6.4 Experimental

General. Melting points were determined on a Mel-Temp apparatus in open capillaries and are uncorrected. IR and UV-vis spectra were recorded on a JASCO A-100 infrared and on a JASCO Ubest V-550 spectrophotometers, respectively. ^1H NMR spectra were obtained on a JEOL GX-270 spectrometer in $CDCl_3$, CD_3OD, or DMSO-d_6 solutions. Chemical shifts are reported in ppm (δ) downfield from internal TMS. Thin layer chromatography (TLC) was performed on silica gel 60 F-254 with a 0.2 mm layer thickness. Column chromatography was carried out with Merck Kieselgel 60 (230-400 mesh). Optical rotations were determined on a JASCO PIP-370 digital polarimeter. CD spectra were measured with a JASCO J-720 spectropolarimeter. HPLC was carried out on a JASCO 880-PU and a 875-UV equipped with a JASCO IT integrator by using a column packed with a Finepak SIL $C_{12}S$. Combustion analyses were performed on a YANACO MT-3 CHN corder.

***N*$^{\alpha}$-(*tert*-butoxycarbonyl)-L-glutamic acid γ-methyl ester (46).** To a solution of L-glutamic acid γ-methyl ester (8.0 g, 50 mmol) and $KHCO_3$ (5 g, 50 mmol) in H_2O (50 mL) was added Boc_2O (12 g, 55 mmol) in dioxane (50 mL) at 0 °C. After stirring overnight, the solvent was evaporated, and then the mixture was acidified with 10% citric acid to pH 2. AcOEt (200 mL) was added, and the organic layer was successively washed with H_2O (2x100 mL) and brine, and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave the crude product, which was purified by

recrystallization from Et_2O-petroleum ether mixture to give the product as colorless crystal (**46**); 10.6 g (82%).

***N*-Benzyloxy-N^{α}-(*tert*-butoxycarbonyl)-L-glutamamide γ-methyl ester (47).** To a solution of **46** (6.78 g, 26 mmol), and Et_3N (2.91 g, 29 mmol) in THF (50 mL) was added isobutyl chloroformate (3.56 g, 26 mmol) in THF (20 mL) at -17 °C. After 15 min, benzyloxyamine (2.96 g, 27 mmol) in THF (20 mL) was added to the mixture at -15 °C, and then the reaction mixture was stirred overnight at room temperature. The precipitated Et_3N·HCl was filtered off and the filtrate was then concentrated. The residue was dissolved in AcOEt (300 mL), and the organic layer was successively washed with 10% citric acid, 4% $NaHCO_3$, and brine, and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave the product (**3**) as colorless crystals; 8.29 g (87%): mp 88-90 °C; IR (KBr) 1720, 1650, 740, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.41 (s, 9H), 2.05-2.39 (m, 4H), 3.65 (s, 3H), 4.07 (br s, 1H), 4.89 (s, 2H), 5.18 (br s, 1H), 7.37 (m, 5H), 9.51 (br s, 1H). Anal. Calcd for $C_{18}H_{26}N_2O_6$: C, 59.00; H, 7.15; N, 7.65. Found: C, 59.24; H, 6.82; N, 7.29.

***N*-Benzyloxy-L-glutamamide γ-methyl ester hydrochloride (48).** Compound **47** (7.8g, 21.3 mmol) was dissolved in 4M HCl in dioxane (100 mL) at 0 °C. Disappearance of **47** was monitored by TLC. The reaction mixture was concentrated to remove HCl and dioxane. Dry benzene was added to the residue and evaporated. Addition and evaporation of benzene were repeated three times to give the product (ca. 100%).

1-Benzyloxy-3-methoxycarbonylethyl-5,6-dimethyl-2(1*H*)-pyrazinone (49). To a solution of **48** (6.83 g, 22.6 mmol) in a MeOH-H_2O (2:1) mixture (60 mL) was added biacetyl (1.94 g, 22.5 mmol) upon

cooling with a dry ice-acetone bath (-30 °C). The pH of the reaction mixture was adjusted to 8 with 4 M NaOH solution and then stirred for 2 h at room temperature. After evaporation of the solvent, the residue was dissolved in $CHCl_3$ (500 mL). The organic layer was successively washed with 10% citric acid, 4% $NaHCO_3$, and brine, and dried over anhydrous Na_2SO_4. The residue was purified by column chromatography on silica gel (eluent: $CHCl_3$-acetone-EtOH=100:5:1) to give the product (**49**) as pale yellow crystals, 3.1g (43%): mp 118-121 °C; IR ($CHCl_3$) 1730,1650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.21 (s, 3H), 2.25 (s, 3H), 2.80 (t, *J* 7 Hz, 2H), 3.20 (t, *J* 7 Hz, 2H), 3.71 (s, 3H), 5.26 (s, 2H), 7.43-7.49 (m, 5H). Anal. Calcd for $C_{17}H_{20}N_2O_4$: C, 64.54; H, 6.37; N, 8.85. Found: C, 64.41; H, 6.26; N, 8.58.

1-Benzyloxy-3-carboxyethyl-5,6-dimethyl-2(1*H*)-pyrazinone (50). To a solution of **49** (350 mg, 1.1 mmol) in MeOH (10 mL) was added 1 M NaOH (1.5 mL, 1.5 mmol) at 0 °C. After stirring for 6.5 h at room temperature, the solvent was evaporated to remove the bulk of MeOH. The pH of the residual aqueous solution was adjusted to 2 with 5M HCl at 0 °C, then the aqueous reaction mixture was extracted with $CHCl_3$ (3x100 mL). The combined organic layers were successively washed with 10% citric acid and brine, and dried over anhydrous $MgSO_4$. Evaporation of the solvent gave the product as pale yellow crystals, 297 mg (89%): ^1H NMR ($CDCl_3$) δ 2.15 (s, 3H), 2.18 (s, 3H), 2.68 (t, *J* 7 Hz, 2H), 3.24 (t, *J* 7 Hz, 2H), 5.20 (s, 2H), 7.40 (m, 5H), 9.79 (br s, 1H). Anal. Calcd for $C_{16}H_{18}N_2O_4$: C, 63.56; H, 6.00; N, 9.27. Found: C, 63.84; H, 5.89; N, 9.02.

General procedure for the coupling of 50 and N^{ω}-Boc-substituted diamine. A typical dxample: *N*-(*tert*-Butoxycarbonyl)-*N'*-3-(1-benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanoyldiaminoethane (51a). WSC·HCl (479 mg, 2.5 mmol) in CH_2Cl_2 (8 mL) was added to a mixture of **50** (756 mg, 2.5 mmol), *N*-Boc-ethylenediamine (400 mg, 2.5 mmol), and HOBt (308 mg, 2.5 mmol) in DMF (4 mL) at -10 °C. The mixture was stirred overnight at room temperature. After removal of DMF under reduced pressure, the residue was dissolved in $CHCl_3$ (200 mL). The organic layer was successively washed with water, 5% $NaHCO_3$, 10% citric acid, and brine, and dried over anhydrous $MgSO_4$. Evaporation of the solvent, followed by recrystallization of the residual solid from a AcOEt-hexane mixture gave the product, 760 mg (85%): mp 149-151 °C; ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H), 2.20 (s, 3H), 2.24 (s, 3H), 2.66 (t, *J* 7 Hz, 2H), 3.17 (t, *J* 7 Hz, 2H), 3.28 (m, 2H), 3.36 (m, 2H), 5.00 (br s, 1H), 5.25 (s, 2H), 6.57 (br s, 1H), 7.39-7.50 (m, 5H). Anal. Calcd for $C_{23}H_{32}N_4O_5$: C, 62.14; H, 7.26; N, 12.60. Found: C, 61.91; H, 7.05; N, 12.57.

***N*-(*tert*-Butoxycarbonyl)-*N'*-3-(1-benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanoyldiaminobutane (51b).** 76%: mp 118-119 °C; ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H), 1.49 (m, 4H), 2.20 (s, 3H), 2.24 (s, 3H), 2.65 (t, *J* 7 Hz, 2H), 3.16 (m, 4H), 3.26 (q, *J* 6 Hz, 2H), 4.60 (br s, 1H), 5.25 (s, 2H), 6.32 (br s, 1H), 7.41-7.49 (m, 5H). Anal. Calcd for $C_{25}H_{36}N_4O_5 \cdot 0.1H_2O$: C, 63.30; H, 7.69; N, 11.81. Found: C, 63.16; H, 7.45; N, 11.71.

***N*-(*tert*-Butoxycarbonyl)-*N'*-3-(1-benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanoyldiaminopentane (51c).** The

residual oil was purified by silica gel column chromatography with $CHCl_3$-acetone-EtOH=100:10:2) to give the product, 25%: mp 60-65 °C; ^1H NMR ($CDCl_3$) δ 1.43 (s, 9H), 1.49 (m, 9H), 2.20 (s, 3H), 2.24 (s, 3H), 2.64 (t, *J* 7 Hz, 2H), 3.12-3.22 (m, 6H), 4.70 (br s, 1H), 5.24 (s, 2H), 6.50 (br s, 1H), 7.40-7.46 (m, 5H). Anal. Calcd for $C_{26}H_{38}N_4O_5$: C, 64.18; H, 7.87; N, 11.51. Found: C, 64.00; H, 8.03; N, 11.77.

***N*-(*tert*-Butoxycarbonyl)-*N'*-3-(1-benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanoyldiaminohexane (51d).** The residual oil was purified by silica gel column chromatography (eluent: $CHCl_3$-acetone-EtOH=100:10:2) to give the product, 75%: mp 97-100 °C; ^1H NMR ($CDCl_3$) δ 1.32 (m, 4H), 1.44 (s, 13H), 2.20 (s, 3H), 2.24 (s, 3H), 2.65 (t, *J* 7 Hz, 2H), 3.09 (q, *J* 7 Hz, 3H), 3.16 (t, *J* 7 Hz, 2H), 3.23 (q, *J* 7 Hz, 2H), 4.50 (br s, 1H), 5.24 (s, 2H), 6.25 (br s, 1H), 7.41-7.47 (m, 5H). Anal. Calcd for $C_{27}H_{40}N_4O_5 \cdot 0.1H_2O$: C, 64.54; H, 8.06; N, 11.15. Found: C, 64.14; H, 8.03; N, 11.04.

***N*-3-(1-Benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanoyldiaminoethane hydrochloride (52a).** To a solution of **51a** (226 mg, 0.48 mmol) in CH_2Cl_2 (4 mL) was added CF_3CO_2H (TFA) (4 mL) dropwise at 0 °C. The reaction mixture was stirred for 30 min at 0 °C, and then the solvent was evaporated. Dry benzene was added to the residue and evaporated. Addition and evaporation of benzene were repeated three times to give the product (**52a**) (ca. 100%), which was directly used for the next reaction.

General procedure for the deprotection of the Boc group of compounds (51b-d). A typical example: *N*-3-(1-benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanoyldiaminobutane

hydrochloride (52b). To a solution of compound **51b** (308 mg, 0.65 mmol) in dry dioxane (6 mL) was added dropwise 4M HCl in dioxane (3 mL) at 0 °C. The reaction mixture was stirred for 20 min at 0 °C, and then the solvent was evaporated. Dry benzene was added to the residue and evaporated. Addition and evaporation of benzene were repeated three times to give the product (**52b**) (ca. 100%), which was directly used for the next reaction.

General procedure for the tripodal compounds (53a-d). A typical example: 1,1,1-Tris[2-[2-[3-[1-(benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl]propanamido]ethylaminocarbonyl] ethyloxymethyl]ethane (53a). A solution of **52a** (490 mg, 1.1 mmol), **36** (200 mg, 0.32 mmol) and NEt_3 (121 mg, 1.2 mmol) in DMF (8 mL) was stirred for 48 h at 38 °C. After removal of the solvent, $CHCl_3$ (200 mL) was added to the residue. The organic layer was successively washed with H_2O, 5% $NaHCO_3$, 10% citric acid, and brine, and dried over anhydrous $MgSO_4$. Purification by column chromatography on silica gel (eluent: $CHCl_3$-MeOH=6:1), followed by gel chromatography on TOYOPEARL HW-40 (eluent: MeOH), gave the product as an amorphous solid (**53a**), 217 mg (51%); ^1H NMR ($CDCl_3$) δ 0.86 (s, 3H), 2.18 (s, 9H), 2.22 (s, 9H), 2.42 (t, *J* 7 Hz, 6H), 2.64 (t, *J* 7Hz, 6H), 3.13 (t, *J* 7 Hz, 6H), 3.24 (m, 6H), 3.37 (s, 12H), 3.64 (t, *J* 7 Hz, 6H), 5.22 (s, 6H), 7.08 (br s, 3H), 7.40-7.55 (m, 18H). Anal. Calcd for $C_{68}H_{90}N_{12}O_{15}\cdot 2H_2O$: C, 60.43; H, 7.04; N, 12.44. Found: C, 60.32; H, 7.34; N, 12.88.

1,1,1-Tris[2-[2-[3-[1-(benzyloxy)-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl]propanamido]butylaminocarbonyl]ethyloxy-methyl]ethane (53b). 82%: mp 104-109 °C (decomp.); ^1H NMR

($CDCl_3$) δ 0.87 (s, 3H), 1.53 (m, 12H), 2.19 (s, 9H), 2.23 (s, 9H), 2.42 (t, *J* 7 Hz, 6H), 2.65 (t, *J* 7 Hz, 6H), 3.14 (t, *J* 7 Hz, 6H), 3.25 (m, 18H), 3.64 (t, *J* 7 Hz, 6H), 5.22 (s, 6H), 6.66 (br s, 3H), 6.90 (br s, 3H), 7.39-7.47 (m, 15H). Anal. Calcd for $C_{76}H_{102}N_{12}O_{15}\cdot 4H_2O$: C, 61.03; H, 7.41; N, 11.24. Found: C, 60.85; H, 7.29; N, 11.49.

1,1,1-Tris[2-[2-[3-[1-(benzyloxy)-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl]propanamido]pentylaminocarbonyl]ethyloxy-methyl]ethane (53c). An amorphous solid, 57%: ^1H NMR ($CDCl_3$) δ 0.88 (s, 3H), 1.32 (m, 6H), 1.50 (m, 12H), 2.20 (s, 6H), 2.34 (s, 6H), 2.41 (t, *J* 7 Hz, 6H), 2.65 (t, *J* 7 Hz, 6H), 3.12-3.24 (m, 18H), 3.65 (t, *J* 7 Hz, 6H), 5.24 (s, 6H), 6.62 (t, *J* 6 Hz, 3H), 6.85 (t, *J* 6 Hz, 3H), 7.41-7.53 (m, 15H). Anal. Calcd for $C_{79}H_{108}N_{12}O_{15}\cdot 7H_2O$: C, 59.61; H, 7.72; N, 10.56. Found: C, 59.69; H, 7.80; N, 10.75.

1,1,1-Tris[2-[2-[3-[1-(benzyloxy)-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl]propan-amido]hexylaminocarbonyl]ethyloxy-methyl]ethane (53d). 54%: mp 115-112 °C (decomp.); ^1H NMR ($CDCl_3$) δ 0.88 (s, 3H), 1.32 (m, 12H), 1.49 (12H, m), 2.20 (s, 9H), 2.23 (s, 9H), 2.40 (t, *J* 7 Hz, 6H), 2.64 (t, *J* 7 Hz, 6H), 3.12-3.23 (m, 24H), 3.64 (t, *J* 7 Hz, 6H), 5.23 (s, 6H), 6.59 (br s, 3H), 6.79 (br s, 3H), 7.39-7.49 (m, 15H). Anal. Calcd for $C_{82}H_{114}N_{12}O_{15}\cdot 3H_2O$: C, 63.06; H, 7.74; N, 10.76. Found: C, 62.92; H, 7.94; N, 10.78.

General procedure for the preparation of target compounds (26). A typical example: 1,1,1-Tris[2-[2-[3-[1-hydroxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl]propanamido]ethylamino carbonyl]ethyloxy-methyl]ethane (26a). A suspension of 10% Pd-C (19 mg) in MeOH (10 mL) was prehydrogenated with H_2 for 0.5 h. To

the suspension was added a solution of compound **53a** (106 mg, 0.08 mmol) in MeOH (20 mL). After hydrogenation with H_2 under atmospheric pressure for 30 min at room temperature, the catalyst was removed by filtration. The filtrate was concentrated to give the residue, which was purified by gel chromatography on Shephadex LH-20 (eluent: MeOH) to afford the product (**26a**), 82 mg (100%): ^{1}H NMR (CD_3OD) δ 0.86 (s, 3H), 2.32 (s, 9H), 2.39 (m, 15H), 2.60 (t, *J* 7 Hz, 6H), 3.04 (t, *J* 7 Hz, 6H), 3.20-3.40 (m, 18H), 3.62 (t, *J* 7 Hz, 6H). Anal. Calcd for $C_{46}H_{72}N_{12}O_{15}\cdot 7H_2O$: C, 48.18; H, 7.40; N, 14.35. Found: C, 48.49; H, 6.94; N, 14.00.

1,1,1-Tris[2-[2-[3-[1-hydroxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl]propanamido]butylaminocarbonyl]ethyloxy-methyl]ethane (26b). 100%: ^{1}H NMR (CD_3OD) δ 0.85 (s, 3H), 1.50 (m, 12H), 2.31 (s, 9H), 2.38 (m, 15H), 2.58 (t, *J* 7 Hz, 6H), 3.03 (t, *J* 7 Hz, 6H), 3.17 (m, 12H), 3.24 (s, 6H), 3.61 (t, *J* 7 Hz, 6H). Anal. Calcd for $C_{53}H_{84}N_{12}O_{15}\cdot 2H_2O$: C, 54.63; H, 7.61; N, 14.42. Found: C, 54.33; H, 7.50; N, 14.23.

1,1,1-Tris[2-[2-[3-[1-hydroxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl]propanamido]pentylaminocarbonyl]ethyloxy-methyl]ethane (26c). 70%: ^{1}H NMR (CD_3OD) δ 0.86 (s, 3H), 1.47 (m, 18H), 2.30 (s, 9H), 2.37 (m, 15H), 2.57 (t, *J* 7 Hz, 6H), 3.02 (t, *J* 7 Hz, 6H), 3.16 (m, 12H), 3.24 (s, 6H), 3.62 (t, *J* 7 Hz, 6H). Anal. Calcd for $C_{56}H_{90}N_{12}O_{15}\cdot 1.5H_2O$: C, 60.80; H, 8.05; N, 10.91. Found: C, 60.99; H, 7.75; N, 10.56.

1,1,1-Tris[2-[2-[3-[1-hydroxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl]propanamido]hexylaminocarbonyl]ethyloxy-

methyl]ethane (26d). 98%: mp 135-143 °C (decomp); ^{1}H NMR (DMSO-d_6) δ 0.77 (s, 3H), 1.23-1.37 (m, 24H), 2.23 (s, 9H), 2.28 (m, 15H), 2.42 (t, *J* 7 Hz, 6H), 2.87 (t, *J* 8 Hz, 6H), 3.02 (m, 12H), 3.14 (s, 6H), 3.52 (t, *J* 7 Hz, 6H), 7.79 (br s, 6H). Anal. Calcd for $C_{59}H_{96}N_{12}O_{15}\cdot 3H_2O$: C, 55.91; H, 8.11; N, 13.26. Found: C, 56.19; H, 8.23; N, 12.91.

***N*-3-(1-Benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanoyl-L-alanine Methyl Ester (54a).** WSC·HCl (1.86 g, 9.7 mmol) in CH_2Cl_2 (40 mL) was added to a mixture of **50** (2.35 g, 7.8 mmol), H-L-Ala-OMe·HCl (1.46 g, 10.4 mmol), *N*-methylmorpholine (1.08 g, 10.7 mmol), and HOBt (1.90 g, 12.4 mmol) in DMF (20 mL) at -10 °C. The mixture was stirred overnight at room temperature. After removal of DMF under reduced pressure, the residue was dissolved in AcOEt (300 mL). The organic layer was successively washed with water, 4% $NaHCO_3$, 10% citric acid, and brine, and dried over anhydrous $MgSO_4$. Evaporation of the solvent, followed by purification by column chromatography on silica gel (eluent: $CHCl_3$-acetone-EtOH=100:10:1) gave the product, 2.2 g (71%): mp 116 -120 °C; ^{1}H NMR ($CDCl_3$) δ 1.38 (d, *J* 7 Hz, 3H), 2.16 (s, 3H), 2.23 (s, 3H), 2.69 (m, 2H), 3.16 (m, 2H), 3.69 (s, 3H, OMe), 4.57 (m, 1H), 5.20 (s, 3H), 7.30-7.44 (m, 5H). Anal. Calcd for $C_{20}H_{25}N_3O_5$: C, 62.00; H, 6.50; N, 10.85. Found: C, 61.69; H, 6.50; N, 10.85.

***N*-3-(1-Benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanoyl-L-leucine Methyl Ester (54b).** The coupling of compound **50** (320 mg, 1.1 mmol) and H-L-Leu-OMe·HCl (278 mg, 1.5 mmol) was carried out by a similar procedure for the preparation **54a** to give the product (**54b**), 350 mg (60%): mp 85-97 °C; ^{1}H NMR ($CDCl_3$) δ

0.93 (d, *J* 6.1 Hz, 6H), 1.54-1.62 (m, 3H), 2.20 (s, 3H), 2.25 (s, 3H), 2.71 (t, *J* 6.6 Hz, 2H), 3.19 (t, *J* 6.6 Hz, 2H), 3.70 (s, 3H), 4.64 (m, 1H), 5.24 (s, 2H), 6.71 (d, *J* 8.0 Hz, 1H), 7.40-7.48 (m, 5H). Anal. Calcd for $C_{23}H_{31}N_3O_5 \cdot 0.5H_2O$: C, 62.99; H, 7.36; N, 9.58. Found: C, 62.86; H, 7.10; N, 9.68.

***N*-3-(1-Benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanoyl-L-alanine (55a).** To a solution of compound **54a** (2.2 g, 5.6 mmol) in MeOH (100 mL) was added 1M NaOH (11 mL, 11 mmol) at 0 °C. After 3 h, the mixture was neutralized and then concentrated. The residual aqueous solution was acidified with 5M HCl at 0 °C, then extracted with $CHCl_3$ (200 mL). The organic layer was successively washed with 10% citric acid, and brine, and dried over anhydrous $MgSO_4$. Evaporation of the solvent, followed by recrystallization from benzene-hexane, gave the product (**55a**) as pale yellow crystals, 1.90 g (92%): mp 158-162 °C; ^{1}H NMR ($CDCl_3$) δ 1.43 (d, *J* 7 Hz, 3H), 2.20 (s, 3H), 2.24 (s, 3H), 2.70 (t, *J* 7 Hz, 2H), 3.18 (t, *J* 7 Hz, 2H), 4.57 (m, 1H), 5.23 (s, 2H), 7.38-7.48 (m, 5H), 7.95 (d, *J* 6 Hz, 1H, NH). Anal. Calcd for $C_{19}H_{23}N_3O_5 \cdot 0.3H_2O$: C, 60.25; H, 6.24; N, 11.10. Found: C, 60.18; H, 6.52; N, 10.99.

***N*-3-(1-Benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanoyl-L-leucine (55b).** The hydrolysis of **54b** (350 mg, 0.81 mmol) with 1M NaOH was carried out by a similar procedure for the preparation of **55a** to give the product (**55b**) as yellow crystals, 330 mg (90%): mp 147-149 °C; ^{1}H NMR ($CDCl_3$) δ 0.92 (d, *J* 5.0 Hz, 6H), 1.55-1.68 (m, 3H), 2.20 (s, 3H), 2.24 (s, 3H), 2.72 (t, *J* 6.8 Hz, 2H), 3.19 (t, *J* 6.8 Hz, 2H), 4.60 (m, 1H), 5.23 (s, 2H), 6.97 (d, *J* 8.0 Hz, 1H), 7.38-7.49 (m,

5H). Anal. Calcd for $C_{22}H_{29}N_3O_5 \cdot 1.0H_2O$: C, 60.96; H, 7.21; N, 9.69. Found: C,61.09; H, 7.23; N, 9.38.

***N*-3-(1-Benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanoyl-L-alanyl–β–alanine methyl ester (56a).** Compound **55a** (1.45 g, 3.89 mmol) was coupled with H-β–Ala-OMe·HCl (706 mg, 5.06 mmol) in the presence of HOBt (1.17 g, 7.65 mmol), *N*-methylmorpholine (529 mg, 5.23 mmol), WSC·HCl (904 mg, 4.71 mmol) by the same procedure for the preparation of **54a**. The crude product was purified by column chromatography on silica gel (eluent: $CHCl_3$-acetone-EtOH=100:40:8) to give the pure product (**56a**), 1.44 g (81%): mp 134-137 °C; $[\alpha]_D^{22}$ -17.3° (c 0.38, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.35 (d, *J* 7Hz, 3H), 2.19 (s, 3H), 2.24 (s, 3H), 2.52 (t, *J* 6 Hz, 2H), 2.67 (t, *J* 6 Hz, 2H), 3.17 (m, 2H), 3.49 (t, *J* 6 Hz, 2H), 3.67 (s, 3H), 4.47 (m, 1H), 5.23 (s, 2H), 7.01 (d, *J* 7 Hz, 1H, NH), 7.09 (br s, 1H, NH), 7.30-7.49 (m, 5H). Anal. Calcd for $C_{23}H_{30}N_4O_6 \cdot 0.5H_2O$: C, 59.08; H, 6.68; N, 11.98. Found: C, 58.97; H, 6.54; N, 12.14.

***N*-3-(1-Benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanoyl-L-leucyl–β–alanine methyl ester (56b).** The coupling of **55b** (330 mg, 0.79 mmol) with H-β–Ala-OMe·HCl (127 mg, 0.91 mmol) was carried out by a similar procedure to the preparation of **56a** to give the product (**56b**), 360 mg (91%): mp 142-148 °C; ^{1}H NMR ($CDCl_3$) δ 0.90 (d, *J* 4.3 Hz, 3H), 0.92 (d, *J* 4.3 Hz, 3H), 1.51-1.70 (m, 3H), 2.20 (s, 3H), 2.25 (s, 3H), 2.51 (t, *J* 6.3 Hz, 2H), 2.68 (t, *J* 7.3 Hz, 2H), 3.17 (t, *J* 7.3 Hz, 2H), 3.48 (m, 2H), 3.68 (s, 3H), 4.40 (m, 1H), 5.24 (s, 2H), 6.70 (d, *J* 9.0 Hz, 1H), 6.82 (br s, 1H), 7.39-7.50 (m, 5H). Anal. Calcd for

$C_{26}H_{36}N_4O_6 \cdot 1.0H_2O$: C, 60.21; H, 7.39; N, 10.80. Found: C, 60.22; H, 7.19; N, 10.79.

***N*-3-(1-Benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanoyl-L-alanyl–β–alanine (57a).** Compound **56a** (350 mg, 0.76 mmol) was hydrolyzed by a similar procedure to the preparation of **55a** to give the product (**57a**), 311 mg (92%); mp 172-173 °C; $[\alpha]_D^{22}$ -17.2° (c 0.52, MeOH); IR (KBr) 3550-2930, 1732, 1658 cm^{-1}; ^1H NMR (CD_3OD) δ 1.34 (m, 3H), 2.27 (s, 6H), 2.52 (m, 2H), 2.71 (m, 2H), 3.12 (m,2H), 3.44 (m, 2H), 4.35 (m, 1H), 5.26 (s, 2H), 7.43-7.52 (m, 5H). Anal. Calcd for $C_{22}H_{28}N_4O_6 \cdot 0.5H_2O$: C, 58.28; H, 6.40; N, 12.36. Found: C, 58.30; H, 6.32; N, 12.25.

***N*-3-(1-Benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanoyl-L-leucyl–β–alanine (57b).** Compound **56b** (230 mg, 0.46 mmol) was hydrolyzed with 1M NaOH (1mL, 1 mmol) by a similar procedure to the preparation of **57a** to give the product (**57b**), 180 mg (80%): mp 144-149 °C; ^1H NMR ($CDCl_3$) δ 0.86 (d, *J* 6.4 Hz, 3H), 0.88 (d, *J* 6.4 Hz, 3H), 1.55 (m, 3H), 2.20 (s, 3H), 2.25 (s, 3H), 2.52 (m, 2H), 2.70 (m, 2H), 3.16 (m, 2H), 3.49 (m, 2H), 4.70 (m, 1H), 5.23 (s, 2H), 7.11 (d, *J* 8.8 Hz, 1H), 7.35-7.48 (m, 5H), 7.66 (m, 1H). Anal. Calcd for $C_{25}H_{34}N_4O_6 \cdot 0.5H_2O$: C, 60.59; H, 7.19; N, 11.31. Found: C, 60.25; H, 7.02; N, 11.20.

***N*-3-(1-Benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanoyl-L-alanyl–β–alanine *O*-succinimide ester (58a).** WSC·HCl (374 mg, 1.95 mmol) in CH_2Cl_2 (9 mL) was added to a mixture of compound **57a** (352 mg, 0.94 mmol) and HOSu (219 mg, 1.9 mmol) in DMF (4.5 mL) at -10 °C. After stirring for 24 h at room temperature, the

solvent was removed under reduced pressure, and the residue was dissolved in $CHCl_3$ (300 mL). The organic layer was successively washed with H_2O, 4% $NaHCO_3$, and brine, and dried over anhydrous $MgSO_4$. Evaporation of the solvent gave the *O*-succinimide ester (**58a**) as colorless crystals, which was used for the next reaction without further purification, 805 mg (100%); IR ($CHCl_3$) 1816, 1792, 1740 cm^{-1}.

***N*-3-(1-Benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanoyl-L-leucyl–β–alanine *O*-succinimide ester (58b).** Compound **57b** (180 mg, 0.37 mmol) was coupled with HOSu (54 mg, 0.47 mmol) by a similar procedure to the preparation of **58a** to give the corresponding *O*-succinimide ester (**58b**) as yellow crystals, which was used directly for the next reaction, 230 mg (ca. 100%): IR ($CHCl_3$) 1810, 1740, 1710 cm^{-1}.

Tris-(2-(3-(2-(3-(1-Benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanamido)-2(*R*)-methylethanamido)-propanamido)ethyl)amine (59a). A solution of compound **58a** (511 mg, 0.94 mmol) and tris(2-aminoethyl)amine (40 mg, 0.27 mmol) in DMF (12 mL) was stirred for 48 h at 38 °C. After removal of the solvent, $CHCl_3$ (400 mL) and 0.1M NaOH (100 mL) was added to the residue. The organic layer was successively washed with H_2O, and brine, and dried over anhydrous Na_2SO_4. Purification by column chromatography on silica gel (eluent: $CHCl_3$-MeOH=6:1), follwed by gel chromatography on TOYOPEARL HW-40 (eluent: MeOH), gave the product (**59a**), 283 mg (74%): mp 158-160 °C (decomp.); $[\alpha]_D^{22}$ -15.6° (c 0.36, MeOH); IR (KBr) 1648 cm^{-1}; ^1H NMR (CD_3OD) δ 1.32 (d, *J* 7Hz, 9H), 2.24 (s, 18H), 2.42 (t, *J* 6 Hz, 6H), 2.60 (m, 6H), 2.67 (t, *J* 7 Hz, 6H), 3.08 (m, 6H), 3.25 (m, 6H),

3.43 (t, 6H), 4.28 (q, *J* 7Hz, 3H), 5.23 (s, 6H), 7.40-7.51 (m, 15H). Anal. Calcd for $C_{72}H_{96}N_{16}O_{15}\cdot 3H_2O$: C, 58.44; H, 6.95; N, 15.15. Found: C, 58.59; H, 7.18; N, 15.50.

Tris-(2-(3-(2-(3-(1-Benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanamido)-2(*R*)-*sec*-butylethanamido)-propanamido)ethyl)amine (59b). The coupling of compound **58b** (1.26 g, 2.16 mmol) with tris(2-aminoethyl)amine (104 mg, 0.71 mmol) in DMF (25 mL) was carried out for 96 h at 38 °C. The crude product was purified by column and gel chromatographies as described for the preparation of **59a** to give the product (**59b**), 940 mg (85%): mp 165-172 °C (decomp.); $[\alpha]_D^{20}$ -4.15° (c 0.58, MeOH); ^{1}H NMR (DMSO-d$_6$) δ 0.80 (d, *J* 6 Hz, 9H), 0.86 (d, *J* 6 Hz, 9H), 1.41 (m, 6H), 1.55 (m, 3H), 2.18 (s, 9H), 2.24 (s, 9H), 2.24 (m, 6H), 2.50 (m, 6H), 2.90 (m, 6H), 3.07 (m, 6H), 3.30 (m, 12 H), 4.22 (m, 3H), 5.20 (s, 6H), 7.42-7.52 (m, 15H), 7.77 (br s, 3H), 7.90 (br s, 3H), 7.95 (d, *J* 8 Hz, 3H). Anal. Calcd for $C_{81}H_{114}N_{16}O_{15}\cdot 3H_2O$: C, 60.58; H, 7.53; N, 13.95. Found: C, 60.44; H, 7.40; N, 13.99.

Tris-(2-(3-(2-(3-(1-Hydroxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanamido)-2(*R*)-methylethanamido)-propanamido)ethyl)amine (27a). A suspension of 10% Pd-C (86 mg) in MeOH (10 mL) was prehydrogenated with H_2 for 0.5 h. To the suspension was added a solution of compound **59a** (157 mg, 0.11 mmol) in MeOH (40 mL). After hydrogenation with H_2 under atmospheric pressure for 1 h under reflux, the catalyst was removed by filtration. The filtrate was concentrated to give the residue. Purification of the residue by gel chromatography on Shephadex LH-20 (eluent: MeOH) afforded the product

(**27a**), 104 mg (84%): $[\alpha]_D^{25}$ -16.5° (c 0.11, MeOH); ^{1}H NMR (CD_3OD) δ 1.33 (d, *J* 7.1 Hz, 9H), 2.31 (s, 9H),2.38 (s, 9H), 2.40 (m, 6H), 2.64 (t, *J* 8.9 Hz, 6H), 3.01-3.05 (m, 12H), 3.43 (m, 12H), 4.27 (q, *J* 7.1 Hz, 3H). Anal. Calcd for $C_{51}H_{78}N_{16}O_{15}\cdot 8.5H_2O$: C, 46.82; H, 7.32; N, 17.13. Found: C, 46.98; H, 6.99; N, 16.79.

Tris-(2-(3-(2-(3-(1-Hydroxy-5,6-dimethyl-2-oxo-1,2-dihydro-pyraz-3-yl)propanamido)-2(*R*)-*sec*-butylethanamido) propanamido)ethyl)amine (27b). Compound **59b** (100 mg, 0.064 mmol) was hydrogenated with 10% Pd-C (45 mg) in MeOH (50 mL) in the presence of concd. HCl (17 μL) for 25 min at room temperature. Purification by gel chromatography on Shephadex LH-20 (eluent: MeOH) gave the product (**27b**), 56 mg (68%): mp 170-190 °C (decomp.); $[\alpha]_D^{22}$ -7.79° (c 1.0, MeOH); ^{1}H NMR (DMSO-d_6) δ 0.80 (d, *J* 7 Hz, 9H), 0.86 (d, *J* 7 Hz, 9H), 1.41 (t, *J* 7 Hz, 6H), 1.54 (m, 3H), 2.22 (s, 9H), 2.27 (s, 9H), 2.27 (m, 6H), 2.52 (m, 6H), 2.75 (m, 6H), 2.89 (m, 6H), 3.15 (m, 6H), 3.27 (m, 6H), 4.21 (q, *J* 7Hz, 3H), 7.95 (br s, 3H), 8.30 (br s, 6H).; (CD_3OD) δ 0.86 (d, *J* 7 Hz, 9H), 0.93 (d, *J* 7 Hz, 9H), 1.55 (m, 9H), 2.30 (s, 9H), 2.40 (s, 15H), 2.66 (m, 6H), 3.05 (m, 6H), 3.16 (m, 6H), 3.42 (m, 12H), 4.30 (t, *J* 7Hz, 3H). Anal. Calcd for $C_{60}H_{96}N_{16}O_{15}\cdot 3.0H_2O$: C, 53.95; H, 7.70. Found: C, 53.86; H, 7.47.

General procedure for the spectral measurement of 1:1 mixtures of iron(III) and hexadentate ligands. A sample (13-15 mg) of each hexadentate ligand was dissolved in deionized water (5.0 mL). The sample solution (1.0 mL) was mixed with an equimolar amount of ferric nitrate solution (3.28 mM) and diluted to 10.0 mL (0.3 mM). The pH of

the solution was adjusted to an appropriate value with 0.1 or 0.01M NaOH or 0.1 or 0.01M HNO_3 before spectral measurement.

General procedure for the mole ratio plots of the hexadentate ligands and iron(III). A sample (3 mg) of each hexadentate ligand was dissolved in deionized water (5.0 mL). 0.5 mL of the sample solution was mixed with an appropriate amount of a standard aqueous ferric nitrate solution (0.25-2.25 mL, 0.33 mM) and 0.5 mL of 0.4M KNO_3. The pH of the mixture was adjusted to 6.0 or 4.0 with 0.01 or 0.1M NaOH and diluted to 5.0 mL with McIlvaine's buffer (pH 6.0) or with acetate buffer (pH 4.0). Then the visible spectra were measured.

Iron(III) exchange reaction. Each iron(III) complex solution (0.195 mM) of the hexadentate ligands was prepared by mixing a stock solution of the ligand (1.3 mM) with an equimolar amount of ferric nitrate solution (3.28 mM) and 0.5 mL of 0.4M KNO_3, and then diluting to 5.0 mL with acetate buffer. An EDTA solution was prepared by dissolving $(EDTA)^{2-}\cdot 2Na^{+}\cdot 2H_2O$ in McIlvaine's buffer solution (ionic strength 0.04, pH 6.0) to give a concentration of 0.3 mM. Iron(III) exchange reaction was monitored by the decrease of absorbance at 450 nm. The relative stability constants of the iron(III) complexes were calculated by using the stability constant of Fe(edta) (logK 25.1),[23] the pKa of the corresponding bidentate ligand (pKa 4.7 of **10b** for **26** and **27**), and the pH of the solution at an equilibrium point at 20 °C.

Gallium complex formation. A sample of each hexadentate ligand (6-8 mg) and $Ga(NO_3)_3$ (4-5 mg) was dissolved in 10% CD_3OD/D_2O (1:9; 0.5 mL). The pD was adjusted to 6 with freshly prepared 0.4% NaOD in D_2O.[24] ^1H NMR spectrum was measured at room temperature. **Ga/27a**: δ

1.31 (d, *J* 7 Hz, 9H), 2.24 (m, 6H), 2.44 (s, 24H), 2.67 (m, 6H), 3.06 (m, 6H), 3.42 (m, 6H), 3.57 (m, 6H), 4.20 (m, 3H); **Ga/27b**: δ 0.86 (d, *J* 6 Hz, 9H), 0.92 (d *J* 6 Hz, 9H), 1.53 (m 6H), 2.23-2.27 (m, 6H), 2.43 (s, 21H), 2.47 (s, 9H), 2.75 (m, 12H), 3.10 (m, 6H), 3.45 (br s, 6H), 3.60 (m, 6H), 4.25 (m, 3H).

Iron removal from transferrin. The stock solutions of each hexadentate ligands (0.2-2.0 mL, 0.2-20 mM, pH 7.4) and **Tf**$_{Fe2.0}$ (2.0 mL, 0.03-0.05 mM) in Tris buffer were combined, and then the absorbance of the solution was monitored at 460 nm (500 nm for **26**, and 460 nm for **27**). The pseudo-first-order-rate constant (k_{obsd}) was calculated from the slope of the plots of log $[(A_\infty\text{-Abs})/(A_\infty\text{-A0})]$ as a function of time.

6.5 References

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Chapter 7

Hexadentate Ligands Bearing 1-Hydroxy-2(1*H*)-pyrazinone and D-Amino Acid Residues

7.1 Introduction

The microbial siderophore desferrioxamine B (DFB: trade name Desferal, Ciba-Geigy) is still the most effective therapeutic agent for removal of toxic quantities of iron from patients that receive regular transfusions.[1] Unfortunately, DFB cannot be administered orally and has a short half-life *in vivo* (5-10 min).[2] Therefore, much effort has been devoted to development of new sequestering agents that would have improved properties.

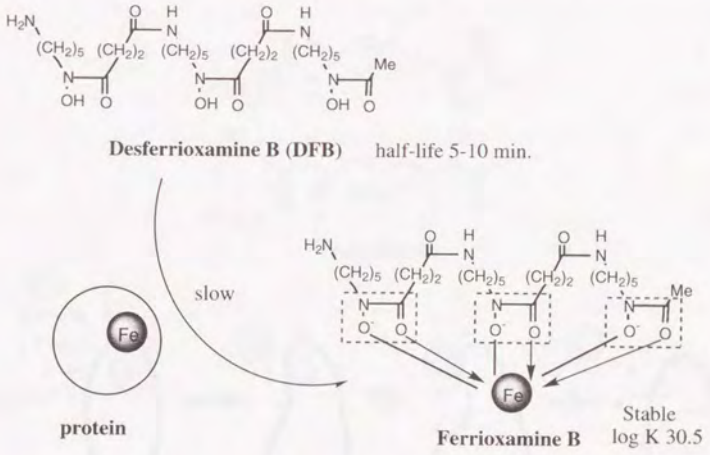
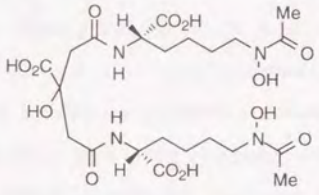


Figure 7-1

One of the problems of its iron chelation is a kinetic barrier in the release of iron from serum transferrin (iron transport protein), which prevents efficient transfer of iron to the chelator as shown in Figure 7-1.[3,4] Thus, the high kinetic efficiency of iron removal from the protein is requisite for acting as a new agent.

The kinetic studies of iron removal from transferrin have been reported,[5-11] and an interaction between chelators and transferrin on iron exchange process was suggested. The process was found to be catalyzed by pyrophosphate, which induced the conformation change of the protein. Neiland and co-workers demonstrated[5,6] that aerobactin can remove iron from transferrin *in vitro*, and they showed spectrophotometric evidence



Aerobactin

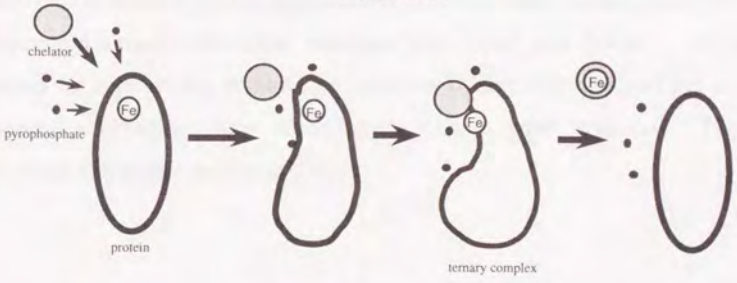


Figure 7-2

for ternary complex formation in the process of iron removal from the protein as shown in Figure 7-2. They suggested that the rate of iron exchange between transferrin and aerobactin is controlled by the rate of the conformational change of transferrin and the rate of dissociation of the complex. Cowart and co-workers also proposed the formation of a complex between the protein and the chelator; the complexation promotes a conformation change of the protein to expose the metal site.[7] On the other hand, Raymond and co-workers suggested that the rate of iron removal from transferrin strongly depends on anionic property of the chelators.[8] Thus, it is likely that the kinetic behavior of the chelators is closely related to not only its anionic property, but also the structural suitability to the protein. However, no paper concerning the relationship between the ligand structure including the chirality and the kinetic behavior has been reported.

As mentioned in Chapter 6, *N*-hydroxyamide-containing pyrazines showed the feasibility for iron sequestering agents under physiological conditions by virtue of their high water solubility and high acidity. It was also elucidated that chiral hexadenate chelators (**27**) composed of 1-hydroxy-5,6-dimethyl-2(1*H*)-pyrazinones, L-amino acid residues, and tris(2-aminoethyl)amine effectively removed iron from transferrin. As an attempt to explore the relationship between ligand structure and the iron removal efficiency, their enantiomers **66a**, **b** were prepared. Their structures are shown in Figure 7-3.

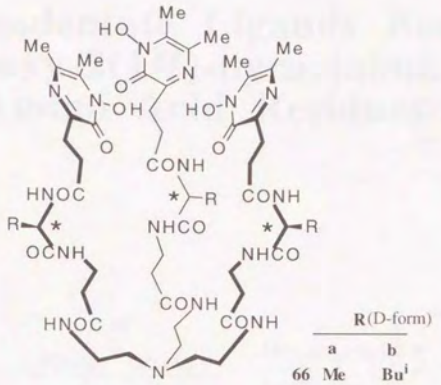
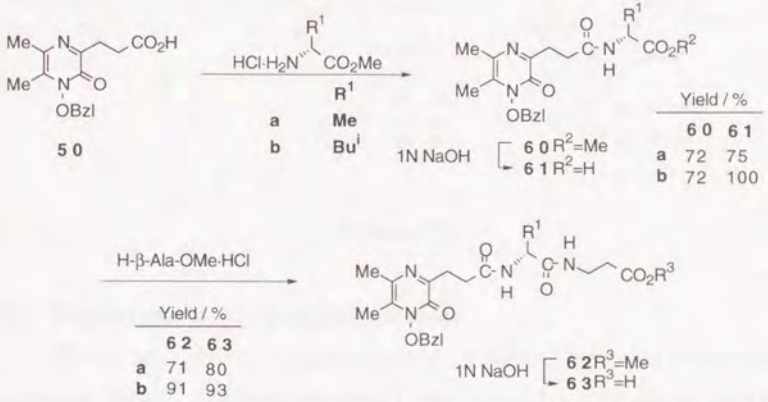


Figure 7-3

In this chapter, the author describes the difference in kinetic efficiency between the enantiomers on iron removal from transferrin.

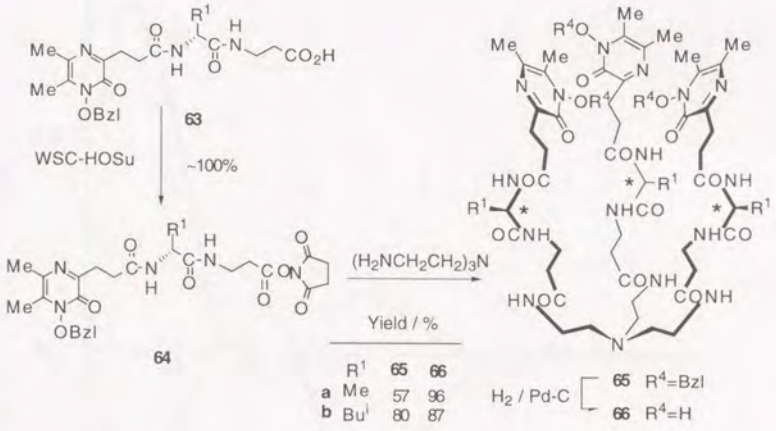
7.2 Hexadentate Ligands Bearing 1-Hydroxy-2(1*H*)-pyrazinone and D-Amino Acid Residues[12]

(a) Synthesis



Scheme 7-1

Synthesis of **66** was achieved by a similar fashion to the preparation of **27**[13] as shown in Schemes 7-1 and 7-2. D-Alanine (or D-leucine) and β-alanine were successively introduced to 1-benzyloxy-3-carboxyethyl-5,6-dimethyl-2(1*H*)-pyrazinone (**50**), and resulting dipeptide-linked pyrazinones **63** were converted to corresponding *O*-succinimide esters **64**. Compounds **64** were condensated with tris(2-aminoethyl)amine, followed by debenzylation by hydrogenation, to give **66**.



Scheme 7-2

(b) Configuration of iron(III) complex

UV-vis spectra of a 1:1 molar mixture of **66** and ferric ion in aqueous solutions showed characteristic LMCT band around at 450 nm (ε 3580 at 450 nm for **66a**; ε 3010 at 455 nm for **66b**), indicating the formation of intramolecular 1:1 iron complexes.

CD spectrum of the Fe/**66a** complex in an aqueous solution is shown in Figure 7-4 with the data of Fe/**27a** complex. The spectrum of Fe/**66a** complex has a negative band at 480 nm which is opposite to the band at 478 nm in Fe/**27a** complex. These bands arise from LMCT of the complexes and are therefore sensitive to the chirality at the metal center. On the basis of the result, the absolute configuration in Fe/**66a** complex should be assigned as Δ form (opposite to Fe/**27a** complex).

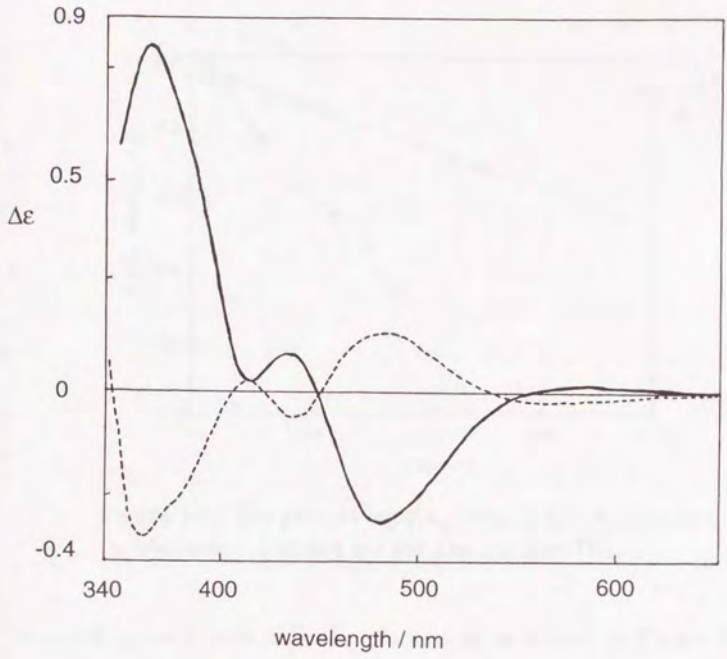


Figure 7-4. CD spectra of iron(III) complex of **66a** in aqueous solutions at pH 4.0: [Fe**66a**]=1.2 mM (-); [Fe**27a**]=0.3 mM (--).

(c) Iron removal from transferrin

Iron removing ability of **66a** from human diferric transferrin (**Tf**$_{Fe2.0}$) was evaluated at pH 7.4 by the same procedure as that for **27a**[13] as described in Chapter 6. The plots of $\log[(A_\infty - Abs)/(A_\infty - A_0)]$ as a

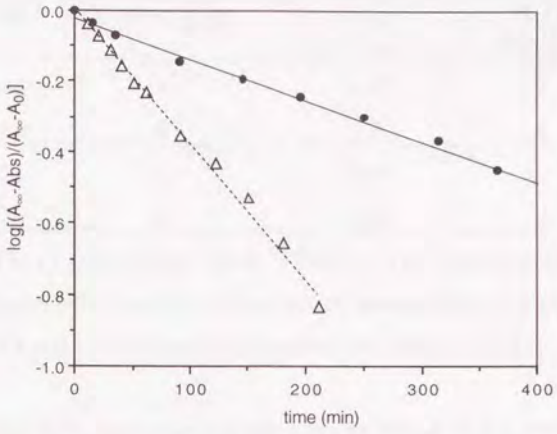


Figure 7-5. The plots of log $[(A_\infty - Abs)]/(A_\infty - A_0)]$ vs time on iron removal of **66a** (•) and **27a** (Δ) from $\mathbf{Tf}_{Fe2.0}$.

function of time gave a linear relationship as shown in Figure 7-5. It indicates that the iron removal from transferrin by **66a** proceeds in the pseudo-first-order kinetics. The k_{obs} was calculated from the slope of the straight line. The kinetic results are summarized in Table 1 with the data of **27a**, **b**. The rates of iron removal by **66a** were greater than that by desferrioxamine B, which is a currently used drug for iron overload (k_{obs} 0.66×10^{-3} min^{-1}; [L]/[$\mathbf{Tf}_{Fe2.0}$]=100),[14] even at a less concentration of the ligand. It is noteworthy that the rates of iron removal by alanine residue-containing ligands were significantly affected by the chirality of the free ligands. The rate of iron removal by **66a** was suppressed to only one fourth of **27a** (Figure 7-5). On the other hand, no apparent difference was observed in the case of leucine residue-containing **66b** and **27b**.

Table 1. Iron Removal from Transferrin at pH 7.4

Ligand	[L]/[**Tf**Fe2.0][a]	k_{obs} ($x10^{-3}$ min^{-1})	% Fe removed[b]
66a	5	1.05	7
66b	6	1.29	8
27a[c]	5	3.80	23
27b[c]	6	0.90	9

[a] [**Tf**Fe2.0]0=0.0368 mM. **Tf**Fe2.0 was prepared from commercially available human serum apotransferrin (Sigma).

[b] At a point 30 min after the reaction was initiated. [c]Ref. 13.

It is the first case demonstrating the influence of the chirality of the ligand upon the rate of iron removal from transferrin. Although the detail remains obscure, a plausible mechanism is proposed as follows.

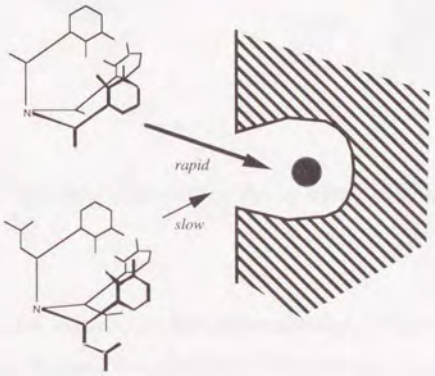


Figure 7-6. Schematic representation of the steric hindrance of the ligand upon the access to the metal site in protein.

The structural feature of the ligand would give an influence on the interaction between the protein and the ligand. A bulky substituent such as an Bu^i group may prevent access of the ligand to exposed metal site of the protein as shown in Figure 7-6. The iron removal process could be affected by the chirality of the ligand itself, or the absolute configuration (i.e. Λ and Δ) of the resulting iron complex of the ligand (Figure 7-7).

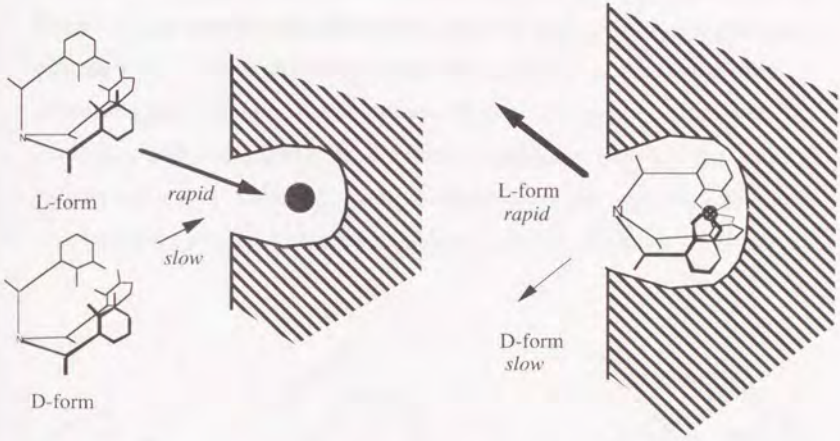


Figure 7-7. Schematic representation of the influence of chirality of the ligand.

It is reported that transferrin has anion-binding sites on its surface and that anion binding triggers the conformational change of the protein.[15-17] On the basis of these evidences, Kretchmar and co-workers[11] proposed that the kinetic efficiency of the reaction is related to the conformational change

of the protein, transferrin, during iron removal by strong sequestering agents. However, desferrioxamine B is no the case. Namely, desferrioxamine B does not act as a synergistic anion; it is thermodynamically capable of removing iron from transferrin but does not bind to the protein and trigger the conformational change.

N-Hydroxyamide-containing diazines, particularly pyrazinones, should be anionic under physiological conditions, due to their low pKa values compared to hydroxamic acid derivatives, such as desferrioxamine B. Therefore, the diazines would interact with the surface of the protein more efficiently than does desferrioxamine B, resulting in the more efficient conformational change of the protein (Figure 7-8 (a)). Moreover, the resulting iron complexes of diazines should be neutral; the neutral complexes easily released from the cavity of the protein, which is surrounded by anionic amino acid residues as shown in Figure 7-8 (b).

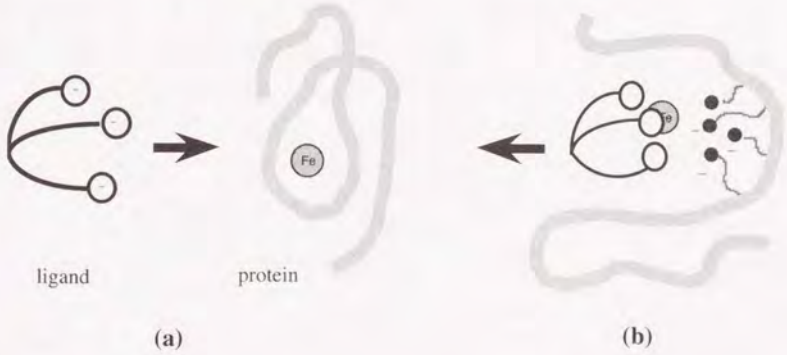


Figure 7-8. Schematic representation of "catch and release" model.

These results suggest that the kinetics of iron removal from transferrin by diazines did not simply depend on the stability of the iron complexes of the diazines; the conformational change of the protein, triggered by the interaction with the ligands, would play an important role in determining the kinetics. The lower pKa value and molecular shape of the ligands characterized by the substituents would give large influence on the process of the ternary complex formation between the ligands, iron, and the protein. Moreover, the fact that L-amino acid residues-containing ligand **27a** removed iron more effectively than the ligand bearing D-amino acid residues **66a** suggests that the chirality of the ligands is one of the important factors determining the kinetics; the ligands having the same chirality as natural environment are more feasible to fit the natural-occurring proteins.

7.3 Experimental

General. Melting points were determined on a Mel-Temp apparatus in open capillaries and are uncorrected. IR and UV-vis spectra were recorded on a JASCO A-100 infrared and on a JASCO Ubest V-550 spectrophotometers, respectively. ^1H NMR spectra were obtained on a JEOL GX-270 spectrometer in $CDCl_3$, DMSO-d_6 or CD_3OD solutions. Chemical shifts are reported in ppm (δ) downfield from internal TMS. Thin layer chromatography (TLC) was performed on silica gel 60 F-254 with a 0.2 mm layer thickness. Column chromatography was carried out with Merck Kieselgel 60 (230-400 mesh). Optical rotations were determined on a JASCO PIP-370 digital polarimeter. CD spectra were measured with a JASCO J-720 spectropolarimeter. HPLC was carried out on a JASCO 880-PU and a 875-UV equipped with a JASCO IT integrator by using a column packed with a Finepak SIL $C_{12}S$. Combustion analyses were performed on a YANACO MT-3 CHN corder.

1-Benzyloxy-3-carboxyethyl-5,6-dimethyl-2(1*H*)-pyrazinone (**50**) was prepared by the same procedure as that described in Chapter 6.

***N*-3-(1-Benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanoyl-D-alanine methyl ester (60a).** A solution of 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (water soluble carbodiimide: WSC·HCl; 1.28 g, 6.1 mmol) in CH_2Cl_2 (10 mL) was added to a mixture of **50** (1.84 g, 6.1 mmol), H-D-Ala-OMe·HCl (934 mg, 6.7 mmol), *N*-methylmorpholine (678 g, 6.7 mmol), and HOBt (1.03 g, 6.7

mmol) in DMF (5 mL) at -10 °C. The mixture was stirred overnight at room temperature. After removal of DMF under reduced pressure, the residue was dissolved in AcOEt (300 mL). The organic layer was successively washed with water, 4% $NaHCO_3$, 10% citric acid, and brine, and dried over anhydrous $MgSO_4$. Evaporation of the solvent, followed by purification by recrystallization from AcOEt-petroleum ether, gave the product; 1.7 g (72%): mp 115-118 °C; $[\alpha]_D^{22}$ +20.5° (c 0.5, MeOH); ^1H NMR ($CDCl_3$) δ 1.39 (d, *J* 7 Hz, 3H), 2.16 (s, 3H), 2.25 (s, 3H), 2.69 (m, 2H), 3.18 (m, 2H), 3.72 (s, 3H, OMe), 4.60 (m, 1H), 5.24 (s, 3H), 6.74 (d, *J* 7 Hz, 1H), 7.38-7.49 (m, 5H). Anal. Calcd for $C_{20}H_{25}N_3O_5 \cdot 0.5H_2O$: C, 60.59; H, 6.61; N, 10.06. Found: C, 60.71; H, 6.36; N, 10.05.

***N*-3-(1-Benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanoyl-D-leucine methyl ester (60b).** The coupling of compound **50** (1.53 g, 5.06 mmol) and H-D-Leu-OMe·HCl (990 mg, 5.45 mmol) was carried out by a similar procedure to the preparation **60a** to give the product (**60b**); 1.56 g (72%): mp 108-110 °C; ^1H NMR ($CDCl_3$) δ 0.93 (d, *J* 7 Hz, 6H), 1.58 (m, 3H), 2.20 (s, 3H), 2.25 (s, 3H), 2.71 (t, *J* 7 Hz, 2H), 3.19 (t, *J* 7 Hz, 2H), 3.71 (s, 3H), 4.61 (m, 1H), 5.25 (s, 2H), 6.61 (d, *J* 7 Hz, 1H), 7.41-7.51 (m, 5H). Anal. Calcd for $C_{23}H_{31}N_3O_5 \cdot 0.5H_2O$: C, 62.99; H, 7.35; N, 9.58. Found: C, 63.32; H, 7.36; N, 9.76.

***N*-3-(1-Benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanoyl-D-alanine (61a).** To a solution of compound **60a** (610 mg, 1.61 mmol) in MeOH (20 mL) was added 1M NaOH (4.8 mL, 4.8 mmol) at 0 °C. After being stirred for 3 min at room temperature, the mixture was neutralized and then concentrated. The residual aqueous solution was acidified with 5M HCl at 0 °C, then extracted with $CHCl_3$ (100

mL). The organic layer was successively washed with 10% citric acid and brine, and dried over anhydrous $MgSO_4$. Evaporation of the solvent, followed by recrystallization from benzene-hexane, gave the product (**61a**) as pale yellow crystals; 440 mg (75%): mp 140-142 °C; $[\alpha]_D^{23}$ +7.4° (c 0.5, MeOH); ^1H NMR ($CDCl_3$) δ 1.46 (d, *J* 7 Hz, 3H), 2.21 (s, 3H), 2.25 (s, 3H), 2.73 (t, *J* 7 Hz, 2H), 3.20 (t, *J* 7 Hz, 2H), 4.54 (m, 1H), 5.24 (s, 2H), 7.15 (d, *J* 5 Hz, 1H), 7.39-7.48 (m, 5H). Anal. Calcd for $C_{19}H_{23}N_3O_5 \cdot 0.7H_2O$: C, 59.12; H, 6.37; N, 10.89. Found: C, 59.30; H, 6.25; N, 10.59.

***N*-3-(1-Benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanoyl-D-leucine (61b).** The hydrolysis of **60b** (1.34 g, 3.12 mmol) with 1M NaOH was carried out by a similar procedure to the preparation of **61a** to give the product (**61b**) as yellow oil; 1.43 g (100%): ^1H NMR ($CDCl_3$) δ 0.92 (d, *J* 6 Hz, 3H), 0.94 (d, *J* 6 Hz, 3H), 1.70 (m, 3H), 2.21 (s, 3H), 2.25 (s, 3H), 2.73 (t, *J* 7 Hz, 2H), 3.19 (t, *J* 7 Hz, 2H), 4.55 (m, 1H), 5.24 (s, 2H), 6.97 (d, *J* 7 Hz, 1H), 7.39-7.49 (m, 5H).

***N*-3-(1-Benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanoyl-D-alanyl–β–alanine methyl ester (62a).** Compound **61a** (502 mg, 1.34 mmol) was coupled with H-β–Ala-OMe·HCl (207 mg, 1.48 mmol) in the presence of HOBt (226 mg, 1.48 mmol), *N*-methylmorpholine (150 mg, 1.48 mmol), WSC·HCl (257 mg, 1.34 mmol) by the same procedure for the preparation of **60a**. The crude product was purified by column chromatography on silica gel (eluent: $CHCl_3$-acetone-EtOH=100:40:8) to give the pure product (**62a**); 440 mg (71%): mp 144-148 °C; $[\alpha]_D^{20}$ +18.7 ° (c 0.67, MeOH); ^1H NMR ($CDCl_3$) δ 1.35 (d, *J* 7Hz, 3H), 2.20 (s, 3H), 2.25 (s, 3H), 2.51 (t, *J* 7 Hz, 2H), 2.70 (t, *J* 7 Hz,

2H), 3.18 (m, 2H), 3.59 (t, *J* 7 Hz, 2H), 3.69 (s, 3H), 4.42 (q, J 7 Hz, 1H), 5.25 (s, 2H), 6.74 (br s, 1H), 6.77 (br s, 1H), 7.30-7.49 (m, 5H). Anal. Calcd for $C_{23}H_{30}N_4O_6 \cdot 0.8H_2O$: C, 58.42; H, 6.73; N, 11.85. Found: C, 58.11; H, 6.31; N, 12.28.

***N*-3-(1-Benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanoyl-D-leucyl–β–alanine methyl ester (62b).** The coupling of **61b** (330 mg, 0.79 mmol) with H-β–Ala-OMe·HCl (127 mg, 0.91 mmol) was carried out by a similar procedure to the preparation of **62a** to give the product (**62b**); 360 mg (91%): mp 142-148 °C; ^{1}H NMR ($CDCl_3$) δ 0.90 (d, *J* 4.3 Hz, 3H), 0.92 (d, *J* 4.3 Hz, 3H), 1.51-1.70 (m, 3H), 2.20 (s, 3H), 2.25 (s, 3H), 2.51 (t, *J* 6.3 Hz, 2H), 2.68 (t, *J* 7.3 Hz, 2H), 3.17 (t, *J* 7.3 Hz, 2H), 3.48 (m, 2H), 3.68 (s, 3H), 4.40 (m, 1H), 5.24 (s, 2H), 6.70 (d, *J* 9.0 Hz, 1H), 6.82 (br s, 1H), 7.39-7.50 (m, 5H). Anal. Calcd for $C_{26}H_{36}N_4O_6 \cdot 1.0H_2O$: C, 60.21; H, 7.39; N, 10.80. Found: C, 60.22; H, 7.19; N, 10.79.

***N*-3-(1-Benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanoyl-D-alanyl–β–alanine (63a).** Compound **62a** (440 mg, 0.96 mmol) was hydrolyzed by a similar procedure to the preparation of **61a** to give the product (**63a**); 340 mg (80%); mp 160-162 °C; $[\alpha]_D^{22}$ +19.8° (c 0.50, MeOH); ^{1}H NMR (CD_3OD) δ 1.33 (d, *J* 7 Hz, 3H), 2.25 (s, 3H), 2.26 (s, 3H), 2.49 (t, *J* 7 Hz, 2H), 2.68 (t, *J* 7 Hz, 2H), 3.10 (t, *J* 7 Hz, 2H), 3.42 (t, *J* 7 Hz, 2H), 4.30 (q, *J* 7 Hz, 1H), 5.25 (s, 2H), 7.39-7.52 (m, 5H). Anal. Calcd for $C_{22}H_{28}N_4O_6 \cdot 0.5H_2O$: C, 58.66; H, 6.49; N, 12.44. Found: C, 58.79; H, 6.69; N, 12.17.

***N*-3-(1-Benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanoyl-D-leucyl–β–alanine (63b).** Compound **62b** (502 mg,

1.0 mmol) was hydrolyzed by a similar procedure to the preparation of **63a** to give the product (**63b**); 454 mg (93%): mp 154-155 °C; ^{1}H NMR ($CDCl_3$) δ 0.86 (d, *J* 6 Hz, 3H), 0.89 (d, *J* 6 Hz, 3H), 1.58 (m, 3H), 2.20 (s, 3H), 2.26 (s, 3H), 2.54 (m, 2H), 2.71 (m, 2H), 3.16 (m, 2H), 3.49 (m, 2H), 4.70 (m, 1H), 5.24 (s, 2H), 6.88 (d, *J* 7 Hz, 1H), 7.41-7.47 (m, 5H), 7.60 (m, 1H). Anal. Calcd for $C_{25}H_{34}N_4O_6 \cdot 0.5H_2O$: C, 60.59; H, 7.19; N, 11.31. Found: C, 60.28; H, 7.00; N, 11.17.

***N*-3-(1-Benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanoyl-D-alanyl–β–alanine *O*-succinimide ester (64a).** WSC·HCl (129 mg, 0.68 mmol) in CH_2Cl_2 (2 mL) was added to a mixture of compound **63a** (300 mg, 0.68 mmol) and HOSu (155 mg, 1.35 mmol) in DMF (3 mL) at -10 °C. After being stirred for 24 h at room temperature, the solvent was removed under reduced pressure, and the residue was dissolved in $CHCl_3$ (200 mL). The organic layer was successively washed with H_2O and brine, and dried over anhydrous $MgSO_4$. Evaporation of the solvent gave the *O*-succinimide ester (**64a**) as pale yellow crystals, which was used for the next reaction without further purification, 300 mg (82 %); IR ($CHCl_3$) 1820, 1790, 1740 cm^{-1}.

***N*-3-(1-Benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanoyl-D-leucyl–β–alanine *O*-succinimide ester (64b).** Compound **63b** (382 mg, 0.79 mmol) was coupled with HOSu (181 mg, 1.58 mmol) by a similar procedure to the preparation of **64a** to give the corresponding *O*-succinimide ester (**64b**) as yellow crystals, which was used directly for the next reaction, 310 mg (68%): IR ($CHCl_3$) 1839, 1800, 1744 cm^{-1}.

Tris-(2-(3-(2-(3-(1-Benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanamido)-2(*S*)-methylethanamido)-propanamido)ethyl)amine (65a). A solution of compound **64a** (300 mg, 0.55 mmol) and tris(2-aminoethyl)amine (25 mg, 0.17 mmol) in DMF (3 mL) was stirred for 48 h at 38 °C. After removal of the solvent, $CHCl_3$ (300 mL) and 0.1M NaOH (50 mL) was added to the residue. The organic layer was successively washed with H_2O, and brine, and dried over anhydrous Na_2SO_4. Purification by column chromatography on silica gel (eluent: $CHCl_3$-MeOH=6:1), follwed by gel chromatography on TOYOPEARL HW-40 (eluent: MeOH), gave the product (**65a**); 140 mg (57%): mp 175-179 °C (decomp.); $[\alpha]_D^{25}$ +15.1 ° (c 0.18, MeOH); ^1H NMR (DMSO-d_6) δ 1.18 (d, *J* 7Hz, 9H), 2.18 (s, 9H), 2.23 (s, 9H), 2.26 (t, *J* 7 Hz, 6H), 2.52 (t, *J* 7 Hz, 6H), 2.94 (t, *J* 7 Hz, 6H), 3.14 (m, 6H), 3.26 (m, 6H), 4.23 (q, *J* 7Hz, 3H), 5.21 (s, 6H), 7.40-7.50 (m, 15H), 7.65 (m, 6H), 7.80 (d, *J* 7 Hz, 3H). Anal. Calcd for $C_{72}H_{96}N_{16}O_{15}\cdot 2.5H_2O$: C, 58.80; H, 6.92; N, 15.24. Found: C, 58.59; H, 7.18; N, 15.76.

Tris-(2-(3-(2-(3-(1-Benzyloxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanamido)-2(*S*)-*sec*-butylethanamido)-propanamido)ethyl)amine (65b). The coupling of compound **64b** (316 mg, 0.54 mmol) with tris(2-aminoethyl)amine (20 mg, 0.14 mmol) in DMF (3 mL) was carried out for 48 h at 38 °C. The crude product was purified by column and gel chromatographies as described for the preparation of **65a** to give the product as an amorphous solid (**65b**); 170 mg (80%): ^1H NMR (CD_3OD) δ 0.88 (d, *J* 6 Hz, 9H), 0.93 (d, *J* 6 Hz, 9H), 1.56 (m, 9H), 2.24 (s, 18H), 2.43 (m, 6H), 2.69 (m, 12H), 3.06 (m, 6H), 3.26 (m, 6H), 3.45 (m, 6H), 4.34 (m, 3H), 5.23 (s, 6H), 7.40-7.49 (m, 15H).

Tris-(2-(3-(2-(3-(1-Hydroxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanamido)-2(*S*)-methylethanamido)-propanamido)ethyl)amine (66a). A suspension of 10% Pd-C (25 mg) in MeOH (10 mL) was prehydrogenated with H_2 for 0.5 h. To the suspension was added a solution of compound **65a** (118 mg, 0.08 mmol) in MeOH (40 mL). After hydrogenation with H_2 under atmospheric pressure for 1 h under reflux, the catalyst was removed by filtration. The filtrate was concentrated to give the residue. Purification of the residue by gel chromatography on Shephadex LH-20 (eluent: MeOH) afforded the product (**66a**), 89 mg (96%): $[\alpha]_D^{22}$ +15.9 ° (c 0.30, MeOH); ^{1}H NMR (CD_3OD) δ 1.33 (d, *J* 7 Hz, 9H), 2.33 (s, 9H),2.41 (s, 9H), 2.44 (t, *J* 7 Hz, 6H), 2.66 (t, *J* 7 Hz, 6H), 3.03 (t, *J* 7 Hz, 6H), 3.35 (m, 12H), 3.59 (m, 6H), 4.34 (q, *J* 7 Hz, 3H). Anal. Calcd for $C_{51}H_{78}N_{16}O_{15}\cdot 5H_2O$: C, 49.19; H, 7.12; N, 18.00. Found: C, 49.45; H, 6.90; N, 17.83.

Tris-(2-(3-(2-(3-(1-Hydroxy-5,6-dimethyl-2-oxo-1,2-dihydropyraz-3-yl)propanamido)-2(*S*)-*sec*-butylethanamido) propanamido)ethyl)amine (66b). Compound **65b** (36 mg, 0.023 mmol) was hydrogenated with 10% Pd-C (10 mg) in MeOH (30 mL) for 1 h at room temperature. Purification by gel chromatography on Shephadex LH-20 (eluent: MeOH) gave the product as an amorphous solid (**66b**), 26 mg (87%): $[\alpha]_D^{25}$ +9.07° (c 0.19, MeOH); ^{1}H NMR (CD_3OD) δ 0.87 (t, *J* 6 Hz, 9H), 0.92 (d, *J* 6 Hz, 9H), 1.55 (m, 9H), 2.32 (s, 9H), 2.39 (s, 9H), 2.40 (m, 6H), 2.66 (t, *J* 7 Hz, 6H), 3.04 (m, 12H), 3.43 (m, 12H), 4.30 (t, *J* 7Hz, 3H). Anal. Calcd for $C_{60}H_{96}N_{16}O_{15}\cdot 3H_2O$: C, 53.96; H, 7.70; N, 16.78. Found: C, 54.05; H, 7.56; N, 17.12.

General procedure for the spectral measurement of 1:1 mixtures of iron(III) and hexadentate ligands. A sample (13-15 mg) of each hexadentate ligand was dissolved in deionized water (5.0 mL). The sample solution (1.0 mL) was mixed with an equimolar amount of ferric nitrate solution (3.28 mM) and diluted to 10.0 mL (0.3 mM). The pH of the solution was adjusted to an appropriate value with 0.1 or 0.01M NaOH or 0.1 or 0.01M HNO_3 before spectral measurement.

Iron removal from transferrin. The stock solutions of each hexadentate ligand (0.2-2.0 mL, 0.2-20 mM, pH 7.4) and **Tf**Fe2.0 (2.0 mL, 0.03-0.05 mM) in Tris buffer were combined, and then the absorbance of the solution was monitored at 460 nm. The pseudo-first-order-rate constant (k_{obsd}) was calculated from the slope of the plots of log $[(A_\infty\text{-Abs})/(A_\infty\text{-}A0)]$ as a function of time.

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Chapter 8

Conclusions

In this thesis, the author described the synthesis, application to organic synthesis, reaction, and iron chelating properties of *N*-hydroxyamide-containing diazines.

This study established the syntheses of 1-hydroxy-2(1*H*)-pyrimidinones and -pyrazinones, which showed remarkable high water solubility and acidity.

This study revealed the nucleophilic and photoreactivities of 1-benzyloxy-2(1*H*)-pyrimidinones and -pyrazinones; the ring transformation of pyrimidinones with hydroxylamine and the N-O bond photocleavage reaction of pyrazinones with high quantum yields.

As an application of 1-hydroxy-2(1*H*)-pyrimidinones and -pyrazinones in organic synthesis, the diazines were used as acylating agents for amines and alcohols and found to be very effective owing to their high acidity and water solubility.

These diazines acted as bidentate ligands for ferric ion in aqueous solutions. However, the stability of their iron complexes was very low. In order to overcome this problem and to apply the diazines to the synthesis of artificial siderophores, tripodal hexadentate ligands, which is the simplest enterobactin model, were synthesized upon introducing a functional group to

the heterocyclic ring system. Their iron removal ability from human transferrin was also elucidated. From the viewpoint of the potential application of the ligands to iron overload disease, two factors are important for the removal of iron from transferrin; the thermodynamic and kinetic abilities of such sequestering agents. Although *N*-hydroxyamide-containing diazines were not suitable for thermodynamic chelation due to their low pKa values, their kinetic ability to remove iron was greater than that of commercially available desferrioxamine B. Thus, the kinetic ability does depend on the interaction process for the formation of the ternary complex between the ligand, iron, and the protein, for which the molecular shape and anionic property of the ligands significantly affect. In other words, the iron removal ability of the ligands from the protein could not be estimated only on the basis of the thermodynamic chelation property. The influence of chirality of the ligands was also observed on the iron removal reaction; L-amino acid residues-containing ligands showed more kinetic iron removal ability than do D-amino acid residues-containing ones; synthetic ligands having the same chirality of natural environment are more feasible to fit the natural-occuring proteins.

In conclusion, this study provided significant data for understanding the fundamental properties of *N*-hydroxyamide-containing diazines. Furthermore, intensive studies on the synthesis and functional evaluation of the novel heterocyclic ligands gave many valuable informations in the field of bioinorganic chemistry.

List of Publications

(1) *N*-Hydroxyamide-Containing Heterocycles. New Effective Additives for Peptide Synthesis by the Dicyclohexylcarbodiimide Method
Akira Katoh, Junko Ohkanda, Yoshifumi Itoh, and Keiryo Mitsuhashi
Chemistry Lett., **1992**, 2009.

(2) *N*-Hydroxyamide-containing Heterocycles. Part 2. Synthesis and Iron(III) Complex-Forming Tendency of 1-Hydroxy-2(1*H*)-pyrimidinone and -pyrazinone
Junko Ohkanda, Takeshi Tokumitsu, Keiryo Mitsuhashi, and Akira Katoh
Bull. Chem. Soc. Jpn., **1993**, *66*, 841.

(3) *N*-Hydroxyamide-Containing Heterocycles. Part 3. The Ring Trasformation of 1-Benzyloxy-2(1*H*)-Pyrimidinones into 2-Isoxazolines with Hydroxylamine
Akira Katoh, Junko Ohkanda, Atsushi Tamura, Yuji Yoshiike, and Keiryo Mitsuhashi
Heterocycles, **1994**, *37*, 1141.

(4) *N*-Hydroxyamide-Containing Heterocycles. 4. Synthesis and Fe^{III}-Chelating Properties of Novel Hexadentate Ligands Composed of *N*-

Hydroxy-2(1*H*)-Pyrazinone, Amino Acid Residues, and Tris(2-aminoethyl)amine
Junko Ohkanda and Akira Katoh
J. Org. Chem., **1995**, *60*, 1583.

(5) *N*-Hydroxyamide-Containing Heterocycles. Part 5. Synthesis of Novel Hexadentate Ligands Composed of *N*-Hydroxypyrazinone, Aliphatic Diamine, and 1,1,1-Tris(carboxyethoxymethyl)ethane and Spectroscopic Study of Their Ferric Complexes
Junko Ohkanda, and Akira Katoh
Tetrahedron, **1995**, *51*, 12995.

(6) *N*-Hydroxyamide-Containing Heterocycles. Part 6. Application of 1-Hydroxy-4,6-dimethyl-2(1*H*)-pyrimidinone to A New Benzyloxycarbonylating Agent
Akira Katoh, Syoichi Kondoh, and Junko Ohkanda
Heterocyclic Commun., **1996**, *2*, 141.

(7) *N*-Hydroxyamide-Containing Heterocycles. Part 7. Preparation and Photochemical Behavior of 1-Benzyloxy-2(1*H*)-pyrazinones
Junko Ohkanda, Toshihiko Kumasaka, Aki Takasu, Tadashi Hasegawa, and Akira Katoh
Heterocycles, **1996**, *43*, 883.

(8) The Influence of the Chirality of Synthetic Iron Chelators Bearing *N*-Hydroxy-2(1*H*)-pyrazinones and Amino Acid Residues upon Iron Removal from Human Transferrin
Junko Ohkanda and Akira Katoh
Chemistry Lett., **1996**, 423.

(9) *N*-Hydroxyamide-Containing Heterocycles. Part 8. Synthesis and Evaluation of Iron Chelating Ability of Novel Effective Iron Sequestering Agent composed of *N*-Hydroxy-2(1*H*)-pyrimidinone
Junko Ohkanda, Jun Kamitani, Takeo Konakahara, Yoko Hida, and Akira Katoh
In preparation.

(10) *N*-Hydroxyamide-Containing Heterocycles. Part 9. Synthesis of Novel Hexadentate Ligands Composed of *N*-Hydroxypyrimidinone, Aliphatic Diamine, and 1,1,1-Tris(carboxyethoxymethyl)ethane and Spectroscopic Study of Their Ferric Complexes
Junko Ohkanda, Yoko Hida, and Akira Katoh
In preparation.

Miscellaneous papers:

(1) Cis-trans Isomerization of Stilbene by Illumination of Sun-light-Teaching Material for Photochemical Experiment in High school
Tadashi Hasegawa, Junko Ohkanda, and Toyokazu Usui
Kagaku to Kyoiku, **1990**, *38*, 72.

(2) Unusual Substituent Effect by the α-Acetyl Group on the Type II Photoreaction of Valerophenones: Strong Wavelength Dependence of Quantum Yields
Tadashi Hasegawa, Junko Ohkanda, Mariko Kobayashi, and Michikazu Yoshioka
J. Photochem. Photobiol. A: Chem., **1992**, *64*, 299.

(3) Structural Aspects of the Mechanical and Thermal Dissociation of the Central Bond in 2,2'-Bis(2,3,4-triarylchromenyl)s
Junko Ohkanda, Yukie Mori, Koko Maeda, and Eiji Osawa
J. Chem. Soc., Perkin Trans. 2, **1992**, 59.

(4) Unusual Reactivity on the Reaction of *N*-Alkoxyureas with β-Diketones
Akira Katoh, Takeshi Tokumitsu, and Junko Ohkanda
Technical Reports of Seikei Univ., **1992**, *53*, 47.

(5) Isolation of the Intermediates and Improved Synthesis of Pyrido[1', 2':1,2]imidazo[4,5-*b*]pyrazines and -quinoxalines

Akira Katoh, Shuhei Ueda, Junko Ohkanda, Mutsumi Hirota, Motohiro Komine, and Keiryo Mitsuhashi
Heterocycles, **1992**, *34*, 1965.

(6) Synthesis of Unnatural Amino Acid Containing 4,6-Dimethyl-2(1*H*)-pyrimidinone
Akira Katoh, Mika Inokawa, Ken Yamazaki, and Junko Ohkanda
Technical Reports of Seikei Univ., **1993**, *55*, 47.

(7) A Convenient Syhthesis of 3-(Aryl)substituted 2,4(1*H*, 3*H*)-Pteridinediones and Their Absorption and Fluorescence Spectroscopic Characteristics
Akira Katoh, Junko Ohkanda, Hiroshi Sato, Tsuyoshi Sakamoto, and Keiryo Mitsuhashi
Heterocycles, **1993**, *35*, 949.

(8) Final stage of the Lab. Work -Cleaning a Lab. Up-
Junko Ohkanda
Kagaku to Kyoiku, **1993**, *41*(8), 526.

(9) Reaction of 2,3-Dichloro-6-nitroquinoxaline with Various Amines
Akira Katoh, Shuhei Ueda, Toshihiko Yahagi, and Junko Ohkanda
Technical Reports of Seikei Univ., **1994**, *31*(1), 35.

(10) Synthesis of 2(1*H*)-Pyrimidinone-containing α-Amino acid Derivatives by Chemical Modification of L-Glutamic Acid

Akira Katoh, Mika Inokawa, Junko Ohkanda, and Keiryo Mitsuhashi
Chem. Pharm. Bull., **1994**, *42*, 1514.

(11) Carboxymethylation of Phenolic Hydroxy Group of L-Tyrosine
Akira Katoh, Sadamitsu Jyumonji, and Junko Ohkanda
Technical Reports of Seikei Univ., **1995**, *32*(2), 53.

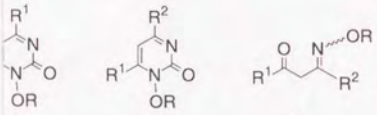
(12) Reaction of 2,3-Dichloro-6-nitroquinoxaline with Amino Acids
Akira Katoh, Yoshiyuki Fujisaki, and Junko Ohkanda
Technical Reports of Seikei Univ., **1995**, *32*(2), 57.

(13) Synthesis of Novel Amphiphilic Compounds Containing Aza-12-crown-4 or D-Glucosamine and Their Ion Permeability
Akira Katoh, Shin-ichi Ishida, Junko Ohkanda, and Masao Washizu
Heterocycles, **1996**, *43*, 589.

(14) Synthesis of 2,3-Substituted 6-Aminoquinoxalines and Their Application to New Fluorescence Derivatization Reagents for Carboxylic Acids
Akira Katoh, Motoki Takahashi, and Junko Ohkanda
Chemistry Lett., **1996**, 369.

(15) Synthesis and Structural Analysis of *N*-(ω-Phenylalkyl)substituted Quinoxalin-2(1*H*)-ones and -thiones
Akira Katoh, Tohru Yoshida, Junko Ohkanda, and Takehiko Nishio
Heterocycles, **1996**, in press.

s of Compounds



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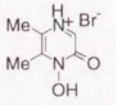
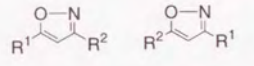


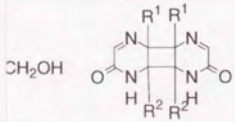
10b·HBr

	R^1	R^2
a	H	H
b	Me	Me
c	Me	H
d	Ph	Ph
e	-$(CH_2)_4$-	



12 12'

	R^1	R^2
b	Me	H
c	Me	Me
d	Et	Me



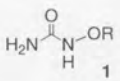
16 17

	R^1	R^2
a	H	H
b	Me	Me
c	Me	H
d	Ph	Ph
e	-$(CH_2)_4$-	

X-R

	XR		
a	$NHCH_2Ph$	g	L-Phe-OMe
b	NHPh	h	OCH_2Ph
c	pyrrolidine	i	OPr^n
d	morpholine	j	$OCH(Et)_2$
e	L-Ala-OH	k	OPh
f	L-Ala-OMe		

General Notations of Compounds



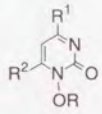
1

R

a Bzl

b Me

	R^1	R^2
a	H	H
b	Me	H
c	Me	Me
d	Et	Me
e	Ph	Me
f	Ph	Me (R=Me)



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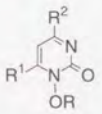

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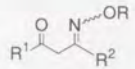

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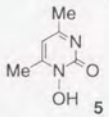

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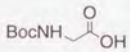

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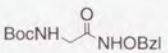

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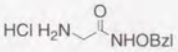

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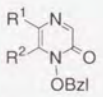

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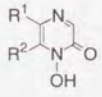

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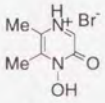
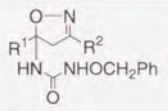


10b·HBr

	R^1	R^2
a	H	H
b	Me	Me
c	Me	H
d	Ph	Ph
e		$-(CH_2)_4-$



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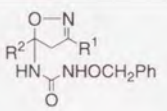

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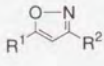

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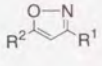
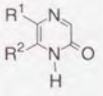


12'

	R^1	R^2
b	Me	H
c	Me	Me
d	Et	Me



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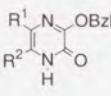

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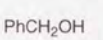

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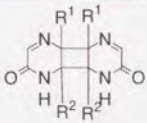
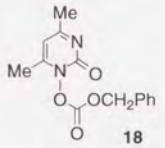


17

	R^1	R^2
a	H	H
b	Me	Me
c	Me	H
d	Ph	Ph
e		$-(CH_2)_4-$



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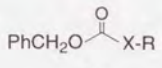
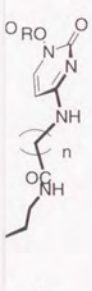
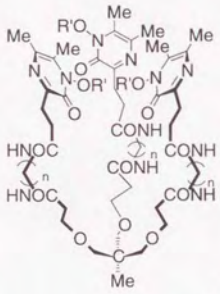


19

	XR		
a	$NHCH_2Ph$	g	L-Phe-OMe
b	NHPh	h	OCH_2Ph
c	pyrrolidine	i	OPr^n
d	morpholine	j	$OCH(Et)_2$
e	L-Ala-OH	k	OPh
f	L-Ala-OMe		

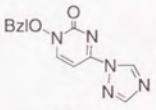


	n
a	5
b	6
c	7



53 R'=Bzl
26 R'=H

	n
a	2
b	4
c	5
d	6



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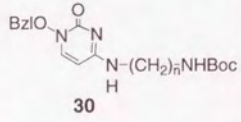

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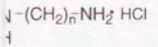
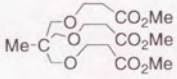


	n
a	2
b	4
c	6

33



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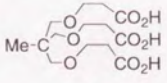

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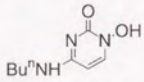

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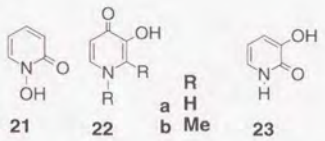
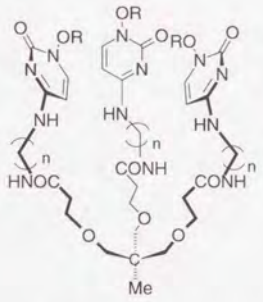
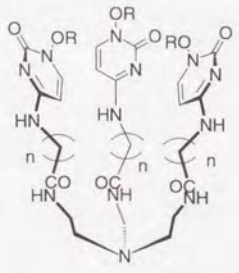


21 22 23

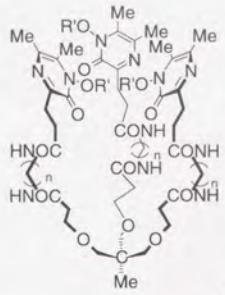
R
a H
b Me



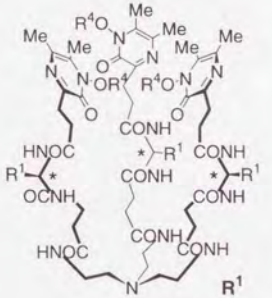
R n
37 Bzl a 2
24 H b 4
c 6



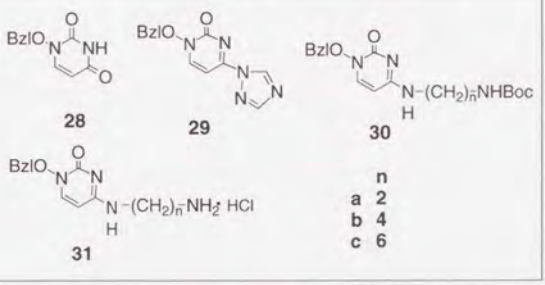
R n
44 Bzl a 5
25 H b 6
c 7



53 R'=Bzl n
26 R'=H a 2
b 4
c 5
d 6



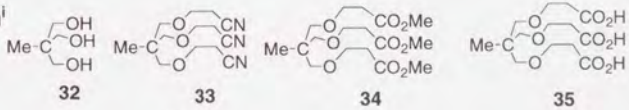
59 R^4=Bzl R1
27 R^4=H a Me
b Bu^i



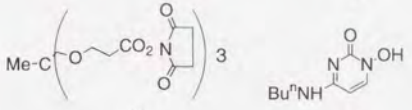
28 29 30

31

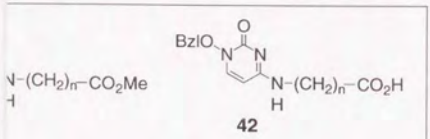
n
a 2
b 4
c 6



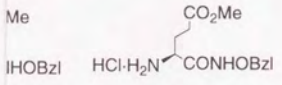
32 33 34 35



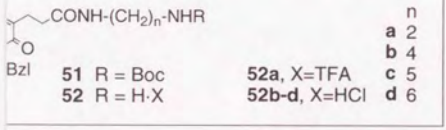
36 38



42

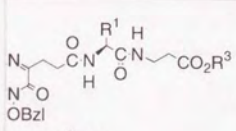


48

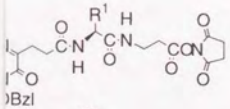


51 R = Boc **52a**, X=TFA
52 R = H·X **52b-d**, X=HCl

	n
a	2
b	4
c	5
d	6



56 R^3=Me
57 R^3=H



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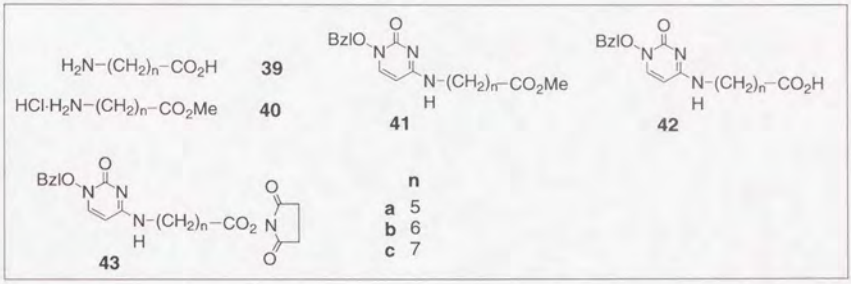
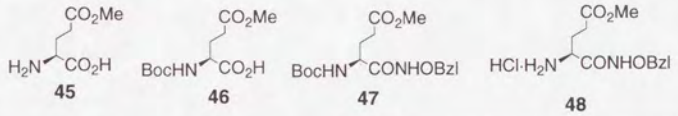
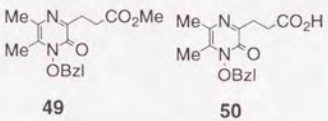
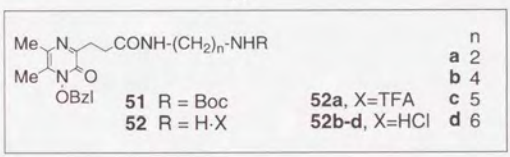
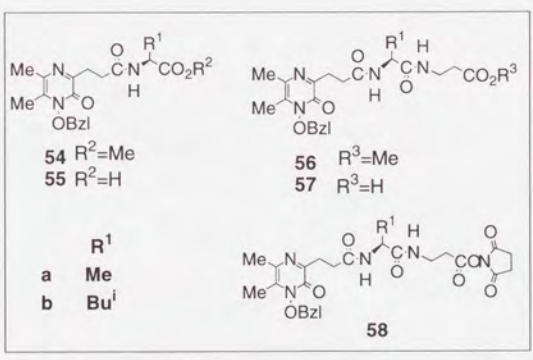
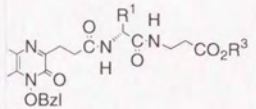

H2N–(CH2)n–CO2H 39
HCl·H2N–(CH2)n–CO2Me 40
BzlO
N–(CH2)n–CO2Me
H
41
N–(CH2)n–CO2H
42
N–(CH2)n–CO2
43
n
a 5
b 6
c 7


CO2Me
H2N CO2H
45
BocHN CO2H
46
BocHN CONHOBzl
47
HCl·H2N CONHOBzl
48

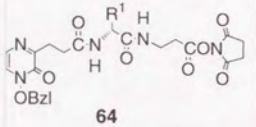

Me
Me
CO2Me
OBzl
49
CO2H
50


CONH-(CH2)n-NHR
OBzl
51 R = Boc
52 R = H·X
52a, X=TFA
52b-d, X=HCl
n
a 2
b 4
c 5
d 6

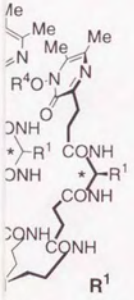

Me
Me
OBzl
54 R2=Me
55 R2=H
56 R3=Me
57 R3=H
R1
a Me
b Bui
58



62 R^3=Me
63 R^3=H

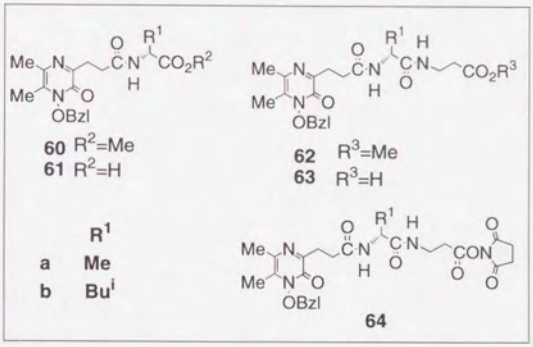


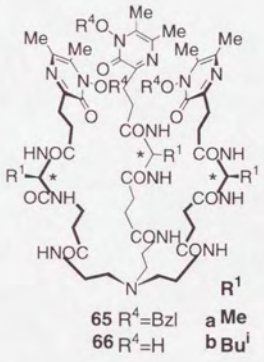
64

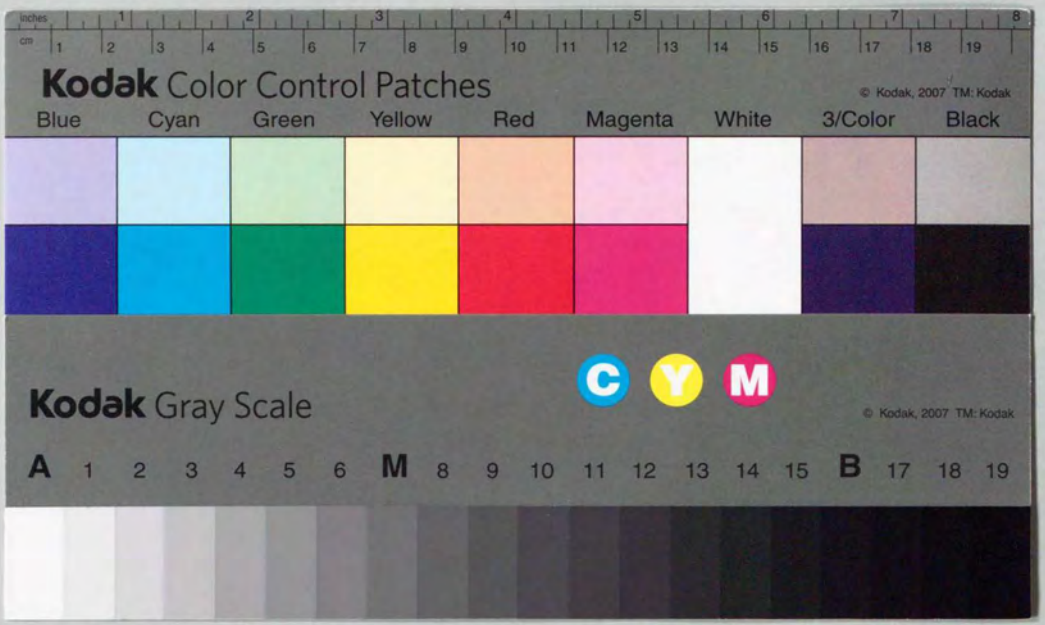


R¹

=Bzl **a** **Me**
=H **b** **Bu^i**


Me
N
O
C
N
H
R1
CO2R2
OBzl
60 R2=Me
61 R2=H
CO2R3
62 R3=Me
63 R3=H
R1
a Me
b Bui
64


R4O
Me
N
OR4
CONH
HNOC
OCNH
R1
65 R4=Bzl
66 R4=H
a Me
b Bui


inches
cm
Kodak Color Control Patches
© Kodak, 2007 TM: Kodak
Blue
Cyan
Green
Yellow
Red
Magenta
White
3/Color
Black
C
Y
M
Kodak Gray Scale
© Kodak, 2007 TM: Kodak
A 1 2 3 4 5 6 M 8 9 10 11 12 13 14 15 B 17 18 19