Efficient Synthesis of a Novel 4-Hydroxy-2,3-dioxocyclobut-1-enyl Group Containing Amino Acids

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Supporting information.

Experimental procedures, spectral data for all compounds, and titration graphs for determination of the pKa values of **2** and **3.**

1. Experimental details for the syntheses of 6-20.

Melting points were determined with a Yanaco MP-21 melting point apparatus and were uncorrected. Optical rotations were taken on a Perkin Elmer 241 polarimeter with a sodium lamp (D line). Infrared spectra (IR) were measured on a HITACHI 270-30 infrared spectrophotometer. ^{1}H NMR spectra were recorded on an either JEOL JNM-LA 300 (300 MHz) spectrometer. Chemical shifts of ^{1}H NMR were reported in perts per million (ppm, δ) relative to tetramethylsilane (δ = 0.00) in $CDCl_3$ or HDO (δ = 4.80) in 6 M DCl. ^{13}C NMR spectra were recorded on a JEOL JNM-LA 300 (75 MHz) spectrometer. Chemical shifts of ^{13}C NMR were reported in ppm (δ) relative to $CHCl_3$ (δ = 77.0) in $CDCl_3$ or dioxane (δ = 68.9) in 6 M DCl. High resolution mass spectra (HRMS) were obtained on an either JEOL JMS-D300 or JEOL JMS-AX500 for fast atom bombardment ionization (FAB) or chemical ionization (CI). All reactions were monitored by thin layer chromatography (TLC), which

was performed with precoated plates (silica gel 60 F-254, 0.25 mm layer thickness, manufactured by Merck). Daisogel IR-60 1002W(40/63 mm) was used for flash column chromatography on silica gel. Reversed phase chromatography was performed on Cosmosil® 140C_{18}-PREP.

1,1-Dimethylethyl 2,3-bis(1-methylethoxy)-1-hydroxy-4-oxo-2-cyclobutene-1-acetate (6a).

To a solution of **5a** (5.30 mL, 39.3 mmol) in THF (50 mL) was added LDA (35.2 mmol) in THF (50 mL) under argon at - 78 °C. The mixture was stirred for 1h. To the mixture was added a solution of **4** (5.00 g, 25.2 mmol) in THF (30 mL) at -78 °C. The mixture was stirred for 2 h, quenched with saturated aqueous NH_4Cl (100 mL), and extracted with EtOAc (3 x 100 mL). The combined organic phase was washed with brine (100 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (EtOAc/hexane = 1/4) to give **6a** (7.9 g, 98%) as a colorless oil.: IR ($CDCl_3$) 3460 (br), 2990, 2940, 1770, 1710, 1630, 1470, 1460, 1390, 1370, 1340, 1320, 1230, 1150, 1100, 1030, 1010 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.90 (sept, J = 6.2 Hz, 1 H), 4.88 (sept, J = 6.2 Hz, 1 H), 4.68 (br s, 1 H), 2.75 (d, J = 15.4 Hz, 1 H), 2.67 (d, J = 15.4 Hz, 1 H), 1.48 (s, 9 H), 1.40 (d, J = 6.2 Hz, 3 H), 1.38 (d, J = 6.2 Hz, 3 H), 1.29 (d, J = 6.2 Hz, 3 H), 1.26 (d, J = 6.2 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 183.9, 171.0, 165.8, 131.6, 83.1, 82.3, 73.6, 38.5, 28.0, 22.75, 22.71, 22.3; HRMS (CI) *m/z* calcd for $C_{16}H_{27}O_6$ $(M+H)^+$ 315.1787, found 315.1797.

1,1-Dimethylethyl 2,3-bis(1-methylethoxy)-α-methyl-1-hydroxy-4-oxo-2-cyclobutene-1-acetate (6b).

To a mixture of **5b** (130 mg, 1.0 mmol) and $CeCl_3$ (246 mg, 1.0 mmol) in THF (5 mL) was added LDA (1.2 mmol) in THF (5 mL) under argon at - 78 °C. After stirring for 1h, **4** (198 mg, 1.0 mmol) in THF (3 mL) was added at - 78 °C. The mixture was stirred for 2 h, diluted with saturated aqueous NH_4Cl (10 mL), and extracted with EtOAc (3 x 10 mL). The combined organic phase was washed with brine (10 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (EtOAc/hexane =

1/2) to give **6b** (234 mg, 71%) as a colorless oil.: IR ($CDCl_3$) 3460 (br), 2990, 2940, 1770, 1740, 1700, 1630, 1460, 1390, 1370, 1340, 1320, 1260, 1220, 1150, 1100, 1030 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.98-4.81 (m, 2 H), 4.59 (br s, 1/2 H), 4.56 (br s, 1/2 H), 3.85-3.74 (m, 1 H), 1.49 (s, 9 H), 1.40-1.36 (m, 6 H), 1.30-1.18 (m, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 183.8, 183.5, 173.8, 173.7, 165.3, 164.8, 132.4, 86.5, 86.1, 82.2, 76.9, 73.5, 73.4, 43.3, 42.6, 27.9, 22.74, 22.72, 22.71, 22.70, 22.55, 22.49, 22.3, 22.2, 13.0, 12.8; HRMS (CI) *m/z* calcd for $C_{17}H_{29}O_6$ $(M+H)^+$ 329.1964, found 329.1954.

1,1-Dimethylethyl 3,4-dioxo-2-(1-methylethoxy)-1-cyclobutene-1-acetate (7a).

To a solution of **6a** (4.12 g, 13.1 mmol) in CH_2Cl_2 (30 mL) was added 12 M HCl (100 μL) at room temperature. The mixture was stirred for 2.5 h, diluted with saturated aqueous $NaHCO_3$ (30 mL), and extracted with EtOAc (3 x 30 mL). The combined organic phase was washed with brine (30 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (EtOAc/hexane = 1/4) to give **7a** (3.10 g, 93%) as a pale yellow oil.: IR (neat) 2990, 2950, 1800, 1760, 1740, 1610, 1420, 1390, 1370, 1330, 1260, 1210, 1160, 1100, 1030 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.45 (sept, J = 6.2 Hz, 1 H), 3.55 (s, 2 H), 1.48 (d, J = 6.2 Hz, 6 H), 1.47 (s, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 198.3, 194.2, 193.6, 175.4, 165.6, 82.8, 79.7, 31.7, 27.9, 22.8; HRMS (CI) *m/z* calcd for $C_{13}H_{19}O_5$ $(M+H)^+$ 255.1233, found 255.1254.

1,1-Dimethylethyl 3,4-dioxo-α-methyl-2-(1-methylethoxy)-1-cyclobutene-1-acetate (7b).

To a solution of **6b** (131 mg, 0.399 mmol) in CH_2Cl_2 (2 mL) was added 12 M HCl (50 μL) at room temperature. The mixture was stirred for 2.5 h, diluted with saturated aqueous $NaHCO_3$ (2 mL), and extracted with EtOAc (3 x 2 mL). The combined organic phase was washed with brine (2 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (EtOAc/hexane = 1/4) to give **7b** (81 mg, 76%) as a pale yellow oil.: IR ($CHCl_3$) 2990, 2940, 1800, 1760, 1730, 1600, 1460, 1400, 1370, 1350, 1330, 1290, 1260, 1230, 1150, 1100 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.46 (sept, J = 6.2 Hz, 1 H), 3.73 (q, J = 7.3 Hz, 1 H), 1.50 (d, J = 7.3 Hz, 3 H), 1.47 (d, J = 6.2

Hz, 6 H), 1.46 (s, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.1, 194.3, 193.2, 180.7, 168.9, 82.3, 79.5, 38.2, 27.8, 22.7, 13.9; HRMS (CI) *m/z* calcd for $C_{14}H_{21}O_5$ $(M+H)^+$ 269.1389, found 269.1403.

3-Hydroxy-4-methylcyclobutene-1,2-dione.

To a solution of **7a** (115 mg, 0.537 mmol) in CH_2Cl_2 (5 mL) was added TFA (5 mL) at 0 °C. The mixture was warmed to room temperature, stirred for 8 h, and concentrated *in vacuo*. To a solution of the residue in CH_2Cl_2 (5 mL) was added Et_3N (120 μL, 1.61 mmol). The mixture was stirred for 15 min and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (EtOAc/hexane = 1/9-1/4) to give **8a** (49 mg, 59%) as a pale yellow oil. To a solution of **8a** (161 mg, 1.04 mmol) in acetone (2 mL) was added 12 M HCl (2 mL). The mixture was stirred for 0.5 h and concentrated *in vacuo*. The residue was purified by flash chromatography on Cosmosil® (H_2O) to give the titled compound (114 mg, 98%).

3-Ethyl-4-hydroxy-cyclobutene-1,2-dione.

To a solution of **7b** (5.7 mg, 0.021 mmol) in CH_2Cl_2 (2 mL) was added TFA (2 mL) at 0 °C. The mixture was warmed to room temperature, stirred for 8 h, and concentrated *in vacuo*. To a solution of the residue in CH_2Cl_2 (2 mL) was added Et_3N (5 μL, 0.063 mmol). The mixture was stirred for 15 min and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (EtOAc/hexane = 1/4) to give **8b** (2.0 mg, 57%) as a pale yellow oil. To a solution of **8b** (90 mg, 0.535 mmol) in acetone (2 mL) was added 12 M HCl (2 mL). The mixture was stirred for 0.5 h and concentrated *in vacuo*. The residue was purified by flash chromatography on Cosmosil® (H_2O) to give the titled compound (66 mg, 98%) .

1,1-Dimethylethyl 2,3-bis(1-methylethoxy)-α-(1,1-dimethylethoxy)carbonylamino-1-hydroxy-4-oxo-2-cyclobutene-1-acetate (6c).

To a solution of **5d** (4.66 g, 20.1 mmol) in THF (100 mL) was added LDA (42.0

mmol) in THF (100 mL) under argon at - 78 °C. The mixture was stirred for 0.5 h. To the mixture was added a solution of **4** (3.71 g, 18.8 mmol) in THF (30 mL) at -78 °C. The mixture was stirred for 1 h, diluted with saturated aqueous NH_4Cl (100 mL), and extracted with EtOAc (3 x 100 mL). The combined organic phase was washed with brine (100 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (EtOAc/hexane = 1/9-1/3) to give **6c** (6.22 g, 72%) as a pale yellow oil.: IR (neat) 3420 (br), 2980, 2940, 1770, 1720, 1630, 1500, 1460, 1390, 1320, 1220, 1150, 1100, 1060, 1030 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.55 (br d, J = 8.0 Hz, 3/5 H), 5.37 (br d, J = 7.9 Hz, 2/5 H), 4.87 (sept, J = 6.2 Hz, 2 H), 4.59 (d, J = 9.0 Hz, 1 H), 4.44 (br s, 1 H), 1.49-1.18 (m, 30 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 181.6, 168.3, 166.8, 163.7, 155.5, 134.3, 132.5, 82.6, 78.6, 76.9, 73.4, 73.3, 57.5, 50.0, 45.4, 28.0, 27.5, 22.5, 22.4, 22.2, 22.1, 22.0, 21.9; HRMS (CI) *m/z* calcd for $C_{21}H_{36}NO_8$ $(M+H)^+$ 430.2440, found 430.2412.

1,1-Dimethylethyl α-1,1-dimethylethoxycarbonylamino-3,4-dioxo-2-(1-methylethoxy)-1-cyclobutene-1-acetate (7c).

To a solution of **6c** (6.73 g, 15.7 mmol) in CH_2Cl_2 (200 mL) was added 12 M HCl (13 mL) at room temperature. The mixture was stirred for 4 h, diluted with saturated aqueous $NaHCO_3$ (200 mL), and extracted with EtOAc (3 x 200 mL). The combined organic phase was washed with brine (200 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (EtOAc/hexane = 1/9-1/2) to give **7c** (4.87 g, 84%) as a pale yellow oil.: IR (neat) 3390, 2990, 2940, 1800, 1760, 1600, 1510, 1390, 1370, 1150, 1100, 1050 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.70 (br d, J = 7.5 Hz, 1 H), 5.47 (sept, J = 6.2 Hz, 1 H), 5.36 (d, J = 7.5 Hz, 1 H), 1.49 (d, J = 6.4 Hz, 3 H), 1.48 (d, J = 6.4 Hz, 3 H), 1.48 (s, 9 H), 1.45 (s, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.3, 193.6, 191.4, 175.3, 165.3, 154.7, 84.2, 80.5, 80.1, 51.1, 28.1, 27.7, 22.7, 22.6; HRMS (CI) *m/z* calcd for $C_{18}H_{28}NO_7$ $(M+H)^+$ 370.1866, found 370.1890.

3-Aminomethyl-4-hydroxy-1-cyclobuten-2,3-dione (1).

To a solution of **7c** (104 mg, 0.28 mmol) in acetone (1 mL) was added 12 M HCl (1

mL). The mixture was stirred for 0.5 h and concentrated *in vacuo*. The residue was purified by flash chromatography on Cosmosil® (H_2O) to give **1** (33 mg, 72%) as a white solid.

1,1-Dimethylethyl (1*R,α*S*,β*R**)- and (1*R**,α*S*,β*S**)-2,3-bis(1-methylethoxy)-β-1,1-dimethylethoxycarbonyl-1-hydroxy-4-oxo-α-phenylmethyloxycarbonylamino-2-cyclobutene-1-propionate (9).**

To a solution of di-*tert*-butyl *N*-Cbz-L-aspartate (2.64 g, 6.95 mmol) and LiCl (2.65 g, 62.5 mmol) in THF (100 mL) was added LHMDS (15.3 mmol) in THF (100 mL) under argon at - 78 °C. The mixture was stirred for 2 h. To the mixture was added a solution of **4** (1.24 g, 6.26 mmol) in THF (50 mL) at - 45 °C. The mixture was stirred for 1 h, diluted with saturated aqueous NH_4Cl (100 mL), and extracted with EtOAc (3 x 100 mL). The combined organic phase was washed with brine (100 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (EtOAc/hexane = 1/9-1/2) to give a mixture of diastereomers of **9** (3.35 g, 92%) as a colorless oil.: IR ($CDCl_3$) 3460, 3300 (br), 2990, 2940, 1770, 1720, 1630, 1520, 1460, 1390, 1370, 1320, 1280, 1230, 1160, 1100, 1030, 1010 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.40-7.31 (m, 5 H), 6.01 (br d, J = 9.8 Hz, 1 H), 5.26 (d, J = 12.4 Hz, 1 H), 5.17 (br s, 1 H), 5.01 (d, J = 12.4 Hz, 1 H), 4.92-4.79 (m, 2 H), 4.61 (dd, J = 9.8, 3.4 Hz, 1 H), 3.76 (d, J = 3.4 Hz, 1 H), 1.46-1.25 (m, 30 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 182.1, 169.6, 169.2, 163.0, 157.5, 136.0, 132.8, 128.5, 128.1, 127.9, 84.4, 83.0, 82.7, 77.1, 73.9, 67.3, 52.4, 51.2, 27.89, 27.87, 22.90, 22.88, 22.4, 22.3; HRMS (CI) *m/z* calcd for $C_{30}H_{44}NO_{10}$ $(M+H)^+$ 578.2965, found 578.2962.

1,1-Dimethylethyl (α*S*,β*R*)- and (α*S*,β*S*)-β-1,1-dimethylethoxycarbonyl-3,4-dioxo-2-(1-methylethoxy)-α-phenylmethyloxycarbonylamino-1-cyclobutene-1-propionate (11a).

To a solution of **9** (290 mg, 0.502 mmol) in CH_2Cl_2 (5 mL) was added 12 M HCl (50 μL) at room temperature. The mixture was stirred for 12 h, diluted with saturated aqueous $NaHCO_3$ (5 mL), and extracted with EtOAc (3 x 5 mL). The combined organic phase was washed with brine (5 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (EtOAc/hexane = 1/4) to give a 1:1 mixture of

diastereomers (based on ^{1}H NMR integrations of the Cβ-H) of **11a** (163 mg, 63%) as a pale yellow oil.: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35-7.30 (m, 5 H), 6.32 (br d, J = 9.9 Hz, 1/2 H), 5.82 (br d, J = 9.7 Hz, 1/2 H), 5.45 (sept, J = 6.2 Hz, 1/2 H), 5.37 (sept, J = 6.1 Hz, 1/2 H), 5.13 (s, 1 H), 5.11 (dd, J = 9.9, 3.9 Hz, 1/2 H), 5.07 (s, 1 H), 4.77 (dd, J = 9.7, 3.9 Hz, 1/2 H), 4.39 (d, J = 3.9 Hz, 1/2 H), 4.30 (d, J = 3.9 Hz, 1/2 H), 1.48-1.34 (m, 24 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 198.1, 197.9, 193.8, 193.3, 192.6, 192.3, 176.2, 175.9, 168.3, 168.0, 167.0, 165.3, 156.4, 156.1, 136.3, 136.1, 128.5, 128.4, 128.1, 128.05, 128.01, 127.95, 83.8, 83.7, 83.18, 83.16, 80.4, 80.0, 67.1, 67.0, 54.4, 53.7, 46.5, 45.4, 27.81, 27.78, 27.69, 27.68, 22.8, 22.74, 22.71, 22.6; HRMS (CI) *m/z* calcd for $C_{27}H_{36}NO_9$ $(M+H)^+$ 518.2390, found 518.2363.

(α*S*)-3,4-Dioxo-2-(1-methylethoxy)-α-phenylmethyloxycarbonylamino-1-cyclobutene-1-propionic acid (11b).

To a solution of **11a** (163 mg, 0.315 mmol) in CH_2Cl_2 (5 mL) was added TFA (5 mL) at 0 °C. The mixture was warmed to room temperature and concentrated *in vacuo*. To a solution of the residue in CH_2Cl_2 (5 mL) was added Et_3N (70 μL, 0.945 mol). The mixture was stirred for 10 min, acidified with 1 M HCl, and extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (EtOAc) to give **11b** (91 mg, 80%) as a pale yellow oil.: $[\alpha]^{22}_D$ -5.0° (*c* 1.0, MeOH); IR ($CHCl_3$) 3440 (br), 3030, 2990, 1800, 1750, 1720, 1590, 1510, 1470, 1460, 1390, 1380, 1330, 1230, 1150, 1090, 1060 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 7.36-7.26 (m, 5 H), 5.35 (sept, J = 6.2 Hz, 1 H), 5.11 (d, J = 11.9 Hz, 1 H), 5.06 (d, J = 11.9 Hz, 1 H), 4.63 (dd, J = 8.3, 5.4 Hz, 1 H), 3.16 (dd, J = 16.0, 5.4 Hz, 1 H) 3.04 (dd, J = 16.0, 8.3 Hz, 1 H), 1.40 (d, J = 6.2 Hz, 3 H) 1.39 (d, J = 6.2 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.8, 196.7, 194.3, 179.4, 173.4, 157.8, 137.7, 129.3, 128.9, 128.6, 80.9, 67.6, 52.1, 28.2, 22.8; HRMS (FAB) *m/z* calcd for $C_{18}H_{20}NO_7$ $(M+H)^+$ 362.1231, found 362.1245.

(*S*)-α-Amino-3,4-dioxo-2-hydroxy-1-cyclobutene-1-propionic acid (2).

To a solution of **11b** (141 mg, 0.390 mmol) in acetone (5 mL) was added 12 M HCl

(5 mL). The mixture was heated to reflux with stirring for 3 h and concentrated *in vacuo*. The residue was purified by flash chromatography on Cosmosil® (H_2O) to give **2** (75 mg, 87%) as a white solid.: mp 170-171 °C (from H_2O); $[\alpha]^{14}_D$ +15.6° (*c* 1.0, 6 M HCl); ^{1}H NMR (300 MHz, 6 M DCl) δ 4.62 (t, J = 6.1 Hz, 1H), 3.32 (d, J = 6.1 Hz, 2H); ^{13}C NMR (75 MHz, 6 M DCl) δ 202.9, 178.5, 172.5, 52.6, 27.9; HRMS (FAB) *m/z* calcd for $C_7H_8NO_5$ $(M+H)^+$ 186.0403, found 186.0387.

Synthesis of 2 from 9 via 13a.

To a solution of **9** (1.11 g, 1.91 mmol) in acetone (10 mL) was added 12 M HCl (10 mL) at room temperature. The mixture was stirred for 7 h, and concentrated *in vacuo*. The residue was purified by flash chromatography on Cosmosil® (H_2O/MeOH = 1/0-2/1) to give **13a** as a pale yellow oil. A solution of **13a** in 6 M HCl (10 mL) was heated to reflux with stirring for 12 h and concentrated *in vacuo*. The residue was purified by flash chromatography on Cosmosil® (H_2O) to give **2** (240 mg, 68%).

Synthesis of racemic 2.

To a solution of **9** (2.77 g, 4.91 mmol) prepared from DL-aspartic acid diester in acetone (100 mL) was added 12 M HCl (100 mL) at room temperature. The mixture was stirred to reflux for 7 h and concentrated *in vacuo*. The residue was purified by flash chromatography on Cosmosil® (H_2O/MeOH = 1/0-2/1) to give racemic **13a** as a pale yellow oil. A solution of racemic **13a** in 6 M HCl (30 mL) was heated to reflux with stirring for 12 h and concentrated *in vacuo*. The residue was purified by flash chromatography on Cosmosil® (H_2O) to give racemic **2** (591 mg, 65%).

1,1-Dimethylethyl (1*R,α*S*,γ*R**)- and (1*R**,α*S*,γ*S**)-2,3-bis(1-methylethoxy)-γ-1,1-dimethylethoxycarbonyl-1-hydroxy-4-oxo-α-phenylmethyloxycarbonylamino-2-cyclobutene-1-butyrate (10).**

To a solution of di-*tert*-butyl *N*-Cbz-L-glutamate (2.73 g, 6.93 mmol) and LiCl (1.76 g, 41.5 mmol) in THF (100 mL) was added LHMDS (15.2 mmol) in THF (100 mL) under

argon at - 78 °C. The mixture was stirred for 2 h. To the mixture was added a solution of **4** (1.37 g, 6.92 mmol) in THF (50 mL) at - 45 °C. The mixture was stirred for 1 h, diluted with saturated aqueous NH_4Cl (100 mL), and extracted with EtOAc (3 x 100 mL). The combined organic phase was washed with brine (100 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (EtOAc/hexane = 1/9-1/2) to give **10** (a mixture of diastereomers, 3.77 g, 92%) as a colorless oil.: IR ($CDCl_3$) 3450 (br), 3000, 2940, 1770, 1720, 1630, 1500, 1470, 1390, 1370, 1320, 1250, 1220, 1150, 1100, 1050 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.40-7.30 (m, 5 H), 5.19 (br s, 1 H), 5.10 (s, 2 H), 4.96-4.80 (m, 2 H), 4.40-4.23 (m, 1 H), 4.18-4.07 (m, 1 H), 2.85-2.78 (m, 1 H), 2.44-2.00 (m, 2 H), 1.50-1.23 (m, 30 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 183.5, 183.4, 172.6, 170.9, 164.4, 136.2, 132.2, 128.4, 128.08, 128.06, 85.6, 82.9, 82.2, 77.0, 73.6, 67.0, 60.4, 53.9, 30.9, 27.9, 27.8, 22.8, 22.7, 22.5, 22.2; HRMS (CI) *m/z* calcd for $C_{31}H_{44}NO_9$ $(M-OH)^+$ 574.3016, found 574.2997.

1,1-Dimethylethyl (α*S*,γ*R*)- and (α*S*,γ*S*)-γ-1,1-dimethylethoxycarbonyl-3,4-dioxo-2-(1-methylethoxy)-α-phenylmethyloxycarbonylamino-1-cyclobutene-1-butyrate (12a).

To a solution of **10** (1.01 g, 1.71 mmol) in CH_2Cl_2 (50 mL) was added 12 M HCl (0.5 mL) at room temperature. The mixture was stirred for 12 h, diluted with saturated aqueous $NaHCO_3$ (50 mL), and extracted with EtOAc (3 x 50 mL). The combined organic phase was washed with brine (50 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (EtOAc/hexane = 1/4-1/2) to give **12a** (a 1:1 mixture of diastereomers, 817 mg, 90%) as a pale yellow oil.: ^1H NMR (300 MHz, $CDCl_3$) δ 7.34 (br s, 5 H), 5.43 (sept, J = 6.2 Hz, 1 H), 5.39-5.27 (m, 1 H), 4.38-4.20 (m, 1 H), 3.83-3.75 (m, 1 H), 2.60-2.51 (m, 1 H), 2.32-2.15 (m, 1 H), 1.47-1.43 (m, 24 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.4, 197.3, 193.9, 193.3, 192.6, 192.3, 178.6, 178.4, 170.4, 170.1, 167.7, 167.5, 156.4, 156.1, 136.1, 136.0, 128.48, 128.46, 128.16, 128.13, 128.10, 128.06, 83.0, 82.9, 82.83, 82.79, 80.0, 79.8, 67.1, 67.0, 52.9, 52.8, 40.9, 40.8, 31.4, 31.3, 27.90, 27.88, 27.81, 27.79, 22.8, 22.7. HRMS (CI) *m/z* calcd for $C_{28}H_{38}NO_9$ $(M+H)^+$ 532.2546, found 532.2527.

(αS)-3,4-Dioxo-2-(1-methylethoxy)-α-phenylmethyloxycarbonylamino-1-cyclobutene-1-butyric acid (12b).

To a solution of **12a** (817 mg, 1.54 mmol) in CH_2Cl_2 (20 mL) was added TFA (5 mL) at 0 °C. The mixture was warmed to room temperature and concentrated *in vacuo*. To a solution of the residue in CH_2Cl_2 (5 mL) was added Et_3N (340 μL, 0.945 mol). The mixture was stirred for 10 min, acidified with 1 M HCl, and extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (EtOAc) to give **12b** (451 mg, 78%) as a pale yellow oil.: $[\alpha]^{22}_D$ -5.6° (*c* 1.0, MeOH); IR ($CHCl_3$) 3440 (br), 3020, 2990, 1800, 1750, 1720, 1590, 1510, 1470, 1450, 1390, 1350, 1200, 1150, 1100, 1060, 1000 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.37-7.25 (m, 5 H), 5.37 (sept, J = 6.2 Hz, 1 H), 5.11 (d, J = 12.8 Hz, 1 H), 5.07 (d, J = 12.8 Hz, 1 H), 4.20 (dd, J = 9.4, 4.7 Hz, 1 H), 2.71 (t, J = 7.4 Hz, 2 H), 2.37-2.26 (m, 1 H), 2.09-1.96 (m, 1 H), 1.43 (d, J = 6.2 Hz, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.5, 197.2, 194.6, 182.5, 174.7, 158.2, 137.8, 129.3, 128.9, 128.6, 80.7, 67.5, 54.6, 28.3, 22.8, 22.4; HRMS (FAB) *m/z* calcd for $C_{19}H_{22}NO_7$ $(M+H)^+$ 376.1392, found 376.1399.

(S)-α-Amino-3,4-dioxo-2-hydroxy-1-cyclobutene-1-butyric acid (3) from 12b.

To a solution of **12b** (115 mg, 0.307 mmol) in acetone (5 mL) was added 12 M HCl (5 mL). The mixture was heated to reflux with stirring for 3 h and concentrated *in vacuo*. The residue was purified by flash chromatography on Cosmosil® (H_2O) to give **3** (71 mg, 98%) as a white solid.: mp 160-163 °C (H_2O); $[\alpha]^{18}_D$ +38.9° (*c* 1.0, 6 M HCl); ^1H NMR (300 MHz, 6 M DCl) δ 4.22 (t, J = 6.4 Hz, 1 H), 2.86 (d, J = 7.2 Hz, 2 H), 2.24-2.04 (m, 2 H); ^{13}C NMR (75 MHz, 6 M DCl) δ 202.0, 199.7, 182.8, 173.1, 54.7, 28.0, 23.2; HRMS (FAB) *m/z* calcd for $C_8H_{10}NO_5$ $(M+H)^+$ 200.0559, found 200.0561.

Synthesis of racemic 3.

To a solution of racemic **10** (1.05 g, 1.82 mmol) prepared from DL-glutamic acid diester in acetone (100 mL) was added 12 M HCl (100 mL) at room temperature. The

mixture was heated to reflux with stirring for 7 h and concentrated *in vacuo*. The residue was purified by flash chromatography on Cosmosil® (H_2O/MeOH = 1/0-2/1) to give racemic **13b** as a pale yellow oil. A solution of racemic **13b** in 6 M HCl (30 mL) was heated to reflux with stirring for 12 h and concentrated *in vacuo*. The residue was purified by flash chromatography on Cosmosil® (H_2O) to give racemic **3** (261 mg, 72%).

General procedure for syntheses of amides and carbamates (14)-(17).

To a mixture of an amino acid (**2** or **3**) and $NaHCO_3$ (9 equiv) in a biphasic solution [Et_2O/H_2O or AcOEt/H_2O, each 10 mL for the substrate (1 mmol)] was added an acylating reagent (3-4 equiv). The mixture was vigorously stirred for 12 h at room temperature, acidified with 6 M HCl, and extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash column chromatography on Cosmosil® (H_2O/MeOH = 1/0-1/1).

(α*S*,2'*R*)-3,4-Dioxo-2-hydroxy-α-(2-methoxy-1-oxo-3,3,3-trifluoro-2-phenylpropyl amino)-1-cyclobutene-1-propionic acid (14a).

According to the general procedure, **2** (17 mg, 0.090 mmol) was treated with MTPACl (90 mg, 0.353 mmol) and $NaHCO_3$ (57 mg, 0.678 mmol) in a biphasic solution [Et_2O/H_2O (each 1 mL)]. The titled compound (35 mg, 80%) was obtained as a pale yellow oil: ^{1}H NMR (300 MHz, D_2O) δ 7.39-7.29 (m, 5 H), 4.80 (dd, J = 8.2, 4.9 Hz, 1 H), 3.19 (s, 3 H), 3.09 (dd, J = 16.4, 8.2 Hz, 1 H), 2.91 (dd, J = 16.4, 4.9 Hz, 1 H).

MTPA Amide from racemic 2.

According to the general procedure, racemic **2** (8 mg, 0.045 mmol) was treated with MTPACl (45 mg, 0.177 mmol) and $NaHCO_3$ (28 mg, 0.339 mmol) in a biphasic solution [Et_2O/H_2O (each 0.5 mL)]. The titled compound (14 mg, 76%) was obtained as a pale yellow oil: ^{1}H NMR (300 MHz, D_2O) δ 7.43-7.24 (m, 5 H), 4.88 (dd, J = 8.8, 5.2 Hz, 1/2 H), 4.80 (dd, J = 8.2, 4.9 Hz, 1/2 H), 3.31 (s, 3/2 H), 3.19 (s, 3/2 H), 3.09 (dd, J = 16.4, 8.2 Hz, 1/2 H), 2.97-2.87 (m, 3/2 H).

(α*S*,2'*R*)-3,4-Dioxo-2-hydroxy-α-(2-methoxy-1-oxo-3,3,3-trifluoro-2-phenylpropyl amino)-1-cyclobutene-1-butyric acid (14b).

According to the general procedure, **3** (18 mg, 0.090 mmol) was treated with MTPACl (90 mg, 0.353 mmol) and $NaHCO_3$ (57 mg, 0.678 mmol) in a biphasic solution [Et_2O/H_2O (each 0.5 mL)]. The titled compound (43 mg, 98%) was obtained as a pale yellow oil: ^1H NMR (300 MHz, D_2O) δ 7.44-7.34 (m, 5 H), 4.38 (dd, J = 9.2, 4.4 Hz, 1 H), 3.23 (s, 3 H), 2.49 (t, J = 7.2 Hz, 2 H), 2.22-2.16 (m, 1 H), 2.07-2.00 (m, 1 H);

MTPA amide of racemic 3.

According to the general procedure, racemic **3** (9 mg, 0.045 mmol) was treated with MTPACl (45 mg, 0.177 mmol) and $NaHCO_3$ (28 mg, 0.339 mmol) in a biphasic solution [Et_2O/H_2O (each 0.5 mL)]. The titled compound (17 mg, 90%) was obtained as a pale yellow oil: ^1H NMR (300 MHz, D_2O) δ 7.33-7.25 (m, 5H), 4.38 (dd, J = 9.2, 4.4 Hz, 1/2H), 4.33 (dd, J = 9.8, 4.6 Hz, 1/2H), 3.31 (s, 3/2H), 3.23 (s, 3/2H), 2.51-2.40 (m, 3/2H), 2.37-2.31 (m, 1/2H), 2.23-2.14 (m, 1H), 2.07-1.96 (m, 1H).

(*S*)-α-(1,1-Dimethylethyloxycarbonylamino)-3,4-dioxo-2-hydroxy-1-cyclobutene-1-propionic acid (15a).

According to the general procedure, **2** (266 mg, 1.44 mmol) was treated with Boc_2O (1.41 g, 6.48 mmol) and $NaHCO_3$ (1.09 g, 13.0 mmol) in a biphasic solution [Et_2O/H_2O (each 15 mL)]. The titled compound (63 mg, 15%) was obtained as a pale yellow oil: ^1H NMR (300 MHz, D_2O) δ 4.18 (dd, J = 9.6, 4.4 Hz, 1 H), 2.88 (dd, J = 14.9, 4.4 Hz, 1 H), 2.77 (dd, J = 14.9, 9.7 Hz, 1 H), 1.27 (s, 9 H).

(*S*)-α-(1,1-Dimethylethyloxycarbonylamino)-3,4-dioxo-2-hydroxy-1-cyclobutene-1-butyric acid (15b).

According to the general procedure, **3** (80 mg, 0.402 mmol) was treated with Boc_2O (396 mg, 1.81 mmol) and $NaHCO_3$ (304 mg, 3.62 mmol) in a biphasic solution [Et_2O/H_2O

(each 5 mL)]. The titled compound (33 mg, 27%) was obtained as a pale yellow oil: ^{1}H NMR (300 MHz, D_2O) δ 3.95 (dd, J = 9.2, 4.1 Hz, 1 H), 2.52 (t, J = 7.2 Hz, 2 H), 2.16-2.05 (m, 1 H), 1.93-1.82 (m, 1 H); ^{13}C NMR (75 MHz, D_2O) δ 212.2, 202.7, 188.8, 180.0, 157.7, 81.4, 56.5, 28.4, 28.8, 21.8; HRMS (FAB) *m/z* calcd for $C_{13}H_{17}NO_7$ $(M)^+$ 299.1005, found 299.1014.

(*S*)-3,4-Dioxo-2-hydroxy-α-phenylmethyloxycarbonylamino-1-cyclobutene-1-propionic acid (16a).

According to the general procedure, **2** (41 mg, 0.221 mmol) was treated with CbzCl (147 mg, 0.862 mmol) and $NaHCO_3$ (138 mg, 1.66 mmol) in a biphasic solution [Et_2O/H_2O (each 2 mL)]. The titled compound (59 mg, 84%) was obtained as a pale yellow oil: ^{1}H NMR (300 MHz, D_2O) δ 7.25-7.12 (m, 5 H), 5.10 (s, 2 H), 4.30 (t, J = 6.5 Hz, 1 H), 2.32 (d, J = 6.5 Hz, 2 H); ^{13}C NMR (75 MHz, D_2O) δ 205.3, 201.4, 180.5, 174.4, 157.6, 136.2, 128.9, 128.5, 127.9, 67.4, 51.8, 27.2.

(*S*)-3,4-Dioxo-2-hydroxy-α-phenylmethyloxycarbonylamino-1-cyclobutene-1-butyric acid (16b).

According to the general procedure, **3** (106 mg, 0.530 mmol) was treated with CbzCl (354 mg, 2.07 mmol) and $NaHCO_3$ (333 mg, 4.00 mmol) in a biphasic solution [Et_2O/H_2O (each 5 mL)]. The titled compound (112 mg, 66%) was obtained as a pale yellow oil: ^{1}H NMR (300 MHz, D_2O) δ 7.48-7.12 (m, 5 H), 5.10 (s, 2 H), 4.21 (dd, J = 9.5, 4.6 Hz, 1 H), 2.65 (t, J = 7.5 Hz, 2 H), 2.32-2.20 (m, 1 H), 2.12-1.96 (m, 1 H); ^{13}C NMR (75 MHz, D_2O) δ 209.1, 203.3, 187.5, 175.8, 158.1, 136.4, 129.0, 128.6, 127.9, 67.4, 54.1, 27.4, 21.0.

(*S*)-3,4-Dioxo-2-hydroxy-α-(*N*-phenylmethyloxycarbonyl-D-phenylalanylamino)-1-cyclobutene-1-propionic acid (17a).

According to the general procedure, **2** (51 mg, 0.231 mmol) was treated with Cbz-D-Phe-OSu (357 mg, 0.901 mmol) and $NaHCO_3$ (147 mg, 1.73 mmol) in a biphasic solution [Et_2O/H_2O (each 2 mL)]. The titled compound (82 mg, 85%) was obtained as a pale yellow oil: ^{1}H NMR (300 MHz, D_2O) δ 7.24-7.09 (m, 10 H), 4.90 (s, 2 H), 4.74-4.68 (m, 1 H), 4.20

(t, J = 7.5 Hz, 1 H), 2.89-2.84 (m, 3 H), 2.58-2.48 (m, 1 H); ^{13}C NMR (75 MHz, D_2O) δ 210.9, 202.6, 182.6, 174.8, 174.2, 158.2, 137.04, 137.01, 130.1, 129.8, 129.6, 129.3, 128.6, 128.3, 67.8, 58.4, 50.2, 38.3, 27.8.

(*S*)-3,4-Dioxo-2-hydroxy-α-((*N*-phenylmethyloxycarbonyl-D-phenylalanyl)-amino)-1-cyclobutene-1-butyric acid (17b).

According to the general procedure, **3** (22 mg, 0.112 mmol) was treated with Cbz-D-Phe-OSu (174 mg, 0.437 mmol) and $NaHCO_3$ (72 mg, 0.84 mmol) in a biphasic solution [Et_2O/H_2O (each 1 mL)]. The titled compound (27 mg, 50%) was obtained as a pale yellow oil: ^{1}H NMR (300 MHz, D_2O) δ 7.29-7.11 (m, 10 H), 4.93 (s, 2 H), 4.30 (t, J = 8.0 Hz, 1 H), 4.24-4.18 (m, 1 H), 2.92-2.87 (m, 2 H), 2.20-1.75 (m, 4 H).

Dipeptide formation of 2.

To a mixture of **2** (116 mg, 0.348 mmol) and L-Val-OMe•HCl (128 mg, 0.766 mmol) in DMF (5 mL) was added EDCI•HCl (167 mg, 0.87 mmol) and Et_3N (110 μL, 0.766 mmol) at 0 °C. The mixture was warmed to room temperature, stirred for 12 h at room temperature, acidified with 1 M HCl, and extracted with EtOAc. The organic phase was washed with saturated $NaHCO_3$ followed by brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (EtOAc/ hexane = 2/1-4/1) to give **18** (13 mg, 7%) as a pale yellow oil.: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40-7.23 (m, 5 H), 6.25 (d, J = 7.5 Hz, 1 H), 5.17 (dd, J = 11.3 Hz, 1 H), 5.12 (dd, J = 11.3 Hz, 1 H), 4.82 (dd, J = 9.4, 4.7 Hz, 1 H), 4.68-4.61 (m, 1 H), 4.40 (dd, J = 8.4, 4.7 Hz, 1 H), 3.80 (s, 1 H), 3.70 (s, 1 H), 3.15 (dd, J = 14.1, 4.7 Hz, 1 H), 2.98 (dd, J = 14.1, 5.9 Hz, 1 H), 2.29-2.05 (m, 2 H), 0.93 (d, J = 6.6 Hz, 3 H), 0.91 (d, J = 6.6 Hz, 3 H), 0.81 (d, J = 6.6 Hz, 3 H), 0.79 (d, J = 6.6 Hz, 3 H).

Methyl (2'*S*)-*N*-(3-(3,4-Dioxo-2-(1-methylethyloxy)-1-cyclobutenyl)-1-oxo-2-phenyl methyloxycarbonylpropyl)-L-valinate (19).

To a mixture of **11b** (45 mg, 0.125 mmol) and L-Val-OMe•HCl (23 mg, 0.138 mmol)

in CH_2Cl_2 (5 mL) was added EDCI•HCl (26 mg, 0.138 mmol) and Et_3N (20 μL, 0.138 mmol) at 0 °C. The mixture was warmed to room temperature, stirred for 12 h, acidified with 1 M HCl, and extracted with EtOAc. The organic phase was washed with saturated $NaHCO_3$ followed by brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (EtOAc/ hexane = 1/1) to give **19** (24 mg, 40%) as a pale yellow oil.: ^{1}H NMR (300 MHz, CD_3OD) δ 7.35-7.27 (m, 5 H), 5.35 (sept, J = 6.3 Hz, 1 H), 5.09 (s, 2 H), 4.68 (dd, J = 8.1, 6.1 Hz, 1 H), 4.32 (d, J = 5.6 Hz, 1 H), 3.69 (s, 3 H), 3.05 (dd, J = 15.9, 6.1 Hz, 1 H), 2.97 (dd, J = 15.9, 8.1 Hz, 1 H), 2.17-2.09 (m, 1 H), 1.41 (d, J = 6.3 Hz, 3 H), 1.39 (d, J = 6.3 Hz, 3 H), 0.91 (d, J = 6.6 Hz, 3 H), 0.90 (d, J = 6.6 Hz, 3 H); ^{13}C NMR (75 MHz, CD_3OD) δ 199.9, 197.0, 194.4, 179.1, 173.2, 172.8, 158.0, 138.0, 129.5, 129.1, 128.9, 81.0, 67.8, 59.2, 53.0, 52.6, 31.8, 28.6, 22.9, 19.4, 18.3.

Methyl (2'*S*)-*N*-(3-(3,4-dioxo-2-hydroxy-1-cyclobutenyl)-1-oxo-2-phenylmethyloxy carbonylpropyl)-L-valinate (20).

To a solution of **19** (79 mg, 0.166 mmol) in acetone (1 mL) was added 1 M HCl (1 mL). The mixture was stirred for 4 h and concentrated *in vacuo*. The residue was purified by flash chromatography on Cosmosil® (H_2O-MeOH) to give **20** (63 mg, 88%) as a pale yellow oil.: ^{1}H NMR (300 MHz, CD_3OD) δ 7.31-7.26 (m, 5 H), 5.07 (s, 2 H), 4.66 (t, J = 6.7 Hz, 1 H), 4.31 (d, J = 5.5 Hz, 1 H), 3.66 (s, 3 H), 3.06 (d, J = 6.7 Hz, 2 H), 2.14-2.08 (m, 1 H), 0.89 (d, J = 5.6 Hz, 6 H).

2. Titration experiments of 2 and 3.

To a solution of an amino acid (**2** or **3**, ca. 5 mg) in D_2O (0.4 mL) was added TSP as an internal standard. The pD value of the solution was adjusted by adding a small amount of DCl (20% solution in D_2O) and/or NaOD (40% in D_2O) through a capillary tube, and recorded by glass electrode pH/ion meter (Iwaki Glass, M-225). The pD dependence of the chemical shift values of **2** or **3** (^{1}H-NMR, 400 MHz) was measured at 20 points in the range of pD 0.5 to 12.5. These results were shown in Figures 1 and 2.

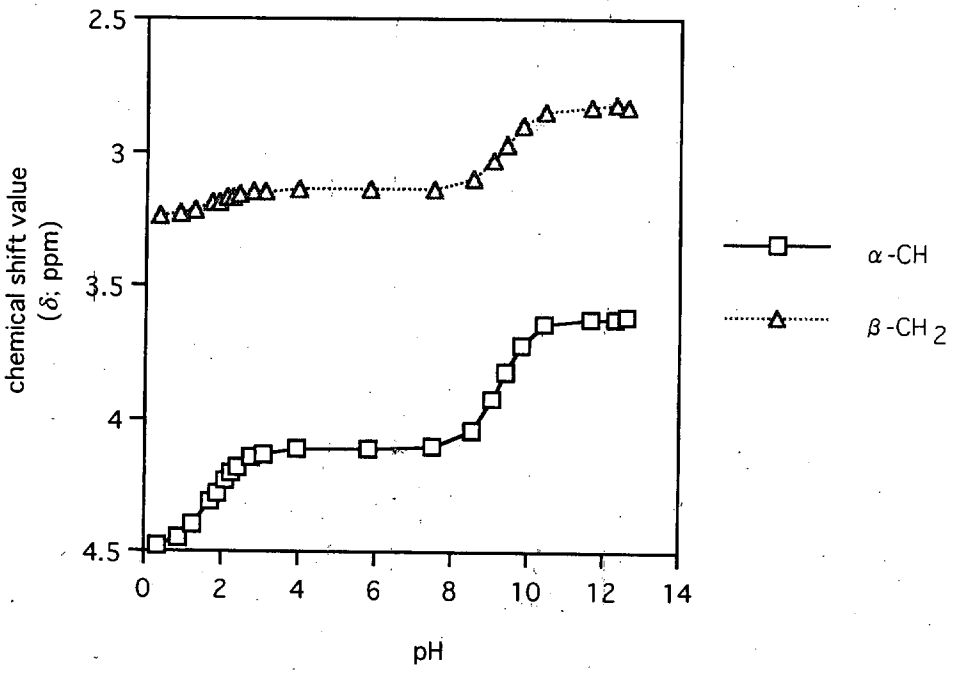

Figure 1. Titration graph of **2**

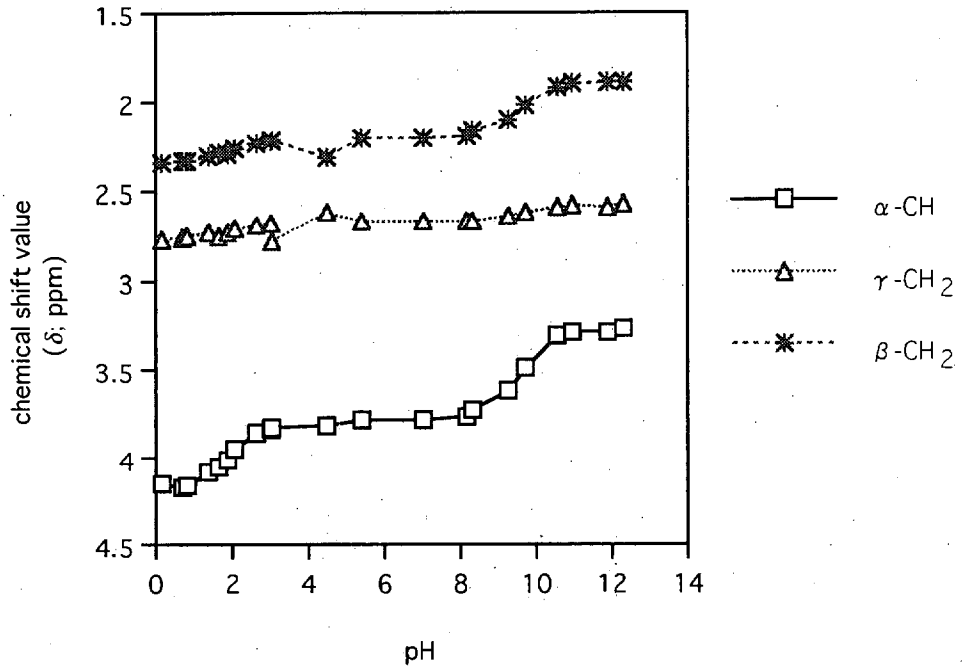

Figure 2. Titration graph of **3**