Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Supplementary Information

Synthesis, Radical Scavenging Activity and Structure-Activity Relationship of Uric Acid Analogs.

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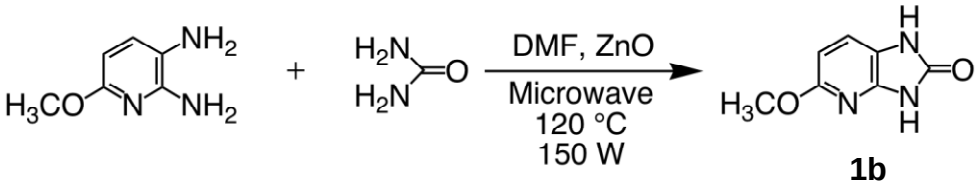
Contents

1. Preparation of Uric acid analogs

1.1. General

^{1}H-NMR spectra (500 MHz) were measured on a JEOL JNM-A500 FT-NMR spectrometer with tetramethylsilane as an internal standard (δ = 0.00) in CD_3OD or DMSO-d_6. ^{13}C-NMR spectra (125 MHz) were obtained on the same spectrometer and the chemical shifts were referenced to the signals of CD_3OD (δ = 33.3) or DMSO-d_6 (δ = 39.5). Melting points were determined using a Yanagimoto MP-J3 micro-melting point apparatus and uncorrected. Column chromatography was performed using Merck Silica gel 60. 6-Hydroxy-2-benzoxazolinone **4**, 5-indanol **7** were purchased from Sigma-Aldrich Chemical Co., 5-hydroxyoxindole **3a** was purchased from Apin Chemical Co. 5-hydroxyindole **5**, 6-hydroxyindole **6**, Benzimidazole **8** and 2-indanone **9** were purchased from Kanto Chemical Co., 1,1-diphenyl-2-picrylhydrazyl, hydroquinone, *p*-aminophenol and uric acid were purchased from Tokyo Kasei Kogyo Co. The regioisomers of **2a** and **3a** were synthesized according to the method reported by R. J. S. Beer *et al* (*J. chem. Soc.*, **1948**, 1605-1609) with some suitable modification.

1-2 Preparation of 1,3-dihydro-7-methoxy-2H-imidazo[4,5-b]pyridine-2-one (**1b**)



1b

To solution of urea (0.45 g, 7.5 mmol) and *o*-6-methoxy-2,3-pyridinediamine (1.59 g, 7.5 mmol) in *N*, *N*-dimethylformamide (12 mL) was added ZnO (442.5 mg, 5.44 mmol) and reacted for 5 hr under microwave irradiation condition (120°C, 150 W). Whole the reaction mixturewas diluted with H_2O, extracted with ethyl acetate twice. The organic layer was washed with sat. NaCl solution, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was recrystalyzed from $CHCl_3$: MeOH to brown crystal (459.5 mg, 37 %).

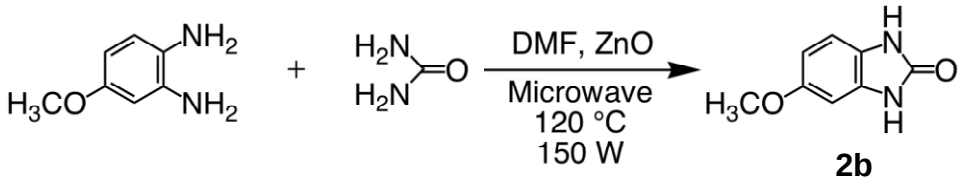
^{1}H-NMR (DMSO-*d*$_6$, 500MHz) δ 3.78 (*s*, 3H, -OC<u>H</u>$_3$), 6.36 (*d*, 1H, *J* = 8.50 Hz, 8-H), 7.21 (*d*, 1H, *J* = 8.00 Hz, 9-H), 10.54 (*brs*, 1H, -N<u>H</u>-), 11.20 (*brs*, 1H, -N<u>H</u>-). .

^{13}C-NMR (DMSO-*d*$_6$, 125 MHz) δ 53 (-O<u>C</u>H$_3$), 101 (8C), 117 (4C), 119 (9C), 142 (5C), 154 (7C), 159 (2C).

FAB-MS: *m/z*=166 $[M+H]^+$ (84 %), 165 $[M]^+$ (64 %), 138 (40 %), 137 (77 %), 136 (100 %), 107 (29 %), 89 (24 %), 77 (21 %).

FAB-HRMS: calcd. for $C_7H_8N_3O_2$ 166.0617, found 166.0633.

1-3. Preparation of 5-methoxy-2-benzimidazolinone **2b**



2b

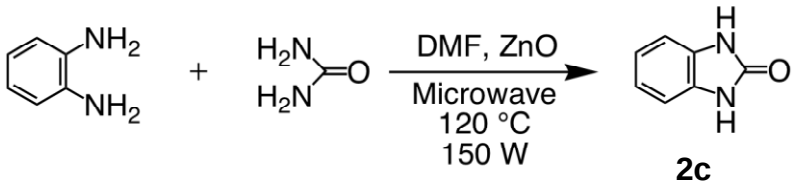
To solution of urea (0.55 g, 9.19 mmol) and 4-methoxy-1,2-phenylenediamine (1.94 g, 9.19 mmol) in *N*, *N*-dimethylformamide (20 mL) was added ZnO (540.0 mg, 6.63 mmol) and reacted for 10 hr under microwave irradiation condition (120°C, 150 W). Whole the reaction mixturewas diluted with H_2O, extracted with ethyl acetate twice. The organic layer was washed with sat. NaCl solution, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was recrystalyzed from $CHCl_3$: MeOH to give yellow crystal (560 mg, 37 %).

^{1}H-NMR (CD_3OD, 500 MHz) δ 3.69 (*s*, 3H, -OC<u>H</u>$_3$), 6.55 (*dd*, 1H, *J* = 8.50 Hz, 2.14 Hz, 6-H), 6.59 (*d*, 1H, *J* = 2.14 Hz, 4-H), 6.85 (*d*, 1H, *J* = 8.54 Hz, 7-H).

^{13}C-NMR (CD_3OD, 125 MHz) δ 56 (-OCH_3), 108 (4C), 110 (6C), 124 (7C), 131 (9C), 157 (5C), 158

(2C).

1-4. Preparation of 2-benzimidazolinone **2c**



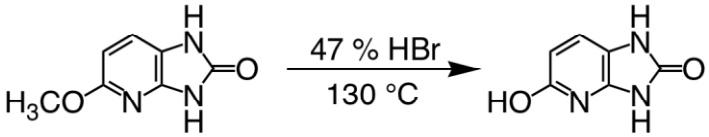
2c

To solution of urea (0.20 g, 3.33 mmol) and *o*-phenylenediamine (0.36 g, 3.33 mmol) in *N*, *N*-dimethylformamide (8 mL) was added ZnO (196.7 mg, 2.42 mmol) and reacted for 6 hr under microwave irradiation condition (120°C, 150 W). Whole the reaction mixturewas diluted with H_2O, extracted with ethyl acetate twice. The organic layer was washed with sat. NaCl solution, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was recrystalyzed from CH_2Cl_2: MeOH to give black crystal (37.3 mg, 8 %).

^{1}H-NMR (CD_3OD, 600 MHz) δ 7.01 (s, 4H, 4,5,6,7-H).

^{13}C-NMR (CD_3OD, 125 MHz) δ 110 (5C, 6C), 122 (4C, 7C), 130 (8C, 9C), 158 (2C).

1-5 Preparation of 1,3-dihydro-7-hydroxy-2H-imidazo[4,5-b]pyridine-2-one (**1a**)



1b **1a**

1b (100.0 mg, 0.61 mmol) was dissolved in 47 % aqueous HBr (1.0 mL) and stirred at 130 °C for 3 hr. After excess HBr and H_2O were removed by evaporation, the crude product (101.2 mg) was purified by column chromatography (SiO_2) to give white solid (10.0 mg, 11%).

^{1}H-NMR (DMSO-d_6, 500MHz) δ 6.17 (*d*, 1H, *J* = 8.30 Hz, 7-H), 7.11 (*d*, 1H, *J* = 8.06 Hz, 8-H), 10.04 (*brs*, 1H, -O<u>H</u>), 10.40 (*s*, 1H, -N<u>H</u>-), 11.02 (*s*, 1H, -N<u>H</u>-).

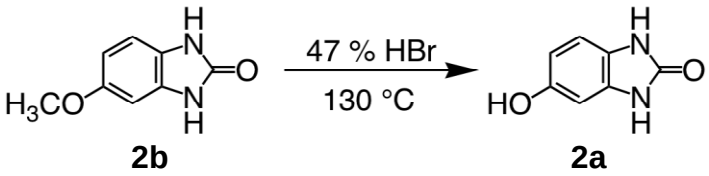
^{13}C-NMR (DMSO-d_6, 125 MHz) δ 100 (8C), 116 (4C), 118 (9C), 142 (5C), 154 (7C), 158 (2C).

FAB-MS: *m/z*=152 $[M+H]^+$ (39 %), 151 $[M]^+$ (19 %), 138 (39 %), 137 (76 %), 136 (100 %), 107 (28 %), 89 (25 %), 77 (21 %).

FAB-HRMS: calcd. for $C_6H_6N_3O_2$ 152.0460, found 152.0472.

Mp. : over 300 °C (decomp.)

1-6. Preparation of 5-hydroxy-2-benzimidazolinone **2a**



2b **2a**

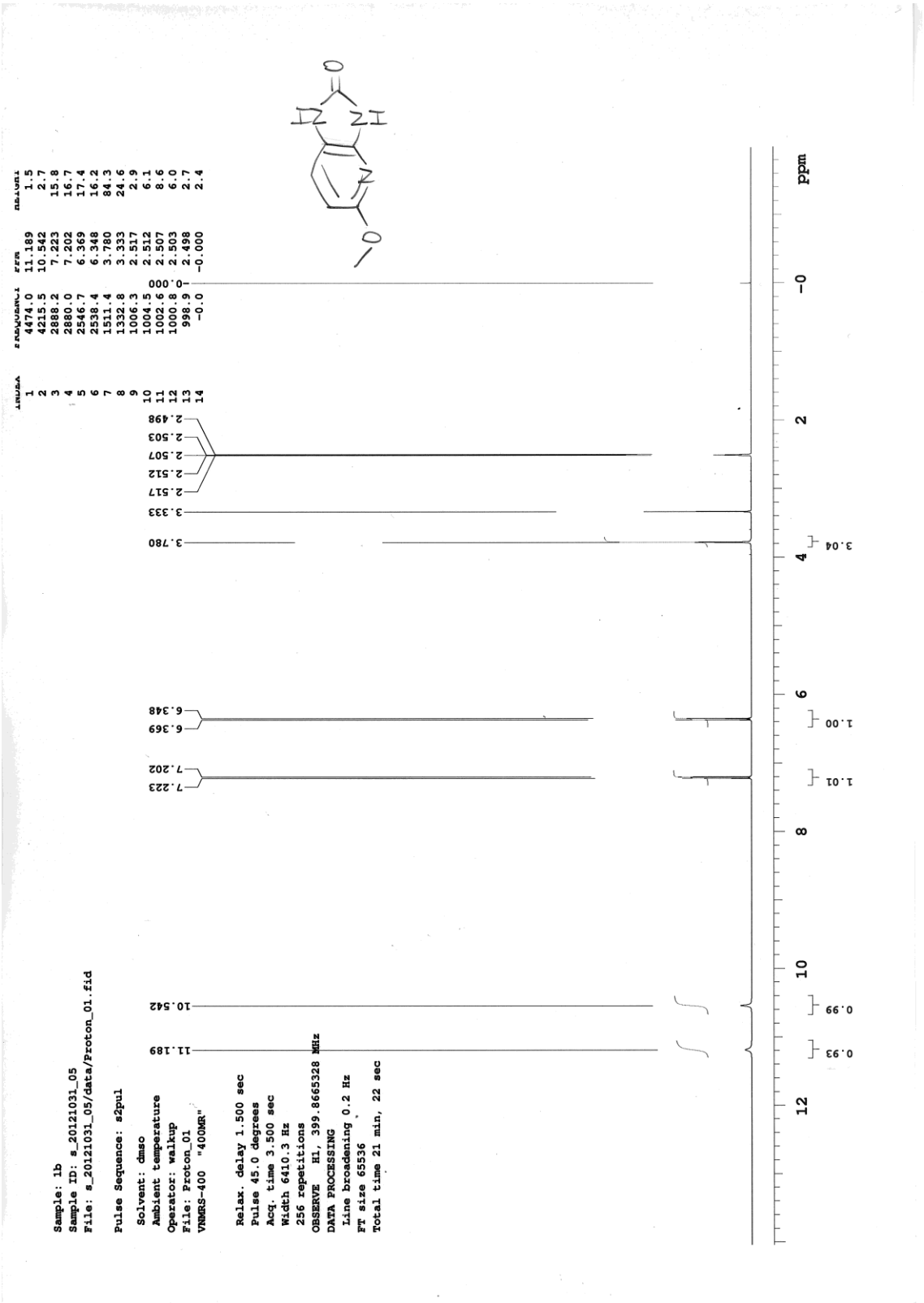
2b (80.6 mg, 0.49 mmol) was dissolved in 47 % aqueous HBr (1.0 mL) and stirred at 130 °C for 3 hr. After excess HBr and H_2O were removed by evaporation, the crude product (81.5 mg) was purified by column chromatography (SiO_2) to give white solid (65.6 mg, 89 %).

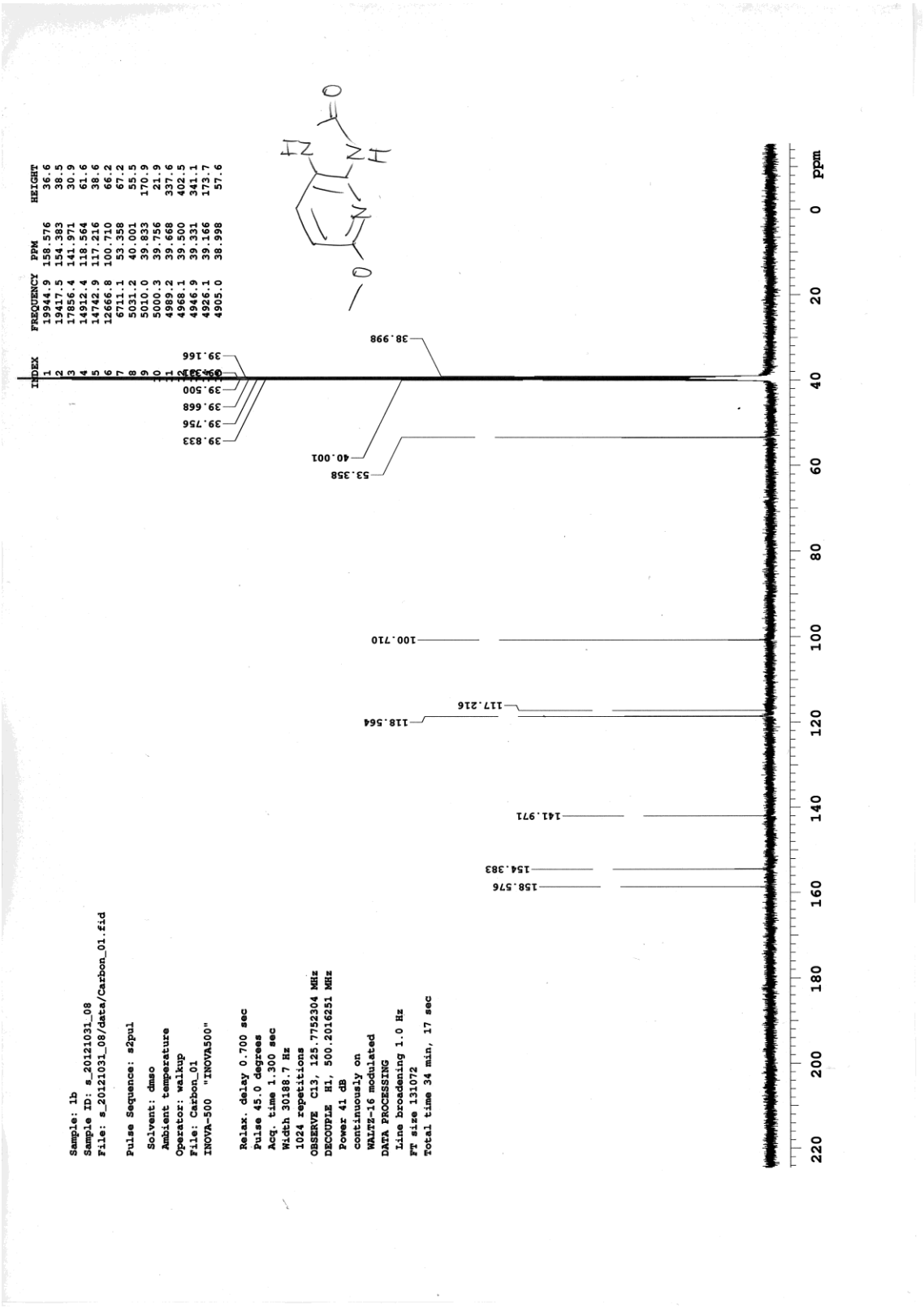
^{1}H-NMR (CD_3OD, 500 MHz) δ 6.49 (*dd*, 1H, *J* = 8.50 Hz, 2.14 Hz, 6-H), 6.53 (*d*, 1H, *J* = 1.83, 4-H), 6.83 (*d*, 1H, *J* = 8.28 Hz, 7-H).

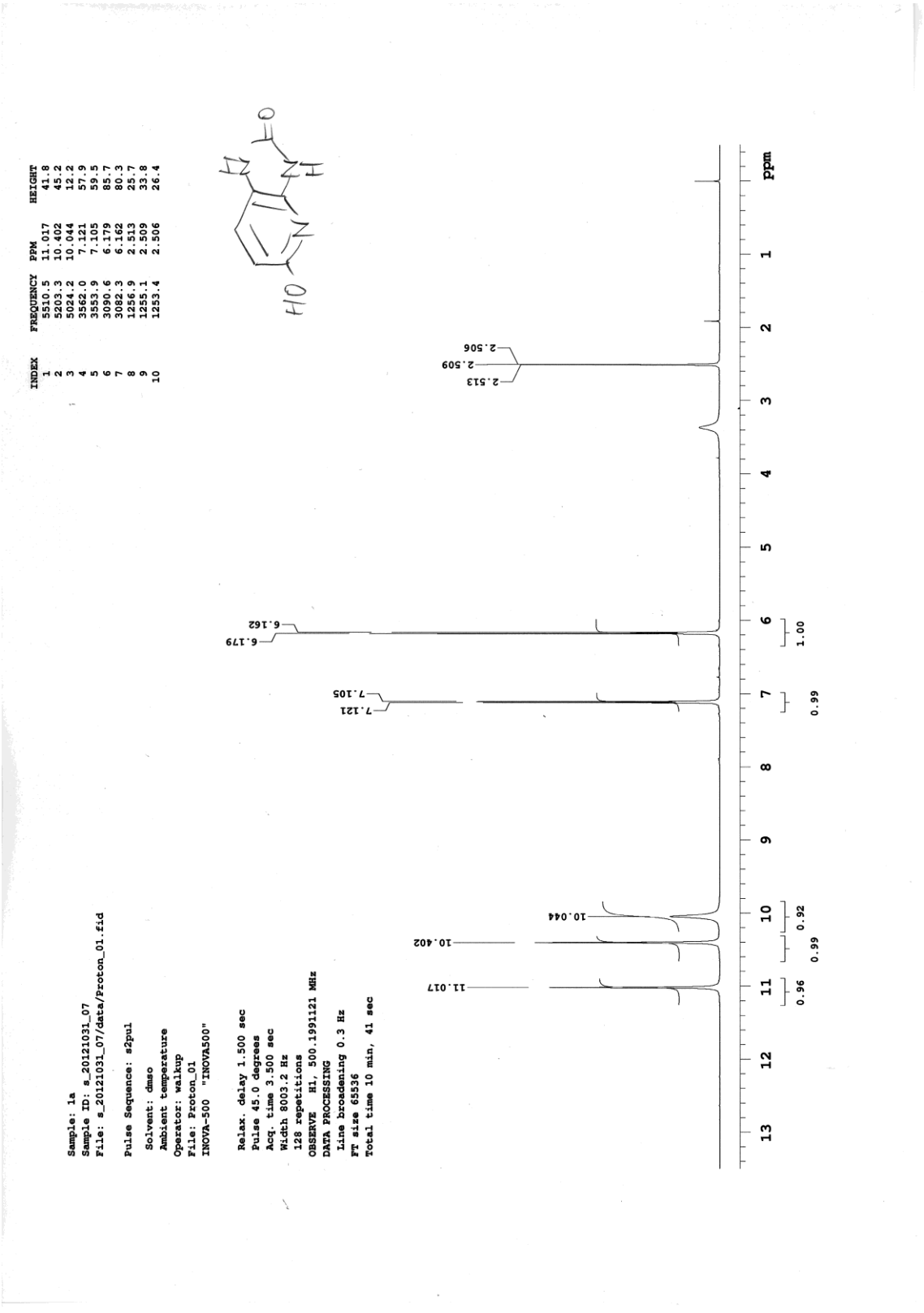
^{13}C-NMR (CD_3OD, 125 MHz) δ 98 (4C), 109 (6C), 110 (1C), 123 (7C), 131 (3C), 153 (5C), 158 (2C).

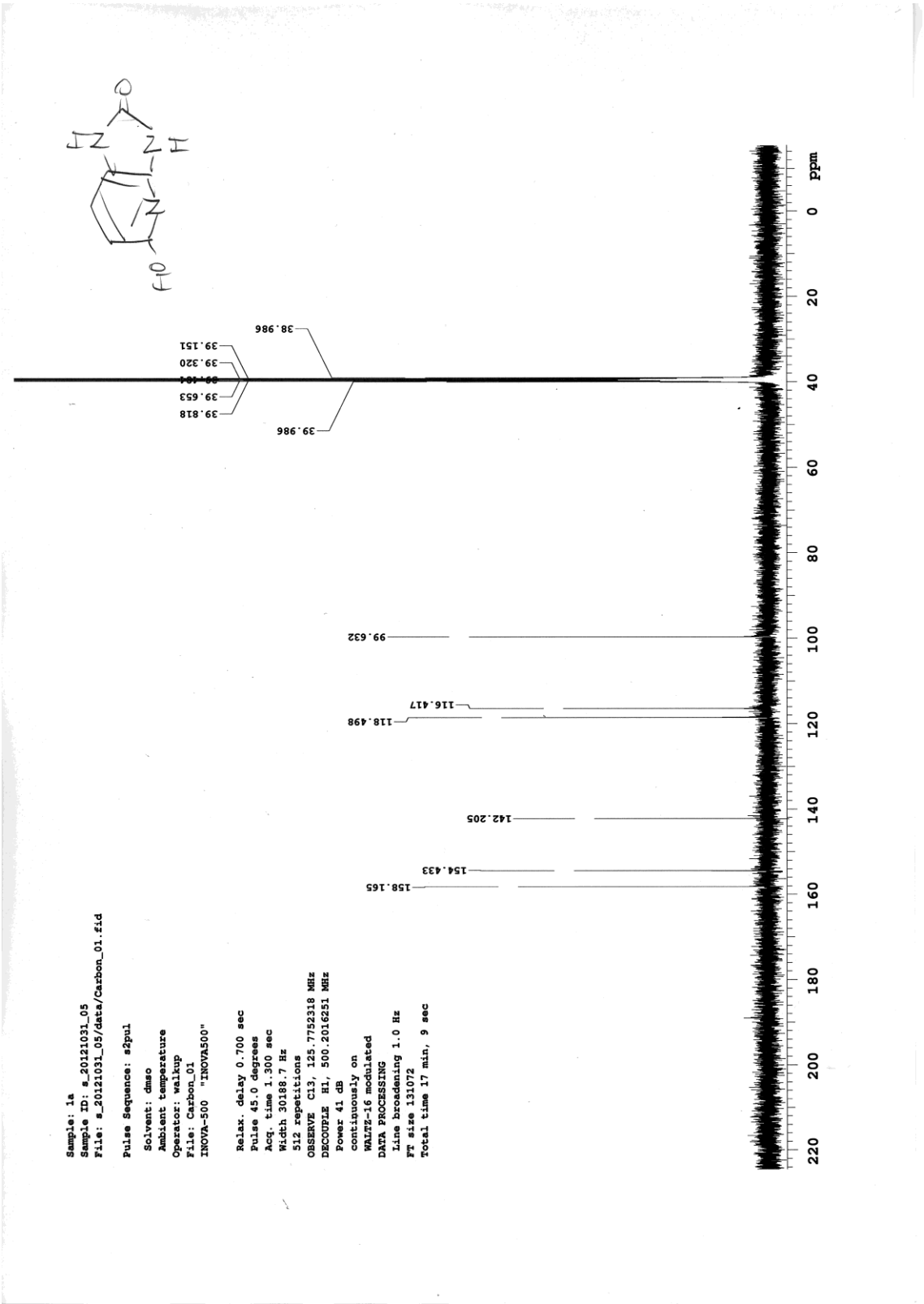
M. p. : over 300 °C (decomp.)

2. Scanned copies of the ^{1}H and ^{13}C NMR spectra









3. DPPH radical scavenging activity

The measurement procedure is a modification of the method of Yamaji *et al* (Yamaji, K.; Sarker, K. P.; Maruyama, I.; Hizukuri, S. *Planta Med*., **2002**, *60*. 16). Sample (500 μM) and DPPH (50 μM) was mixed in a solution of MES buffer (pH 7.4) and ethanol (3:2) at 25 °C. The decrease in absorbance at 517 nm was recorded on a stopped-flow Rapid-Scan Spectrophotometer RSP-1000 (UNISOKU Co., Ltd.) for 60 sec. The second-order rate constant was calculated based on a decreasing curve fitting method (UNISOKU Spectroscopy & Kinetics, NISOKU Co., Ltd.).

4. Prediction of p*K*a values

Apparent p*K*a values of all UA analogs were theoretically calculated by using ACD/pKa DB (version 9.0) software.

4. Determination of cytotoxicity.

HL-60 cells ($5x10^5$ cells/mL) were plated onto a six-well multi-plate and incubated with the test compound in DMSO (0.5-100 μM) at 37 °C for 24 h under a 5% CO_2 atmosphere. Only the cells treated with DMSO were used as a non-treated control. The concentration of DMSO was set at 1 v/v %. The incubation mixture was centrifuged at 1,000 rpm for 5 min, and the pellet was suspended in 2 mL of PBS(-). The cells were stained with trypan blue, and the viable cells were counted by a Vi-CELL™ cell viability analyzer (Beckman Coulter Inc.). Cell viability was calculated by the following equation. Cell Viability (%) = (treated viable cells)/(non-treated control viable cells) x 100.