Supporting Information

for

Substrate/Product-Targeted NMR Monitoring of Pyrimidine Catabolism and Its Inhibition by a Clinical Drug

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1. Preparation

General. Reagents and solvents were purchased from standard suppliers and used without further purification. NMR spectra were obtained on a JEOL JNM-A500 (500 MHz) NMR or a JEOL JNM-ECX400 (400 MHz) spectrometer. The coupling constants (*J* values) are reported in Hertz. The EI mass spectra were recorded on a JEOL JMS SX102A mass spectrometer.

[^{13}C3]-Ethyl *N*-(2-cyano-3-ethoxyacryloyl)carbamate (2): A solution of ethyl *N*-(2-cyanoacetyl)carbamate (**1**) (1.57 g, 10.1 mmol) and labeled triethyl orthoformate ($H^{13}C(OCH_2CH_3)_3$) (99%, ^{13}C) (1.5 g, 10.1 mmol) in acetic anhydride (4 mL) was refluxed for 7 h. After the reaction mixture was allowed to cool to room temperature, the insoluble solid was collected by filtration and washed with petroleum ether and cold diethyl ether to give compound **2** (1.84 g, 86%) as a white solid; ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 10.58 (s, 1H), 8.57, 8.20 (d, J = 187.0 Hz, 1H), 4.37 (m, 2H), 4.13 (q, J = 7.0 Hz, 2H), 1.32 (t, J = 7.0 Hz, 3H), 1.22 (t, J = 7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 173.8, 158.8, 149.9, 113.2, 86.9 (d, J_{CC} = 85.8 Hz), 74.6, 62.5, 15.2, 14.1; HRMS (EI): *m*/*z* calcd. for $C_8{}^{13}CH_{12}O_4N_2$ [M^+] 213.0831, found 213.0834.

[^{15}N1, ^{13}C6]-5-Cyanouracil (3): A solution of compound **2** (1.74 g, 8.18 mmol) and labeled ammonium chloride ($^{15}NH_4Cl$) (99%, ^{15}N) (490 mg, 8.99 mmol) in 0.25 M aqueous NaOH (46 mL) was stirred for 4 h at room temperature, and then refluxed for 5 h. The reaction mixture was concentrated in vacuo. The crude residue was recrystallized from water to give compound **3** (1.05 g, 93%) as a white solid; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.14 (dd, J = 178.5, 7.5 Hz, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 161.1, 152.1 (d, J_{CN} = 12.0 Hz), 150.0 (d, J_{CN} = 17.7 Hz), 114.6, 87.1 (d, J_{CC} = 70.9 Hz); ^{15}N NMR (DMSO-d_6, 40 MHz) for labeled ^{15}N δ 198.6; HRMS (EI): *m*/*z* calcd. for $C_4{}^{13}CH_3O_2N_2{}^{15}N$ [M^+] 139.0229, found 139.0229.

[^{15}N1, ^{13}C6]-Uracil: A solution of compound **3** (1.38 g, 9.89 mmol) in aqueous 6N HCl (60 mL) was refluxed for 27 h. The reaction mixture was concentrated in vacuo. The crude residue was recrystallized from water to give doubly labeled [^{15}N1, ^{13}C6]-uracil (1.07 g, 95%) as a white solid; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.0 (s, NH), 10.8 (d, J = 97.2 Hz, NH), 7.37 (ddd, J = 182.4, 7.8,

3.6 Hz, 1H), 5.45-5.41 (1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 164.3, 151.0 (d, J_{CN} = 11.0 Hz), 142.1 (d, J_{CN} = 10.5 Hz), 100.2 (d, J_{CC} = 66.1 Hz); ^{15}N NMR (DMSO-d_6, 40 MHz) labeled for ^{15}N δ 127.4 (d, J_{CN} = 10.4 Hz); HRMS (EI): *m*/*z* calcd. for $C_3{}^{13}CH_4O_2N^{15}N$ [M^+] 114.0277, found 114.0277.

The NMR spectra for compounds **2** and **3** and [^{15}N1, ^{13}C6]-uracil are shown below in Figures S2-S9.

2. Pulse Scheme for One-dimensional ^{1}H-{^{13}C-^{15}N} Triple Resonance NMR

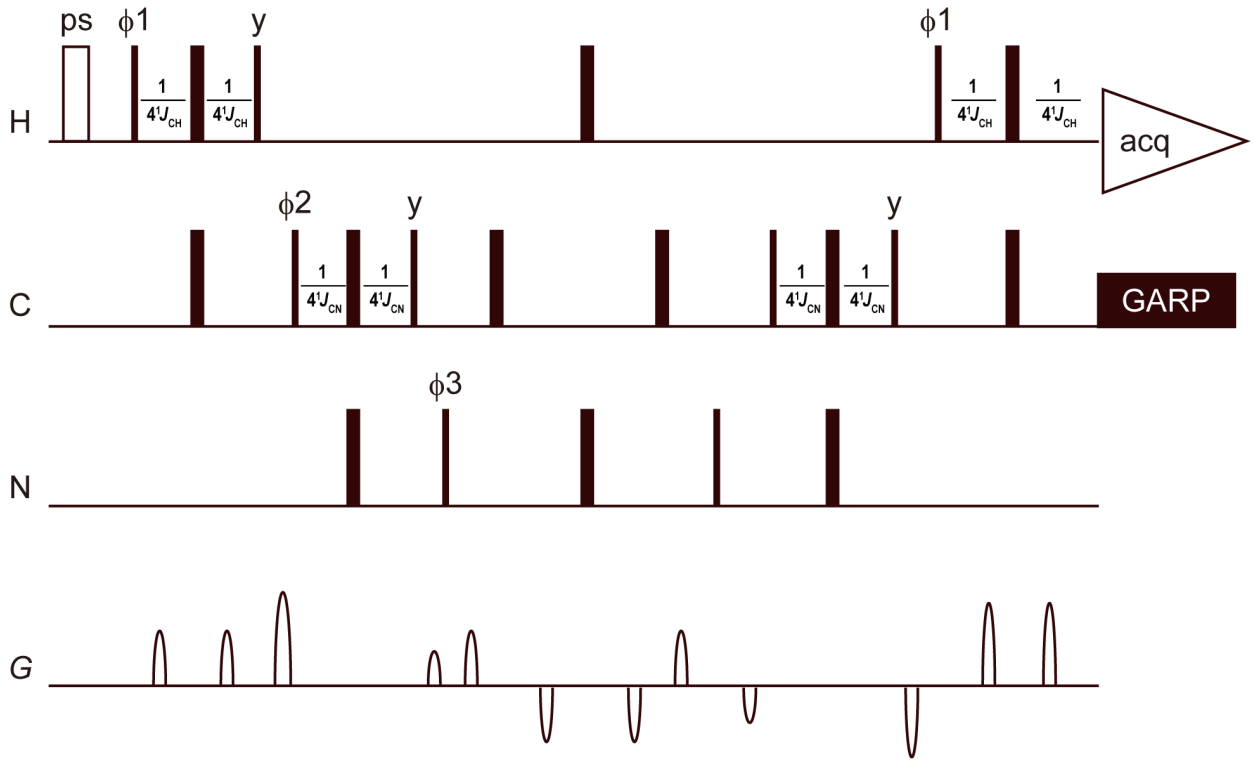

Figure S1. Pulse scheme of one-dimensional ^{1}H-{^{13}C-^{15}N} triple resonance NMR experiments used in this study. The narrow and broad filled bars represent 90° and 180° pulses, respectively. All pulses have phase = *x* unless otherwise indicated. Parameters for detection of ^{1}H-^{13}C-^{15}N in labeled uracil were transmitter offsets of ^{13}C = 143 ppm and ^{15}N = 110 ppm, and delay intervals of $1/4^{1}J_{CH}$ = 1.36 ms and $1/4^{1}J_{CN}$ = 32 ms. Those for detection of ^{1}H-^{13}C-^{15}N in labeled β-alanine were ^{13}C = 36.3 ppm, ^{15}N = 30.5 ppm, $1/4^{1}J_{CH}$ = 1.72 ms, and $1/4^{1}J_{CN}$ = 48 ms. PS denotes a pre-saturation pulse (1.5 ms) used for water suppression. The phase cycle is ϕ1 = *x*, -*x*; ϕ2 =2(*x*), 2(-*x*); ϕ3 = 4(*y*), 4(-*y*) and receiver = 2(*y*), 4(-*y*), 2(*y*). During the detection period, ^{13}C GARP decoupling is used. All gradients were applied along the *z* axis.

3. NMR Spectra of Labeled Compounds

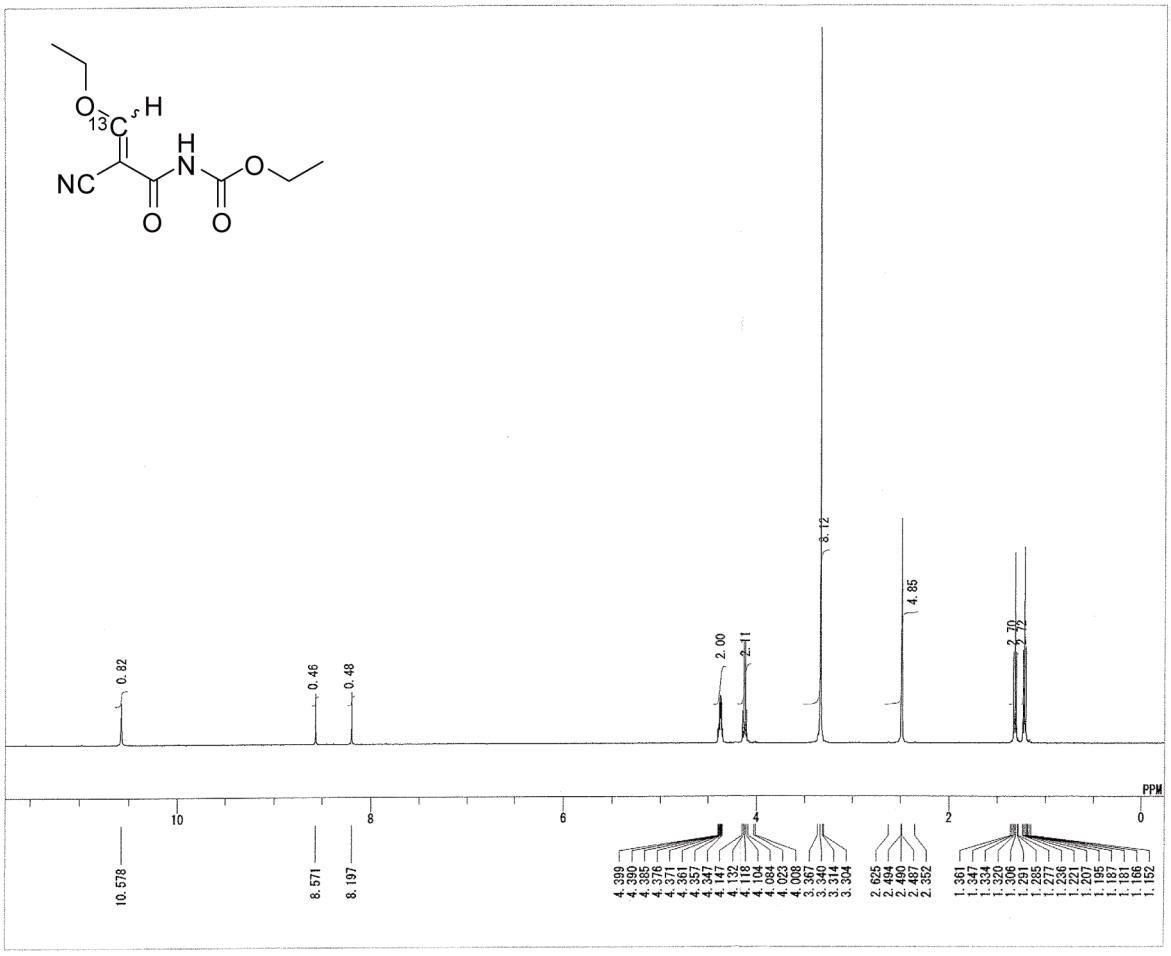

Figure S2. ^{1}H NMR spectrum of compound **2** in DMSO-d_6 (500 MHz).

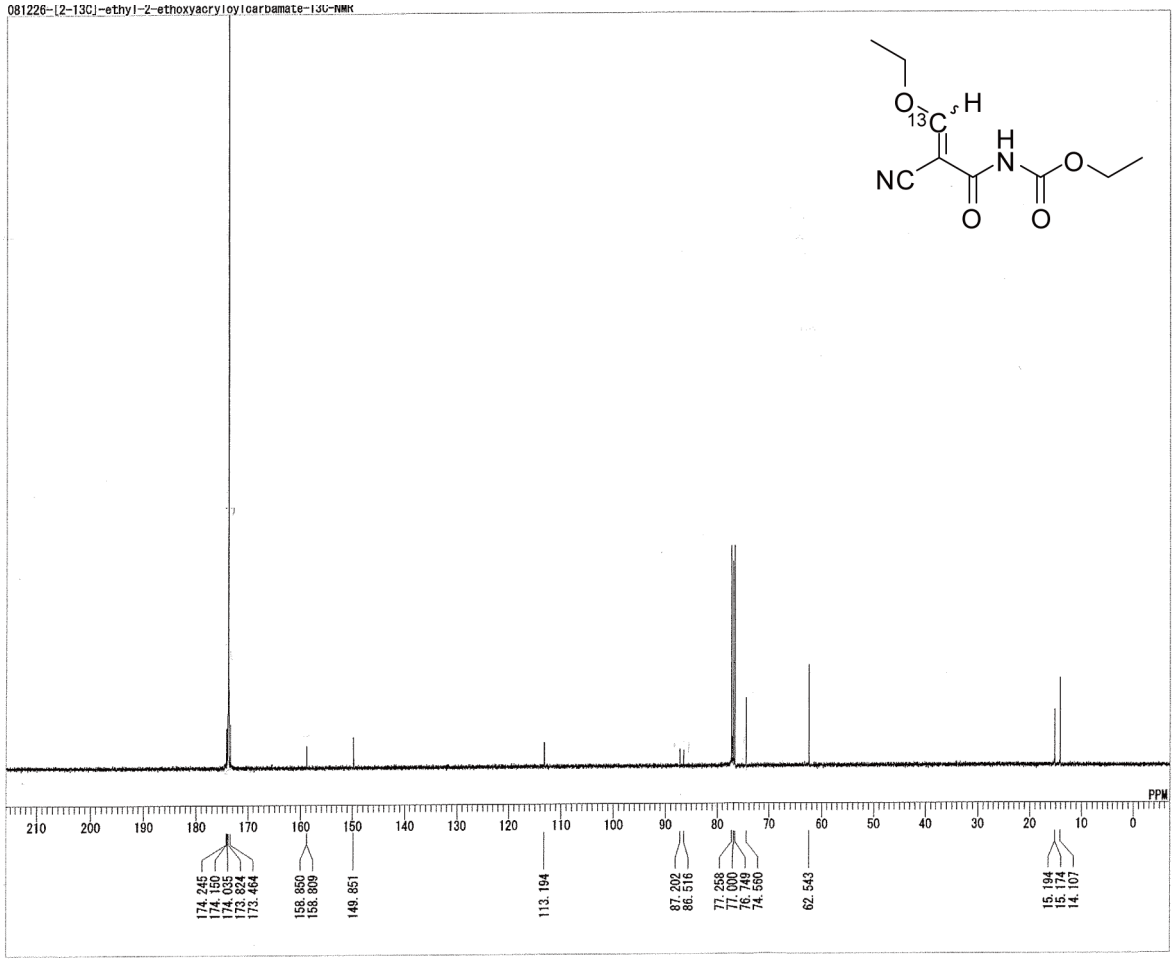

Figure S3. ^{13}C NMR spectrum of compound **2** in DMSO-d_6 (125 MHz).

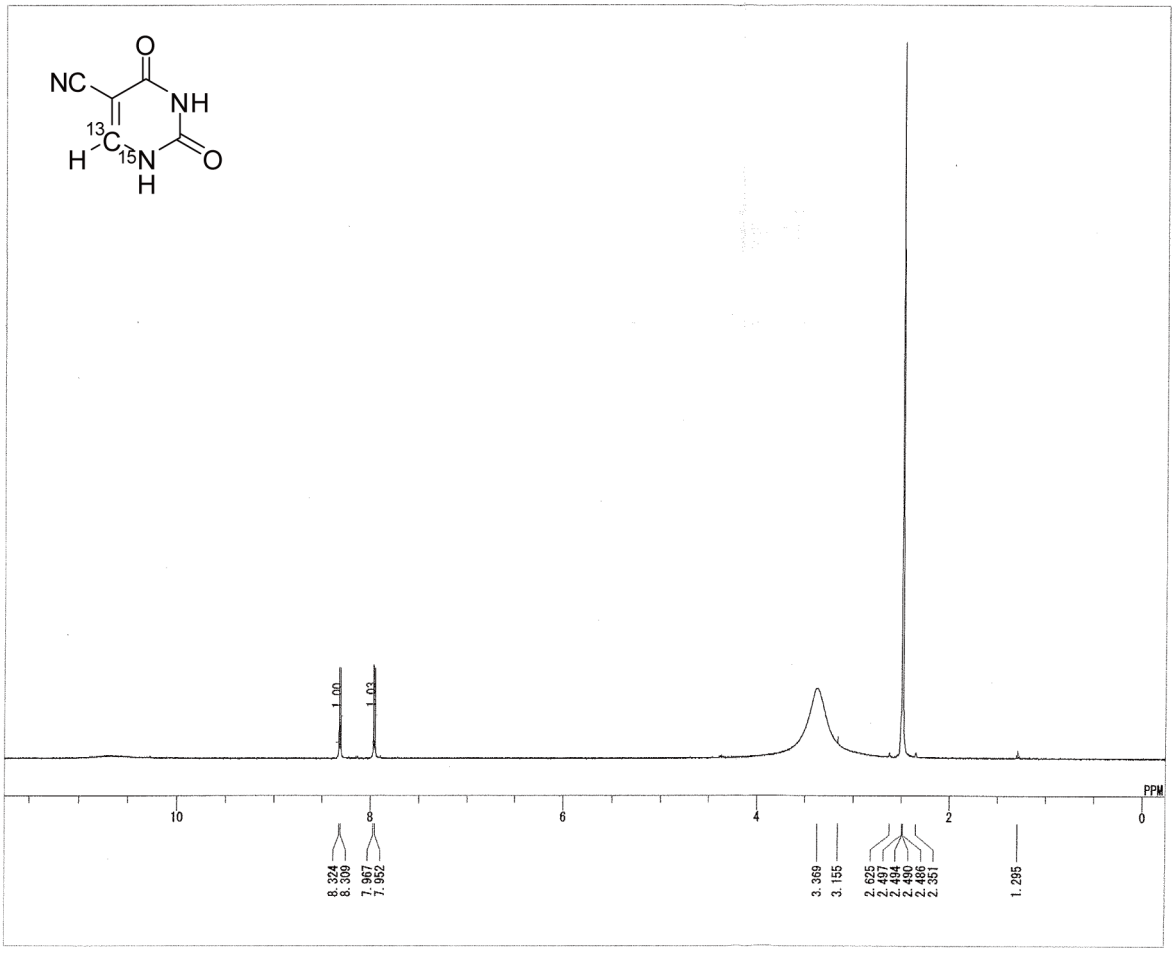

Figure S4. ^{1}H NMR spectrum of compound **3** in DMSO-d_6 (500 MHz).

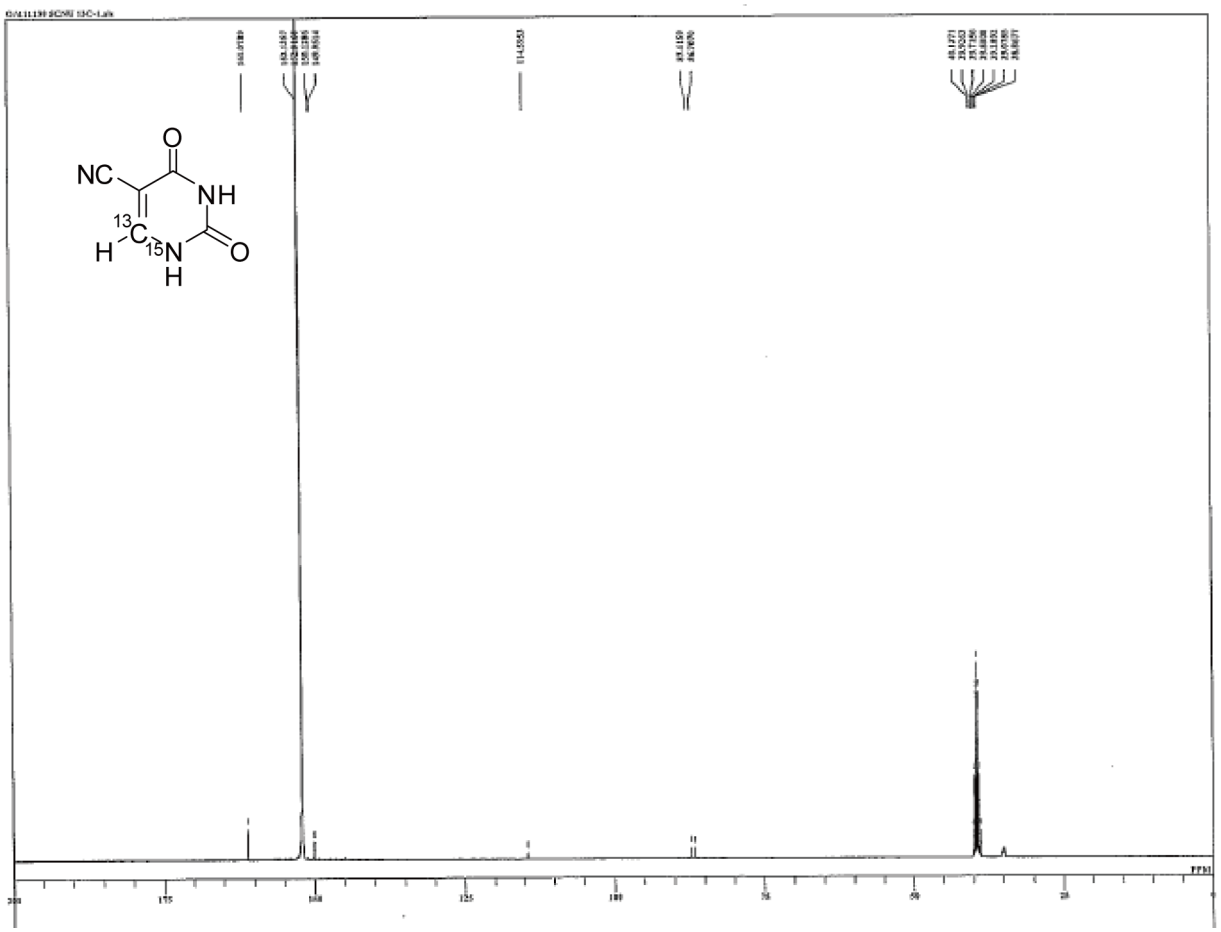

Figure S5. ^{13}C NMR spectrum of compound **3** in DMSO-d_6 (100 MHz).

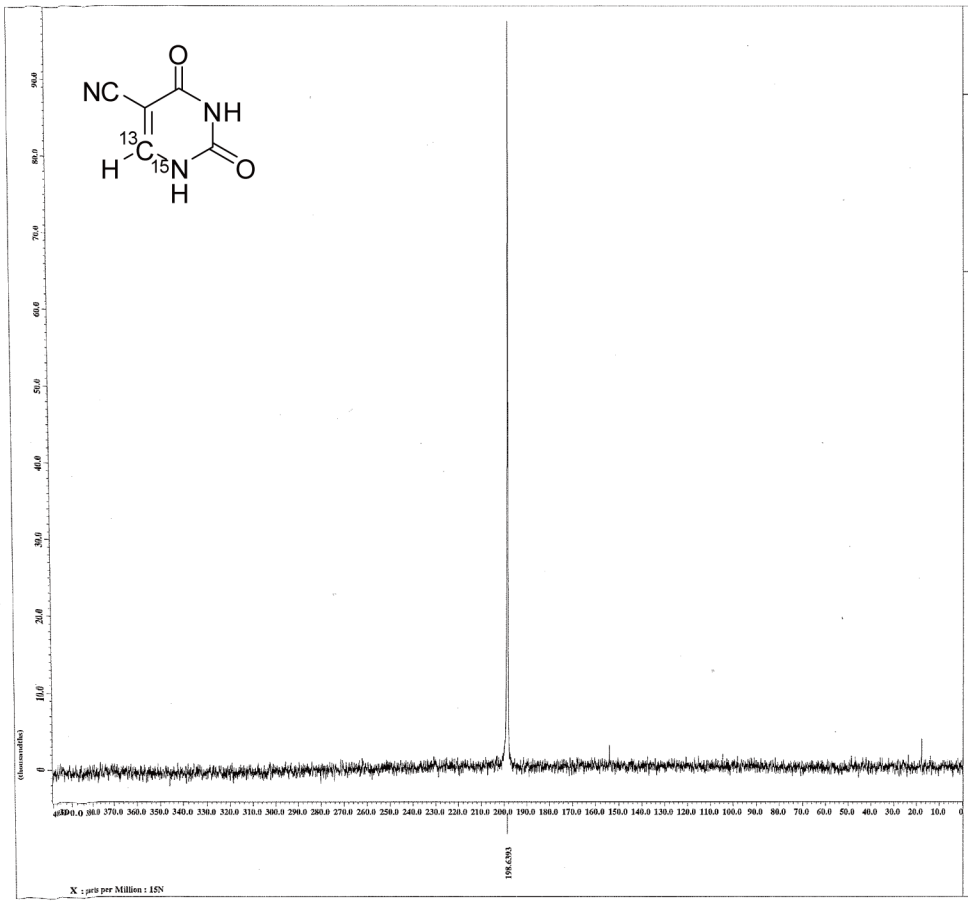

Figure S6. ^{15}N NMR spectrum of compound **3** in DMSO-d_6 (40 MHz).

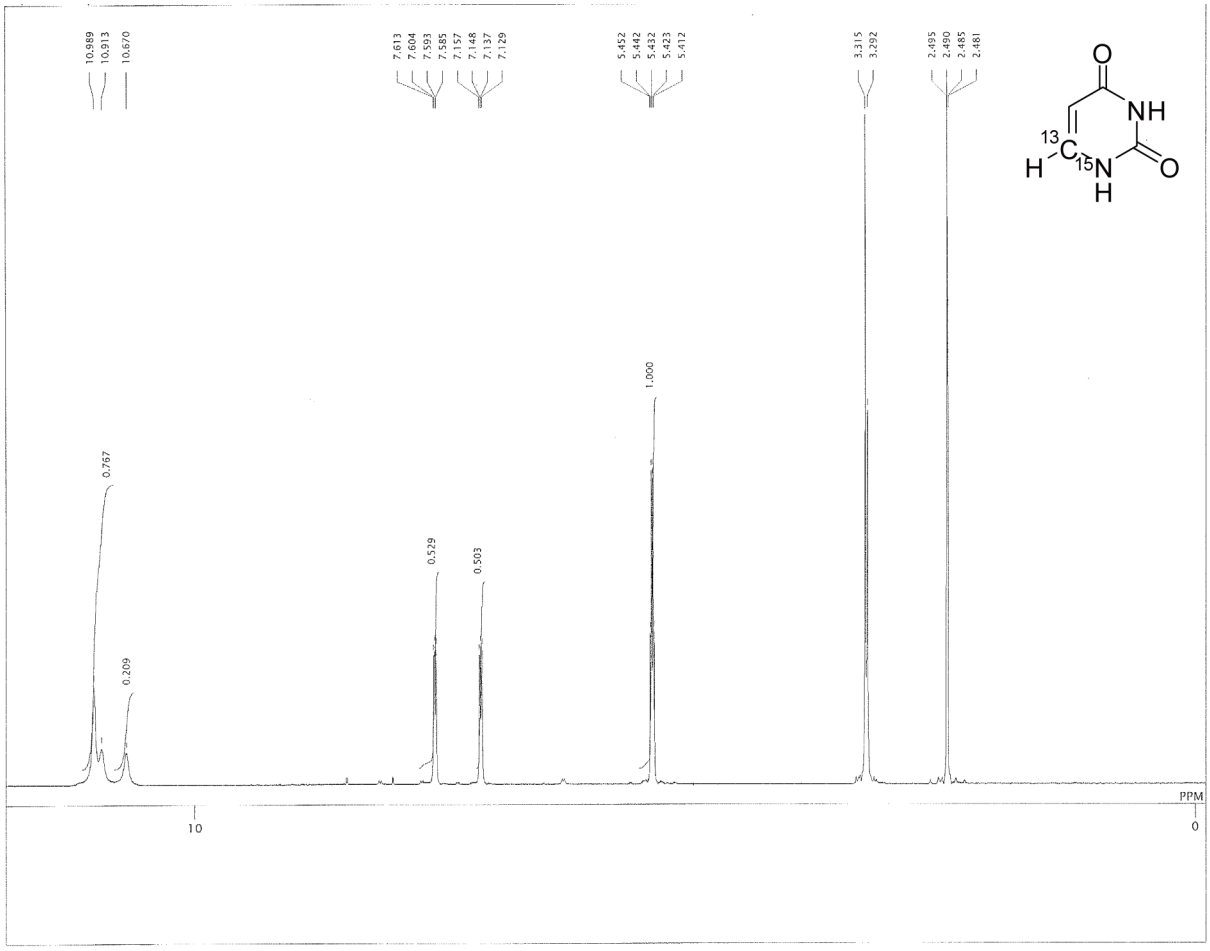

Figure S7. ^{1}H NMR spectrum of [^{15}N1, ^{13}C6]-uracil in DMSO-d_6 (400 MHz).

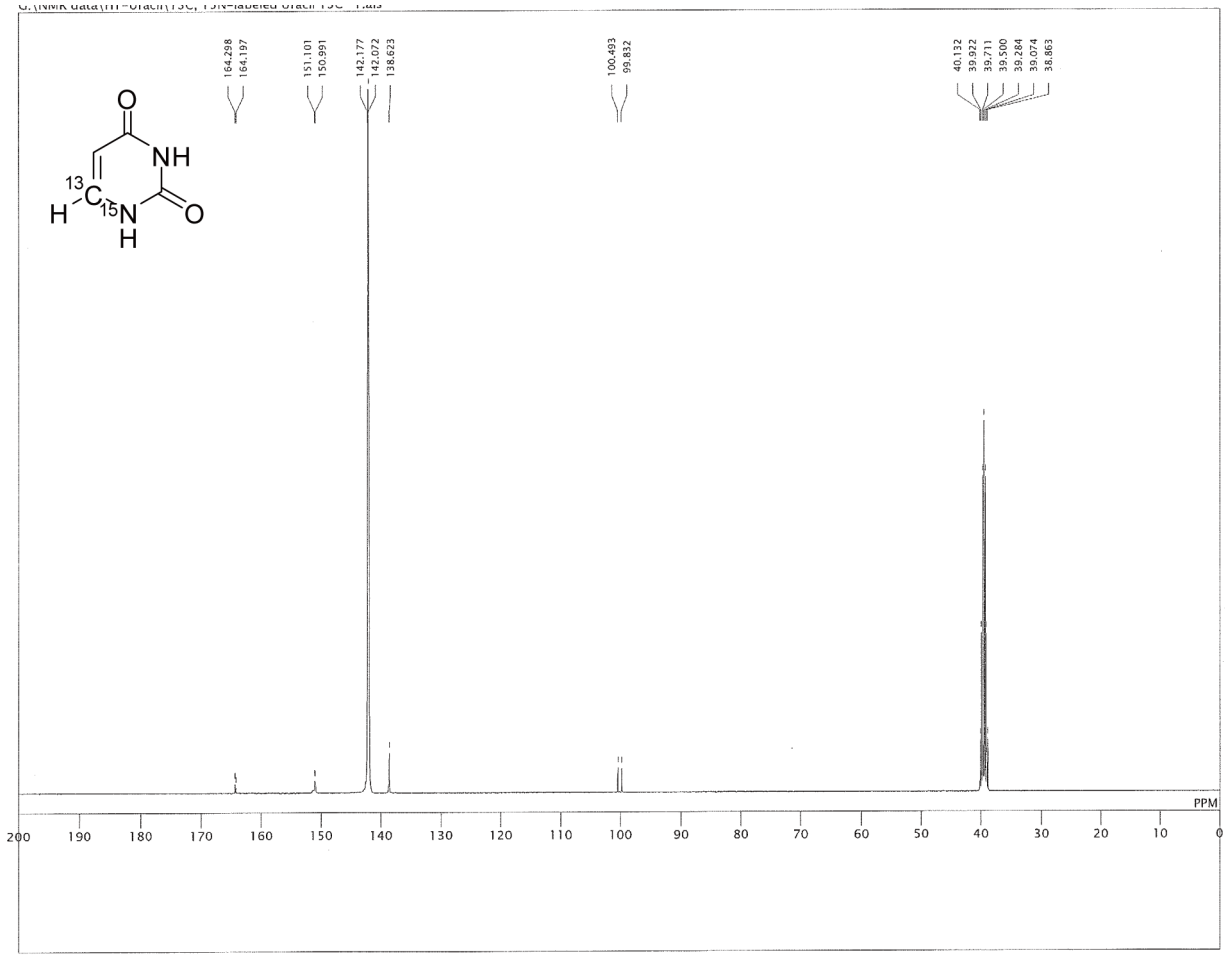

Figure S8. ^{13}C NMR spectrum of [$^{15}N1$, $^{13}C6$]-uracil in DMSO-d_6 (100 MHz).

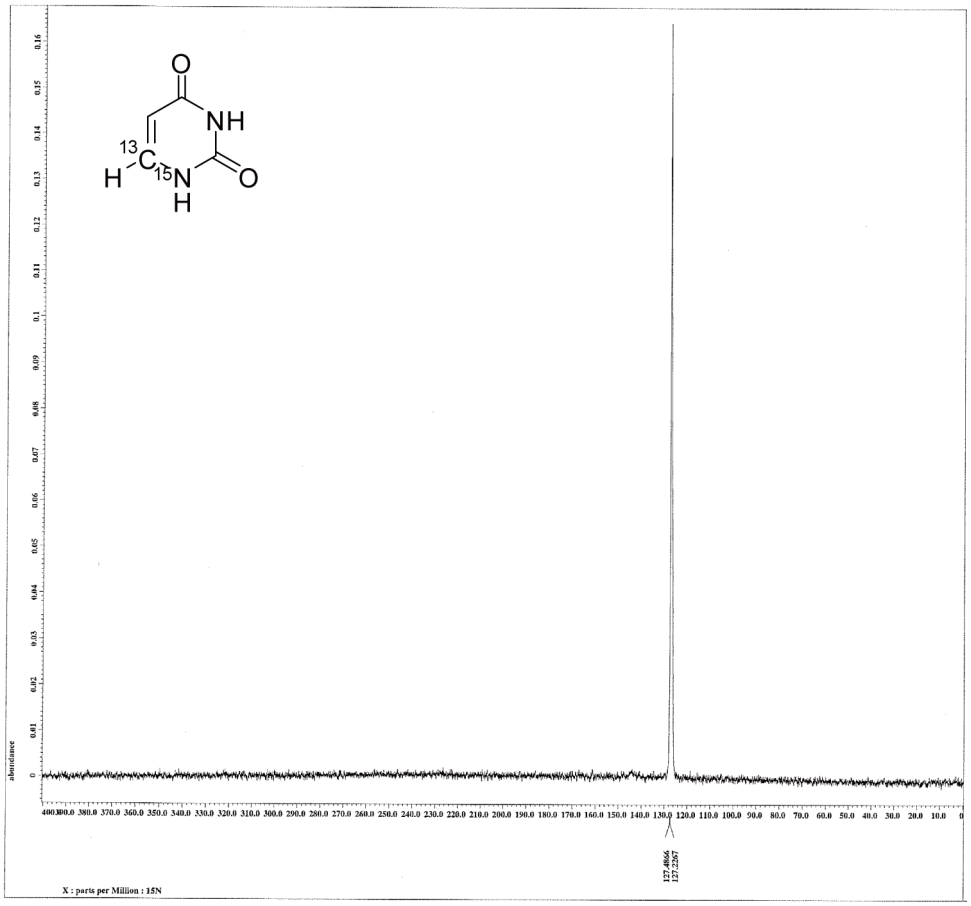

Figure S9. ^{15}N NMR spectrum of [^{15}N1, ^{13}C6]-uracil in DMSO-d_6 (40 MHz).

4. Uracil-Optimized 1D ^{1}H-{^{13}C} HSQC Spectrum

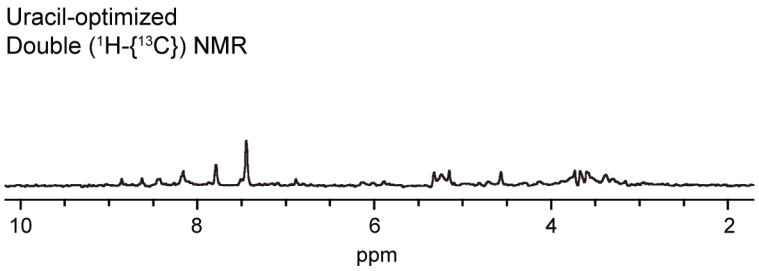

Figure S10. Uracil-optimized 1D ^{1}H–{^{13}C} double-resonance NMR spectrum of a mouse liver lysate (10% v/v) containing [^{15}N1, ^{13}C6]-labeled uracil (0.5 mM) in 10 mM Tris-HCl (pH 8.0), 0.5 mM EDTA, 0.5 mM 2-mercaptoethanol, 2 mM dithiothreitol, 5 mM $MgCl_2$, 2 mM NADPH, incubated at 37 °C for 1 h. Uracil-optimized parameters: chemical-shift (^{13}C = 142.5 ppm) and coupling-constant data ($J_{C–H}$ = 184 Hz).

5. 2D ^{1}H-^{13}C HSQC Spectra

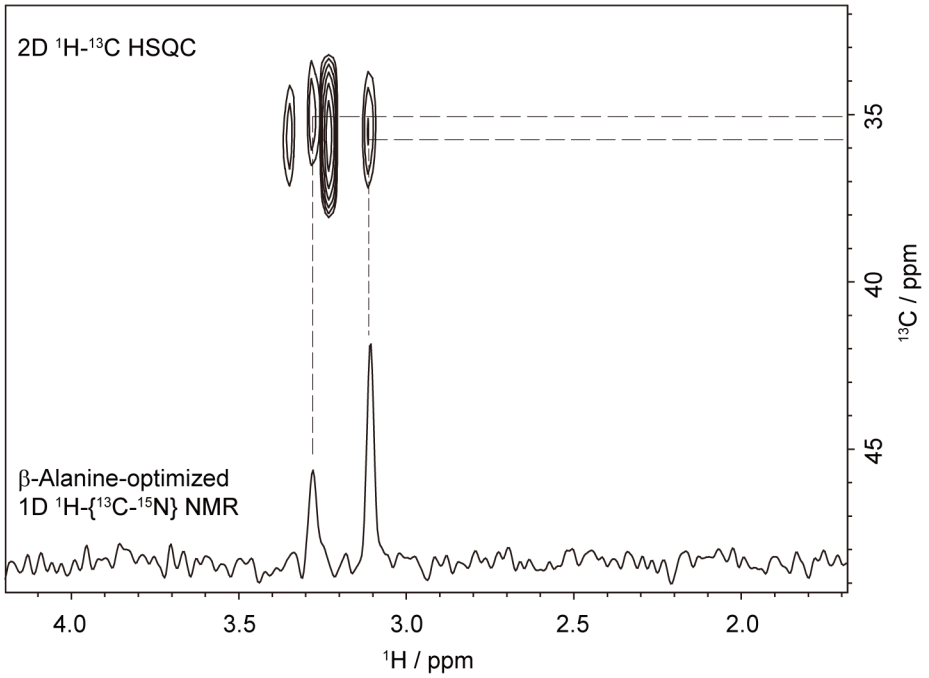

Figure S11. 2D ^{1}H–^{13}C HSQC spectrum of the extracts from kidney of a mouse injected intraperitoneally with a solution of [^{15}N1, ^{13}C6]-labeled uracil (300 μg g^{-1} body weight). The 2D HSQC spectrum is merged with the β-alanine-optimized 1D triple-resonance NMR spectrum of the kidney extracts (Figure 5A).

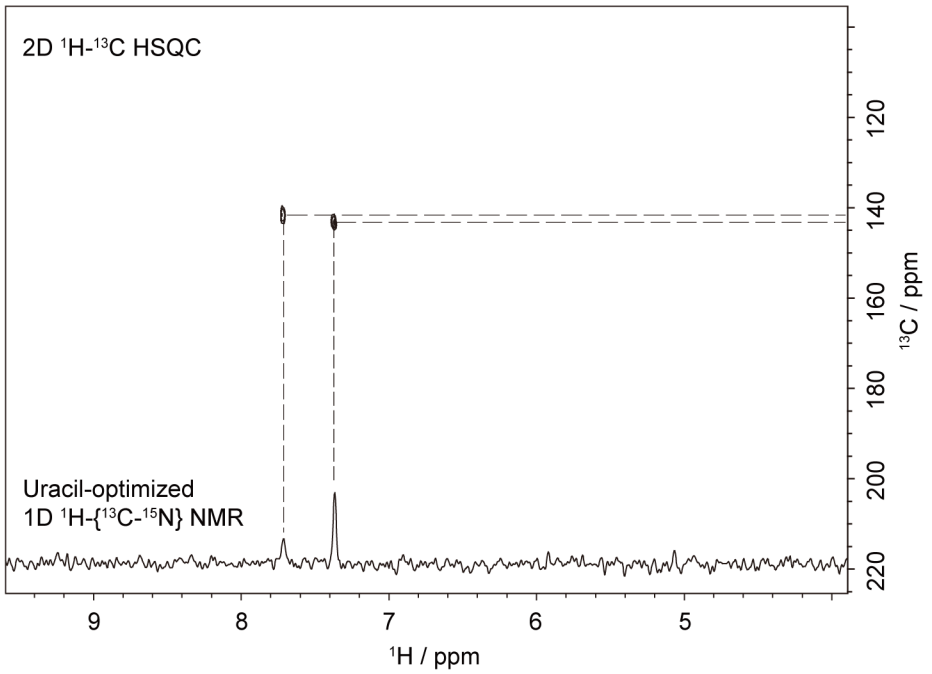

Figure S12. 2D ^{1}H–^{13}C HSQC spectrum of the extracts from liver of a mouse injected intraperitoneally with a solution of [^{15}N1, ^{13}C6]-labeled uracil (300 μg g^{-1} body weight). The 2D HSQC spectrum is merged with the uracil-optimized 1D triple-resonance NMR spectrum of the liver extracts (Figure 5B).

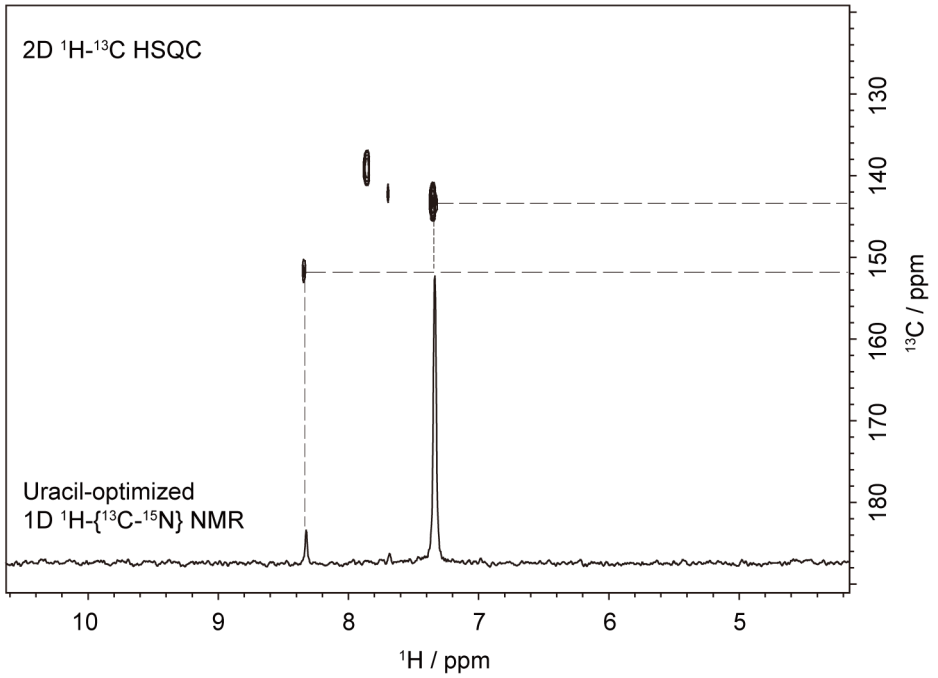

Figure S13. 2D ^{1}H–^{13}C HSQC spectrum of the extracts from kidney of a mouse injected intraperitoneally with a solution of [$^{15}N1$, $^{13}C6$]-labeled uracil (300 μg g^{-1} body weight). The 2D HSQC spectrum is merged with the uracil-optimized 1D triple-resonance NMR spectrum of the kidney extracts (Figure 5B).