Supporting Information for:

Synthesis and identification of proposed biosynthetic intermediates of saxitoxin in the cyanobacterium *Anabaena circinalis* (TA04) and the dinoflagellate *Alexandrium tamarense* (Axat-2)

Shigeki Tsuchiya,[a] Yuko Cho,[a] Keiichi Konoki,[a] Kazuo Nagasawa,[b] Yasukatsu Oshima[c] and Mari Yotsu-Yamashita*[a]

[a]*Graduate School of Agricultural Science, Tohoku University 1-1 Tsutsumidori-Amamiya, Aoba-ku, Sendai 981-8555, Japan.*
Email: myama@biochem.tohoku.ac.jp; Tel: (+81)22-717-8922

[b]*Faculty of Technology, Tokyo University of Agriculture and Technology 2-24-16 Naka-cho, Koganei-shi, Tokyo 184-8588, Japan*

[c]*Graduate School of Life Sciences, Tohoku University1-1 Katahira, Aoba-ku, Sendai 980-8577, Japan*

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1. General information

All chemical synthetic experiments were conducted under nitrogen. The dry solvents used for organic synthesis were purchased from Wako Chemicals (Osaka, Japan). The reagents were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA), Wako Chemicals, Tokyo Chemical Industry Co. (Tokyo, Japan) and Nacalai Tesque Co. (Kyoto, Japan). LC-MS grade acetonitrile (Wako Chemicals), ammmonium formate and formic acid (Optima™ LC/MS Grade, Fisher Scientific, Waltham, MA, USA) were used for LC-Q-TOF MS. Distilled and purified water (MilliQ) by Simplicity UV (Millipore, Billerica, MA, USA) was used for all of the experiments. FT-IR (film: ATR, Zn-Se) and optical rotation were measured with a JASCO 4100 spectrometer JASCO DIP-370 digital polarimeter (JASCO, Tokyo, Japan), respectively. NMR spectra were recorded with the Agilent 600 MHz NMR spectrometer (Agilent Technologies, Santa Clara, CA, USA) and $CDCl_3$ or CD_3OD as solvent and internal standard, respectively. The spectra were referenced to residual solvent signals with resonances at $\delta_{H/C}$=3.30/49.0 ppm (CD_3OD) and $\delta_{H/C}$=7.26/77.0 ppm ($CDCl_3$). LC-MS was performed with micrOTOF-Q II (ESI, Q-TOF) (Bruker Daltonics, Billerica, MS, USA) and API2000 (AB SCIEX, Foster City, CA, USA). High resolution-MS was measured with a micrOTOF-Q II (ESI) and a JEOL JMS700 MS Station (JEOL, Akishima, Japan) (FAB, glycerol and *m*-nitrobenzyl alcohol matrix).

2. Synthetic procedures

Tri-Boc-protected L-arginine (10)[9]: Basic cupric carbonate (65.2 mg, 0.294 mmol) was added to the L-ornithine hydrochloride solution (24.5 mg, 0.145 mmol) in water (1 mL), and the mixture was boiled for 1 h. After removal of the excess cupric carbonate by filtration, the filtrates were concentrated *in vacuo* to produce a blue solid. *N,N*-diisopropylethylamine (DIEA, 59.0 μL, 0.339 mmol) and *N,N'*-bis(tert-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine (45.6 mg, 0.147 mmol) were added to the blue solid in dry *p*-dioxane/formamide (1:2, v/v, 430 μL) under nitrogen atmosphere at room temperature. After stirring for 5 h, water (30 mL) was added to the reaction mixture, and the mixture was extracted with EtOAc (30 mL) three times. The organic layer was washed with brine and water and was concentrated *in vacuo*. EDTA 2Na (34.6 mg, 0.092 mmol), $NaHCO_3$ (36.8 mg, 0.438 mmol) and water (280 μL) were added to the residue and stirred. Then, di-*tert*-butyl dicarbonate (72.5 mg, 0.332 mmol) and acetone (1.4 mL) were added to the mixture. After vigorous stirring for 4 h at room temperature, acetone in the reaction mixture was evaporated *in vacuo*. Distilled water (30 mL) was added to the mixture and was extracted with EtOAc (30 mL) three times. The organic layer was washed with brine (10 mL) and water (10 mL) and then concentrated *in vacuo*. The residue was purified by Cosmosil 140C18-OPN (Nacalai tesque, Kyoto, Japan) column chromatography (water, then MeOH/water 1:1 to 3:1) to produce **10** (25.9 mg, 37%, 3 steps). ^{1}H NMR (600 MHz, $CDCl_3$): δ=11.48 (brs, 1H), 8.46 (s, 1H), 5.34 (d, $J_{H,H}$ = 4.8 Hz, 1H), 4.34 (s, 1H), 3.42 (s, 2H), 1.92 (s, 1H), 1.72-1.69 (m, 3H), 1.49 (m, 9H), 1.48 (s, 9H), 1.44 ppm (s, 9H); ^{13}C NMR (151 MHz, $CDCl_3$): δ=174.7, 162.91, 156.32, 155.82, 153.19, 83.44, 80.21, 79.83, 50.86, 40.35, 29.59, 28.32, 28.18, 28.04, 27.52, 25.30 ppm; HRMS (FAB) (*m/z*): $[M+H]^+$ calcd. for $C_{21}H_{39}N_4O_8$, 475.2762; found, 475.2769.

Tri-Boc-protected arginine Weinreb amide (11): 1-hydroxybenzotriazole monohydrate (HOBt, 7.2 mg, 0.053 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, 12.6 mg, 0.066 mmol) in dry dichloromethane (220 μL) were added to a solution of tri-Boc-protected arginine **10** (25.9 mg, 0.054 mmol) at 0°C. After stirring for 15 min, *N,O*-dimethylhydroxylamine hydrochloride salt (6.2 mg, 0.064 mmol) and *N*-methyl morpholine (7 μL, 0.064 mmol) were added to the reaction mixture, and the resultants were stirred overnight at room temperature. Then, the solvent was removed *in vaccuo*, and the resulting residue was partitioned between 1 m HCl aq. (30 mL) and EtOAc (30 mL). The organic layer was washed with saturated $NaHCO_3$ (10 mL), brine (10 mL) and water (10 mL) and concentrated. The residue was purified by Cosmosil 140C18-OPN (Nacalai tesque, Kyoto, Japan) column chromatography (water, then MeOH:water = 3:1 to 1:0) to produce **11** (27.1 mg, 0.052 mmol, 95%).^{1}H NMR (600 MHz, $CDCl_3$): δ=11.49 (s, 1H), 8.35 (s, 1H), 5.22 (d, $J_{H,H}$ = 9.0 Hz, 1H),

4.68 (s, 1H), 3.77 (s, 3H), 3.44 (s, 2H), 3.20 (s, 3H), 1.77 (m,1H), 1.68-1.57 (m,3H),1.50 (s, 9H), 1.49 (s, 9H), 1.44 ppm (s, 9H); ^{13}C NMR (151 MHz, $CDCl_3$): δ=172.69, 163.49, 156.08, 155.52, 153.22, 83.18, 79.67, 79.35, 61.64, 50.12, 40.44, 32.06, 30.08, 28.35, 28.27, 28.05, 25.13 ppm; HRMS (FAB) (*m/z*): $[M+H]^+$ calcd. for $C_{23}H_{44}N_5O_8$, 518.3184; found, 518.3193.

Tri-Boc-protected arginine ethyl ketone (12): Ethylmagnesium bromide (3.0 M in ether, 155 μL, 0.475 mmol) was added to the Weinreb amide solution **11** (22.2 mg, 0.043 mmol) in dry THF (470 μL) on ice, and the resulting reaction mixture was stirred overnight at room temperature. The reaction was quenched with 0.1 M HCl aq. (10 mL) and was extracted with EtOAc (30 mL) three times. The organic layer was washed with brine (10 mL) and water (10 mL) and concentrated. The residue was purified by reverse phase HPLC (column; Mightysil RP-18 GP column, 5 μm, 4.6x250 mm, Kanto Chemical, Tokyo, Japan, solvent; MeOH/water 80:20, v/v, with the flow rate of 0.5 mL/min, 33 min; ESI-MS detection) to produce **12** (6.2 mg, 30%). ^{1}H NMR (600 MHz, $CDCl_3$): δ= 11.48 (s, 1H), 8.35 (s, 1H), 5.38 (d, $J_{H,H}$ = 7.2 Hz, 1H), 4.34 (dd, $J_{H,H}$ = 11.4, 7.2 1H), 3.43 (d, $J_{H,H}$ = 4.8 Hz, 2H), 2.53 (m, 2H), 1.88 (m, 1H), 1.63 (m, 1H), 1.58 (m, 1H), 1.55 (m, 1H), 1.49 (s, 9H), 1.48 (s, 9H), 1.43 (s, 9H), 1.07 ppm(t, $J_{H,H}$ = 7.2 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$): δ=209.82, 163.43, 156.18, 155.50, 153.26, 83.26, 79.77, 79.37, 58.73, 40.31, 32.91, 28.80, 28.34, 28.26, 28.04, 25.03, 7.59 ppm; HRMS (*m/z*) (FAB): $[M+H]^+$ calcd. for $C_{23}H_{43}N_4O_7$, 487.3126; found, 487.3132.

Int-A' (2): Trifluoroacetic acid (TFA, 500 μL) was added to the ethyl ketone solution **12** (3.2 mg, 0.007 mmol) in dichloromethane (500 μL). After stirring for 1 h at room temperature, the solvent was removed by the stream of nitrogen gas affording analytically pure Int-A' (**2**) as 2 TFA salt (2.5 mg, 0.006 mmol, 92%). $[\alpha]_D^{20}$ = 30.6 (*c* = 0.18, MeOH); IR (ATR): 3353, 3187, 2989, 1676, 1203, 1138 cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD): δ=4.19 (H-5, dd, $J_{H,H}$ = 7.8, 4.2, 1H), 3.24 (H-2, td, $J_{H,H}$ = 7.2, 1.8, 2H), 2.70 (H-7, m, 1H), 2.59 (H-7, m, 1H), 2.04 (H-4, m, 1H), 1.87 (H-4, m, 1H), 1.70 (H-3, m, 1H), 1.59 (H-3, m, 1H), 1.10 ppm (H-8, t, $J_{H,H}$ = 7.2 Hz, 3H); ^{13}C NMR (151 MHz, CD_3OD): δ=207.99 (C-6), 159.54 (C-1), 60.15 (C-5), 42.54 (C-2), 34.06 (C-7), 28.74 (C-4), 26.13 (C-3), 8.32 ppm (C-8); HRMS (ESI^+) (*m/z*): $[M+H]^+$ calcd. for $C_8H_{19}N_4O$, 187.1553; found, 187.1553.

Tetra-Boc-protected bis-guanidine carboxylic acid (13): To a solution of L-ornithine hydrochloride (105.3 mg, 0.624 mmol) in dry *p*-dioxane/ formamide (3:1, v/v, 4.8 mL) was added DIEA (220 μL) and *N,N'*-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine (407 mg, 1.26 mmol = 2.02 eq) at room temperature. After stirring for 24 h under nitrogen atmosphere, 0.1 M HCl aq (50 mL) was added to the reaction mixture, and extracted with EtOAc (50 mL) three times. The organic layer was washed with brine (30 mL) and water (30 mL), and concentrated *in vacuo.* The residue was purified by Cosmosil 140C18-OPN column chromatography (water, then MeOH:water=3:2 to 1:0) to produce **13** (214.1 mg, 0.347 mmol, 56%). ^{1}H NMR (600 MHz, $CDCl_3$): δ=11.49 (brs, 1H), 11.32 (s, 1H), 8.71 (d, $J_{H,H}$ = 5.2 Hz, 1H), 8.44 (s, 1H), 4.50 (dd, $J_{H,H}$ = 13.2, 6 Hz, 1H), 3.50 (m, 2H), 2.11 (m, 1H), 1.84 (m, 1H), 1.72 (m, 2H), 1.51 (s, 9H), 1.50 (s, 9H), 1.49 (s, 9H), 1.48 ppm (s, 9H) (one N-H proton was not found probably due to exchange with solvent or sever broadening); ^{13}C NMR (151 MHz, $CDCl_3$): δ=171.14, 161.23, 156.80, 156.15, 153.18, 152.66, 84.51, 83.35, 80.70, 54.49, 40.18, 28.23, 28.05, 28.04, 27.97, 27.61, 25.32 ppm (one sp^2 carbon signal and one sp^3 carbon signal were missing, probably due to overlapping with other close signals); HRMS (FAB) (*m/z*): $[M+H]^+$ calcd. for $C_{27}H_{49}N_6O_{10}$, 617.3505; found, 617.3511.

Tetra-Boc protected bis-guanidine Weinreb amide (14): *N,O*-dimethylhydroxylamine hydrochloride salt (15.1 mg, 0.155 mmol), EDC (31.2 mg, 0.163 mmol), and *N*-methyl morpholine (16 μL, 0.145 mmol) were added to a solution of **13** (84.0 mg, 0.136 mmol) in dry dichloromethane (1 mL) at 0°C. Then, the reaction mixture was stirred at room temperature for overnight. After evaporation of the solvent *in vaccuo*, the resulting residue was partitioned between 1 m HCl aq (50 mL) and EtOAc (50 mL). The organic layer was washed with saturated $NaHCO_3$ (30 mL), brine (30 mL) and water (30 mL), and concentrated. The residue was purified by Cosmosil 140C18-OPN column chromatography (water, then MeOH:water=4:1 to 1:0) to produce **14** (63.8 mg, 0.097 mmol, 71%). ^{1}H NMR (600 MHz, $CDCl_3$): δ=11.50 (s, 1H), 11.30 (s, 1H), 8.80 (brs, 1H), 8.35 (s, 1H), 5.19 (drs, 1H), 3.87 (s, 3H), 3.46 (brs, 2H), 3.22 (s, 3H), 1.89 (m, 1H), 1.73 (m, 1H), 1.65 (m, 2H), 1.50 (m, 9H), 1.49 (s, 9H), 1.49 (s, 9H), 1.45 ppm (s, 9H); ^{13}C NMR (151 MHz, $CDCl_3$): δ=171.79, 163.52, 163.15, 156.10, 155.48, 153.2, 152.71, 83.12, 83.06, 79.25, 78.79, 61.44, 50.28, 40.40, 31.96, 29.12, 28.26, 28.20, 28.06, 28.04, 24.79 ppm; HRMS (FAB) (*m/z*): $[M+H]^+$ calcd. for $C_{29}H_{54}N_7O_{10}$, 660.3927; found, 660.3932.

Tetra-Boc-protected bis-guanidine ethyl ketone (15): Ethylmagnesium bromide (3.0 m in ether, 400 μL, 1.200 mmol) was added to a solution of Weinreb amide **14** (13.9 mg, 0.021 mmol) in dry THF (1 mL) on ice under nitrogen atmosphere, then the reaction mixture was stirred at 40-50°C for 1 h. The reaction was quenched with 0.1 m HCl aq (30 mL) and extracted

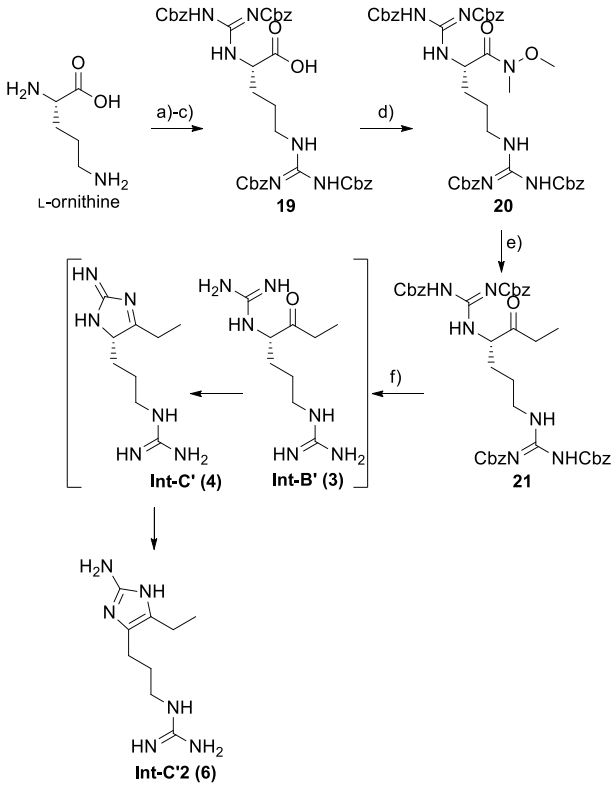
with EtOAc (30 mL) three times. The organic layer was washed with brine (10 mL) and water (10 mL), and concentrated. The residue was purified by reversed phase HPLC (column; Mightysil RP-18 GP column, 5 μm, 4.6x250 mm, Kanto Chemical, Tokyo, Japan, solvent; MeOH/water (90:10, v/v) with the flow rate of 0.5 mL/min, 15min; ESI-MS detection) to produce **15** (5.2 mg, 0.008 mmol, 40%). ^{1}H NMR (600 MHz, $CDCl_3$): δ=11.49 (s, 1H), 11.38 (s, 1H), 8.95 (brs, 1H), 8.34 (brs, 1H), 4.87 (drs, 1H), 3.44 (s, 2H), 2.54 (m, 2H), 2.03 (m, 1H), 1.71 (m, 1H), 1.60 (m, 2H), 1.50 (s, 9H), 1.49 (s, 9H), 1.49 (s, 9H), 1.47 ppm (s. 9H), 1.08 (t, 7.2 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$): δ=208.63, 163.31 (trace, the signal corresponding to another guanidine carbon was not shown), 156.09, 155.45, 153.23, 152.84, 83.32, 79.31 (83.32, 79.31: probably for four quaternary carbons in BOC groups), 58.72, 40.41, 32.95, 28.44, 28.24, 28.07, 28.04, 24.47, 7.52 ppm; HRMS (FAB) (*m/z*): $[M+H]^+$ calcd. for $C_{29}H_{53}N_6O_9$, 629.3869; found, 629.3879.

Int-C'2 (6): Trifluoroacetic acid (TFA, 500 μL) was added to a solution of ethyl ketone **15** (6.3 mg, 0.010 mmol) in dichloromethane (500 μL), then the mixture was stirred for 1 h at room temperature. The solvent was evaporated by the stream of nitrogen gas affording analytically pure Int-C'2 (**6**) as 2 TFA salt (4.1 mg, 0.009 mmol, 93%). ^{1}H NMR (600 MHz, CD_3OD): δ=3.18 (H-2, t, $J_{H,H}$ = 7.2 Hz, 2H), 2.53 (H-4, t, $J_{H,H}$ = 7.8 Hz, 2H), 2.48 (H-8, q, $J_{H,H}$ = 7.8 Hz, 2H), 1.83 (H-3, quin, $J_{H,H}$ = 7.2 Hz, 2H), 1.17 ppm (H-9, t, $J_{H,H}$ = 7.2 Hz, 3H); ^{13}C NMR (151 MHz, CD_3OD): δ=159.54 (C-1), 148.82 (C-6), 126.16 (C-7), 121.85 (C-5), 42.27 (C-2), 30.03 (C-3), 22.10 (C-4), 18.47 (C-8), 15.04 ppm (C-9); 2FTA. IR (ATR): 3359, 3191, 2982, 2933, 1774, 1684, 1204, 1146 cm^{-1}; HRMS (FAB) (*m/z*): $[M+H]^+$ calcd. for $C_9H_{19}N_6$, 211.1666; found, 211.1675.

Tetra-Boc-protected bis-guanidine vinyl ketone (16): Vinylmagnesium bromide (1.0 M in THF, 1.5 mL) was added to a solution of Weinreb amide **14** (19.8 mg, 0.030 mmol) in dry THF (1.5 mL) on ice, then the reaction mixture was stirred for 1 h at 40-50°C. The resulting mixture was quenched with saturated 0.1 M HCl aq (30 mL) and extracted with EtOAc (30 mL) three times. The organic layer was washed with brine (10 mL) and water (10 mL), and concentrated *in vaccuo*. The residue was purified by reversed phase HPLC (column; Mightysil RP-18 GP, 5 μm, 4.6x250 mm, (Kanto Chemical, Tokyo, Japan), solvent; MeOH/water (90:10, v/v) with the flow rate of 0.5 mL/min, 21 min; ESI-MS detection) to produce **16** (5.4 mg, 0.009 mmol, 29%).^{1}H NMR (600 MHz, $CDCl_3$): δ=11.48 (s, 1H), 11.42 (s, 1H), 9.07 (brs, 1H), 8.31 (brs, 1H), 6.48 (m, 2H), 5.87 (d, $J_{H,H}$ = 10.8 Hz, 1H), 5.16 (brs, 1H), 3.43 (brs, 2H), 2.08 (m, 1H), 1.72 (m, 1H), 1.60 (m, 2H), 1.51 (s, 9H), 1.49 (s, 9H), 1.48 ppm (s, 18H); ^{13}C NMR (151 MHz, $CDCl_3$): δ=196.78, 163.38 (trace, the signal corresponding to another guanidine carbon was not

shown), 156.10, 155.46, 153.22, 152.83, 132.43, 130.68, 83.33, 79.36 (83.33, 79.36: probably for four quaternary carbons in BOC groups), 57.27, 40.41, 28.70, 28.27, 28.25, 28.09, 28.05, 24.14 ppm; HRMS (FAB) (*m/z*): $[M+H]^+$ calcd. for $C_{29}H_{51}N_6O_9$, 627.3712; found,627.3723

D"2 (7): To a solution of vinyl ketone **16** (6.0 mg, 0.010 mmol) in dichloromethane (600 μL) was added TFA (600 μL), then the reaction mixture was stirred for 1 h at room temperature. The solvent was evaporated by the stream of nitrogen gas affording D"2 (**7**) as 2 TFA salt (3.0 mg, 0.007 mmol, 72%).^{1}H NMR (600 MHz, CD_3OD): δ 6.50 ppm (H-8, dd, $J_{H,H}$ = 17.4, 12 Hz, 1H), 5.41 (H-9, d, $J_{H,H}$ = 17.4 Hz, 1H), 5.14 (H-9, d, $J_{H,H}$ = 11.4 Hz, 1H), 3.17 (H-2, dd, $J_{H,H}$ = 14.4, 7.2 Hz, 2H), 2.60 (H-4, m, 2H), 1.84 ppm (H-3, m, 2H); ^{13}C NMR (151 MHz, CD_3OD): δ=159.53 (C-1), 149.56 (C-6, trace), 125.66 (C-7), 123.19 (C-5), 123.09 (C-8), 114.15 (C-9), 42.19 (C-2), 29.78 (C-3), 22.18 ppm (C-4); IR (ATR): 3354, 3193, 1685, 1204, 1138 cm^{-1}; HRMS (ESI^+) (*m/z*): $[M+H]^+$ calcd. for $C_9H_{17}N_6$, 209.1509; found, 209.1510. Impurity and minor byproduct were shown in the ^{1}H NMR spectrum, even after purification of **7**. Low signal to noise ratio of the ^{1}H and ^{13}C NMR signals might be due to equilibrium mixture of **7**.



Scheme S1. Reagents and conditions: a) $CuCO_3$, H_2O. b) *N,N'*-bis(benzyloxycarbonyl)-1*H*-pyrazole-1-carboxamidine (2.03 eq), DIEA, formamide, 1,4-dioxane. C) EDTA2Na, H_2O, 57% over three steps. d) *N,O*-dimethylhydroxylamine hydrochloride salt, EDC, HOBt, NMM, CH_2Cl_2, 54%. e) EtMgBr, THF, 40-50°C, 12%. f) 10% Pd/C, H_2, EtOAc, MeOH, 63% (crude mixture).

Tetra-Cbz-protected bis-guanidine carboxylic acid (19): Basic cupric carbonate (310.9 mg, approx. 1.401 mmol) was added to a solution of L-orinithine hydrochloride (101.8 mg, 0.604 mmol) in water (10 mL), and the mixture was boiled for 2 h. After removal of the excess cupric carbonate by filtration, the filtrates were concentrated *in vaccuo* to produce blue solid. DIEA (250 µL, 1.435 mmol) and *N,N'*-Bis(carbobenzoxy)-1*H*-pyrazole-1-carboxamidine (465.0 mg, 1.229 mmol = 2.03 eq) were added to the solution of the solid in dried *p*-dioxane/formamide (57:120, v/v, 1.77 mL) under nitrogen atmosphere at room temperature. After stirring for overnight, water (10 mL) was added to the reaction mixture, and extracted with EtOAc (10 mL) three times. The organic layer was washed with brine (10 mL) and water (10 mL), and concentrated *in vaccuo*. To the residue were added EDTA 2Na (146.7 mg, 0.394 mmol) and water (30 mL), and the mixture was stirred for 1 h at room temperature. Then, the mixture was extracted with EtOAc (50 mL) three times, and the organic layer was washed with brine (30 mL) and water (30 mL), and concentrated *in vaccuo*. The residue was purified by column chromatography

on Cosmosil 140C18-OPN (water, MeOH:water = 7:3, then MeCN) to produce **19** (258.7 mg, 0.3437 mmol, 57% (3 steps)). ^{1}H NMR (600 MHz, $CDCl_3$): δ=11.72 (brs, 1H), 11.63 (s, 1H), 8.78 (d, $J_{H,H}$ = 6.6 Hz, 1H), 8.39 (s, 1H), 7.41-7.26 (m, 20H), 5.18 (s, 2H), 5.16 (s, 2H), 5.11 (s, 4H), 4.69 (d, $J_{H,H}$ = 5.4 Hz, 1H), 3.46 (m, 2H), 2.05 (m, 1H), 1.82 (m, 1H), 1.68 ppm (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$): δ=172.39, 163.47, 162.60, 156.14, 156.05, 153.78, 153.51, 136.62, 136.16, 134.49, 134.18, 128.92, 128.78, 128.72, 128.67, 128.63, 128.46, 128.38, 128.06, 128.03, 127.96, 127.90, 68.64, 68.22, 67.42, 67.18, 53.64, 40.33, 28.28, 24.85 ppm; HRMS (FAB) (*m/z*): $[M+H]^+$ calcd. for $C_{39}H_{41}N_6O_{10}$, 753.2879; found, 753.2888.

Tetra-Cbz-protected bis-guanidine Weinreb amide (20): *N,O*-dimethylhydroxylamine hydrochloride salt (38.6 mg, 0.396 mmol), EDC (83.1 mg, 0.433 mmol), and *N*-methyl morpholine (42 μL, 0.382 mmol) was added to a solution of **19** (245.2 mg, 0.326 mmol) in dry dichloromethane (1.3 mL) at 0°C. Then, the reaction mixture was stirred at room temperature for overnight. After the solvent was removed *in vaccuo*, the resulting residue was partitioned between 1 M HCl aq (50 mL) and EtOAc (50 mL). The organic layer was washed with saturated $NaHCO_3$ (30 mL), brine (30 mL) and water (30 mL), and concentrated. The residue was purified by reverse-phase column chromatography on Cosmosil 140C18-OPN (water, methanol:water = 7:3, then MeCN) to produce **20** (180.1 mg, 0.226 mmol, 69%). ^{1}H NMR (600 MHz, $CDCl_3$): δ=11.72 (s, 1H), 11.63 (s, 1H), 8.97 (d, $J_{H,H}$ = 7.2 Hz, 1H), 8.34 (s, 1H), 7.40-7.27 (m, 20H), 5.19 (s, 2H), 5.16 (s, 2H), 5.15 (s, 2H), 5.12-5.10 (m, 3H), 3.77 (s, 3H), 3.45 (dd, $J_{H,H}$ = 13.2, 6.0 Hz, 2H), 3.20 (s, 3H), 1.92 (m, 1H), 1.76 (m, 1H), 1.64 ppm (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$): δ=171.16, 163.53, 163.47, 155.93, 155.43, 153.75, 153.44, 136.76, 136.70, 134.57, 128.73, 128.66, 128.64, 128.61, 128.45, 128.39, 128.11, 127.90, 127.83, 127.74, 68.22, 68.14, 67.15, 66.95, 61.56, 50.65, 40.58, 32.01, 29.06, 24.46 ppm; HRMS (FAB) (*m/z*): $[M+H]^+$ calcd. for $C_{41}H_{46}N_7O_{10}$, 796.3301; found, 796.3310.

Tetra-Cbz-protected bis-guanidine ethyl ketone (21): Ethyl magnesium bromide (3.0 M in ether, 820 μL, 2.4 mmol) was added to a solution of Weinreb amide **20** (50.5 mg, 0.063 mmol) in dry THF (1.26 mL) on ice under nitrogen atomsphere, then the reaction mixture was stirred for 1 h at 40-50°C. The reaction was quenched with 0.1 M HCl aq (30 mL) and extracted with EtOAc (30 mL) three times. The organic layer was washed with brine (10 mL) and water (10 mL), and concentrated *in vaccuo*. The residue was purified by reversed phase HPLC (column; Mightysil RP-18 GP, 5 μm, 4.6x250 mm, (Kanto Chemical, Tokyo, Japan), solvent; MeCN/water (85:15, v/v) with the flow rate of 0.5 mL/min, 24 min; ESI-MS detection) to give **21** (5.7 mg, 0.007 mmol, 12%). ^{1}H NMR (600 MHz, $CDCl_3$): δ=11.71 (s, 1H), 11.66 (s, 1H), 9.03 (d, $J_{H,H}$ = 6.6 Hz, 1H), 8.34 (s, 1H), 7.39-7.27 (m, 20H), 5.19 (s, 2H), 5.15 (s, 2H), 5.11 (s,

2H), 5.10 (s, 1H), 5.09 (s, 1H), 4.88 (dd, $J_{H,H}$ = 11.4, 6.6 Hz, 1H), 3.44 (dd, $J_{H,H}$ = 12.6, 6.6 Hz, 2H), 2.51 (m, 2H), 2.04 (m, 1H), 1.72 (m, 1H), 1.59 (m, 1H), 1.53 (m, 1H), 1.06 ppm (t, $J_{H,H}$ = 7.2 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$): δ=207.93, 163.34, 155.97, 155.42, 153.79, 153.53, 136.60, 134.51, 134.48, 128.80, 128.68, 128.63, 128.49, 128.42, 128.39, 128.10, 127.98, 127.93, 68.33, 68.23, 67.19, 58.87, 40.51, 32.90, 28.35, 24.30, 7.50 ppm; HRMS (FAB) (*m/z*): $[M+H]^+$ calcd. for $C_{41}H_{45}N_6O_9$, 765.3243; found, 765.3253.

Int-C'2 (6) from Cbz-protected ethyl ketone (21): 10% Pd/C (9.2 mg) was added to a solution of ethyl ketone **21** (4.2 mg, 0.005 mmol) in EtOAc/MeOH=2:1 (v/v, 2.4 mL) under nitrogen atmosphere, and hydrogen was introduced to the reaction mixture. After stirring at room temperature for 15 min, the mixture was filtered through a pad of celite. The filtrates were concentrated *in vaccuo* affording Int-C'2 (**6**) as a mixture with a structurally unidentified compound (63%, by ^{1}H NMR, crude). Isolation of the byproduct using reversed phase HPLC was difficult due to the low recovery from the column. The byproduct was suggested to be a bis-guanidine compound by the crude NMR data. **6**: ^{1}H NMR (600 MHz, CD_3OD): δ=3.17 (H-2, t, $J_{H,H}$ = 7.2 Hz, 2H), 2.53 (H-4, t, $J_{H,H}$ = 7.2 Hz, 2H), 2.47 (H-8, q, $J_{H,H}$ = 7.8 Hz, 2H), 1.83 (H-3, quin, $J_{H,H}$ = 7.2 Hz, 2H), 1.17 ppm (H-9, t, $J_{H,H}$ = 7.8 Hz, 3H); ^{13}C NMR (151 MHz, CD_3OD): δ=159.61 (C-1), 149.03 (C-6), 125.99 (C-7), 121.95 (C-5), 42.27 (C-2), 30.04 (C-3), 22.16 (C-4), 18.62 (C-8), 15.11 ppm (C-9); HRMS (FAB) (*m/z*): $[M+H]^+$ calcd. for $C_9H_{19}N_6$, 211.1666; found, 211.1675.

Int-C'2 (6) from Cbz-protected ethyl ketone (21) ($Pd(OH)_2$/C): 20% $Pd(OH)_2$/C (2.1 mg) was added to a solution of ethyl ketone **21** (4.4 mg, 0.006 mmol) in MeOH (2.0 mL) under nitrogen atmosphere, and hydrogen was introduced to the reaction mixture. After stirring at room temperature for 15 min, the mixture was filtered through a pad of celite. The filtrates were concentrated *in vaccuo* affording Int-C'2 (**6**) as a mixture with a structurally unidentified compound. Int-B' (**3**) was not detected by ESI-MS. The ^{1}H NMR chemical shifts of some signals of Int-C'2 (**6**) were slightly different from those of Int-C'2 (**6**) deprotected by Pd/C, probably due to difference of pH of the solvent.

3. The cyanobacterium and dinoflagellate strains

The toxic strain of the freshwater cyanobacterium *A. circinalis* used in this study is a non-axenic strain TA04.[10] The field sample of *A. circinalis* was collected at the Tullaroop reservoir, Victoria, Australia, and the TA04strain was one of single-trichome isolates prepared by Negri *et al*.[3g] The non-toxic strain of *A. circinalis* Rabenhorst ex Bornet et Flahault (NIES-1645) was obtained from the National Institute of Environmental Studies (Tsukuba, Japan). This strain was isolated from the Innba pond in Chiba Prefecture, Japan, in 2001.[11] Both strains were cultured in CB' medium ($Ca(NO_3)_2 \cdot 4H_2O$ 15 mg, KNO_3 10 mg, β–Na_2glycerophosphate $\cdot 5H_2O$ 5 mg, $MgSO_4 \cdot 7H_2O$ 4 mg, Vitamin B12 0.01 µg, Biotin 0.01 µg, Thiamine HCl 1 µg, PIV metals ($Na_2EDTA \cdot 2H_2O$ 100 mg, $FeCl_3 \cdot 6H_2O$ 19.6 mg, $MnCl_2 \cdot 4H_2O$ 3.6 mg, $ZnCl_2$ 1.04 mg, $CoCl_2 \cdot 6H_2O$ 0.4 mg, $Na_2MoO_4 \cdot 2H_2O$ 0.25 mg, distilled water 100 mL) 0.3 mL, Bicine 50 mg, distilled water 99.7 mL. pH 9.0 adjusted with 2N NaOH) in a 250 mL polystyrene tissue culture flask (cat#137787) with a 0.2 µm vented blue plug seal cap (cat#353136) (BD Falcon, Franklin Lakes, NJ, USA). The cyanobacteria were cultured on a 16-h-light/8-h-dark cycle at 17°C. The light was provided using cool white bulbs (25 µmol photons m^{-2} s^{-1}).

The *A. tamarense* Balech Axat-2 toxic strain of the marine dinoflagellate was used in this study. Strain Axat-2 was re-isolated by Dr. Omura at Tokyo University of Marine Science and Technology in 1996 from the OF935-AT6 strain, which was isolated by Dr. Ogata of Kitasato University from sea water collected at Ofunato, Iwate Prefecture, Japan in 1993.[12] As the non-toxic strain of *A. tamarense*, a subclone [UAT-014-009] derived from the toxic clonal culture of above *A. tamarense* Balech strain, OF935-AT6 was used. The cultures of toxic and non-toxic strains of *A. tamarense* were maintained and grown in modified T_1-medium at 15°C in 250 mL tissue culture flasks under a 12-h-light/12-h-dark photo cycle; using the light provided by cool white bulbs (100-150 µmol photons m^{-2} s^{-1}).

4. Sample preparation for HR-LC-MS and HR-LC-MS/MS (Q-TOF)

The 190 mL of 38 days culture of *A. circinalis* (TA04), 165 mL of 47 days culture of *A. circinalis* (N-1645), 200 mL of 24 days culture of *A. tamarense* (Axat-2), and 170 mL of 20 days culture of *A. tamarense* (UAT-014-009) was harvested. Aliquots of harvested samples were used to obtain cell count by microscopy (dinoflagellates). The cultures were centrifuged at 6,300 *g* for 5 min at 4°C (cyanobacteria) or 1700 x *g* for 5 min at ambient temperature (dinoflagellates) to pellet the cells. Then, after removal of the supernatant, the pellet was transferred to a new microtube and centrifuged at 6,300 *g* for 5 min at 4°C (cyanobacteria) or 1700 *g* for 5 min at ambient temperature (dinoflagellates) again to pellet the cells. After removal of supernatant, the pellet was lyophilised (dry-weight: TA04 32 mg, N-1645 16 mg, Axat-2 22 mg, UAT-014-009 22 mg) and re-suspended with 400 µL of the extract solution (CH_3CN/water/HCOOH 80:20:0.1, v/v/v). After sonication for 30 s three times on ice, the homogenate was centrifuged at 20,000 *g* for 5 min at 4°C. The supernatant was transferred to a new tube. The residue was re-extracted with 200 µL of the extract solution twice more and the supernatants were combined. The resultant supernatant was treated with ZIC-HILIC SPE cartridge (100 mg) (Merck Millipore) in HILIC mode, which had been previously conditioned with 2 mL of CH_3CN/water/HCOOH (10:90:0.1, v/v/v) and 2 mL of CH_3CN/water/HCOOH (80:20:0.1, v/v/v). A part of the crude extract (300-700 µL) was loaded, followed by a wash of 2 mL of CH_3CN/water/HCOOH (80:20:0.1, v/v/v). The STX intermediates were eluted with 1 mL of CH_3CN/water/HCOOH (50:50:0.1, v/v/v). The recoveries of arginine, Int-A' (**2**) and Int-C2' (**6**) from SPE determined by spiked test solution were approximately 60%, 15% and 5%, respectively. The recovery of D"2 (**7**) was not determined but was assumed to be 5% as same as Int-C2' (**6**).

5. Sample preparation for quantitative LC-MS/MS (MRM)

The cells of *A. circinalis* (TA04) (40 mL culture, dry-weight 9.28 mg, cultured for 29 days) were collected by filtration using the glass fiber filter (GA100, 0.6 µm, Advantec, Tokyo, Japan). Aliquots of harvested cultures were used to obtain cell density by fluorescent microplate reader (cyanobacteria). The cells of *A. tamarense* (Axat-2) (10 mL culture, cultured for 23 days) were collected by centrifugation at 1,700 *g* for 5 min at ambient temperature. The dinoflagellates cells were transferred to a new microtube and peletted by centrifugation at 1,700 *g* for 5 min at ambient temperature. Aliquots of harvested cultures were used to obtain cell count by microscopy (dinoflagellates). The cells were extracted with 1 mL (AT04) or 300 µL (Axat-2) of MeOH/water (1:9, v/v), ultrasonicated, and centrifuged at 20,600 *g* for 5 min at 4°C. The supernatants were filtered through Cosmospin filter H (Nakalai Tesque, Kyoto, Japan). The diluted filtrates were directly applied to LC-MS/MS.

6. HR-LC-MS and LC-MS/MS analysis of intermediates

The liquid chromatography system used for analysis was a Shimadzu Nexera UHPLC System (Shimadzu, Kyoto, Japan). The autosampler (SIL-30AC, Shimadzu) was maintained at 5°C. Liquid chromatography was performed on a 2.1 i.d. × 150 mm (130Å, 1.7 μm) ACQUITY UPLC BEH Amide column (Waters, USA). The mobile phase A was 200 mM $HCOONH_4$/200 mm HCOOH/water (2.5:2.5:95, v/v, pH 3.9), and the mobile phase B was 200 mM $HCOONH_4$/200 mM HCOOH/water/CH_3CN (2.5:2.5:1.5:95, v/v). A gradient elution program was applied as follows: 0-3 min 85% B, 3-11 min 85%-70% B,11-13 min 70% B, 13-20 min 85% B. The flow rate was 0.3 mL/min. The oven temperature was 40°C. The liquid chromatography system was connected to a Q-TOF mass spectrometer, MicrOTOFQII, equipped with an ESI source. The mass spectrometer conditions were as follows: positive ionization mode, dry gas: nitrogen 10 L/min, dry temperature: 180°C, nebulizer: 2.2 Bar, capillary: −4500 V. High resolution LC-MS/MS was performed in MRM mode setting $[M+H]^+$ as the precursor ions. The precursor ions were *m/z* 175.12 width 2Da for arginine, *m/z* 187.20 width 2Da for Int-A' (**2**) and *m/z* 211.20 width 2Da for Int-C'2 (**6**). The sweeping collision ener0gy was 40-160 eV.

7. Spiking of the authentic compounds into the sample solutions prepared from the cells: HR-LC-MS

The mixture of the authentic compounds (Arginine (10 μM), **2** (2 μM), and **6** (2 μM)) was combined with the sample solution (see, Section 4) in the ratio of 1:7 (v/v) for *A. circinalis* (TA04) and 1:3 (v/v) for *A. tamarense* (Axat-2), and an aliquot of the mixture for *A. circinalis* (12 μL) and *A. tamarense* (7 μL) was applied to HR-LC-MS. The analytical condition was same as described above (Section 6). The result was shown in Figure S-1, on page S15.

8. Quantitation of arginine, Int-A' (2) and Int-C'2 (6) using LC-MS/MS (MRM)

A column-switching LC-MS/MS method was utilized to quantify arginine, Int-A' (**2)** and Int-C'2 (**6)** in the microorganisms extracts using an API2000 triple quadrupole tandem mass spectrometer equipped with ESI ion source. Three guard column cartridges, Develosil C8-UG (4.0x10 mm, Nomura Chemical, Seto, Japan), TSKgel guardgel Amide-80 (5 μm, 2.0x10 mm, Tosoh, Tokyo, Japan) and SeQuant® ZIC®-HILIC Guard (2.1x20 mm, Merck KGaA, Damstadt, Germany) were connected in tandem for the solid- phase extraction (SPE) process; this apparatus was connected between the LC-pump (Shimadzu LC-10AD) and the valco valve of the mass spectrometer. A SeQuant® ZIC®-HILIC (2.1x150 mm, 3.5 μm) column used for liquid chromatography was connected between the valco valve and the mass spectrometer. The position of the valve was set to A (to waste) from 0 to1 min, B (to column) from 1 to 50 min and A

from 50 to 62 min. The mobile phase A was 10 mM $HCOONH_4$/10 mM HCOOH/water, and the mobile phase B was CH_3CN/water containing 9.9 mM $HCOONH_4$ and 9.9 mM HCOOH (95:6.5, v/v). A gradient elution program was applied as follows: 0-3.1 min 100% B, 3.1-6 min 85% B, 6-14 min 85-70% B, 14-16 min 70% B, 16.1-30 min 85% B, 30.1-35 min 45%B, 35.1-49.9 min 85%B, 50-51 min 40%B, 51.1-62 min 100%B. The flow rate was initially set at 0.2 mL/min, changed gradually to 0.3 mL/min (0-3.05 min) and then kept at 0.3 mL/min (3.05-62 min). The mass spectrometer conditions were as follows: positive ionization mode, dry gas: nitrogen, spray voltage: 5.5 kV; capillary temperature: 500°C; curtain gas: 40 (arbitrary units). The target compound calibration was conducted at the concentration ranges 0.05 to 10 µM (10 µL injection) for arginine and 1 to 5 µM for **2** and **6**. In the multiple reaction monitoring mode (MRM), the precursor ions to the product ions (Q1/Q3) and collision energy (CE in eV) were chosen as *m/z* 175.1/70.1 (25) for arginine, *m/z* 187.2/128.1 (22) for **2**, and *m/z* 211.2/152.1 (25) for **6**. The parameters for the mass spectrometer were set as DP (20), FP (160), EP 10.5, CEP (13), CXP (4). Quantification was conducted using the external standard method. Linearity (R^2>0.99) was observed over the calibration range. The standards of **2** and **6** were stored in MeOH/water (1:9, v/v) at -30°C. The LODs for arginine, **2** and **6** were 30 fmol, 6.7 pmol and 0.5 pmol, respectively. The retention times of arginine, **2** and **6** were approximately 28.9, 23.2 and 20.2 min, respectively.

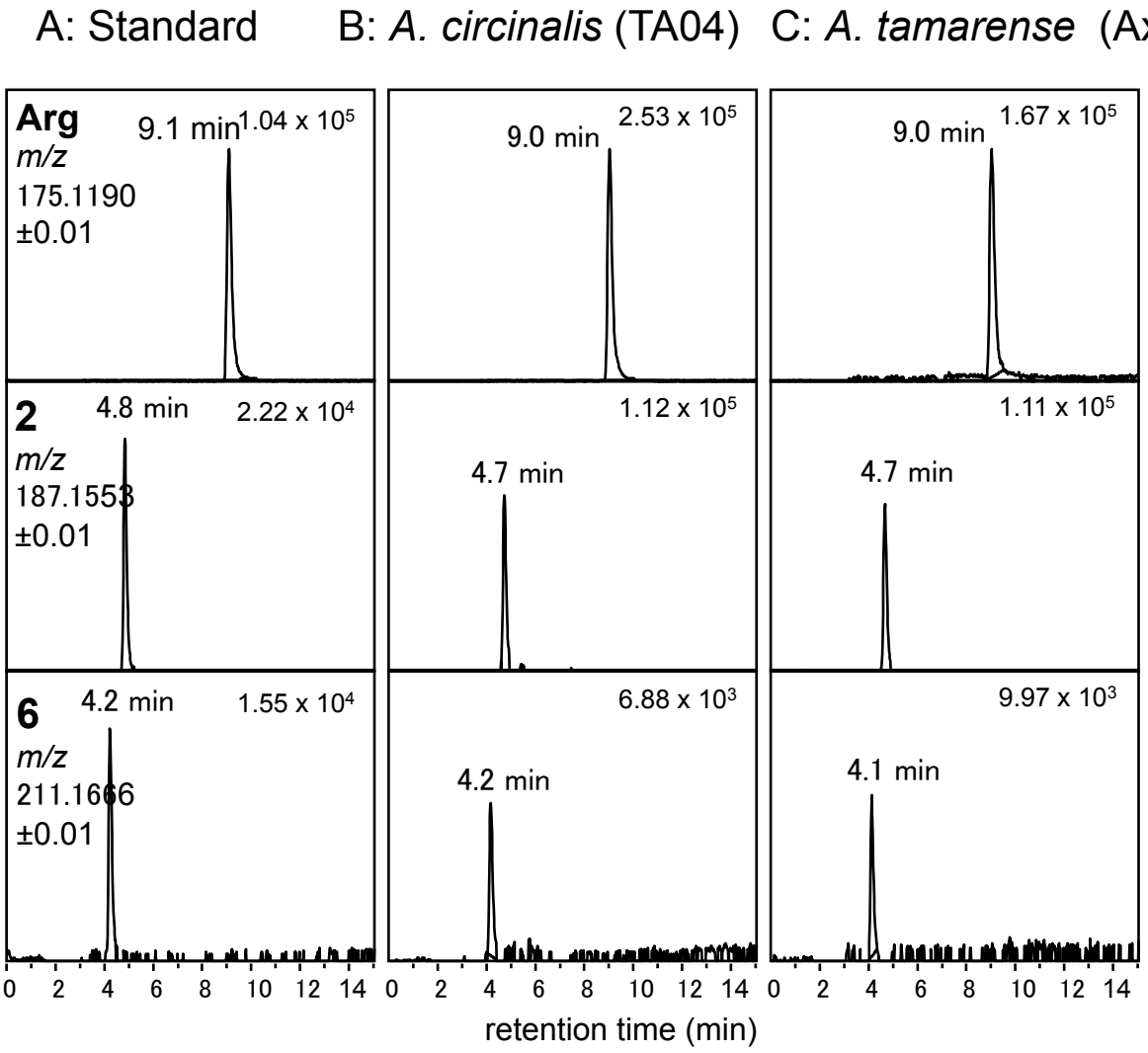
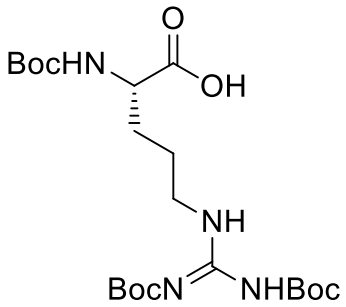


Fig. S1
HR-LC-MS extracted ion chromatograms of the authentic compounds (Arg 50 pmol, 2:10 pmol, 6:10 pmol in 5 µL) (A), and the sample solutions from the *A. circinalis* (TA04) (B) *and A. tamarense* (Axat-2) (C) spiked with the authentic compounds. See, page S13 for details.

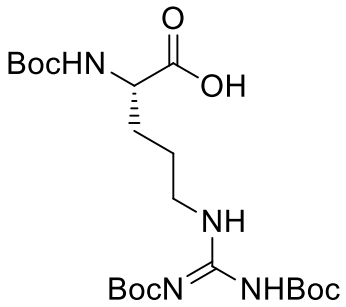
Tri-Boc-protected L-arginine (**10**)

^{1}H NMR (600 MHz, $CDCl_3$)

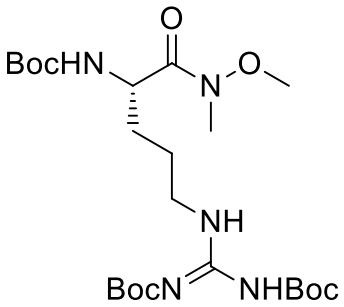


Tri-Boc-protected L-arginine (**10**)

^{13}C NMR (151 MHz, $CDCl_3$)

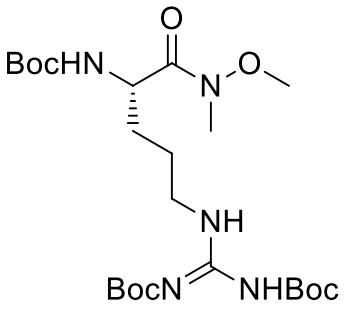


Tri-Boc-protected arginine weinreb amide (**11**)
^{1}H NMR (600 MHz, $CDCl_3$)

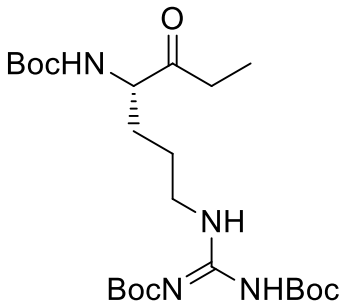


Tri-Boc-protected arginine weinreb amide (**11**)

^{13}C NMR (151 MHz, $CDCl_3$)

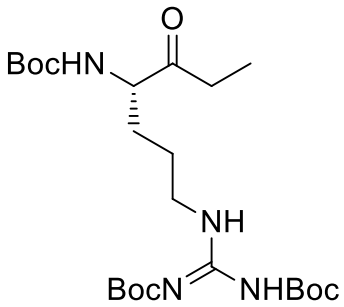


Tri-Boc-protected arginine ethyl ketone (**12**)
^{1}H NMR (600 MHz, $CDCl_3$)



Tri-Boc-protected arginine ethyl ketone (**12**)

^{13}C NMR (151 MHz, $CDCl_3$)



tsuchiya A' cd3od nt=8 2013.9.9

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Solvent: cd3od
Temp. 20.0 C / 293.1 K
Operator: vnmr1
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VNMRS-600 "varian2"

Relax. delay 1.592 sec
Pulse 45.0 degrees
Acq. time 4.561 sec
Width 7183.9 Hz
8 repetitions
OBSERVE H1, 599.8706626 MHz
DATA PROCESSING
Line broadening 0.3 Hz
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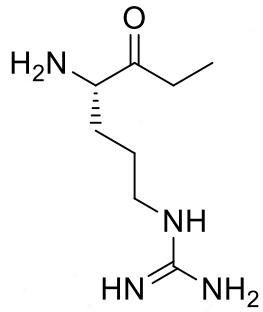
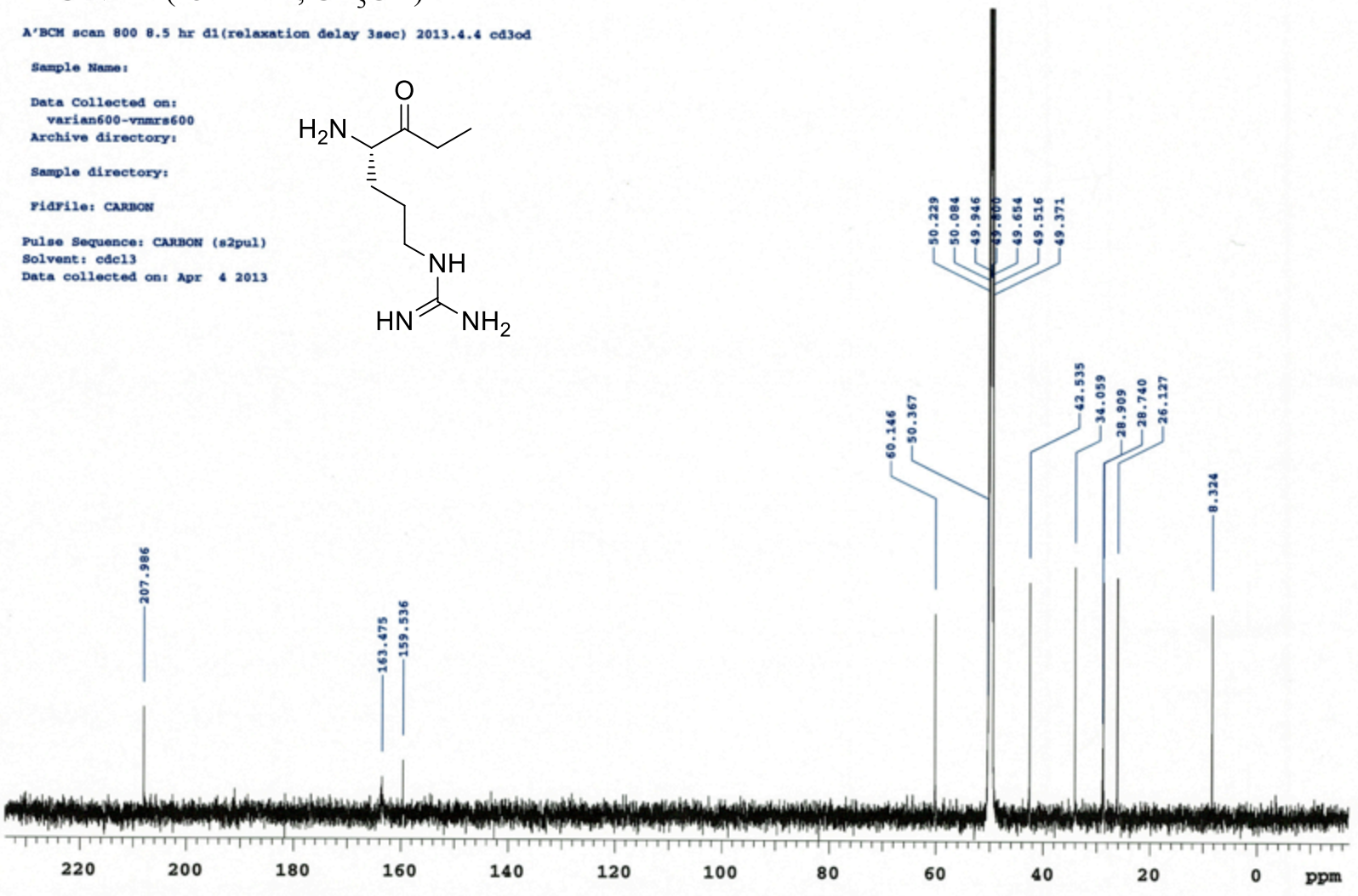
IntermediateA' (**2**)
^{1}H NMR (600 MHz, CD_3OD)

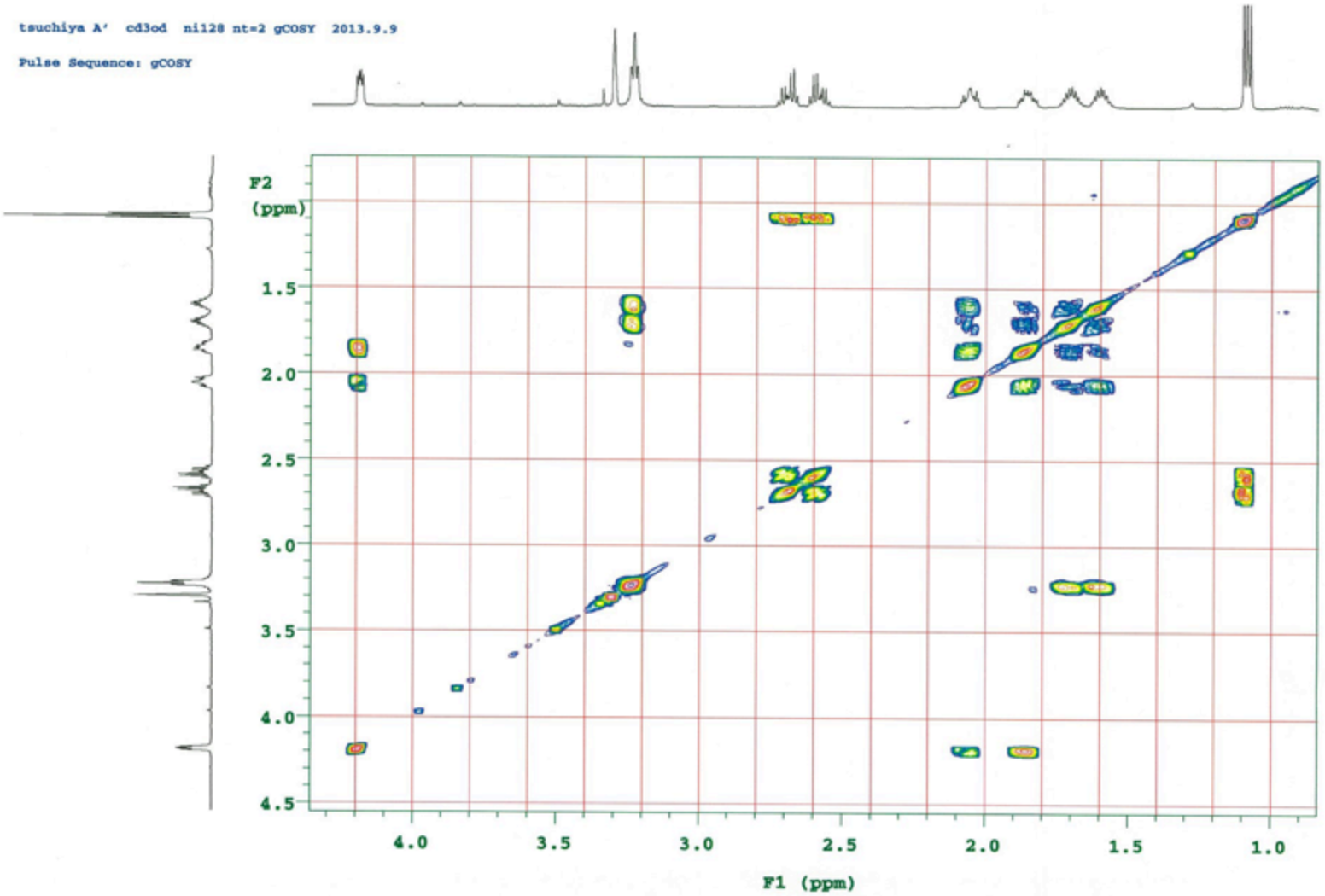


IntermediateA' (**2**)

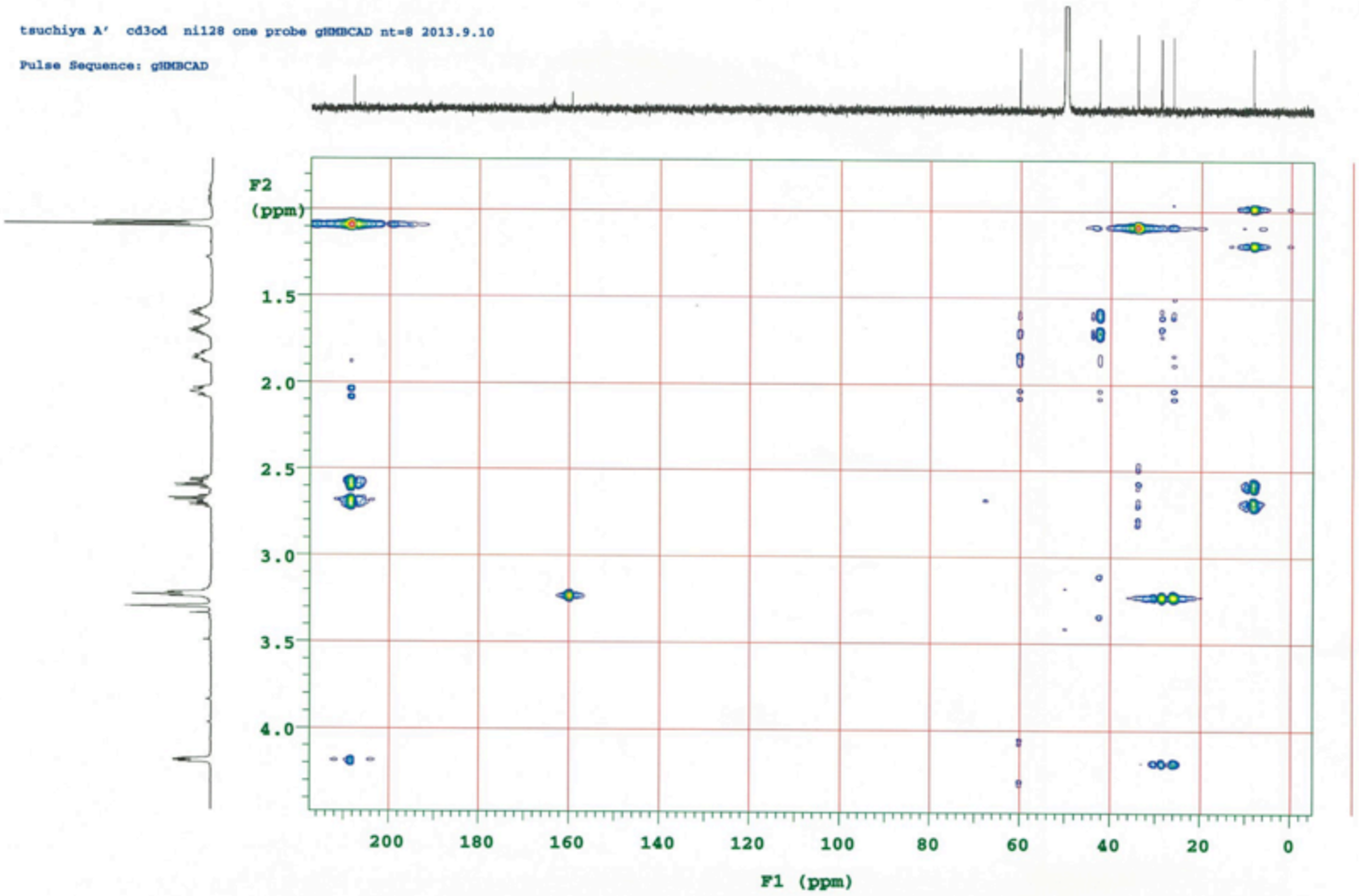
^{13}C NMR (151 MHz, CD_3OD)



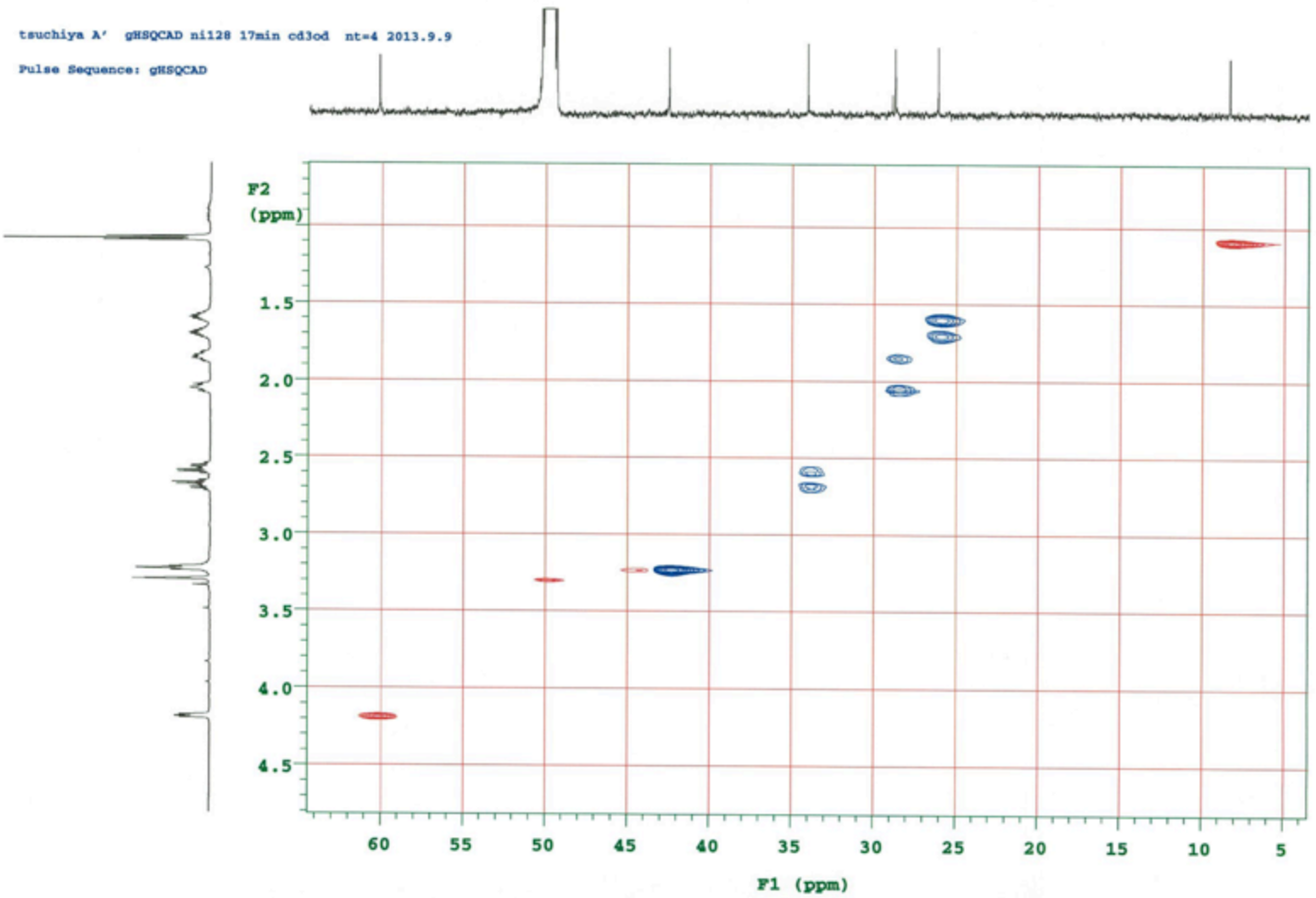
IntermediateA' (**2**) COSY(600 MHz, CD_3OD)

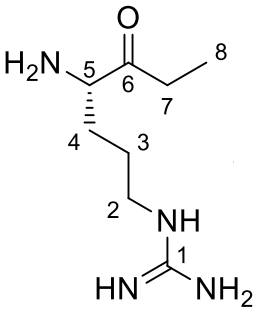


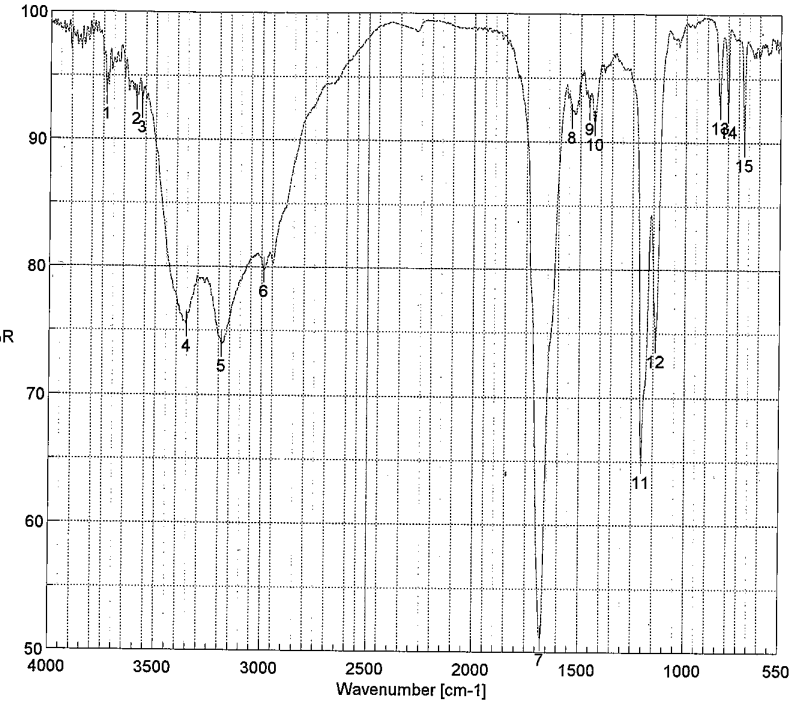
IntermediateA' (**2**) HMBC (600 MHz, CD_3OD)



IntermediateA' (**2**) HSQC (600 MHz, CD_3OD)







ピーク検出結果

No.	位置	強度	No.	位置	強度
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5	3186.79	73.92	6	2989.12	79.76
7	1675.84	51.12	8	1540.85	91.98
9	1456.96	92.69	10	1433.82	91.48
11	1203.36	65.06	12	1137.8	74.50
13	838.883	92.82	14	801.278	92.49
15	723.175	89.90			

[コメント情報]

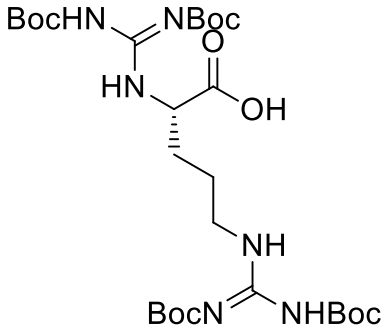
試料名	
コメント	ATR(ZnSe)
測定者	
所属	
会社	東北大学

[測定情報]

機種名	FT/IR-4100typeA
シリアル番号	B032661016
付属品名	ATR PRO410-S
付属品シリアルNo.	A017961044
光源	標準光源
検出器	TGS
積算回数	60
分解	4 cm-1
ゼロフィリング	On
アポダイゼーション	Cosine
ゲイン	Auto (8)
アパーチャー	Auto (7.1 mm)
スキャンスピード	Auto (2 mm/sec)
フィルタ	Auto (30000 Hz)

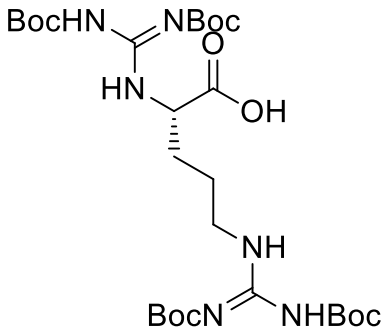
Tetra-Boc-protected bis-guanidine carboxylic acid (**13**)

^{1}H NMR (600 MHz, $CDCl_3$)



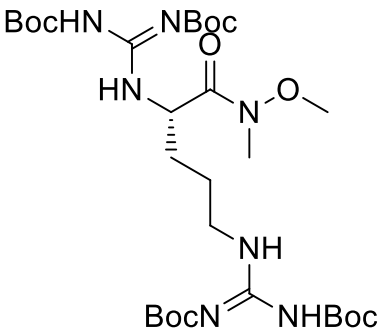
Tetra-Boc-protected bis-guanidine carboxylic acid (**13**)

^{13}C NMR (151 MHz, $CDCl_3$)



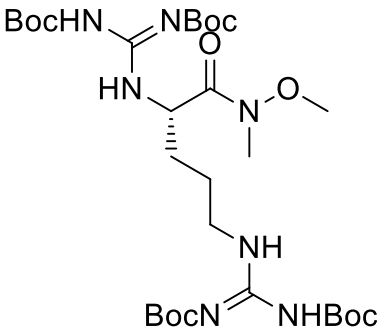
Tetra-Boc-protected bis-guanidine weinreb amide (**14**)

^{1}H NMR (600 MHz, $CDCl_3$)



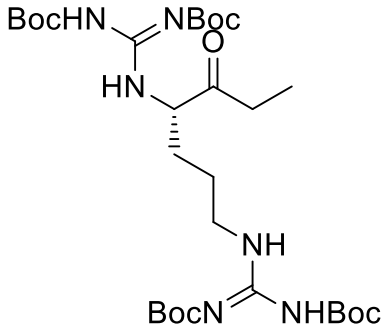
Tetra-Boc-protected bis-guanidine weinreb amide (**14**)

^{13}C NMR (151 MHz, $CDCl_3$)



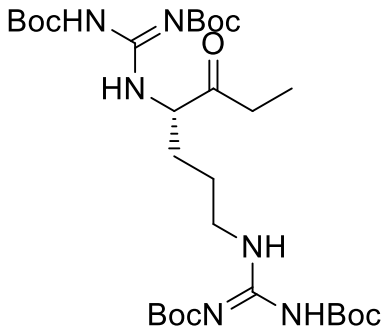
Tetra-Boc-protected bis-guanidine etyl ketone (**15**)

^{1}H NMR (600 MHz, $CDCl_3$)



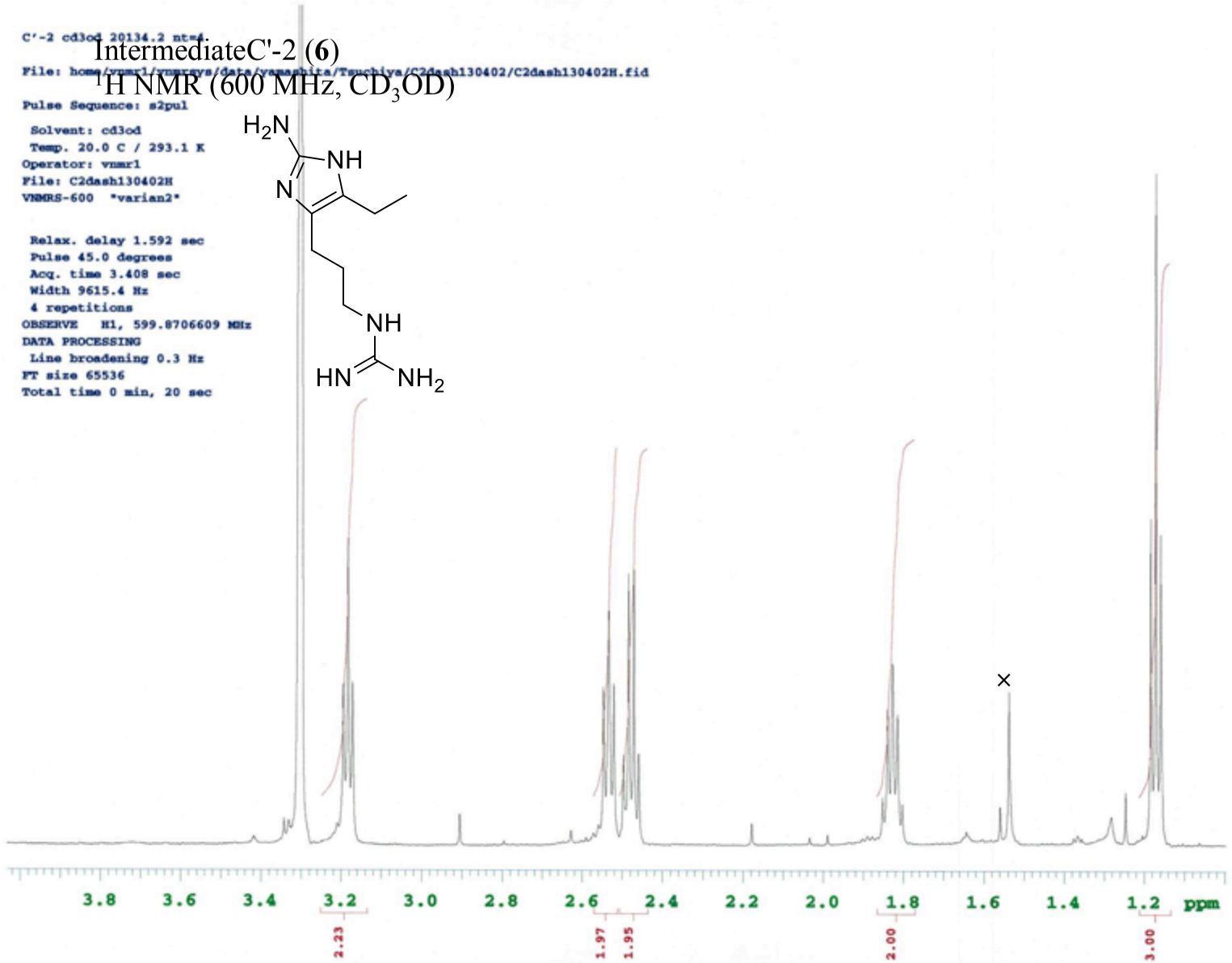
Tetra-Boc-protected bis-guanidine etyl ketone (**15**)

^{13}C NMR (151 MHz, $CDCl_3$)



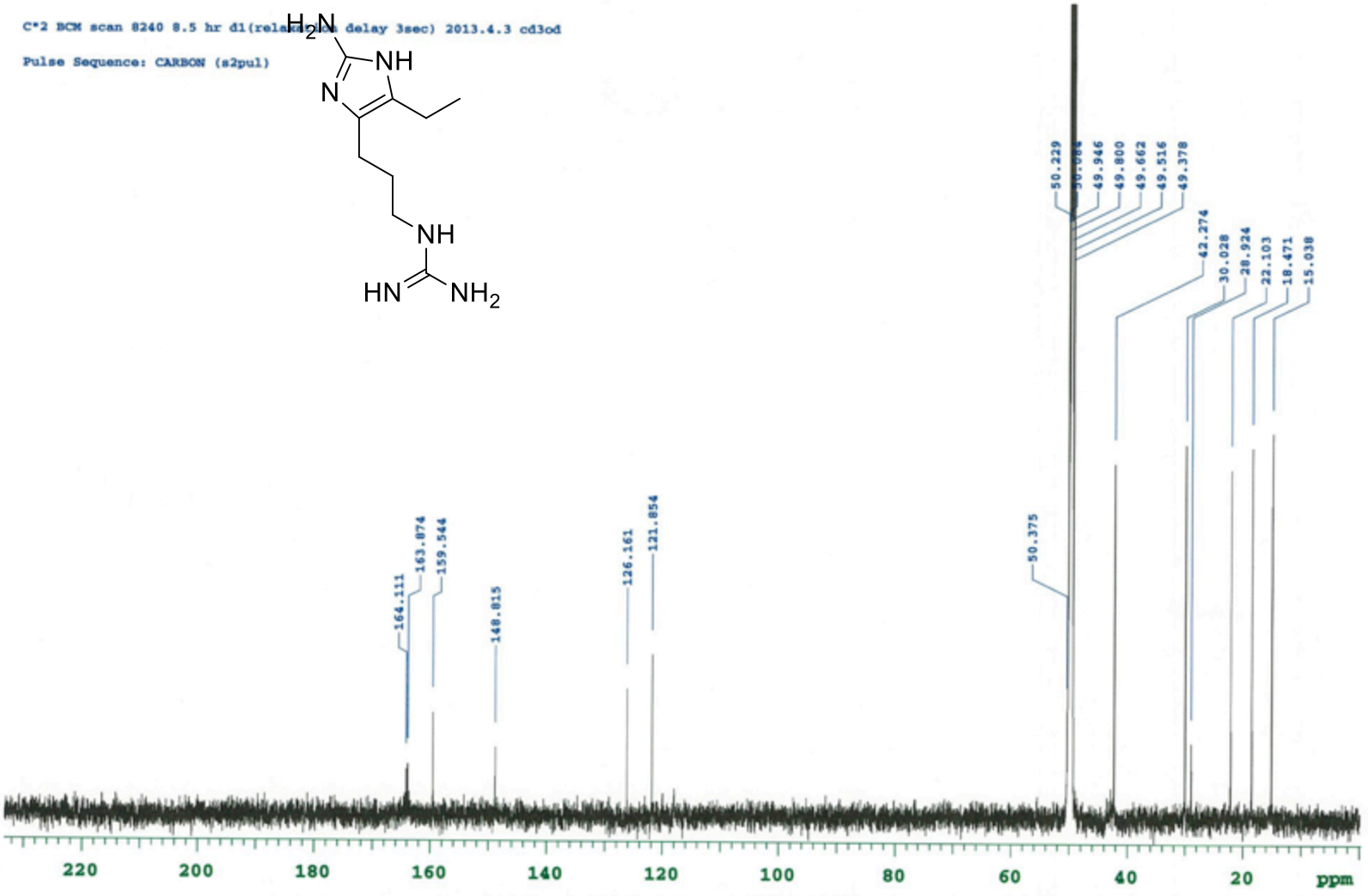
IntermediateC'-2 (**6**)

^{1}H NMR (600 MHz, CD_3OD)

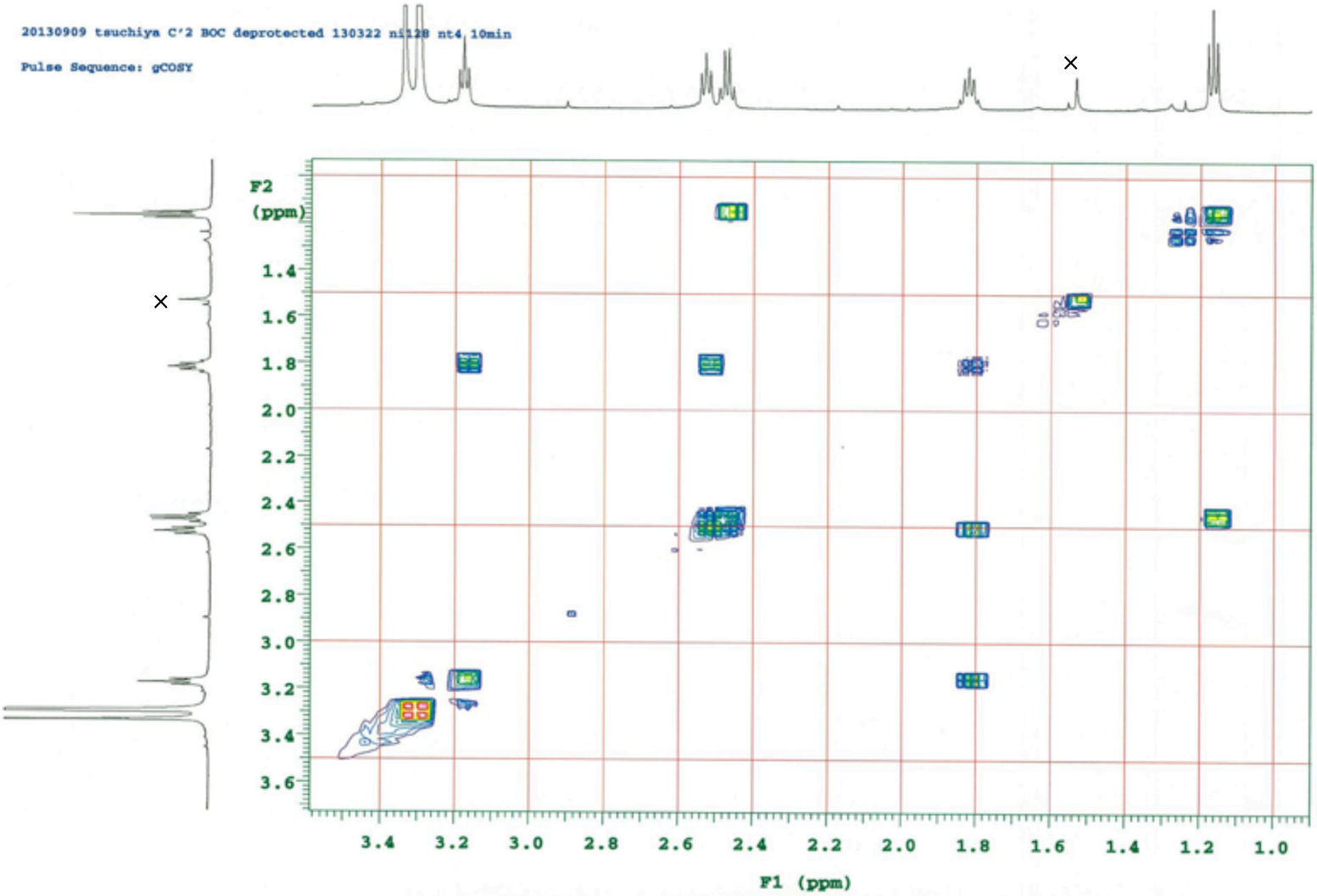


IntermediateC'-2 (**6**)

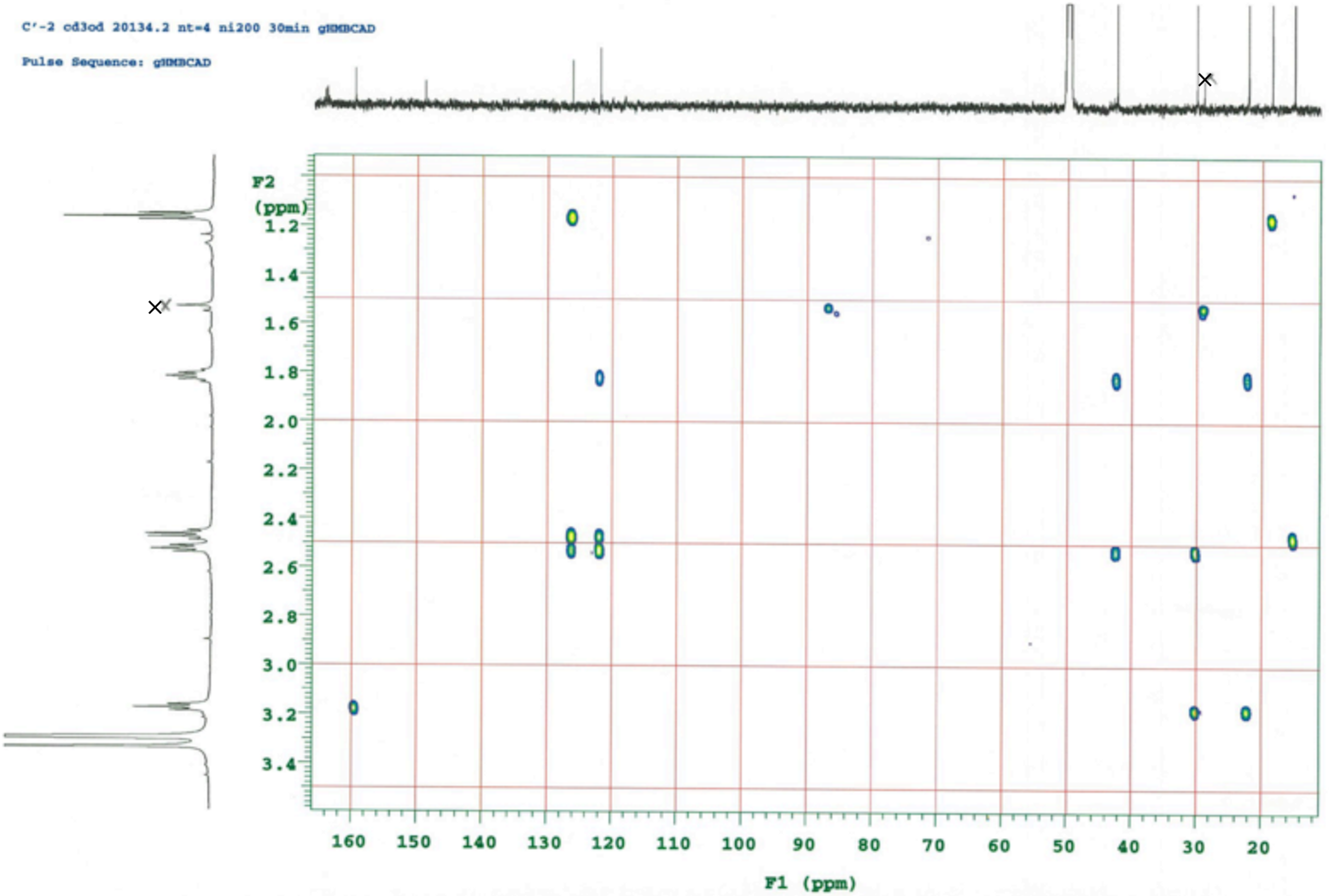
^{13}C NMR (151 MHz, CD_3OD)



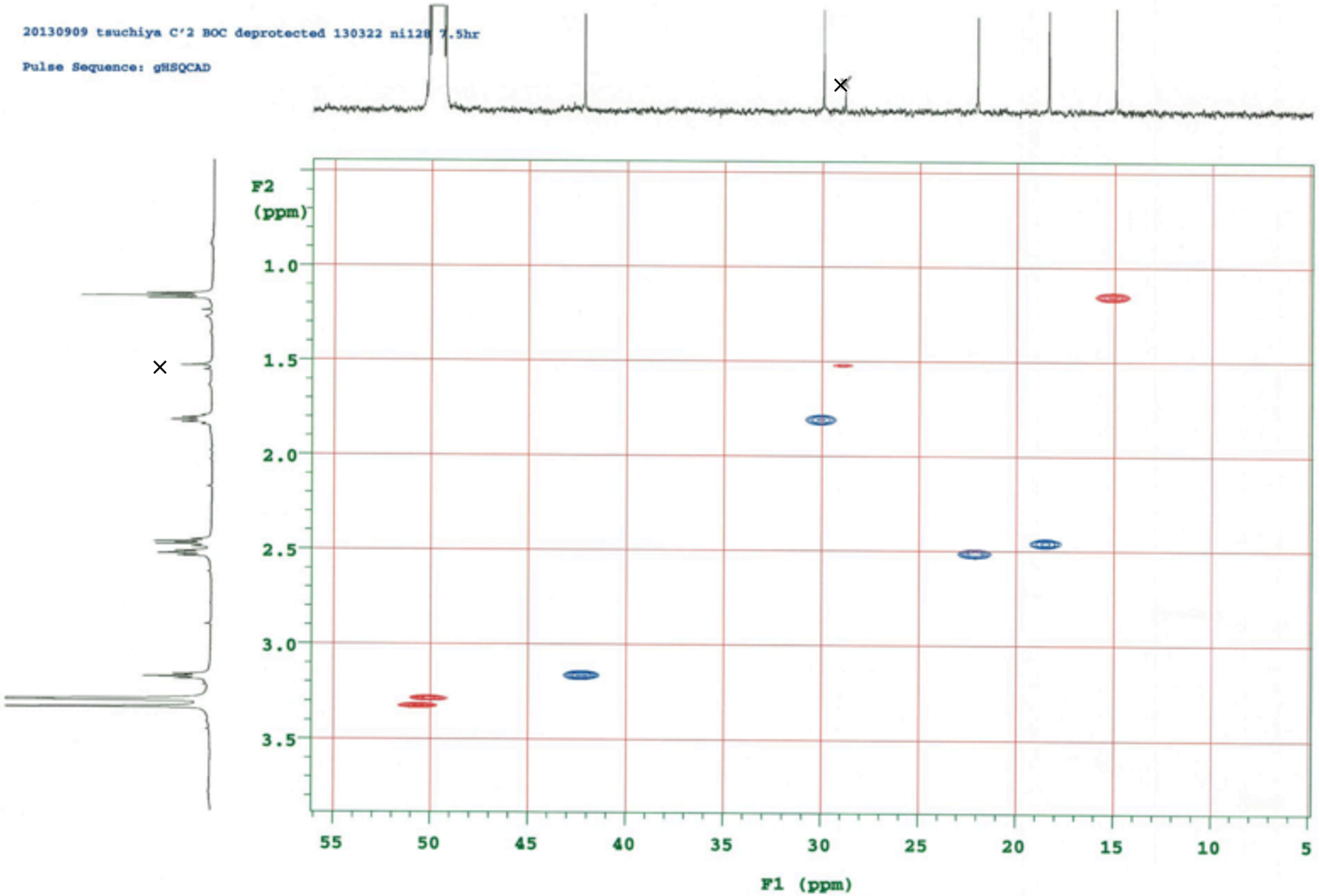
IntermediateC'-2 (**6**) COSY (600 MHz, CD_3OD)

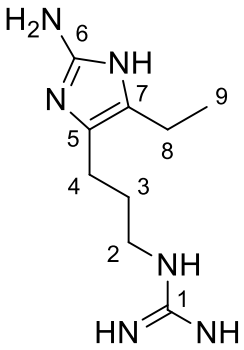


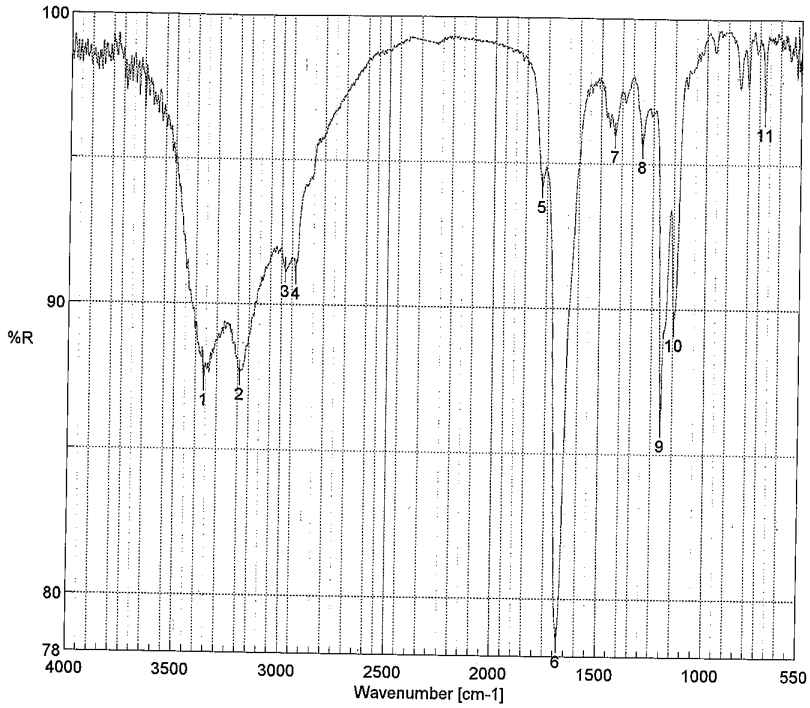
IntermediateC'-2 (**6**) HMBC (600 MHz, CD_3OD)



IntermediateC'-2 (**6**) HSQC (600 MHz, CD_3OD)







ピーク検出結果

No.	位置	強度	No.	位置	強度
1	3359.39	87.42	2	3190.65	87.63
3	2982.37	91.19	4	2933.2	91.12
5	1774.19	94.21	6	1683.55	78.56
7	1433.82	95.98	8	1303.64	95.62
9	1204.33	86.03	10	1145.51	89.51
11	722.211	96.78			

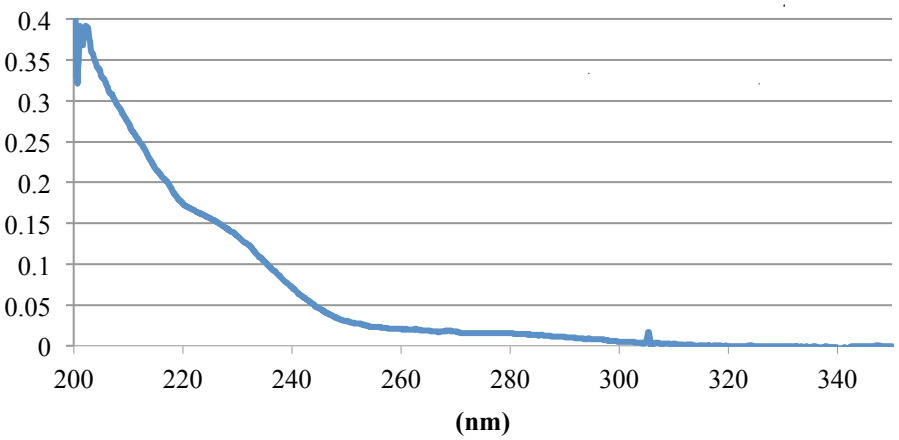
[コメント情報]

試料名	
コメント	ATR(ZnSe)
測定者	
所属	
会社	東北大学

[測定情報]

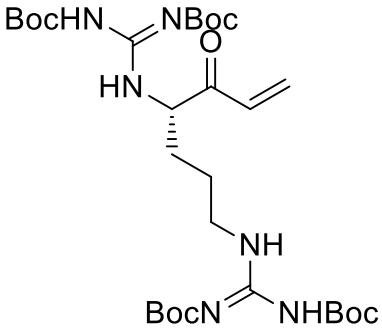
機種名	FT/IR-4100typeA
シリアル番号	B032661016
付属品名	ATR PRO410-S
付属品シリアルNo.	A017961044
光源	標準光源
検出器	TGS
積算回数	60
分解	4 cm-1
ゼロフィリング	On
アポダイゼーション	Cosine
ゲイン	Auto (8)
アパーチャー	Auto (7.1 mm)
スキャンスピード	Auto (2 mm/sec)
フィルタ	Auto (30000 Hz)

UV



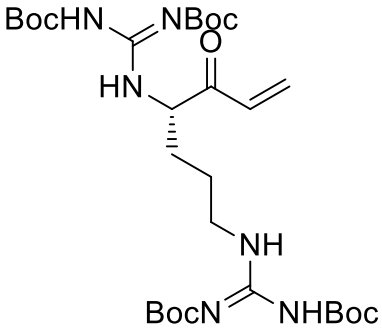
Tetra-Boc-protected bis-guanidine vinyl ketone (**16**)

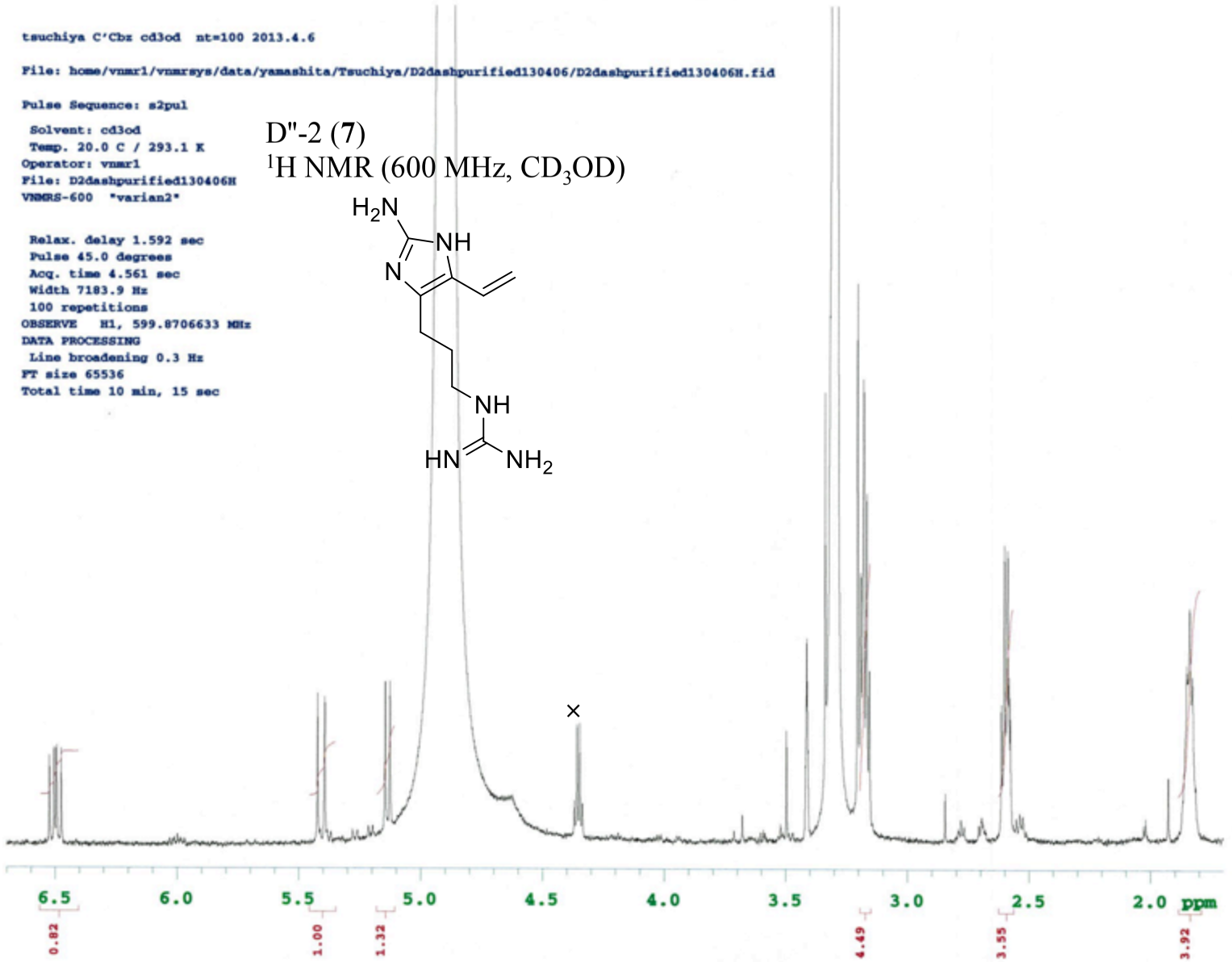
^{1}H NMR (600 MHz, $CDCl_3$)



Tetra-Boc-protected bis-guanidine vinyl ketone (**16**)

^{13}C NMR (151 MHz, $CDCl_3$)



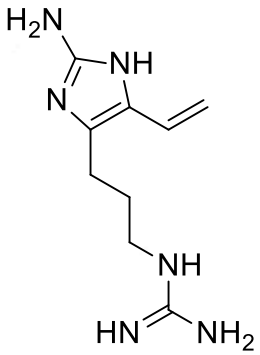

tsuchiya C'Cbz cd3od nt=100 2013.4.6
File: home/vnmr1/vnmrsys/data/yamashita/Tsuchiya/D2dashpurified130406/D2dashpurified130406H.fid
Pulse Sequence: s2pul
Solvent: cd3od
Temp. 20.0 C / 293.1 K
Operator: vnmr1
File: D2dashpurified130406H
VNMRS-600 "varian2"
Relax. delay 1.592 sec
Pulse 45.0 degrees
Acq. time 4.561 sec
Width 7183.9 Hz
100 repetitions
OBSERVE H1, 599.8706633 MHz
DATA PROCESSING
Line broadening 0.3 Hz
FT size 65536
Total time 10 min, 15 sec
D"-2 (7)
1H NMR (600 MHz, CD3OD)
H2N
NH
N
NH
HN
NH2
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm
0.82
1.00
1.32
4.49
3.55
3.92

D"2 BCM scan 21000 24 hr d1(relaxation delay 3sec) 2013.3.31 cd3od

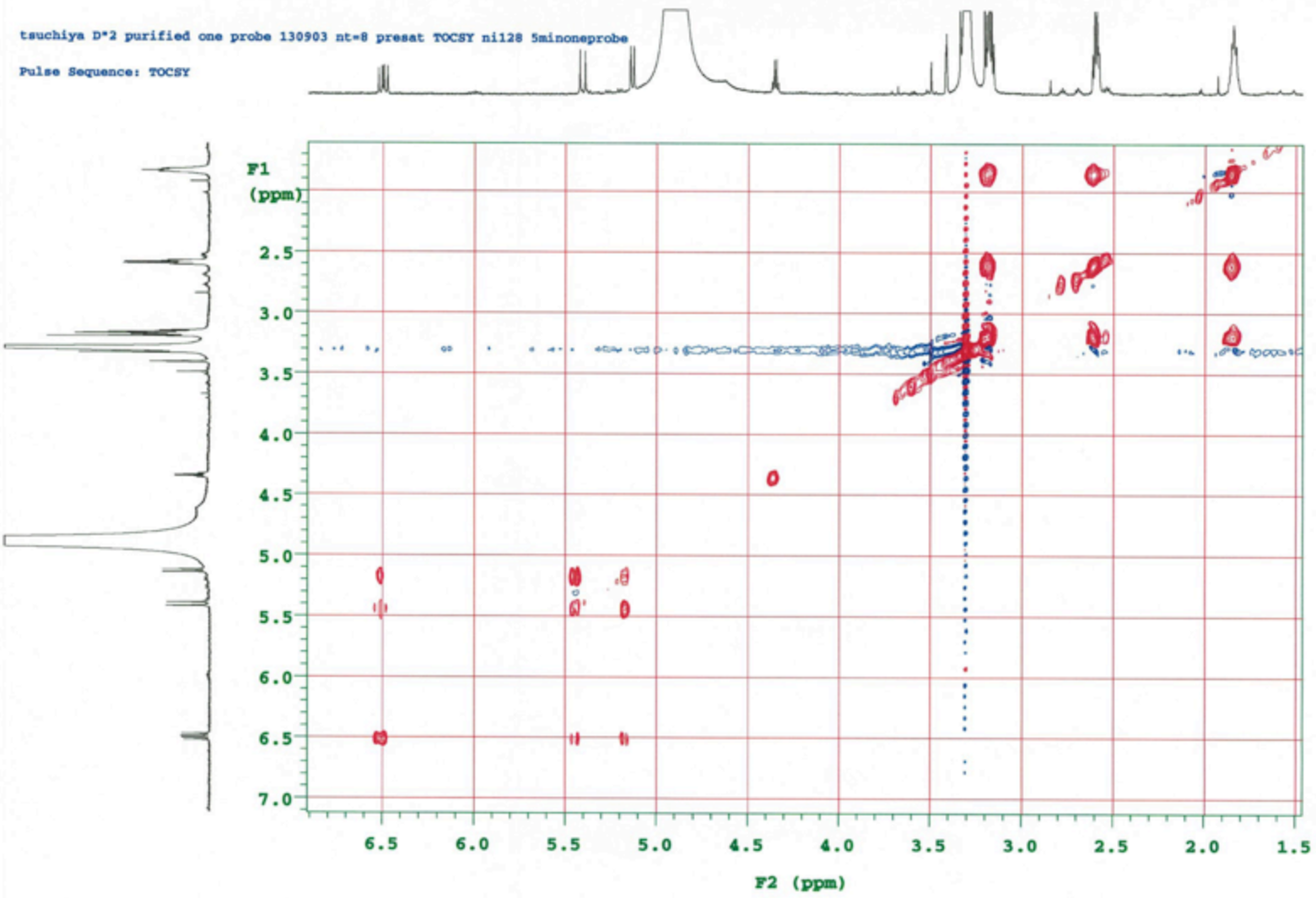
Pulse Sequence: CARBON (s2pul)

D"-2 (**7**)

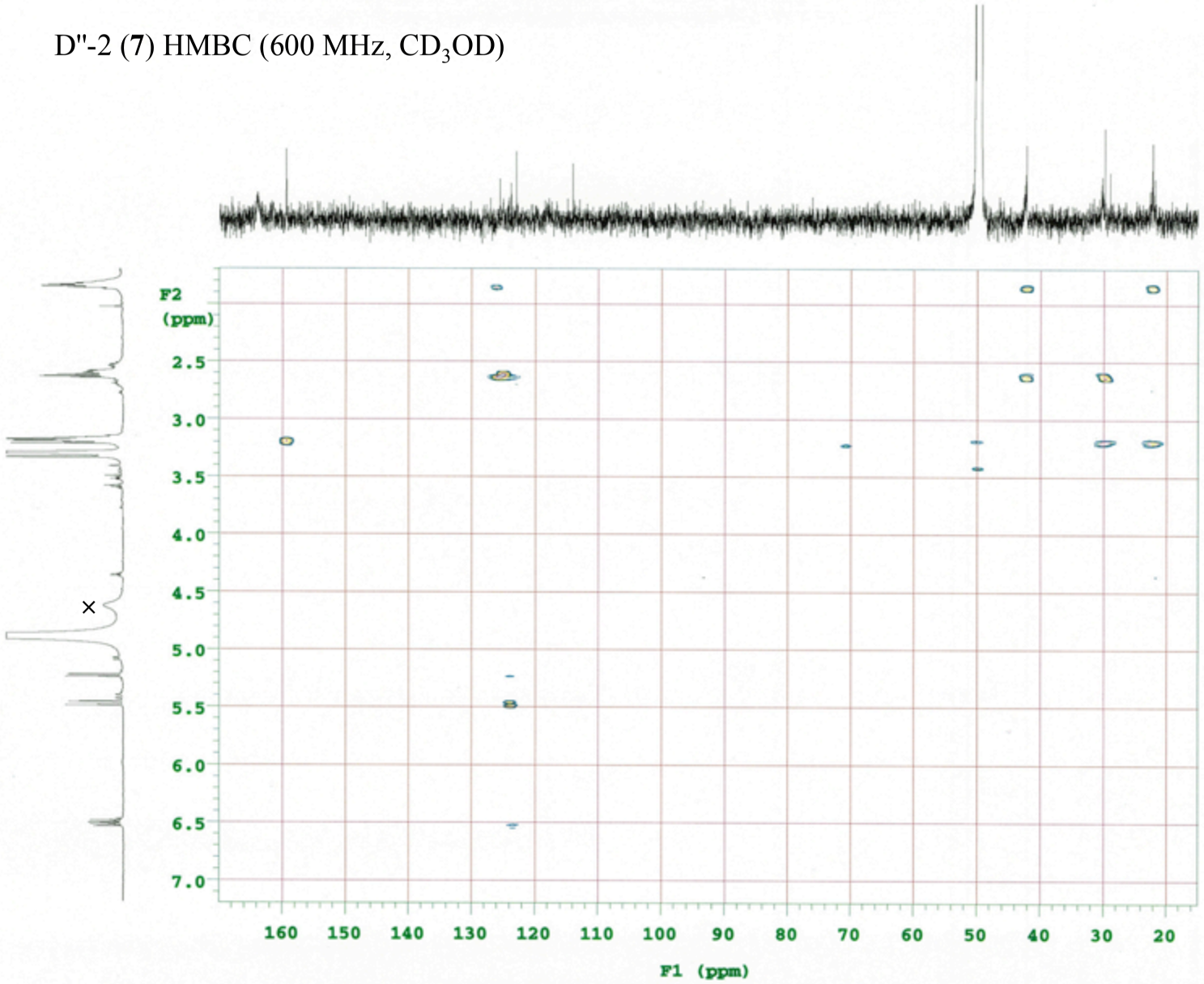
^{13}C NMR (151 MHz, CD_3OD)



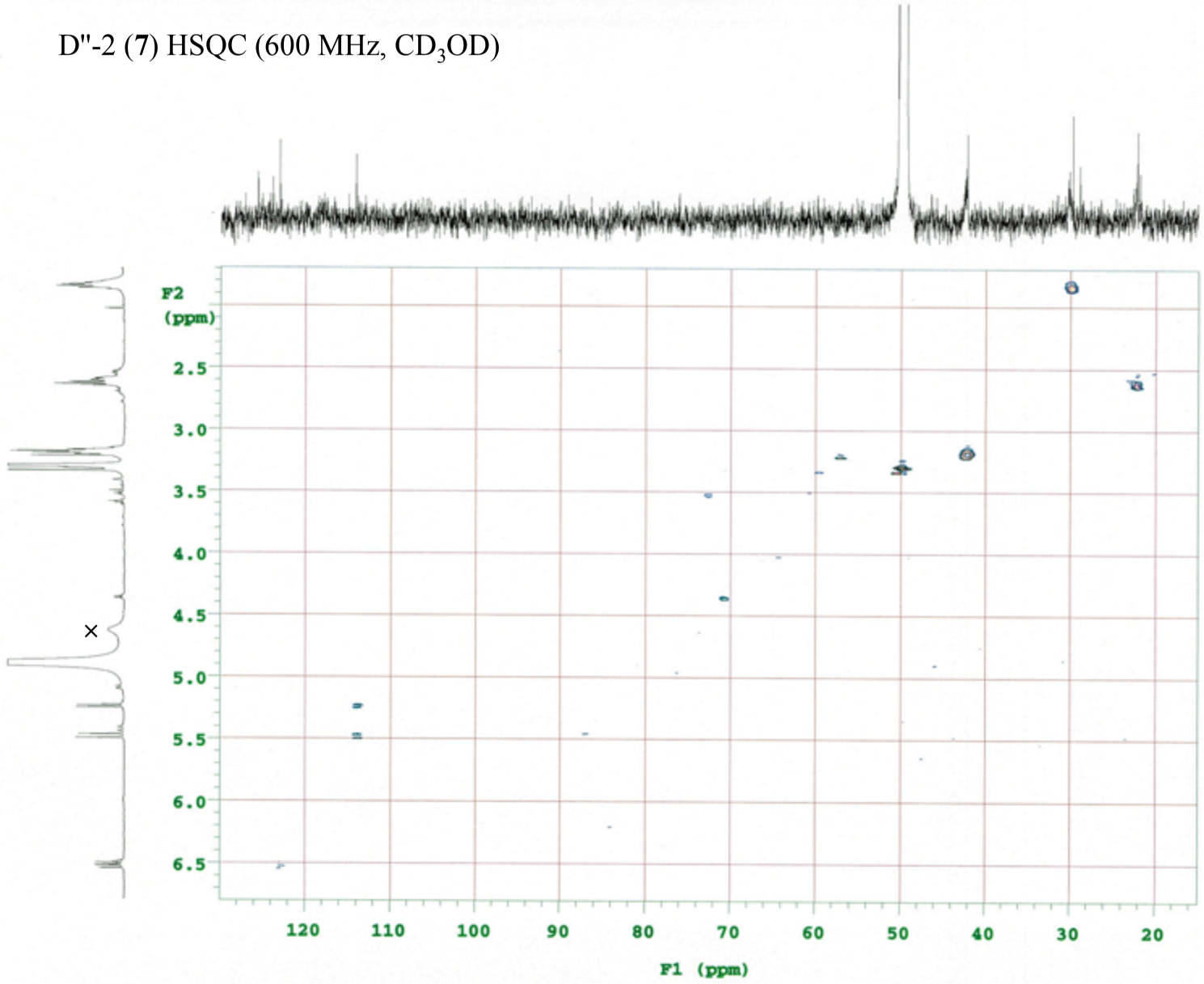
D"-2 (**7**) TOCSY (600 MHz, CD_3OD)

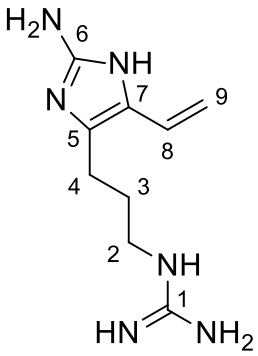


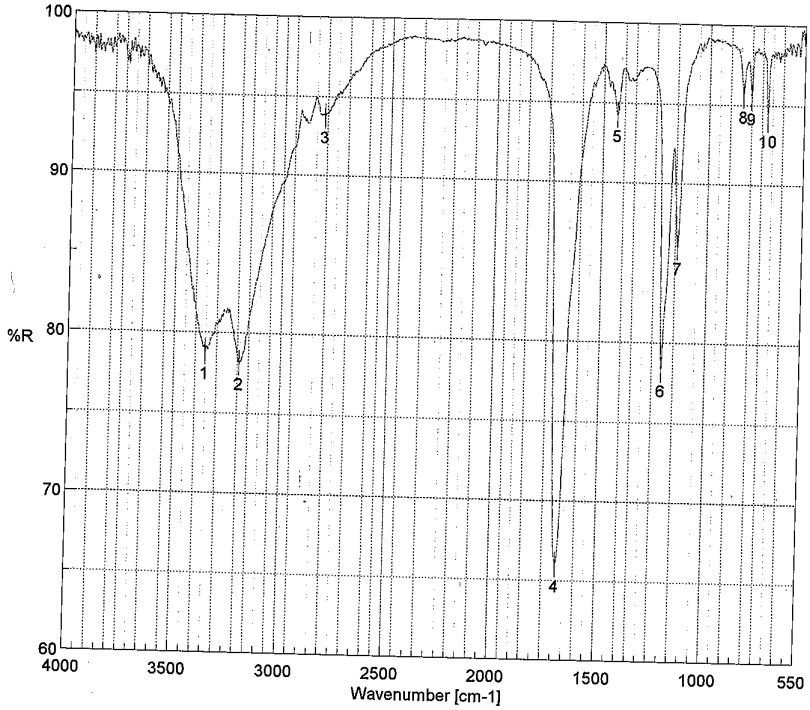
D"-2 (**7**) HMBC (600 MHz, CD_3OD)



D''-2 (7) HSQC (600 MHz, CD_3OD)







ピーク検出結果

No.	位置	強度	No.	位置	強度
1	3353.6	78.78	2	3192.58	78.13
3	2813.63	93.75	4	1684.52	65.98
5	1435.74	94.24	6	1204.33	78.33
7	1137.8	86.00	8	839.847	95.59
9	802.242	95.48	10	723.175	94.09

[コメント情報]

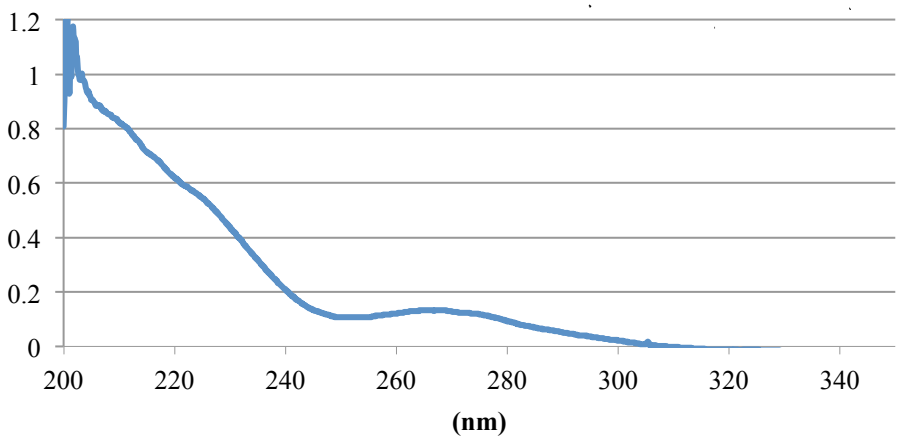
試料名	
コメント	ATR(ZnSe)
測定者	
所属	
会社	東北大学

[測定情報]

機種名	FT/IR-4100typeA
シリアル番号	B032661016
付属品名	ATR PRO410-S
付属品シリアルNo.	A017961044
光源	標準光源
検出器	TGS
積算回数	60
分解	4 cm-1
ゼロフィリング	On
アポダイゼーション	Cosine
ゲイン	Auto (8)
アパーチャー	Auto (7.1 mm)
スキャンスピード	Auto (2 mm/sec)
フィルタ	Auto (30000 Hz)

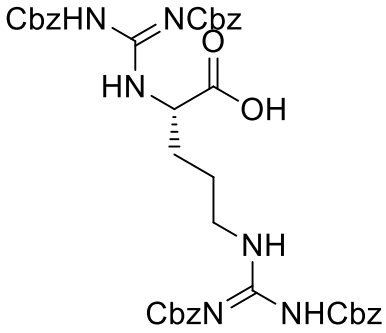
UV

D''-2



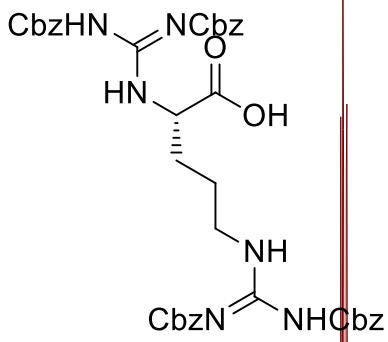
Tetra-Cbz-protected bis-guanidine carboxylic acid (**19**)

^{1}H NMR (600 MHz, $CDCl_3$)



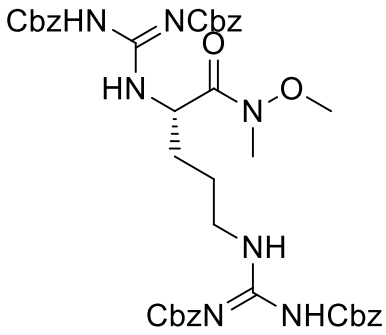
Tetra-Cbz-protected bis-guanidine carboxylic acid (**19**)

^{13}C NMR (151 MHz, $CDCl_3$)



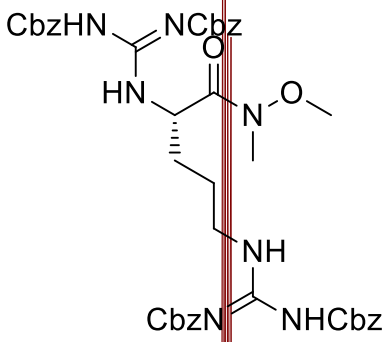
Tetra-Cbz-protected bis-guanidine weinreb amide (**20**)

^{1}H NMR (600 MHz, $CDCl_3$)



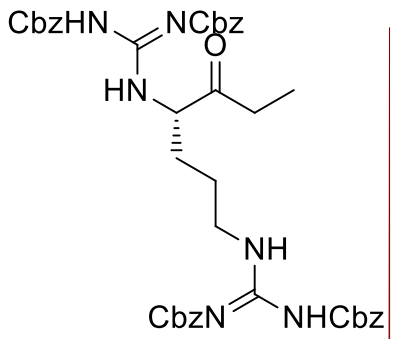
Tetra-Cbz-protected bis-guanidine weinreb amide (**20**)

^{13}C NMR (151 MHz, $CDCl_3$)



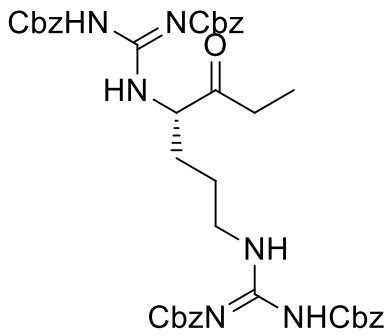
Tetra-Cbz-protected bis-guanidine etyl ketone (**21**)

^{1}H NMR (600 MHz, $CDCl_3$)



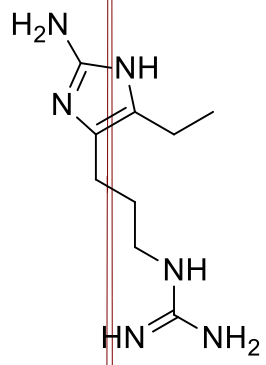
Tetra-Cbz-protected bis-guanidine etyl ketone (**21**)

^{13}C NMR (151 MHz, $CDCl_3$)



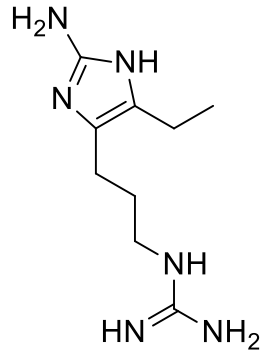
IntermediateC'2 (**6**) from Cbz-protected ethyl ketone (**21**) by Pd/C

^{1}H NMR (600 MHz, CD_3OD)



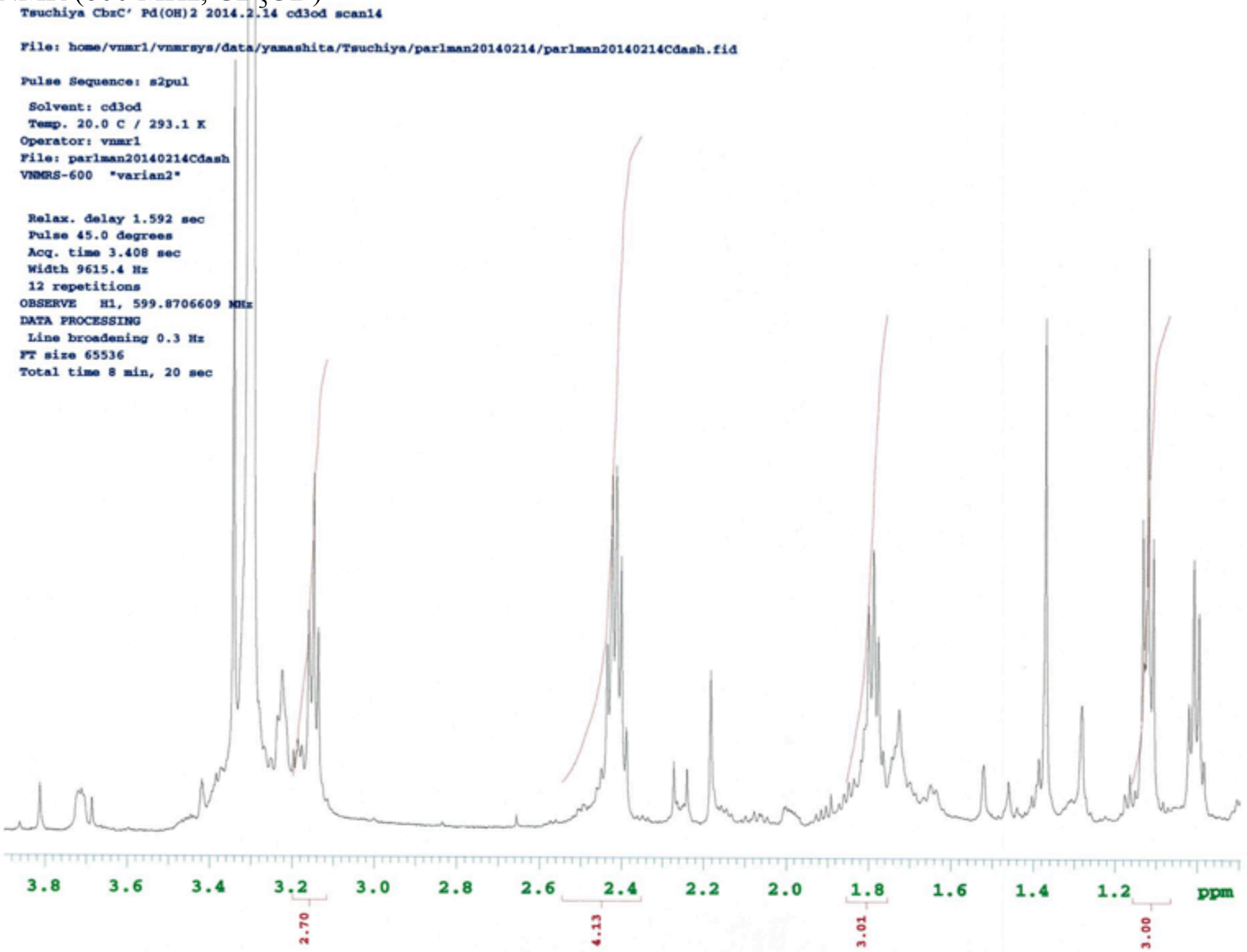
IntermediateC'2 (**6**) from Cbz-protected ethyl ketone (**21**) by Pd/C

^{13}C NMR (151 MHz, CD_3OD)



Intermediate C‘2 (**6**) crude from Cbz-protected ethyl ketone (**21**) by $Pd(OH)_2/C$

^1H NMR (600 MHz, CD_3OD)



Intermediate C‘2 (**6**) crude from Cbz-protected ethyl ketone (**21**) by $Pd(OH)_2/C$
ESI-Mass spectrum of the product mixture