Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

69451 Weinheim, Germany

A Truly Practical Synthesis of a Phytosiderophore 2’-Deoxymugineic Acid. A Key to the Mechanistic Study of Iron Acquisition by Graminaceous Plants

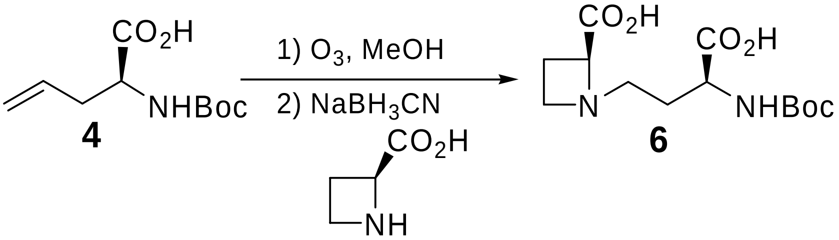
Kosuke Namba, Yoshiko Murata, Manabu Horikawa, Takashi Iwashita, and Shoichi Kusumoto*
Suntory Institute for Bioorganic Research
1-1-1 Wakayamadai, Shimamoto, Mishima, Osaka, 618-8503 (JAPAN)
Tel: (+81) 75-962-3749 Fax: (+81) 75-962-2115
E-mail: skus@sunbor.or.jp

General Procedures and Methods

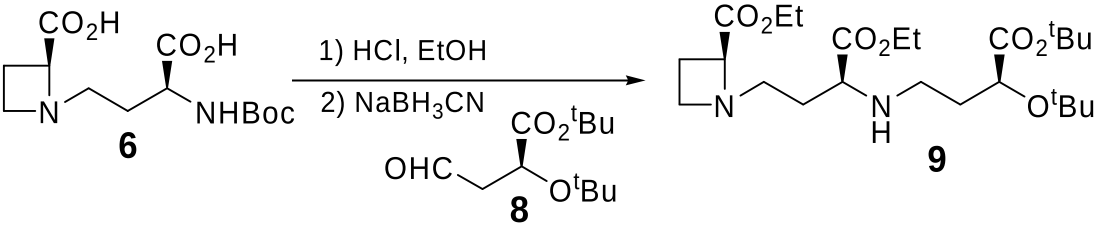
Melting points were determined with a Yanaco MP-21 melting point apparatus and were uncorrected. Optical rotations were taken on a JASCO DIP-1000 with a sodium lump (D line). FTIR spectra were measured on a JASCO FT/IR-410 infrared spectrophotometer. NMR spectra were recorded on a JEOL EX-400 or Varian mercury 300 spectrometer. Chemical shifts are reported in parts per million (ppm). For ^{1}H NMR spectra ($CDCl_3$, CD_3OD, and D_2O), the central residual solvent peak was used as the internal reference (7.24, 3.35, and 4.65 ppm, respectively), whereas the central solvent peak as the reference (77.0 and 49.3 ppm, respectively) for ^{13}C NMR spectra ($CDCl_3$ and CD_3OD). Elecrtospray mass spectra were recorded on a Bruker Daltonics Apex-Q94e FT-ICR-MS (9.4 T magnet). Analytical thin layer chromatography (TLC) was performed with E. Merck pre-coated TLC plates, silica gel 60F-254, layer thickness 0.25 mm. Flash chromatography was performed on E. Merck kieselgel 60 (230-400 mesh) silica gel. Reagents and solvents are commercial grade and were used as supplied. The starting materials (L-allylglycine, L-azetidine-2-carboxylic acid, and L-(–)-malic acid) were purchased from Aldrich and used as supplied.

All PCR experiments were performed with Taq^{EX} polymerase (Takara) on a thermal cycler, GeneAmp PCR system 9600 (Applied Biosystems). Subcloned inserts were sequenced on a genetic analyzer, ABI PRISMTM 310 (Applied Biosystems) with use of a Big-Dye sequencing kit (Applied Biosystem). The open reading frame (ORF) region of *HvYS1* or *ZmYS1* cDNA was inserted into the *Xenopus* expression vector pSP64 polyA (Promega). The plasmid was linearized with *Bam*H1, and cRNA for *HvYS1* or *ZmYS1* was transcribed *in vitro* with SP6 RNA polymerase by mMESSAGE mMACHINE kit (Ambion). The cRNA was injected in *Xenopus* oocytes with a microdispenser injector (Drumond). Electric currents were measured by two-electrode voltage clamp analysis on –60 mV at room temperature with an Oocyte clamp OC725C instrument (Warner Instrument) and Digital 1322A data acquisition system (Axon Instruments).

Stepwise synthesis of 9 **from** 4

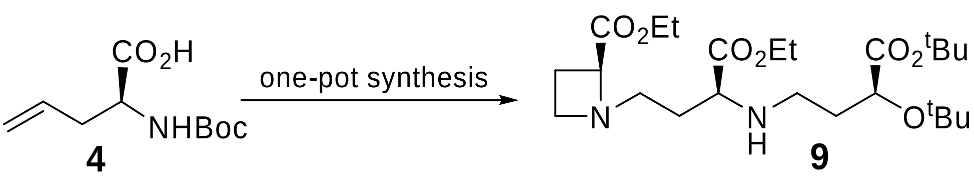


Ozone was bubbled through a solution of Boc-L-allylglycine (1.6 g, 7.4 mmol) in methanol (25 mL) at –78 °C until the color of solution changed to blue. After bubbling of nitrogen until the blue color was gone, to the solution were added L-azetidine-2-carboxylic acid (752 mg, 7.4 mmol) and $NaBH_3CN$ (470 mg, 7.4 mmol). The mixture was stirred for 1 h and concentrated under reduced pressure. The residue was purified by short-pass flash column chromatography on silica gel (elution with ethyl acetate/methanol = 5/1 to only methanol) to give **6** (2.1 g, 93%). IR (neat): ν = 3397, 2976, 1697, 1614, 1393, 1365 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 4.52 (t, J = 9.2 Hz, 1H), 3.85-4.30 (m, 2H), 3.82 (q, J = 9.2 Hz, 1H), 3.32 (m, 1H), 3.20 (m, 1H), 2.65 (m, 1H), 2.45 (m, 1H), 1.85-2.08 (m, 2H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 177.27, 172.79, 157.37, 80.37, 68.29, 53.75, 50.84, 49.85, 29.42, 28.76, 22.36; HRMS: m/z calcd for $C_{13}H_{23}N_2O_6^+$ $[M+H]^+$: 303.1556, found: 303.1551; $[\alpha]_D^{20}$ = –44.0 (c = 1.0, MeOH).



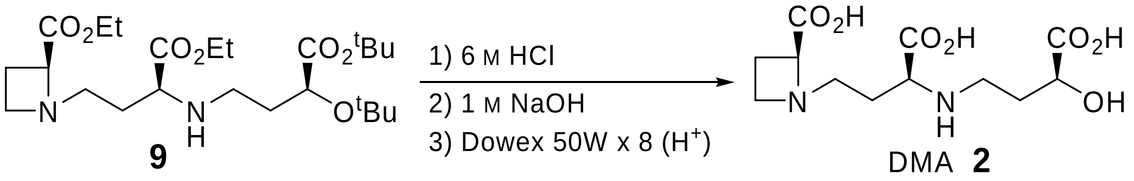
The suspension of **6** (1.7 g, 5.6 mmol) in cooled dry HCl/EtOH (prepared from 4.0 mL of acetyl chloride and 160 mL of ethanol) was stirred for 2 h at 0 °C, stirred for 15 h at room temperature, concentrated under reduced pressure, dehydrated by toluene azeotropy, and vacuumed for several hours. To the mixture of the residue in methanol (30 mL) were added aldehyde **8** (1.3 g, 5.6 mmol) and $NaBH_3CN$ (354 mg, 5.6 mmol) at room temperature. The mixture was stirred for 4 h, quenched with satd. $NaHCO_3$, and extracted with ethyl acetate (200 mL x 3). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (elution with hexane/ethyl acetate = 2/1 to only ethyl acetate containing 0.1 % triethylamine) to give protected DMA **9** (1.8 g, 68%) as colorless oil. IR (neat): ν = 3329, 2975, 1744, 1474, 1391, 1192 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 4.12-4.22 (m, 4H), 3.91 (t, *J* = 6.4 Hz, 1H), 3.58 (t, *J* = 8.8 Hz, 1H), 3.39 (br t, *J* = 6.0 Hz, 1H), 3.25 (br t, *J* = 6.0 Hz, 1H), 2.82 (q, *J* = 7.6 Hz, 1H), 2.61-2.75 (m, 2H), 2.55-2.59 (m, 2H), 2.32 (m, 1H), 2.21 (m, 1H), 1.58-1.80 (m, 4H), 1.45 (s, 9H), 1.27 (t, *J* = 7.2 Hz, 3H), 1.26 (t, *J* = 7.2 Hz, 3H), 1.16 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ =174.38, 173.36, 172.07, 80.07, 74.11, 69.68, 64.77, 60.16, 60.11, 59.21, 55.06, 50.68, 43.71, 33.93, 30.65, 27.56, 27.49, 20.96, 13.95, 13.86; HRMS: m/z calcd for $C_{24}H_{44}N_2O_7^+$ $[M+H]^+$: 473.3229, found: 473.3221; $[\alpha]_D^{20}$ = –92.8 (*c* = 1.0, $CHCl_3$).

One-pot synthesis of 9 **from** 4



Ozone was bubbled through a solution of Boc-L-allylglycine (1.2 g, 5.7 mmol) in methanol (40 mL) at –78 °C until the color of solution changed to blue. After bubbling of nitrogen until the blue color was gone, to the solution was added L-azetidine-2-carboxylic acid (564 mg, 5.7 mmol) and $NaBH_3CN$ (351 mg, 5.7 mmol). The mixture was stirred for 2 h at room temperature, and concentrated under reduced pressure. The suspension of the residue in cooled dry HCl /EtOH (prepared from 4.5 mL of acetyl chloride and 100 mL of ethanol) was stirred for 2 h at 0 °C, stirred for 15 h at room temperature, concentrated under reduced pressure, dehydrated by toluene azeotropy, and vacuumed for several hours. To the mixture of the residue in methanol (50 mL) was added aldehyde **8** (1.3 g, 5.7 mmol) and $NaBH_3CN$ (351 mg, 5.7 mmol) at room temperature. The mixture was stirred for 4 h, quenched with satd. $NaHCO_3$, and extracted with ethyl acetate (x 3). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (elution with hexane/ethyl acetate = 2/1 to only ethyl acetate containing 0.1 % triethylamine) to give protected DMA **9** (1.44 g, 55%) as colorless oil.

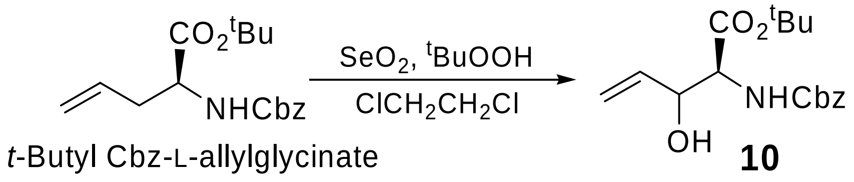
Deprotection of 9 to DMA 2



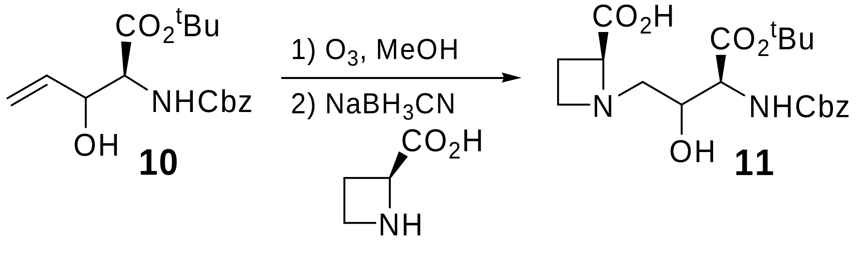
A suspension of **9** (2.1 g, 4.4 mmol) in cooled 6 M HCl (40 mL) was stirred for 10 h at room tempetrature and concentrated under reduced pressure. The mixture of the residue in 1 M NaOH (40 mL) was stirred for 10 h at room temperature. The mixture was neutralized by 1 M HCl and concentrated under reduced pressure. The residue was purified by column chromatography on ion-exchange resin (Dowex 50W x 8) (elution with water to 1 M NH_3) to give DMA ammonium salts (1.3 g, 97%) as white solid. Further purification by recrystallization (from H_2O-MeOH-EtOH) gave pure DMA **2** (950 mg, 71%). IR (neat): ν = 3381, 3041, 1644, 1414, 1093 cm^{-1}; ^1H NMR (300 MHz, D_2O): δ = 4.63 (t, J = 9.6 Hz, 1H), 4.01 (dd, J = 6.6, 4.5 Hz, 1H), 3.97 (td, J = 9.6, 4.2 Hz, 1H), 3.83 (app. q, J = 9.6 Hz, 1H), 3.64 (dd, J = 7.8, 3.6 Hz, 1H), 3.18-3.38 (m, 2H), 2.96-3.14 (m, 2H), 2.60 (qd, J = 9.6, 4.2 Hz, 1H), 2.41 (dt, J = 11.7, 9.3 Hz, 1H), 1.70-2.18 (m, 4H); ^{13}C NMR (75 MHz, D_2O): δ = 180.05, 173.21, 172.17, 70.32, 66.90, 59.61, 51.24, 50.50, 44.25, 30.45, 24.54, 21.12; HRMS: m/z calcd for $C_{12}H_{19}N_2O_7^-$

[M-H]$^-$: 303.1200, found: 303.1198; $[\alpha]_D^{22}$ = –69.2 (*c* = 1.0, H_2O, pH = 6.5) (lit = –70.5[6]).

Synthesis of MA 1 and 2’-epi-MA 3 from *t*-butyl Cbz-L-allylglycinate

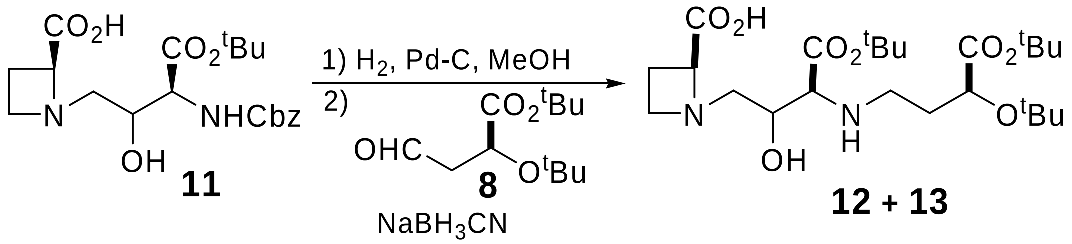


To a solution of *t*-butyl Cbz-L-allylglycinate (1.4 g, 4.6 mmol) in 1,2-dichloroethane (30 mL) was added selenium dioxide (510 mg, 4.6 mmol) and ^{t}BuOOH (3.3 mL of 5.5 M solution in decane, 18.4 mmol). The mixture was stirred for 15 h at 70 °C, quenched with satd. $NaHCO_3$, and extracted with ethyl acetate (x 2). The combined organic layers were washed with satd. $Na_2S_2O_3$, dried over anhydrous $MgSO_4$, filtered, concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (elution with hexane/ethyl acetate = 9/1 to 4/1) to give **10** (812 mg, 55%) as colorless oil. IR (neat): ν = 3422, 2978, 1718, 1509, 1455, 1369 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.30-7.45 (m, 5H), 5.90 (ddd, *J* = 16.8, 10.4, 5.2 Hz, 1H), 5.46 (br d, *J* = 7.2 Hz, 1H), 5.36 (d, *J* = 16.8 Hz, 1H), 5.24 (d, *J* = 10.4 Hz, 1H), 5.12 (s, 2H), 4.60 (br s, 1H), 4.36 (d, *J* = 6.8 Hz, 1H), 2.19 (s, 1H), 1.49 (s, 9H) as a major isomer; δ = 7.30-7.45 (m, 5H), 5.81 (ddd, *J* = 16.4, 10.8, 5.6 Hz, 1H), 5.59 (br s, 1H), 5.36 (d, *J* = 16.4 Hz, 1H), 5.24 (d, *J* = 10.8 Hz, 1H), 5.12 (s, 2H), 4.55 (br s, 1H), 4.47 (br d, *J* = 6.8 Hz, 1H), 3.15 (br s, 1H), 1.46 (s, 9H) as the minor isomer; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.32, 156.31, 136.35, 128.29, 128.23, 127.98, 127.86, 116.54, 82.50, 72.69, 66.87, 58.56, 27.90 as the major isomer; δ = 169.32, 156.31, 136.08, 128.23, 127.98, 127.86, 127.78, 117.01, 82.50, 72.69, 67.13, 27.90 as a minor isomer; HRMS: m/z calcd for $C_{17}H_{23}NO_5^+$ $[M+Na]^+$: 344.1471, found : 344.1468.

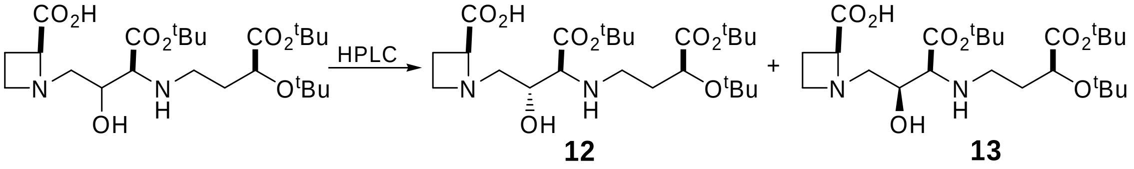


Ozone was bubbled through a solution of **10** (550 mg, 1.7 mmol) in methanol

(15 mL) at –78 °C until the color of the solution changed to blue. After bubbling of nitrogen until the blue color was gone, to the solution was added L-azetidine-2-carboxylic acid (173 mg, 1.7 mmol) and $NaBH_3CN$ (107 mg, 1.7 mmol). The mixture was stirred for 2 h and concentrated under reduced pressure. The residue was purified by short-pass flash column chromatography on silica gel (elution with ethyl acetate/methanol = 5/1 to only methanol) to give **6** (595 mg, 86%) as colorless oil. IR (neat): ν = 3290, 2978, 1729, 1633, 1516 cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ = 7.25-7.40 (m, 5H), 5.10 (s, 2H), 4.74 (t, J = 9.6 Hz, 1H), 4.48 (d, J = 10.8 Hz, 1H), 4.27 (br s, 1H), 4.01 (td, J = 9.6, 4.0 Hz, 1H), 3.89 (q, J = 9.6 Hz, 1H), 3.32 (d, J = 12.4 Hz, 1H), 3.17 (t, J = 12.4 Hz, 1H), 2.48-2.68 (m, 2H), 1.45 (s, 9H) as a major isomer; δ 7.25-7.40 (m, 5H), 5.10 (s, 2H), 4.74 (t, J = 9.6 Hz, 1H), 4.48 (d, J = 10.8 Hz, 1H), 4.17 (br s, 1H), 4.01 (td, J = 9.6, 4.0 Hz, 1H), 3.89 (q, J = 9.6 Hz, 1H), 3.41 (t, J = 12.4 Hz, 1H), 3.32 (d, J = 12.4 Hz, 1H), 2.48-2.68 (m, 2H), 1.45 (s, 9H) as a minor isomer; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.92, 170.04, 158.61, 137.88, 129.37, 128.98, 128.86, 83.74, 69.08, 68.40, 67.89, 67.42, 58.83, 52.55, 28.31, 23.17; HRMS: m/z calcd for $C_{20}H_{28}N_2O_7^+$ $[M+H]^+$: 409.1976, found: 409.1969.

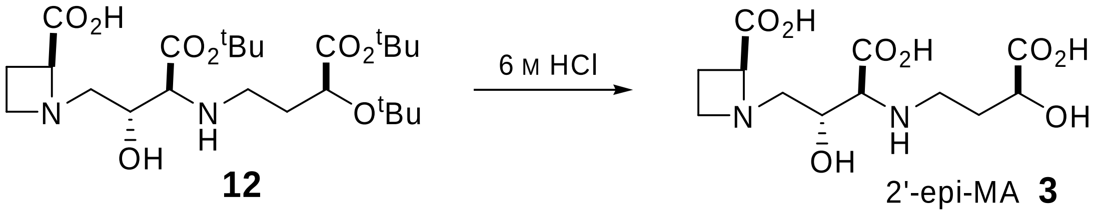


To a solution of **11** (450 mg, 1.1 mmol) in methanol (10 mL) was added 10% palladium on carbon (90 mg, 20 wt%). The mixture was stirred for 2 h under hydrogen, flashed with argon, filtered, and concentrated under reduced pressure. To a solution of the residue in methanol (10 mL) were added acetic acid for adjusting to pH 4 ~ 6 (66 μL, 1.1 mmol), aldehyde **8** (254 mg, 1.1 mmol), and $NaBH_3CN$ (70 mg, 1.1 mmol). The mixture was stirred for 2 h at room temperature and then concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (elution with ethyl acetate/methanol = 4/1 to chloroform/methanol = 4/1) to give a mixture of **12** and **13** (480 mg, 89%) as colorless oil. IR (neat): ν = 3290, 2975, 1731, 1634, 1455 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 4.52 (t, J = 9.2 Hz, 1H), 4.15 (dt, J = 6.8, 3.2 Hz, 1H), 4.04 (dd, J = 7.6, 5.2 Hz, 1H), 3.93 (td, J = 9.2, 4.4 Hz, 1H), 3.77 (q, *J* = 9.2 Hz, 1H), 3.25-3.40 (m, 3H), 2.89 (dt, *J* = 12.0, 7.6 Hz, 1H), 2.55-2.75 (m, 2H), 2.46 (m, 1H), 1.78-1.87 (m, 2H), 1.50 (s, 9H), 1.47 (s, 9H), 1.19 (s, 9H) as a major isomer **12**; δ 4.60 (t, *J* = 9.2 Hz, 1H), 3.90-4.06 (m, 3H), 3.81 (q, *J* = 9.2 Hz, 1H), 3.25-3.40 (m, 3H), 2.80 (m, 1H), 2.40-2.75 (m, 3H), 1.78-1.87 (m, 2H), 1.50 (s, 9H), 1.47 (s, 9H), 1.19 (s, 9H) as a minor isomer **13**; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 175.46, 174.25, 171.63, 83.48, 82.31, 76.10, 71.25, 69.49, 68.36, 65.71, 60.38, 52.58, 45.68, 34.66, 28.39, 28.27, 28.24, 23.36 as a major isomer **12**; δ = 175.28, 173.65, 171.41, 83.83, 82.34, 76.17, 71.31, 69.22, 68.40, 66.62, 59.46, 51.90, 45.48, 34.24, 28.44, 28.31, 28.21, 23.07 as a minor isomer **13**; HRMS: m/z calcd for $C_{24}H_{44}N_2O_8^+$ $[M+H]^+$: 489.3170, found: 489.3170.

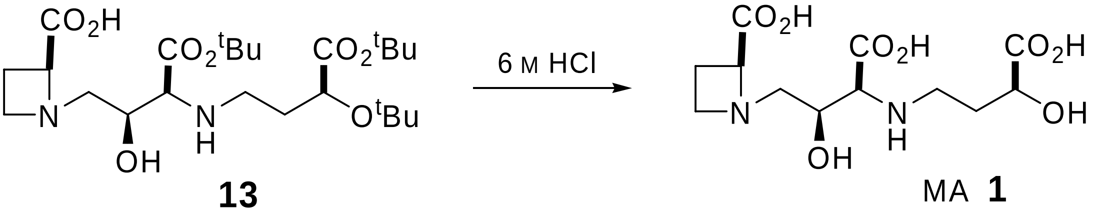


HPLC conditions for separation of **12** and **13:** column: CAPCELLPAK C18-UG80 (5 μm) (Shiseido); flow rate: 9.999 mL/min; elution: 35% CH_3CN-H_2O (1% AcOH); retention times: minor **13**: 14.0 min, major **12**: 16.3 min.

The respective fractions were concentrated separately under reduce pressure to give **12** and **13**, respectively.

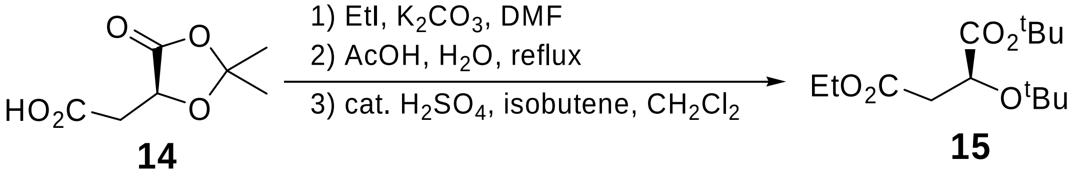


A mixture of **12** (122 mg, 0.25 mmol) in 6 M HCl (20 mL) was stirred for 6 h, concentrated under reduced pressure. The residue was purified by column chromatography on ion-exchange resin (Dowex 50W x 8) (elution with water to 5% NH_4OH) to give 2’-epi-MA ammonium salts as white solid. Further purification by DIANION HP20 (elution with water) was followed by lyophilization to give pure 2’-epi-MA **3** (81 mg, quant.) as white powder. IR (film, cm^{-1}) 3213, 3051, 2839, 1610, 1412, 1329, 1238, 1107, 934, 773; ^{1}H NMR (400 MHz, D_2O, pH = 4.53 adjusted by the addition of 1 M DCl): δ = 1.96-2.05 (1H, m), 2.10-2.20 (1H, m), 2.51-2.61 (1H, m), 2.65-2.75 (1H, m), 3.16 (1H, dt, J = 12.7, 7.1 Hz), 3.30 (1H, dt, J = 12.7, 7.1 Hz), 3.41 (1H, dd, J = 9.8, 13.2 Hz), 3.57 (1H, dd, J = 2.7, 13.2 Hz), 3.63 (1H, d, J = 8.3 Hz), 4.06 (1H, app. q, J = 9.7 Hz), 4.07-4.21 (1H, m), 4.16 (1H, dd, J = 4.4, 7.6 Hz), 4.22 (1H, ddd, J = 2.7, 8.3, 9.8 Hz), 4.89 (1H, t, J = 9.5 Hz); ^{13}C NMR (100 MHz, D_2O, pH = 4.53 adjusted by the addition of 1 M DCl): δ = 24.5, 32.7, 48.1, 54.4, 60.0, 67.4, 67.5, 69.9, 73.2, 172.4, 175.7, 182.1 ppm; HRMS: m/z calcd for $C_{12}H_{21}N_2O_8^+$ $[M+H]^+$: 321.1298, found : 321.1298 ; $[\alpha]_D^{24}$ = –48.8 (c = 0.57, H_2O).

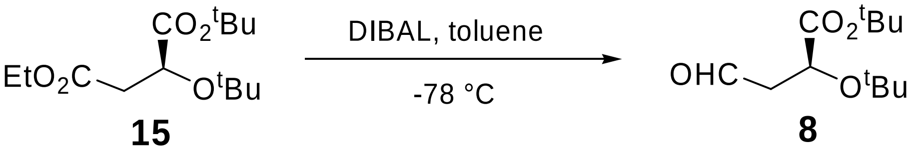


According to a similar procedure mentioned above for 2’-epi-MA **3**, MA **1** was obtained (20 mg, quant.) from **13** (30 mg, 0.063 mmol). IR (film, cm^{-1}): ν = 3215, 3061, 2855, 1618, 1412, 1319, 1236, 1115, 1092, 976, 773; ^{1}H NMR (400 MHz, D_2O, pH = 4.53 adjusted by the addition of 1 M DCl): δ = 1.99-2.09 (1H, m), 2.14-2.24 (1H, m), 2.52-2.62 (1H, m), 2.67-2.77 (1H, m), 3.16-3.25 (1H, m), 3.25-3.33 (1H, m), 3.43 (1H, dd, J = 2.7, 13.7 Hz), 3.56 (1H, dd, J = 9.5, 13.7 Hz), 3.85 (1H, d, J = 2.9 Hz), 4.03 (1H, app. q, J = 9.7 Hz), 4.10 (1H, dt, J = 4.2, 10.1 Hz), 4.17 (1H, dd, J = 4.6, 7.3 Hz), 4.44 (1H, dt, J = 9.3, 2.9 Hz), 4.88 (1H, t, J = 9.5 Hz); ^{13}C NMR (100MHz, D_2O, pH=4.53 adjusted by the addition of 1 M DCl): δ = 24.6, 32.9, 47.9, 53.9, 59.0, 67.4 (2C), 70.6, 73.2, 171.8, 175.8, 182.3; HRMS: m/z calcd for $C_{12}H_{21}N_2O_8^+$ $[M+H]^+$: 321.1298, found: 321.1292; $[\alpha]_D^{24}$ = –63.5 (c = 0.31, H_2O) (lit: –70.7,[2] –64.6[8h)]).

Synthesis of aldehyde 8



To a solution of **14** (17.5 g, 100 mmol) in DMF (40 mL) were added K_2CO_3 (41 g, 300 mmol) and ethyl iodide (12.1 mL, 150 mmol) successively. The mixture was stirred for 2 h, quenched with saturated ammonium chloride, and extracted with diethyl ether (x 2). The combine organic layers were washed with water, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The solution of the residue in AcOH (80 mL) and H_2O (20 mL) was heated under reflux for 30 min, concentrated under reduced pressure, and vacuumed for 3 h. Isobutene gas was bubbled through the mixture of the residue and concd H_2SO_4 (1 mL) in CH_2Cl_2 (100 mL) for 1 h. The mixture was stirred for 16 h in a stoppered flask, quenched with satd. $NaHCO_3$, and extracted with ethyl acetate (x 2). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (elution with hexane/ethyl acetate = 9/1 to 4/1) to give **15** (16.7 g, 61%) as colorless oil. IR (neat): ν = 2976, 2935, 1725, 1475, 1367 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 4.31 (dd, *J* = 8.1, 5.4 Hz, 1H), 4.14 (m, 2H), 2.63 (dd, *J* = 15.3, 5.4 Hz, 1H), 2.57 (dd, *J* = 15.3, 8.1 Hz, 1H), 1.45 (s, 9H), 1.26 (t, *J* = 7.2 Hz, 3H), 1.19 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.37, 170.42, 81.22, 75.26, 68.98, 60.65, 39.49, 27.84, 27.78, 14.17; HRMS: m/z calcd for $C_{14}H_{26}O_5^+$ $[M+H]^+$: 275.1859, found: 275.1863; $[\alpha]_D^{21}$ = –37.3 (*c* = 1.5, $CHCl_3$).



To a solution of **15** (10.0 g, 36 mmol) in toluene (130 mL) was added DIBAL (39 mL, 1 M solution in toluene) at –78 °C. The mixture was stirred for 20 min, quenched with saturated ammonium chloride (11.5 mL), diluted with diethyl ether (200 mL), and stirred for 2 h at room temperature. The mixture was added anhydrous $MgSO_4$, stirred for 20 min, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (elution with hexane/ethyl acetate = 9/1 to 4/1) to give **8** (7.3 g, 88%) as colorless oil. IR (neat): ν = 2977, 2935, 1737, 1459, 1367 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 9.76 (t, *J* = 2.1 Hz, 1H), 4.39 (dd, *J* = 8.1, 4.8 Hz, 1H), 2.74 (ddd, *J* = 16.2, 8.1, 2.1 Hz, 1H), 2.64 (ddd, *J* = 16.2, 4.8, 2.1 Hz, 1H), 1.47 (s, 9H), 1.21 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 200.00, 172.58, 81.89, 75.82, 67.70, 47.48, 28.09, 28.03; HRMS m/z calcd for $C_{12}H_{22}O_4^+$ $[M+H]^+$: 231.1597, found: 231.1596; $[\alpha]_D^{21}$ = –52.9 (*c* = 1.0, $CHCl_3$).

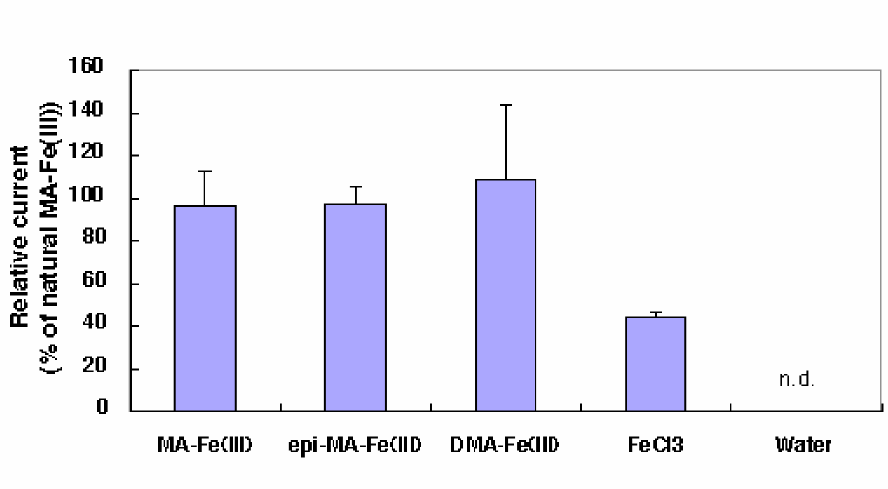
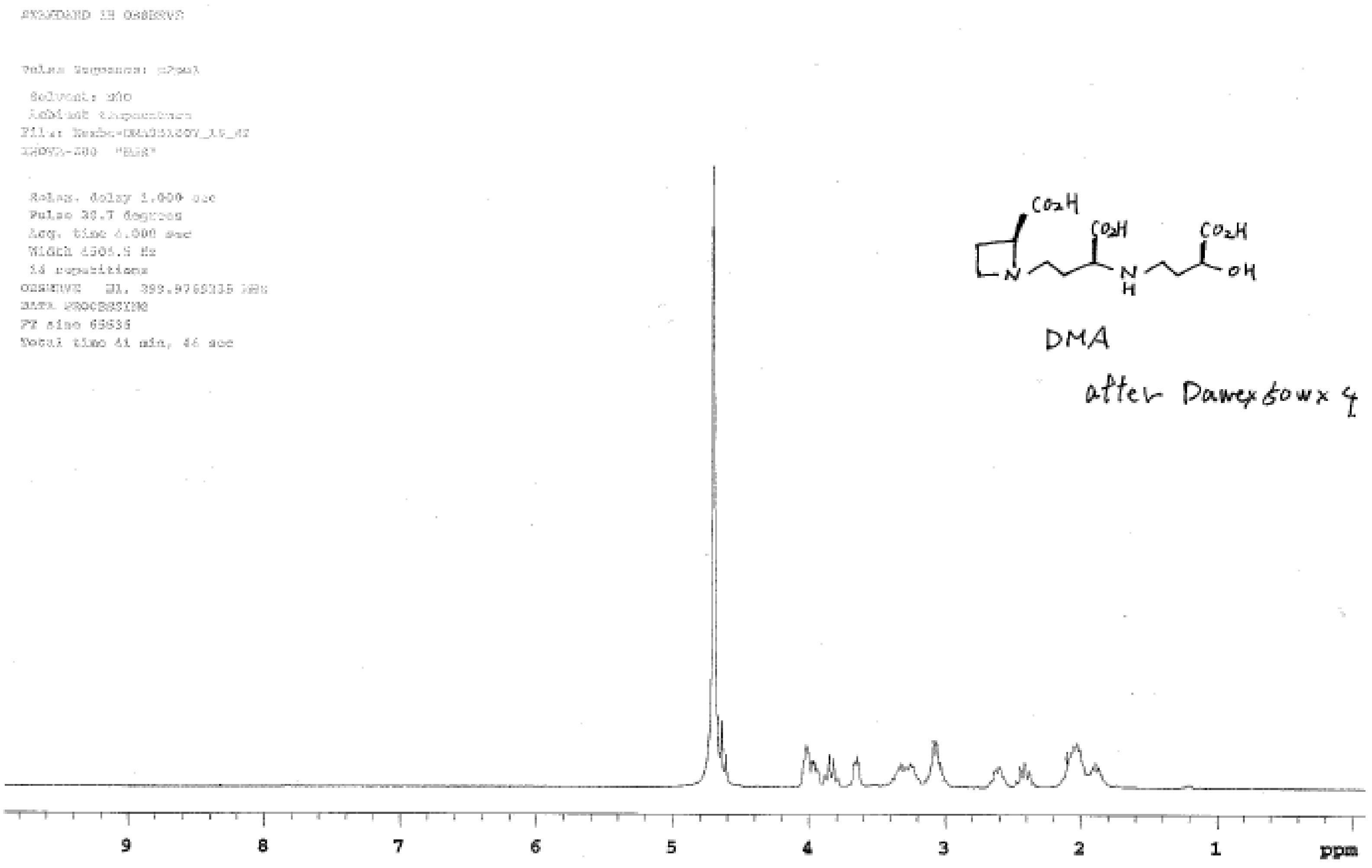
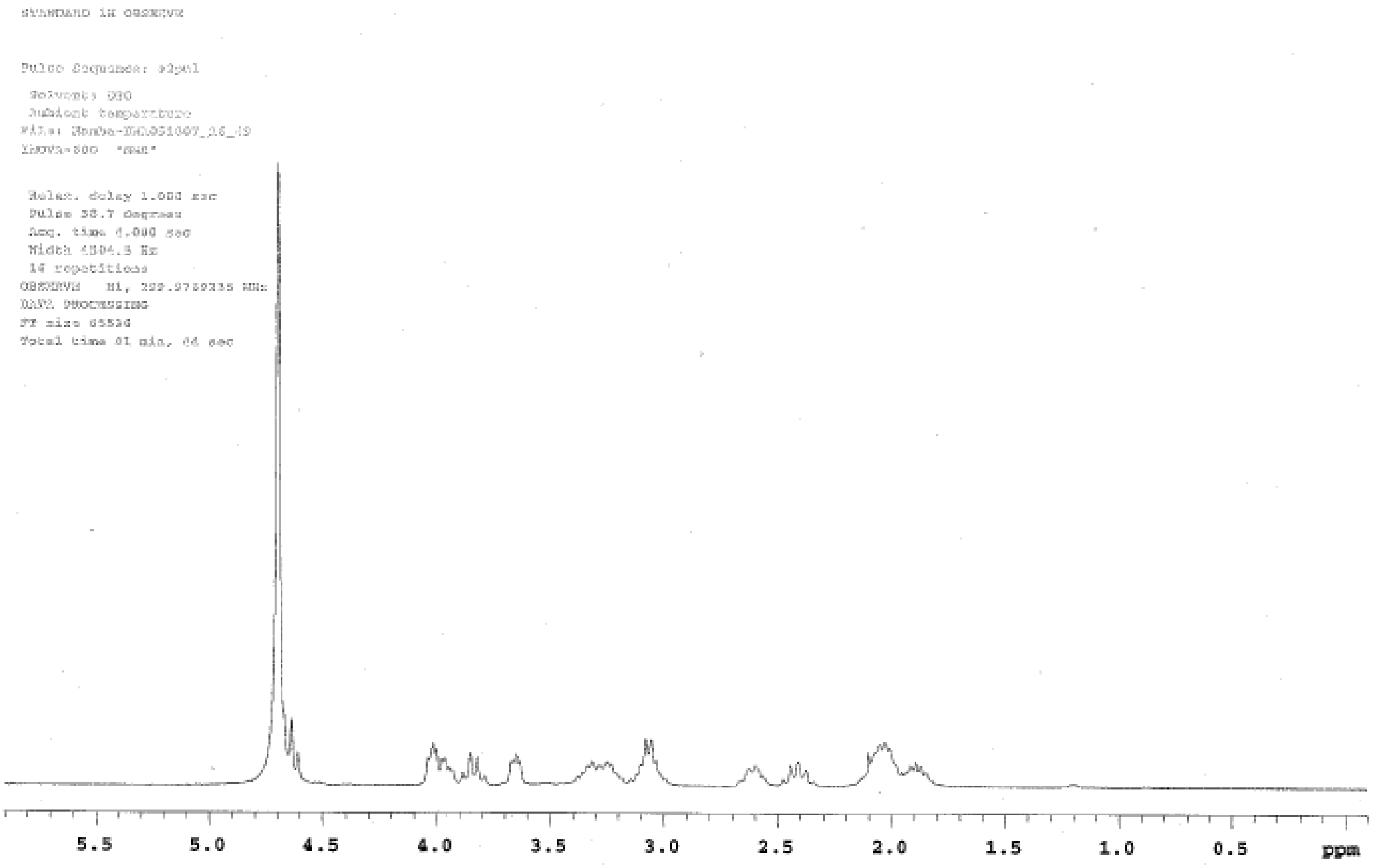


Figure S1: Iron transporting activities of synthetic MA-, 2’-epi-MA-, DMA-iron(III) and natural MA-iron(III) with *Xenopus laevis* oocytes expressing ZmYS1 by the two-electrobe voltage clamp method. Currents relative to natural MA-iron(III) are shown.



DMA

after Dowex 50wx4


STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: D2O
Ambient temperature
File: Namba-DHL051007_16_49
INOVA-300 "nmr"
Relax. delay 1.000 sec
Pulse 38.7 degrees
Acq. time 4.000 sec
Width 4504.5 Hz
16 repetitions
OBSERVE H1, 299.9789235 MHz
DATA PROCESSING
FT size 65536
Total time 01 min, 04 sec
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm

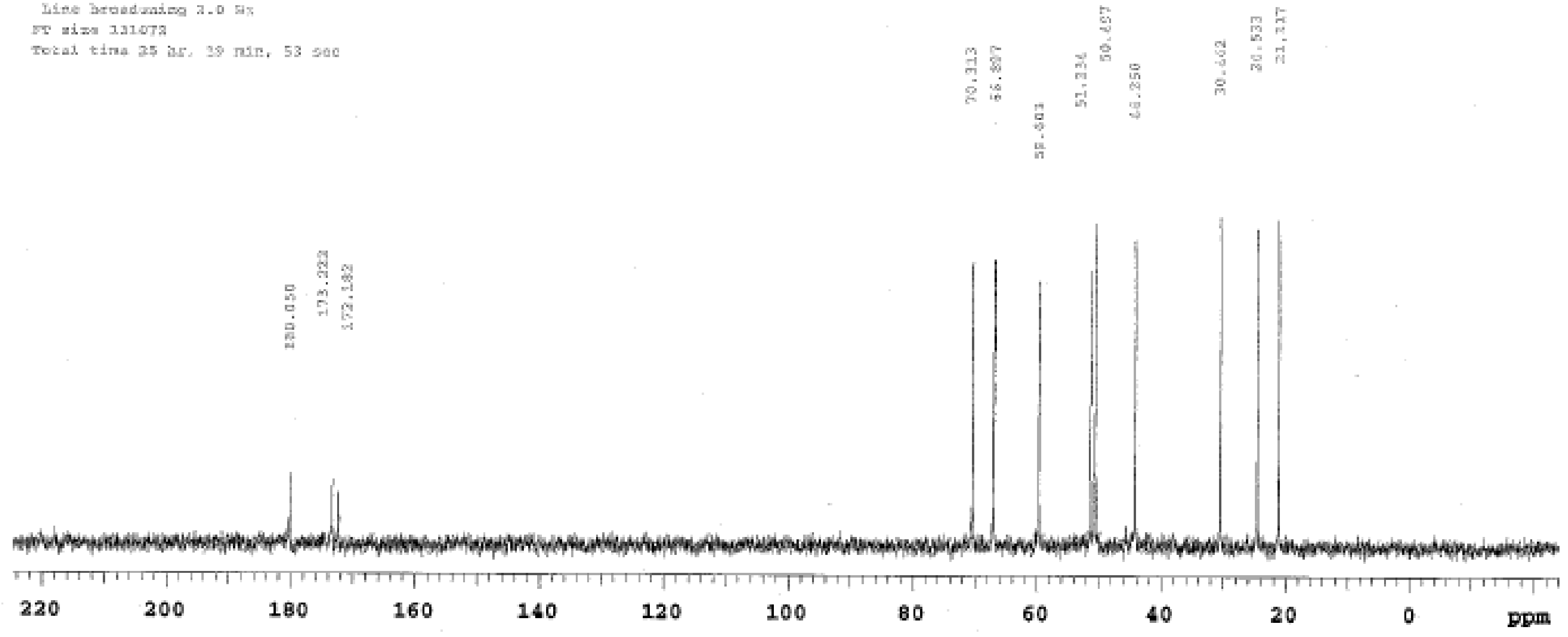
13C OBSERVE

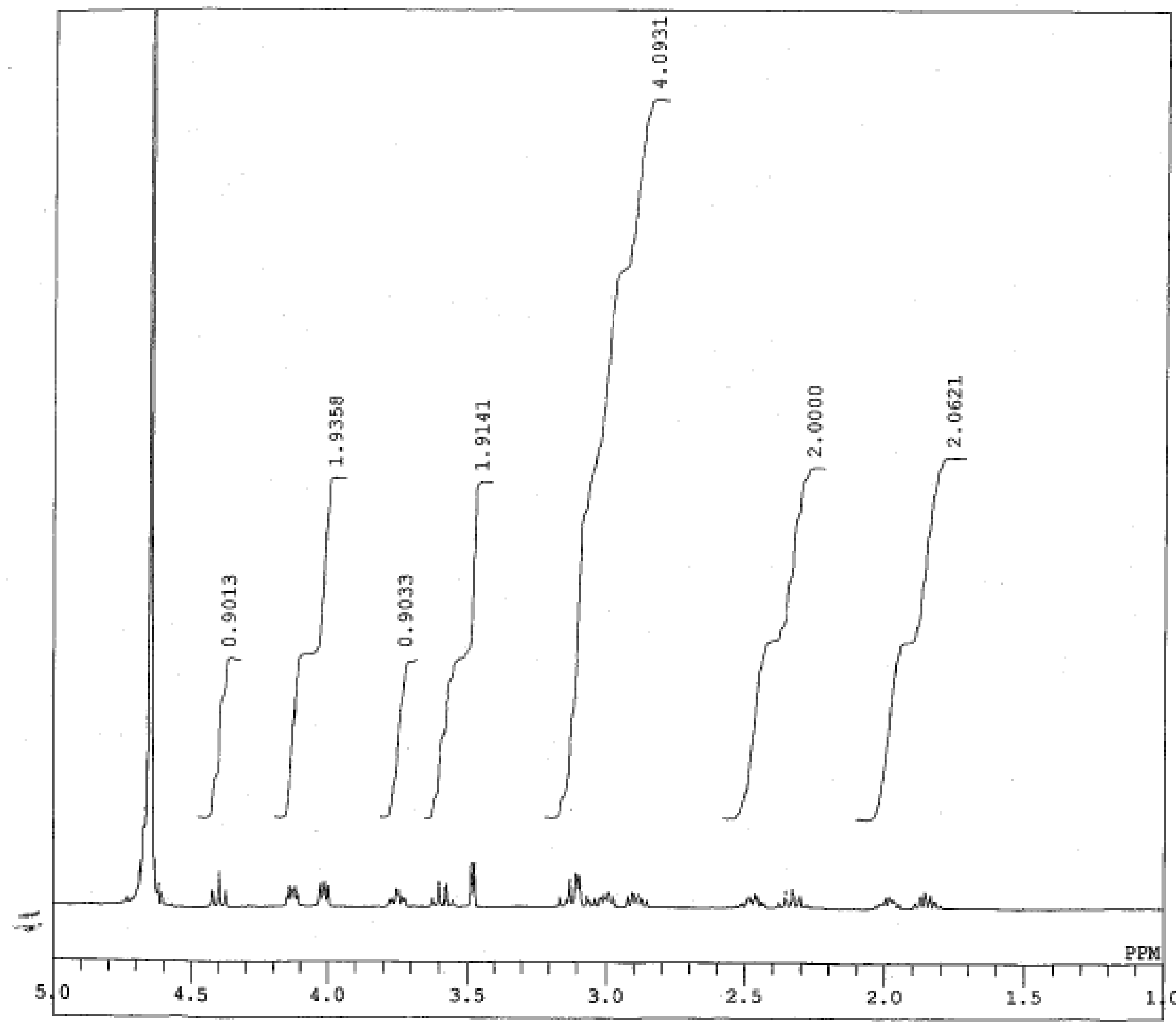
Pulse Sequence: s2pul

Solvent: D2O
Ambient temperature
File: [illegible]
INOVA-300 "[illegible]"

Pulse 54.2 degrees
Acq. time 1.815 sec
Width 18761.7 Hz
272 repetitions
OBSERVE C13, 75.4282029 MHz
DECOUPLE H1, 299.9724877 MHz
Power 39 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 3.0 Hz
FT size 131072
Total time 25 hr, 39 min, 53 sec

DMA
after Dowex 50W×4.

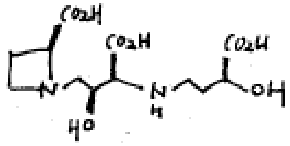


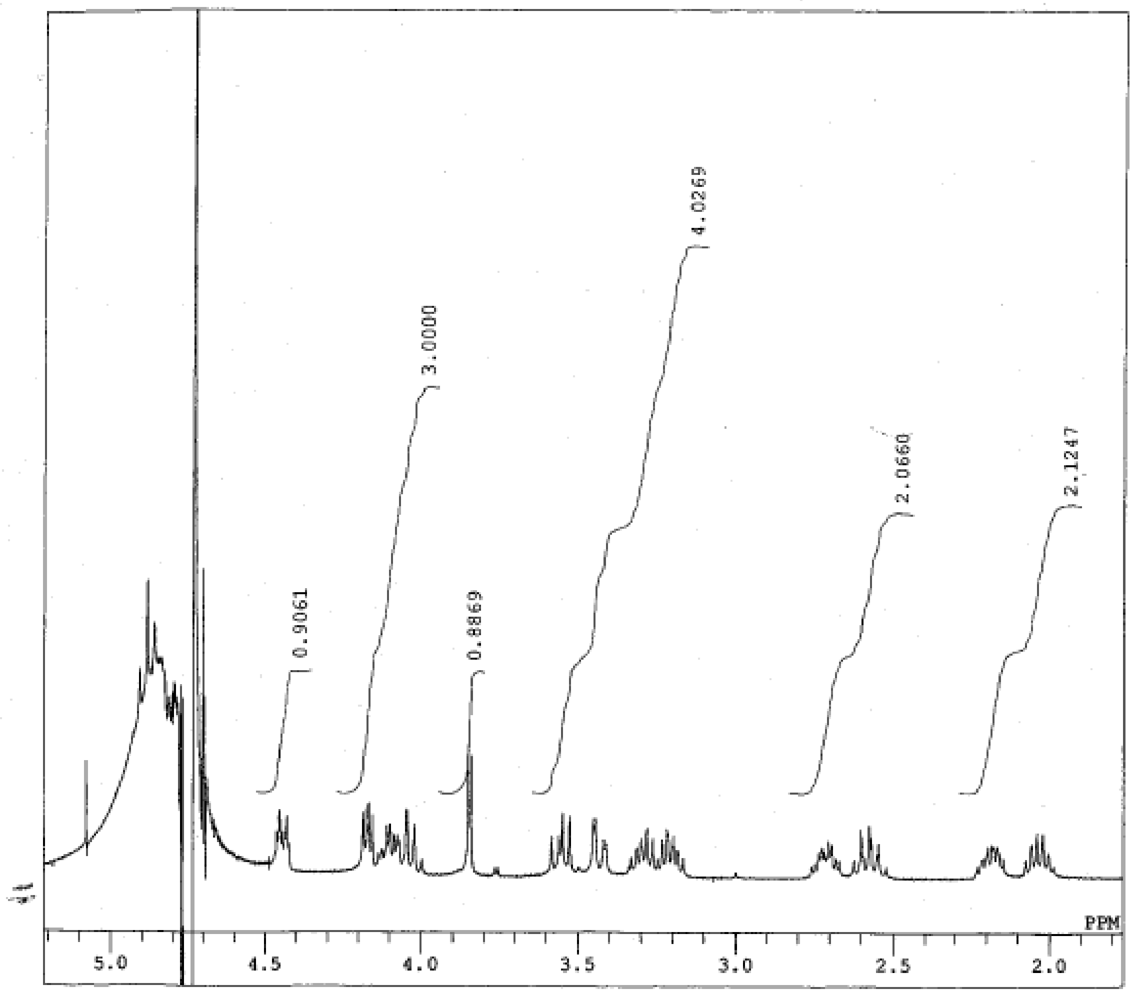


```
DFILE  C:\WINNMR95\COMMON\_DEFAULT.P
COMNT
DATIM  Mon Sep 25 13:25:43 2006
OBNUC  1H
EXMOD  NON
OBFRQ        399.65 MHz
OBSET        124.00 KHz
OBFIN       10500.0 Hz
POINT         32768
FREQU        8000.0 Hz
SCANS            32
ACQTM         4.096 sec
PD            2.904 sec
PW1             6.0 us
IRNUC  1H
CTEMP          27.0 c
SLVNT  D2O
EXREF          4.65 ppm
BF             0.12 Hz
RGAIN            18
```

pH 8.15

natural MA



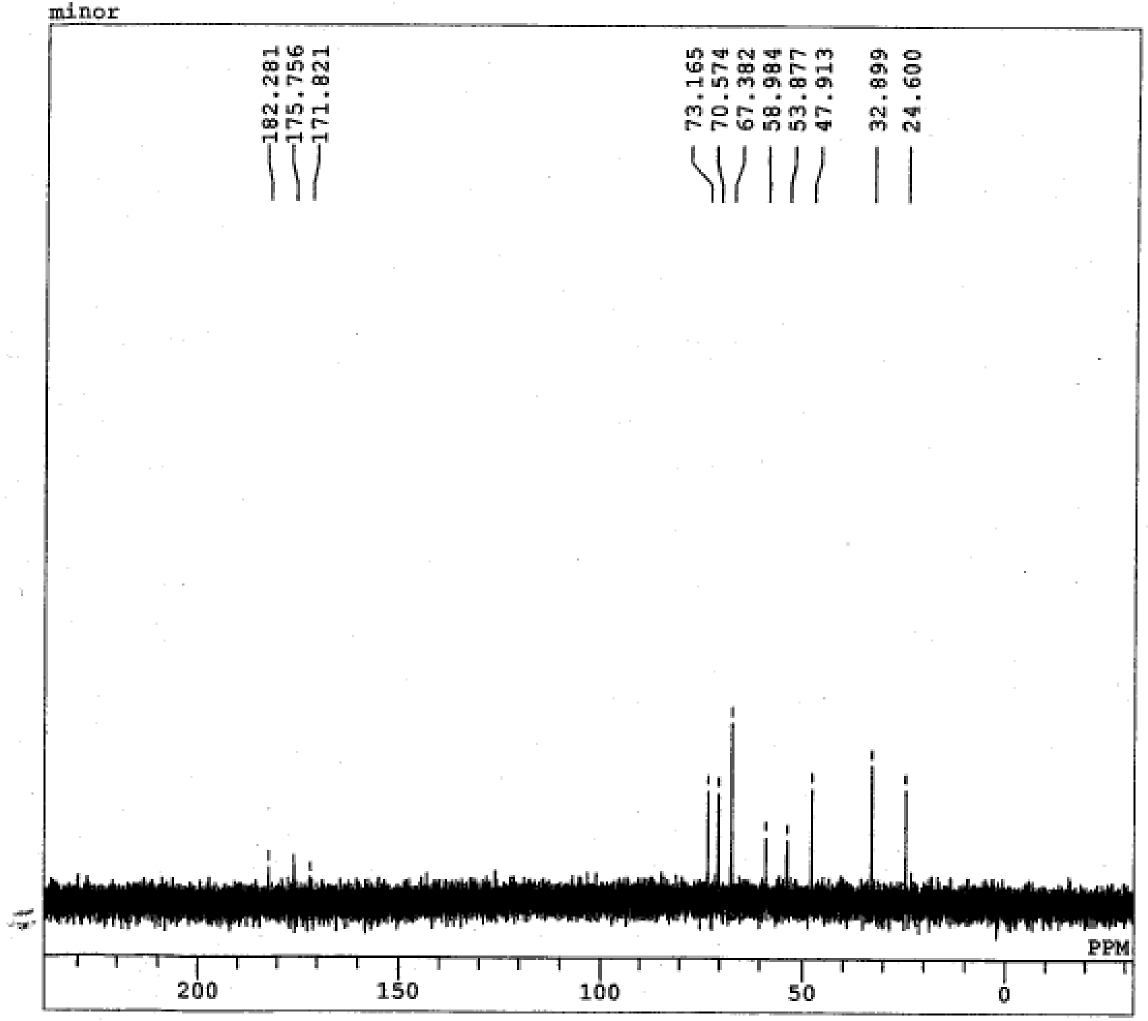


```
DFILE  C:\My Documents\horikawa\0 78
COMNT
DATIM  Tue Sep 26 13:13:41 2006
OBNUC  1H
EXMOD  HOM
OBFRQ       399.65 MHz
OBSET       124.00 KHz
OBFIN      10500.0 Hz
POINT        32768
FREQU       8000.0 Hz
SCANS           16
ACQTM        4.096 sec
PD           2.904 sec
PW1            6.0 us
IRNUC  1H
CTEMP         25.4 c
SLVNT  D2O
EXREF         4.44 ppm
BF            0.12 Hz
RGAIN           21
```

pH 4.53.

MA

```
C:\WINNMR98\COMMON\_DEFAULT.ALS
minor
```

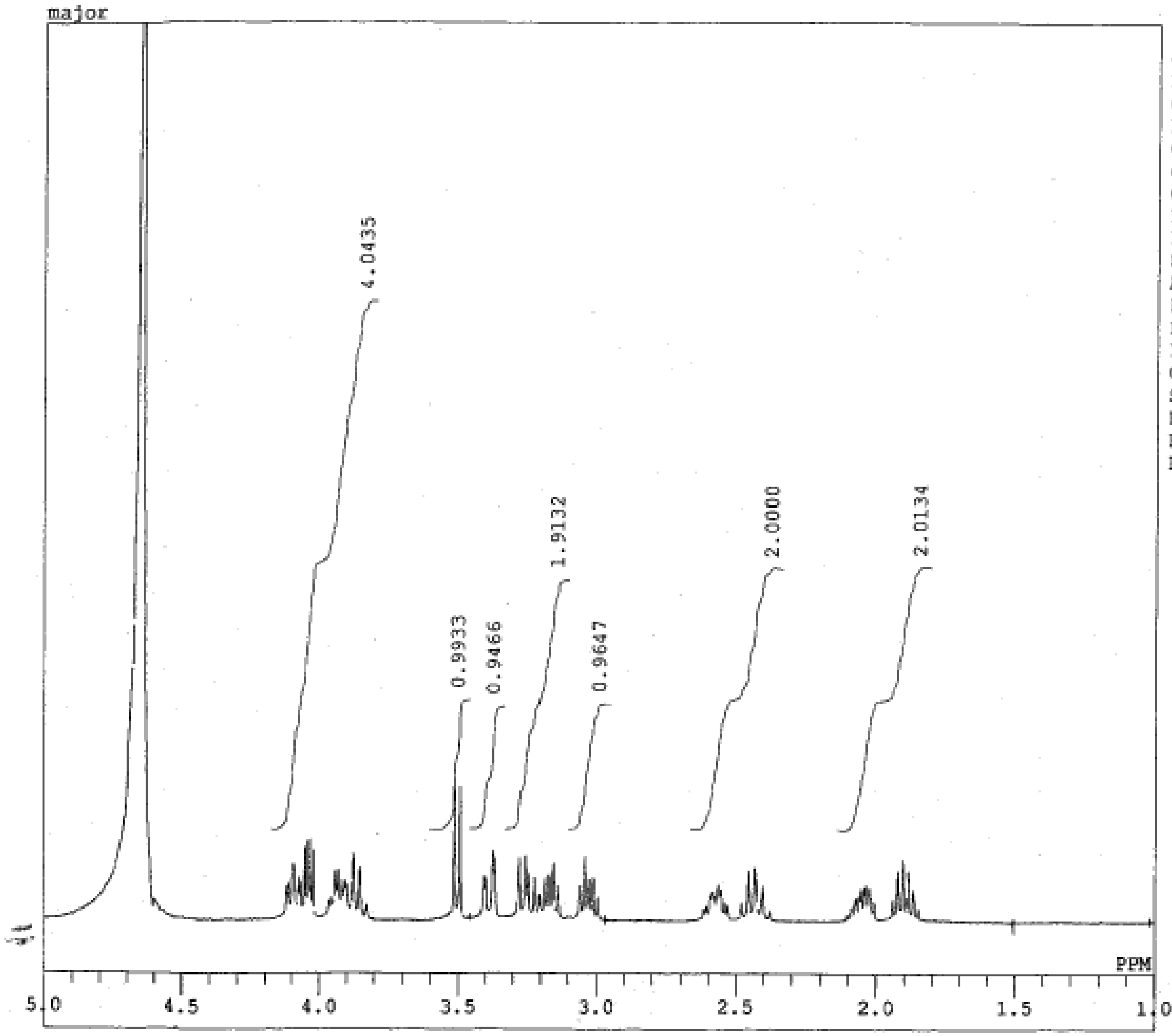


```
DFILE  C:\WINNMR98\COMMON\
COMNT  minor
DATIM  Thu Sep 28 16:02:16
OBNUC  13C
EXMOD  BCM
OBFRQ       100.40 MHz
OBSET       125.00 KHz
OBFIN      10500.0 Hz
POINT        32768
FREQU      27173.9 Hz
SCANS         7319
ACQTM        1.206 sec
PD           1.794 sec
PW1            5.1 us
IRNUC  1H
CTEMP         25.8 c
SLVNT  D2O
EXREF         0.00 ppm
BF            0.10 Hz
RGAIN           25
```

pH 4.53

natural form

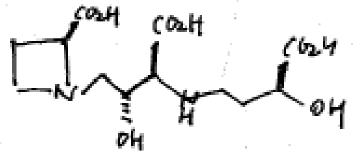
minor

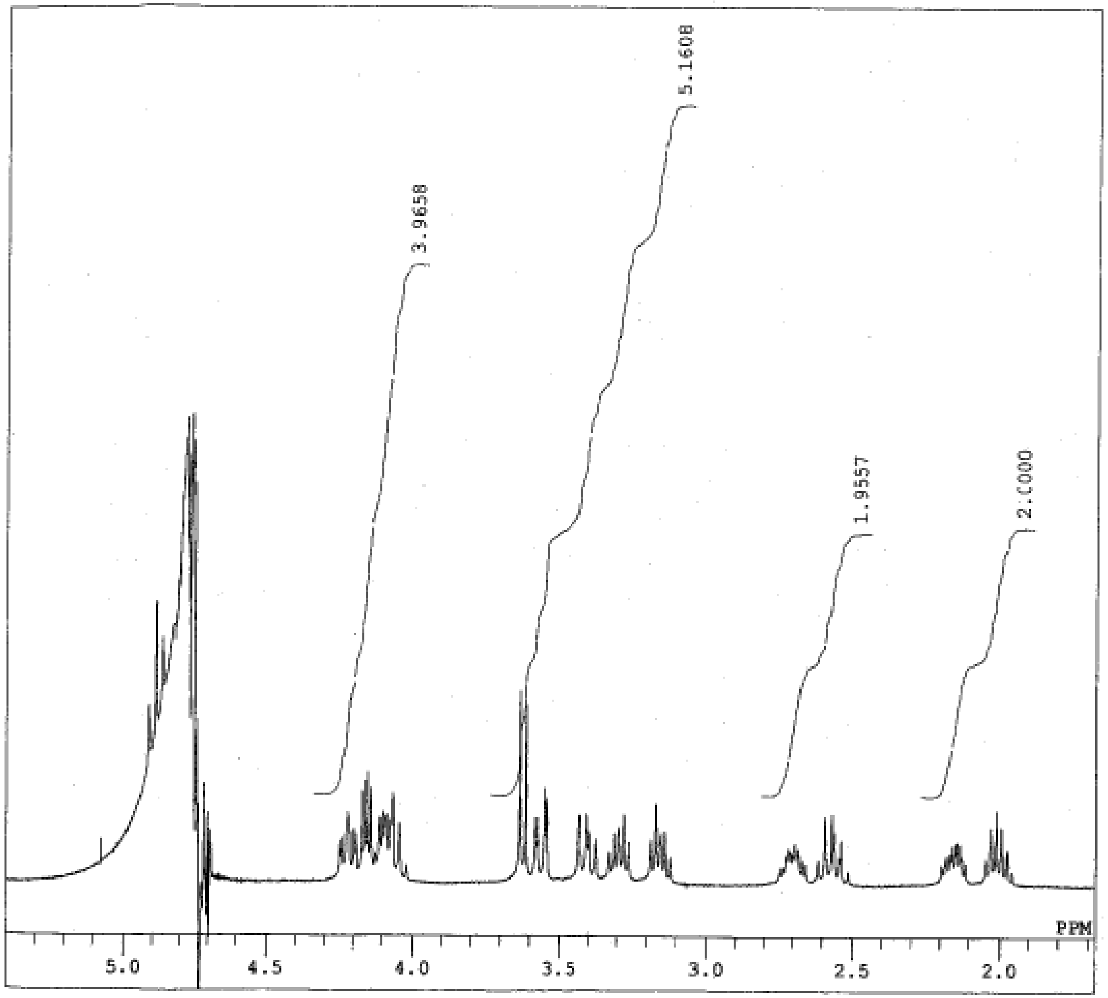


```
DFILE  C:\WINNMR95\COMMON\_DEFAULT.A
COMNT  major
DATIM  Mon Sep 25 13:56:52 2006
OBNUC  1H
EXMOD  NON
OBFRQ        399.65 MHz
OBSET        124.00 KHz
OBFIN       10500.0 Hz
POINT         32768
FREQU        8000.0 Hz
SCANS            32
ACQTM         4.096 sec
PD            2.904 sec
PW1             6.0 us
IRNUC  1H
CTEMP          26.0 c
SLVNT  D2O
EXREF          4.65 ppm
BF             0.12 Hz
RGAIN            19
```

pH 7.5

2'-epi-MA



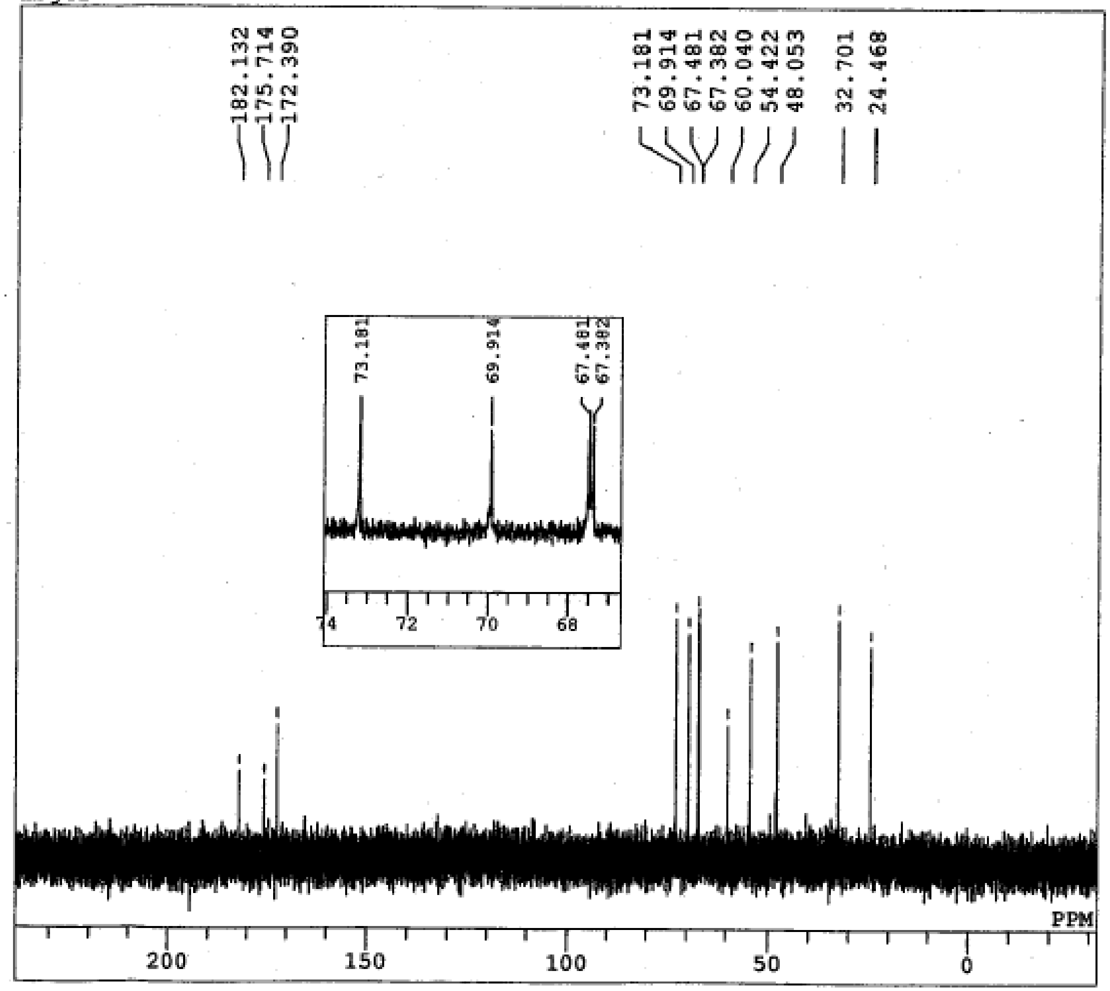


```
DFILE  C:\WINNMR95\COMMON\_DEFAULT.
COMNT
DATIM  Tue Sep 26 13:46:42 2006
OBNUC  1H
EXMOD  HOM
OBFRQ        399.65 MHz
OBSET        124.00 KHz
OBFIN       10500.0 Hz
POINT         32768
FREQU        8000.0 Hz
SCANS            32
ACQTM         4.096 sec
PD            2.904 sec
PW1             6.0 us
IRNUC  1H
CTEMP          25.4 c
SLVNT  D2O
EXREF          4.44 ppm
BF             0.12 Hz
RGAIN            21
```

pH 4.50

2'-epi-MA

```
C:\WINNMR98\COMMON\_DEFAULT.ALS
major
```



```
DFILE  C:\WINNMR98\COMMON\
COMNT  major
DATIM  Thu Sep 28 02:24:10
OBNUC  13C
EXMOD  BCM
OBFRQ      100.40 MHz
OBSET      125.00 KHz
OBFIN     10500.0 Hz
POINT       32768
FREQU     27173.9 Hz
SCANS       16384
ACQTM       1.206 sec
PD          1.794 sec
PW1           5.1 us
IRNUC  1H
CTEMP        25.2 c
SLVNT  D2O
EXREF        0.00 ppm
BF           0.10 Hz
RGAIN          24
```

pH 4.50

epi 体

major