Supplementary Information

Three-Dimensional Structure-Activity Relationship Study of Belactosin A and its Stereo- and Regioisomers: Development of Potent Proteasome Inhibitors by the Stereochemical Diversity-Oriented Strategy

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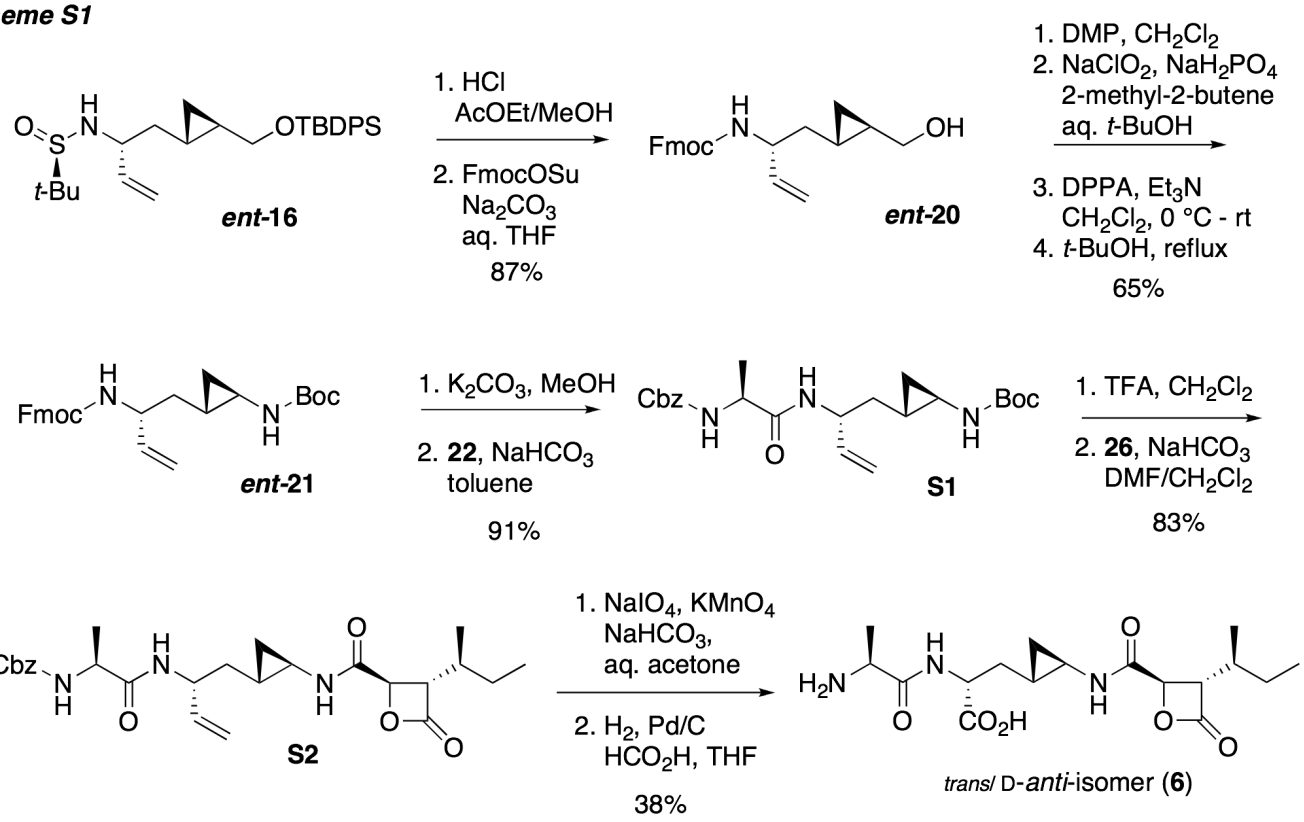
Contents: Experimental details for the synthesis of the stereoisomers **6–9** (S2–S8), ^{1}H NMR charts (S9–S17), ^{13}C NMR charts (S18–S22), and HPLC charts and data (S23–S27) of the synthesized belactosin A (**2**), the stereo- and regioisomers **6**–**10**, and the precursors **11–13**).

Experimental

Scheme S1



(1*S*,2*R*)-1-[(2*R*)-2-(9-Fluorenylmethoxycarbonylamino)-3-butenyl]-2-hydroxymethylcyclopropane (*ent*-20). Yield 87%; $[\alpha]^{22}_{D}$ –14.55 (*c* 0.95, MeOH); HRMS (EI) calcd for $C_{23}H_{25}NO_3$: 363.1834 (M^+), found 363.1832 (M^+).

(1*S*,2*R*)-2-*tert*-Butoxycarbonylamino-1-[(2*R*)-2-(9-fluorenylmethoxycarbonylamino)-3-butenyl]cyclopropane (*ent*-21). Yield 65%; $[\alpha]^{23}_{D}$ +14.75 (*c* 1.05, $CHCl_3$); HRMS (EI) calcd for $C_{27}H_{32}N_2O_4$: 448.2362 (M^+), found 448.2360 (M^+).

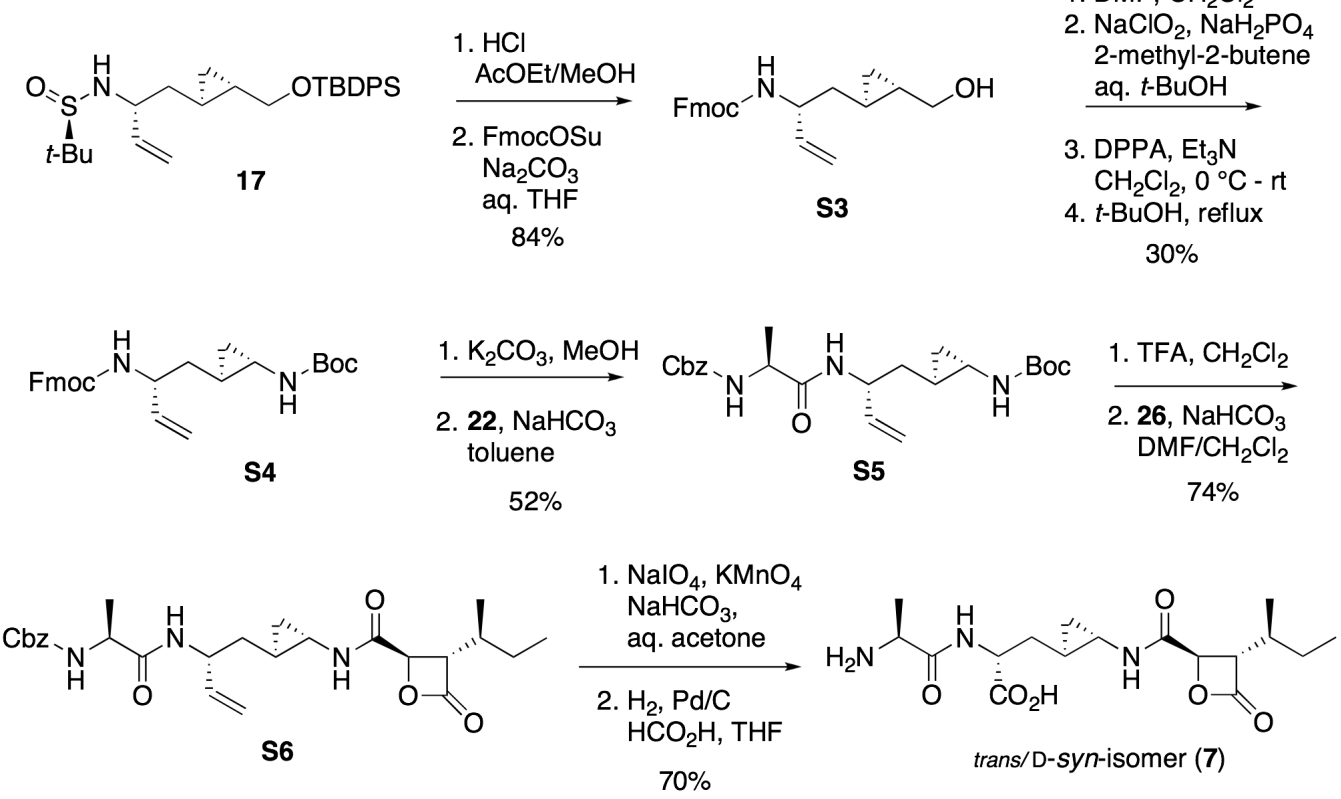
(1*S*,2*R*)-2-*tert*-Butoxycarbonylamino-1-[(2*R*)-2-(*N*-Cbz-L-alanyl)amino-3-butenyl]cyclopropane (S1). Compound **S1** (256 mg, 91%, white amorphous solid) was prepared from ***ent*-21** (290 mg, 0.65 mmol) as described for the synthesis of **23**: $[\alpha]^{22}_{D}$ +26.87 (*c* 1.02, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.41 (1 H, m), 0.55 (1 H, m), 0.91 (1 H, m), 1.31 (1 H, m), 1.42-1.43 (12 H, m), 2.07 (2 H, m), 4.46 (1 H, t, *J* = 7.2 Hz), 4.79 (2 H, br s), 5.08-5.21 (4 H, m), 5.84-5.89 (2 H, m), 7.25-7.35 (5 H, m), 9.08 (1 H, d, *J* = 9.2 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 10.52, 16.25, 19.98, 28.36, 30.16, 36.12, 49.91, 50.86, 66.50, 80.54, 114.54, 127.88, 128.00, 128.33, 136.61, 137.40, 155.40, 157.89, 172.09; HRMS (EI) calcd for $C_{23}H_{33}N_3O_5$: 431.2420 (M^+), found 431.2422 (M^+).

Compound S2. Compound **S2** (94 mg, 83%, white amorphous solid) was prepared from **S1** (100 mg, 231 μmol) as described for the synthesis of **11**: $[\alpha]^{18}_{D}$ +55.36 (*c* 1.02, $CHCl_3$); ^{1}H-NMR (500 MHz, DMSO-d_6) δ –0.29 (1 H, dd, *J* = 5.1, 12.6 Hz), –0.15-0,09 (2 H, br s), –0.01 (3 H, t, *J* = 7.4 Hz), 0.08 (3 H, d, *J* = 6.8 Hz), 0.32-0.37 (4 H, m), 0.49 (1 H, m), 0.63-0.71 (2 H, m), 1.05 (1 H, m), 1.56 (1 H, m),

2.81 (1 H, dd, J = 4.0, 7.4 Hz), 3.23 (1 H, m), 3.58 (1 H, m), 3.91 (1 H, m), 4.10-4.32(4 H, m), 5.01 (1 H, m), 6.46-6.53 (6 H, m), 7.41 (1 H, d, J = 8,6 Hz), 7.78 (1 H, d, J = 3.4 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 10.16, 10.99, 15.95, 16.15, 19.60, 26.62, 29.33, 33.56, 36.02, 49.63, 51.18, 62.96, 66.73, 70.08, 114.80, 128.13, 128.47, 128.58, 136.24, 137.17, 155.31, 168.52, 171.01, 172.41; HRMS (EI) calcd for $C_{26}H_{35}N_3O_6$: 485.2525 (M^+), found 485.2527 (M^+).

***Trans*/D-*Anti*-Isomer (6).** Compound **6** (11 mg, 38%, white amorphous solid) was prepared from **S2** (40 mg, 82 μmol) as described for the synthesis of **2**: $[\alpha]^{17}_D$ +3.80 (*c* 0.82, H_2O); ^{1}H-NMR (500 MHz, D_2O) δ 0.64 (1 H, m), 0.76 (1 H, m), 0.80-0.84 (4 H, m), 0.93 (3 H, d), 1.26 (1 H, m), 1.42 (1 H, m), 1.46 (3 H, d, J = 6.8 Hz), 1.52 (1 H, m), 1.86-1.98 (2 H, m), 2.43 (1 H, m), 3.79 (1 H, dd, J = 4.6, 7.4 Hz), 4.10 (1 H, dd, J = 6.8, 14.3 Hz), 4.32 (1 H, t, J = 5.4 Hz), 4.81 (1 H, d, J = 4.0); ^{13}C-NMR (125 MHz, D_2O) δ 10.86, 11.70, 15.95, 16.59, 17.10, 26.80, 28.86, 33.23, 34.40, 49.79, 55.38, 62.45, 71.26, 170.63, 172.61, 173.20, 178.69; LRMS (FAB) *m/z* 370 [(M+H)$^+$]; HRMS (FAB) calcd for $C_{17}H_{27}N_3O_6$: 370.1978 [(M+H)$^+$], found 370.1976 [(M+H)$^+$].

Scheme S2



(1*R*,2*S*)-1-[(2*R*)-2-(9-Fluorenylmethoxycarbonylamino)-3-butenyl]-2-hydroxymethylcyclopropane (S3). Compound **S3** (182 mg, 84%, white amorphous solid) was prepared from **17** (280 mg, 590 μmol) as described for the synthesis of **20**: $[\alpha]^{21}_D$ –11.65 (*c* 1.04, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.35 (1 H, m), 0.42 (1 H, m), 0.66 (1 H, br s), 0.91 (1 H, br s), 1.35 (1 H, m), 1.56 (1 H, br s), 1.64 (1 H, br s), 3.32 (1 H, br s), 3.52 (1 H, br s), 4.22-4.30 (2 H, m), 4.46 (2 H, m), 4.81 (1 H, br s), 5.15 (2 H, m),

5.83 (1 H, br s), 7.30-7.33 (2 H, m), 7.38-7.42 (2 H, m), 7.52-7.61 (2 H, m), 7.76-7.78 (2 H, m); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 9.63, 13.89, 21.08, 39.01, 47.25, 47.34, 53.73, 66.54, 66.70, 114.68, 119.86, 124.90, 126.93, 127.57, 138.44, 141.23, 143.84, 155.96; HRMS (EI) calcd for $C_{23}H_{25}NO_3$: 363.1834 (M^+), found 363.1834 (M^+).

(1*R*,2*S*)-2-*tert*-Butoxycarbonylamino-1-[(2*R*)-2-(9-fluorenylmethoxycarbonylamino)-3-butenyl]cyclopropane (S4). Compound **S4** (86 mg, 30%, white amorphous solid) was prepared from **S3** (230 mg, 632 μmol) as described for the synthesis of **21**: $[\alpha]^{21}_D$ –3.42 (*c* 0.95, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.50 (1 H, dd, *J* = 6.1, 12.3 Hz), 0.67 (1 H, m), 0.92 (1 H, br s), 1.41-1.47 (10 H, m), 1.78 (1 H, br s), 2.29 (1 H, br s), 4.10-4.39 (4 H, m), 4.79 (1 H, br s), 5.09 (1 H, d, *J* = 10.3 Hz), 5.17 (1 H, d, *J* = 17.1 Hz), 5.86 (2 H, br s), 7.27-7.31 (2 H, m), 7.36-7.40 (2 H, m), 7.60-7.64 (2 H, m), 7.74-7.76 (2 H, m); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 13.40, 17.58, 28.39, 29.19, 37.37, 47.37, 53.35, 66.57, 79.50, 114.57, 119.82, 124.99, 126.92, 127.51, 137.79, 141,19, 143.91, 144.01, 155.96, 156.46; HRMS (EI) calcd for $C_{27}H_{32}N_2O_4$: 448.2362 (M^+), found 448.2366 (M^+).

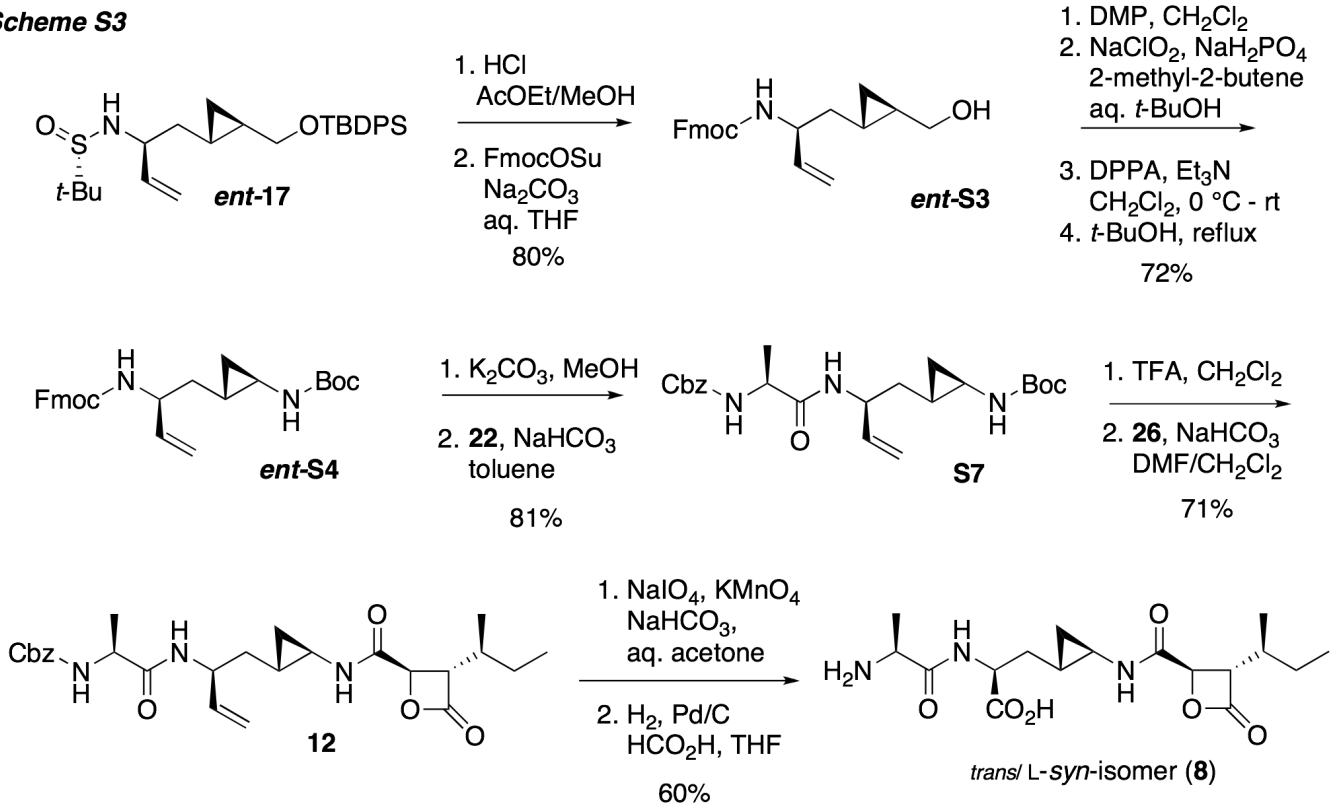
(1*R*,2*S*)-2-*tert*-Butoxycarbonylamino-1-[(2*R*)-2-(*N*-Cbz-L-alanyl)amino-3-butenyl]cyclopropane (S5). Compound **S5** (39 mg, 52%, white amorphous solid) was prepared from **S4** (78 mg, 173 μmol) as described for the synthesis of **23**: $[\alpha]^{22}_D$ –29.83 (*c* 1.03, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.47 (1 H, m), 0.62 (1 H, m), 0.92 (1 H, m), 1.07 (1 H, br s), 1.42-1.45 (12 H, m), 1.98 (1 H, br s), 2.31 (1 H, br s), 4.38 (1 H, br s), 4.56 (1 H, br s), 4.93 (1 H, br s), 5.03-5.15 (4 H, m), 5.82 (1 H, m), 5.97 (1 H, br s), 7.29-7.34 (5 H, m), 7.53 (1 H, br s); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 13.17, 17.39, 19.33, 28.39, 28.80, 36.44, 50.57, 51.35, 66.72, 79.80, 114.48, 127.89, 127.94, 128.36, 136.29, 138.83, 156.64, 156.81, 171.86; LRMS (EI) *m/z* 431 (M^+); HRMS (EI) calcd for $C_{23}H_{33}N_3O_5$: 431.2420 (M^+), found 431.2422 (M^+).

Compound S6. Compound **S6** (32 mg, 74%, white amorphous solid) was prepared from **S5** (38 mg, 89 μmol) as described for the synthesis of **11**: $[\alpha]^{25}_D$ +10.33 (*c* 1.00, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.56 (1 H, m), 0.73 (1 H, m), 0.85 (1 H, br s), 0.93 (3 H, t, *J* = 7.3 Hz), 1.00-1.06 (4 H, m), 1.33 (1 H, m), 1.46 (3 H, d, *J* = 6.7 Hz), 1.62 (1 H, m), 1.97 (1 H, m), 2.09 (1 H, d, *J* = 14.5), 2.41 (1 H, br s), 3.49 (1 H, dd, *J* = 4.7, 7.3 Hz), 4.40 (1 H, m), 4.63 (1 H, d, *J* = 4.7 Hz), 4.85 (1 H, br s), 5.08-5.17 (4 H, m), 5.74 (1 H, d, *J* = 7.7 Hz), 5.82 (1 H, m), 6.65 (1 H, s), 7.29-7.34 (5 H, m), 9.02 (1 H, d, *J* = 8.8 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 10.14, 11.15, 16.40, 16.41, 20.06, 26.86, 29.54, 33.75, 36.01, 49.58, 50.57, 62.98, 66.56, 70.10, 114.31, 127.77, 127.82, 128.29, 136.50, 137.44, 155.44, 168.49, 170.78, 171.85; HRMS (EI) calcd for $C_{26}H_{35}N_3O_6$: 485.2525 (M^+), found 485.2525 (M^+).

***Trans*/D-*Syn*-Isomer (7).** Compound **7** (17 mg, 70%, white amorphous solid) was prepared from **S6** (32 mg, 65 μmol) as described for the synthesis of **2**: $[\alpha]^{18}_D$ +22.51 (*c* 0.82, H_2O); ^{1}H-NMR (500 MHz, D_2O) δ 0.66 (1 H, dd, *J* = 6.3, 13.2 Hz), 0.72 (1 H, m), 0.80-0.83 (4 H, m), 0.93 (3 H, d, *J* = 6.8 Hz), 1.24 (1 H, m), 1.42-1.47 (4 H, m), 1.63 (1 H, m), 1.79 (1 H, m), 1.97 (1 H, m), 2.48 (1 H, m), 3.75 (1 H, dd, *J* = 4.6, 7.4 Hz), 4.05 (1 H, dd, *J* = 6.8, 14.3 Hz), 4.19 (1 H, dd, *J* = 5.1, 8.6 Hz, 4.79 (1 H, d, *J* = 4.0); ^{13}C-NMR (125 MHz, D_2O) δ 10.83, 12.74, 15.98, 16.76, 17.15, 26.79, 28.85, 33.30, 35.46, 49.77,

55.83, 62.42, 71.38, 170.83, 172.22, 173.16, 178.90; LRMS (FAB) *m/z* 370 [(M+H)$^+$]; HRMS (FAB) calcd for $C_{17}H_{27}N_3O_6$: 370.1978 [(M+H)$^+$], found 370.1972 [(M+H)$^+$].

Scheme S3



(1*S*,2*R*)-1-[(2*S*)-2-(9-Fluorenylmethoxycarbonylamino)-3-butenyl]-2-hydroxymethylcyclopropane (*ent*-S3). Yield 80%; $[\alpha]^{24}_D$ +11.91 (*c* 1.01, $CHCl_3$); HRMS (EI) calcd for $C_{23}H_{25}NO_3$: 363.1834 (M^+), found 363.1843 (M^+).

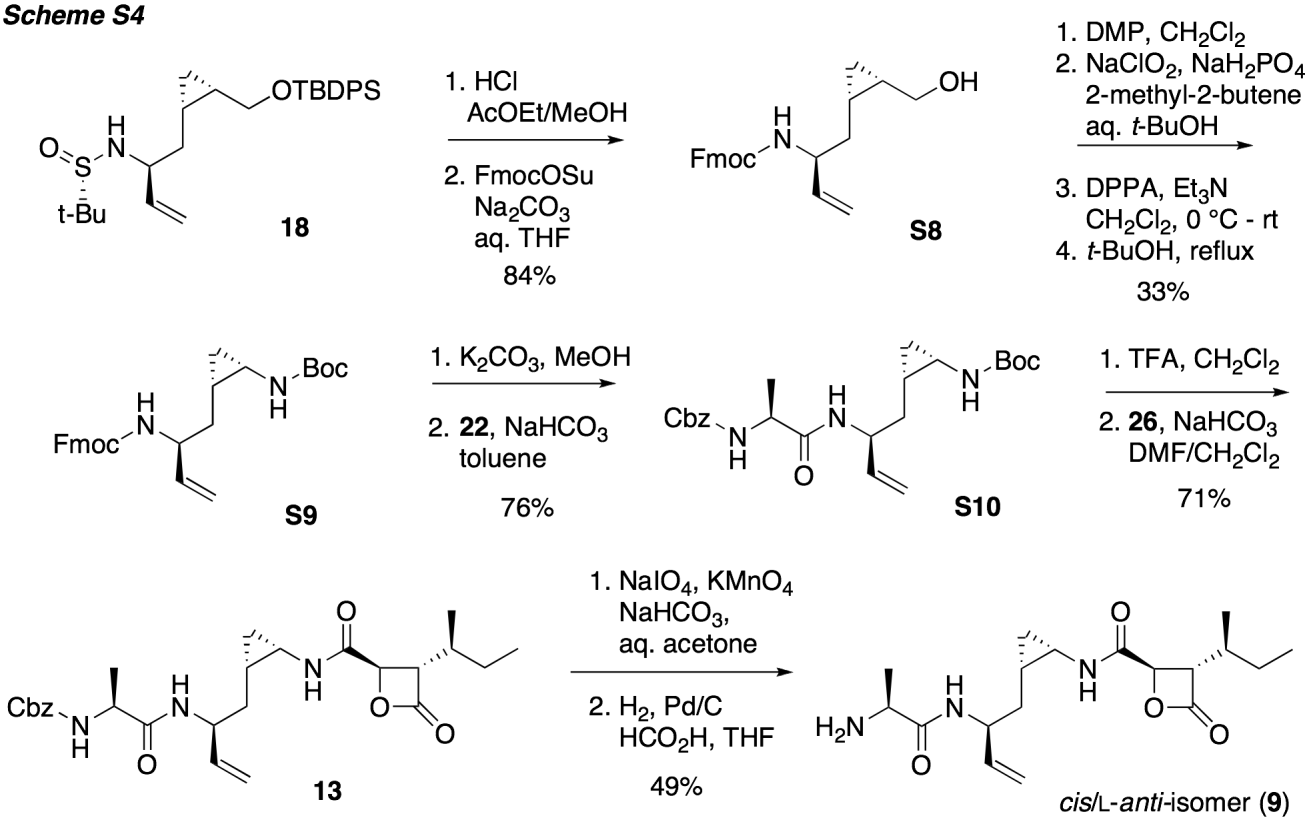
(1*S*,2*R*)-2-*tert*-Butoxycarbonylamino-1-[(2*S*)-2-(9-fluorenylmethoxycarbonylamino)-3-butenyl]cyclopropane (*ent*-S4). Yield 72%; $[\alpha]^{23}_D$ +3.44 (*c* 1.20, $CHCl_3$); HRMS (EI) calcd for $C_{27}H_{32}N_2O_4$: 448.2362 (M^+), found 448.2371 (M^+).

(1*S*,2*R*)-2-*tert*-Butoxycarbonylamino-1-[(2*S*)-2-(*N*-Cbz-L-alanyl)amino-3-butenyl]cyclopropane (S7). Compound **S7** (47 mg, 81%, white amorphous solid) was prepared from ***ent*-S4** (60 mg, 133 μmol) as described for the synthesis of **23**: $[\alpha]^{23}_D$ –9.76 (*c* 1.03, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.47 (1 H, dd, *J* = 5.7, 12.8 Hz), 0.62 (1 H, m), 0.92 (1 H, m), 1.07 (1 H, br s), 1.42-1.45 (12 H, m), 1.98 (1 H, br s), 2.31 (1 H, br s), 4.38 (1 H, br s), 4.56 (1 H, br s), 4.93 (1 H, br s), 5.03-5.15 (4 H, m), 5.82 (1 H, m), 5.97 (1 H, br s), 7.29-7.34 (5 H, m), 7.53 (1 H, br s); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 12.82, 17.50, 19.38, 28.47, 29.60, 36.70, 50.60, 51.23, 66.64, 79.88, 114.12, 127.78, 127.82, 128.26, 136.35, 138.83, 155.63, 156.78, 171.95; HRMS (EI) calcd for $C_{23}H_{33}N_3O_5$: 431.2420 (M^+), found 431.2424 (M^+).

Compound 12. Compound **12** (35 mg, 71%, white amorphous solid) was prepared from **S7** (46 mg, 100 μmol) as described for the synthesis of **11**: $[\alpha]^{23}_{D}$ +42.52 (*c* 1.00, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.61 (1 H, m), 0.74 (1 H, br s), 0.91-0.95 (4 H, m), 1.03 (3 H, d), 1.14 (1 H, m), 1.29 (1 H, m), 1.40 (3 H, d, *J* = 6.9 Hz), 1.63 (1 H, m), 1.94-1.95 (2 H, m), 2.49 (1 H, br s), 3.57 (1 H, m), 4.37 (1 H, br s), 4.57 (2 H, m), 5.05-5.15 (4 H, m), 5.80 (2 H, m), 6.73 (1 H, br s), 7.19 (1 H, d, *J* = 7.5 Hz), 7.29-7.34 (5 H, m); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 11.04, 12.16, 16.30, 17.21, 19.14, 26.67, 29.20, 33.78, 36.56, 50.40, 51.13, 62.73, 66.75, 70.53, 114.47, 127.89, 128.01, 128.40, 136.28, 138.36, 155.78, 168.87, 169.66, 172.21; HRMS (EI) calcd for $C_{26}H_{35}N_3O_6$: 485.2525 (M^+), found 485.2528 (M^+).

***Trans*/L-*syn*-Isomer (8).** Compound **8** (21 mg, 60%, white amorphous solid) was prepared from **12** (46 mg, 100 μmol) as described for the synthesis of **3**: $[\alpha]^{17}_{D}$ –24.46 (*c* 1.06, H_2O); ^{1}H-NMR (500 MHz, D_2O) δ 0.68 (1 H, dd, J = 6.0, 13.4 Hz), 0.75 (1 H, m), 0.83 (3 H, t, J = 7.4 Hz), 0.94 (3 H, d, *J* = 6.3 Hz), 0.97 (1 H, m), 1.25 (1 H, m), 1.46 (1 H, m), 1.50 (3 H, d, *J* = 7.4 Hz), 1.61 (1 H, m), 1.80 (1 H, m), 1.97 (1 H, m), 2.50 (1 H, m), 3.75 (1 H, dd, *J* = 3.6, 7.3 Hz), 4.07 (1 H, q, *J* = 6.8 Hz), 4.16 (1 H, dd, *J* = 5.7, 8.0 Hz), 4.81 (1 H, d, *J* = 4.6); ^{13}C-NMR (125 MHz, D_2O) δ 10.85, 12.56, 15.99), 16.82, 16.98, 26.81, 28.79, 33.33, 34.38, 49.63, 56.08, 62.45, 71.38, 170.63, 172.31, 173.15, 178.74; HRMS (FAB) calcd for $C_{17}H_{27}N_3O_6$: 370.1978 [$(M+H)^+$], found 370.1979 [$(M+H)^+$].

Scheme S4



(1*S*,2*S*)-1-[(2*S*)-2-(9-Fluorenylmethoxycarbonylamino)-3-butenyl]-2-hydroxymethylcyclopropane (S8). Compound **S8** (519 mg, 84%, white amorphous solid) was prepared from **18** (825 mg, 1.7

mmol) as described for the synthesis of **20**: $[\alpha]^{21}_{D}$ –1.34 (*c* 1.04, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ –0.06 (1 H, dd, *J* = 5.2, 10.3 Hz), 0.70 (1 H, m), 0.82 (1 H, m), 1.13 (1 H, m), 1.38 (1 H, m), 1.78 (1 H, m), 2.05 (1 H, br s), 3.26 (1 H, m), 3.96 (1 H, m), 4.20-4.24 (2 H, m), 4.40-4.51 (1 H, m), 5.11 (1 H, d, *J* = 10.3 Hz), 5.21 (1 H, d, *J* = 17.1 Hz), 5.79-5.81 (2 H, m), 7.29-7.33 (2 H, m), 7.38-7.42 (2 H, m), 7.60-7.62 (2 H, m), 7.76-7.77 (2 H, m); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 8.09, 13.09, 17.82, 24.19, 33.11, 47.35, 54.30, 62.35, 66.16, 114.71, 119.79, 124.96, 126.88, 127.48, 138.41, 141.18, 143.85, 156.20; HRMS (EI) calcd for $C_{23}H_{25}NO_3$: 363.1834 (M^+), found 363.1832 (M^+).

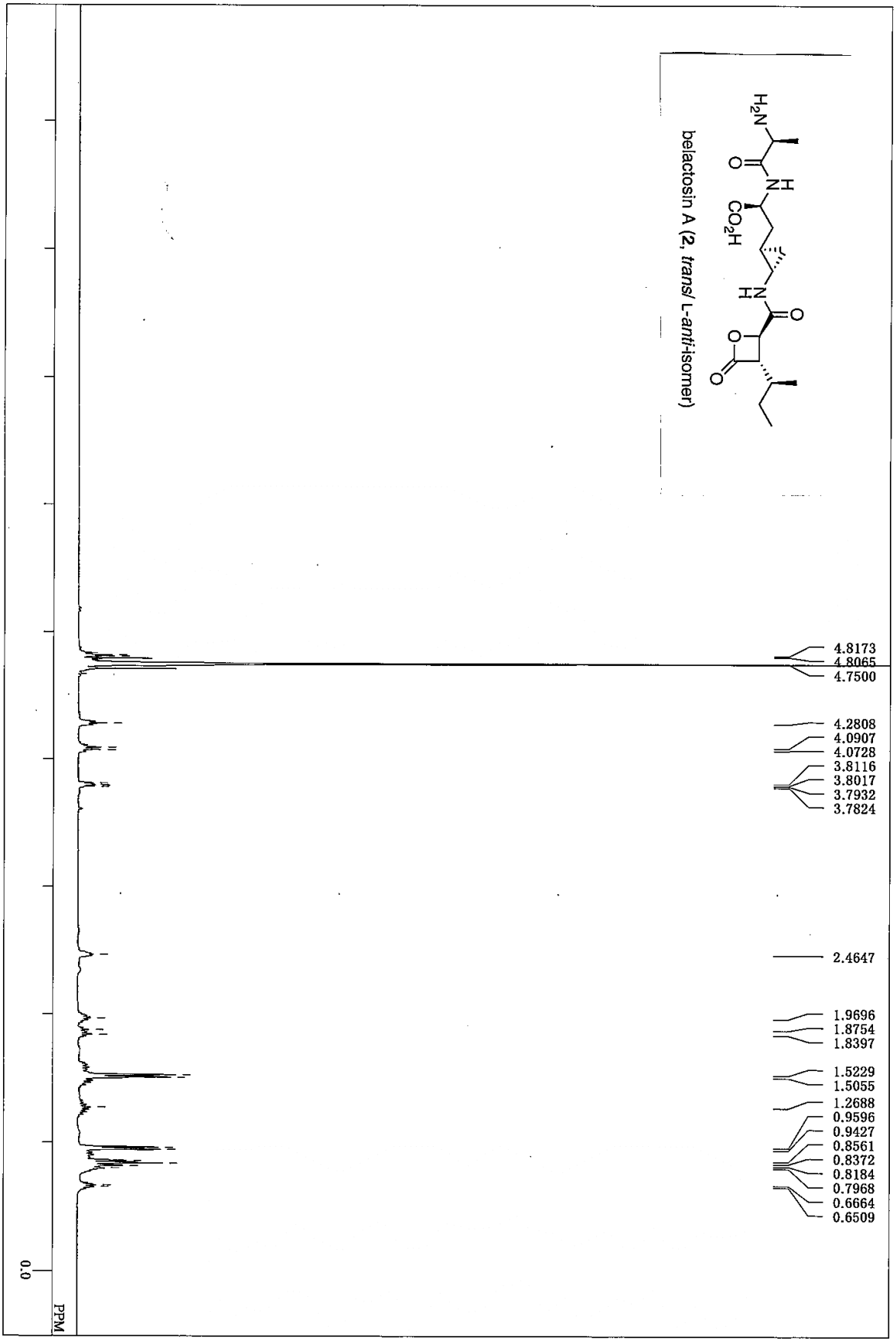
(1*S*,2*S*)-2-*tert*-Butoxycarbonylamino-1-[(2*S*)-2-(9-fluorenylmethoxycarbonylamino)-3-butenyl]cyclopropane (S9). Compound **S9** (373 mg, 33%, white amorphous solid) was prepared from **S8** (909 mg, 2.5 mmol) as described for the synthesis of **21**: $[\alpha]^{23}_{D}$ +14.75 (*c* 1.05, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.11 (1 H, dd, *J* = 4.9, 9.9 Hz), 0.90 (2 H, m), 1.26 (1 H, br s), 1.49 (9 H, s), 1.62 (1 H, br s), 2.65 (1 H, br s), 4.23-4.45 (4H, m), 4.80 (1 H, br s), 5.07 (1 H, d, *J* = 10.3 Hz), 5.18 (1 H, d, *J* = 17.3 Hz), 5.79 (1 H, m), 6.26 (1 H, br s), 7.23-7.31 (2 H, m), 7.36-7.40 (2 H, m), 7.68-7.76 (4 H, m); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 11.46, 14.89, 27.58, 28.38, 31.56, 47.42, 53.45, 66.42, 79.88, 114.16, 119.80, 125.24, 126.90, 127.49, 137.96, 141.23, 144.04, 144.12, 156.27, 157.10; HRMS (EI) calcd for $C_{27}H_{32}N_2O_4$: 448.2362 (M^+), found 448.2352 (M^+).

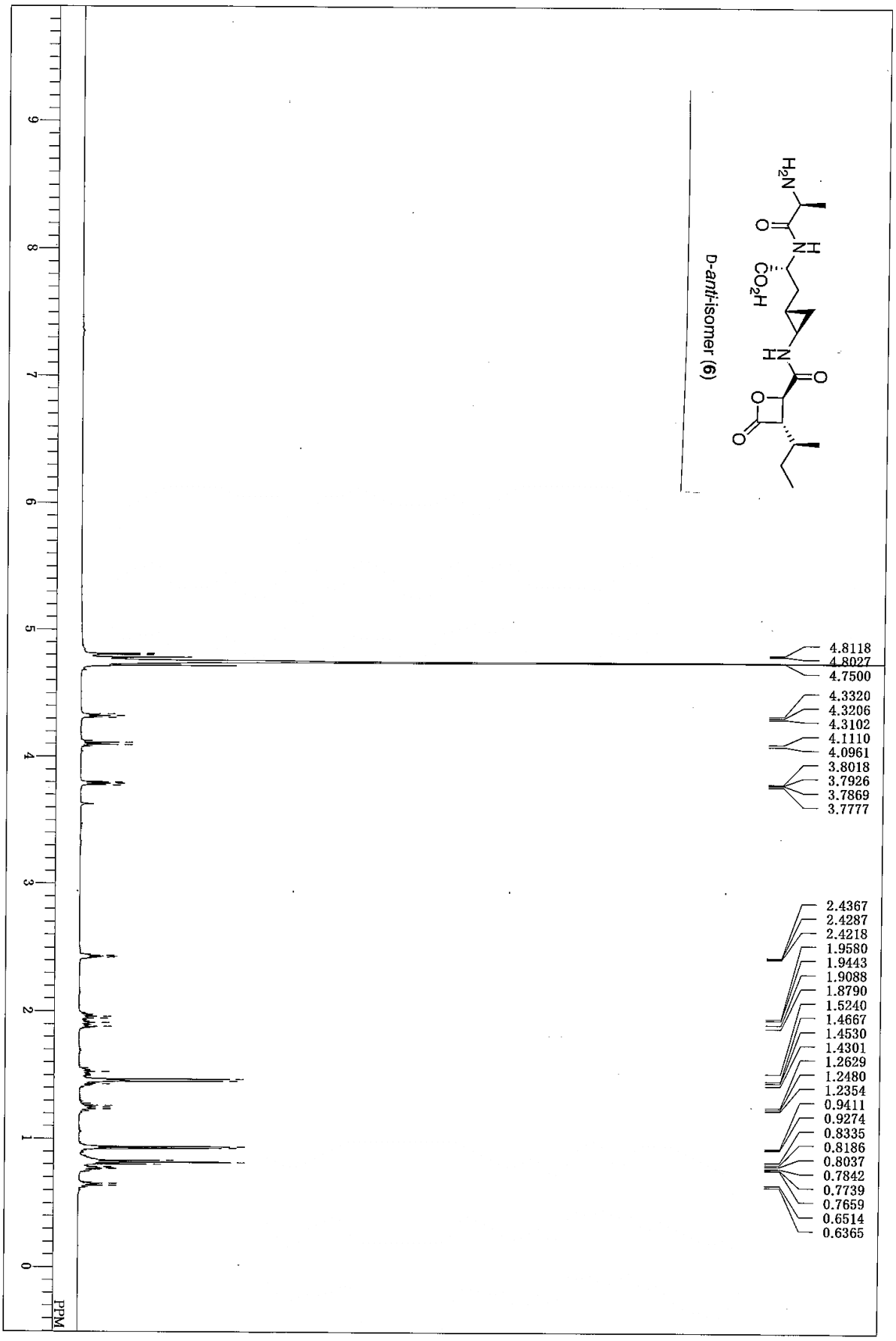
(1*S*,2*S*)-2-*tert*-Butoxycarbonylamino-1-[(2*S*)-2-(*N*-Cbz-L-alanyl)amino-3-butenyl]cyclopropan e (S10). Compound **S10** (269 mg, 76%, white amorphous solid) was prepared from **S9** (370 mg, 0.82 mmol) as described for the synthesis of **23**: $[\alpha]^{23}_{D}$ –20.67 (*c* 1.04, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.41 (1 H, m), 0.55 (1 H, m), 0.91 (1 H, m), 1.31 (1 H, m), 1.42-1.43 (12 H, m), 2.07 (2 H, m), 4.46 (1 H, t, *J* = 7.2 Hz), 4.79 (2 H, br s), 5.08-5.21 (4 H, m), 5.84-5.89 (2 H, m), 7.25-7.35 (5 H, m), 9.08 (1 H, d, *J* = 9.2 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 10.71, 15.39, 19.96, 27.93, 28.33, 30.42, 50.66, 51.16, 66.66, 80.30, 113.50, 127.83, 127.95, 128.25, 136.39, 138.38, 155.47, 157.20, 173.33; HRMS (EI) calcd for $C_{23}H_{33}N_3O_5$: 431.2420 (M^+), found 431.2411 (M^+).

Compound 13. Compound **13** (24 mg, 71%, white amorphous solid) was prepared from **S15** (29 mg, 69 μmol) as described for the synthesis of **11**: $[\alpha]^{24}_{D}$ –0.52 (*c* 1.01, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.26 (1 H, dd, *J* = 5.7, 10.5 Hz), 0.88-1.04 (8 H, m), 1.18 (1 H, m), 1.29 (1 H, m), 1.41 (3 H, d, *J* = 6.9 Hz), 1.62 (1 H, m), 1.84 (1 H, m), 1.96 (1 H, m), 2.80 (1 H, br s), 3.62 (1 H, dd, *J* = 4.5, 7.7 Hz), 4.34-4.43 (2 H, m), 4.65 (1 H, d, *J* = 4.5 Hz), 5.05-5.18 (4 H, m), 5.66 (1 H, br s, N<u>H</u>), 5.77 (1 H, m), 6.99 (2 H, br s), 7.29-7.36 (5 H, m); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 11.13, 11.47, 14.56, 16.44, 19.20, 26.80, 27.08, 31.97, 33.71, 50.62, 51.62, 63.00, 66.82, 70.73, 115.11, 127.79, 127.86, 128.32, 136.26, 137.27, 155.68, 168.96, 170.10, 172.07; HRMS (EI) calcd for $C_{26}H_{35}N_3O_6$: 485.2525 (M^+), found 485.2529 (M^+).

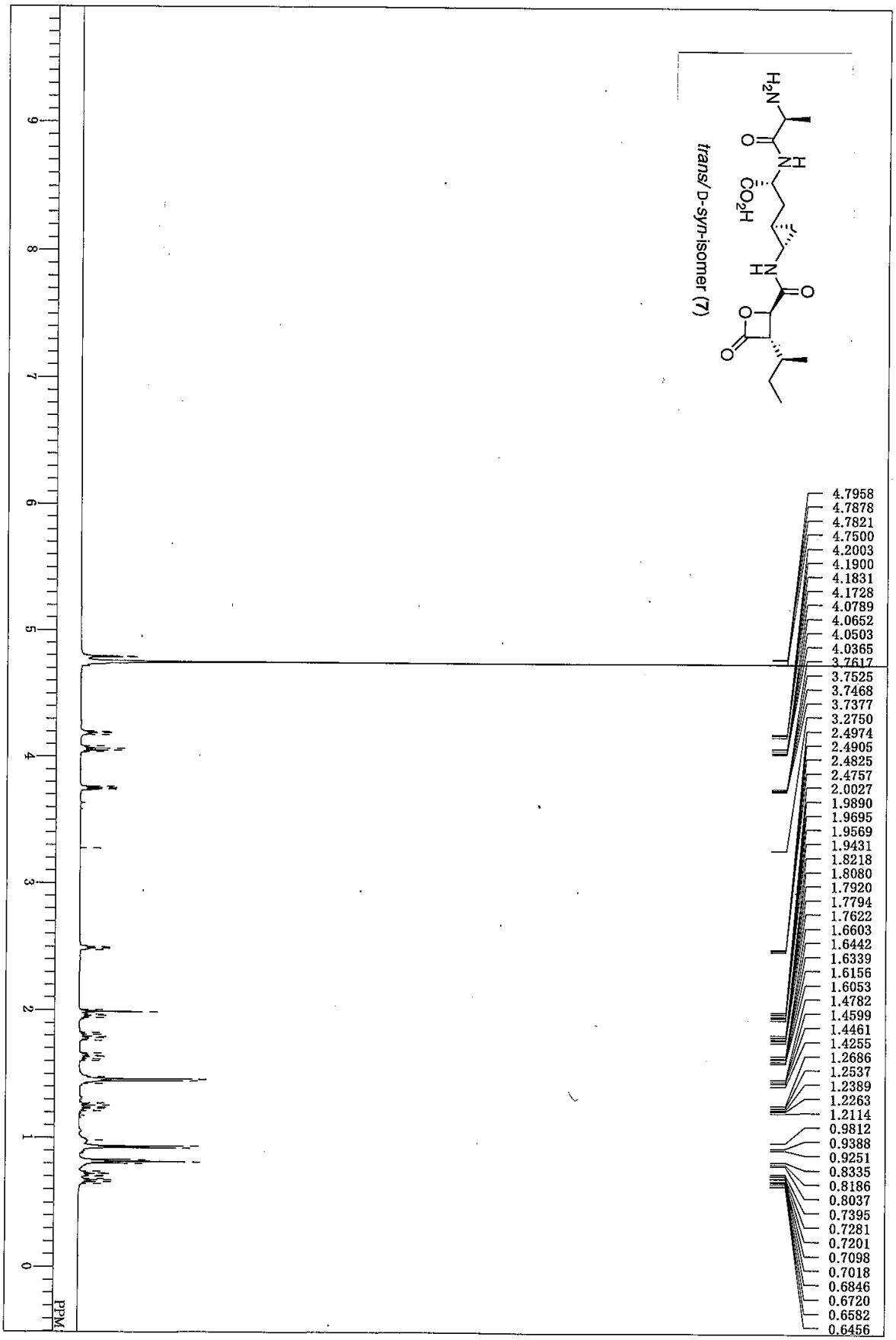
***Cis*/L-*anti*-Isomer (9).** Compound **9** (20 mg, 49%, white amorphous solid) was prepared from **13** (55 mg, 113 μmol) as described for the synthesis of **2**: $[\alpha]^{20}_{D}$ –149.21 (*c* 1.02, H_2O); ^{1}H-NMR (500 MHz, D_2O) δ 0.40 (1 H, dd, *J* = 5.7, 10.8 Hz), 0.82 (3 H, t, *J* = 7.4 Hz), 0.94 (3 H, d, *J* = 6.8 Hz), 0.98-1.06 (2 H, m), 1.25 (1 H, m), 1.43-1.52 (5 H, m), 1.80 (1 H, m), 1.97 (1 H, m), 2.68 (1 H, m), 3.79 (1 H, dd, *J* =

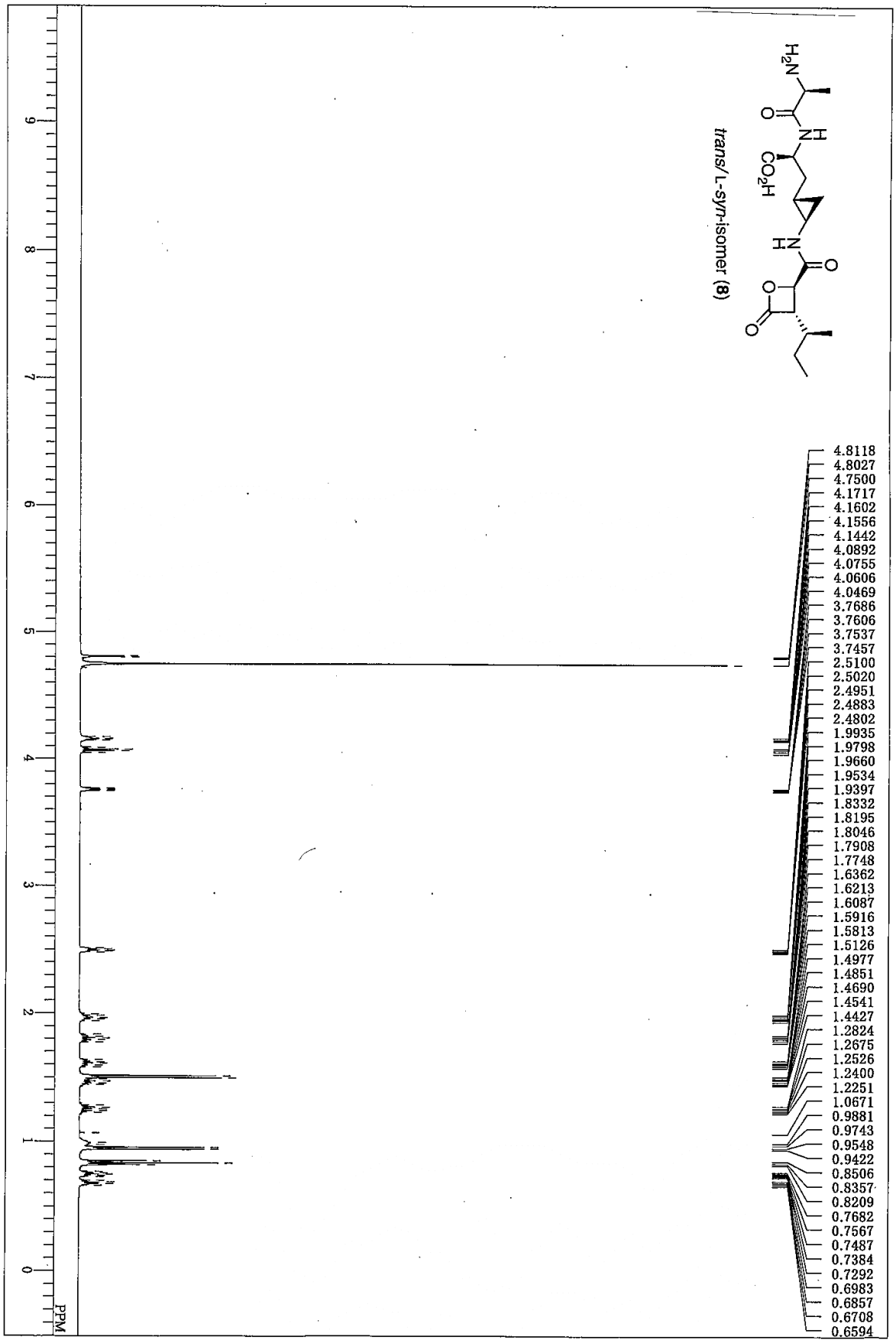
4.0, 7.4 Hz), 4.03 (1 H, q, *J* = 6.8 Hz), 4.16 (1 H, dd, *J* = 5.1, 8.9 Hz), 4.86 (1 H, d, *J* = 4.6); ^{13}C-NMR (125 MHz, D_2O) δ 10.85, 10.92, 14.70, 15.97, 16.99, 26.82, 28.79, 30.17, 33.31, 49.60, 56.17, 62.65, 71.32, 170.58, 173.04, 173.12, 178.69; LRMS (FAB) *m/z* 370 [$(M+H)^+$]; HRMS (FAB) calcd for $C_{17}H_{27}N_3O_6$: 370.1978 [$(M+H)^+$], found 370.1976 [$(M+H)^+$].

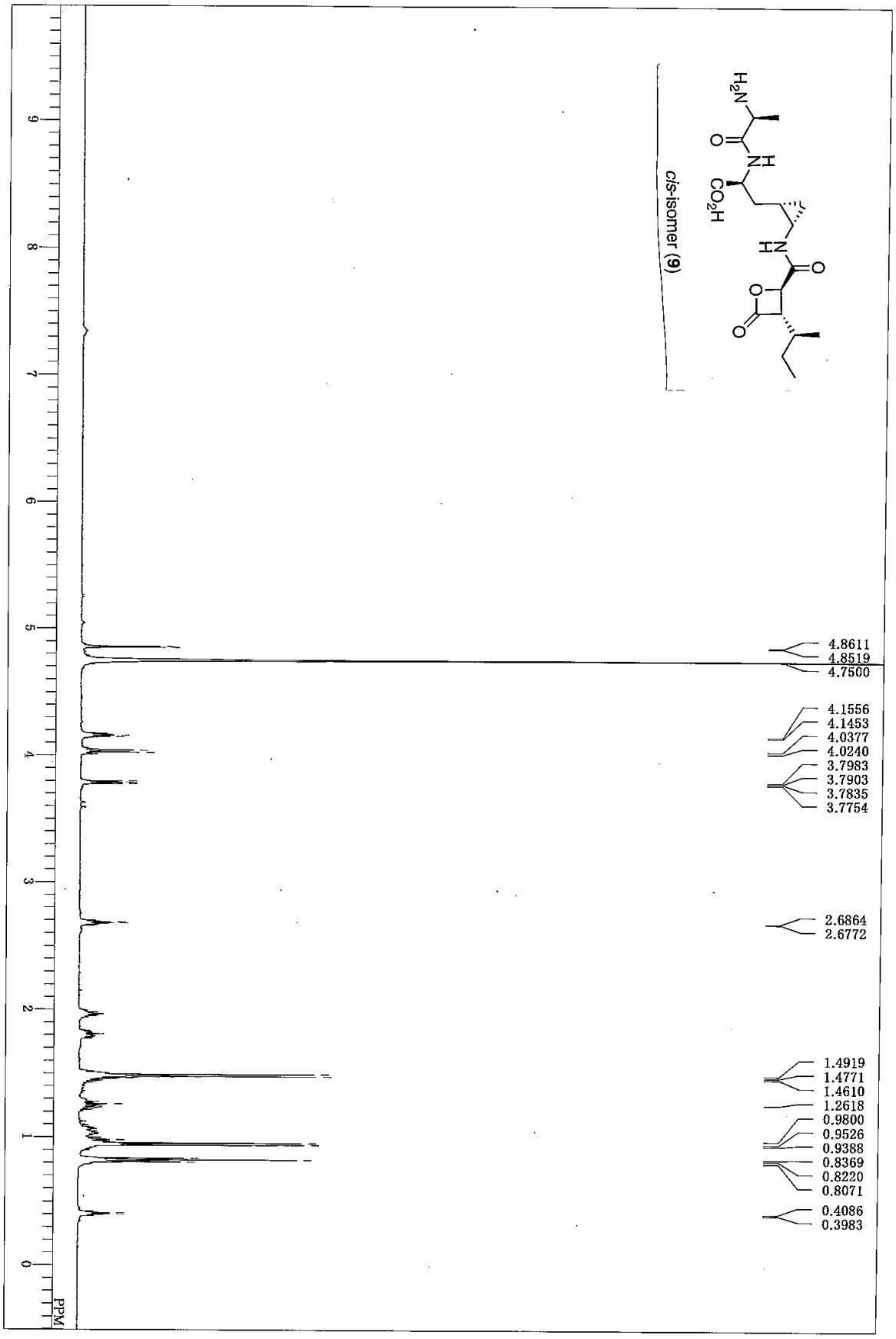

belactosin A (2, trans/ L-anti-isomer)
4.8173
4.8065
4.7500
4.2808
4.0907
4.0728
3.8116
3.8017
3.7932
3.7824
2.4647
1.9696
1.8754
1.8397
1.5229
1.5055
1.2688
0.9596
0.9427
0.8561
0.8372
0.8184
0.7968
0.6664
0.6509
0.0
PPM


D-anti-isomer (6)
4.8118
4.8027
4.7500
4.3320
4.3206
4.3102
4.1110
4.0961
3.8018
3.7926
3.7869
3.7777
2.4367
2.4287
2.4218
1.9580
1.9443
1.9088
1.8790
1.5240
1.4667
1.4530
1.4301
1.2629
1.2480
1.2354
0.9411
0.9274
0.8335
0.8186
0.8037
0.7842
0.7739
0.7659
0.6514
0.6365
PPM

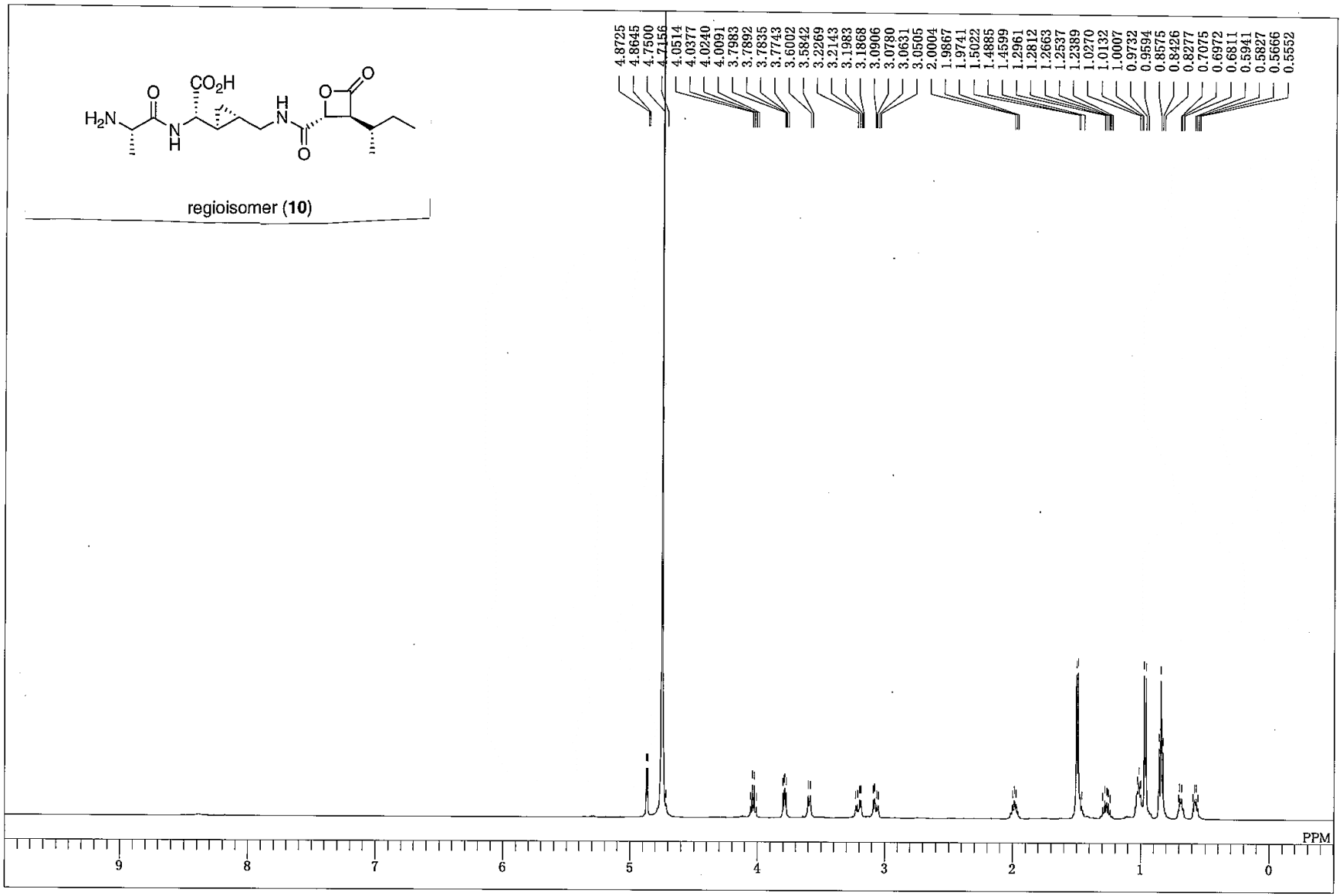
single_pulse



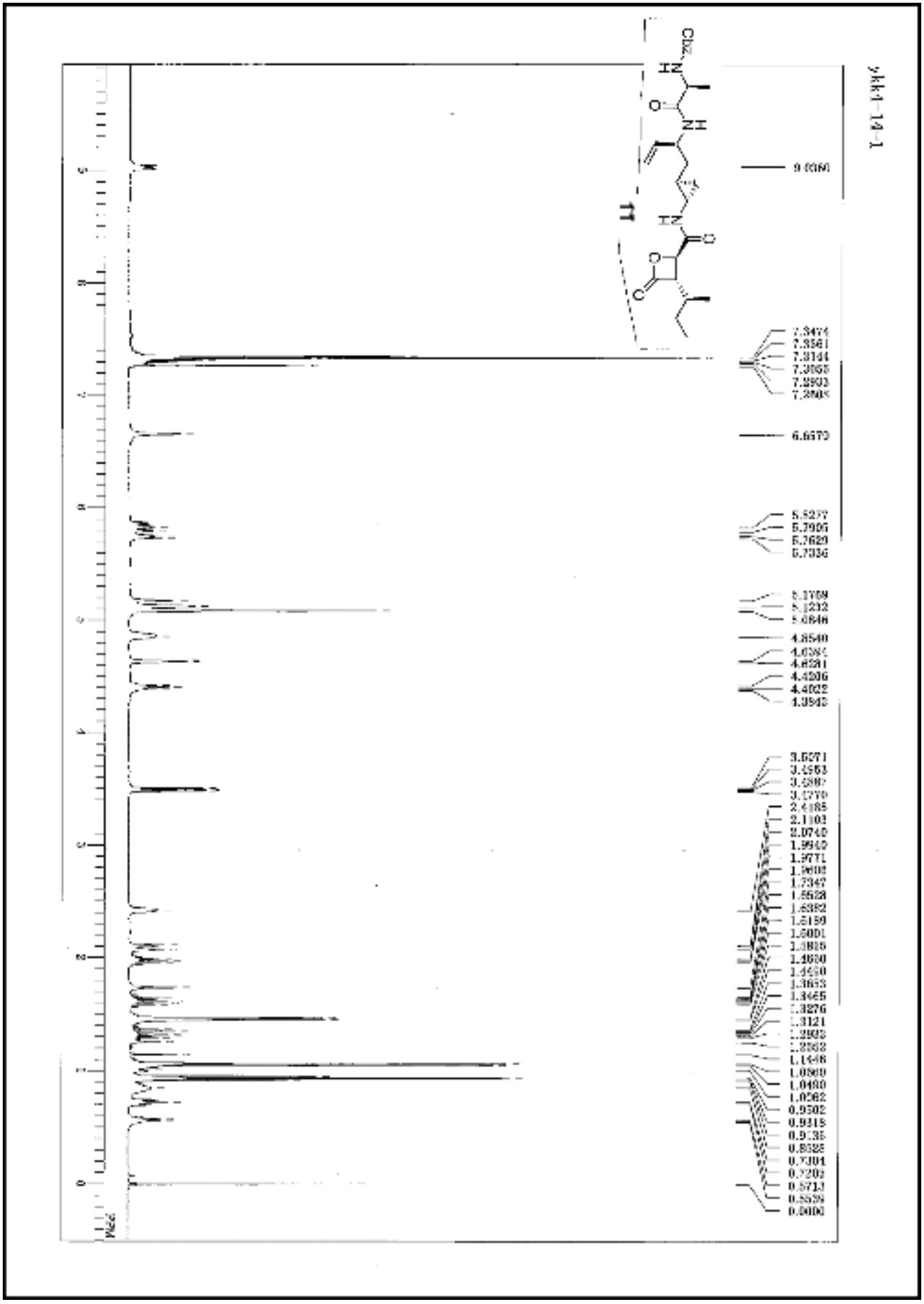

trans/L-syn-isomer (8)
4.8118
4.8027
4.7500
4.1717
4.1602
4.1556
4.1442
4.0892
4.0755
4.0606
4.0469
3.7686
3.7606
3.7537
3.7457
2.5100
2.5020
2.4951
2.4883
2.4802
1.9935
1.9798
1.9660
1.9534
1.9397
1.8332
1.8195
1.8046
1.7908
1.7748
1.6362
1.6213
1.6087
1.5916
1.5813
1.5126
1.4977
1.4851
1.4690
1.4541
1.4427
1.2824
1.2675
1.2526
1.2400
1.2251
1.0671
0.9881
0.9743
0.9548
0.9422
0.8506
0.8357
0.8209
0.7682
0.7567
0.7487
0.7384
0.7292
0.6983
0.6857
0.6708
0.6594
PPM

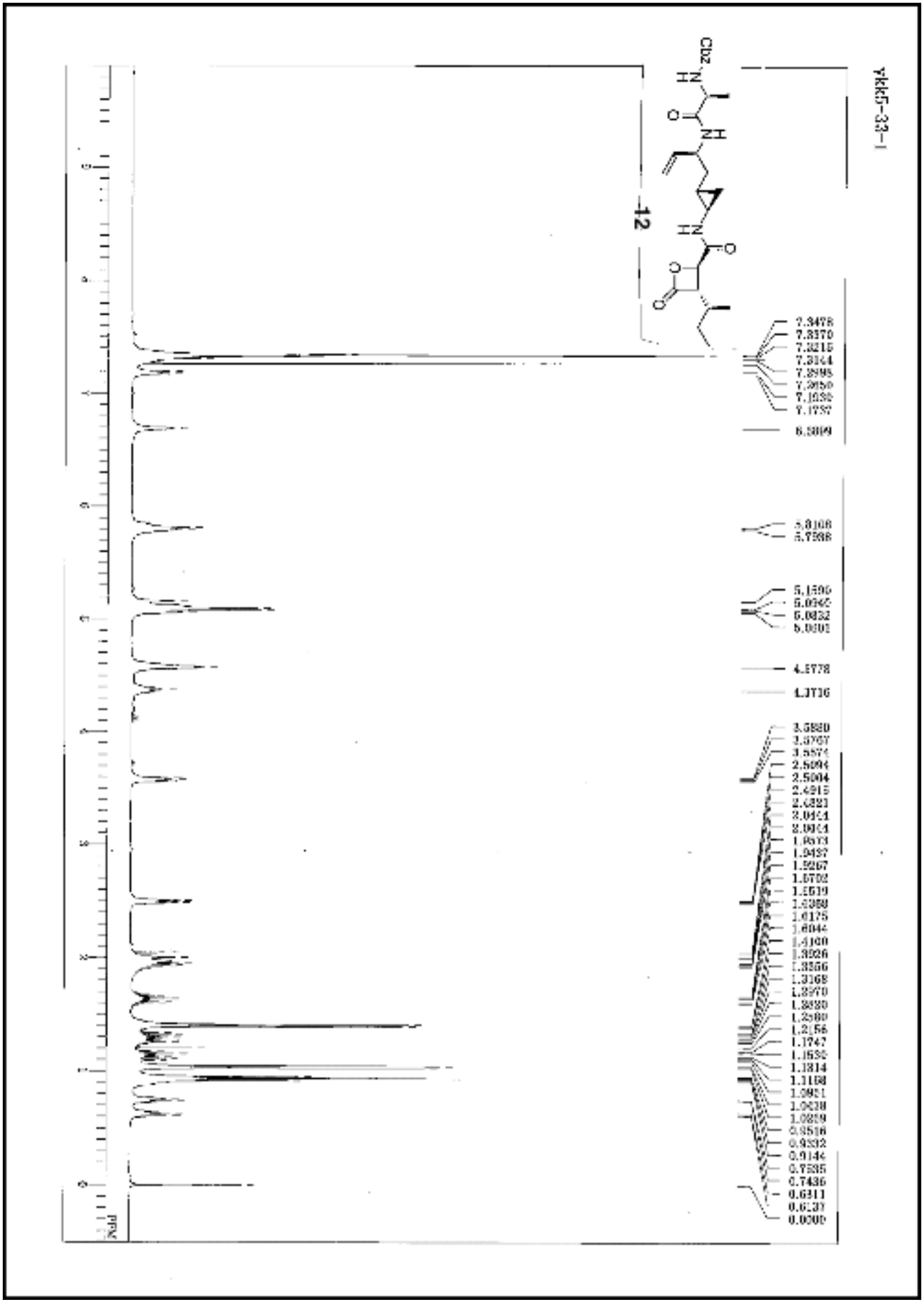

cis-isomer (9)
4.8611
4.8519
4.7500
4.1556
4.1453
4.0377
4.0240
3.7983
3.7903
3.7835
3.7754
2.6864
2.6772
1.4919
1.4771
1.4610
1.2618
0.9800
0.9526
0.9388
0.8369
0.8220
0.8071
0.4086
0.3983
PPM

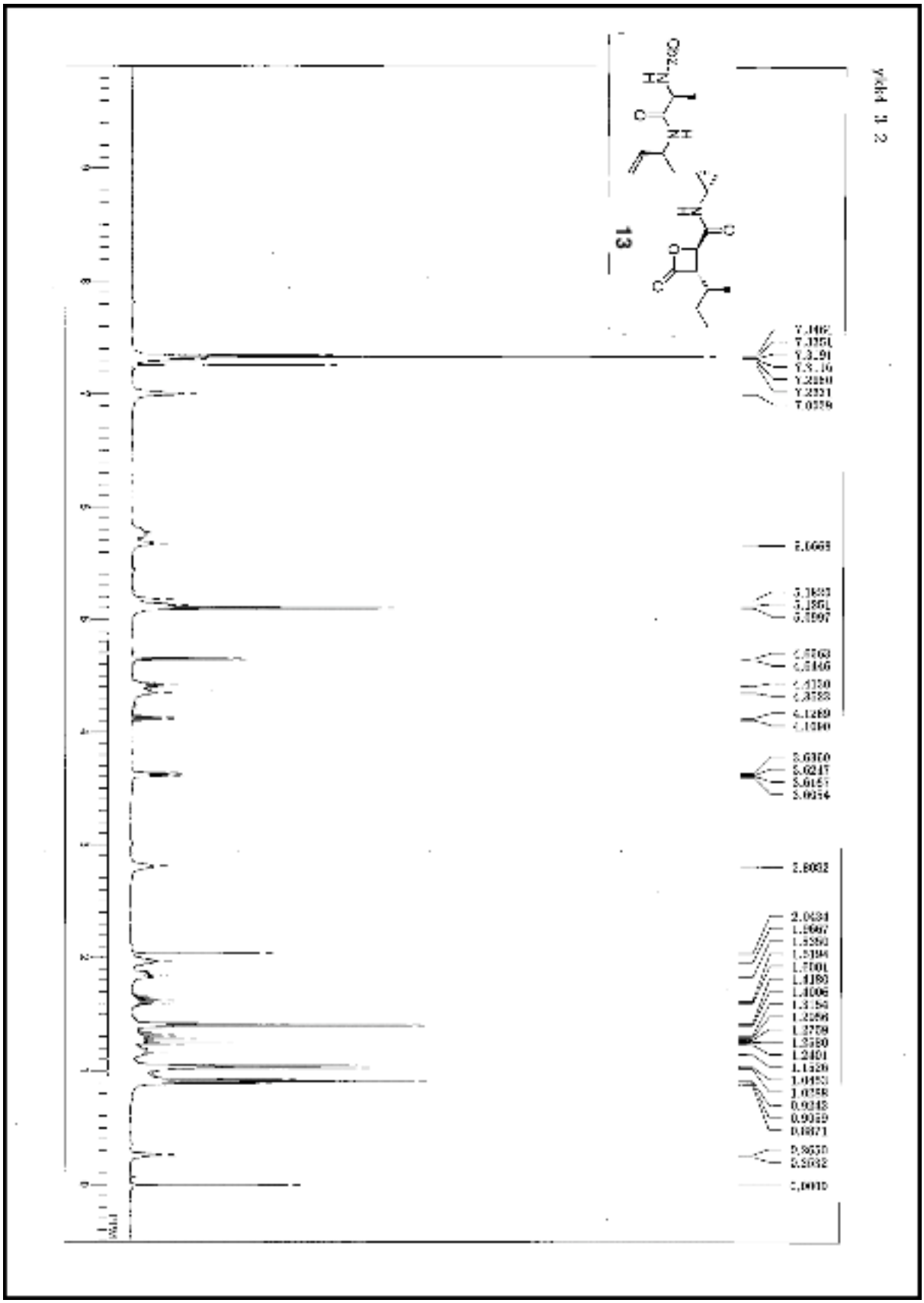
ykk5-39-1



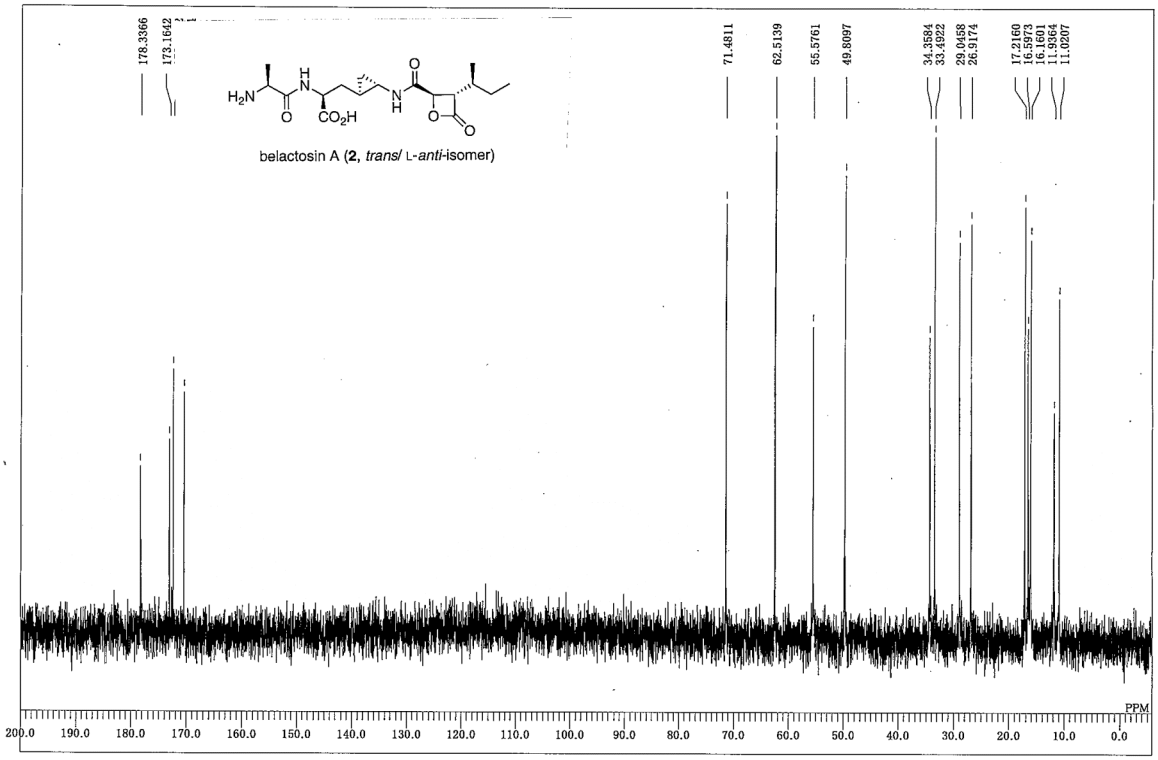
regioisomer (**10**)


yhh1-14-1
9.0000
7.3474
7.3541
7.3184
7.3068
7.2903
7.2805
6.6670
5.5277
5.3906
5.2629
5.2036
5.1708
5.1232
5.0848
4.8540
4.6384
4.6281
4.4206
4.4022
4.3943
3.5271
3.4953
3.4807
3.4779
2.4185
2.1103
2.0740
1.9942
1.9771
1.9408
1.7347
1.6528
1.6382
1.6159
1.6001
1.3815
1.3690
1.3650
1.3653
1.3465
1.3276
1.3121
1.2903
1.2868
1.1446
1.0860
1.0480
1.0262
0.9502
0.9315
0.9136
0.8925
0.7301
0.7202
0.5713
0.5539
0.0000
PPM

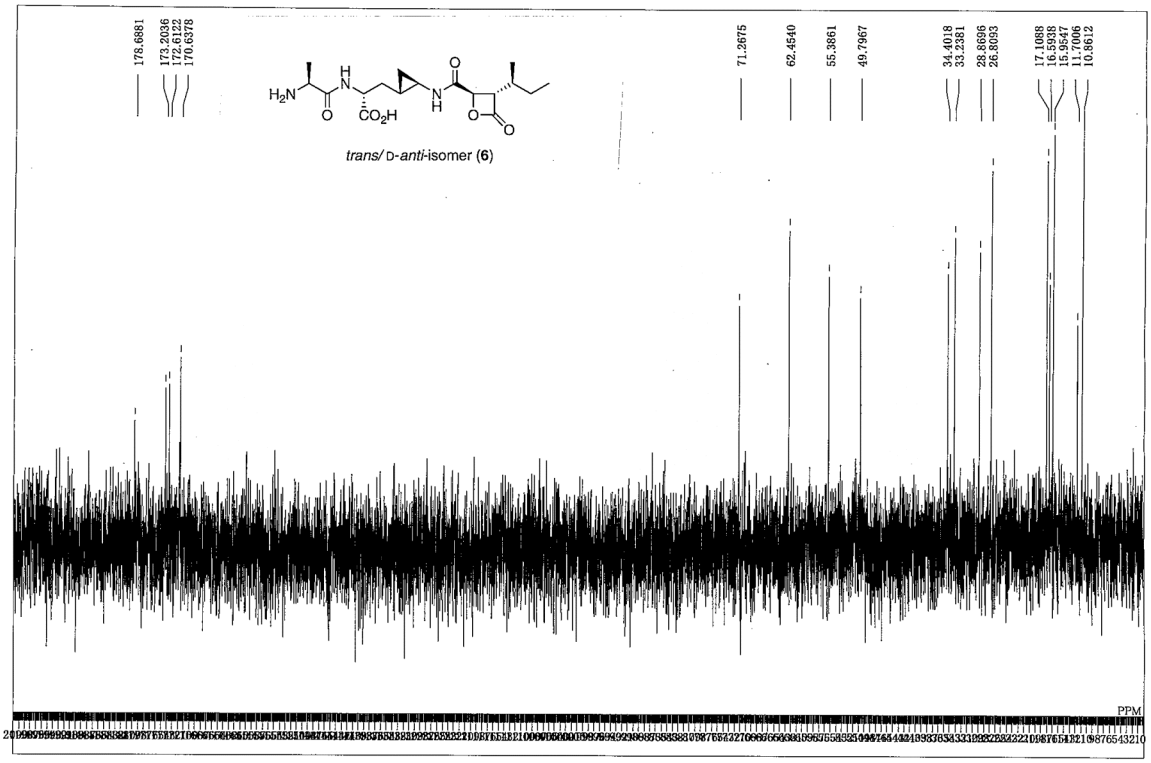

ykk5-33-1
-12



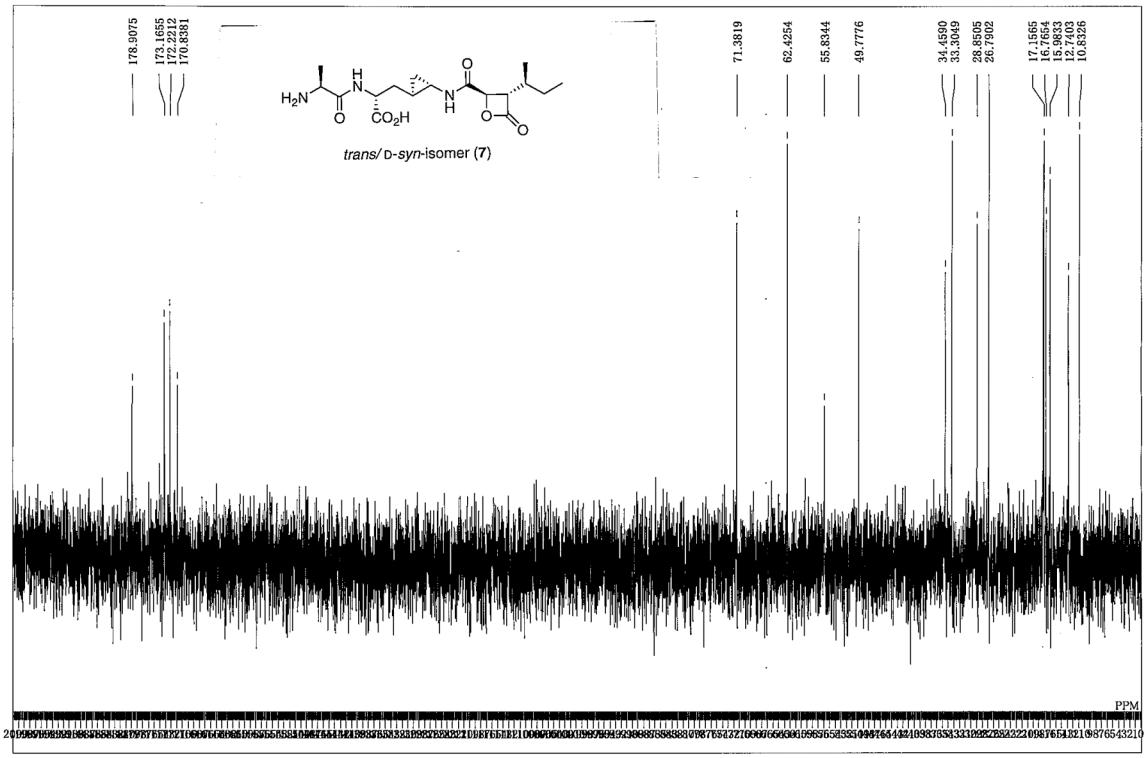
ykk5-15-1



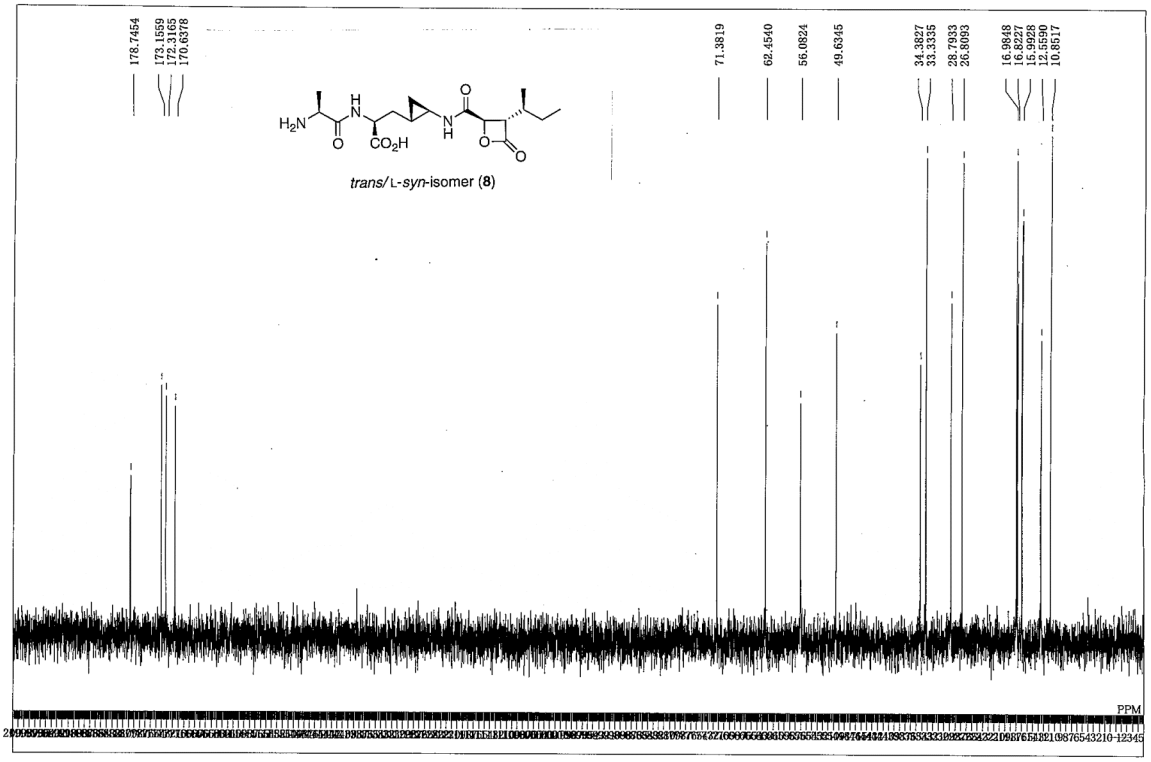
ykk5-62-1



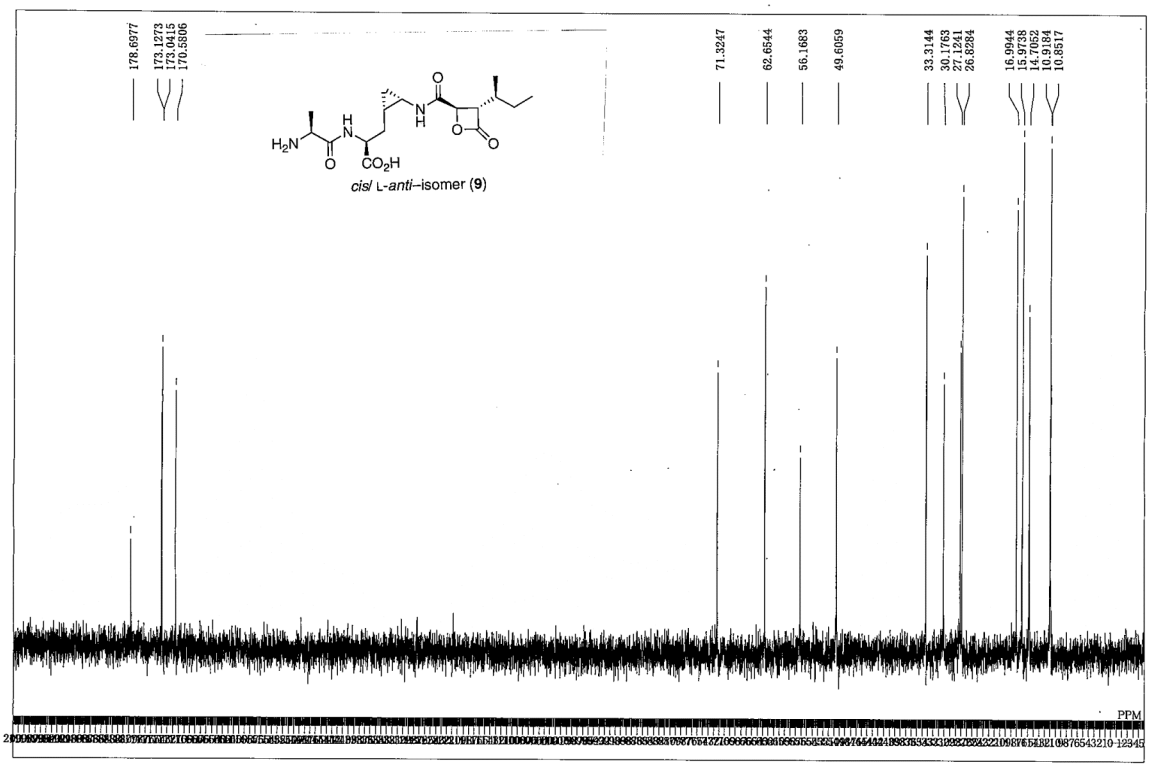
single pulse decoupled gated NOE



ykk5-43-1

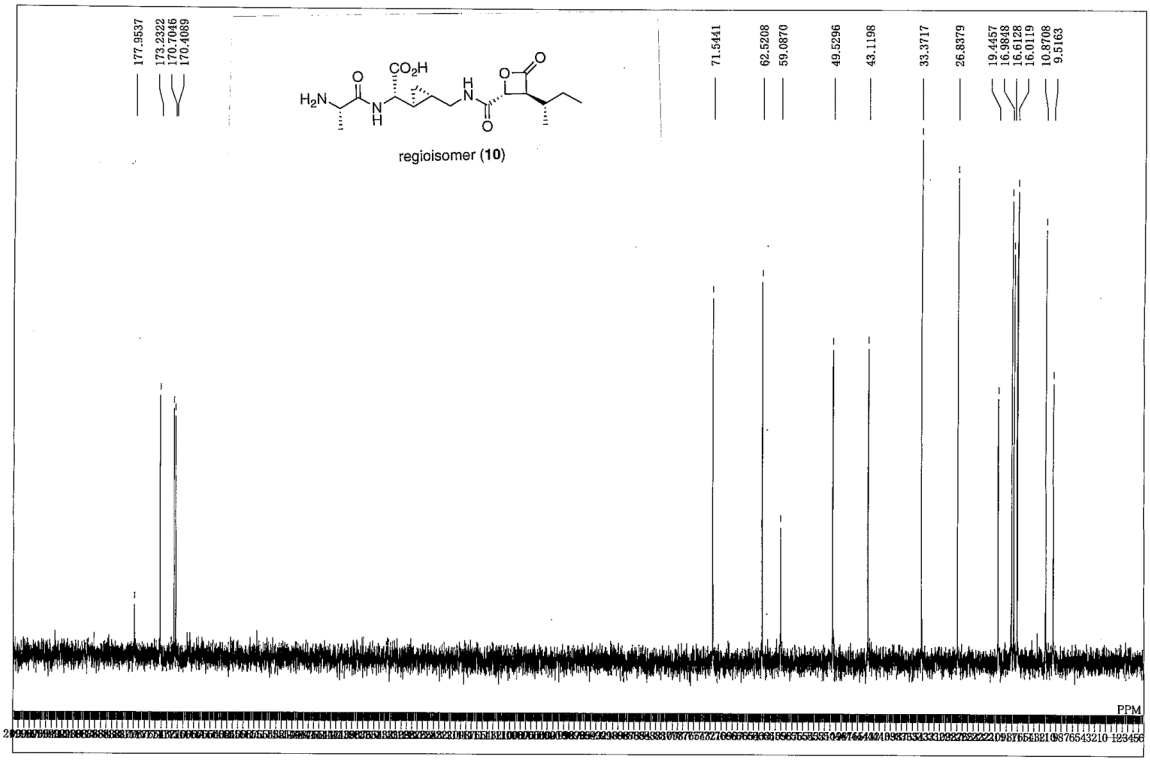


ykk5-37-1



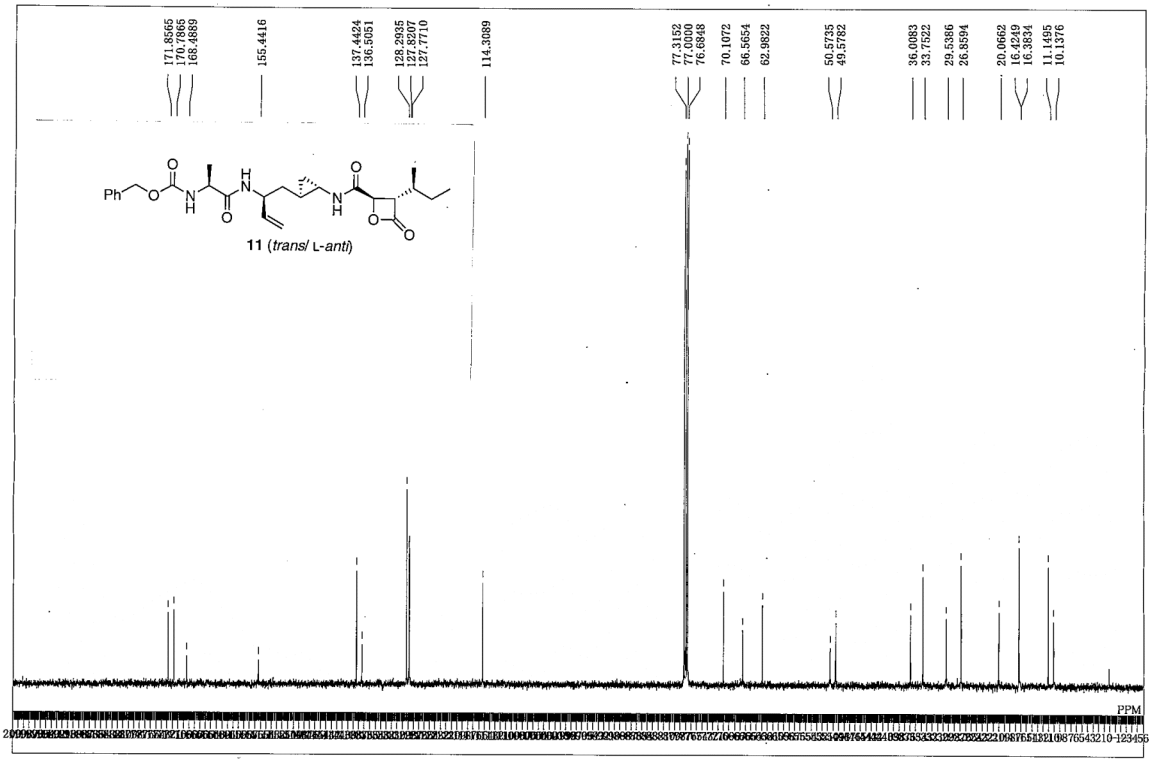
cis/ L-*anti*–isomer (**9**)

ykk5-39-1



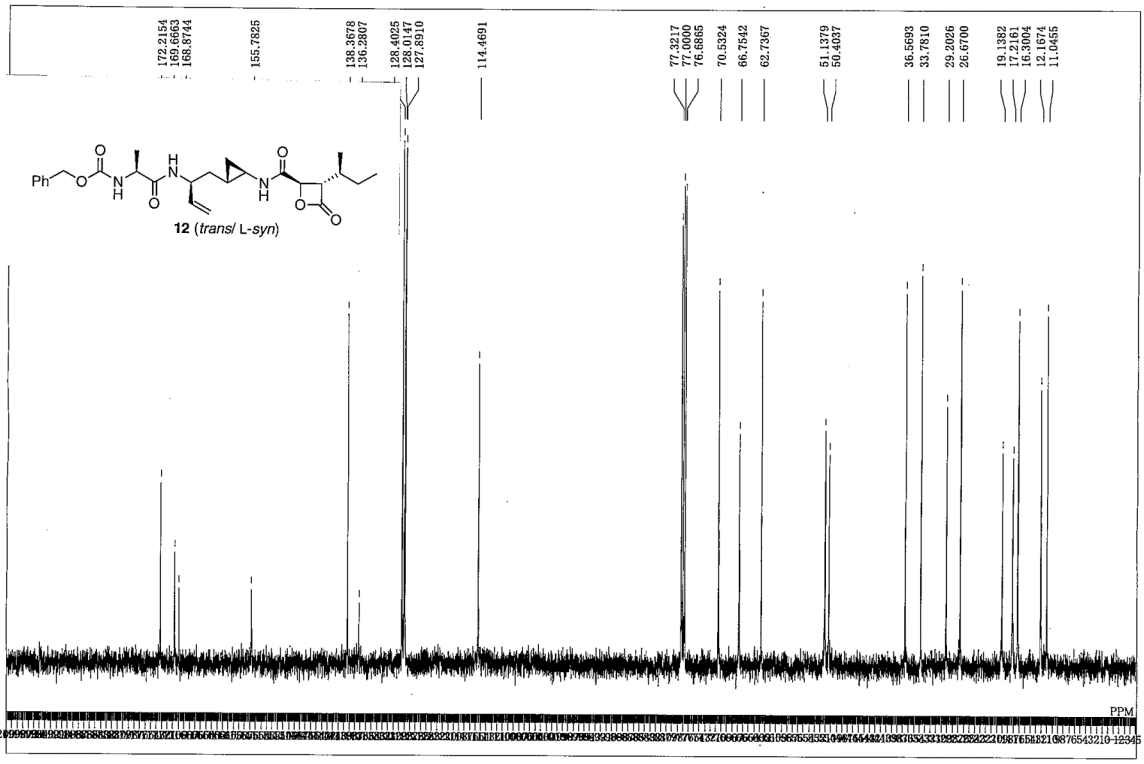
regioisomer (**10**)

ykk4-14-1



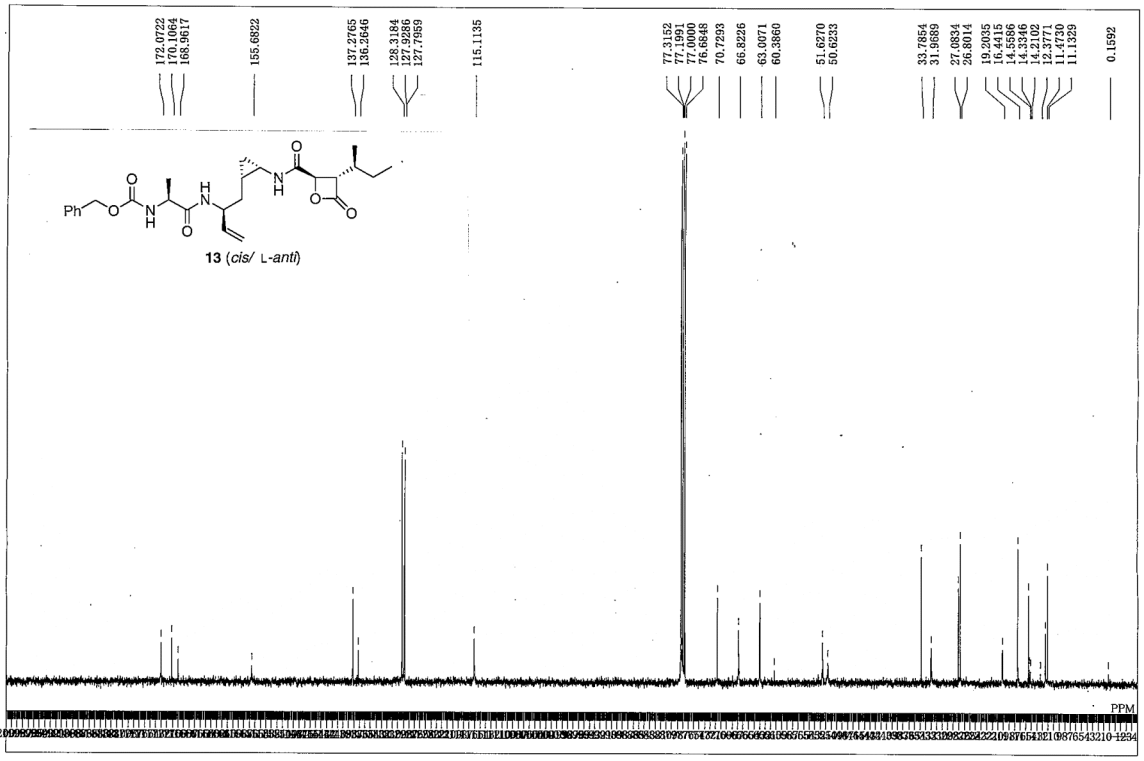
11 (*trans*/ L-*anti*)

ykk5-33-1



12 (*trans*/ L-*syn*)

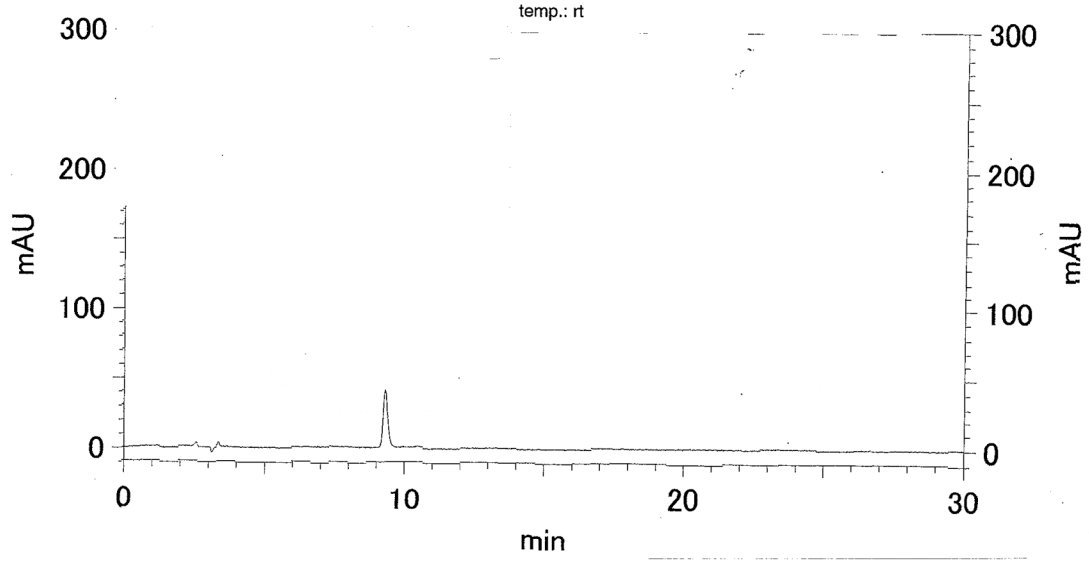
ykk4-3-2



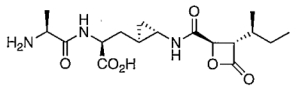
13 (*cis*/ L-*anti*)

HPLC Chart of **2**

column: Kanto Mightsil, RP-18 GP250-4.6 (5 μm)
eluent: aq. CH_3OH (10%) containing TFA (0.1%)
detection: 210 nm
temp.: rt



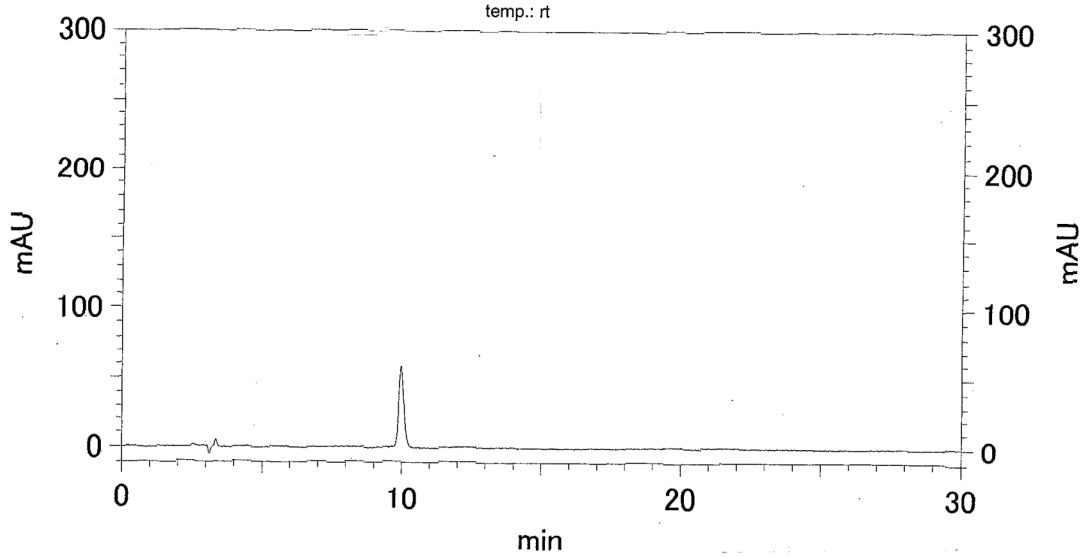
C:¥CLASS-VP¥DATA¥Public¥yoshida¥ykk5-15-1, 検出器 A-210 nm



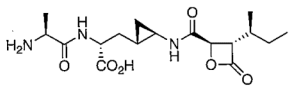
belactosin A (**2**, *trans*/ L-*anti*-isomer)

HPLC Chart of **6**

column: Kanto Mightsil, RP-18 GP250-4.6 (5 μm)
eluent: aq. CH_3OH (10%) containing TFA (0.1%)
detection: 210 nm
temp.: rt



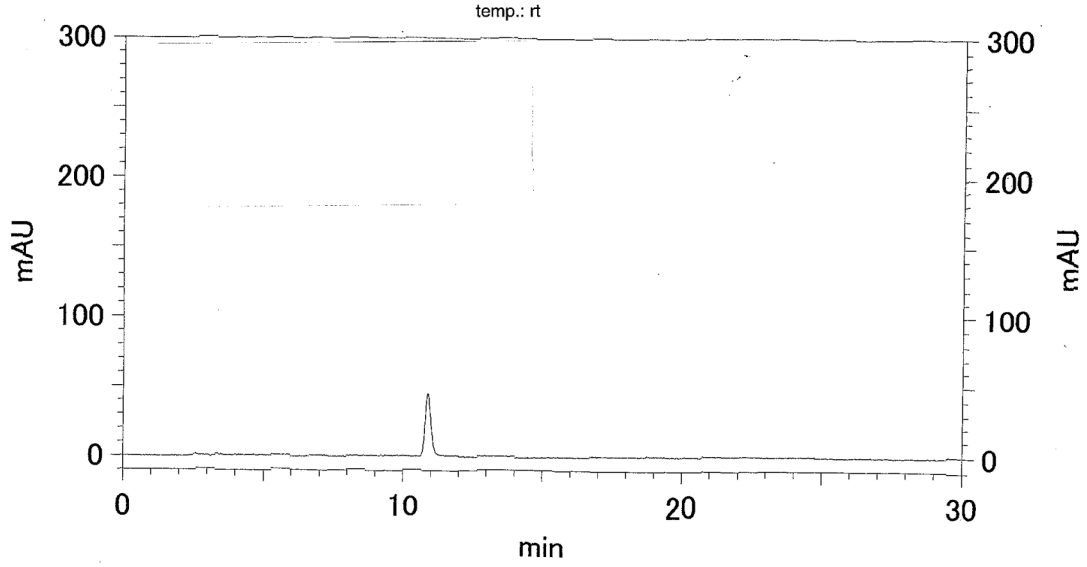
C:¥CLASS-VP¥DATA¥Public¥yoshida¥ykk5-62-1, 検出器 A-210 nm



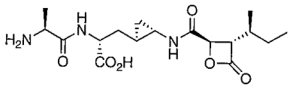
trans/ D-*anti*-isomer (**6**)

HPLC Chart of **7**

column: Kanto Mightsil, RP-18 GP250-4.6 (5 μm)
eluent: aq. CH_3OH (10%) containing TFA (0.1%)
detection: 210 nm
temp.: rt



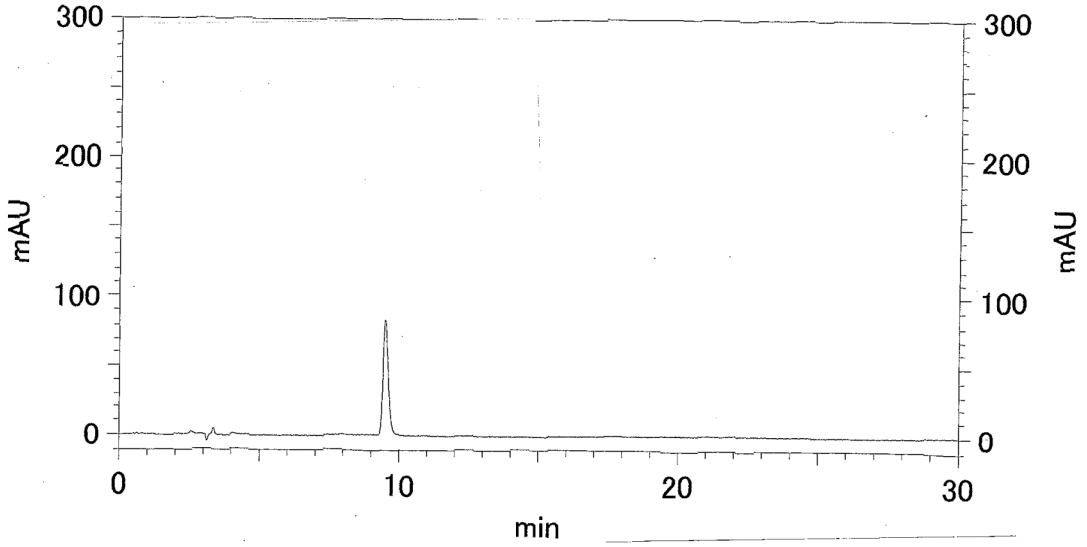
C:¥CLASS-VP¥DATA¥Public¥yoshida¥ykk5-29-1, 検出器 A-210 nm



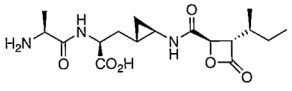
trans/D-*syn*-isomer (**7**)

HPLC Chart of **8**

column: Kanto Mightsil, RP-18 GP250-4.6 (5 μm)
eluent: aq. CH_3OH (10%) containing TFA (0.1%)
detection: 210 nm
temp.: rt



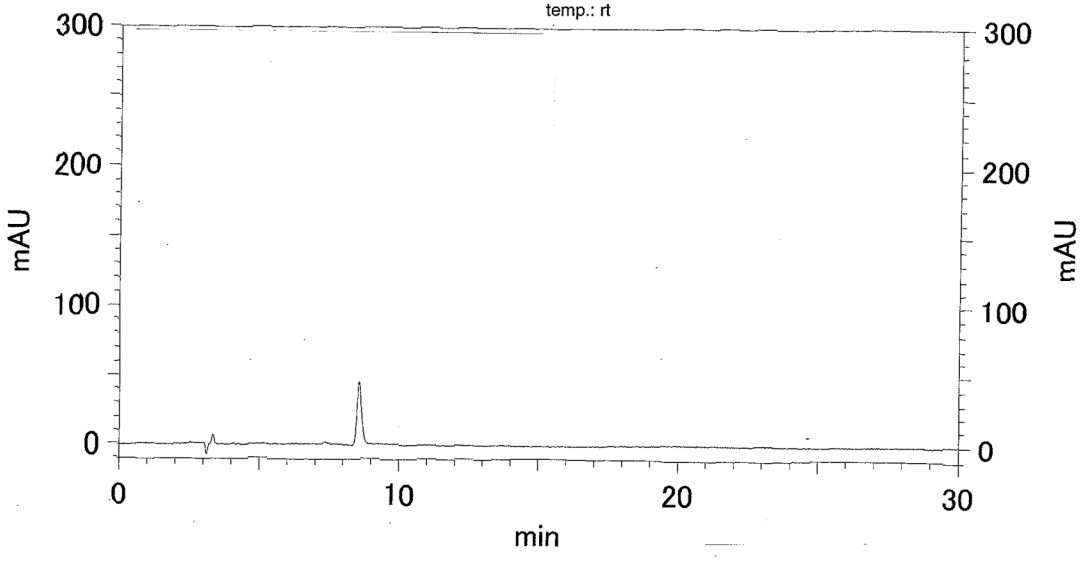
C:¥CLASS-VP¥DATA¥Public¥yoshida¥ykk5-43-1, 検出器 A-210 nm



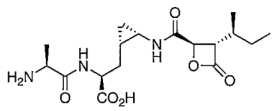
trans/L-*syn*-isomer (**8**)

HPLC Chart of **9**

column: Kanto Mightsil, RP-18 GP250-4.6 (5 μm)
eluent: aq. CH_3OH (10%) containing TFA (0.1%)
detection: 210 nm
temp.: rt



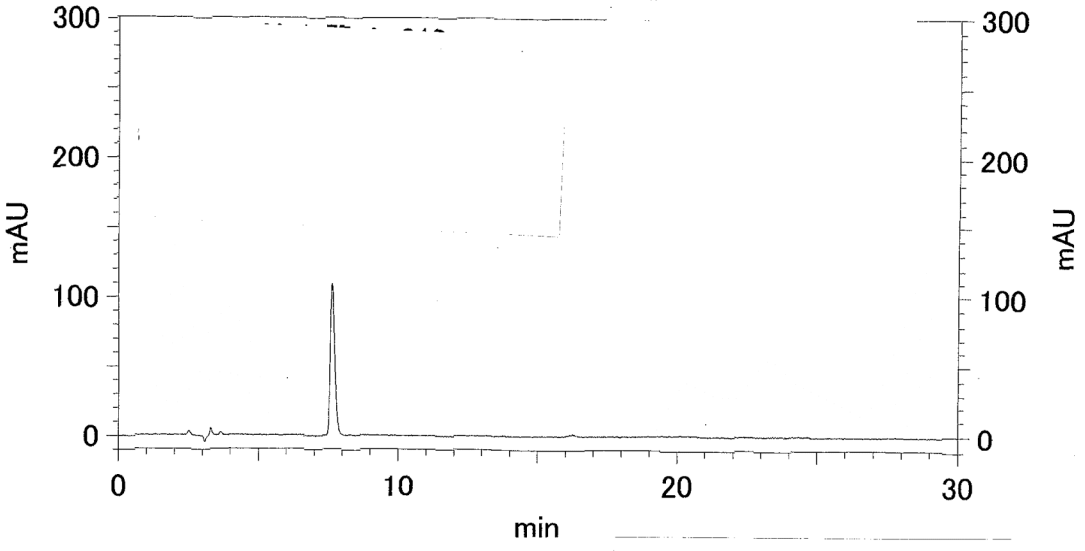
C:¥CLASS-VP¥DATA¥Public¥yoshida¥ykk5-37-1, 検出器 A-210 nm



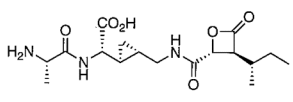
cis/ L-*anti*–isomer (**9**)

HPLC Chart of **10**

column: Kanto Mightsil, RP-18 GP250-4.6 (5 μm)
eluent: aq. CH_3OH (10%) containing TFA (0.1%)
detection: 210 nm



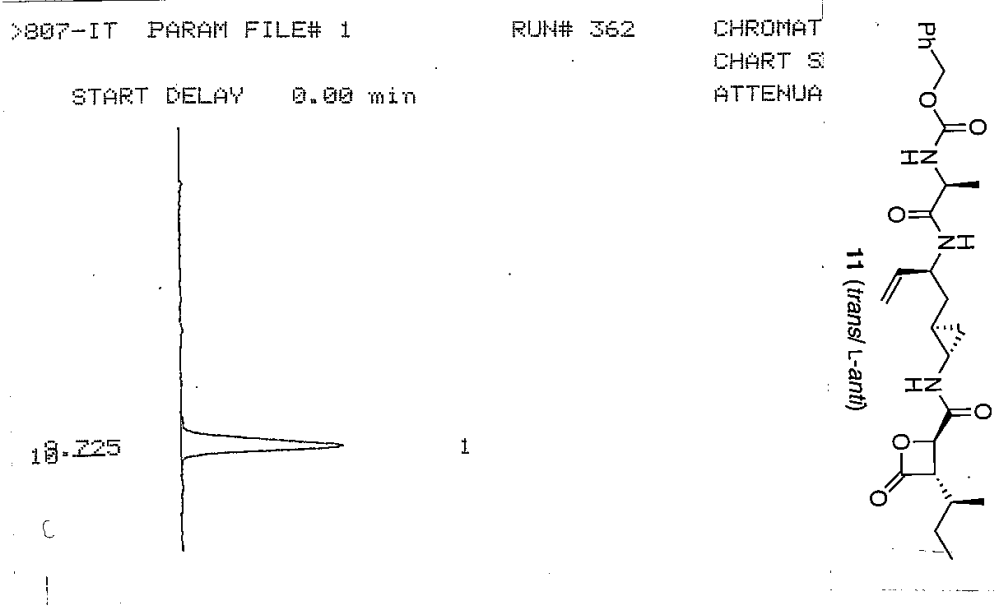
C:¥CLASS-VP¥DATA¥Public¥yoshida¥ykk5-39-1, 検出器 A-210 nm



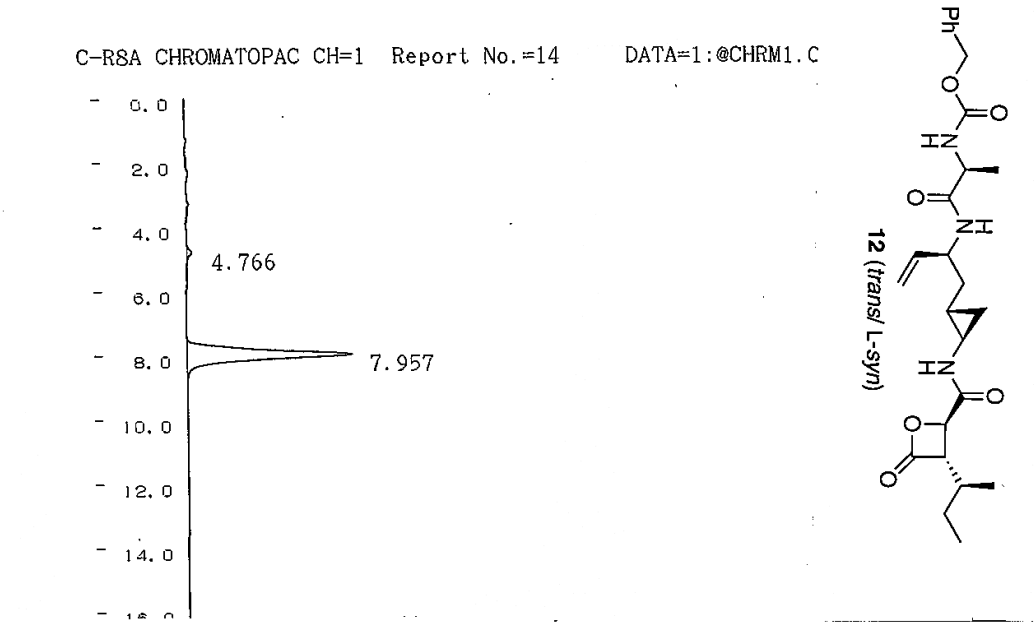
regioisomer (**10**)

HPLC Charts of **11**, **12**, and **13**

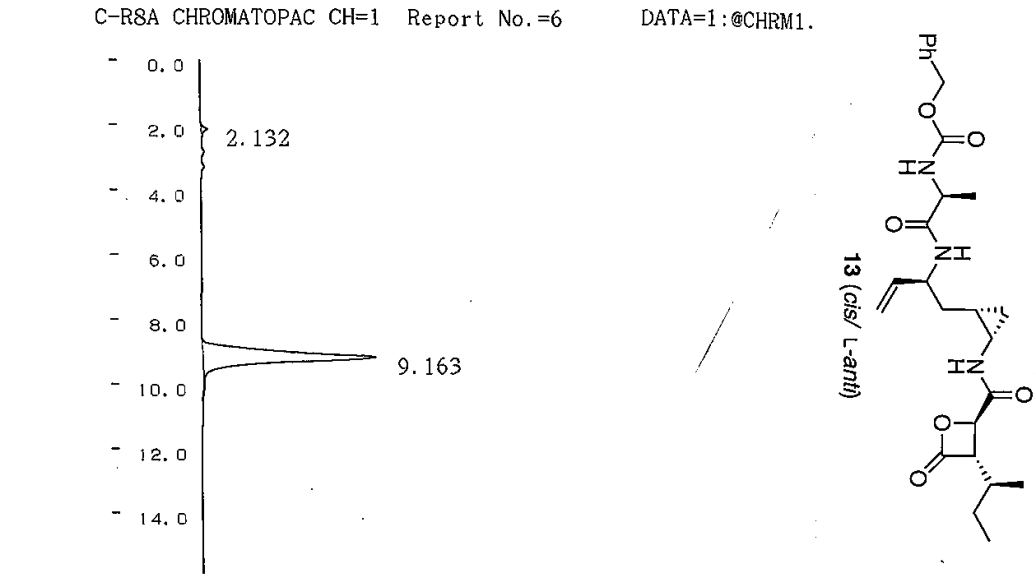
column: Kanto Mightsil, RP-18 GP250-4.6 (5 μm)
eluent: aq. CH_3CN (55%)
detection: 256 nm
temp.: rt



11 (*trans*/ L-*anti*)



12 (*trans*/ L-*syn*)



13 (*cis*/ L-*anti*)

Table S1. HPLC Analysis Data of Compounds.[a]

Compound	eluent	detection	t_R	purity
2	A	210 nm	9.3 min	>98%
6	A	210 nm	10.0 min	>98%
7	A	210 nm	10.9 min	>98%
8	A	210 nm	9.4 min	>98%
9	A	210 nm	8.5 min	>98%
10	A	210 nm	7.6 min	>98%
11	B	256 nm	9.7 min	>98%
12	B	256 nm	8.0 min	>98%
13	B	256 nm	9.2 min	>98%

[a]Column, Kanto Mightysil RP-18 GP250-4.6 (5 μm); eluent, A (aqueous 10% CH_3OH containing 0.1% TFA, 1.0 mL/min) or B (aqueous 55% CH_3CN, 1.0 mL/min); room temperature.