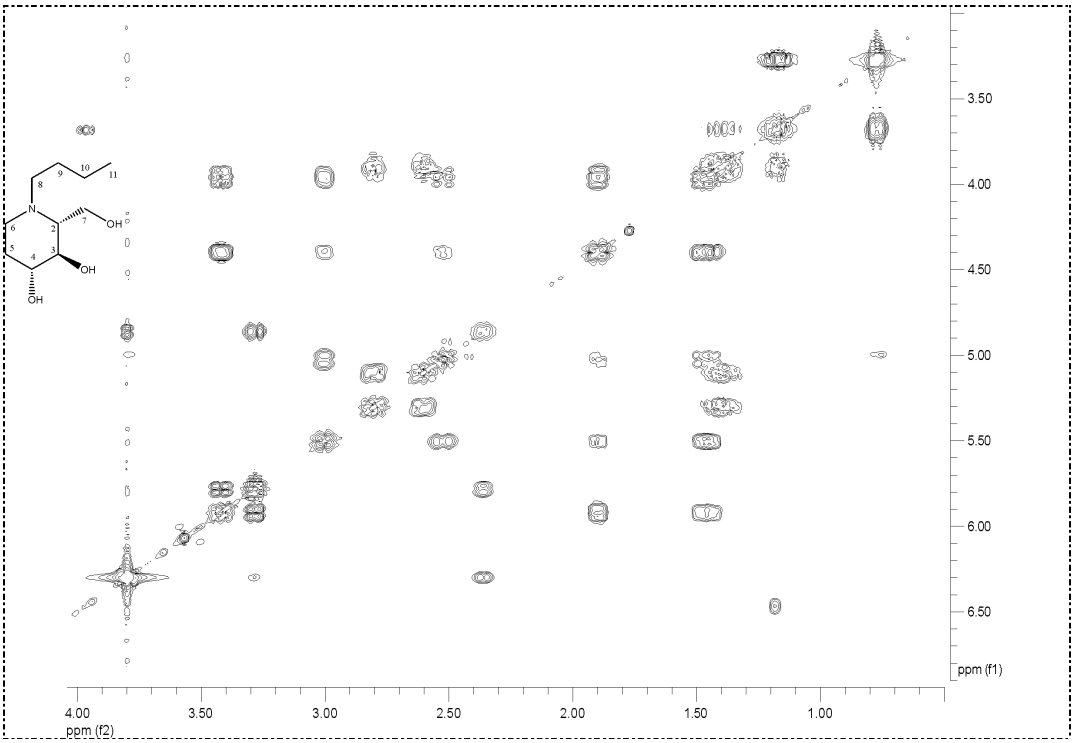
^{1}H-NMR spectrum of *N*-butyl-D-fagomine **4a** in D_2O:



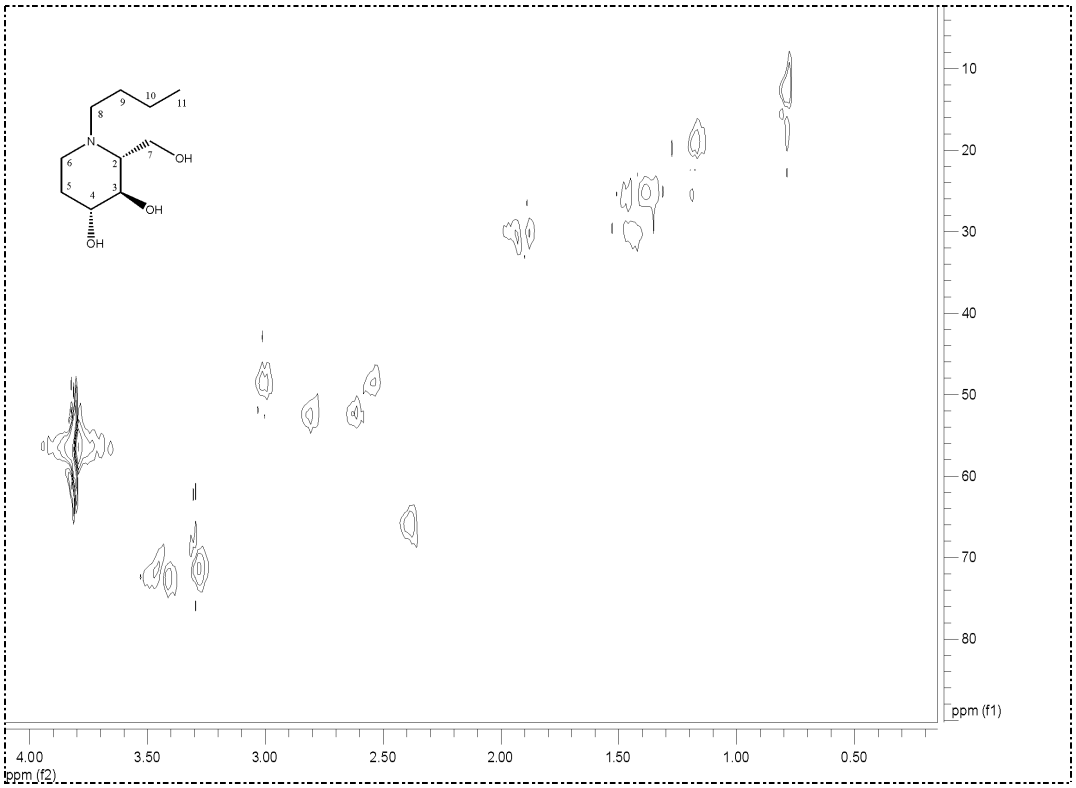
^{13}C-NMR spectrum of *N*-butyl-D-fagomine **4a** in D_2O:



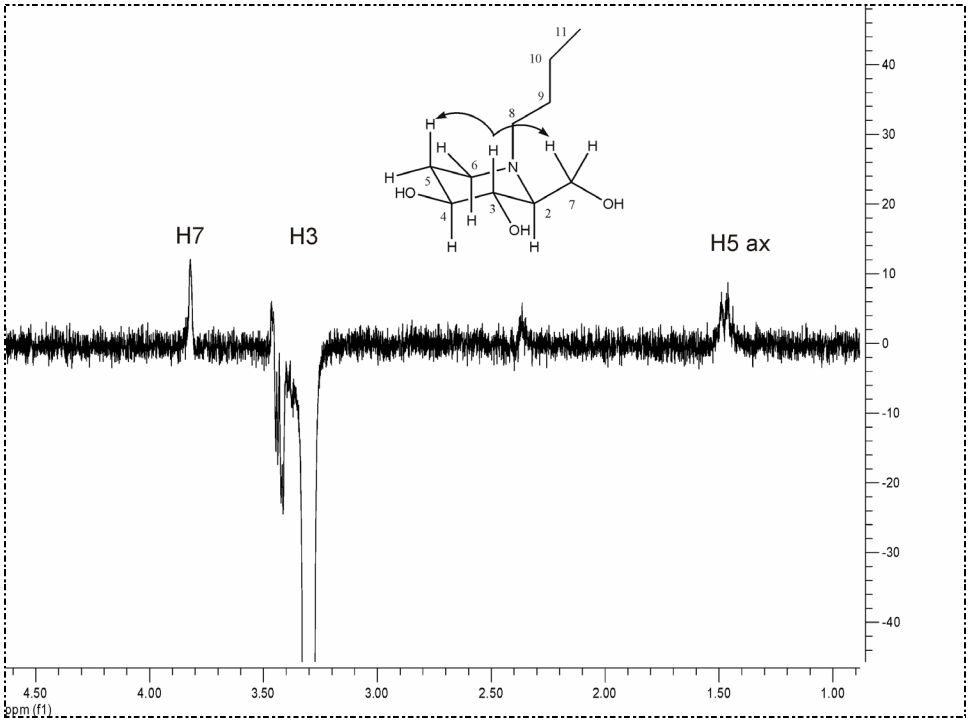
COSY-NMR spectrum of *N*-butyl-D-fagomine **4a** in D_2O:



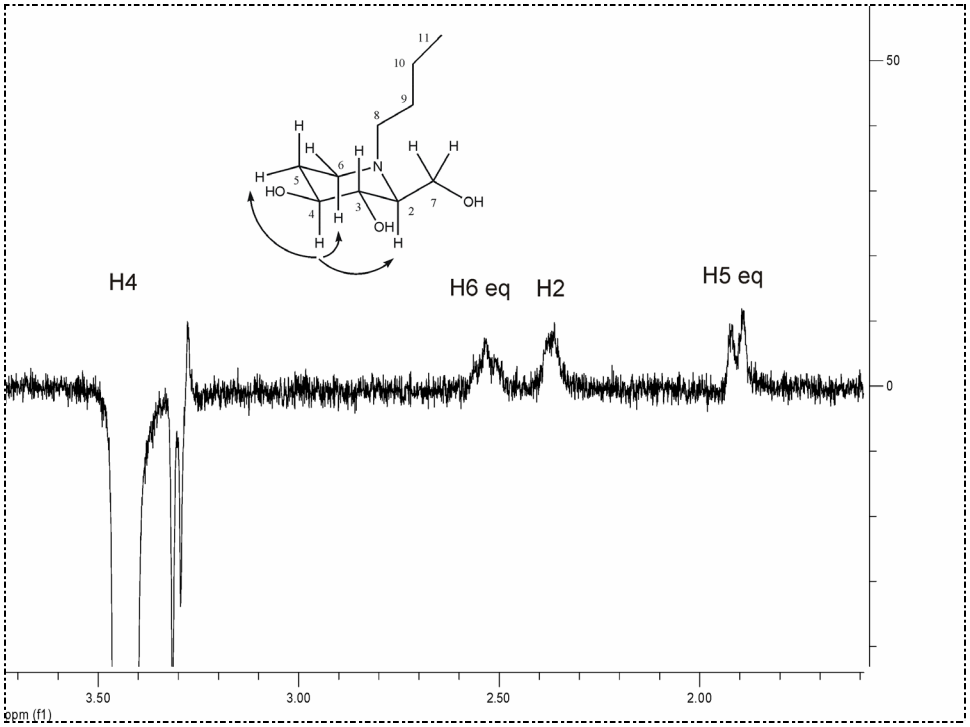
HSQC-NMR spectrum of *N*-butyl-D-fagomine **4a** in D_2O:



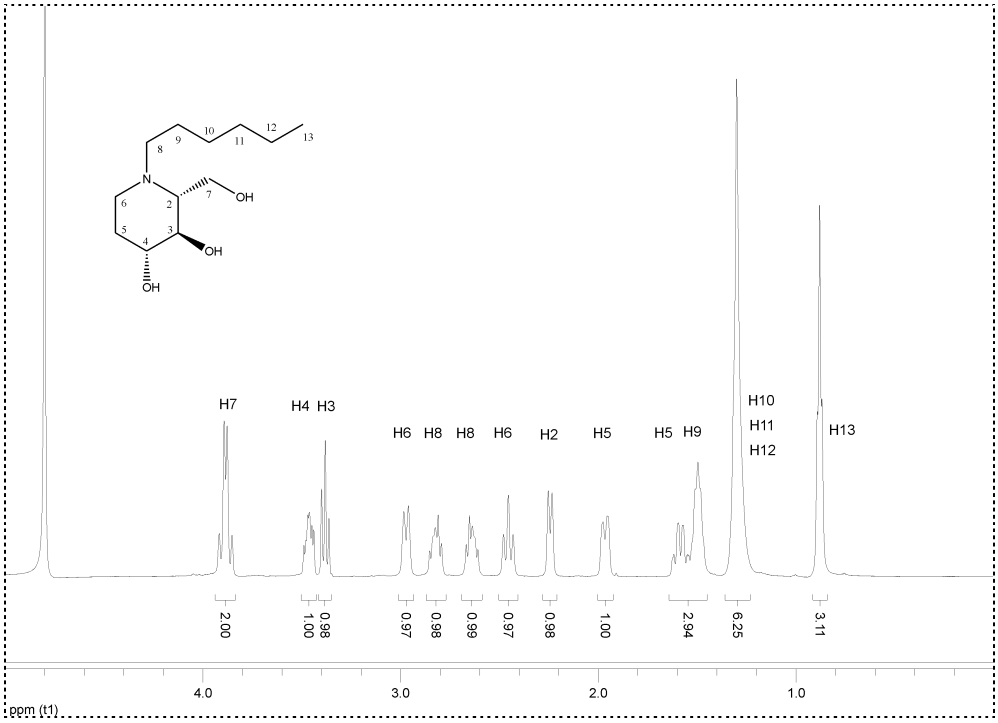
NOE-NMR spectrum of *N*-butyl-D-fagomine **4a** in D_2O:



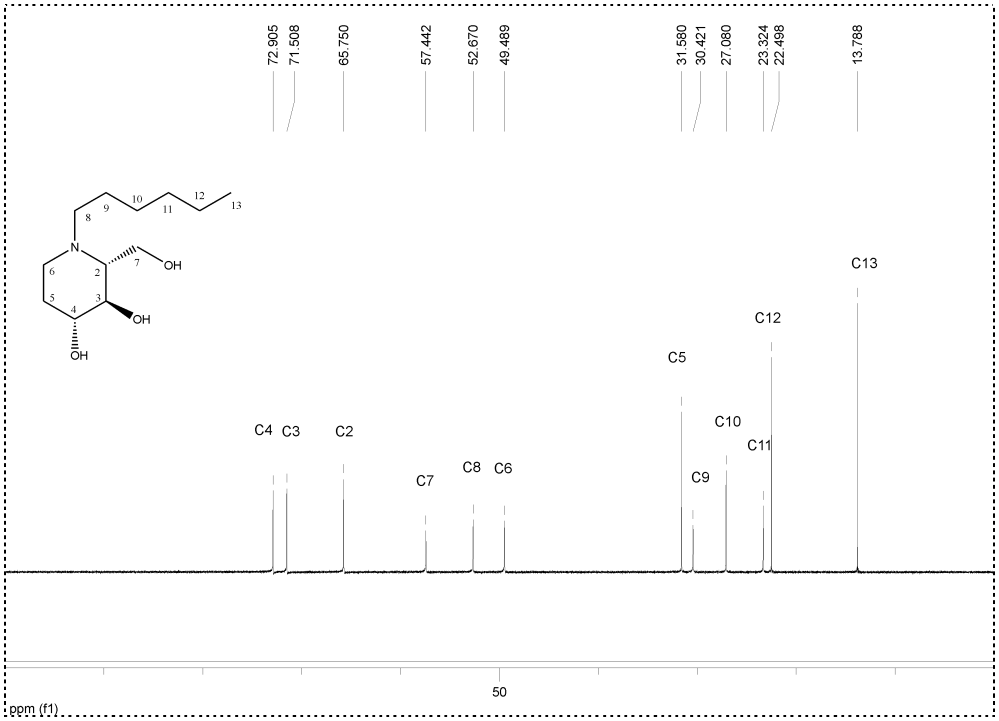
NOE-NMR spectrum of butyl-D-fagomine **4a** in D_2O:



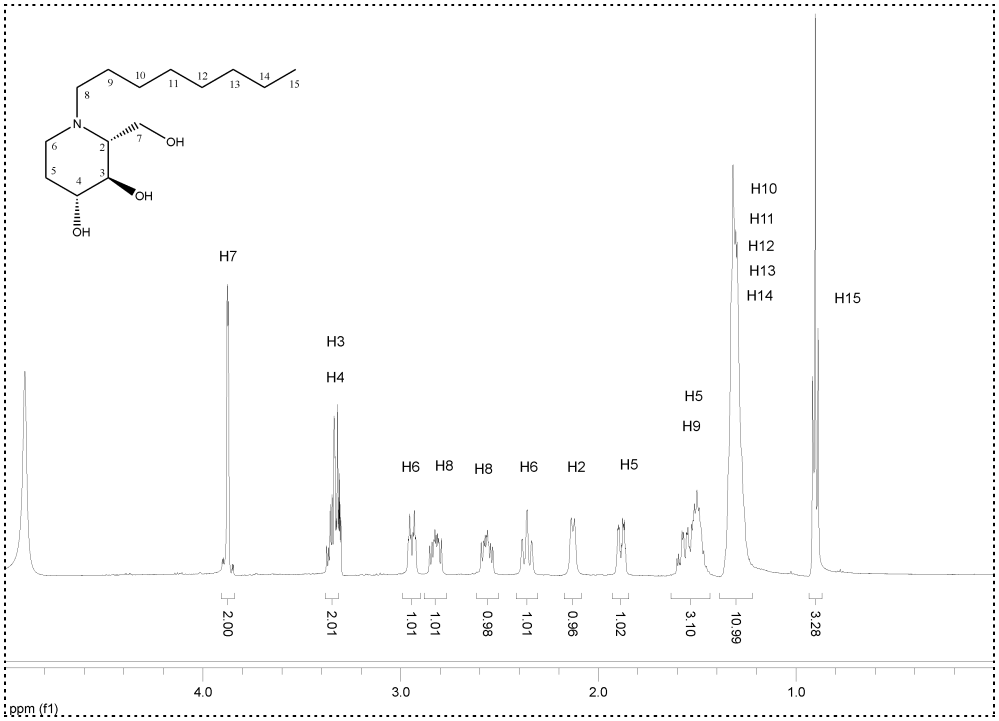
^{1}H-NMR spectrum of *N*-hexyl-D-fagomine **4b** in D_2O :



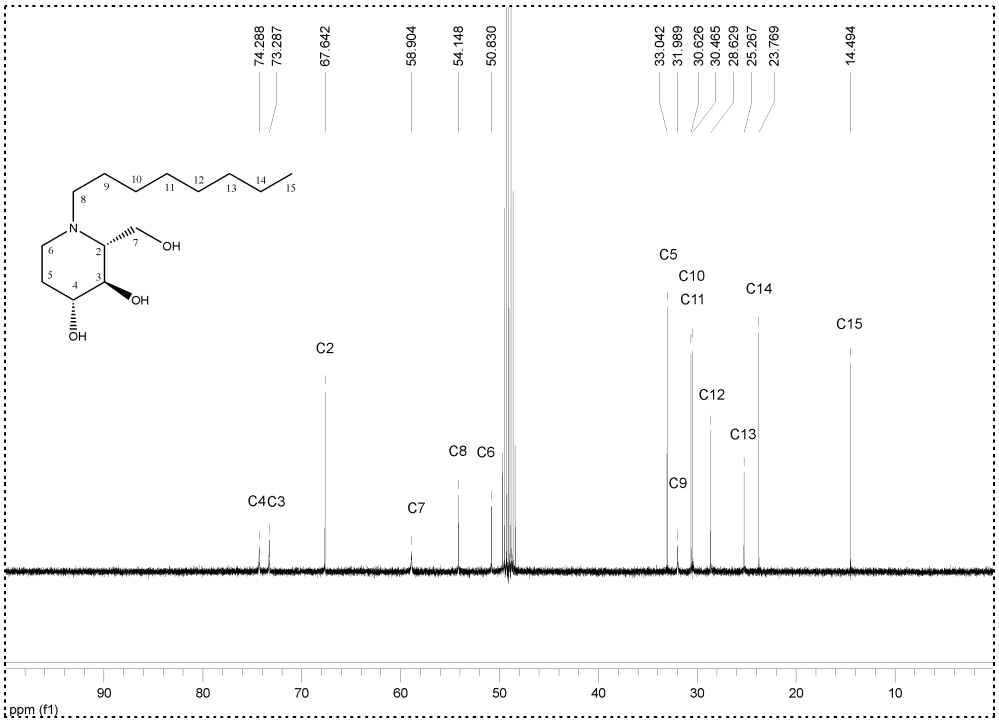
^{13}C-NMR spectrum of *N*-hexyl-D-fagomine **4b** in D_2O:



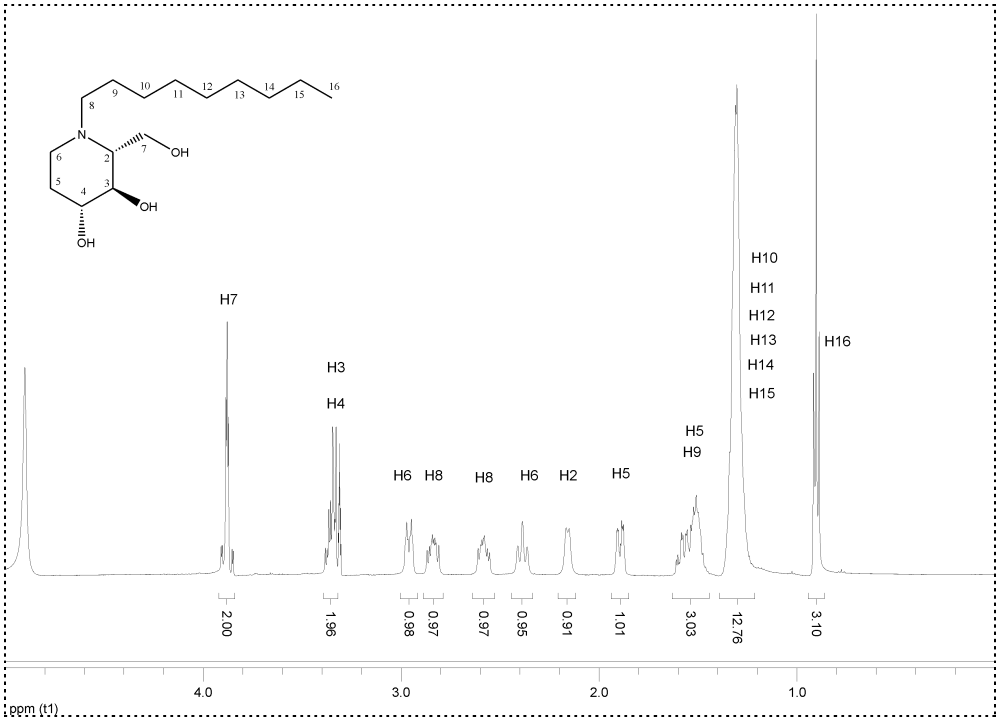
^{1}H-NMR spectrum of *N*-octyl-D-fagomine **4c** in CD_3OD:



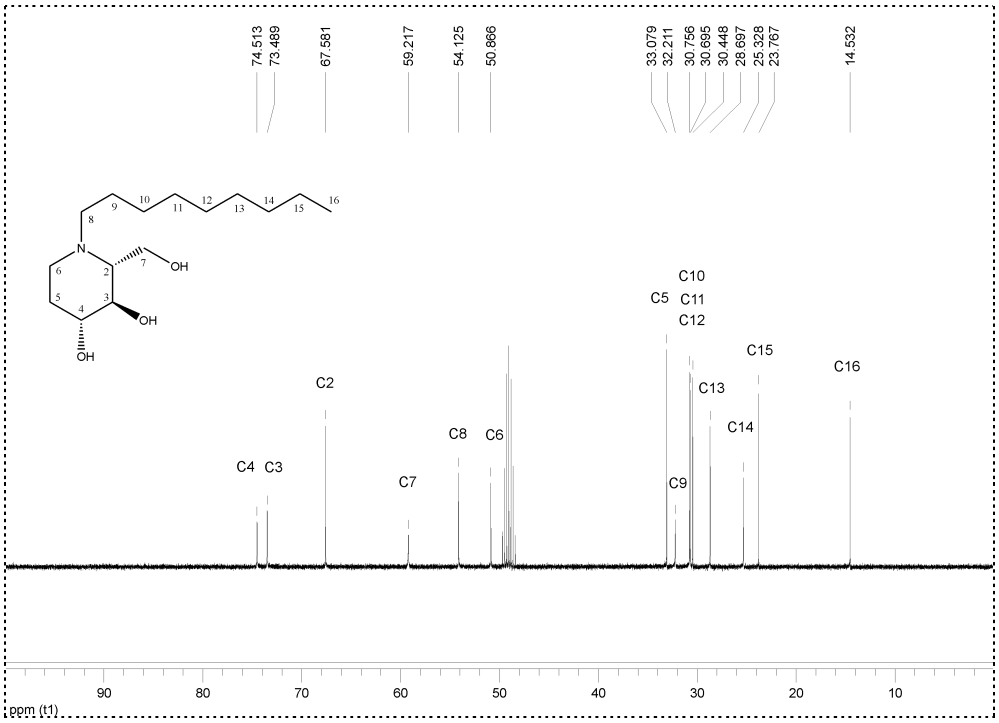
^{13}C-NMR spectrum of *N*-octyl-D-fagomine of **4c** in CD_3OD:



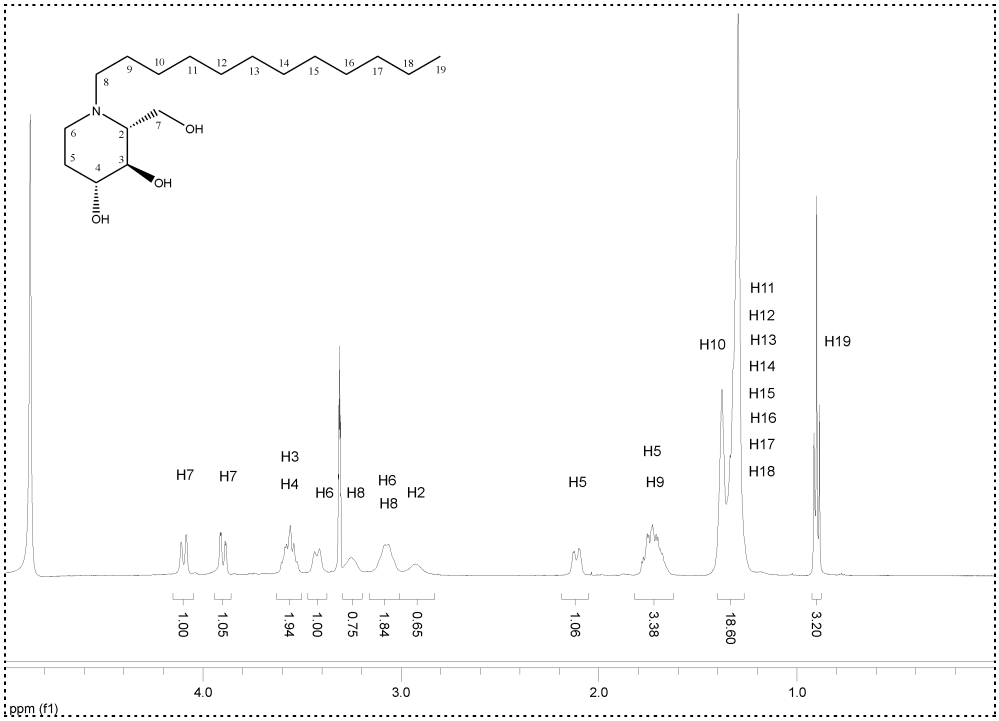
^{1}H-NMR spectrum of *N*-nonyl-D-fagomine **4d** in CD_3OD:



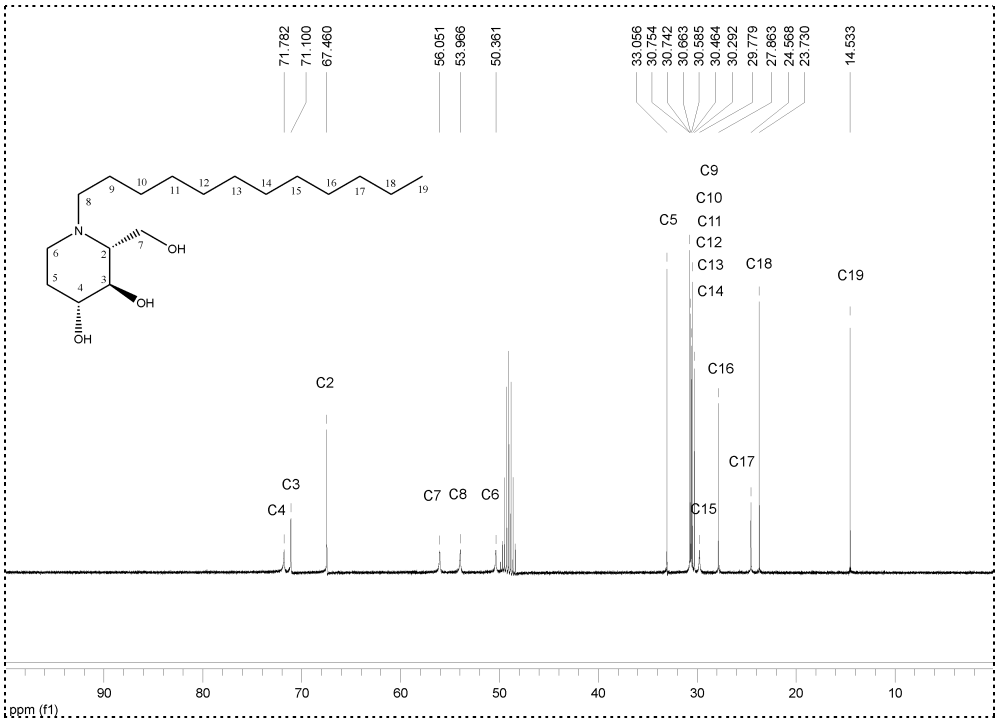
^{13}C-NMR spectrum of *N*-nonyl-D-fagomine **4d** in CD_3OD):



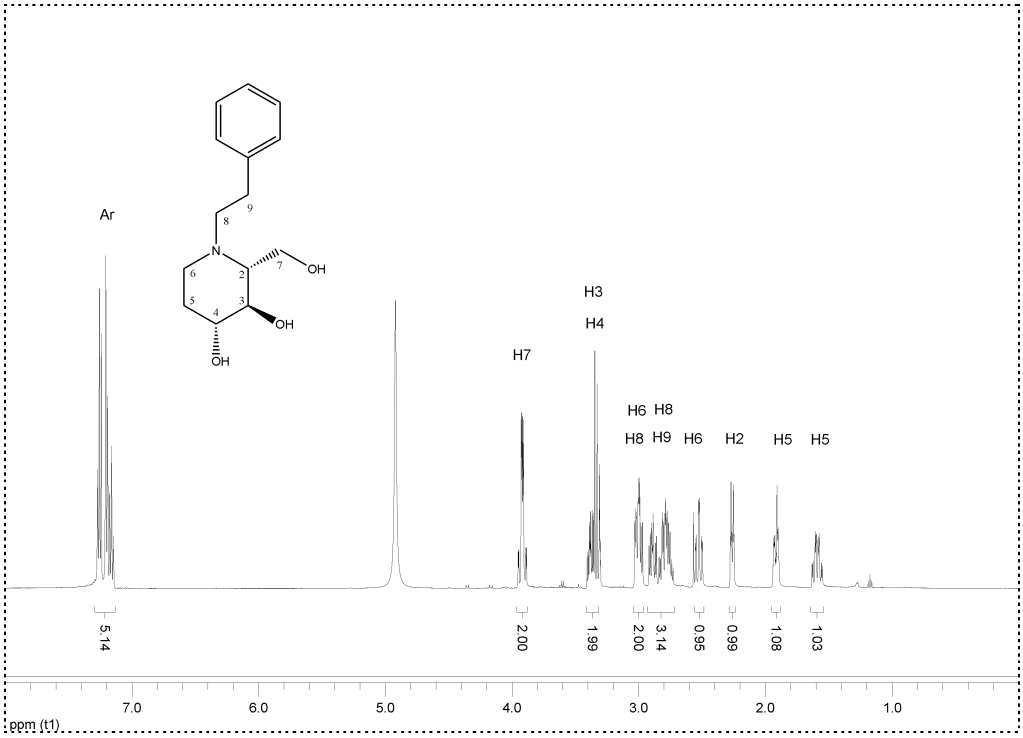
^{1}H-NMR spectrum of *N*-dodecyl-D-fagomine **4e** in CD_3OD:



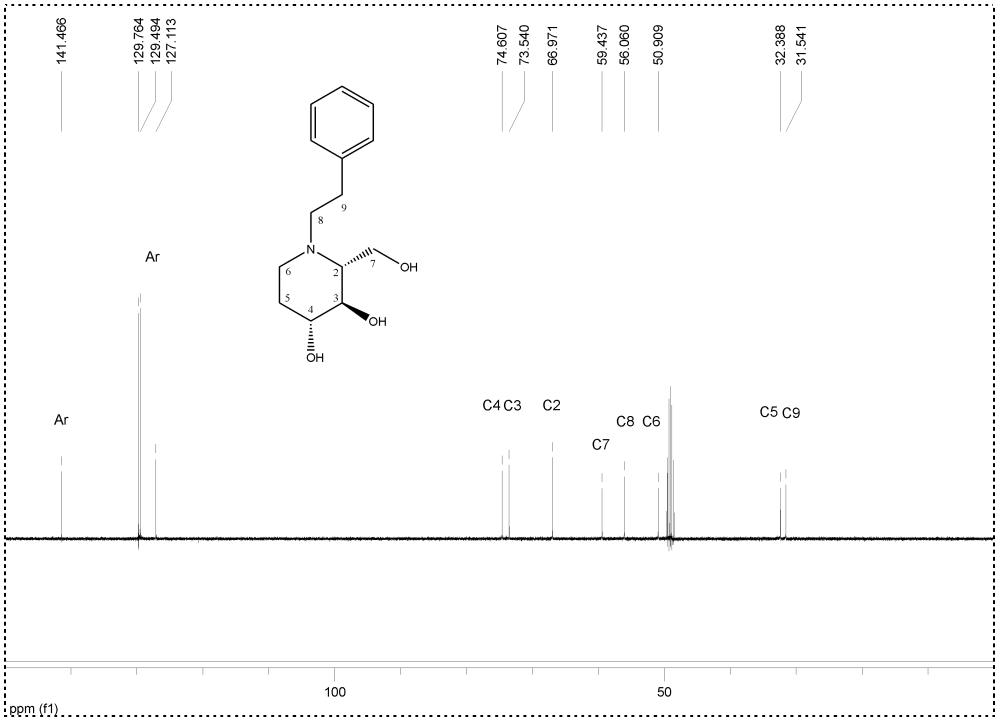
^{13}C-NMR spectrum of *N*-dodecyl-D-fagomine **4e** in CD_3OD:



^{1}H-NMR spectrum of *N*-phenylethyl-D-fagomine **4f** in CD_3OD:



^{13}C-NMR spectrum of *N*-phenylethyl-D-fagomine **4f** in CD_3OD:



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[1] M. Schürmann, G. A. Sprenger, *J. Biol. Chem.* **2001**, *276*, 11055-11061.

[2] *N*-benzyloxycarbonyl-3-aminopropanal was prepared by a two-step procedure following a previously described methodology: L. Espelt, T. Parella, J. Bujons, C. Solans, J. Joglar, A. Delgado, P. Clapés, *Chem.-Eur. J.* **2003**, *9*, 4887-4899; M. Ocejo, J. L. Vicario, D. Badia, L. Carrillo, E. Reyes, *Synlett* **2005**, 2110-2112.

[3] N. Asano, H. Kuroi, K. Ikeda, H. Kizu, Y. Kameda, A. Kato, I. Adachi, A. A. Watson, R. J. Nash, G. W. J. Fleet, *Tetrahedron: Asymmetry* **2000**, *11*, 1-8.

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[5] M. Mitjans, V. Martínez, P. Clapés, L. Pérez, M. R. Infante, M. P. Vinardell, *Pharm. Res.* **2003**, *20*, 1697-1701.