STUDIES ON SYNTHESIS, REACTION, AND IRON-CHELATING PROPERTY OF *N*-HYDROXYAMIDE-CONTAINING HETEROCYCLES

N-ヒドロキシアミド含有複素環の合成、反応および鉄キレート特性に関する研究

JUNKO OHKANDA

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by

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1996

Preface

This thesis was described to apply the Ph. D at the Faculty of Engineering, The University of Tokyo in 1996.

The author has been studying the synthetic organic chemistry of nitrogen-containing heterocycles, as a research associate at the Department of Industrial Chemistry, Faculty of Engineering, Seikei University since April 1991. In order not only to develope the synthetic procedure of new heterocycles, but also to create new materials which are applicable to various fields, the research which have been performed at the laboratory in this period are; 1) synthesis and evaluation of metal chelating property of *N*-hydroxyamide-containing heterocycles, 2) reaction of 1-benzyloxy-2(1*H*)-pyrimidinones with various nucleophiles, 3) synthesis of heterocycle-containing unnatural amino acids, 4) synthesis of crown ethers or peptides having amphiphilic double chains and their ion channel forming tendency, 5) synthesis of dihydropyridines bearing amino acids and their enantioselective reducing ability, 6) synthesis of unsymmetrical bipyridine derivatives and their ruthenium complexes, and 7) synthesis and reactivity of quinoxaline dearivatives and application to fluorescent derivatization reagents. Specifically, the author has mainly studied *N*-hydroxyamide-containing pyrimidines and pyrazines; synthesis, reactivity, and application to iron-chelating agents to clinical agents for the iron overload disease. Therefore, the results on the course of studies and discussion are reviewed here.

The thesis consists of 8 chapters. The background which motivated the author to start the studies was described as general introduction in Chapter 1. Chapter 2 dealed with the synthesis of *N*-hydroxyamide-containing

heterocycles, 1-hydroxy-2(1*H*)-pyrimidinones and -pyrazinones. In Chapter 3, their reactivities were described; (1) reaction with nucleophiles and photochemical behavior in solutions, (2) the application to organic synthesis as new additives for peptide synthesis, and (3) benzyloxycarbonylating agents for various amines, amino acids, and alcohols. On the basis of the results of the thermodynamic and kinetic studies, application of 1-hydroxy-2(1*H*)-pyrimidinones and -pyrazinones to new iron chelators in aqueous solutions was also discussed in Chapter 4 to Chapter 7.

The author sincerely wishes to express her deepest gratitude to Professor Akira Katoh for his constant guidance and encouragement throughout the course of the study. Furthermore, the author greatly appreciates the opportunity he gave her to start an attractive study in the field not only of synthetic heterocyclic chemistry, but also of applied chemistry, including supramolecular chemistry.

Grateful acknowledgment is made to Professor Kazuhiko Saigo of The University of Tokyo for his many helpful and valuable suggestions throughout the preparation of the thesis.

The author sincerely devotes her appreciation to Emeritus Professor Koko Maeda of Ochanomizu University, and Professor Tadashi Hasegawa of Tokyo Gakugei University for their helpful advice and continuous encouragement. The author also thanks to Dr. Yukie Mori of Ochanomizu University for her many suggestions and constant support since the author was a student in Ochanomizu University.

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The author wishes to thank Associate Professor Takeo Konakahara of Science University of Tokyo for MM and MD calculations, Mr. Takashi

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The sincere appreciation is extended to all the students of Katoh Laboratory for their kind help, especially to Takeshi Tokumitsu, Yoshifumi Itoh, Toshihiko Kumasaka, Atsushi Tamura, Aki Takasu, Shoichi Kondoh, Yuji Yoshiike, Jun Kamitani, and Yoko Hida for their experimental aids.

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Finally, to her parents and family, the author wishes to devote her deep appreciation for their constant encouragement and support during this work.

Tokyo, Spring 1996

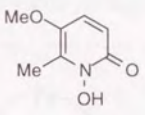
Junko Ohkanda

Contents

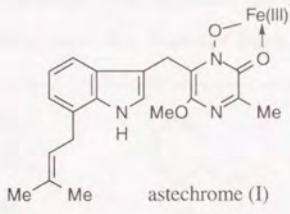
Chapter 1

General Introduction

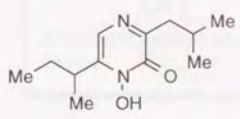
Nitrogen-containing heterocycles are essential compounds in the engineering, clinical, and agricultural fields, because of their great important roles in application to the dyes, pigments, pesticides, and pharmaceuticals. In addition, it is noteworthy that nowadays their applications are rapidly advanced in the synthesis of new functional materials. Highly oxidized heterocycles have been found in natural



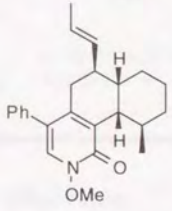
G1549



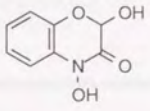
astechrome (I)



aspergillic acid



Leporin A



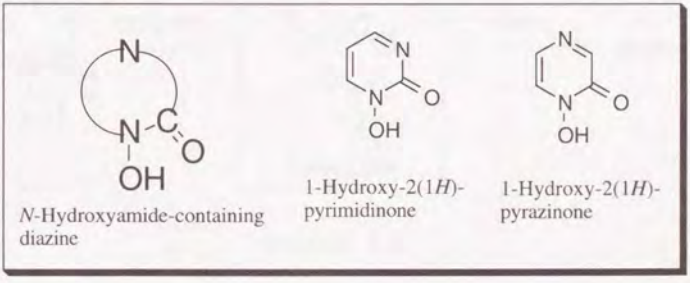
DIBOA

Scheme 1-1

products, for example, antibiotic G1549[1] which was isolated from *Pseudomonas alcaligenes*, astechrome (I)[2] which was a metabolic product of *Aspergillus terreus,* bactericidal antibiotic aspergillic acid, Leporin A[3] isolated from *Aspergillus leporis*, and DIBOA[4] which occurs in cereal plants (Scheme 1-1).

It is important that the common structure of these natural compounds is cyclic hydroxamic acid, and it allows the compounds to show effective biological activity with affinity to metal ion in aqueous media under physiological conditions. Although the first isolation of aspergillic acid was carried out over half a century ago,[5] study concerning the properties and applications of such highly oxidized heterocycles have been limited. Thus, these fascinating molecules should be the object of interests due to their potential applications to various areas.

As one of typical series of highly oxidized heterocycles, the author would like to focus on the *N*-hydroxyamide-containing diazines depicted in



N-Hydroxyamide-containing diazine

1-Hydroxy-2(1*H*)-pyrimidinone

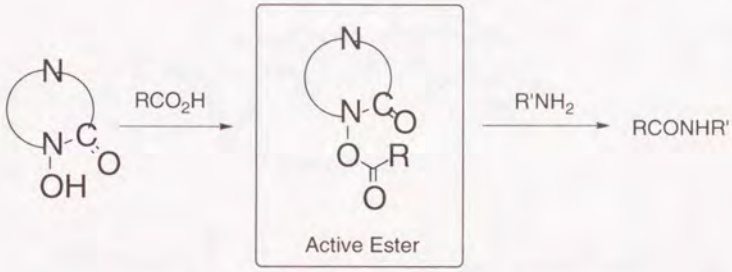
1-Hydroxy-2(1*H*)-pyrazinone

- low pKa value
- high water solubility
- metal chelating ability

Scheme 1-2

Scheme 1-2. It should be predictable that oxidized nitrogens in π-electron deficient ring systems may lead remarkable acidity of the OH groups. Further, the molecular shapes can be regarded as cyclic hydroxamic acids. On the basis of these facts, therefore, three principal properties would be expected: (1) low pKa value, (2) high water solubility, and (3) metal chelating ability. Because of these properties, these compounds may be applicable in several fields as new fascinating materials.

Although large numbers of papers concerning reactivity of heterocycles have been reported,[6-8] less attention has focused on the chemical reaction of *N*-hydroxyamide-containing heterocycles. The electron withdrawing group, *N*-OR moiety should activate the ring system to receive a nucleophile attack. Consequently, a study concerning the chemical reaction of *N*-hydroxyamide-containing heterocycles and their derivatives would be interest.

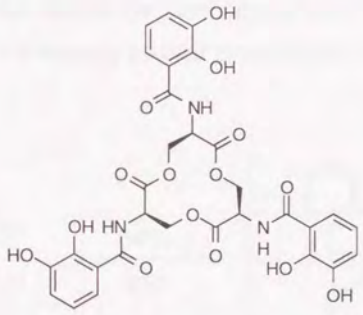


Scheme 1-3

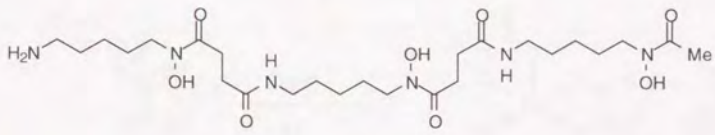
On the other hand, in current organic synthesis, there are many kinds of heterocycles, which are used as effective reagents to form intermediary active esters.[9,10] Principal properties such as (1) low pKa value and (2) high water solubility are essential for additives being effective. The former

property will be important in acceleration of the formation of a desired active ester, and the latter one will be promise in easy work up. It seems likely that the pKa value of a N-OH directly attached to a π-deficient diazine ring system is fairly lower than that of a N-OH group in monoazines owing to existence of two electron withdrawing nitrogens in the ring. Thus, their acyl derivatives can be regarded as active esters, so that these diazines are expected to be new acylating reagents in organic synthesis (Scheme 1-3).

Iron is one of the most abundant transition metals in earth and yet is highly inaccessible under aerobic conditions because of the insolubility of



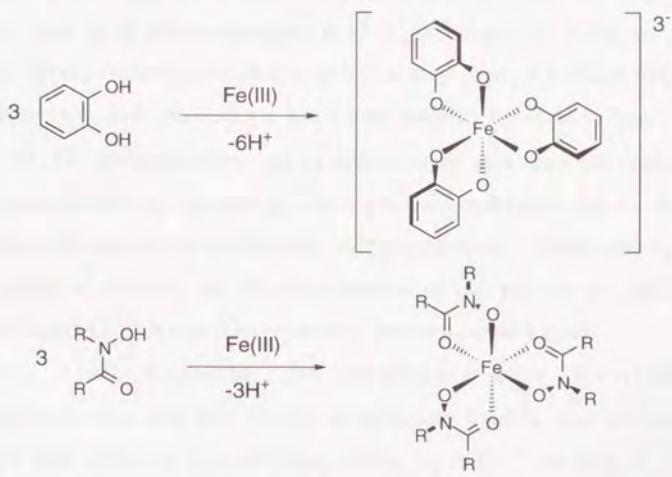
Enterobactin



Desferrioxamine B

Scheme 1-4

$Fe(OH)_3$ (K_{sp}~10^{-38}). However, for all plants and animals, and for virtually all microbes, life without iron is impossible. In order to solubilize and uptake such low water soluble iron, the microorganisms secrete highly affinitive iron-transporting compounds, called siderophores[11] (from the Greek, "iron carrier"). More than a hundred naturally-occurring siderophores have been isolated,[12] and they are classified into two classes on the basis of their general structures: catecholates and hydroxamates, typified by enterobactin and desferrioxamine B, respectively (Scheme 1-4). These hexadentate iron chelators form stable octahedral iron complexes (Scheme 1-5). The ongoing search for new siderophores and synthesis of their analogues are driven strongly by their potential for clinical use.[13, 14]

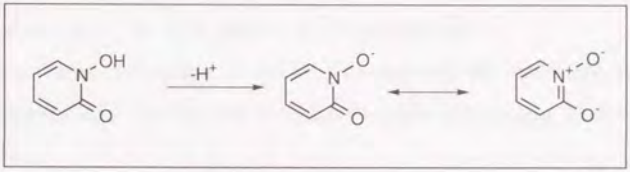


Scheme 1-5. Iron complex formation with catechol and hydroxamic acid.

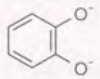
Iron deficiency and overload are significant health problems. The patients typified by β-thalassemia, a genetic disease also called Cooly's anemia, require frequent blood transfusions to survive. However, the resulting excess iron in the body cannot be removed entirely by normal pathway. Therefore, iron build-up is a serious problem leading to deposition of the metal in a number of organs, causing tissue damage and early death. Thus, there is a need to develop efficient and practical iron-chelating agents for the treatment of iron overload. A currently used drug of trihydroxamate-type natural siderophore, desferrioxamine B, is still the most effective drug for removal of toxic quantity of iron from patients. Unfortunately, this compound cannot be orally administered and has a short half-life *in vivo*. Furthermore, it has severe side effect such as septicemia. Thus, many chemists have been devoted much effort to develop new chelators instead of desferrioxamine B.[14] Catecholates are much stronger bidentate ligands than hydroxamates owing to their high pKa values (pKa_{cat} ~11, pKa_{ha} ~9), and enterobactin has a high stability constant to ferric iron (log K 49).[15] Unfortunately, use of enterobactin as a drug for chelation therapy is precluded by extreme propensity toward hydrolysis and by strong promoting the growth of pathogenic microorganisms. Furthermore, the weak acidity of catechol and the required loss of two protons per catechol unit at neutral pH limit the effectiveness of catechol-based ligands.

Since 1980 Raymond and co-workers have investigated hydroxypyridinones and their family as bidentate ligands, and the results indicated their effective iron chelating ability upon the formation of a 3:1 complex.[16] Moreover, at least under neutral conditions, they were superior to hydroxamate and catecholate derivatives. 1-Hydroxy-2(1*H*)-pyridonate

and catecholate anions are isoelectronic and isostructural; thus these attractive compounds can be viewed as either aromatic hydroxamic acids or as catechol analogues as shown in Scheme 1-6. Their strong acidity would allow to form iron complexes more effectively under neutral conditions than hydroxamic acids and catechols. Furthermore, 1,2-dimethyl-3-hydroxy-4(1*H*)-pyridinone has been recently found to be a potential compound as an orally active iron chelator.[17-23] These results strongly suggest the feasibility of application of *N*-hydroxyamide-containing heterocycles as artificial siderophores.



Hydroxamic acid



catecholate

Scheme 1-6

N-Hydroxyamide-containing diazines can be regarded as aza analogues of monoazines. Thus, they would be expected to sequester a metal ion and to form a 3:1 octahedral complex with the *N*-hydroxyamide group in the molecule as well as the corresponding monoazines. Furthermore, their lower pKa value and higher water solubility than the corresponding monoazines should be advantageous to chelate a metal ion under physiological conditions.

In addition, by introducing functional groups in the ring system of the compounds, highly molecular design is possible to give multidentate ligands composed of *N*-hydroxyamide-containing diazines. Especially, hexadentate ligands, in which bidentate ligands are connected to an anchor part by spacer groups, were strongly attracted the author's interest, because they would have advantages for metal chelating due to the cooperative effect by the three bidentate parts in a dilute solution.

As described above, there is great interest in applications of *N*-hydroxyamide-containing heterocycles as summarized in Figure 1-1. Their bright prospect in developing the new field of heterocyclic chemistry prompted the author to start studies on the compounds.

In this thesis, the author would like to describe the synthesis, reaction, and evaluation of functions of *N*-hydroxyamide-containing heterocycles, especially:

Chapter 2

General preparative method of 1-hydroxy-2(1*H*)-pyrimidinones and -pyrazinones.

Chapter 3

(1) Reaction of 1-benzyloxy-2(1*H*)-pyrimidinones with hydroxylamine.

(2) Photochemical reaction of 1-benzyloxy-2(1*H*)-pyrazinones.

(3) Application of 1-hydroxy-2(1*H*)-pyrimidinones and -pyrazinones to organic synthesis as acylating agents.

Chapter 4

Metal chelating properties of 1-hydroxy-2(1*H*)-pyrimidinone and -pyrazinone as bidentate ligands in an aqueous solution.

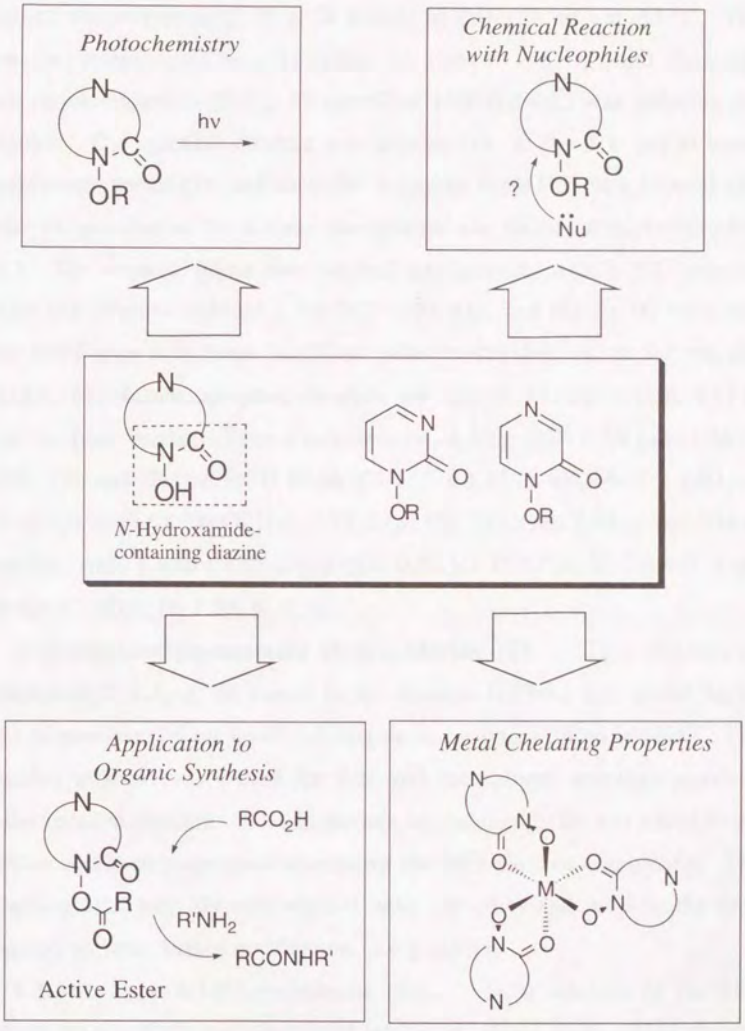

Photochemistry
hv
Chemical Reaction with Nucleophiles
?
Nu
N-Hydroxamide-containing diazine
Application to Organic Synthesis
RCO2H
R'NH2
RCONHR'
Active Ester
Metal Chelating Properties

Figure 1-1

isobutyl chloroformate (3.28 g, 24 mmol) in THF (20 mL) at -17 °C. The reaction temperature was kept for 15 min at -15 °C, and then *O*-benzylhydroxylamine (2.5 g, 23 mmol) in THF (10 mL) was added to the mixture. The reaction mixture was kept at -15 °C for 3 h and at room temperature overnight, and then the resulting Et_3N·HCl was filtered off. After evaporation of the solvent, the residue was dissolved in AcOEt (200 mL). The organic phase was washed successively with a 5% aqueous $NaHCO_3$ solution (100 mL), 5% HCl (100 mL), and H_2O (100 mL), and then dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was chromatographed on silica gel (eluent: hexane-AcOEt=1:1) to give the pure product (**7**) as a colorless oil, 4.36 g (77%): IR (neat) 3400, 1690, 740, and 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.45 (s, 9H, Me_3C), 3.60 (m, 2H, CH_2), 4.80 (s, 2H,OCH_2), 5.75 (br s, 1H, $NHCO_2$), 7.40 (s, 6H, NH-O and Ph). Anal. Calcd for $C_{14}H_{20}N_2O_4$ $0.7H_2O$: C, 57.96; H, 7.43; N, 9.66. Found: C, 58.08; H, 7.29; N, 9.34.

***N*-(Benzyloxy)glycinamide Hydrochloride (8).** To a solution of compound **7** (8.41 g, 30 mmol) in dry dioxane (12 mL) was added 5.8 M HCl in dioxane (26 mL) with stirring upon cooling with an ice bath. The reaction mixture was stirred for 1 h, and the solvent was then removed under reduced pressure. A small amount of absolute EtOH was added to the residue and then evaporated to remove the HCl-dioxane completely. The resulting HCl salt (**8**) was washed with dry ether and used in the next reaction without further purification, 5.4 g (83%).

1-Benzyloxy-2(1*H*)-pyrazinone (9a). To a solution of the HCl salt (**8**, 10.6 g, 0.05 mol) in MeOH-H_2O (1:1, 80 mL) was added glyoxal (7.5 g, 0.05 mol) at -30 °C. The pH of the reaction mixture was adjusted to

Chapter 5

(1) Synthesis of tripodal hexadentate ligands composed of 1-hydroxy-2(1*H*)-pyrimidinones, spacers, and an anchor.

(3) Metal chelating properties of tripodal hexadentate ligands in aqueous solutions and stability of their iron complexes.

(4) Kinetic studies on iron removal ability from human serum iron transport protein under physiological conditions.

Chapter 6

(1) Synthesis of tripodal hexadentate ligands composed of 1-hydroxy-2(1*H*)-pyrazinones, spacers, and an anchor.

(3) Metal chelating properties of tripodal hexadentate ligands in aqueous solutions and stability of their iron complexes.

(4) Kinetic studies on iron removal ability from human serum iron transport protein under physiological conditions.

(5) Absolute configuration analysis of iron(III) complexes of the chiral ligands by means of CD spectra.

Chapter 7

(1) Synthesis of tripodal hexadentate ligands bearing 1-hydroxy-2(1*H*)-pyrazinone, D-amino acid residues as spacers, and tris(2-aminoethyl)amine as an anchor.

(2) Kinetic studies on iron removal from transferrin under physiological pH, and comparison of the kinetic efficiency between the enantiomers.

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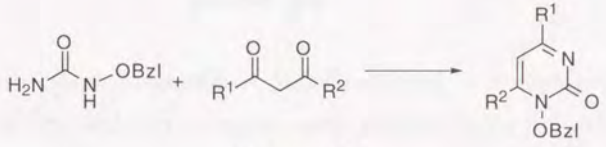
Chapter 2

Synthesis of 1-Hydroxy-2(1*H*)-pyrimidinones and -pyrazinones

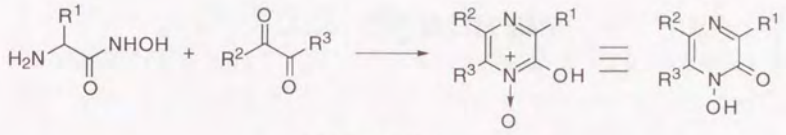
2.1 Introduction

Many successful preparative methods for *N*-substituted-2(1*H*)-pyrimidinones have been already reported.[1-3] Most of the synthetic methods are condensations of *N*-substituted ureas and β-diketones under acidic conditions to afford *N*-alkyl or *N*-aryl substituted pyrimidinones. Concerning the synthesis of 1-alkoxy-2-(1*H*)-pyrimidinones, three papers have been reported.[4-6] Interestingly, only 2,4-pentanedione was employed as a β-diketone. Because debenzylation by hydrogenation is known to proceed under mild conditions in quantitative yield, 1-benzyloxy-2(1*H*)-pyrimidinones could be useful precursors of 1-hydroxy-2(1*H*)-pyrimidinones. In this study, 1-benzyloxy-2(1*H*)-pyrimidinones were synthesized by the condensation of *N*-benzyloxyurea with various β-diketones under appropriate acidic conditions (Scheme 2-1).



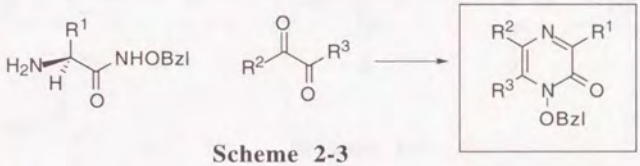
Scheme 2-1

Synthesis of 2(1*H*)-pyrazinones have been extensively studied as much as that of pyrimidinone derivatives. Dunn and co-workers[7,8] at first reported the reaction of α-aminohydroxamic acids with 1,2-dicarbonyl compounds to give 2-hydroxypyrazine 1-oxides (Scheme 2-2).



Scheme 2-2

This reaction seems to be applicable to the synthesis of 1-benzyloxy-2(1*H*)-pyrazinones using *O*-benzyl-α-aminohydroxamic acids, which are easily prepared by the condensation of natural L-amino acids with benzyloxyamine, in the place of α-aminohydroxamic acids. Furthermore, the introducion of another functional groups to pyrazinone ring systems may be possible upon using acidic or basic amino acids as starting materials as shown in Scheme 2-3.

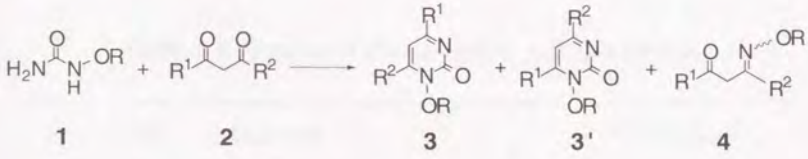


Scheme 2-3

In this chapter, synthetic procedures for 1-benzyloxy-2(1*H*)-pyrimidinones and -pyrazinones, and debenzylation to afford the corresponding 1-hydroxy derivatives were described.[9-11]

2.2 Synthesis

A benzyl group was selected as a protecting of the hydroxyl group in the *N*-hydroxyamide moiety. 1-Benzyloxy-2(1*H*)-pyrimidinones (**3a-d** and **3'b**) were synthesized by the condensation of *N*-benzyloxyurea (**1,** R=Bzl), which was derived from *O*-benzylhydroxylamine and sodium cyanate, with 1,1,3,3-tetraethoxypropane, 4,4-dimethoxy-2-butanone, 2,4-pentanedione, and 2,4-hexanedione, respectively, under various acidic conditions (Scheme 2-4). The results are summarized in Table 2-1.



Scheme 2-4

The reaction with 4,4-dimethoxy-2-butanone gave a mixture of 1-benzyloxy-4-methyl- (**3b**) and 1-benzyloxy-6-methyl-2(1*H*)-pyrimidinone (**3'b**) which was easily separated by column chromatography on silica gel. The structures of the two isomers **3b** and **3'b** were assigned on the basis of the following data: The signals of the 4- and 6-methyl protons of 1-methoxy-4,6-dimethyl-2(1*H*)-pyrimidinone[5] appeared at δ 2.31 and 2.39 ppm, respectively. On the other hand, the signals of methyl protones of the two isomers appeared at δ 2.16 and 2.35 ppm, respectively. Upon

comparison of the chemical shifts, the isomers with methyl signals at lower and higher chemical fields were assigned to be **3b** and **3'b**, respectively. Moreover, $\Delta\delta$ ($\delta_{4\text{-Me}}$-$\delta_{6\text{-Me}}$) of 1-methoxy-4,6-dimethyl-2(1*H*)-pyrimidinone was only 0.08 ppm, while the $\Delta\delta$ value between **3b** and **3'b** was 0.19 ppm. This enlargement in $\Delta\delta$ value may be attributable to the anisotropic effect of the benzene ring at the N-1 position as reported previously for 1-aryl-4,6-dimethyl-2(1*H*)-pyrimidinones.[12] On the other hand, 2,4-hexanedione afforded only one isomer, 1-benzyloxy-4-ethyl-6-methyl-2(1*H*)-pyrimidinone (**3d**). The yield of **3d** was fairly lower than that of **3c**, although it was not improved even under reflux overnight.

Table 2-1. Reaction of *N*-alkoxyureas with β-diketones

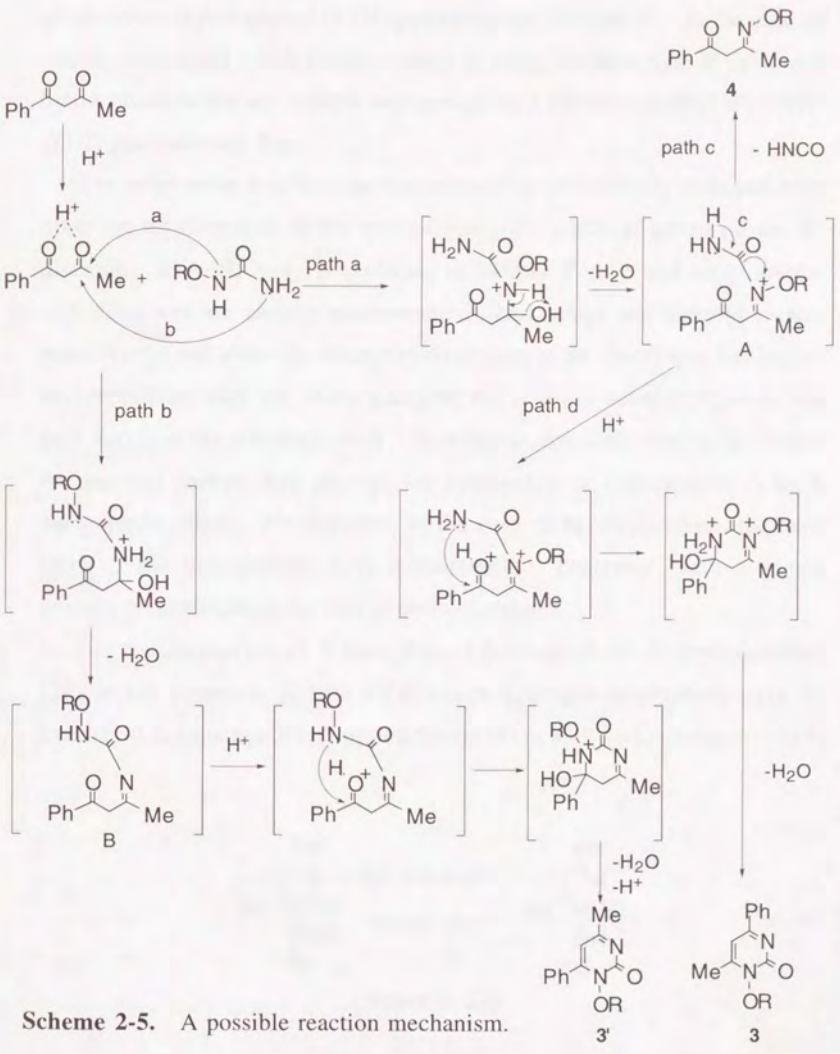
	Urea **1**	β-Diketone **2**			Yields/%		
	R	R^1	R^2	Conditions	**3**	**3'**	**4**
a	Bzl	H	H[a)]	10 M HCl/EtOH/r.t.(3 d)	33		0
b	Bzl	Me	H[b)]	H_2SO_4/EtOH/reflux (1h)	44	16	0
c	Bzl	Me	Me	H_2SO_4/EtOH/reflux (2h)	42		0
d	Bzl	Et	Me	H_2SO_4/EtOH/reflux (2h)	19	0	0
e	Bzl	Ph	Me	H_2SO_4/Et_2O/r.t. (2h)	2	trace	45
f	Me	Ph	Me	6% HCl/EtOH/reflux (28h)	5	0	28

a) 1,1,3,3-tetraethoxypropane. b) 4,4-dimethoxy-2-butanone.

In contrast, the reaction of *N*-benzyloxyurea (**1,** R=Bzl) with 1-phenyl-1,3-butanedione gave 1-benzyloxy-6-methyl-4-phenyl-2(1*H*)-pyrimidinone (**3e**) in only 2% yield with a trace amount of 1-benzyloxy-4-methyl-6-phenyl-2(1*H*)-pyrimidinone (**3'e**), and 3-benzyloxyimino-1-phenyl-1-butanone (**4e**) was isolated as a major product in 45% yield. The structures of the two isomers **3e** and **3'e** were assigned by means of ^{1}H NMR, as done for **3b** and **3'b**. 4-Phenyl derivative **3e** showed a typical benzoyl pattern, and an olefinic proton signal at the C-5 position of **3'e** appeared at δ 6.50 ppm which was ca 0.6 ppm lower field than that of 4,6-dimethyl derivative **3c.** These characteristics in the NMR spectrum would be attributable to the anisotropic effect of the phenyl group,[13] which exists nearly coplanar to the pyrimidinone ring. The structure of **4e** was confirmed by comparision of its spectral data with those of an authentic sample, prepared by the condensation of 1-phenyl-1,3-butanedione with *O*-benzylhydroxylamine in the presence of *p*-toluenesulfonic acid.

A similar result was observed in the reaction using *N*-methoxyurea (**1,** R=Me) in the place of *N*-benzyloxyurea; 3-methoxyimino-1-phenyl-1-butanone (**4f**) was obtained in 28% yield, accompanying 1-methoxy-6-methyl-4-phenyl-2(1*H*)-pyrimidinone (**3f**) in only 5% yield.

A possible reaction mechanism is shown in Scheme 2-5. Two kinds of lone electron pairs of the urea have possibility to attack the protonated acetyl carbonyl carbon to give intermediates A and B (paths a and b). In the case of path a, the elimination of isocyanic acid from intermediate A affords the 3-(*N*-alkoxy)imino ketone derivative **4** (path c), while the attack of the lone electron pair of the other nitrogen to the benzoyl carbonyl carbon affords 1-

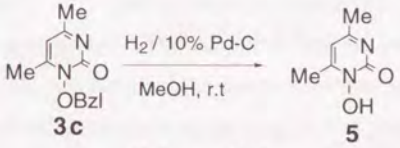


Scheme 2-5. A possible reaction mechanism.

alkoxy-6-methyl-4-phenyl-2(1*H*)-pyrimidinone **3e** (path d). In the case of path b, nucleophilic attack, dehydration to afford intermediate B, followed by the attack of the alkoxylated nitrogen, gives 1-alkoxy-4-methyl-6-phenyl-2(1*H*)-pyrimidinone **3'e**.

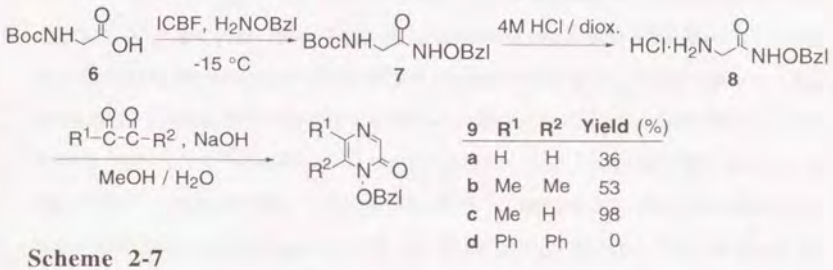
It is noteworthy that the reaction pathway is dramatically changed only upon the displacement of the methyl group by a phenyl group on the β-diketone. This difference is explained as follows: Formyl and acetyl groups of β-diketones are usually more reactive than acetyl and benzoyl group, respectively, and since the alkoxylated nitrogen of an alkoxyurea has higher nucleophilicity than the other nitrogen, the reaction mainly proceeds *via* path a to give the intermediate A. In addition, the steric hindrance around the benzoyl carbon may prevent the cyclization of intermediate A by a nucleophilic attack. Furthermore, in the case of benzoylacetone, the enol form of the intermediate A is considerable. Therefore, path c would become predominant in the case of benzoylacetone.

The hydrogenation of 1-benzyloxy-4,6-dimethyl-2(1*H*)-pyrimidinone (**3c**) in the presence of 10% Pd-C under hydrogen atmosphere gave 1-hydroxy-4,6-dimethyl-2(1*H*)-pyrimidinone (**5**) in 83% yield (Scheme 2-6).[5]



3c **5**

Scheme 2-6

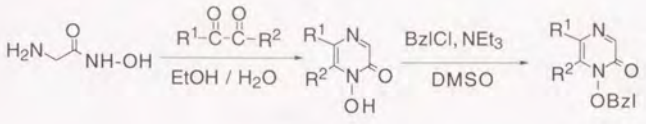


9	R^1	R^2	Yield (%)
a	H	H	36
b	Me	Me	53
c	Me	H	98
d	Ph	Ph	0

Scheme 2-7

The synthetic procedure for 1-benzyloxy-2(1*H*)-pyrazinones (**9**) is depicted in Scheme 2-7. *N*-(*t*-Butoxycarbonyl)glycine (**6**) was coupled with *O*-benzylhydroxylamine by means of the mixed anhydride method,[14] and the Boc group of **7** was removed with 4 M HCl in dioxane to give *N*-(benzyloxy)glycinamide hydrochloride (**8**). The condensation of the salt **8** with glyoxal, 2,3-butanedione, or pyruvic aldehyde under basic conditions at -30 °C afforded 1-benzyloxy-2(1*H*)-pyrazinone (**9a**), 1-benzyloxy-5,6-dimethyl-2(1*H*)-pyrazinone (**9b**), and 1-benzyloxy-5-methyl-2(1*H*)-pyrazinone (**9c**) in 28, 53, and 98% yields, respectively. Two isomers, 5-methyl and 6-methyl derivatives, are possible in the case of pyruvic aldehyde. However, only one isomer, 1-benzyloxy-5-methyl-2(1*H*)-pyrazinone (**9c**), was obtained. The chemical shift of the methyl group at the C-6 position of **9b** was found to be lower than that at the C-5 position due to the anisotropic effect of the benzene ring at N-1 position. The signal attributable to the 5-methyl group of **9c** was observed at δ 2.15 ppm, and the chemical shift is quite close to that observed for the methyl proton signal of the 5-methyl group (δ 2.18 ppm) rather than that of the 6-methyl group

(δ 2.27 ppm) in **9b**. The reason why a sole isomer is generated can be explained by the preferential attack of the lone electron pair of the nitrogen of benzyloxy amide rather than of the α-amino group to formyl carbon. An attempt to cyclize *N*-(benzyloxy)glycinamide hydrochloride with benzil was unsuccessful, but benzilic acid rearrangement was observed due to strong basic reaction conditions. The failure of the condensation was considered to arise from the steric congestion of the three phenyl groups. On the basis of this consideration, in order to introduce aromatic substituents at C-5 and C-6 positions, glycinohydroxamic acid was condensed with benzil under reflux. As expected, the reaction afforded 1-hydroxy-5,6-diphenyl-2(1*H*)-pyrazinone (**10d**) in 42% yield (Scheme 2-8). Subsequently, **10d** was treated with benzyl chloride in order to obtain the desired product **9d**. Cyclohexane-condensed pyrazinone **9e** was also prepared by the same procedure using 1,2-cyclohexanedione.



10

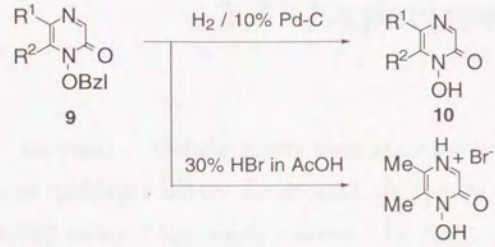
	R^1	R^2	Yield (%)
d	Ph	Ph	42
e		-$(CH_2)_4$-	27

9

	Yield (%)
d	98
e	58

Scheme 2-8

The deprotection of **9a** and **9b** with 10% Pd-C in a hydrogen atmosphere gave 1-hydroxy-2(1*H*)-pyrazinone (**10a**) and 1-hydroxy-5,6-dimethyl-2(1*H*)-pyrazinone (**10b**) as shown in Scheme 2-9.



10	R^1	R^2	Yield (%)
a	H	H	70
b	Me	Me	76

Scheme 2-9 **10b·HBr** 81 %

The treatment of compound **9b** with 30% HBr in acetic acid[5] gave the hydrobromide salt (**10b·HBr**).

The high water solubility of these diazines, **5**, **10a**, and **10b** is notable.

2.3 Experimental

General. Melting points were recorded on a Mel-Temp apparatus in open capillaries and are uncorrected. IR spectra were recorded on a JASCO A-100 Infrared Spectrophotometer. ^{1}H NMR spectra were recorded on a JEOL JNM-PMX60 spectrometer or a JEOL GX-270 NMR Spectrometer in $CDCl_3$, DMSO-d_6, or D_2O and are reported in ppm (δ) downfield from internal Me_4Si or 3-trimethylsilyl-1-propanesulfonic acid sodium salt. Thin-layer chromatography (TLC) analyses were performed on silica gel 60F-254 with a 0.2 mm layer thickness. Column chromatography was carried out with Merck Kieselgel 60 (230-400 mesh). High-performance liquid chromatography (HPLC) was carried out with a JASCO 880-PU and an 875-UV equipped with a JASCO 807-IT integrator by using a column packed with Finepak SIL $C_{12}S$. Combustion analyses were performed on a Yanaco MT-3 CHN CORDER.

N-Benzyloxyurea (**1**, R=Bzl)[15] and *N*-methoxyurea (**1**, R=Me)[16] were prepared according to the literature methods.

1-Benzyloxy-2(1*H*)-pyrimidinone (3a). A mixture of *N*-benzyloxyurea (500 mg, 3 mmol), 1,1,3,3-tetraethoxypropane (525 mg, 2.4 mmol), EtOH (3 mL), and 10 M hydrochloric acid (0.6 mL) was stirred for 3 days at room temperature. After evaporation of the solvent, the residue was dissolved in water. The pH of the aqueous solution was adjusted to 11 with an aqueous NaOH solution and extracted with $CHCl_3$ (3x50 mL). The combined extracts were dried over anhydrous $MgSO_4$. The crude product was purified by column chromatography on silica gel (eluent: $CHCl_3$-

acetone-EtOH=100:20:4) to give the pure product (**3a**), 150 mg (33%): mp 86-90 °C; IR (KBr) 1670, 740, and 690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.32 (s, 2H, CH_2), 6.04 (dd, *J* 4 and 6 Hz, 1H, 5-H), 7.36 (s, 5H, Ph), 7.40 (dd, *J* 2 and 6 Hz, 1H, 6-H) 8.45 (dd, *J* 2 and 4Hz, 1H, 4-H). Anal. Calcd for $C_{11}H_{10}N_2O_2 \cdot 0.5H_2O$: C, 62.56; H, 5.21; N, 13.27. Found: C, 62.64; H, 5.27; N, 13.05.

1-Benzyloxy-4-methyl- (3b) and 1-Benzyloxy-6-methyl-2(1*H*)-pyrimidinone (3'b). To a solution of *N*-benzyloxyurea (700 mg, 4.2 mmol) in absolute EtOH (7 mL) were added 4,4-dimethoxy-2-butanone (560 mg, 4.2 mmol) and concd H_2SO_4 (0.5 mL) at room temperature. The reaction mixture was refluxed for 1 h. After evaporation of the solvent, H_2O (10 mL) was added to the residue. The pH of the aqueous solution was adjusted to 11 with a saturated $NaHCO_3$ solution and extracted with CH_2Cl_2 (3x50 mL). The combined extracts were washed with brine (50 mL), and dried over anhydrous $MgSO_4$. The crude product was chromatographed on silica gel (eluent: $CHCl_3$-acetone-EtOH=100:20:4). The first fraction (R_f=0.39) was 1-benzyloxy-4-methyl-2(1*H*)-pyrimidinone (**3b**), 400 mg (44%): mp 135.5-138 °C; IR (KBr) 1650, 745, and 695 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.35 (s, 3H, Me), 5.30 (s, 2H, CH_2), 5.95 (d, *J* 7 Hz, 1H, 5-H), 7.28 (d, *J* 7 Hz, 1H, 6-H) 7.34 (s, 5H, Ph). The second fraction (R_f=0.31) was 1-benzyloxy-6-methyl-2(1*H*)-pyrimidinone (**3'b**), 145 mg (16%): mp 136-139 °C; IR (KBr) 1650, 740, and 690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.16 (s, 3H, Me), 5.31 (s, 2H, CH_2), 6.03 (d, *J* 5 Hz, 1H, 5-H), 7.40 (s, 5H, Ph), 8.31 (d, *J* 5 Hz, 1H, 4-H). Anal. Calcd for $C_{12}H_{12}N_2O_2$ $0.1H_2O$: C, 66.10; H, 5.64; N, 12.85. Found (a mixture of **3b** and **3'b**): C, 66.37; H, 5.72; N, 12.58.

1-Benzyloxy-4,6-dimethyl-2(1*H*)-pyrimidinone (3c). The reaction of *N*-benzyloxyurea with 2,4-pentanedione gave the product (**3c**) (42%): mp 130-131 °C (lit[17] mp 131-132 °C); ^{1}H NMR ($CDCl_3$) δ 2.18 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 5.32 (s, 2H, CH_2), 5.92 (s, 1H, 5-H), 7.45 (m, 5H, Ph).

1-Benzyloxy-4-ethyl-6-methyl-2(1*H*)-pyrimidinone (3d). To a solution of *N*-benzyloxyurea (**1**) (1.66 g, 10 mmol) and 2,4-hexanedione (1.42 g, 12 mmol) in EtOH (15 mL) was cautiously added concd H_2SO_4 (1.2 mL) at room temperature. The reaction mixture was refluxed for 2 h. After evaporation of the solvent, H_2O (10 mL) was added to the residue. The pH of the aqueous solution was adjusted to 10 with 4M NaOH solution and then extracted with $CHCl_3$ (3x80 mL). The conbined organic layers were successively washed with H_2O (60 mL) and saturated NaCl solution (60 mL), and then dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was chromatographed on silica gel (eluent: AcOEt) to give the product (**3d**, 0.19 g) in 19% yield, mp 105-108 °C; UV λ_{max} (log ε in EtOH): 205 (4.27) and 305 nm (3.83); IR (KBr): 1660 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.24 (t, *J* 7 Hz, 3H), 2.12 (s, 3H), 2.58 (q, *J* 7 Hz, 2H), 5.31 (s, 2H), 5.94 (s, 1H), 7.37-7.45 (m, 5H). Anal. Calcd for $C_{14}H_{16}N_2O_2$: C, 68.83; H, 6.60; N, 11.47. Found: C, 68.55; H, 6.65; N, 11.39.

1-Benzyloxy-6-methyl-4-phenyl- (3e) and 1-Benzyloxy-4-methyl-6-phenyl-2(1*H*)-pyrimidinone (3'e), and 3-Benzyloxyimino-1-phenyl-1-butanone (4e). To a solution of 1-phenyl-1,3-butanedione (1.45 g, 9 mmol) in dry ether (15 mL) were added *N*-benzyloxyurea (1.49 g, 9 mmol) and concd H_2SO_4 (1 mL). After stirring for 19 h at room temperature, the solvent was evaporated, and H_2O

(30 mL) was added to the residue. The pH of the aqueous solution was adjusted to 11 with an aqueous NaOH solution and extracted with CH_2Cl_2 (4x30 mL). The combined extracts were dried over anhydrous $MgSO_4$. After evaporation of the solvent, the residue was chromatographed on silica gel (eluent: $CHCl_3$-acetone-EtOH=100:20:1). The first fraction (R_f=0.7) was 3-benzyloxyimino-1-phenyl-1-butanone (**4e**), 1.07 g (45%): IR (neat) 1690, 755, and 700 cm^{-1}; ^1H NMR ($CDCl_3$, a mixture of *E* and *Z*) δ 1.92 and 1.94 (s, 3H, Me), 3.85 and 4.03 (s, 2H, CH_2), 5.12 and 5.15 (s, 2H, OCH_2), 7.25-7.65 (m, 8H, Ph), 7.8-8.1 (2H, m, Ph). Anal. Calcd for $C_{17}H_{17}NO_2 \cdot 0.2H_2O$: C, 75.39; H, 6.43; N, 5.17. Found: C, 75.09; H, 6.07; N, 4.93. Compound **4e** was identical with the product, which was obtained by the reaction of 1-phenyl-1,3-butanedione with *O*-benzylhydroxylamine in the presence of *p*-toluenesulfonic acid in benzene under reflux. A trace of the second fraction (R_f=0.51) was 1-benzyloxy-4-methyl-6-phenyl-2(1*H*)-pyrimidinone (**3'e**); ^1H NMR ($CDCl_3$) δ 2.21 (3H, s, CH_3), 5.38 (2H, s, CH_2), 6.50 (s, 1H, CH), 7.30-7.55 (10H, m, 2xPh). The third fraction (R_f=0.26) was 1-benzyloxy-6-methyl-4-phenyl-2(1*H*)-pyrimidinone (**3e**), 0.05 g (2%): mp 129-130 °C; IR (KBr) 1660, 750, and 680 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.40 (s, 3H, Me), 4.96 (s, 2H, CH_2), 6.03 (s, 1H, 5-H), 6.6-7.65 (m, 10H, 2xPh). Anal. Calcd for $C_{18}H_{16}N_2O_2 \cdot 0.3H_2O$: C, 72.61; H, 5.62; N, 9.41. Found: C, 72.57; H, 5.89; N, 9.30.

1-Methoxy-6-methyl-4-phenyl-2(1*H*)-pyrimidinone (3f) and 3-Methoxyimino-1-phenyl-1-butanone (4f). A mixture of *N*-methoxyurea (2.4 g, 0.03 mol) and 1-phenyl-1,3-butanedione in a 6% ethanolic HCl solution (20 mL) was refluxed for 28 h. The reaction mixture was adjusted to pH 11 with an aqueous NaOH solution and extracted with

CH_2Cl_2 (50 mLx3). The organic layer was washed with brine (50 mL) and dried over anhydrous $MgSO_4$. The products were chromatographed on silica gel with a hexane-AcOEt (9:1) mixture. The first fraction (R_f=0.18) was 3-methoxyimino-1-phenyl-1-butanone (**4f**), 570 mg (28%): IR (neat)1680, 750, and 685 cm^{-1}; ^1H NMR ($CDCl_3$, a mixture of *E* and *Z*) δ 1.94 and 1.96 (s, 3H, Me), 3.85 (s, 3H, OMe), 3.80 and 4.03 (s, 2H, CH_2), 7.25-7.66 (m, 3H, Ph), 7.83-8.06 (m, 2H, Ph). Compound **4f** was identical with the product, which was obtained from the reaction of 1-phenyl-1,3-butanedione and *O*-methylhydroxylamine in the presence of *p*-toluenesulfonic acid in benzene under reflux. Further chromatography by changing the eluent (eluent: $CHCl_3$-acetone-EtOH=100:10:2) afforded 1-methoxy-6-methyl-4-phenyl-2(1*H*)-pyrimidinone (**3f**), 95 mg (5%). ^1H NMR ($CDCl_3$) δ 2.5 (s, 3H, Me), 4.12 (s, 3H, OMe), 6.6 (s, 1H, 5-H), 7.3-7.7 (m, 3H, Ph), 7.8-8.2 (m, 2H, Ph). Found: C, 55.60; H, 6.56; N, 11.46. Anal. Calcd for $C_{12}H_{12}N_2O_2$:C, 55.55; H, 6.53; N, 10.79.

1-Hydroxy-4,6-dimethyl-2(1*H*)-pyrimidinone (5). Compound **3c** (460 mg, 2 mmol) was hydrogenated in absolute MeOH (20 mL) with 10% Pd-C (80 mg) for 20 min. After removal of the catalyst, the product **5** was obtained by recrystallization from EtOH, 230 mg (83%): mp 179-181 °C; IR (KBr) 3350 (broad) and 1720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.30 (s, 3H, 4-Me), 2.45 (s, 3H, 6-Me), 6.15 (s, 1H, 5-H), 8.30 (br s, 1H, OH). Anal. Calcd for $C_6H_{12}N_2O_2$ $0.5H_2O$: C, 51.10; H, 5.75; N, 19.87. Found: C, 51.29; H, 5.72; N, 19.94.

***N*-Benzyloxy-N^{α}-(*t*-butoxycarbonyl)glycinamide (7).** To a mixture of *N*-(*t*-butoxycarbonyl)glycine (**6**, 3.97 g, 23 mmol) and Et_3N (2.38 g, 24 mmol) in THF (40 mL) was added dropwise a solution of

isobutyl chloroformate (3.28 g, 24 mmol) in THF (20 mL) at -17 °C. The reaction temperature was kept for 15 min at -15 °C, and then *O*-benzylhydroxylamine (2.5 g, 23 mmol) in THF (10 mL) was added to the mixture. The reaction mixture was kept at -15 °C for 3 h and at room temperature overnight, and then the resulting Et_3N·HCl was filtered off. After evaporation of the solvent, the residue was dissolved in AcOEt (200 mL). The organic phase was washed successively with a 5% aqueous $NaHCO_3$ solution (100 mL), 5% HCl (100 mL), and H_2O (100 mL), and then dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was chromatographed on silica gel (eluent: hexane-AcOEt=1:1) to give the pure product (**7**) as a colorless oil, 4.36 g (77%): IR (neat) 3400, 1690, 740, and 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.45 (s, 9H, Me_3C), 3.60 (m, 2H, CH_2), 4.80 (s, 2H,OCH_2), 5.75 (br s, 1H, $NHCO_2$), 7.40 (s, 6H, NH-O and Ph). Anal. Calcd for $C_{14}H_{20}N_2O_4$ $0.7H_2O$: C, 57.96; H, 7.43; N, 9.66. Found: C, 58.08; H, 7.29; N, 9.34.

***N*-(Benzyloxy)glycinamide Hydrochloride (8).** To a solution of compound **7** (8.41 g, 30 mmol) in dry dioxane (12 mL) was added 5.8 M HCl in dioxane (26 mL) with stirring upon cooling with an ice bath. The reaction mixture was stirred for 1 h, and the solvent was then removed under reduced pressure. A small amount of absolute EtOH was added to the residue and then evaporated to remove the HCl-dioxane completely. The resulting HCl salt (**8**) was washed with dry ether and used in the next reaction without further purification, 5.4 g (83%).

1-Benzyloxy-2(1*H*)-pyrazinone (9a). To a solution of the HCl salt (**8**, 10.6 g, 0.05 mol) in MeOH-H_2O (1:1, 80 mL) was added glyoxal (7.5 g, 0.05 mol) at -30 °C. The pH of the reaction mixture was adjusted to

8 with a 2 M aqueous NaOH solution and then stirred overnight at room temperature. After evaporation of the solvents, the residue was dissolved in $CHCl_3$ (100 mL). The organic phase was washed with H_2O (20 mL), dried over anhydrous $MgSO_4$, and then evaporated. The crude product was purified by column chromatography on silica gel (eluent: AcOEt-hexane=1:1) to give the pure product **9a**, 2.9 g (28%): mp 88-90 °C; IR (KBr) 1670 cm^{-1}; ^1H HMR ($CDCl_3$) δ 5.32 (s, 2H, CH_2), 6.98 (d *J* 2Hz, 1H, 5-H), 7.10 (d *J* 2 Hz, 1H, 6-H), 7.39 (m, 5H, Ph), 8.29 (s, 1H, 3-H). Anal. Calcd for $C_{11}H_{10}N_2O_2$: C, 65.33; H, 4.98; N, 13.86. Found: C, 65.56; H, 5.36; N, 13.92.

1-Benzyloxy-5,6-dimethyl-2(1*H*)-pyrazinone (9b). Similar reaction of the HCl salt (**8**) (3.1 g, 14.3 mmol) with 2,3-butanedione (1.5 g, 17 mmol) afforded the product **9b**, 1.8 g (53%): mp 118-121 °C; IR (KBr) 1650, 740, and 690 cm^{-1}; ^1H HMR ($CDCl_3$) δ 2.18 (s, 3H, 5-Me), 2.27 (s, 3H, 6-Me), 5.29 (s, 2H, CH_2), 7.45 (s, 5H, Ph), 8.14 (s, 1H, 3-H). Anal. Calcd for $C_{13}H_{14}N_2O_2$: C, 67.81; H, 6.13; N, 12.17. Found: C, 67.75; H, 6.17; N, 11.84.

1-Benzyloxy-5-methyl-2(1*H*)-pyrazinone (9c). The reaction of *N*-(benzyloxy)glycinamide hydrochloride (580 mg, 2.69 mmol) with pyruvic aldehyde (40 % in H_2O: 623 mg, 3.46 mmol) by a similar procedure for the preparation of **9a** gave the product (**9c**), 292 mg (19 %): mp 111-113 °C; ^1H NMR ($CDCl_3$) δ 2.15 (s, 3H), 5.30 (s, 2H), 6.82 (s, 1H), 7.40 (m, 5H), 8.25 (s, 1H); Calcd for $C_{12}H_{12}N_2O_2 \cdot 0.3H_2O$: C, 65.02; H,5.72; N, 12.64. Found: C, 65.13; H, 5.82; N, 12.52.

1-Benzyloxy-5,6-diphenyl-2(1*H*)-pyrazinone (9d). To a solution of 1-hydroxy-5,6-diphenyl-2(1*H*)-pyrazinone (**10d**)[6] (1.1 g, 4.2 mmol) and

Et_3N (0.5 g, 5.0 mmol) in DMSO (5 mL) was added benzyl chloride (0.8 g, 6.5 mmol). After stirring for 24 h at room temperature, the resulting precipitate was collected by filtration, and successively washed with H_2O and Et_2O. Purification by column chromatography on silica gel ($CHCl_3$:acetone:EtOH=100:20:4) afforded the product (**9d**), 1.4 g (98%); mp 160-162 °C; ^1H NMR ($CDCl_3$) δ 4.92 (s, 2H), 7.18-7.45 (m, 15H), 8.43 (s, 1H); Calcd for $C_{23}H_{18}N_2O_2 \cdot 0.5H_2O$: C, 76.02; H, 5.27; N, 7.71. Found: C, 76.04; H, 5.15; N, 7.29.

1-Benzyloxy-5,6,7,8-tetrahydro-2(1*H*)-quinoxalinone (9e). 1-Hydroxy-5,6,7,8-tetrahydro-2(1*H*)-quinoxalinone (**10e**) was prepared from *N*-(hydroxy)glycinamide and 1,2-cyclohexanedione by a similar procedure for the synthesis of **10d**. The crude product was recrystallized from a MeOH-Et_2O mixture. Compound **9e** was prepared from **10e** by a similar procedure for the synthesis of **9d**.

10e: 41%: mp 199-200 °C; ^1H NMR (DMSO-d_6) δ 1.75 (m, 4H), 2.65 (t, *J* 6 Hz, 2H), 2.75 (t, *J* 6 Hz, 2H), 7.88 (s, 1H); Calcd for $C_8H_{10}N_2O_2 \cdot 0.1H_2O$: C, 57.20; H, 6.12; N, 16.67. Found: C, 56.99; H, 6.23; N, 16.28.

9e: 58%: mp 127-127.5 °C; ^1H-NMR ($CDCl_3$) δ 1.70 (m, 4H,), 2.60 (t, *J* 6 Hz, 2H), 2.70 (t, *J* 6 Hz, 2H), 5.30 (s, 2H), 7.38-7.49 (m, 5H), 8.15 (s, 1H). Calcd for $C_{15}H_{16}N_2O_2$:C, 69.80; H, 6.33; N, 10.85. Found: C, 69.58; H, 6.13; N, 10.74.

1-Hydroxy-2(1*H*)-pyrazinone (10a). Compound **9a** (500 mg, 2.5 mmol) was hydrogenated in absolute MeOH (15 mL) with 10% Pd-C (60 mg) for 20 min by the same procedure as hydrogenation of **3c** to **5**. The product **10a** was obtained by column chromatography on silica gel (eluent:

$CHCl_3$-MeOH=6:1), 194 mg (70%): mp 168-170 °C (lit.[18] mp 166-168 °C); IR (KBr) 3420-3310 and 1630 cm^{-1}; ^1H NMR (D_2O) δ 7.58 (d *J* 5.8 Hz, 1H, 5-H), 8.06 (d *J* 5.8 Hz, 1H, 6-H), and 8.10 (s, 1H, 3-H).

1-Hydroxy-5,6-dimethyl-2(1*H*)-pyrazinone (10b). Compound **9b** (345 mg, 1.5 mmol) was hydrogenated in absolute MeOH (20 mL) with 10% Pd-C (60 mg) for 20 min. The product **10b** was obtained by recrystallization from an EtOH-hexane mixture, 160 mg (76%): mp 147-149 °C (lit[6] mp 145-149 °C).

1-Hydroxy-5,6-dimethyl-2(1*H*)-pyrazinone hydrobromide (10b·HBr). A solution of compound **9b** (143 mg, 0.62 mmol) in 30% HBr in acetic acid (3 mL) was stirred under reflux for 10 min. To the mixture was added Et_2O (15 mL). The resulting precipitate was collected by filtration, washed with Et_2O, and then dried *in vacuo* to give a pale red solid, 111mg (81%): ^1H NMR (D_2O) δ 2.47 (s, 3H, Me), 2.48 (s, 3H, Me), 7.71 (s, 1H, 3-H). Anal. Calcd for $C_6H_9BrN_2O_2$ H_2O:C, 30.14; H, 4.65; N, 11.72. Found: C, 30.12; H, 4.85; N, 11.65.

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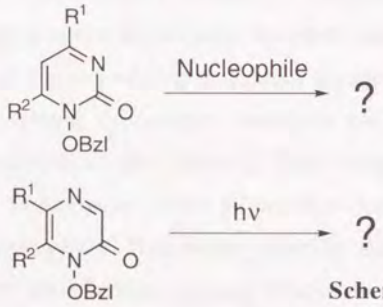
Chapter 3

Reaction of 1-Hydroxy-2(1*H*)-pyrimidinones and -pyrazinones

3.1 Introduction

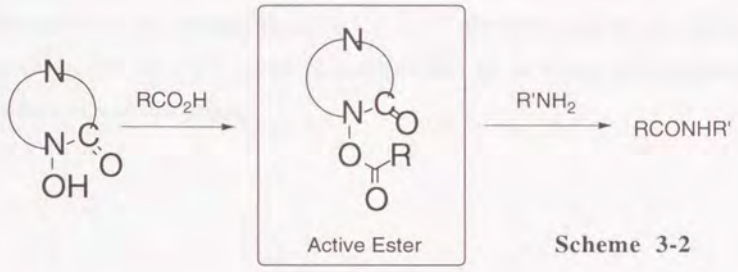
Reaction of diazines, especially with nucleohpiles have been well studied. It has been reported that the ring transformation of 1-aryl-2(1*H*)-pyrimidinones with hydroxylamine afforded the corresponding isoxazoles in good yields.[1] The replacement of the aryl group at the N-1 position of the pyrimidinone ring by benzyloxy group would be expected to change the reactivity toward nucleophiles (Scheme 3-1). Similarly, many aspects of the photochemistry of heterocycles have been established. Their photochemical reactions, which undergo under mild conditions quantitatively, for example ring transformation and cycloaddition, are often useful in organic synthesis. However, there are only a few papers focused on photochemical behavior of *N*-hydroxyamide-containing heterocycles.



Scheme 3-1

Recently, the application of heterocycles to organic syntheses has received considerable attention. However, the application of *N*-hydroxyamide-containing heterocycles has been limited. In 1987 Okuwaki

and co-workers[2] reported that when 1-benzoyloxy-2(1*H*)-pyrazinone, derived from 1-hydroxy-2(1*H*)-pyrazinone and benzoyl chloride, was treated with amines and aliphatic alcohols, the corresponding *N*-benzoylamines and alkylbenzoates were obtained in high yields, respectively.



Scheme 3-2

The electron density of the ring system of *N*-hydroxyamide-containing diazines, such as 1-hydroxy-4,6-dimethyl-2(1*H*)-pyrimidinone **5** and 1-hydroxy-2(1*H*)-pyrazinones **10**, seems to be lower than monoazines due to the introduction of the second electronegative nitrogen atom into the ring system. Thus, several interesting functions are expected in these compounds. As mentioned in Chapter 2, these compounds showed high water solubility. Furthermore, **5** and **10** are expected to show lower pKa values than do monoazines. High water solubility and low pKa value are essential properties for effective reagents in acylation reactions. Namely, the former property will promise an easy work up, and the latter one will be important in acceleration of the formation of a desired active ester. *O*-Acyl derivatives of *N*-hydroxyamide-containing heterocycles are regarded as active esters and then should be applicable as new mild acylating agents as

shown in Scheme 3-2. Consequently, there is great interest in the application of **5** and **10** to organic synthesis, especially to acylation.

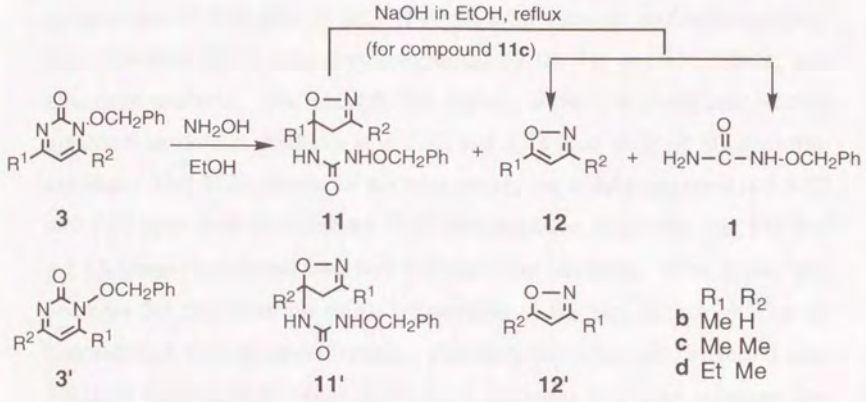
In this chapter, reaction of 1-benzyloxy-2(1*H*)-pyrimidinones (**3**) with *N*-hydroxylamine and photochemical behavior of 1-benzyloxy-2(1*H*)-pyrazinones (**9**) in a benzene solution are described. Furthermore, the application of 1-hydroxy-4,6-dimethyl-2(1*H*)-pyrimidinone (**5**) and -5,6-dimethyl-2(1*H*)-pyrazinone (**10b**) as new effective reagents for peptide synthesis by the DCC-additive method and as new benzyloxycarbonyl carriers is also discussed.

3.2 Reactivities

3.2.1 The Ring Transformation of 1-Benzyloxy-2(1*H*)-pyrimidinones[3]

The synthesis of a variety of new heterocycles by ring transformation of easily accesible heterocycles has received considerable attention, and a number of papers have appeared in literatures.[4,5] It has been reported that the ring transformation of 1-aryl-2(1*H*)-pyrimidinones with hydroxylamine afforded the corresponding isoxazoles in good yields.[1] The replacement of the aryl group at the N-1 position of the pyrimidinone ring by an electronegative benzyloxy group would be expected to change the reactivity toward nucleophiles. Therefore, the author was interested in the reaction of 1-benzyloxy-2(1*H*)-pyrimidinones with nucleophiles, especially with hydroxylamine from the viewpoint of the development of a new ring transformation.

When 1-benzyloxy-4,6-dimethyl-2(1*H*)-pyrimidinone (**3c**) was allowed to react with hydroxylamine hydrochloride in the presence of NaOH in absolute EtOH under reflux, 5-[*N*-(benzyloxy)urea]-attached 2-isoxazoline (**11c**) was isolated in 24% yield in addition to 3,5-dimethylisoxazole (**12c**) and *N*-benzyloxyurea (**1**) as shown in Scheme 3-3. The results are summarized in Table 3-1. Because isolation of **12c** was difficult due to its low boiling point, the yield was estimated from the ^{1}H NMR spectrum of the crude product. The structures of **1** and **12c** were determined by



Scheme 3-3

Table 3-1. Reaction of 1-benzyloxy-2(1*H*)-pyrimidinones and hydroxylamine[a]

Pyrimidinone	Yield[b] / %				
	11	**11'**	**12**	**12'**	**1**
3b	15	-	-	-	29
3'b	-	24	-	-	21
3c	24	-	15	-	18
3d	0		20	0	10

[a]In absolute EtOH with 6 times excess of hydroxylamine, and 6 times excess of NaOH under reflux overnight. [b]Yields were estimated from ^{1}H NMR spectra.

comparison of their data of IR, ^{1}H NMR with those of authentic samples. The structure of **11c** was also determined by IR, ^{1}H and ^{13}C NMR, and elemental analysis. For example, the signals of the C-4 methylene protons appeared as double doublets at δ 2.82 and 3.43 ppm with 18 Hz coupling constant. Two N-H protons of the urea moiety separately appeared at δ 6.02 and 7.03 ppm, both of which are D_2O exchangeable, indicating that **11c** had a 5-[*N*-(benzyloxy)urea]-attached 2-isoxazoline skeleton. This is the first example for the isolation of an isoxazoline in the ring transformation of heterocycles with hydroxylamine. Furthermore, **11c** was converted into 3,5-dimethylisoxazole (**12c**) and *N*-benzyloxyurea (**1**) upon refluxing the solution in the presence of NaOH in absolute EtOH, indicating that **11c** is a reaction intermediate in the formation of **12c** (Scheme 3-3). Similarly, 1-benzyloxy-4-methyl- (**3b**) and 1-benzyloxy-6-methyl-2(1*H*)-pyrimidinones (**3'b**) underwent the ring transformation to afford 2-isoxazoline derivatives (**11b** and **11'b**) in 15 and 24% yields, respectively. In these cases, the formation of the corresponding isoxazole derivatives was not detected in the ^{1}H NMR spectra of the crude products. It may be attributed to their low boiling points.

By the reaction of **3d**, the desired 2-isoxazoline could not be isolated. In the ^{1}H NMR spectrum, however, only one of two possible structural isomers of isoxazole, i.e., 5-ethyl-3-methylisoxazole (**12d**)[6,7] was detected even in the crude reaction mixture, suggesting that hydroxylamine regioselectively attacks the C-6 carbon of the pyrimidinone ring.

In order to clarify the reaction mechanism, the LUMO coefficients of **3c** were estimated by the MNDO molecular orbital calculation.[8] As a result, the calculation indicated that the LUMO coefficient of the C-6 carbon is

greater than that of the C-4 (Figure 3-1). Thus, the nucleophile would preferentially attack C-6.

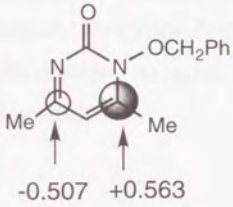
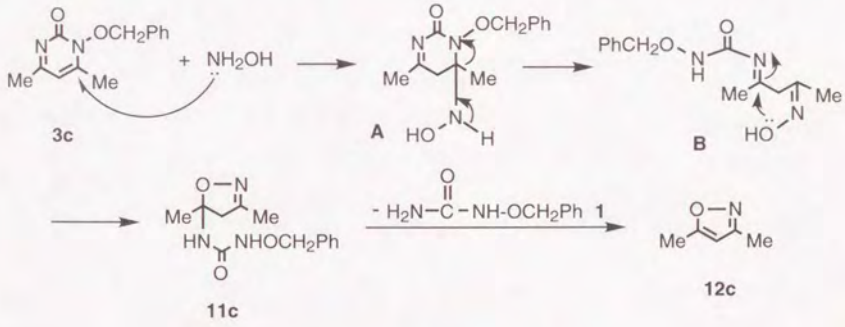


Figure 3-1. Estimated LUMO coefficients by the MNDO method.



Scheme 3-4. A possible reaction mechanism.

From these data, a possible reaction mechanism for the ring transformation is depicted in Scheme 3-4. The lone pair electron of the nitrogen of hydroxylamine predominantly attacks C-6 carbon to afford intermediate A, and then the ring opening occurs to give intermediate B.

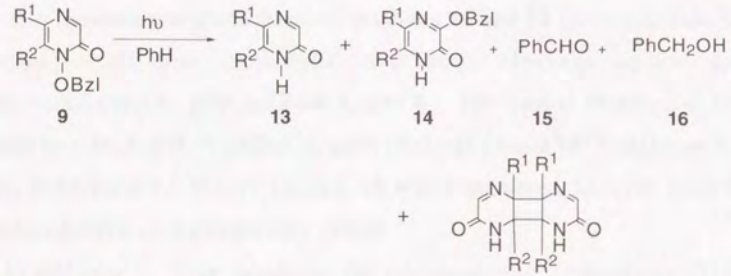
The following attack of the lone electron pair of the oxygen to the imino carbon yields the 2-isoxazoline derivative **11c**. The elimination of *N*-benzyloxyurea (**1**) gives 3,5-dimethylisoxazole (**12c**).

In conclusion, 1-benzyloxy-2(1*H*)-pyrimidinones underwent the ring transformation with hydroxylamine to give a new type of 5-[*N*-(benzyloxy)urea]-attached 2-isoxazolines.

3.2.2 Photochemical Reaction of 1-Benzyloxy-2(1*H*)-pyrazinones[9]

Since *N*-hydroxyamide-containing heterocycles can be regarded as cyclic hydroxamic acid, they are very attractive compounds from the viewpoint of application to the biomimetic chemistry by virtue of their strong metal binding ability and high water solubility. In fact, quite recently, the considerable works focused on their application to iron chelators in clinical usage have been published.[10] Therefore, it is very important to elucidate their chemical properties and reactivities in detail. However extensive work has been focused on their chemical behavior in the ground state except for some reports concerning photolysis of *N*-alkoxy-2(1*H*)-pyridones.[11-13] Furthermore, photochemical reactivity of *N*-hydroxyamide-containing diazine derivatives is still unknown. Here, the author would like to describe the synthesis of 1-benzyloxy-2(1*H*)-pyrazinones having bulky substituents at C-5 and C-6 of the ring and their photochemical behaviors in a benzene solution, including the rearrangement of benzyloxy group to C-3 of the ring.

Irradiation of a benzene solution of 1-benzyloxy-2(1*H*)-pyrazinone (**9a**) with a 450-W high-pressure mercury lamp through a Pyrex filter under a nitrogen atmosphere at room temperature for 1.5 h gave 2(1*H*)-pyrazinone (**13a**), benzaldehyde (**15**), and benzyl alcohol (**16**) in 53%, 31%, and 18% yields, respectively (Scheme 3-5).



9 13 14 15 16

17

Scheme 3-5

Photolysis of **9b-e** in benzene gave mixtures of similar products. The results are summarized in Table 3-2.

Table 3-2. Chemical and quantum yields

	9		Φ_{dis}[a]	Yield / %[b]				
	R^1	R^2		13	14	15	16	17
a	**H**	**H**	1.00	53	-	31	18	-
b	**Me**	**Me**	0.95	50	5	26	-	-
c	**Me**	**H**	0.71	48	-	24	-	-
d	**Ph**	**Ph**	0.90	61	10	25	-	-
e	**-(CH2)4-**		1.00	64	16	77	-	4

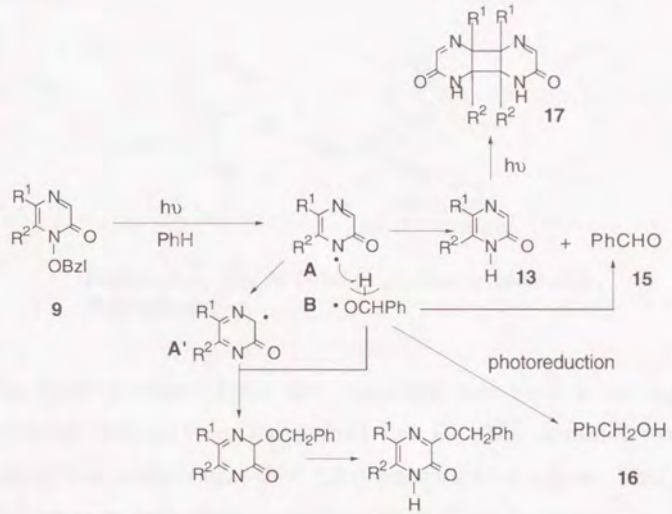
[a]Quantum yields for disappearance of **9**. [b]Theoretical yields should be 200%.

The formation of photochemical products **13** and **15** can be explained as follows. At first, homolytic N-O bond cleavage occurs upon photoirradiation to give radicals A and B. The proton abstraction from benzyloxy radical B by radical A gave products **13** and **15**[11] (Scheme 3-6). The generation of benzyl alcohol **16** was considered to arise from the photoreduction of the benzyloxy radical.

In addition to these products, the corresponding 3-benzyloxy-2(1*H*)-pyrazinones (**14**) were isolated in the cases of **9b**, **9d** and **9e**. Their structures were determined on the basis of their ^{1}H NMR spectra; the singlet of the 3-H of **9** at around 8 ppm disappeared, and that singlet of the benzyl methylene protones was shifted to a lower field ($\Delta\delta$ 0.1-0.6 ppm). Thus, the result suggests that the rearrangement of the benzyloxy group from the N-1 to the C-3 of the ring was competitively occurred with N-O bond scission, followed by coupling of radical B with radical A', which is an isomer of radical A, and proton transfer (Scheme 3-6).

Furthermore, slight benzene insoluble precipitate was obtained in the case of **9e**. From spectroscopic analyses, the structure of the compound was assigned to be cyclobutano[1,2-*e*:1,2-*e*']dipyrazinone derivative (**17e**). IR spectrum showed the amide carbonyl stretching absorption around 1650 cm^{-1}, and the ^{1}H NMR spectrum showed a singlet at δ 7.75 ppm assignable to the 3-H, suggesting that the pyrazinone ring system was remained. Furthermore, the ^{13}C NMR spectrum showed peaks at 30.5 (s), 30.6 (s), 128.1 (d), and 156.1 (s) ppm, which would correspond to the carbons at 5-(or 6-), 6-(or 5-), and 3- positions, and the carbonyl carbon respectively. In order to clarify the reaction mechanism for the photoreaction of 2(1*H*)-pyrazinone, **13e** was irradiated in a $CHCl_3$ solution. As a result, **17e** was

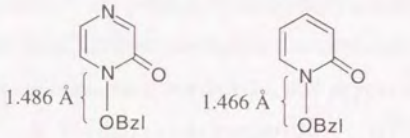
obtained in 24% yield. Thus, it is concluded that cyclobutane derivative **17e** was yielded by the [2+2] cycloaddition of 2(1*H*)-pyrazinone **13e** (Scheme 3-6).



Scheme 3-6

The quantum yields for disappearance of **9a-e** were measured using valerophenone as an actinometer at 313 nm. The results are summarized in Table 3-2. The quantum yields ranged from 0.71 to 1.0, suggesting the reaction occurred more efficiently than that of 1-benzyloxy-2(1*H*)-pyridinone (Φ_{dis} 0.27).[11] These high quantum yields may be attributable to weakness of the N-O bond owing to the π-electron deficient ring system

compared to the monoazine. On PM3 calculations,[14] estimated N-O bond distances of **9a** (1.486 Å) was slightly longer than that of pyridinone (1.466 Å) as shown in Figure 3-2. This result indicates that the electron deficient ring system of **9a** causes the N-O bond to be weak compared to that of pyridinone.



9a 1-Benzyloxy-2(1*H*)-pyridinone

Figure 3-2. The N-O bond distance estimated by PM3 calculation.

The high quantum yields also suggested that there is no apparent deactivation process from the excited states. The reactions were not quenched with a triplet quencher, 2,5-dimethyl-2,4-hexadiene. This means that the reactions exclusively proceed *via* the $\pi\,\pi^*$ singlet states.

3.3 Application to Organic Synthesis

3.3.1 DCC-Additive Method for Peptide Synthesis[15]

A variety of methods for peptide bond formation have been developed.[16] Among them, the DCC-additive method is the most convenient because of its mild reaction conditions, easy operations, and suppression of racemization (Figure 3-3). *N*-Hydroxysuccinimide (HOSu)[17,18] and 1-hydroxybenzotriazole (HOBt)[7] are widely used as additives, but they have some disadvantages. In this section, the author describes the application of 1-hydroxy-4,6-dimethyl-2(1*H*)-pyrimidinone **5** and -5,6-dimethyl-2(1*H*)-pyrazinone **10b** as new effective additives for peptide synthesis by the DCC-additive method.

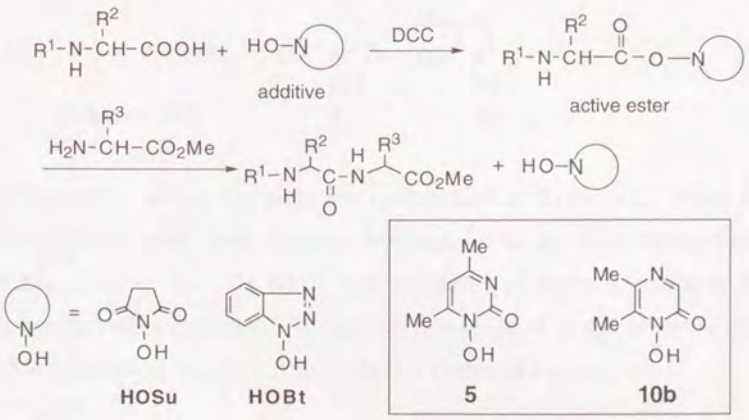
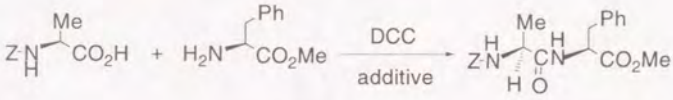
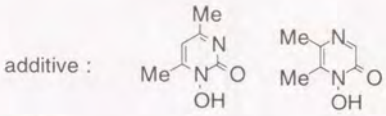


Figure 3-3. DCC-additive method.

Compounds **5** and **10b** were prepared according to the method described in Chapter 2.[20] The high water solubility of both compounds is one of the most important properties as additives for peptide synthesis.

The usefulness of **5** and **10b** as additives was investigated by coupling reaction between *N*-benzyloxycarbonyl-L-alanine (Z-Ala-OH) and L-phenylalanine methyl ester (H-Phe-OMe) as shown in Scheme 3-7, which is known to be one of the most racemizable combination. In this case, the occurrence of racemization during the coupling reaction is easily estimated by means of ^{1}H NMR, according to the method reported by Weinstein et al.[21,22] and Kawasaki et al.;[23] the methyl proton signals of D-Ala and L-Ala separately appeared at δ 1.28 and 1.33 ppm, respectively.





Scheme 3-7 **5** **10b**

The yields of the dipeptide are summarized in Table 3-3. From the viewpoint of yield, both diazines are superior to the most conventional HOBt as additives. ^{1}H NMR was measured in order to estimate the occurrence of racemization. The methyl proton signal of Ala of Z-Ala-Phe-OMe prepared by the DCC-**10b** method is shown in Figure 3-3.

Table 3-3. Coupling yields of dipeptides by the DCC-additive method

		Additive		
Dipeptide(%)	None	HOBt	**5**	**10b**
ZAlaPheOMe	53	78	83	85
ZPheAlaOMe	-	-	81	86
ZAlaSerOMe	-	-	90	-
BocGlu(OMe)GlyOBzl	-	-	98	-

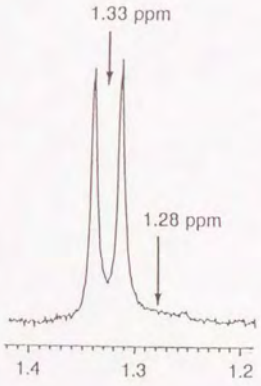


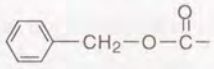
Figure 3-3. ^{1}H NMR spectrum of the methyl signal of Ala in Z-Ala-Phe-OMe prepared by the DCC-**10b** method.

In the case of **5**, the same spectrum was recorded. No signal was observed at δ 1.28 ppm, indicating that a measurable racemization did not occur during the coupling reaction. Similarly, the coupling reaction of several L-amino acids in the presence of diazines **5** and **10b** by the DCC-additive method afforded the corresponding dipeptides in high yields as shown in Table 3-3.

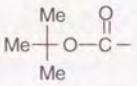
It is concluded from these results that diazines **5** and **10b** formed active ester, and acted as effective new additives for peptide synthesis by the DCC-additive method.

3.3.2 Benzyloxycarbonylating Agent for Amines, Amino Acids, and Alcohols[24]

Benzyloxycarbonyl (Z) group is one of the most important amino protecting groups as well as *t*-butoxycarbonyl (Boc) group, because these carbamate-type protecting groups are resistant to racemization during peptide syntheses (Figure 3-4).[25,26] Moreover, Z-protecting group is easily removed by the catalytic hydrogenation in the presence of palladium.



Z: benzyloxycarbonyl



Boc: *tert*-butyloxycarbonyl

Figure 3-4. Amino protecting group

Recently, the application of heterocycles to organic synthesis has received considerable attention. For example, pyridine,[27] pyrimidine,[28] and pyrazine[2,29,30] have been demonstrated to be useful *tert*-butoxycarbonylating agents. The utilization of 2-hydroxy- and 2-mercaptopyrazines as benzyloxycarbonylating agents has been reported by Ohta and co-workers,[31] while no paper concerning 1-hydroxy-2(1*H*)-pyrimidinone has been reported. Since the pKa value of 1-hydroxy-4,6-dimethyl-2(1*H*)-pyrimidinone (**5**) was estimated to be 6.1,[32] its *O*-acyl derivatives are regarded as active esters and should be applicable as new mild acylating agents. As a part of the studies concerning the application of

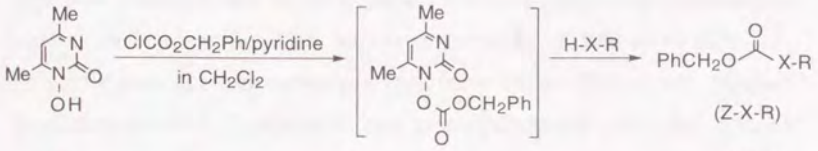
N-hydroxyamide-containing heterocycles to organic synthesis,[15] the utilization of 1-hydroxy-4,6-dimethyl-2(1*H*)-pyrimidinone (**5**) as a new benzyloxycarbonyl carrier was investigated.

Compound **5** was treated with benzyl chloroformate in the presence of pyridine in CH_2Cl_2 at 0 °C to give the corresponding *O*-benzyloxycarbonyl-2(1*H*)-pyrimidinone (**18**). In the IR spectrum, **18** showed two absorption bands at 1810 and 1755 cm^{-1}, characteristics of an active ester. Since the partial decomposition of **18** was observed during isolation, a one-pot reaction was carried out by treatment of the active ester with various amines. The results are summarized in Table 3-4.

Ohta and co-workers have reported that the benzyloxycarbonylation of aniline with 2-benzyloxycarbonyloxy- or 2-benzyloxycarbonylthiopyrazine did not proceed and resulted in recovery of the starting materials.[31] In contrast, the reaction of aniline with the active ester **18** gave *N*-benzyloxycarbonylaniline **19b** in 63% yield through normal benzyloxycarbonylation. These results indicate the advantage of **18** as a benzyloxycarbonylating agent.

The one-pot benzyloxycarbonylation of L-amino acid was also investigated. In the case of L-alanine, Z-Ala-OH (**19e**) was obtained in 87% yield. The specific rotation of the product **19e** was identical with the reported value,[33] indicating that a measurable racemization did not occur during this reaction. Similarly, two amino acids, L-alanine methyl ester and L-phenylalanine methyl ester, were also subjected to the one-pot benzyloxycarbonylation to give the corresponding Z-amino acids, **19f** and **19g**, in good yields.

Table 3-4. The Benzyloxycarbonylation of Amines, Amino Acids, and Alcohols



5 **18** **19a-k**

Substrate (H-X-R)	Reaction conditions		Product (Z-X-R)	Yield (%)
H_2NCH_2Ph	room temp/3 h	**19a**	Z-$NHCH_2Ph$	85
H_2NPh	room temp/3 h	**19b**	Z-NHPh	63
pyrrolidine	room temp/3 h	**19c**	Z-pyrrolidine	84
morpholine	room temp/3 h	**19d**	Z-morpholine	97
H-L-Ala-OH	room temp/24 h	**19e**	Z-L-Ala-OH	87
H-L-Ala-OMe	room temp/3 h	**19f**	Z-L-Ala-OMe	78
H-L-Phe-OMe	room temp/3 h	**19g**	Z-L-Phe-OMe	75
$HOCH_2Ph$	room temp/20 h	**19h**	Z-OCH_2Ph	44
$HOCH_2Ph$	reflux/4 h [a]	**19h**	Z-OCH_2Ph	56
$HOCH_2Ph$	reflux/20 h[a]	**19h**	Z-OCH_2Ph	85
$HOPr^n$	reflux/20 h[a]	**19i**	Z-OPr^n	73
$HOCH(Et)_2$	reflux/ 48 h[a]	**19j**	Z-$OCH(Et)_2$	0
HOPh [b]	NaH, reflux/ 48 h[a]	**19k**	Z-OPh	0

[a]In benzene. [b]Attempts to prepare Z-OPh in the presence / absence of Et_3N were also unsuccessful, and the starting material was completely recovered.

The benzyloxycarbonylation of benzyl alcohol was carried out at room temperature in a similar fashion to that of amines, but the desired carbonate **19h** was obtained only in 44% yield. However, refluxing the reaction mixture in benzene for 20 h improved the yield of **19h** up to 85%. 1-Propanol gave the corresponding carbonate **19i** in 73% yield. Neither secondary alcohol, 3-pentanol, nor aromatic phenol gave the desired carbonate even under more drastic reaction conditions. The results indicate that the steric hindrance and the acidity of these alcohols may suppress benzyloxycarbonylation.

In conclusion, 1-hydroxy-4,6-dimethyl-2(1*H*)-pyrimidinone (**5**) was found to be a new efficient benzyloxycarbonylating agent for amines, amino acids, and alcohols.

3.4 Experimental

General. Melting points were determined on a Mel-Temp apparatus in open capillaries and are uncorrected. IR spectra were recorded on a JASCO A-100 Infrared Spectrophotometer, and UV-vis spectra were measured with a JASCO Ubest V-550 spectrophotometer. ^{1}H NMR spectra were obtained for $CDCl_3$ or DMSO-d_6 solutions on a 270 MHz JEOL GX-270. Chemical shifts are reported in ppm (δ) downfield from internal TMS. Optical rotations were determined on a JASCO PIP-370 digital polarimeter. Thin layer chromatography (TLC) analyses were 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). 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. An Ushio 100 W or 450 W high-pressure mercury lamp was used as an irradiation source.

Compounds 1-benzyloxy-4-methyl- (**3b**), -6-methyl- (**3'b**), -4,6-dimethyl- (**3c**), -4-ethyl-6-methyl-2(1*H*)-pyrimidinone (**3d**), 1-hydroxy-4,6-dimethyl-2(1*H*)-pyrimidinone (**5**), 1-benzyloxy- (**9a**), -5,6-dimethyl- (**9b**), -5-methyl- (**9c**), -5,6-diphenyl-2(1*H*)-pyrazinone (**9d**), 1-benzyloxy-5,6,7,8-tetrahydro-2(1*H*)-quinoxalinone (**9e**), and 1-hydroxy-5,6-dimethyl-2(1*H*)-pyrazinone (**10b**) were prepared by the methods mentioned in Chapter 2.

A typical procedure for the reaction of a 2(1*H*)-pyrimidinone derivative with hydroxylamine. A mixture of 1-benzyloxy-4,6-dimethyl-2(1*H*)-pyrimidinone (**3c**) (368 mg, 1.6 mmol), hydroxylamine

hydrochloride (665 mg, 9.6 mmol), and NaOH (405 mg, 9.6 mmol), in absolute EtOH (30 mL) was refluxed for 21 h. After removal of the solvent, the residue was dissolved in H_2O (50 mL) and extracted with $CHCl_3$ (3x50 mL). The combined extracts were washed with H_2O (50 mL), and then dried over anhydrous Na_2SO_4. After evaparation of the solvent, the residue was chromatographed on silica gel (eluent: $CHCl_3$:acetone:EtOH=100:5:1) to give *N*-benzyloxy-*N'*-(3,5-dimethyl-2-isoxazolin-5-yl)urea (**11c**); Rf=0.26 ($CHCl_3$:acetone:EtOH=100:5:1); mp 93-94 °C; λ_{max} (log ε in EtOH): 207 (4.07) and 258 nm (2.16); IR (KBr) 3200, 1660, 1533, 754, and 711 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.63 (s, 3H), 1.97 (s, 3H), 2.82 and 3.43 (dd, *J* 18 Hz, 2H), 4.77 (s, 2H), 6.02 (br s, 1H, D_2O exchangeable), 7.03 (br s, 1H, D_2O exchangeable), and 7.40 (m, 5H); ^{13}C NMR ($CDCl_3$) δ: 13.2 (q), 25.6 (q), 49.0 (t), 78.7 (t), 92.1 (s), 128.8 (d), 129.0 (s), 129.4 (d), 135.3 (s), 156.9 (s), 158.2 (d). Anal. Calcd for $C_{13}H_{17}N_3O_3$: C, 59.32; H, 6.46; N, 15.70. Found: C, 59.32; H, 6.49; N, 15.69. 3,5-Dimethylisoxazole (**12c**) and *N*-benzyloxyurea (**1**) were identified by comparision of ^1H NMR with that of an authentic sample and by the mixed melting method with an authentic sample, respectively.

***N*-Benzyloxy-*N'*-(5-methyl-2-isoxazolin-5-yl)urea (11b).** An attempt to purify **11b** and **11'b** by column chromatography was unsuccessful due to their partial decomposition. A viscous oil: Rf=0.4 ($CHCl_3$:acetone:EtOH=100:10:2); yield: 15%; IR (neat) 1691, 1520, 775, and 741 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 1.65 (s, 3H), 2.85 and 3.54 (dd, *J* 18 Hz, 2H), 4.78 (s, 2H), 6.01 (br s, 1H), 6.95 (br s, 1H), 7.16 (s, 1H), 7.35-7.45 (m, 5H).

***N*-Benzyloxy-*N'*-(3-methyl-2-isoxazolin-5-yl)urea (11'b).** A viscous oil; Rf=0.32 ($CHCl_3$:acetone:EtOH=100:10:2); yield: 24%; IR (neat) 1691, 1522, 781, and 737 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.02 (s, 3H), 2.48-2.58 (dd, *J* 5 and 17 Hz, 1H), 3.15-3.25 (dd, *J* 8 and 17 Hz, 1H), 4.76 and 4.85 (dd, *J* 11 Hz, 2H), 6.1-6.2 (m, 2H), 7.07 (br s, 1H), 7.3-7.5 (m, 5H).

A typical procedure for the photolysis of 9. A solution of **9a** (200 mg, 1.00 mmol) in benzene (30 mL) was irradiated with a 450 W high-pressure mercury lamp under a nitrogen atmosphere for 1.5 h. After removal of the solvent, products were isolated by flash chromatography under a nitrogen atmosphere on a silica gel ($CHCl_3$-acetone-EtOH=100:20:4). 2(1*H*)-Pyrazinone (**13a**), benzyaldehyde (**15**), and benzylalcohol (**16**) were identified by conparison of ^1H NMR with that of an authentic sample, respectively.

13b: mp 208-210 °C (lit[34] 201-202 °C); ^1H NMR ($CDCl_3$) δ 2.31 (s, 3H), 2.32 (s, 3H), 8.02 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 16.8, 18.7, 132.0,134.0,143.8, and 158.8.

13c: mp 122-124 °C (lit[34] 126-128 °C).

13d: mp 239-240 °C (lit[34]: 243-244 °C); ^1H NMR ($CDCl_3$) δ 7.20-7.60 (m, 11H), 8.27 (s, 1H).

13e: mp 235-237 °C; ^1H NMR ($CDCl_3$) δ 1.78-1.88 (m, 4H), 2.65-2.73 (m, 4H), 8.03 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 20.84, 22.04, 25.94, 28.26, 130.46, 135.00, 143.55, and 156.20. Calcd for $C_8H_{10}N_2O \cdot 0.1H_2O$: C, 63.22; H, 6.76; N, 18.43. Found: C, 63.31; H, 6.65; N, 18.20.

14b: mp 212-215 °C; ^1H NMR ($CDCl_3$) δ 2.12 (s, 3H), 2.21 (s, 1H), 5.92 (s, 2H), 7.30-7.40 (m, 5H). Calcd for $C_{15}H_{16}N_2O_2$:C, 62.89; H, 6.50; N, 11.28. Found: C, 63.16; H, 6.10; N, 10.87.

14d: ^{1}H NMR ($CDCl_3$) δ 5.5 (s, 2H), 7.20-7.60 (m, 11H). Calcd for $C_{23}H_{18}N_2O_2$:C, 74.63; H, 5.27; N, 7.25. Found: C, 74.88; H, 5.42; N, 7.19.

14e: mp 235-237 °C; ^{1}H NMR ($CDCl_3$) δ 1.72-1.82 (m, 4H), 2.48-2.58 (m, 4H), 5.40 (s, 2H), 7.29-7.50 (m, 5H). Calcd for $C_{15}H_{16}N_2O_2$: C, 69.80; H, 6.33; N, 10.85. Found: C, 70.01; H, 6.30; N, 10.63.

17e: mp 260-261 °C; IR (KBr) 1653 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.70-1.80 (m, 4H), 2.53-2.60 (m, 4H), 7.75 (s, 1H); ^{13}C-NMR (DMSO-d_6) δ 20.94, 22.11, 26.14, 28.40, 30.52, 128.11, and 156.09; MS *m/z* 150 ($M^+/2$); Calcd for $C_{16}H_{20}N_4O_2$:C, 72.71; H, 5.08; N, 14.13. Found: C, 72.99; H, 4.78; N, 13.83.

Quantum yield determination. A benzene solution of **9** (ca. 50 mM), containing a known concentration of eicosane (ca. 1 mM) as a caliburant, was placed in a 15x150 mm Pyrex culture tube. The tube was degassed with three freeze-pump-thaw cycles and then sealed. Irradiation was performed on a "merry-go-round" apparatus with a 450 W high-pressure mercury lamp. A potassium chromate filter solution was used to isolate the 313 nm line. Analysis was performed on a Shimazu GC-4B gas chromatograph apparatus equipped with a flame ionization detector using a 2 m column containing SE-30. The quantum yield was determined by using a valerophenone actinometer.

A typical procedure for coupling of Z-Ala-OH and H-Phe-OMe. To a mixture of Z-Ala-OH (1.15 g, 5 mmol), HCl·H-Phe-OMe (1.08 g, 5 mmol), *N*-methylmorpholine (505 mg, 5 mmol), and 1-hydroxy-5,6-dimethyl-2(1*H*)-pyrazinone (**10b**) (700 mg, 5 mmol) in THF (20 mL) was added DCC (1.03 g, 5 mmol) at -5 °C. The mixture was stirred for 3 h at 0

°C and then 20 h at room temperature, and then the resulting *N, N'*-dicyclohexylurea was removed by filtration. After usual workup, recrystallization from AcOEt-petroleum ether afforded Z-Ala-Phe-OMe (85%): mp 100-102 °C (lit.[35] 99-100 °C); $[\alpha]_D^{22}$ -14.9° (c 1.0, EtOH) (lit.[23] $[\alpha]_D^{26}$ -10.4 ° (c 1.0 EtOH)).

Coupling of Z-Phe-OH and H-Ala-OMe. Synthetic procedure of dipeptide Z-Phe-Ala-OMe was similar as described above (81% for in the case of **5** and 86% in the case of **10b**): mp 128-129 °C (lit.[36] 130-131 °C); $[\alpha]_D^{29}$ -24 ° (c 1.0, MeOH).

Coupling of Z-Ala-OH and H-Ser-OMe. Synthetic procedure of dipeptide Z-Ala-Ser-OMe by using of **5** was similar as described above (90%): mp 128 °C (lit.[37] 134.5-135.5 °C); $[\alpha]_D^{23}$ -16.8 ° (c 1.7, MeOH). (lit.[37] $[\alpha]_D$ -17 ° (c 3.43, MeOH)).

Coupling of Boc-Glu(OMe)-OH and H-Gly-OBzl. Synthetic procedure of dipeptide Boc-Glu(OMe)-Gly-OBzl by using of **5** was similar as described above (98%): mp 63-65 °C (lit.[38] 67-69 °C); $[\alpha]_D^{23}$ -17.9 ° (c 2.0, MeOH). (lit.[38] $[\alpha]_D$ -18 ° (c 4.0, MeOH)).

A typical procedure for benzyloxycarbonylation. *N*-Benzyloxycarbonylbenzylamine (19a). To a solution of **5** (280 mg, 2 mmol) and pyridine (175 mg, 2.2 mmol) in CH_2Cl_2 (10 mL) was added dropwise a solution of benzyl chloroformate (360 mg, 2.1 mmol) in CH_2Cl_2 (5 mL) at 0 °C, and then the solution was stirred for 1 h until the starting materials disappeared on TLC. To the solution was added a solution of benzylamine (215 mg, 2 mmol) in CH_2Cl_2 (5 mL), and the reaction mixture was stirred for another 3h at room temperature. The CH_2Cl_2 layer was successively washed with 10% KOH (10 mL), 10% HCl

(10 mL), and H_2O (10 mL), and then dried over anhydrous Na_2SO_4. Column chromatography on silica gel (eluent: $CHCl_3$) afforded *N*-benzyloxycarbonybenzylamine **19a**[33](85%): IR($CHCl_3$) 3448 and 1718 cm^{-1}; ^1H NMR($CDCl_3$) δ 4.38 (d, *J* 8 Hz, 2H), 5.05 (br s, 1H), 5.14 (s, 2H), 7.38 (10H, m).

19b: IR($CHCl_3$) 3435 and 1736 cm^{-1}; ^1H NMR($CDCl_3$) δ 5.21 (s, 2H), 7.05 (br s,1H), 7.34 (m, 10H). Anal. Calcd for $C_{14}H_{13}NO_2$: C, 73.67; H, 5.79. Found C, 73.99; H, 5.77.

19c[29]: IR($CHCl_3$) 1720 cm^{-1}; ^1H NMR($CDCl_3$) δ 1.86 (m, 4H), 3.37 (m, 4H), 5.12 (s, 2H), 7.36 (5H, m).

19d[39]: IR($CHCl_3$) 1720 cm^{-1}; ^1H NMR($CDCl_3$) δ 3.49 (m, 4H), 3.65 (m, 4H), 5.12 (s, 2H), 7.31 (m, 5H).

19e[33]: IR(neat) 3480-2600, 1720, and 1700 cm^{-1}; ^1H NMR($CDCl_3$) δ 1.43 (d, *J* 7 Hz, 3H), 4.40 (m, 1H), 5.10 (s, 2H), 5.41 (br s, 1H), 7.34 (m, 5H); $[\alpha]_D^{23}$ -17.9° (c 0.23 in AcOH) (lit.[33] $[\alpha]_D^{27}$ -14.5° (c 0.21 in AcOH)).

19f: IR($CHCl_3$) 3435, 1740, and 1720 cm^{-1}; ^1H NMR($CDCl_3$) δ 1.20 (d, *J* 7 Hz, 3H), 3.50 (s, 3H), 4.2 (quint, *J* 7 Hz, 1H), 4.92 (s, 2H), 5.30 (br s, 1H), 7.18 (m, 5H). Anal. Calcd for $C_{12}H_{15}NO_4$: C, 60.75; H, 6.37. Found C, 60.81; H, 6.52.

19g: IR($CHCl_3$) 3430, 1740, and 1717 cm^{-1}; ^1H NMR($CDCl_3$) δ 3.0-3.2 (m, 2H), 3.64 (s, 3H), 4.51 (m, 1H), 5.10 (s, 2H), 5.33 (br s, 1H), 7.25 (m, 10H); $[\alpha]_D^{25}$ +6.4° (c 1.0 in AcOH). Anal. Calcd for $C_{18}H_{19}NO_4$: C, 68.03; H, 6.26. Found C, 68.09; H, 6.11.

19h[40-42]: IR($CHCl_3$) 1709 cm^{-1}; ^1H NMR($CDCl_3$) δ 5.14 (s, 4H) 7.38 (m, 10H).

19i[41]: IR(neat) 1760 cm^{-1}; ^1H NMR($CDCl_3$) δ 0.94 (t, *J* 7 Hz, 3H), 1.67 (q, *J* 7 Hz, 2H), 4.09 (t, *J* 7 Hz, 2H), 5.13 (s, 2H), 7.34 (m, 5H).

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Chapter 4

Bidentate Ligands: Iron Chelating Properties of 1-Hydroxy-2(1*H*)-pyrimidinones and -pyrazinones

4.1 Introduction

Hydroxamic acid is one of essential functional components of natural products, and many derivatives are found in low-molecular-weight iron chelators, called siderophores (from thd Greek, "iron carrier"), secreted by microbes. An X-ray crystallographic analysis of acetohydroxamic acid hemihydrate (Figure 4-1)[1] shows that atoms C-1, C-2, N, O-1, and O-2 are almost coplanar with a Z-form stabilized by two hydrogen bonds.

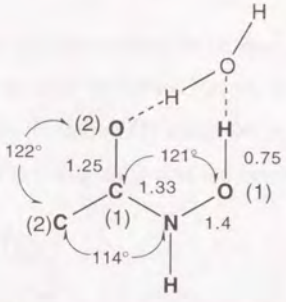

Figure 4-1. Bond length (Å) and bond angles for crystalline acetohydroxamic acid hemihydrate

Hydroxamic acids act as bidentate ligands towards many metal ions, such as Fe(III), Cu(II), Al(III), V(V), Mn(II), U(III), and Sn(II) (Figure 4-2).[2,3] The resultant complexes are generally neutral and are highly colored (red or violet). Therefore, they are useful in colorimetric analyses of metal ions. Violet coloration on treatment of any compound containing a

-C(O)N-OH group with ferric chloride is a well-known spot test for hydroxamic acids.[4]

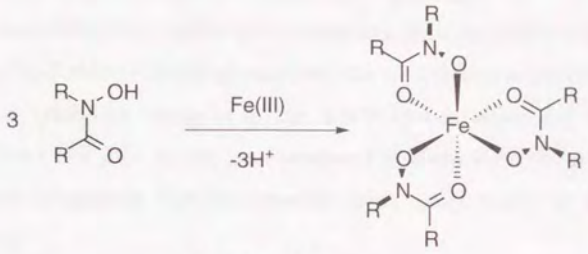

Figure 4-2

Recently, Raymond and co-workers have been focused their interest on the hydroxypyridones as new bidentate ligands to iron.[5,6] They reported that 1-hydroxy-2(1*H*)-pyridinone (**21**) could be an efficient ligand to iron in an aqueous solution. It is noteworthy that the heterocycle can be regarded as

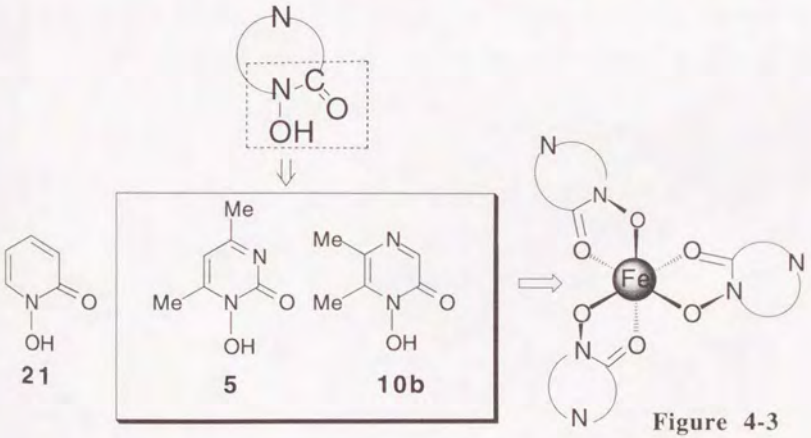

Figure 4-3

an aromatic hydroxamic acid. The fact prompted the author to start the study concerning the application of *N*-hydroxyamide-containing diazines to new iron chelators as shown in Figure 4-3. In Chapter 3, the author have already described that 1-hydroxy-4,6-dimethyl-2(1*H*)-pyrimidinone **5** and 1-hydroxy-5,6-dimethyl-2(1*H*)-pyrazinone **10b** were useful as acylating agents in organic syntheses owing to higher solubilities in water and lower pKa values. Their low pKa values are considered to allow them to form 3:1 iron complexes effectively than hydroxamic acids under acidic to neutral pH conditions.

In this chapter, iron chelating ability of **5** and **10a-b** as bidentate ligands in an aqueous solution is described.

4.2 Iron Chelating Properties of 1-Hydroxy-2(1*H*)-pyrimidinones and -pyrazinones[7]

1-Hydroxy-4,6-dimethyl-2(1*H*)-pyrimidinone **5**, 1-hydroxy- **10a**, -5,6-dimethyl-2(1*H*)-pyrazinone **10b** were prepared by the method mentioned in Chapter 2.

(a) Measurement of their pK_a values

The pK_a values of **5** and **10a,b** were measured by titration with 0.1M NaOH under an Ar atmosphere in aqueous solutions. The results are shown in Table 4-1 with reported values of monoazines, **21** and **22a** (Figure 4-4).[6] The relative acidity decreases in the order of **10a** >**10b** > **21** > **5** >> **22a**. Pyrazinone derivatives **10a,b** show higher acidity than does the pyrimidinone derivative **5** having an ureido-type carbonyl group. As expected, the pKa values of diazines, particularly those of pyrazinones, are much lower than that of monoazines.

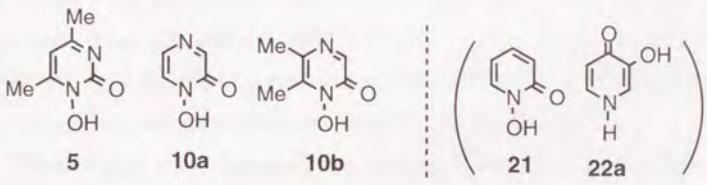

Figure 4-4

Table 4-1. pK_a Values of 1-hydroxy-2(1*H*)-diazines

Ligand	pKa
5[a]	6.1
10a	4.4
10b	4.7
21[b]	5.8
22a[b]	9.0

[a]Ref. 8 [b]Ref. 6.

(b) Iron complex formation

The UV-vis spectra of a 3:1 molar mixture of **10b** and ferric ion in aqueous solutions under various pH conditions are shown in Figure 4-5. The absorption maximum due to the ligand-to-metal charge transfer (LMCT band) was observed at 400-500 nm. In the acidic region, the increase of pH caused blue-shift of the absorption maximum with an increase of absorbance, reflecting the transformation of the 1:1 complex into a 2:1 and then into a 3:1 complex. At pH 4.0, the λ_{max} and ε value of **10b**-Fe(III) complex were 425 nm and 4237 M^{-1} cm^{-1}, respectively. Similar results were obtained for **10a** (λ_{max}=445 nm and ε=2980 M^{-1} cm^{-1} at pH 2.2) and **5** (λ_{max}=405 nm and ε=3470 dm^3 mol^{-1} cm^{-1} at pH 6.0).

These λ_{max} and ε values of the iron(III) complexes are comparable to those of **21**-Fe(III)=3:1 complex previously reported by Raymond and co-workers.[6]

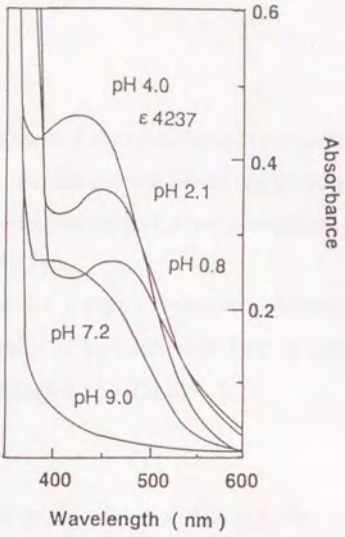

Figure 4-5. UV-vis spectra of a [**10b**]:[Fe(III)]=3:1 mixture in aqeous solution under various pH conditions: [Fe(III)]=1.0×10^{-4} M. (1) pH 4.0 (ε 4237), (2) pH 2.1, (3) pH 0.8, (4) pH 7.2, (5) pH 9.0.

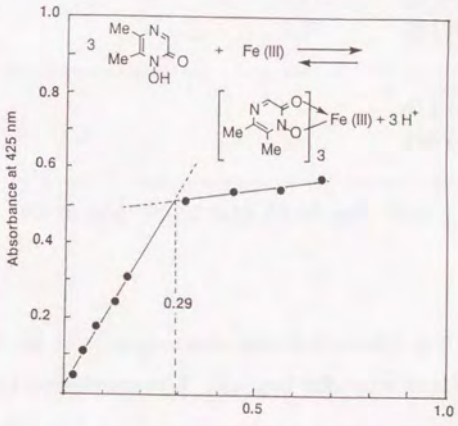

Figure 4-6. Plot of absorbance at 425 nm vs. ratio of Fe(III) to **10b** in H_2O at pH 4.0. [**10b**]=0.475 mM.

These facts indicate that these diazinones form 3:1 iron(III) complexes in the acidic region. However, the absorption bands completely disappeared in the basic region (>pH 9.0), suggesting that these complexes are not sufficiently stable to the attack of OH^- ion.

In order to confirm the 3:1 complex formation, the absorbance at λ_{max} as a function of the mole ratio of iron(III) to **5**, **10a**, or **10b** was plotted. The results are shown in Figure 4-6 and Table 4-2.

Table 4-2. Mole ratio for Iron(III) complex formation

Ligand	pH	Mole ratio	λ_{max}/nm (ε)
5[a]	6	0.31	405 (3470)
10a[b]	3	0.31	445 (2980)
10b[c]	4	0.29	425 (4237)
21[d]	-	-	412 (4310)
22a[d]	-	-	446 (4320)

[a]0.46 mM [b]0.52 mM. [c]0.48 mM. [d]Ref.6

In each case, an intersection was provided nearly at 0.3, indicating the formation of 3:1 complexes of **5**, **10a**, and **10b** with iron(III), respectively, as shown in Figure 4-7.

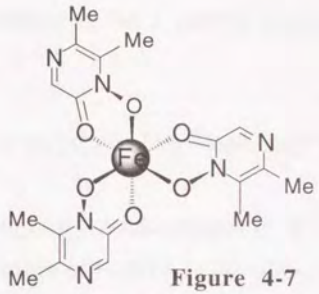

Figure 4-7

(c) Stability constants of the Iron(III) complexes

The formation of an iron(III) complex with a bidentate ligand is composed of three stepwise reactions as shown in Eq. 1. The log β_3 is obtained by summation of the logarithms of each of the equilibrium constants, K_1, K_2, and K_3 as shown in Eq. 2. In order to estimate the stability constants of the iron(III) complexes of **5**, **10a** and **10b**, the competitive reaction between nitrilotriacetic acid (NTA) and these ligands were carried out using a mixture of [Fe (Ligand)$_3$]:[NTA]=1.0:1.0.

$$\left.\begin{aligned} Fe^{3+} + L^- &\overset{K_1}{\rightleftharpoons} FeL^{2+} \\ FeL^{2+} + L^- &\overset{K_2}{\rightleftharpoons} FeL_2^{+} \\ FeL_2^{+} + L^- &\overset{K_3}{\rightleftharpoons} FeL_3 \end{aligned}\right\} \quad (1)$$

$$\log \beta_3 = \log K_1 + \log K_2 + \log K_3 \quad (2)$$

$$Fe^{3+} + 3L^- \overset{\beta_3}{\rightleftharpoons} FeL_3 \quad (3)$$

Equation 1 is also expressed by Eq. 3, and the spectral data were analyzed according to Eq. 4,[9]

$$\beta_3=[Z/(1-Z)][(E_t-(1-Z)M_t)/(L_t-3xZM_t)^3](\alpha_L{}^3/\alpha_Y)K_E \quad (4)$$

where $Z=(A-A_E)/(A_L-A_E)$, A=absorbance of the competing systems at equilibrium; A_E=absorbance of Fe-NTA in the absence of the sample ligand; A_L=absorbance of FeL_3 in the absence of NTA; α_L and α_Y have the form of $1 + \Sigma_{i=1} (H^+)^i/K_{aj}$, where K_{aj} is the three acid dissociation constants of the *N*-hydroxyamide groups of **5**, **10a**, or **10b**, or those of NTA;[10] E_t, L_t, and M_t are the total analytical concentrations of NTA, ligand, and metal ion, respectively; and K_E is the stability constant of Fe-NTA.[11]

The results are summarized in Table 4-3.

Table 4-3. Stability constants of Iron(III) complexes[a]

Ligand	pKa	log β_3
5	6.1	22.1
10a	4.4	18.2
10b	4.7	20.2
22a[b]	9.0	35.1

[a]Initial concentrations of Fe(ligand)3 and NTA, 0.15 mM; in acetate buffer (pH 4.0) at 24 °C.; NTA: pKa 10.7, 3.07, 3.03.; log K 15.9. [b]Ref.6.

The stability constant of the complex with **5** was greater than those with **10a** and **10b**, reflecting the difference in pKa between those diazine compounds. However, the stability constants of their iron complexes are far below that of the complex with monoazine, **22a**,[6] due to their high acidity compared to that of the monoazine.

In conclusion, *N*-hydroxyamide-containing diazines have remarkable iron chelating ability under neutral to acidic conditions in an aqueous solution. However, the stability of 3:1 iron complexes are lower than that of monoazines.

The result prompted the author to construct another effective ligand bearing a diazine moiety. In the next chapter, the construction of hexadentate ligands and their iron chelating properties will be described.

4.3 Experimental

General. UV-vis spectra were measured with a JASCO Ubest V-550 spectrophotometer. pK_a values were determined from the data obtained by measurement on a Horiba F-12 pH meter.

1-Hydroxy-4,6-dimethyl-2(1*H*)-pyrimidinone (**5**), 1-hydroxy-(**10a**) and -5,6-dimethyl-2(1*H*)-pyrazinone (**10b**) was prepared by the method mentioned in Chapter 2.

A typical procedure for measurement of the pK_a value. Pyrazinone **10b** (47 mg) was dissolved in deionized water (30 mL). The pH of the solution was monitored during titration after every 0.1 mL-addition of a 0.08 M NaOH solution at room temperature under an argon atmosphere. The pK_a was calculated from the pH at the midpoint of neutralization.

A typical procedure for measurement of the UV-vis spectra of iron(III) complexes. The pH of an aqueous solution of [**10b**]:[Fe(III)]=3:1 mixture ([Fe(III)]=0.10 mM, which was prepared from iron(III) nitrate solution) was adjusted to an appropriate value with 0.01 or 0.1 M NaOH and 0.01 or 0.1 M HNO_3 solutions. The UV-vis spectra were measured at room temperature.

A typical procedure for iron(III)-binding ratio. To an aqueous solution of **10b** (9.28 mM, 0.5 mL) was added an appropriate amount of a standarized aqueous iron(III) nitrate solution (0.328 mM). The pH of the solution was adjusted to 6.0 with 0.01 or 0.1 M NaOH and then diluted to

10.0 mL. After 1 h, the visible spectrum of each solution was measured. The absorbance at 405 nm was plotted as a function of the mole ratio of iron(III) to **10b**.

A typical procedure for iron(III) exchange reaction. The pH of a solution of **10b** (9 mM, 1.0 mL), Fe(III) (3 mM, 1.0 mL), and KNO_3 (0.4 M, 1.0 mL) was adjusted to 4.0 with aqueous NaOH and diluted to 10.0 mL with an acetate buffer solution, [**10b**-Fe(III) complex]=0.3 mM. An NTA solution was prepared by dilution of a mixture of 1.0 mL of an aqueous NTA stock solution (3.0 mM) and 1.0 mL of an aqueous KNO_3 solution (0.4 M) to 10.0 mL with an acetate buffer solution. The reaction was initiated by adding 1.0 mL of the NTA solution into 1.0 mL of the **10b**-iron complex solution. The spectral change was monitored by the decrease in absorbance at 425 nm in a 10 mm quartz cell. The sample solution was maintained at 24 °C for a week to attain equilibrium. The stability constant β_3 for **10b**-iron complex was calculated according to Eq. 4.

4.4 References

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Chapter 5

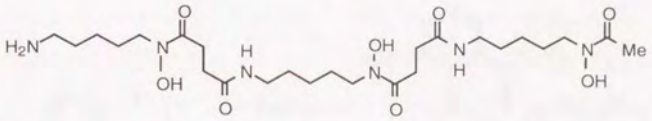
Hexadentate Ligands: Iron Chelating Properties of 1-Hydroxy-2(1*H*)-pyrimidinones

5.1 Introduction

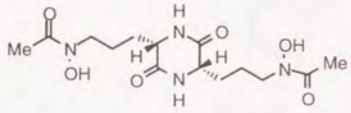
Life without iron is essentially unknown. Although many organisms are auxotrophic for Fe(III) because of the insolubility of $Fe(OH)_3$, nature has developed rather sophisticated iron chelating and transporting systems in order to utilize the metal. Siderophores are low-molecular-weight iron chelators secreted by microbes, and sequester iron needed for essential intracellular physiological precesses. A large number of different siderophores have been isolated and identified.[1] Typical examples are shown in Figure 5-1. They can be primarily classfied into two general structural classes; catecholates and hydroxamates, typified by enterobactin and desferrioxamine B, respectively. These hexadentate iron chelators form stable octahedral Fe(III) complexes as shown in Figure 5-2.

Iron deficiency and overload are serious health problems. Considerable effort has been invested in the development of new therapeutics for managing thalassemia. Currently used drug of microbial siderophore, desferrioxamine B (Desferal®, DFB) is still the most effective drug for removal of overloaded iron from patients which receive regular blood transfusion. Unfortunately, this compound cannot be orally administered[2-4] and has a short half-life time *in vivo*.[5] Furthermore, it has a severe side effect such as septicemia.[6] Thus, many chemists have been devoted much effort to develope a new chelator other than desferrioxamine B.[7]

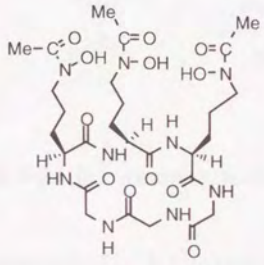
Recently, hydroxy group-containing heterocycles have received considerable attention owing to their strong acidity compared to that of catechol. These include hydroxypyridines,[8-10] 1-hydroxy-2(1*H*)-



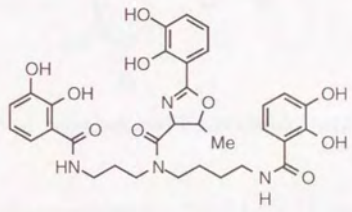
Desferrioxamine B (DFB)



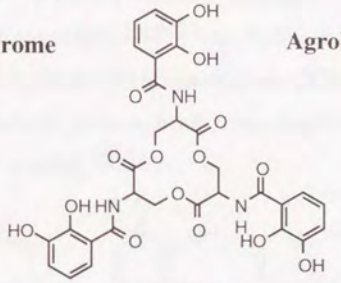
Rhodotorulic acid



Ferrichrome



Agrobactin



Enterobactin

Figure 5-1. Natural-occuring siderophores

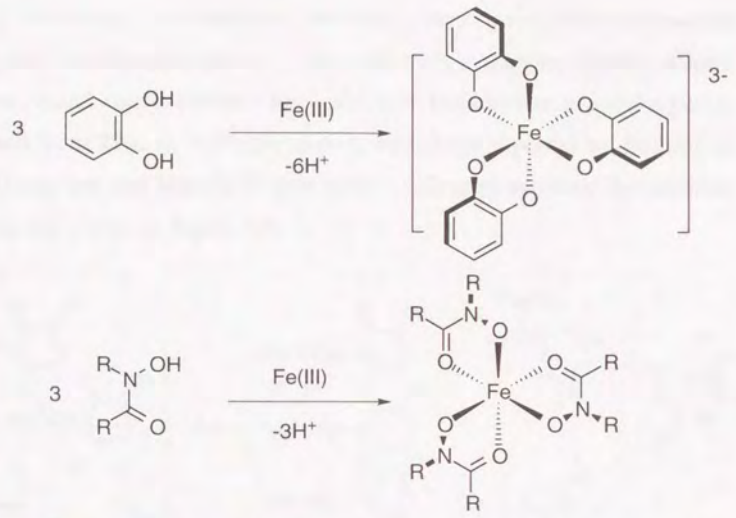
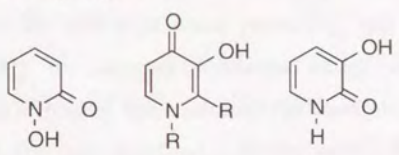


Figure 5-2. Iron complex formation of catechol and hydroxamic acid

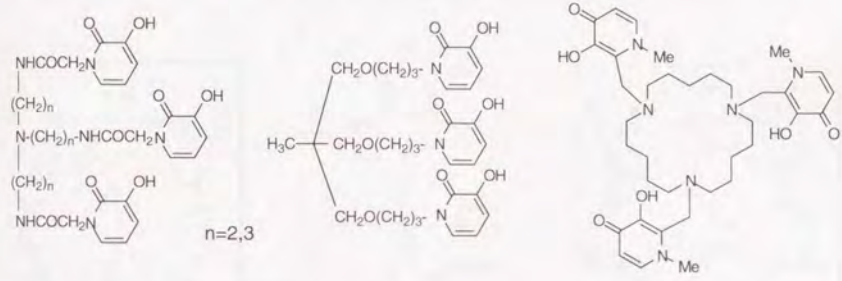
pyridinone (**21**),[11,12] 3-hydroxy-4(1*H*)-pyridinones, (**22**),[11,13-18] and 3-hydroxy-2(1*H*)-pyridinone(**23**)[11,19-21] as shown in Figure 5-3. Among them, 1,2-dimethyl-3-hydroxy-4(1*H*)-pyridinone (**22b**) was found to be an orally active iron chelator, although all toxicology and other preclinical safety studies are still ongoing.[22-24]



21 **22** R **23**
a H
b Me

Figure 5-3

Tetradentate or hexadentate derivatives may have certain advantages over simpler bidentate ligands, in particular such as a higher binding affinity at low ligand concentration. Recently, new hexadentate tripodal ligands, derived from **22a**, or hydroxypyridine, have been reported by Streater et al.,[20] and Sun and Martell,[25] respectively. Several synthetic hexadentate ligands are shown in Figure 5-4.



Streater et al (1990) Sun and Martell (1993) Sun and Martell (1993)

Figure 5-4

As mentioned in Chapter 4, 1-hydroxy-4,6-dimethyl-2(1*H*)-pyrimidinone (**5**), an *N*-hydroxyamide-containing diazine, was found to have a low pKa value and high water solubility, and to form a ferric complex effectively in aqueous solutions under acidic to neutral conditions.[26] These results demonstrated the feasibility of **5** to an iron sequestering agent for iron overload. It was noted, however, that the stability constant of its ferric complex was not so high ($\log\beta_3$ 22), compared to that of monoazines ($\log\beta_3$ 27-36), since the pKa value was lower than that of the monoazines. In order to develope iron squestering agents bearing **5**

in a thermodynamically favorable iron chelation, two types of tripodal hexadentate ligands (**24** and **25**), composed of **5**, were prepared. The structures are shown in Figure 5-5. In order to introduce a spacer group to the heterocycle system, 1-benzyloxy-4-(1,2,4-triazol-1-yl)-2(1*H*)-pyrimidinone was used as a starting material. Three units of **5** are linked with tricarboxylic acid through amide bonds in **24**, and with tris(2-aminoethyl)amine (TREN) in **25**, by three alkyl chains, respectively.

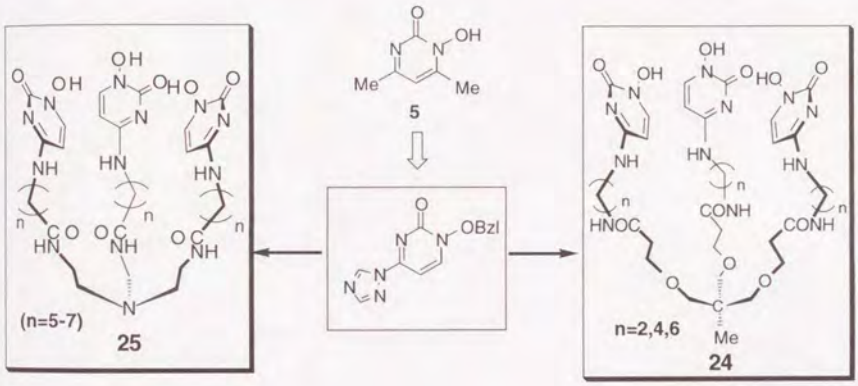


Figure 5-5

Their principal advantages are

(1) The *C*3-symmetrical structures of the hexadentate ligands would enhance the stability of the ferric complexes,

(2) The binding moieties with a low pKa value and high water solubility would allow the investigation of iron chelating properties at physiological pH,

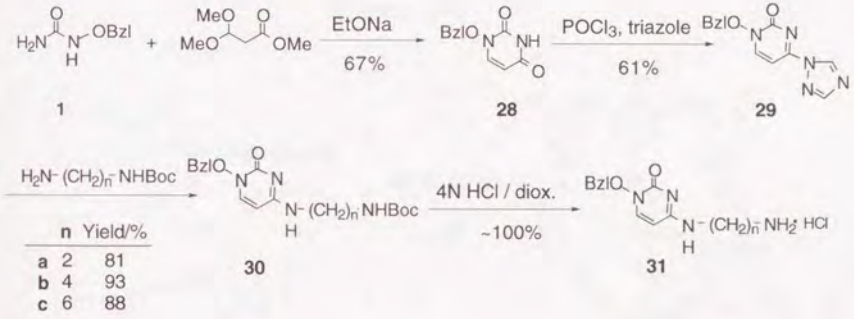
(3) The aliphatic diamine would allow to investigate the relationship between the structural features of the free ligands and iron binding properties.

In this chapter, the synthesis and iron chelating ability of the hexadentate ligands bearing 1-hydroxy-2(1*H*)-pyrimidinones are described.

5.2 Hexadentate Ligands Bearing sp^3 Carbon as an Anchor

(a) Synthesis

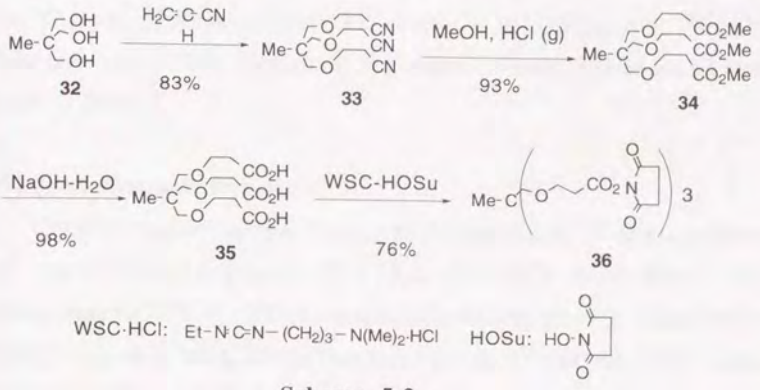
The reaction of *N*-benzyloxyurea (**1**) with methyl 3,3-dimethoxypropionate in the presence of sodium ethoxide gave 1-benzyloxyuracil (**28**)[27] in 67% yield (Scheme 5-1).



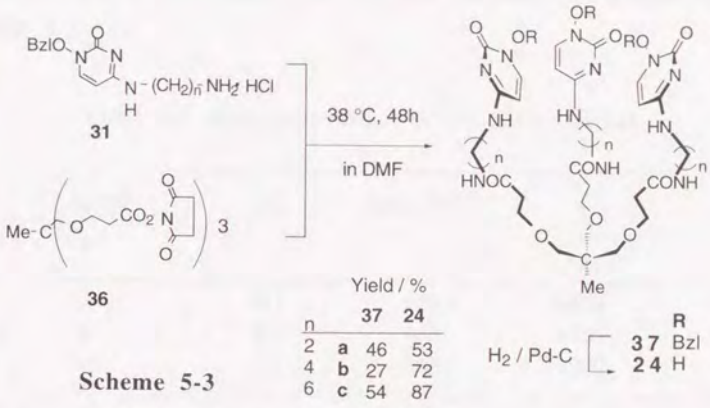
	n	Yield/%
a	2	81
b	4	93
c	6	88

Scheme 5-1

In the ^1H NMR, two characteristic signals of olefinic protons at the C-5 and C-6 positions were observed at δ 5.38 and 6.96 ppm, respectively. In addition, NH and carbonyl stretching vibrations at 3234 and 1633 cm^{-1} were observed in the IR spectrum. Treatment of **28** with 1,2,4-triazole in the presence of phosphorous oxychloride and triethylamine in dry acetonitrile gave 1-benzyloxy-4-(1,2,4-triazol-1-yl)-2(1*H*)-pyrimidinone (**29**)[28] in 61% yield. The coupling of **29** with N^{ω}–*tert*-butoxycarbonyl (Boc)-protected



Scheme 5-2



n		Yield / % 37	24
2	**a**	46	53
4	**b**	27	72
6	**c**	54	87

Scheme 5-3

aliphatic diamines, followed by deprotection of the Boc group under acidic conditions, gave salts **31**. On the other hand, tricarboxylic acid **35** were derived from 1,1,1-tris(hydroxymethyl)ethane **32**[29] *via* 3 steps (Scheme 5-2). Conversion of **35** to the corresponding *O*-succinimide ester **36**, followed by coupling with **31** upon stirring the mixture in DMF for 48 h at

38 °C, gave tripodal products **37**, as shown in Scheme 5-3. Finally, debenzylation of **37** by catalytic hydrogenation afforded desired hexadentate ligands, **24a-c**.

(b) Iron complex formation

The UV-vis spectroscopic features of the octahedral 3:1 iron complexes of hydroxypyridinones (**21**, **22**, and **23**) have been well documented.[11,19,30] These complexes generally show a characteristic LMCT (ligand to metal charge transfer) band at 400-500 nm. The visible spectra of 1:1 molar mixtures of **24a-c** and ferric ion in aqueous solutions showed an absorption maximum at 460 nm. The results are summarized in Table 5-1.

Table 5-1. Spectroscopic data of iron(III) complexes

Ligand **24**	pH	λ_{max}/nm	ε
a	6.1	460	4400
b	6.3	466	4761
c	6.8	463	4900
5[a]	6.1	405	3470

[a] Bidentate ligand.

The observed λ_{max} and ε values, which were comparable to those of **5**-Fe(III) 3:1 complex (ε 3470 at 405 nm),[26] suggest the formation of an intramolecular 1:1 complex. The spectral change under various pH conditions of a **24a**-Fe(III) solution is shown in Figure 5-6. Interestingly, no apparent change in λ_{max} and absorbance was observed over a wide pH range, especially under acidic to neutral conditions, in contrast that of corresponding bidentate ligand, **5**. This observation suggests that hexadentate ligand, **24a**, can form a stable 1:1 complex by virtue of its structural effect as shown in Figure 5-7. However, the absorbance decreased under basic conditions, which would arise from the decomposition of the iron complex by the attack of hydroxyl ion.

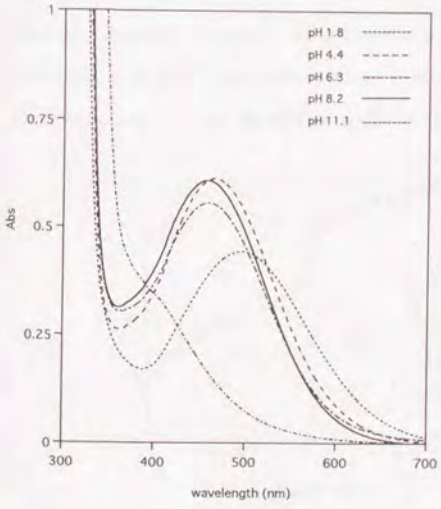


Figure 5-6. Spectral change of the Fe-**24a** in aqueous solution under various pH conditions.

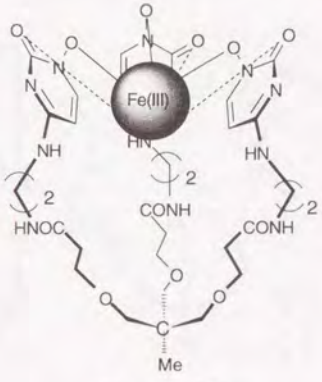


Figure 5-7

(c) Stability of iron complex

The stability constant of the complex of the hexadentate ligand with ferric ion is defined by the following equilibrium.

$$Fe^{3+} + L^{3-} \underset{}{\overset{K}{\rightleftharpoons}} FeL \qquad K = \frac{[FeL]}{[Fe^{3+}][L^{3-}]}$$

In order to obtain the stability constants of the complexes of **24a-c** with iron(III), the competitive reactions between EDTA and these ligands were carried out. Although three pKa values of **24a-c** are necessary for the calculation, the measurement of these pKa values was impossible due to expermental limitation that these values were too close to measure by the normal titration method. Subsequently, 1-hydroxy-4-butylamino-2(1*H*)-pyrimidinone (**38**) was prepared as a model compound, and its pKa value was measured. From the titration curve shown in Figure 5-8, the pKa was

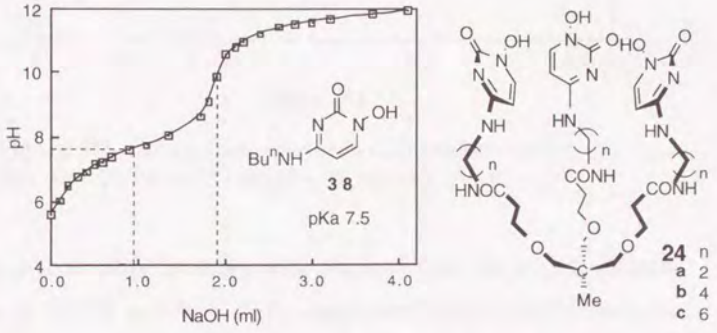


Figure 5-8. Titration curve of **38**.

estimated to be 7.5. This pKa value was used for the present calculations. This relatively higher pKa than that of **5** (6.1) is probably due to the inductive effect of the electron donating amino group at the C-4 position of the pyrimidinone ring. The competition reactions of **24a-c** with EDTA were carried out in 40% aqueous MeOH. The reactions in aqueous solutions were unsuccessful due to low solubility of the ligands in water. The relationship of the absorbance change at 460 nm *vs.* time is shown in Figure 5-9 in the case of **24a**.

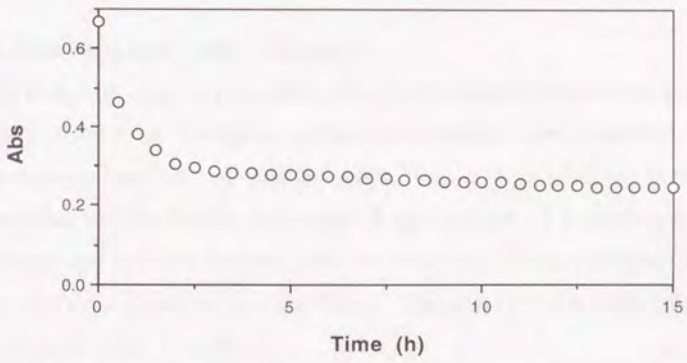


Figure 5-9. Absorbance change in competition reaction of **24a** with EDTA at 450 nm in 40% aqueous MeOH.

The relative stability constants were obtained from the acid dissociation constants of EDTA and the stability constant of Fe(III)-EDTA complex. The results are summarized in Table 5-2. The stability constants were found to be logK~25 with no apparent effect of the spacer length, indicating that the spacer length was too long to effect the stability.

Table 5-2. Stability constants of iron(III) complexes[a]

Ligand **24**	log K
a	25.1
b	25.3
c	24.9

[a] Initial concentrations of Fe-**24** and EDTA, 0.30 mM; in acetate buffer (pH 4.0) at 24 °C.

(d) Iron removal from transferrin

In order to apply **24** as drugs for iron overload, their iron removal ability from iron transport protein, transferrin, was evaluated under physiological conditions. Human serum transferrin is a bilobal protein of molecular weight 78000, and recent X-ray analysis of a single crystal[31] revealed that the iron binding sites are composed of two phenolate groups (tyrosine), one nitrogen (histidine), and carboxylate oxygen (aspartate) with a carbonate (Figure 5-10).

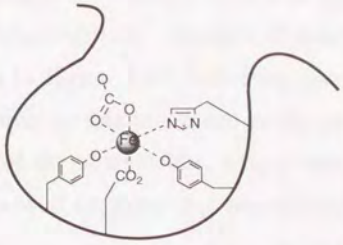


Figure 5-10

Diferric transferrin (**Tf**Fe2.0) was prepared from commercially available human apo-transferrin (apo**Tf**) according to the mentioned in literatures.[12,32]

It is known that the iron removal from transferrin undergoes two-stepwise reaction (Eqs. 1 and 2). Davis and coworkers reported[33] that the dissociation constant of the monoferric transferrin complex was approximately 100-fold larger than that of the diferric complex. Consequently, the reaction of equation (3) would obey pseudo-first-order-kinetics, when an excess of the ligand was used.

$$\mathbf{Tf}_{Fe2.0} + L \xrightarrow{k_1} \mathbf{Tf}_{Fe} + FeL \qquad (1)$$

$$\mathbf{Tf}_{Fe} + L \xrightarrow{k_2} apo\mathbf{Tf} + FeL \qquad (2)$$

$$\mathbf{Tf}_{Fe2.0} + 2\ \mathbf{24} \xrightarrow{k_{obs}} apo\mathbf{Tf} + 2\ Fe\text{-}\mathbf{24} \qquad (3)$$

The iron exchange reaction between **Tf**Fe2.0 and **24a** was carried out at pH 7.4. After mixing a buffered solution of **Tf**Fe2.0 with 20-fold excess of **24a**, the change in absorbance at 460 nm was monitored. The change of the absorbance with time is shown in Figure 5-11. The plots of $\log[(A_\infty - Abs)/(A_\infty - A0)]$ as a function of time gave a good linear relationship as shown in Figure 5-12, indicating that the reaction of iron removal from transferrin by **24a** proceeded in the pseudo-first-order-kinetics. From the slope of the straight line, k_{obsd} was obtained. The kinetic results are summarized in Table 5-3 together with the data of desferrioxamine B (DFB).

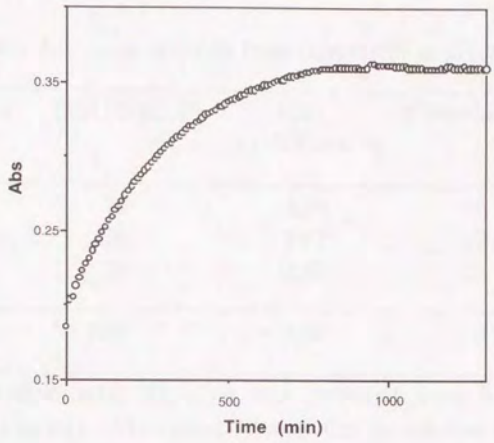


Figure 5-11. Absorbance change at 460 nm in iron removal reaction of **24a** from transferrin at pH 7.4

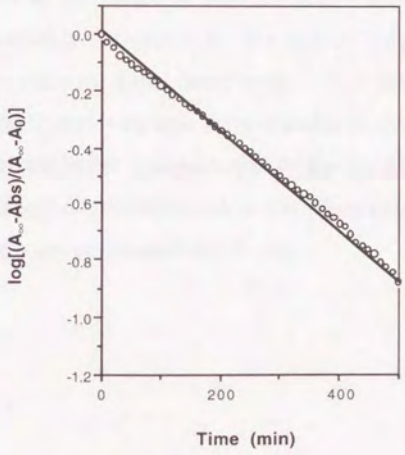


Figure 5-12. The plots of $\log[(A_\infty\text{-Abs})] / [(A_\infty\text{-}A_0)]$ vs time on iron removal of **24a** from $\mathbf{Tf}_{Fe2.0}$.

Table 5-3. Iron removal from transferrin at pH 7.4

Ligand **24**	[L]/[**Tf**Fe2.0][a]	k_{obs} ($x10^{-3}$ min^{-1})	% Fe removed[b]
a	20	2.30	16
b	20	2.62	17
c	20	2.69	23
DFB	100	0.66	5 (5)[c]

[a] [**Tf**Fe2.0]0=0.05 mM, **Tf**Fe2.0 was prepared from human serum apotransferrin (Sigma). [b]At a point 30 min after the reaction was initiated. [c] Ref. 34.

The rates were in the range of 2.30-2.69$x10^{-3}$ min^{-1}, indicating that there is no remarkable influence of the spacer length on the kinetic efficiency of iron removal from transferrin. It is noteworthy, however, that **24a-c** efficiently removed iron from transferrin over 4 times as much as DFB did, even at a lower concentration of the ligand than that of DFB. This difference is apparently observed in the percentages of iron removal after the reactions were performed for 30 min.

5.3 Hexadentate Ligands Bearing Tertiary Amine as an Anchor

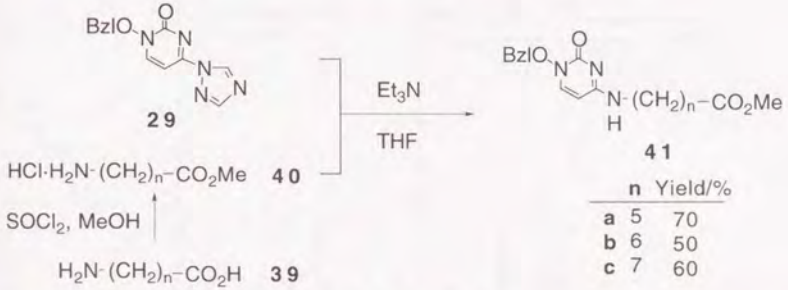
As described in the former section, tripodal hexadentate ligands (**24a-c**), bearing 1-hydroxy-2(1*H*)-pyrimidinones (**5**), aliphatic diamine, and a tricarboxylic acid, were found to form 1:1 iron complexes with high stability constants in aqueous solutions. Furthermore, their iron removal ability form human transferrin was much higher than that of desferrioxamine B. However, it was observed that the iron complexes of **24a-c**, especially that of the ligand having long alkyl chains, tended to become insoluble in water. This limited a study concerning the functional evaluation in aqueous solutions.

The result prompted the author to improve the structure in order to increase water solubility by using a tertiary amine for an anchor. Moreover, the author considered that a more flexible sp^3 nitrogen than a sp^3 carbon would be favorable for the induced fitting to iron. On the basis of these considerations, other tripodal hexadentate ligands (**25a-c**) were designed. In these cases, ω-amino carboxylic acid and tris(2-aminoethyl)amine (TREN) were used as a spacer and an anchor, respectively. The length of methylene chain (n=5-7) should affect on the stability of the ferric complexes. The spacer length of **25c** (n=7) corresponds to that of **24b** (n=4).

In this section, the synthesis and functional evaluation of the tripodal hexadentate ligands **25a-c**, containing **5**, are described.

(a) Synthesis

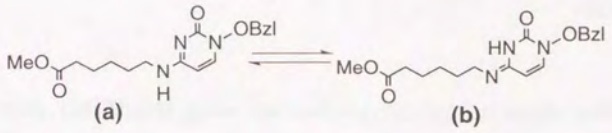
1-Benzyloxy-4-(1,2,4-triazol-1-yl)-2(1*H*)-pyrimidinone (**29**) were prepared by a similar procedure mentioned in the former section 5.2. Commercially available ω-aminocarboxylic acids (**39a-c**; n=5-7) were converted to the corresponding methyl esters (**40a-c**) by treatment with thionyl chloride in MeOH, and the resulting hydrochloride salt were allowed to react with **29** to afford compound **41a-c** (Scheme 5-4).



	n	Yield/%
a	5	70
b	6	50
c	7	60

Scheme 5-4

Interestingly, two types of signals at 3.19 and 3.43 ppm due to -CH_2-N protons were observed in the ^{1}H NMR (Figure 5-13) of **41a** in $CDCl_3$, suggesting that **41a** existed in a tautomeric equilibrium between 4-amino (a) and 4-imino forms (b). The major peak of -CH_2-N at 3.43 ppm, appeared as a quartet, indicates that **41a** predominantly exists in the 4-amino form. Two NH proton signals at δ 6.14 and 6.75 ppm were D_2O-exchangeable with changing the quartet of -CH_2-N into a triplet. In addition, **41a** exclusively existed in the 4-amino form (a) in DMSO-d_6. The hydrolysis of



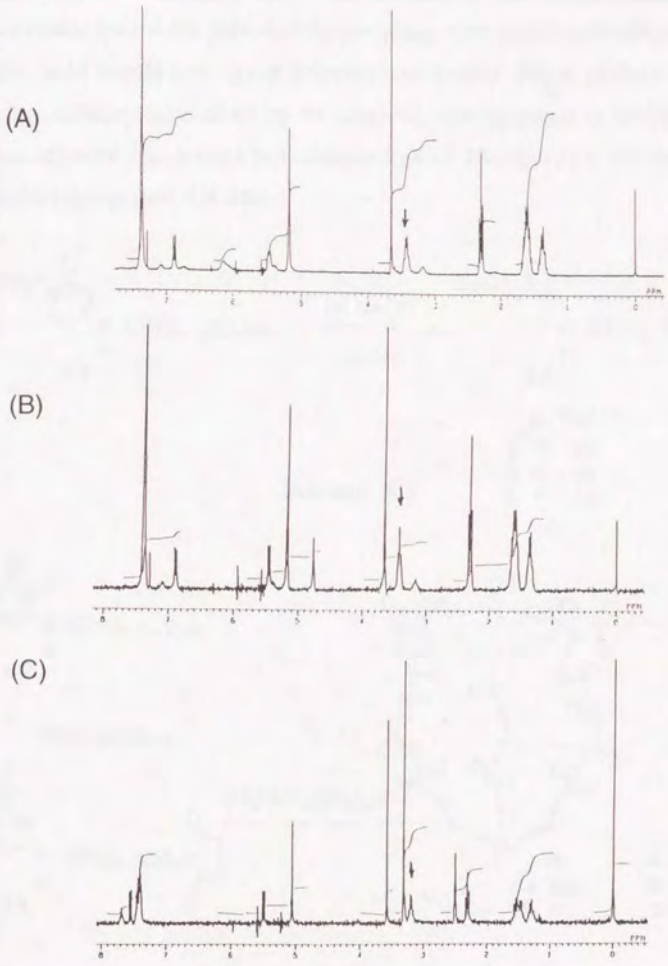
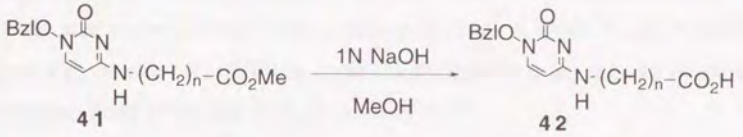


Figure 5-13. ^{1}H NMR spectra of **25a**. (A) in $CDCl_3$; (B) in $CDCl_3/D_2O$; (C) in DMSO-d_6.

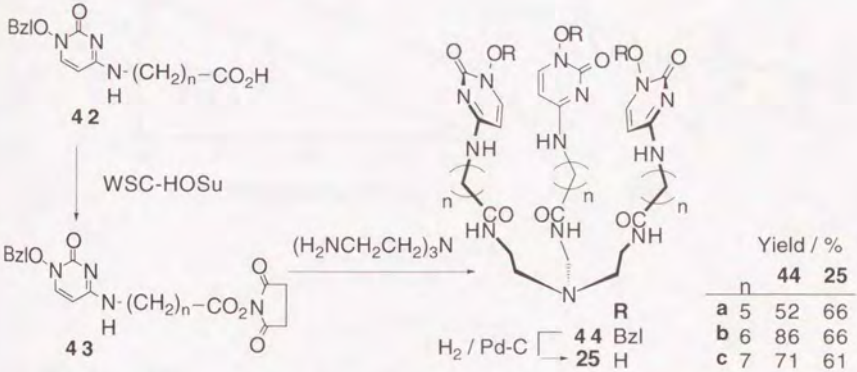
8a-c with 1M NaOH gave the corresponding carboxylic acids **42a-c** (Scheme 5-5). Subsequently, conversion to the corresponding *O*-succinimide esters **43**, followed by coupling with tris(2-aminoethyl)amine under mild conditions, gave tripodal compound **44a-c** (Scheme 5-6). Finally, debenzylation of **44** by the catalytic hydrogenation in MeOH under reflux afforded the desired hexadentate ligand, **25a-c**, which showed more water solubility than did **24a-c**.



41 42

	n	Yield/%
a	5	93
b	6	69
c	7	80

Scheme 5-5



	n	Yield / % 44	25
a	5	52	66
b	6	86	66
c	7	71	61

Scheme 5-6

In order to clarify whether intramolecular hydrogen bonds exsist in the free ligands or not, the temperature dependence of the ^1H NMR chemical shifts was examined for the amide (a) and amino (b) protons of **25a**. The ^1H NMR spectra of **25a** in DMSO-d6 at various temperatures from 23 °C to 90 °C exhibited one set of signals, indicating that **25a** possesses the pseudo-*C*3-symmetrical structure at a range of the temperatures. The plots of chemical shifts of the protons (a) and (b) versus the temperatures gave straight lines as shown in Figure 5-14. The temperature coefficients for the amide and amino protons were calculated to be -4.6×10^{-3} and -5.1×10^{-3} ppm/deg., respectively. These large coefficients indicate that no particular hydrogen bond exists in a DMSO-d6 solution.[35]

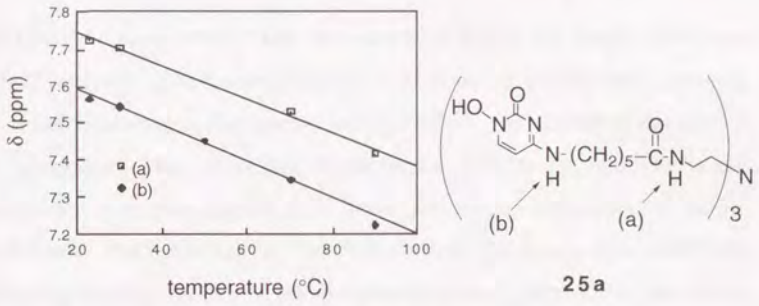


Figure 5-14

(b) Iron(III) complex formation

The visible spectra of 1:1 molar mixtures of **25a-c** and ferric ion in aqueous solutions were measured. In each case, the characteristic LMCT

band of a ferric complex was observed around at 465 nm, as similar to the complexes of **24a-c**. Their λ_{max} and ε are summarized in Table 5-4.

Table 5-4. Spectroscopic data of iron(III) complexes of **25a-c**.

Ligand **25**	pH	λ_{max}/nm	ε
a	3.8	472	4340
b	5.6	465	4550
c	5.7	464	4380
5[a]	6.1	405	3470

[a] Bidentate ligand.

The observed λ_{max} and ε value are somewhat longer and larger than those of Fe/**5** complex. The spectral change of the complex of **25a** upon changing the pH of a solution is also shown in Figure 5-15. As similar to the case of the complex of **24a**, mentioned in the former section, the intensity of the absorbance was maintained in a wide pH range, especially in acidic conditions. Furthermore, no apparent shift of the λ_{max} was observed, indicating that the stability of the complex increased compared to that of the bidentate ligand. In order to confirm a 1:1 ferric complex formation, mole ratio plots were carried out. The results are shown in Figure 5-16 and Table 5-5. Each of **25a-c** gave inflection point at nearly 1.0, strongly indicating 1:1 complex formation.

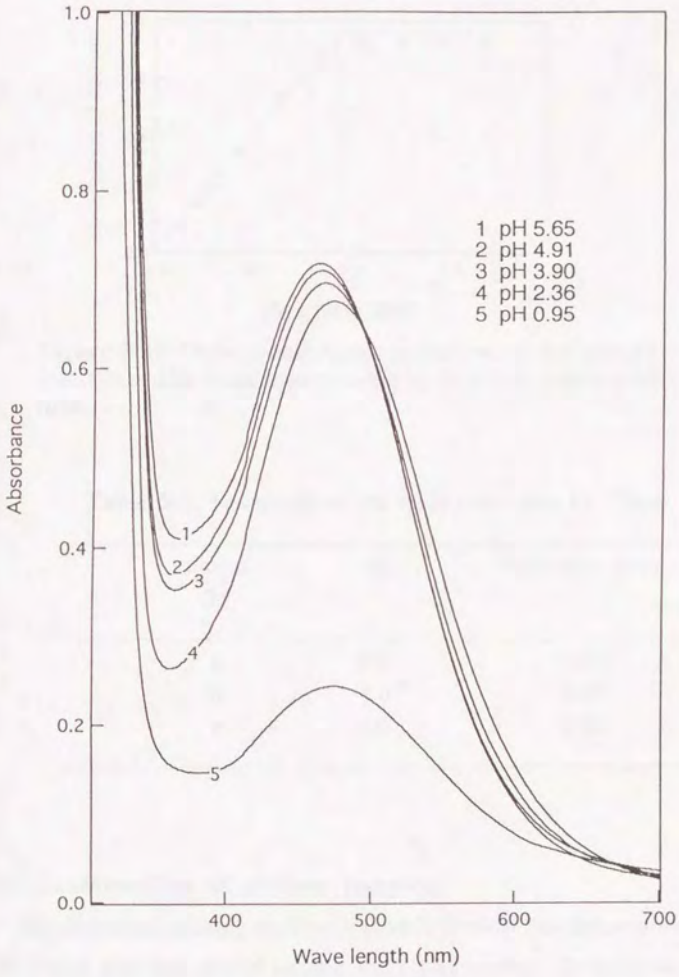


Figure 5-15. Spectral change of the Fe-**25a** in aqueous solution under various pH conditions.

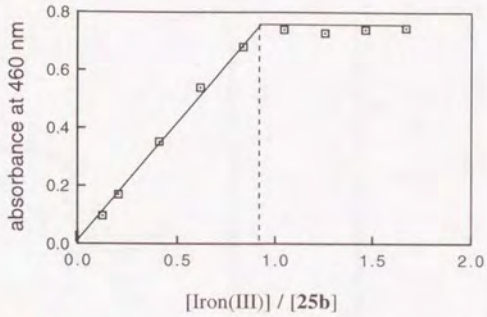


Figure 5-16. Plots of absorbance at 460 nm vs. the ratio of iron(III) to **25b** in an aqueous solution at pH4.0; [**25b**]=0.16 mM.

Table 5-5. The results of the mole ratio plots for **25a-c**.

ligand **25**	pH	inflection point
a	4.0	0.93
b	4.0	0.95
c	4.0	0.90

(c) Conformation of gallium complex

The examination using molecular models showed that the conformation of the Fe/**25** complex should possess the C_3-symmetry. In order to examine the conformation in a solution, Ga(III) complex was prepared from **25a** and $Ga(NO_3)_3$, and the ^1H NMR spectrum of the complex was measured in CD_3OD at room temperature, since the ^1H NMR measurement

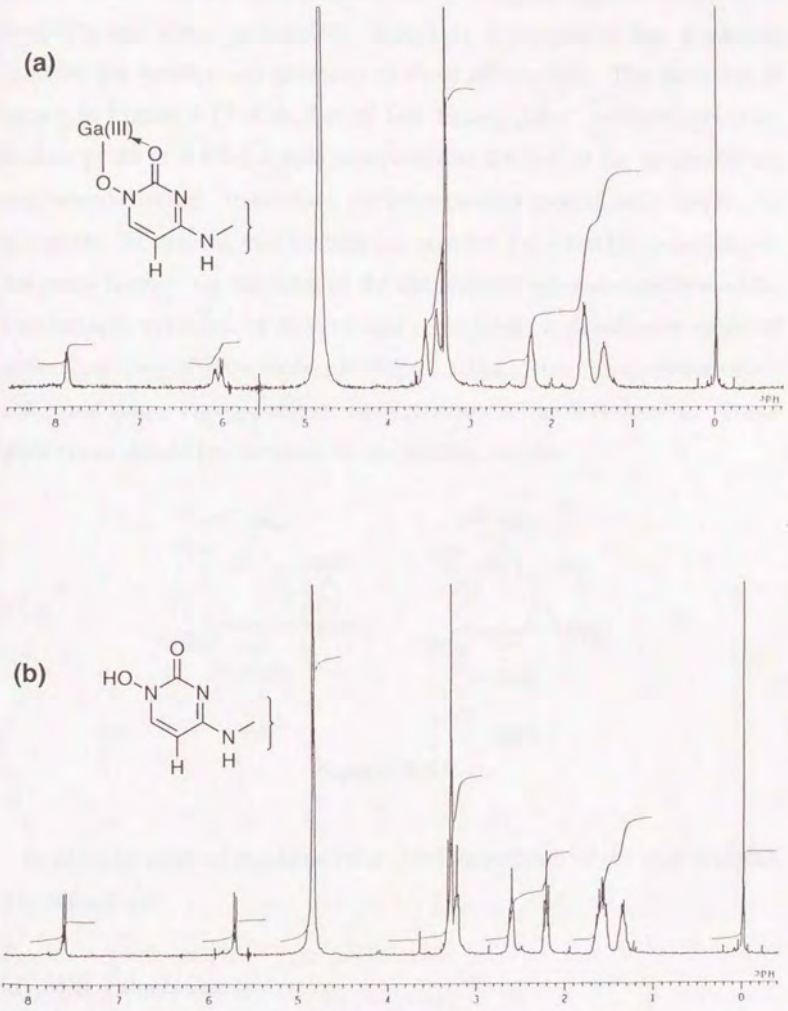


Figure 5-17. ^{1}H NMR spectra of Ga(III)-**25a** complex (a) and that of free ligand, **25a** (b) in CD_3OD, respectively.

of the Fe/**25** complex is impossible due to the paramagnetic property of iron(III), and since gallium(III), which is diamagnetic, has a similar coordination number and geometry to those of iron(III). The spectrum is shown in Figure 5-17 with that of free ligand, **25a**. Interestingly, two doublet peaks at δ 6.0-6.2 ppm, assignable to the 5-H of the pyrimidinone ring, were observed. In contrast, the other protons showed only simple sets of signals. In general, two isomers are possible for a Ga(III) complex, *cis* and *trans* forms. On the basis of the examination using molecular models, simultaneous existence of both *cis* and *trans* forms is possible by virtue of rather long spacers in the molecule (Figure 5-18). Thus, the existence of *cis* and *trans* isomers would reflect the separation of the 5-H doublet. These phenomena should be true even for the Fe/**25a** complex.

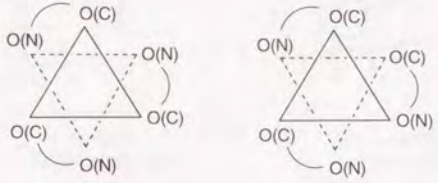


Figure 5-18

In order to confirm this hypothesis, MM calculation of the iron complex was carried out.

(d) MM calculation

The conformations of *cis* and *trans* isomers of Fe/**25a** complex were optimized by MM and MD calculations.[36] The X-ray crystallographic data

of Fe(**1,2-opo**)3 (i.e., **21**-iron(III)=3:1 complex)[19] were used for the cartesian coordinate of each atom around iron. The optimized structures of the isomers are shown in Figure 5-19.

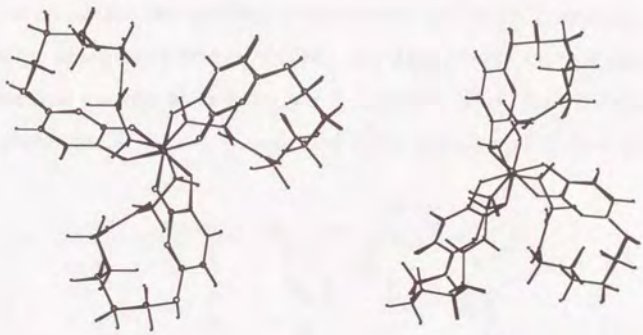


Figure 5-19. Optimized structures of iron(III)-**25a** complexes by MM calculation.

The optimized structure of the *cis*-form completely possessed C_3 symmetry, while that of the *trans*-form was distorted. The heats of formation were -96.34 and -92.23 kcal/mol for *cis* and *trans* isomers, respectively. Energy gap of 4 kcal/mol between the isomers indicated that the *cis* isomer is superior to *trans* one at least in vacuum system. However,

considering other factors determining the stability of the complex in solution, such as the interaction with the solvent, the difference in heat of formation is regarded not enough to allow the formation of the *cis* isomer predominantly in solution. Therefore, the two isomers may be in the equilibrium in the system, as was observed in the ^{1}H NMR spectrum.

(e) Stability of iron(III) complex

In order to obtain the stability constants of the Fe/**25** complexes, the competition reactions between EDTA and **25a-c** were carried out by a similar method mentioned in Section 5.2. In these cases, the pKa value of model compound, **38** (pKa 7.5), was used in the calculations (Figure 5-20).

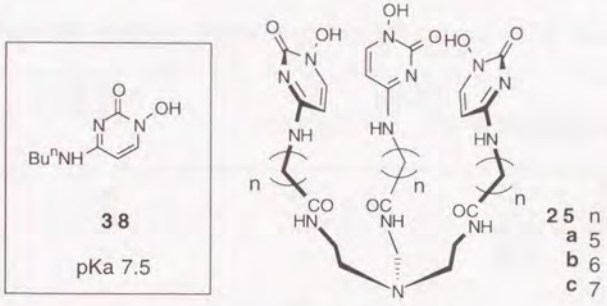


Figure 5-20

The competition reaction of **25a**,**b** with EDTA were carried out in water. The measurement of stability of Fe/**25c** complex in water was precluded due to poor water solubility of **25c**. Thus, the reactions were also carried out in 40% aqueous MeOH in the same fashion of those in water, and the results were compared with each other. The absorbance change, monitored at 460

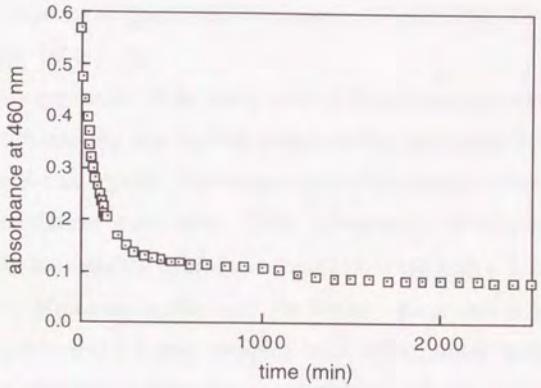


Figure 5-21. Absorbance change in competition reaction of **25a** with EDTA at 460 nm in an aqueous solution.

Table 5-6. Stability constants of Iron(III) complexes of **25a-c**.[a]

Ligand **25**	log K in H_2O	log K in 40% aq.MeOH
a	25.7	25.1
b	26.9	27.0
c	-	27.1
5[b]	22.1	-
DFB[c]	30.5	-

[a] Initial concentrations of Fe-**25** and EDTA, 0.30 mM; in acetate buffer (pH 4.0) at 20°C.; [b] $\log\beta_3$. [c] desferrioxamine B: Ref.37.

nm for the complex of **25a**, is shown in Figure 5-21. The relative stability constants were obtained from the acid dissociation constants of EDTA and

the stability constant of Fe(III)-EDTA complex (logK 25.1). The results are summarized in Table 5-6.

The stability constants of the complexes of **25a-c** were in a range of 25 to 27 in logK; the stability was slightly influenced by the alkyl chain length of the spacer in the molecule. The longer spacer was more advantageous for the stable chelation with iron. This observation disagrees with the prediction that the stability of chelates should increase with a decrease of the spacer length. However, in this case, the longer spacer would be favorable to form an octahedral 3:1 iron complex with hydroxamate moieties in the molecule, because the spacer group is substituted at the para position to the N-OH, not at the ortho or meta position.

(f) Iron removal from transferrin

Evaluation of iron removal ability of **25a-c** from transferrin was carried out at pH 7.4 by a similar procedure mentioned in Section 5.2 for **24a-c**.

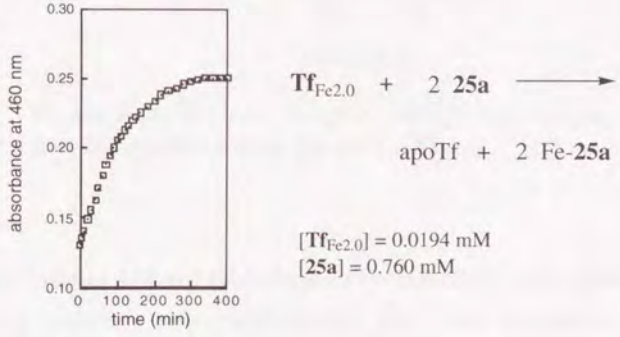


Figure 5-22. Absorbance change at 460 nm in iron removal reaction of **25a** from transferrin at pH 7.4.

After mixing a buffered solution of $\mathbf{Tf}_{Fe2.0}$ and 60-fold excess of **25a**, the change in absorbance at 460 nm was monitored (Figure 5-22). The plots of $\log[(A_\infty - Abs)/(A_\infty - A_0)]$ as a function of time gave a good linear relationship as shown in Figure 5-23. From the slope of the straight line, k_{obsd} was obtained. The kinetic results are summarized in Table 5-7 together with data of desferrioxamine B (DFB).

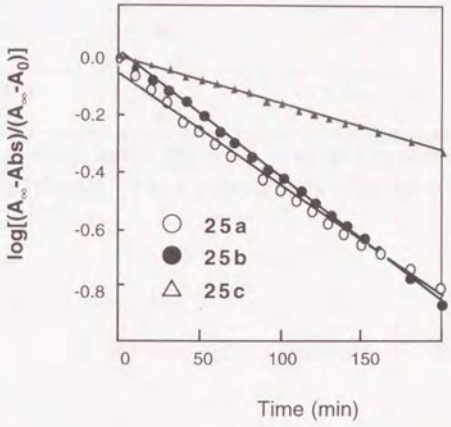


Figure 5-23. The plots of $\log[(A_\infty - Abs)] / [(A_\infty - A_0)]$ vs time on iron removal of **25a-c** from $\mathbf{Tf}_{Fe2.0}$.

In the cases of **25a-c**, higher ligand concentrations were required than **24a-c** in order to effectively remove iron from transferrin. Upon comparing the kinetic constants, **25a-c** were found to remove iron from

transferrin more effectively than did naturally-occuring siderophore, desferrioxamine B.

Table 5-7. Iron removal from transferrin at pH 7.4

Ligand **25**	[L]/[**Tf**Fe2.0][a]	k_{obs} ($x10^{-3}$ min^{-1})	% Fe removed[b]
a	60	4.5	27
b	60	4.0	24
c	60	1.7	11
DFB	100	0.66	5 (5)[c]

[a] [**Tf**Fe2.0]0=0.02 mM, **Tf**Fe2.0 was prepared from human serum apotransferrin (Sigma). [b]At a point 30 min after the reaction was initiated. [c] Ref.34.

5.4 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). 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. MM calculation were carried out on a Macintosh quadra 800 using Cashe ver 2.0. *N*-Benzyloxyurea was prepared by the same procedure described in Chapter 2.

1-Benzyloxyuracil (28). *N*-Benzyloxyurea (9.97 g, 0.06 mol) was added to a solution of sodium (1.5 g, 0.07 mol) in absolute EtOH (90 mL) at 30 °C under nitrogen atmosphere. After addition of 3,3-dimethoxypropionic acid methyl ester (9.78 g, 0.07 mol), the reaction mixture was stirred for 3 h at 20 °C, and then refluxed for another 17 h, and finally cooled to 10 °C and maintained at the temperature for 4 h. The precipitated sdium salt was collected by filtration and dissolved in H_2O (120 mL). The pH of the aqueous solution was adjusted to 4 with AcOH. The

resulting precipitate was collected by filtration and recrystallyzed from EtOH-H_2O (1:1) to give the product (**28**); 1.52 g (67%): mp 180-183 °C; IR (KBr) 3234, 1633, 738, 699 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.18 (s, 2H), 5.40 (d, *J* 9 Hz, 1H), 6.98 (d, *J* 9 Hz, 1H), 7.41 (s, 5H).

1-Benzyloxy-4-(1,2,4-triazol-1-yl)-2(1*H*)-pyrimidinone (29). A solution of 1,2,4-triazole (6.22 g, 90 mmol) in dry acetonitrile (25 mL) was treated with $POCl_3$ (2.5 mL, 27 mmol) and Et_3N (12.5 mL, 90 mmol) at 0 °C. A solution of **28** (1.96 g, 9 mmol) in dry acetonitrile (100 mL) was added to the mixture at 0 °C. The pH of the solution was adjusted to around 9 by adding Et_3N (ca. 5 mL), and the reaction mixture was stirred for 24 h at room temperature. Et_3N (12.5 mL) and H_2O (5 mL) were successively added to the reaction mixture in order to quench the reaction, and MeOH was added to the emulsion until the solution became clear. After removal of the solvents, the residue was washed with cold water (2x40 mL) and recrystallized from EtOH to give the product (**29**); 1.47 g (61%): mp 206-209 °C; IR (KBr) 1680, 740, 695 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.36 (s, 2H), 6.75 (d, *J* 8 Hz, 1H), 7.40 (s, 5H), 7.55 (d, *J* 8 Hz, 1H), 8.07 (s, 1H), 9.22 (s, 1H).

General procedure for the coupling of 29 and N^{ω}-Boc-substituted diamine. A typical example: *N'*-(*tert*-Butoxycarbonyl)amino-N^4-(1-benzyloxy-2-oxo-1,2-dihydropyrimidi-4-yl)aminoethane (30a). *N*-Boc-ethylenediamine (713 mg, 4.5 mmol) was added to a solution of **29** (1.0 g, 3.7 mmol) in dry THF (20 mL). The pH of the solution was adjusted to above 7 with Et_3N. The reaction mixture was refluxed for 9 h with minitoring of the disappearance of the starting material by TLC. After removal of the solvent

by evaporation, H_2O (6 mL) was added to the residue. The aqueous layer was extracted with $CHCl_3$ (5x30 mL), and the combined $CHCl_3$ layers were successively washed with 5% citric acid (20 mL) and brine (20 mL), and dried over anhydous Na_2SO_4. After removal of the solvent, the residue was purified by recrystallization from AcOEt or by column chromatography on silica gel (eluent: $CHCl_3$-acetone-EtOH=100:20:4), to give the product as yellow solid; 994 mg (81%): mp 136-137°C; IR (KBr) 3390, 1706, 1639, 754, and 701 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.37 (s, 9H, -Boc) , 3.06 (q, 2H, -NH-$\underline{CH_2}$-) , 3.26 (q, 2H, -$\underline{CH_2}$-NH-) , 5.09 (s, 2H, Ph-$\underline{CH_2}$-) , 5.52(d, 1H, *J* 7.3 Hz, 5-H) , 6.87 (m, 1H, NH) , 7.35-7.49 (m, 5H, $\underline{Ph}$-CH_2-) , 7.58 (d, *J* 7.3 Hz, 1H, 6-H) , and 7.75 (m, 1H, NH). Calcd for $C_{18}H_{24}N_4O_4$: C 59.99, H 6.71, N 15.55 Found : C 59.94, H 6.74, N 15.51.

***N'*-(*tert*-Butoxycarbonyl)amino-N^4-(1-benzyloxy-2-oxo-1,2-dihydropyrimidi-4-yl)aminobutane (30b).** 93%: mp 132-136°C; IR (KBr) 1690, 1636 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.43 (s, 9H), 1.48-1.68 (m, 4H), 3.05-3.24 (m, 2H),3.41-3.52 (m, 2H), 4.63-4.9 (m, 1H), 5.20 (s, 2H), 5.35 (d, *J* 8 Hz, 1H), 5.86-5.99 (m, 1H), 6.87 (d, *J* 8 Hz, 1H), 7.38 (s, 5H). Calcd for $C_{22}H_{32}N_4O_4$: C, 63.44; H, 7.74; N, 13.45. Found: C, 63.17; H, 8.00; N, 13.69.

***N'*-(*tert*-Butoxycarbonyl)amino-N^6-(1-benzyloxy-2-oxo-1,2-dihydropyrimidi-4-yl)aminohexane (30c).** 88%: mp 145-148 °C; IR (neat) 3308, 1690, 1636 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.36 (m, 4H), 1.43 (s, 9H), 3.10 (m, 2H), 3.44 (m, 2H), 5.22 (s, 2H), 5.29 (d, *J* 8 Hz, 1H), 5.89 (d, *J* 8 Hz, 1H), 7.39 (s, 5H). Calcd for $C_{22}H_{32}N_4O_4$: C, 63.44; H, 7.74; N, 13.45. Found: C, 63.17; H, 8.00; N, 13.69.

General procedure for the deprotection of the Boc group of 30a-c. A typical example: N^4-(1-Benzyloxy-2-oxo-1,2-dihydropyrimidi-4-yl)diaminoethane hydrochloride (31a). Compound **30a** (534 mg, 1.37 mmol) was dissolved in 4M HCl in dioxane (9 mL), and the solution was stirred at 0 °C for 30 min. After removal solvent, dry EtOH (100 mL) was added to the residue and evaporated to remove HCl completely. Addition and evaporation of EtOH (100 mL) were repeated three times to give the product (**3a**) as yellow solid; 756 mg (100 %).

1,1,1-Tris(cyanoethoxymethyl)ethane (33).[29] 1,1,1-Tris(hydroxymethyl)ethane (24 g, 0.2 mol) was added to dioxane (25 mL) containing 40% KOH aqueous solution (1.2 mL). To this suspension, acrylonitrile (32.2 g, 0.2 mol) was added dropwise with vigorous stirring. After stirring at room temperature for 24 h, 2.5M HCl (3.8 mL) was added to the reaction mixture, and then the solvents were evaporated. CH_2Cl_2 (40 mL) was added to the residue, and then the organic layer was washed with H_2O (10 mL), and dried over anhydrous $MgSO_4$. The crude product was purified by column chromatography on neutral alumina (eluent: benezene) to give the product (**33**) in 83% yield.

1,1,1-Tris(carboxyethoxymethyl)ethane trimethyl ester (34).[29] 1,1,1-Tris(cyanoethoxymethyl)ethane (7 g, 0.028 mol) was dissolved in MeOH (93 mL), and the solution was saturated with HCl gas which was produced by addition of concentrated H_2SO_4 (70 mL) to concetrated HCl (80 mL). No attempt was made to cool the solution during this operation. HCl gas was introduced into the solution for 1 h. The reaction mixture was then heated to reflux for an additional 1 h, and then

was stirred for 18 h at room temperature. Ammonium chloride produced was removed by filtration, and MeOH was removed by evaporation. To the residue obtained were successively added benzene (60 mL) and concd. H_2SO_4 (2-3 drops), and the reaction mixture was brought to reflux and distilled azoetropically until no water was produced. After drying the organic layer over anhydrous Na_2SO_4, subsequent evaporation of the solvent gave the product as pale yellow oil; 8.73 g (93 %): IR (neat) 1730, 1100 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85 (s, 3H), 2.55 (t, *J* 6 Hz, 6H), 3.24 (s, 6H), 3.65 (t, *J* 6 Hz, 6H), 3.69 (s, 9H).

1,1,1-Tris(carboxyethoxymethyl)ethane (35).[29] To compound (**34**) (17 g, 5.29 mmol) was added 1M NaOH solution (270 mL). The solution was stirred at room temperature fo 15 h, and then heated on an oil bath for 24 h until a clear aqueous solution was obtained. The pH of the reaction mixture was carefully adjusted with concd. HCl (56 mL) to 1. After removal of water by evaporation, Et_2O (4x80 mL) was added to extract the product. The combined organic layers were dried over anhydrous $MgSO_4$ and then removed by evaporation to give the product as yellow oil; 14.8 g (98 %): IR (neat) 3350-2600, 1710 cm^{-1}; ^1H NMR (D_2O) 0.86 (s, 3H), 2.63 (t, *J* 7 Hz, 6H), 3.34 (s, 6H),, 3.73 (t, *J* 7 Hz, 6H).

1,1,1-Tris(succinimidoxycarbonylethoxymethyl)ethane (36). To a solution of compound **35** (104 mg, 0.31 mmol), HOSu (150 mg, 1.30 mmol) in THF (10 mL) was added WSC·HCl (190 mg, 0.99 mmol) in CH_2Cl_2 (10 mL) at -10 °C. After stirring for 24 h at room temperature, the solvent was evaporated, and the residue was dissolved in AcOEt (300 mL). The organic layer was successively washed with H_2O, 5% $NaHCO_3$, and brine, and dried over anhydrous $MgSO_4$. Evaporation of the solvent

gave the *O*-succinimide ester (**36**) as colorless oil, which was used for the next reaction without further purification; 147 mg (76%); IR (neat) 1812, 1788, 1744 cm^{-1}.

General procedure for the preparation of tripodal compounds (37a-c). A typical example: 1,1,1-Tris(2-(N^2-(1-benzyloxy-2-oxo-1,2-dihydropyrimid-4-yl)diaminoethylcarbonyl) ethyloxymethyl)ethane (37a). A solution of compound **31a** (580 mg, 2.0 mmol), compound **36** (370 mg, 0.6 mmol), and Et_3N (482 mg, 4.8 mmol) in DMF (15 mL) was stirred at 38 °C for 48 h. After removal of DMF under reduced pressure, $CHCl_3$ (400 mL) was added to the residue. The organic layer was successively washed with H_2O (4x50 mL), 5% $NaHCO_3$ (50 mL), 5% citric acid (50 mL), H_2O (50 mL), and brine (50 mL), and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was purified by column chromatography on silica gel (eluent: $CHCl_3$-MeOH=8:1), followed by gel chromatography on Toyopearl HW-40 (eluent: MeOH), to give the product as colorless amorphous solid (**37a**); 286 mg (46%). IR (KBr) : 3284 , 1654 ($\nu_{C=O}$ NCON), 1637 ($\nu_{C=O}$ NHCO), 764 and 700 cm^{-1} (δ_{CH}Ph); ^{1}H NMR (DMSO-d_6) δ 0.76 (s, 3H, -CH_3), 2.50 (m, 6H, $COCH_2$), 3.14 (s, 6H, -CH_2O-), 3.20 (m, 6H, -$\underline{CH_2}$NH-), 3.27 (m, 6H, -$\underline{CH_2}$NH-), 3.53 (m, 6H, -OCH_2-), 5.07 (s, 6H, -$\underline{CH_2}$Ph), 5.52 (d, *J* 8 Hz, 3H, 5-H), 7.38-7.46 (m, 15H, Ph), 7.60 (d, *J* 8 Hz, 3H, 6-H), 7.79 (m, 3H, NH), and 7.97 ppm (m, 3H, NH); ($CDCl_3$) δ 0.77 (s, 3H, -CH_3), 2.42 (m, 6H, $COCH_2$), 3.18 (s, 6H, -CH_2O-), 3.35-3.71 (m, 18H, 2(-$\underline{CH_2}$NH-), -OCH_2-), 5.56 and 5.59 (s, 6H, -$\underline{CH_2}$Ph), 5.36, 5.49, 5.58, and 5.65 (d, 3H, 5-H), 6.93, 6.97, 7.07, and 7.14 (d, 3H, 6-H), 7.37 and 7.40 (s, 15H, Ph).

1,1,1-Tris(2-(N^4-(1-benzyloxy-2-oxo-1,2-dihydropyrimid-4-yl)diaminobutylcarbonyl)ethyloxymethyl)ethane (37b). 27%: mp 121-127 °C; IR (KBr) 3284, 1650, 1104, 754 cm^{-1}: ^1H NMR ($CDCl_3$) δ 0.82 (s, 3H), 1.27 (m, 2H), 1.36-1.60 (m, 12H), 2.43 (m, 6H), 3.06-3.25 (m, 12H), 3.32 (m, 6H), 3.61 (m, 6H),5.11 (s, 6H), 5.67 (d, *J* 6 Hz, 3H), 6.91 (d, *J* 6 Hz, 3H), 7.32 (s, 21H). Calcd for $C_{65}H_{90}N_{12}O_{12}\cdot 1.0H_2O$: C, 62.48; H, 7.42; N, 13.45. Found: C, 62.38; H, 7.54; N, 13.22.

1,1,1-Tris(2-(N^6-(1-benzyloxy-2-oxo-1,2-dihydropyrimid-4-yl)diaminohexylcarbonyl)ethyloxymehyl)ethane (37c). 54%: mp 121-127 °C; IR (KBr) 3284, 1650, 1104, 754 cm^{-1}: ^1H NMR ($CDCl_3$) δ 0.82 (s, 3H), 1.27 (m, 2H), 1.36-1.60 (m, 12H), 2.43 (m, 6H), 3.06-3.25 (m, 12H), 3.32 (m, 6H), 3.61 (m, 6H),5.11 (s, 6H), 5.67 (d, *J* 6 Hz, 3H), 6.91 (d, *J* 6 Hz, 3H), 7.32 (s, 21H). Calcd for $C_{65}H_{90}N_{12}O_{12}\cdot 1.0H_2O$: C, 62.48; H, 7.42; N, 13.45. Found: C, 62.38; H, 7.54; N, 13.22.

General procedure for the preparation of hexadentate ligands (24a-c). A typical example: 1,1,1-Tris(2-(N^2-(1-hydroxy-2-oxo-1,2-dihydropyrimid-4-yl)diaminoethylcarbonyl)ethyloxymethyl) ethane (24a). 10% Pd-C (20 mg) suspended in MeOH (20 mL) was prehydrogenated with H_2 for 0.5 h. To the suspention was added a solution of compound **37a** (223 mg, 0.2 mmol) in MeOH (20 mL). The reaction mixture was stirred for 2 h under a hydrogen atmosphere. After hydrogenation, the catalyst was remove by filtration. After the filtrate was concentrated, the resulting residue was purified by gel chromatography on Shephadex LH-20 (eluent: MeOH) to give the product as colorless amorphous solid; 88 mg (53%): IR (KBr) : 3284 , 1637 cm^{-1}; ^1H NMR (CD_3OD) δ 0.80 (s, 3H, $-CH_3$), 2.40 (m, 6H, CH_2CO), 3.18 (s, 6H, CH_2O),

3.40 (m, 6H, NCH_2), 3.48 (m, 6H, NCH_2), 3.66 (m, 6H, OCH_2), 5.76 (d, *J* 8 Hz, 3H, 5-H), 7.66 (d, *J* 8 Hz, 3H, 6-H). Calcd for $C_{32}H_{48}N_{12}O_{12}\cdot H_2O$: C 47.40, H 6.23, N 20.70. Found: C 47.43, H 6.40, N 20.61.

1,1,1-Tris(2-(N^4-(1-hydroxy-2-oxo-1,2-dihydropyrimid-4-yl)diaminobutylcarbonyl)ethyloxymethyl)ethane (24b). 72%: IR (KBr) : 3284, and 1636 cm^{-1}; ^1H NMR (CD_3OD) δ 0.82 (s, 3H, $-CH_3$), 1.58 (m, 12H, $-(CH_2)_2-$), 2.40 (m, 6H, CH_2CO), 3.21 (m, 12H, $NHCH_2$, CH_2CO), 3.35 (m, 6H, $NHCH_2$), 3.60 (m, 6H, OCH_2), 5.73 (d, *J* 8 Hz, 3H, 5-H), 7.62 (d, *J* 8 Hz, 3H, 6-H). Calcd for $C_{38}H_{60}N_{12}O_{12}\cdot 1.5H_2O$: C 50.49, H 7.02, N 18.59. Found: C 50.53, H 7.13, N 18.83.

1,1,1-Tris(2-(N^6-(1-hydroxy-2-oxo-1,2-dihydropyrimid-4-yl)diaminohexylcarbonyl)ethyloxymethyl)ethane (24c). 87%: IR (KBr) : 3284, and 1636 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85 (s, 3H), 1.37 (m, 12H), 1.48-1.62 (m, 12H), 2.39 (m, 6H), 3.12-3.38 (m, 18H), 3.63 (m, 6H), 5.71 (d, *J* 7 Hz, 3H), 7.62 (d, *J* 7 Hz, 3H). Calcd for $C_{44}H_{72}N_{12}O_{12}\cdot 1.5H_2O$: C, 53.48; H, 7.65; N, 17.01. Found: C, 53.64; H, 7.36; N, 17.11.

1-Hydroxy-4-*N*-butylamino-2(1*H*)-pyrimidinone (38). A solution of **29** (0.63 g, 2.34 mmol) and butylamine (0.21 g, 2.87 mmol) in dry THF (20 mL) was refluxed for 4 h. The solvent was evaporated, and H_2O (10 mL) was added to the residue. The aqueous solution was extracted with $CHCl_3$ (4x50 mL). The combined organic layers were dried over anhydrous Na_2SO_4. After removal of the solvent, the crude product was recrystallized from EtOH to give 1-benzyloxy-4-*N*-butylamino-2(1*H*)-pyrimidinone, 0.48 g (75%). A suspension of 10% Pd-C (25 mg) in distilled MeOH (10 ml) was prehydrogenated with H_2 for 0.5 h. A solution

of 1-benzyloxy-4-*N*-butylamino-2(1*H*)-pyrimidinone (252 mg, 0.92 mmol) in distilled MeOH (20 ml) was added to the suspension. After hydrogenation with H_2 under reflux for 2 h, the catalyst was removed by filtration. The filtrate was evaporated to give the product **38**.

1-Benzyloxy-4-*N*-butylamino-2(1*H*)-pyrimidinone: mp 160-162 °C; IR (KBr) 3246, 1644, 747, 699 cm^{-1}: ^1H NMR ($CDCl_3$) δ 0.94 (s, 3H), 1.3-1.41 (m, 2H), 1.50-1.60 (m, 2H), 3.10-3.50 (m, 2H), 5.22 (s, 3H, Bzl and 5-H), 6.90 (d, *J* 8 Hz, 1H), 7.40 (s, 5H). Anal. Calcd for $C_{15}H_{19}N_3O_2$: C, 65.91; H, 7.01; N, 15.37. Found: C, 65.80; H, 7.18; N, 15.46.

38: 120-123 °C. IR (KBr) 3300-2700, 3284, and 1636 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.85 (t, 3H, CH_3), 1.25-1.51 (m, 4H, $CH_3\underline{CH_2}\underline{CH_2}CH_2NH$), 3.20 (q, 2H, CH_2N), 5.55 (d, *J* 7.3 Hz, 1H, 5-H), 7.51 (br s, 1H, NH) 7.65 (d, *J* 7.3 Hz, 1H, 6-H), and 10.85 (br s, 1H, OH). Anal. Calcd for $C_8H_{13}N_3O_2 \cdot 0.2H_2O$: C 51.44, H 7.23, N 22.49. Found: C 51.62, H 7.09, N 22.75.

General procedure for the estification of amino carboxylic acid (40a-c). A typical example: Methyl 6-aminohexanoate hydrochloride (40a). Dry-HCl gas, which was produced by adding concd. HCl (30 mL) into concd. H_2SO_4 (140 mL), was introduced into a solution of 6-aminohexanoic acid (9.18 g, 0.07 mol) in absolute MeOH (140 mL) on an ice-salt bath. When the reaction mixture became clear, the solvent was evaporated to give the residue. Dry benzene (3x20 mL) was added to the residue in portions and evaporated to remove HCl gas and water completely. The crude solid was recrystallized from MeOH-Et_2O to give the product **40a**; 8.14 g (64%): mp 81-85 °C; IR (KBr) 3000 and 1730

cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.30-2.10 (m, 6H), 2.35 (t, 2H), 2.70-3.60 (m, 2H), 3.65 (s, 3H), 8.10 (br s, 2H).

Methyl 7-aminoheptanoate hydrochloride (40b). 100%: IR (KBr) 2944 and 1737 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.35-1.45 (m, 4H), 1.60-1.70 (m, 2H), 1.73-1.83 (m, 2H), 2.32 (t, 2H),3.00 (m, 2H), 3.68 (s, 3H), 8.20 (br s, 2H).

Methyl 8-aminooctanoate hydrochloride (40c). 100%: IR (KBr) 2980 and 1735 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.30-1.40 (m, 6H), 1.57-1.67 (m, 2H), 1.73-1.83 (m, 2H), 2.32 (t, 2H), 3.02 (m, 2H), 3.68 (s, 3H), 8.20 (br s, 2H).

General procedure for the coupling of 29 with methyl aminocarboxylate hydrochloride (40a-c). A typical example: 4-(5-Methoxycarbonylpentyl)amino-1-benzyloxy-2(1*H*)-pyrimidinone (41a). Compound **40a** (2.12 g, 11.7 mmol) and triethylamine (1.18 g, 11.7 mmol) were added to a solution of **29** (2.7 g, 10 mmol) in dry THF (60 mL). The reaction mixture was refluxed for 44 h, and concentrated. H_2O (30 mL) was added to the residue, and the mixture was extracted with $CHCl_3$ (5x50 mL). The combined organic layers were dried over anhydous $MgSO_4$. After evaporation of the solvent, the resulting residual solid was recrystallized from EtOH to give the product **41a**; 2.41 g (70%): mp 144-146 °C; IR (KBr) 3020, 1670, 1630, 750, 700 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.25-1.38 (m, 2H), 1.40-1.60 (m, 4H), 2.3 (t, 2H), 3.18 (q, 2H), 3.58 (s, 3H), 5.05 (s, 2H), 5.45 (d, *J* 8 Hz, 1H),7.35-7.46 (m, 5H), 7.55 (d, *J* 8 Hz, 1H), and 7.67 (br s, 1H). Anal. Calcd for $C_{18}H_{23}N_3O_4$: C, 62.59; H, 6.71; N, 12.17. Found: C, 62.61; H, 6.74; N, 12.22.

4-(6-Methoxycarbonylhexyl)amino-1-benzyloxy-2(1*H*)-pyrimidinone (41b). 50%: mp 110-111 °C; IR (KBr) 3252, 1737, 1645, 702 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.25-1.35 (m, 4H), 1.52-1.64 (m, 4H), 2.33 (t, 2H), 3.15 (br s, 2/5H), 3.40 (m, 8/5H), 3.65 (s, 3H), 5.20 (s, 2H), 5.55 (d, *J* 8 Hz, 1H), 6.41 (br s, 1H), 6.68 (br s, 1/5H), 6.89 (d, *J* 8 Hz, 4/5H), 7.10 (d, *J* 8 Hz, 1/5H), 7.37 (s, 5H). Anal. Calcd for $C_{19}H_{25}N_3O_4$: C, 63.49; H, 7.01; N, 11.69. Found: C, 63.46; H, 7.21; N, 11.66.

4-(7-Methoxycarbonylheptyl)amino-1-benzyloxy-2(1*H*)-pyrimidinone (41c). The crude product was purified by column chromatography on silica gel (eluent: $CHCl_3$-acetone-EtOH=100:10:2) to afford the pure product; 60%: mp 132-135 °C; IR (KBr) 3250, 1737, 1671, 1642, 749, 702 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.25-1.35 (m, 6H), 1.53-1.65 (m, 4H), 2.32 (t, 2H), 3.16 (br s, 2/5H), 3.41 (m, 8/5H), 3.66 (s, 3H), 5.19 (s, 2H), 5.40 (d, *J* 8 Hz, 1/5H), 5.56 (d, *J* 8 Hz, 4/5H), 6.41 (br s, 4/5H), 6.68 (br s, 1/5H), 6.88 (d, *J* 8 Hz, 4/5H), 7.10 (d, *J* 8 Hz, 1/5H), 7.37 (s, 5H). Anal. Calcd for $C_{20}H_{27}N_3O_4$: C, 64.32; H, 7.29; N, 11.25. Found: C, 64.26; H, 7.30; N, 11.02.

General procedure for the preparation of 42a-c. A typical example: 4-(5-Carboxypentyl)amino-1-benzyloxy-2(1*H*)-pyrimidinone (42a). To a solution of compound **41a** (2.41 g, 6.98 mmol) in MeOH (100 mL) was added 1M NaOH (7.7 mL, 7.7 mmol), and the reaction mixture was stirred for 1 h at room temperature. An additional 1M NaOH (15.4 mL, 15.4 mmol) was added to the mixture, and the reaction mixture was stirred for another 14 h at room temperature. After evaporation of the solvent, the residue was dissolved in H_2O (40 mL). The pH of the aqueous solution was adjusted to 3 with 5% HCl. The resulting

crude solid precipitated was recrystallized from EtOH to give the product **42a**; 2.16 g (93 %): mp 192-193 °C; IR (KBr) 3200-2800, 1710, 1642, 752, 701 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.18-1.39 (m, 2H), 1.43-1.61 (m, 4H), 2.19 (t, 2H), 3.17-3.30 (m, 2H), 5.17 (s, 2H), 5.51 (d, *J* 7 Hz, 1H), 7.32-7.47 (m, 6H), 7.67 (br s, 1H). Anal. Calcd for $C_{17}H_{21}N_3O_4 \cdot 0.3H_2O$: C, 60.80; H, 6.58; N, 12.45. Found: C, 60.63; H, 6.46; N, 12.45.

4-(6-Carboxyhexyl)amino-1-benzyloxy-2(1*H*)-pyrimidinone (42b). 69%: mp 134-137 °C; IR (KBr) 3200-2700, 1716, 1673, 739, 700 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.28-1.48 (m, 8H), 2.20 (t, 2H), 5.08 (s, 2H), 5.63 (d, *J* 8 Hz, 1H), 7.40 (s, 5H), 7.68 (d, *J* 8 Hz, 1H). Anal. Calcd for $C_{18}H_{23}N_3O_4$: C, 56.68; H,7.13; N, 11.02. Found: C, 56.73; H, 6.77; N, 11.19.

4-(7-Carboxyheptyl)amino-1-benzyloxy-2(1*H*)-pyrimidinone (42c). 80%: IR (KBr) 3200-2700, 1739, 1636, 739, 703 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.28-1.46 (m, 10H), 2.19 (t, 2H), 3.13-3.18 (m, 2H), 5.06 (s, 2H), 5.50 (d, *J* 8 Hz, 1H), 7.42 (s, 5H), 7.55 (d, *J* 8 Hz, 1H), 7.68 (br s, 1H), 11.95 (br s, 1H). Anal. Calcd for $C_{19}H_{25}N_3O_4 \cdot 0.5H_2O$: C, 61.94; H,7.11; N, 11.41. Found: C, 62.07; H, 7.07; N, 11.15.

General procedure for the preparation of *O*-succinimide ester hexadentate ligands (43a-c). A typical example: 4-(5-Carboxypentyl)amino-1-benzyloxy-2(1*H*)-pyrimidinone *O*-succinimide ester (43a). To a solution of compound **42a** (700 mg, 2.11 mmol) and HOSu (486 mg, 4.22 mmol) in dry DMF (10 mL) was added a solution of WSC·HCl (810 mg, 4.22 mmol) in CH_2Cl_2 (20 mL) at -10 °C. The reaction mixture was stirred for 14 h at room temperature, and the solvent was evaporated. The resulting solid was dissolved in $CHCl_3$ (150

mL), and then the organic layer was washed with H_2O (50 mLx2) and dried over anhydous Na_2SO_4. After removal of the solvent, the *O*-succinimide ester was obtained and used for the next reaction without further purification; 740 mg (82%): IR ($CDCl_3$) 1810, 1780, 1720, 1620, 740, 690 cm^{-1}.

4-(6-Carboxy-hexyl)amino-1-benzyloxy-2(1*H*)-pyrimidinone *O*-succinimide ester (43b). 100%: IR ($CDCl_3$) 1810, 1770, 1720 cm^{-1}.

4-(7-Carboxy-heptyl)amino-1-benzyloxy-2(1*H*)-pyrimidinone *O*-succinimide ester (43c). 100%: IR ($CDCl_3$) 1808, 1772, 1720 cm^{-1}.

General procedure for the preparation of tripodal compounds (44a-c). A typical example: Tris(2-(6-(1-(1-benzyloxy-2-oxo-1,2-dihydropyrimidi-4-yl)amino)hexamido) ethyl)amine (44a). A solution of tris(2-aminoethyl)amine (76 mg, 0.52 mmol) in DMF (5 mL) was added to a solution of compound **43a** (740 mg, 0.52 mmol) in DMF (15 mL), and then the mixture was stirred at 38 °C for 48 h. After removal of the solvent, the residue was purified by column chromatography on silica gel (eluent: $CHCl_3$-MeOH=5:1), followed by gel chromatography on Toyopearl HW-40 (eluent: MeOH), to give the pure product **44a**; 296 mg (52%): IR (KBr) 3500, 2940, 2860, 1760, 1630, 750, and 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.18-1.32 (m, 6H), 1.45-1.65 (m, 12H), 2.15-2.25 (m, 6H), 2.55-2.70 (m, 6H), 3.25-3.40 (m, 12H), 5.15 (s, 6H), 5.65 (d, *J* 8 Hz, 3H), 6.90 (d, *J* 8 Hz, 3H), 7.25 (s, 15H), 7.72 (br s, 3H). Anal. Calcd for $C_{57}H_{75}N_{13}O_9 \cdot 3H_2O$: C, 60.04; H, 7.16; N, 15.97. Found: C, 60.22; H, 6.98; N, 15.71.

Tris(2-(6-(1-(1-benzyloxy-2-oxo-1,2-dihydropyrimidi-4-yl)amino)heptamido)ethyl)amine (44b). 86%: IR (KBr) 3284, 1636,

753, 702 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.18-1.32 (m, 12H), 1.48-1.65 (m, 12H), 2.22 (m, 6H), 2.58-2.68 (m, 6H), 3.20-3.35 (m, 12H), 5.12 (s, 6H), 5.71 (d, *J* 8 Hz, 3H), 6.92 (d, *J* 8 Hz, 3H), 7.36 (s, 15H), 7.51 (br s, 3H), 7.71 (br s, 3H). Anal. Calcd for $C_{60}H_{81}N_{13}O_9 \cdot 3H_2O$: C, 60.94; H, 7.42 N, 15.40. Found: C, 61.14; H, 7.61; N, 15.46.

Tris(2-(6-(1-(1-benzyloxy-2-oxo-1,2-dihydropyrimidi-4-yl)amino)octamido)ethyl)amine (44c). 71%: IR (KBr) 3284, 1636, 753, 703 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.20-1.32 (m, 18H), 1.45-1.65 (m, 12H), 2.22 (m, 6H), 2.48-2.55 (m, 6H), 3.20-3.40 (m, 12H), 5.12 (s, 6H), 5.72 (d, *J* 8 Hz, 3H), 6.93 (d, *J* 8 Hz, 3H), 7.35 (s, 15H), 7.64 (br s, 3H), 7.71 (br s, 3H). Anal. Calcd for $C_{63}H_{87}N_{13}O_9 \cdot 0.5H_2O$: C, 64.16; H, 7.52 N, 15.44. Found: C, 63.99; H, 7.50; N, 15.58.

General procedure for the preparation of hexadentate ligands (25a-c). A typical example: Tris(2-(6-(1-(1-hydroxy-2-oxo-1,2-dihydropyrimidi-4-yl)amino)hexamido)ethyl)amine (25a). A suspension of 10% Pd-C (35 mg) suspended in MeOH (20 mL) was prehydrogenated with H_2 for 0.5 h. To the suspension was added a solution of compound **44a** (300 mg, 0.28 mmol) in MeOH (10 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, which was purified by gel chromatography on Shephadex LH-20 (eluent: MeOH) to afford the product **44a**; 150 mg (66%): IR (KBr) 3500-3200, 3282, 1636 cm^{-1}: ^1H NMR (DMSO-d_6) δ 1.20-1.30 (m, 6H), 1.41-1.53 (m, 12H), 2.08 (t, 6H), 2.40-2.52 (m, 6H), 3.05-3.22 (m, 12H), 5.55 (d, *J* 8 Hz, 3H), 7.55 (br s, 3H), 7.65 (d, *J* 8 Hz, 3H), 7.73 (br s, 3H). Anal.

Calcd for $C_{36}H_{57}N_{13}O_9 \cdot 0.5H_2O$: C, 52.42; H, 7.09; N, 22.07. Found: C, 52.29; H, 7.37; N, 22.23.

Tris(2-(6-(1-(1-hydroxy-2-oxo-1,2-dihydropyrimidi-4-yl)amino)heptamido)ethyl)amine (25b). 66%: IR (KBr) 3500-3200, 3284, 1635 cm^{-1}: ^1H NMR (DMSO-d_6) δ 1.21-1.32 (m, 12H), 1.40-1.55 (m, 12H), 2.10 (t, 6H), 3.05-3.25 (m, 12H), 5.55 (d, *J* 8 Hz, 3H), 7.55 (br s, 3H), 7.65 (d, *J* 8 Hz, 3H), 7.70 (br s, 3H), 10.90 (br s, 3H, -OH). Anal. Calcd for $C_{39}H_{63}N_{13}O_9 \cdot 1.8H_2O$: C, 52.61; H, 7.54; N, 20.45. Found: C, 52.73; H, 7.73; N, 20.25.

Tris(2-(6-(1-(1-hydroxy-2-oxo-1,2-dihydropyrimidi-4-yl)amino)ctamido)ethyl)amine (25c). 61%: IR (KBr) 3500-3200, 3284, 1636 cm^{-1}: ^1H NMR (DMSO-d_6) δ 1.16-1.30 (m, 12H), 1.40-1.55 (m, 12H), 2.08 (t, 6H), 3.05-3.25 (m, 12H), 5.57 (d, *J* 8 Hz, 3H), 7.56 (br s, 3H), 7.65 (d, *J* 8 Hz, 3H), 7.65 (br s, 3H), 10.86 (br s, 3H, -OH). Anal. Calcd for $C_{42}H_{69}N_{13}O_9 \cdot 2H_2O$: C, 53.89; H, 7.86; N, 19.45. Found: C, 53.83; H, 7.85; N, 19.39.

Measurement of the pKa value of 38. Compound **38** (30 mg) was dissolved in deionized water (30 mL). The pH of the solution was measured after every 0.1 mL addition of 0.08M NaOH solution at room temperature under an Ar atmosphere. The pKa value was calculated from the pH value at the midpoint of neutralization.

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),[38] the pKa of the corresponding bidentate ligand (pKa 7.5 of **38** for **24** and **25**), and the pH of the solution at an equilibrium point at 20 °C.

Gallium complex formation. Compound **25a**(6 mg) and $Ga(NO_3)_3$ (4 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.[39] ^1H NMR spectrum was measured at room temperature. **Ga/25a.** δ 1.35 (m,

6H), 1.60 (m, 12H), 2.25 (m, 6H), 3.40 (m, 6H), 3.60 (m, 12H), 5.55 (d, *J* 8 Hz, 3H), 7.55 (br s, 3H), 7.65 (d, *J* 8 Hz, 3H), 7.73 (br s, 3H).

Preparation of a stock solution of $FeCl_3$. A 0.01 M EDTA and 0.01 M Bi^{3+} solution were prepared as the standard solution (f=1.05194 and 1.05615, respectively). $FeCl_3 \cdot 6H_2O$ (3.82 g) was dissolved in distilled water (250 mL). To a mixed solution of $FeCl_3 \cdot 6H_2O$ (1.0 mL) and EDTA (7.0 mL) was added a few drops of Xylenol orange as and indicator. The solution was titrated with the standard Bi^{3+} solution (0.05536 M). This stock solution was only used for the sbusequent experiment.

Prearation of the stock solution of diferric transferrin ($Tf_{Fe2.0}$). $Tf_{Fe2.0}$ was prepared according to the method reported in detail by Raymond.[12,32] A commercially available human serum apotransferrin (98%, Sigma) was used. An approximate 0.2 mM solution of apotransferrin (83 mg) was prepared with Tris chloride buffer (pH 7.4, 0.1M; 4.5 mL) and 0.5M $KHCO_3$ (0.5 mL). $Tf_{Fe2.0}$ was prepared by titration of the stock solution of apotransferrrin (1 mL) with 1:3 iron(III)/NTA solution (0.00104 M iron(III))[40] monitoring absorbance at 460 nm. The titration sample was recombined with the rest of the original apo transferrin solution and the correct amount of iron(III)/NTA solution was added to give $Tf_{Fe2.0}$, at a level that corresponds to 110 to 115% saturation. The solution was allowed to quilibrate 30 min, and then passed down a column of Sephadex G-25 (eluent: 0.10 M $NaClO_4$). The orange bands were collected, and the fractions were concentrated to near dryness, redissolved in ca. 1 mL of distilled water. The solution was passed down the Sephadex G-25 column with Tris chloride buffer. The orange bands were

given as the stock solution of $Tf_{Fe2.0}$. The extinction coefficient of $Tf_{Fe2.0}$ (9.23×10^4 M^{-1} at λ_{max} 279 nm)[40] was used to determine the concentration.

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 mixed, 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 $[(A_\infty\text{-Abs})/(A_\infty\text{-}A_0)]$ as a function of time.

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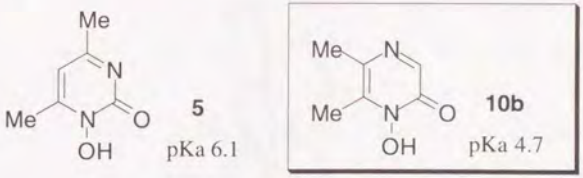
Chapter 6

Hexadentate Ligands: Iron Chelating Properties of 1-Hydroxy-2(1*H*)-pyrazinones

6.1 Introduction

Since 1,2-dimethyl-4-hydroxy-2(1*H*)-pyridinone **22b** was used by Hider[1] in 1982, the application of nitrogen-containing heterocycles to iron chelators has been one of the important issues in the preparation of therapeutic agents for the iron overload disease from the view point of search for new nontoxic and orally active agents.[2] As described in Chapter 5, the synthesis of tripodal hexadentate ligands bearing 1-hydroxy-4,6-dimethyl-2(1*H*)-pyrimidinone **5** was achieved by using alkyl chains as spacer groups and tricarboxylic acid or tris(2-aminoethyl)amine (TREN) as an anchor group. These ligands showed good iron chelating abilities with high stability constants (logK 25-27) in solutions. However, low solubility of the ligands in water is one of the problems for the evaluation of their iron chelating ability under physiological conditions, particular in the cases of the ligands having rather long alkyl chains.



Scheme 6-1

As mentioned in Chapter 4, the other diazine, 1-hydroxy-5,6-dimethyl-2(1*H*)-pyrazinone **10b**, showed a lower pKa value than that of **5** (Scheme 6-

1).[3] Thus, **10b** would be expected to show higher water solubility at around pH 7.4, since the negative charged (deprotonated) cyclic hydroxamate form of **10b** would exist as the major species, compared to the protonated neutral hydroxamic acid form under the conditions. Therefore, the author was interested in the application of **10b** to the synthesis of hexadentate ligands with the expectation that the new ligands would be more water soluble and effective iron chelator under acidic to neutral conditions than **24** and **25**.

When some kinds of microoganisms uptake iron as the complex with a siderophore, through an iron transport system in membrane, an external receptor in membrane recognizes chiral environment around the iron of the complex (i.e. Λ and Δ) and uptakes it stereoselectively.[4] For example, a receptor of *Escherichia coli* displays a high chiral recognition. Its siderophore, enterobactin, which is a cyclic lactone of L-serine, forms an iron complex in a Δ form selectivity[5,6] as shown in Figure 6-1. The racemic enterobactin analogue, derived from D, L-serine, showed the biological activity in only half of that of native enterobactin.[8]

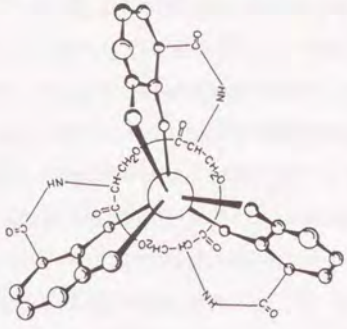


Figure 6-1. Δ-*cis* Enterobactin Fe-complex[7]

It is obvious that chiral environment around the iron atom plays a quite important role in nature. If chiral moiety could be incorporated in a ligand, a seteroselective iron complex formation would be expected.

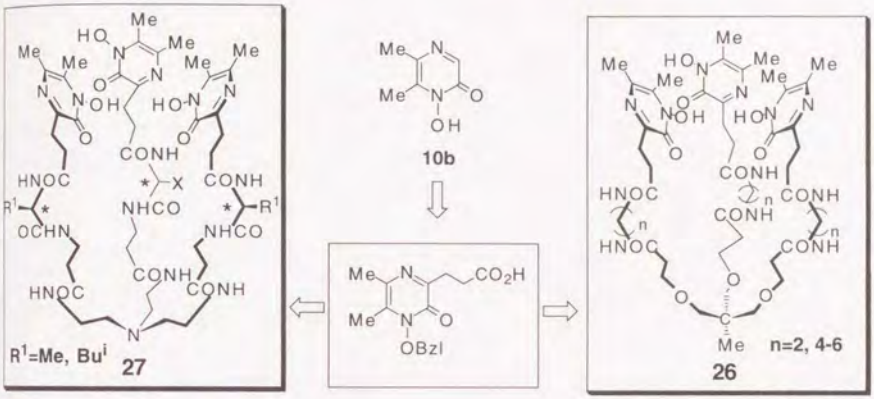


Figure 6-2

In this Chapter, the author describes the synthesis of two types of novel heterocyclic hexadentate ligands (**26** and **27**), in which three units of **10b** are linked to an anchor group by appropriate spacer groups as shown in Figure 6-2. For **26**, alkyl chains and tripodal tricarboxylic acid were used as the spacer and anchor, respectively. In the case of **26**, the effect of the spacer length on iron chelating ability will be investigated. On the other hand, dipetide, composed of a natural L-amino acid and β-alanine, and tris(2-aminoethyl)amine (TREN) were used for **27**. In the case of **27**-iron complex, the chiral environment around the iron may be controlled by the chiral spacer bearing L-amino acid residues. In order to introduce a spacer

group to the heterocycle ring system, 1-benzyloxy-5,6-dimethyl-2(1*H*)-pyrazinone **50** was used as a starting material.

6.2 Hexadentate Ligands Bearing sp^3 Carbon as an Anchor[9]

In order to investigate the influence of molecular structure on iron chelating property, hexadenate ligands **26a-d**, in which each of three 1-hydroxy-5,6-dimethyl-2(1*H*)-pyrazinone (**10b**) moiety is linked to an anchor having a sp^3 carbon by an aliphatic diamine through amide bonds, were prepared (Figure 6-3). The sp^3 carbon in the anchor would make the structure rigid to some extent, in contrast to the fact that a tertiary amine easily undergoes the inversion of the lone electorn pair.

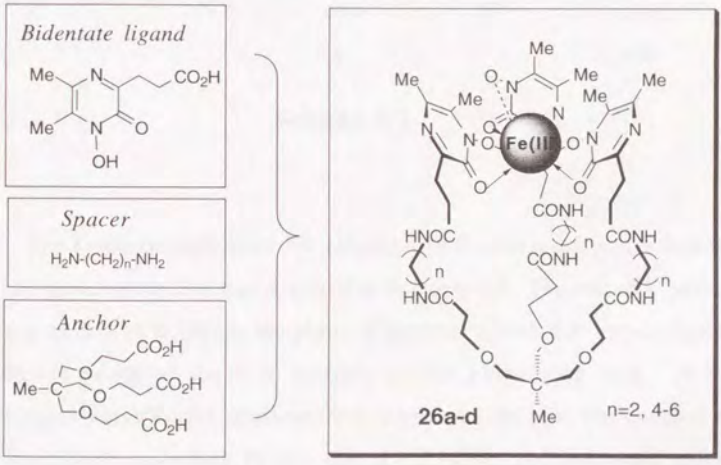
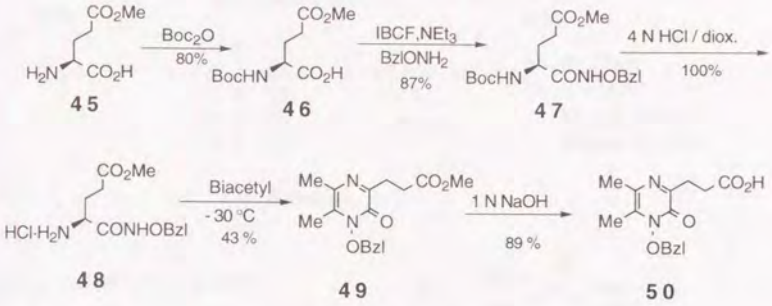


Figure 6-3

Furthermore, the aliphatic diamine would be suitable in order to investigate the relationship between the spacer length and iron binding property. A kinetic study concerning iron removal from transferrin at physiological pH was also carried out.

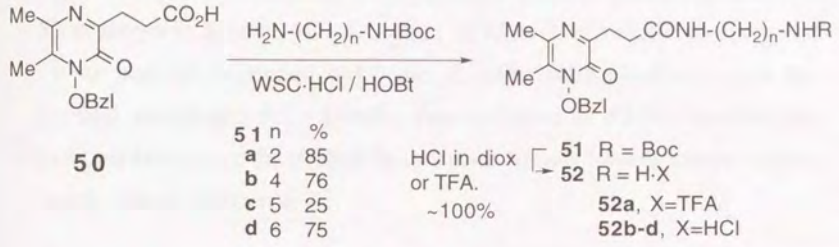
(a) Synthesis



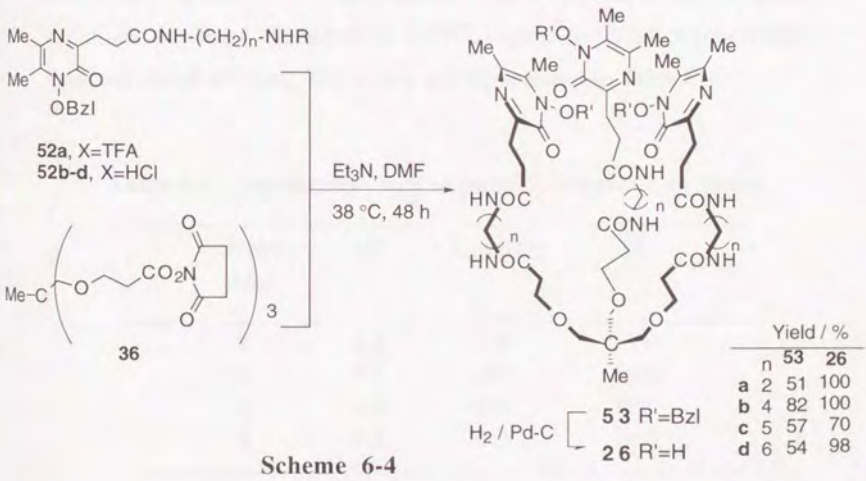
Scheme 6-2

The synthetic procedure for 1-benzyloxy-3-carboxyethyl-5,6-dimethyl-2(1*H*)-pyrazinone (**50**) was depicted in Scheme 6-2. The use of L-glutamic acid γ-methyl ester (**45**) in the place of glycine[3] allows the introduction of a carboxyl group at the C-3 position of the pyrazinone ring. *N-tert*-Butoxycarbonyl(Boc)-L-glutamic acid γ-methyl ester (**46**) was coupled with *O*-benzylhydroxylamine by the mixed anhydride method using isobutyl chloroformate (IBCF) to give the corresponding benzyloxyamide **47**.[10] Deprotection of the Boc group with 4M HCl in dioxane, followed by condensation of the hydrochloride salt **48** with biacetyl at -30 °C under basic

conditions, gave 1-benzyloxy-3-methoxycarbonylethyl-5,6-dimethyl-2(1*H*)-pyrazinone (**49**), which was easily converted to the corresponding carboxylic acid **50** by treatment with 1 M NaOH in MeOH.



Scheme 6-3



Scheme 6-4

The coupling of the carboxyl group at the C-3 position of **50** with N^{ω}–*tert*-butoxycarbonyl (Boc)-protected aliphatic diamine by using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (water soluble carbodiimide: WSC·HCl) and 1-hydroxybenzotriazole (HOBt), followed by deprotection of the Boc group under appropriate acidic conditions, gave salt **52** as shown in Scheme 6-3. Coupling of **52** with **36**, which was prepared by the method mentioned in Chapter 5, under mild conditions gave the tripodal compound **53**. Finally, debenzylation of **53** by the catalytic hydrogenation gave the desired hexadentate ligands having various spacer length, **26a-d** (Scheme 6-4).

(b) Iron complex formation

The visible spectra of 1:1 molar mixtures of **26** and ferric ion in aqueous solutions showed a characteristic LMCT (ligand to metal charge transfer) band around at 450 nm. The results are summarized in Table 6-1.

Table 6-1. Spectroscopic data of iron(III) complexes of **26a-d**.

Ligand **26**	pH	λ_{max}/nm	ε
a	4.8	455	3350
b	5.8	450	3600
c	4.7	450	3856
d	4.5	450	3103
10b[a]	4.0	425	4237

[a] Bidentate ligand.

The observed λ_{max} and ε values, which were comparable to those of the corresponding bidentate ligand, **10b** (ε 4237 at 425 nm),[3] suggest the formation of intramolecular 1:1 complexes. The 1:1 complex formation was also confirmed by the mole ratio plot. The result of the complex of **26b** is shown in Figure 6-4. The plots of the absorbance at 450 nm vs mole ratio of iron/**26b** gave an infection point at 0.90. The value was close to 1.00 that was expected for 1:1 intramolecular complex formation. Thus, the result strongly supports 1:1 complex formation, in the case of **26b**.

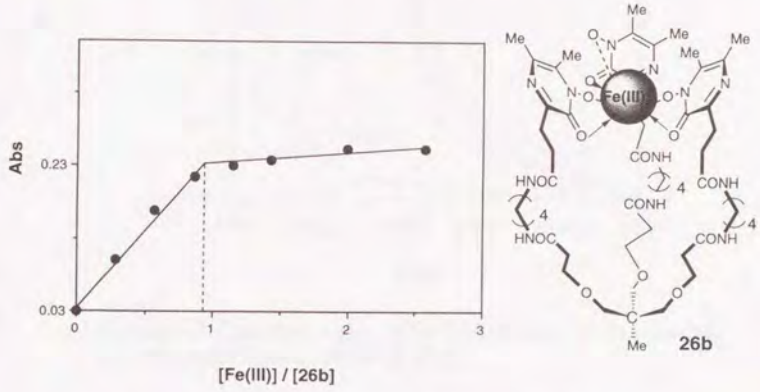


Figure 6-4. Mole ratio plot of **26b** at pH 6: [**26b**] 5.4×10^{-5} M.

The spectral change under various pH conditions of the complex of **26b** is shown in Figure 6-5. No apparent change in the intensity of absorbance was observed in a wide pH range, especially in acidic to neutral conditions, indicating that **26b** could form a stable 1:1 complex under these conditions, in contrast to the case of 1-hydroxy-2(1*H*)-pyridinone (**21**).[11] In contrast,

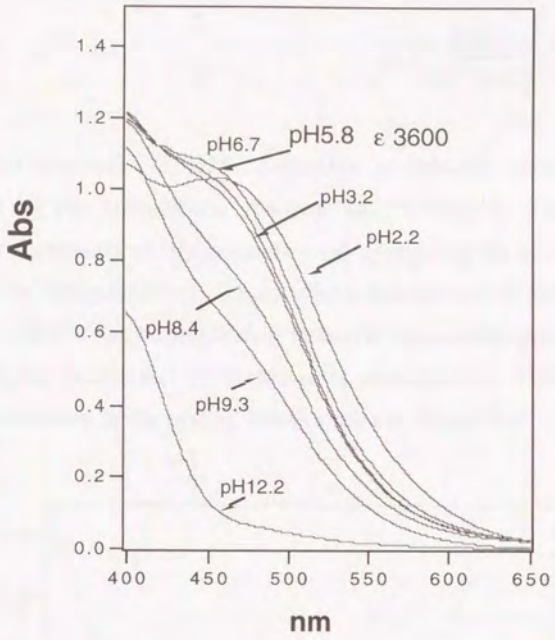


Figure 6-5. Spectral change of the Fe/**26b** mixture in aqueous solution under various pH conditions.

the absorbance decreased under basic conditions, which would be attributable to the decomposition of the iron complex by the attack of hydroxyl ion.

(c) Stability of iron complex.

The stability constant of a complex of a hexadentate ligand with iron is defined by the following equilibrium.

$$Fe^{3+} + L^{3-} \underset{}{\overset{K}{\rightleftharpoons}} FeL \qquad K = \frac{[FeL]}{[Fe^{3+}]\,[L^{3-}]}$$

The stability constant of Fe/**26** complexes in aqueous solutions were determined by the competition reaction with EDTA.[12] Three proton dissociation constants of hydroxamic acid groups in **26** are important parameters for this calculation. These values, however, were approximated by the pKa value of the corresponding bidentate ligand **10b** (pKa 4.7)[3] due to experimental limitations. The change of absorbance at 450 nm in the competition reaction in the case of **26b** is shown in Figure 6-6.

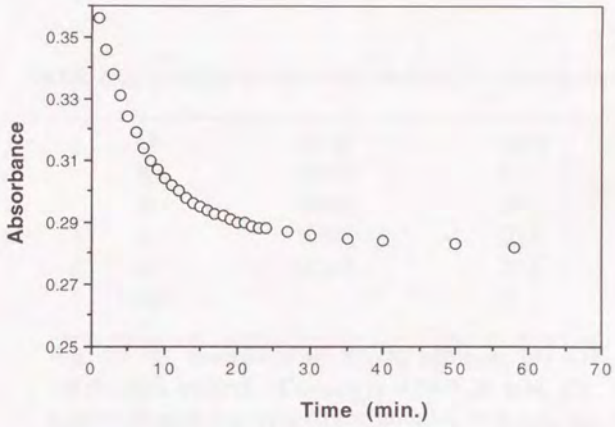


Figure 6-6. Absorbance change at 450 nm in competition reaction of **26b** with EDTA in an aqueous solution. [Fe**26b**]= 0.11 mM, [EDTA]=0.21 mM.

After determination of an equilibrium point for the iron exhange reaction, the relative stability constants were calculated using the acid dissociation constants and the stability constant of Fe(edta).[13] The results are summarized in Table 6-2. The stability constants of **26** were in the range from 20.6 to 21.7 in logK. The stability constant slightly increased with decreasing the spacer length, suggesting that the shorter spacer was more advantageous for stabilizing the complex by virtue of the increased chelating effect; the stability constant of **26b** was almost 1 order greater than that of **26d**. However, the stability constants of the iron complexes were far below that of desferrioxamine B, since the pKa value of 1-hydroxy-2(1*H*)-pyrazinone was considerably lower than that of hydroxamic acids ($pK_{1,2,3}$ 9.70, 9.03, 8.39).[14]

Table 6-2. Stability constants of **26**-iron(III) complexes[a]

26	Keq[b]	log K
a	0.062	21.5
b	0.042	21.7
c	0.452	20.7
d	0.168	20.6
DFB[c]	-	30.5

[a] T=20 °C, m=0.04 with KNO_3 solution, pH 6.0 (Mcvallin's buffer). $C_{iron(III)}$ 0.04-0.20 mM, C_L 0.04-0.20 mM, C_{EDTA} 0.08-0.40 mM. [b] Keq is the equilibrium constant for the reaction Fe(**26**) + H_2EDTA^{2-} + H^+ ⇌ $FeEDTA^-$ + **26**. [c] Ref.14

(d) Iron removal from transferrin

The iron removal ability of **26** from iron transport protein, transferrin, was evaluated under physiological conditions by the same fashion described in Section 5.2. After mixing a buffered solution of $\mathbf{Tf}_{Fe2.0}$ with 40 times excess of **26d**, the change in absorbance at 500 nm was monitored. The plots of $\log[(A_\infty\text{-Abs})/(A_\infty\text{-}A0)]$ as a function of time gave a good linear relationship as shown in Figure 6-7. This relationship indicates that the reaction of iron removal from transferrin by **26d** proceeds in the pseudo-first-order-kinetics. From the slope of the straight line, k_{obsd} was obtained. The kinetic results are summarized in Table 6-3 with the data of DFB.

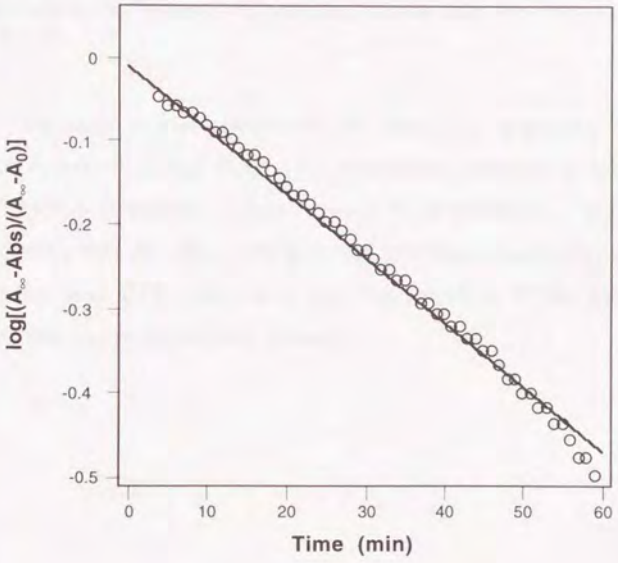


Figure 6-7. The plots of $\log[(A_\infty\text{-Abs})] / [(A_\infty\text{-}A_0)]$ vs time on iron removal of **26d** from $\mathbf{Tf}_{Fe2.0}$.

Table 6-3. Iron Removal from Transferrin at pH 7.4

Ligand **26**	[L]/[**Tf**Fe2.0][a]	k_{obs} ($x10^{-3}$ min^{-1})	% Fe removed[b]
a	40	1.98	13
b	40	3.76	23
c	40	3.20	21
d	40	3.17	20
DFB	100	0.66	5 (5)[c]

[a] [**Tf**Fe2.0]0=0.05 mM, **Tf**Fe2.0 was prepared from human serum apotransferrin (Sigma). [b]At a point 30 min after the reaction was initiated. [c] Ref.15.

The rates of iron removal by **26** were in a range of 3.76-1.98 $x10^{-3}$ min^{-1}, indicating that there is no remarkable influence of spacer length on the kinetic efficiency of iron removal from transferrin. It is noteworthy, however, that **26** efficiently removed iron from transferrin about 4 times greater than DFB, even at a less concentration of the ligand after the reaction was performed for 30 min.

6.3 Hexadentate Ligands Bearing Amino Acids and Tertiary Amine[16]

Chiral hexadentate ligand **27** was constructed as shown in Figure 6-8. A L-amino acid was used for the spacer group. 1-Hydroxy-5,6-dimethyl-2(1*H*)-pyrazinone (**10b**) units were linked to tris(2-aminoethyl)amine through dipeptide chains bearing a L-amino acid and β-alanine. The spacer length, i.e. the number of atoms from the nitrogen atom to the C-3 of the pyrazinone ring, was comparable to that of **26a**.

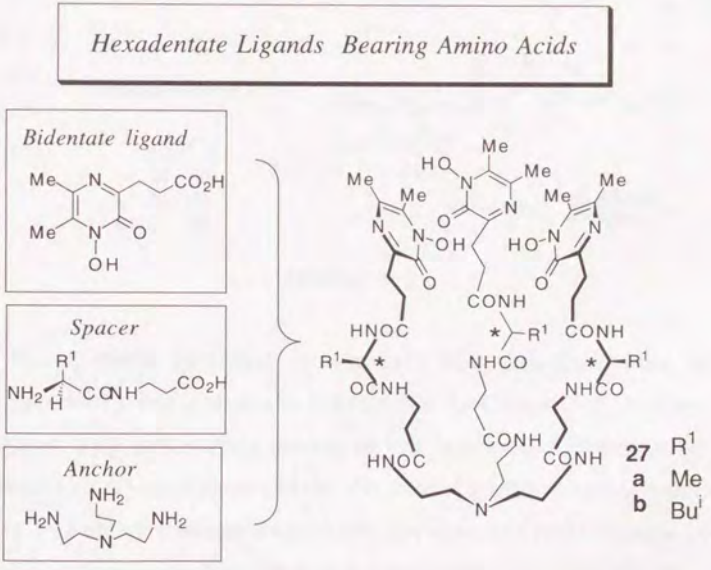
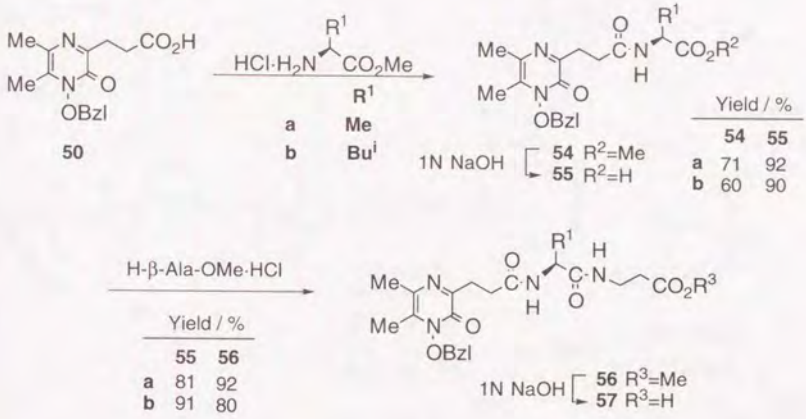


Figure 6-8

In this section, the synthesis, iron chelating properties, and iron removal abilities at pH 7.4 of **27** are described. In addition, the author discusses the absolute configuration around iron, which is controlled by the chiral L-amino acid residues.

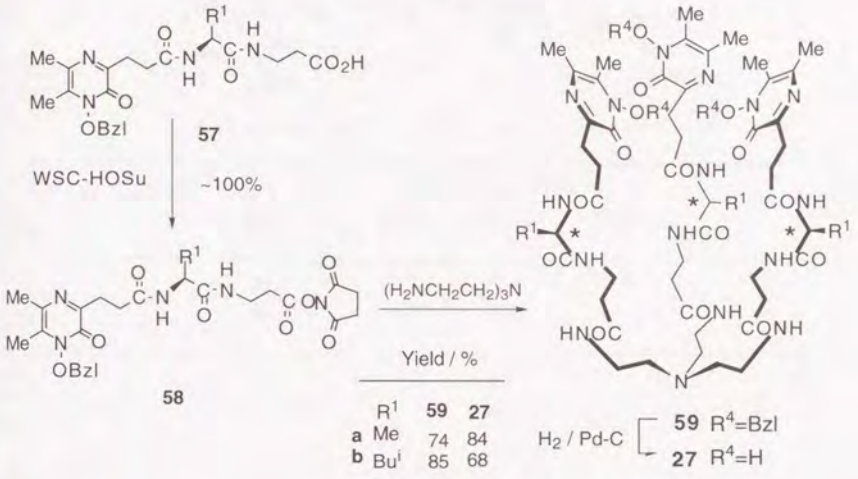
(a) Synthesis



Scheme 6-5

The synthetic procedure to introduce of a dipeptide chain to the heterocyclic system is shown in Scheme 6-5. L-Alanine (or L-leucine) and β-alanine were successively introduced to 1-benzyloxy-3-carboxyethyl-5,6-dimethyl-2(1*H*)-pyrazinone (**50**) by the general peptide coupling method[10] using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (water soluble carbodiimide: WSC·HCl) and 1-hydroxybenzotriazole (HOBt). As shown in Scheme 6-6, dipeptide-linked pyrazinones **57** were converted to

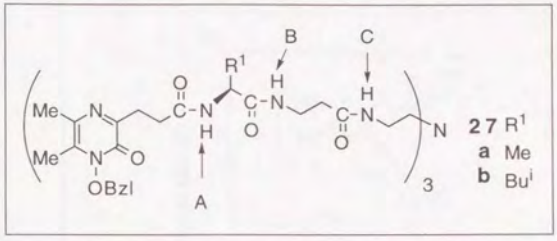
the corresponding *O*-succinimide esters **58**, and then coupled with tris(2-aminoethyl)amine (TREN) to give *O*-protected tripodal compounds **59**. Debenzylation by catalytic hydrogenation on 10% Pd-C and subsequent gel chromatographic purification afforded the desired hexadentate ligands **27**.



Scheme 6-6

The ^{1}H NMR spectra of compound **59** and **27** in DMSO-d$_6$ at room temperature exhibited three sets of amide protons (δ 8.0-7.7); two of them were triplets and the other was a doublet, indicating that these molecules possess pseudo-*C*$_3$-symmetry. The temperature dependence of the amide proton chemical shifts[17] was measured in a range from 22 to 90 °C. The plots gave a straight line (-4.6~-5.4x10^{-3} ppm K^{-1}), and no particular hydrogen bond was observed in a DMSO-d$_6$ solution (Table 6-3).

Table 6-3. Temperature coefficients of amide protons.



	ppm / deg. ($x10^{-3}$)		
27	A(d)	B(t)	C(t)
a	-4.9	-5.8	-4.6
b	-5.27	-5.26	-

(b) Iron complex formation

UV-vis spectra of 1:1 molar mixtures of Fe(III) and **27a** in aqueous solutions under various pH conditions is shown in Figure 6-9. As observed for **26**, the characteristic LMCT band was observed around 450 nm in a wide pH range from 2 to 8 (pH 4.0, ε 3000 at 450 nm), suggesting the formation of a 1:1 complex of **27a** with iron. Formation of the complex was also confirmed by the mole ratio plot (Figure 6-10).

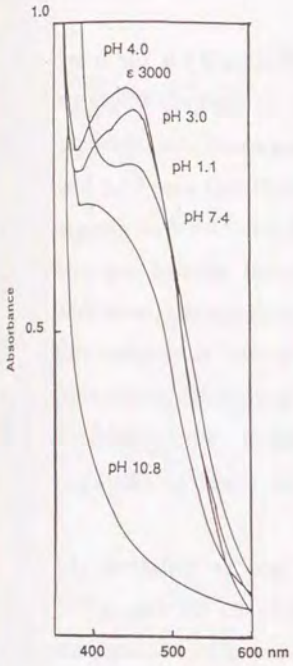


Figure 6-9. Spectral change of the Fe/**27a** in aqueous solution under various pH conditions.

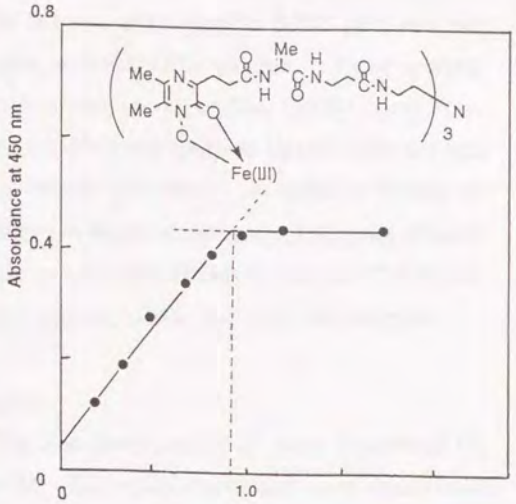


Figure 6-10. Mole ratio plot: A plot of absorbance at 450 nm vs. ratio of iron(III) to **27a** in an aqueous solution at pH 4.0. [**27a**]= 0.15 mM.

(c) Conformation of Gallium complex

In order to obtain an information on the conformation of the complexes in solution, the ^{1}H NMR spectra of diamagnetic Ga(III) complexes of **27** were measured in CD_3OD/D_2O (1:9, pD 6) at room temperature. Two singlets due to the methyl protons apparently shifted to a lower chemical field compared to those of free ligands; $\Delta\delta$ 0.13 for the 5-Me and 0.06 for the 6-Me in a Ga(III)/**27a** mixture, and $\Delta\delta$ 0.13 for the 5-Me and 0.07 for

the 6-Me in a Ga(III)/**27b** mixture. The overlapped signal due to two kinds methylene protons of the -CONH-CH_2- moieties in β-Ala and TREN was separated into two signals; a signal at δ 3.43 ppm into two signals at δ 3.42 and 3.57 ppm in a Ga(III)/**27a** mixture, and a signal at δ 3.42 ppm into two signals at δ 3.45 and 3.60 ppm in Ga(III)/**27b** mixture. These spectral changes indicate the formation of the corresponding Ga(III) complexes. Moreover, the simplicity of the set of signals for these ligands indicates that the complexes have a C_3-symmetric structure. In order to obtain an information concerning the hydrogen bonds in the complexes, more detailed ^{1}H NMR studies on the complexes in an aprotic solvent such as $CDCl_3$ was required. However, the attempt was impossible due to its low solubility.

(d) Stability of iron complex

The stability constants of the iron complexes of **27** were determined by the method similar to that for **26**. The stability constants were determined on the basis of competition with EDTA and three proton dissociation constants of hydroxamic acid groups in a molecule of **27**, which were estimated on the basis of the pKa value of the bidentate ligand **10b** (pKa 4.7)[3] due to experimental limitation. The results are shown in Table 6-4. The stability of Fe/**27b** complex was comparable to that of Fe/**26a** complex. This phenomenon is responsible for almost the same length of the spacer groups in those ligands. The stability constant of Fe/**27a** complex was one order greater than that of Fe/**27b** complex, suggesting that the substituent at α-carbon of the amino acid residue affected the stability of the complex. However, these stability constants were below that of ferrioxamine B.[14]

Table 6-4. Stability Constants with iron(III) complexes of **27**[a]

Ligand (**27**)	Keq[b]	log K
a	0.019	22.5
b	0.167	21.7
DFB[c]	-	30.5

[a] T=20 °C, μ=0.04 with KNO_3 solution, pH 6.0 (Mcvallin's buffer). $C_{Fe(III)}$ 0.07-0.36 mM, C_L 0.07-0.36 mM, C_{EDTA} 0.15-0.20 mM. [b] Keq is the equillibrium constant for the reaction $Fe(\mathbf{27}) + H_2EDTA^{2-} + H^+ \rightleftharpoons FeEDTA^- + \mathbf{27}$. [c] Ref.14

(e) Iron removal from transferrin

The possibility of iron removal by **27** from human diferric transferrin (**Tf**$_{Fe2.0}$) was evaluated at pH 7.4 and 6.0 by the same procedure applied for **26**. The reaction was initiated by mixing a solution of **27** (0.2 mM, 2 mL) with a solution of **Tf**$_{Fe2.0}$ (0.04 mM, 2 mL), and absorbance at 430 nm was monitored. Interestingly, only 5 times excess of ligand concentration was required in contrast to **26**, of which at least 40 times excess of ligand concentration was used. The plots of log $[(A_\infty\text{-Abs})/(A_\infty\text{-}A_0)]$ as a function of time at pH 7.4 gave a straight line as shown in Figure 6-11, indicating that the reaction of Fe(III) removal from transferrin by **27a** proceeded in the pseudo-first-order kinetics. From the slope, k_{obsd} was obtained. The results are summarized in Table 6-5. In the case of **27a**, the rate of Fe(III) removal at pH 6.0 was 15 times faster than that at pH 7.4.

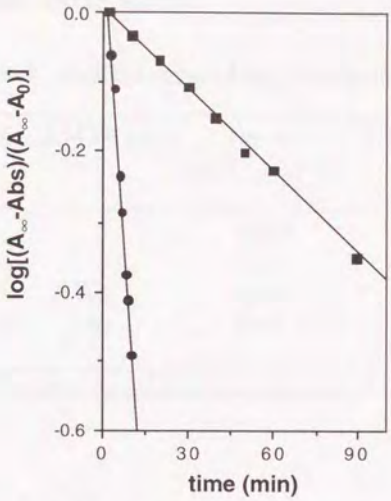


Figure 6-11. The plots of log $[(A_\infty - Abs)]/(A_\infty - A_0)]$ vs time on iron removal of **27a** from $Tf_{Fe2.0}$; pH 6.0 (•), pH 7.4 (♦). The absorbance at 430 nm was taken at 30 sec after mixing **27a** in Tris buffer solution with $Tf_{Fe2.0}$ stock solution. The absorbance at 300 min was used for A_∞.

This observation strongly suggests that the low pKa value of 1-hydroxy-2(1*H*)-pyrazinone is advantageous in order to complete the reaction with transferrin. The presence of bulky substituents in **27b** lowers the pseudo-first-order rate constant to one fourth of that of **27a**. It is noteworthy that **27a** efficiently removes iron from transferrin compared to desferrioxamine B, even when a small excess of the ligand to transferrin ([L]/[**Tf**$_{Fe2.0}$] 5~6) was used and the reaction was performed only for 30 min.

In conclusion, the present ligands, **27**, act as more efficient iron removal agents than conventional desferrioxamine B, and than the other hexadentate ligands, **24**, **25**, and **26**.

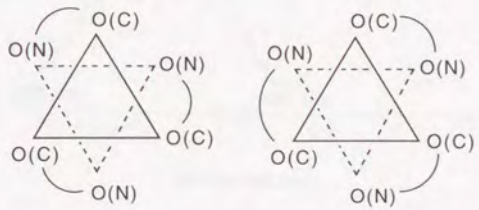
Table 6-5. Fe(III) removal from transferrin at pH 7.4

Ligand (**27**)	[L]/[**Tf**Fe2.0]	k_{obsd} ($\times 10^{-2}$ min^{-1})	% Fe(III) removed[a]
a	5	6.12[b]	
	5	0.38	23
b	6	0.09	9
DFB[c]	100	0.07	5

[a] At a point 30 min after the reaction was initiated. [b] pH 6.0 [c]Ref. 6

(f) Configuration of iron complex

The Λ–*cis* configuration of hydroxamate ligands around the central iron has been proved for ferrichrome by means of X-ray crystallographic and CD analyses both in the solid state and in solution.[18-24]



Λ Δ

Figure 6-12

From molecular model examination of the metal complex of **27**, the *trans* isomer can be precluded due to the steric hindrance, and Δ and Λ configurations are equally possible for the *cis* isomer (Figure 6-12). Therefore, the absolute configuration of the iron complexes of **27** in solution was examined by CD spectroscopy. The spectra of the iron complexes of **27** in water are shown in Figure 6-13.

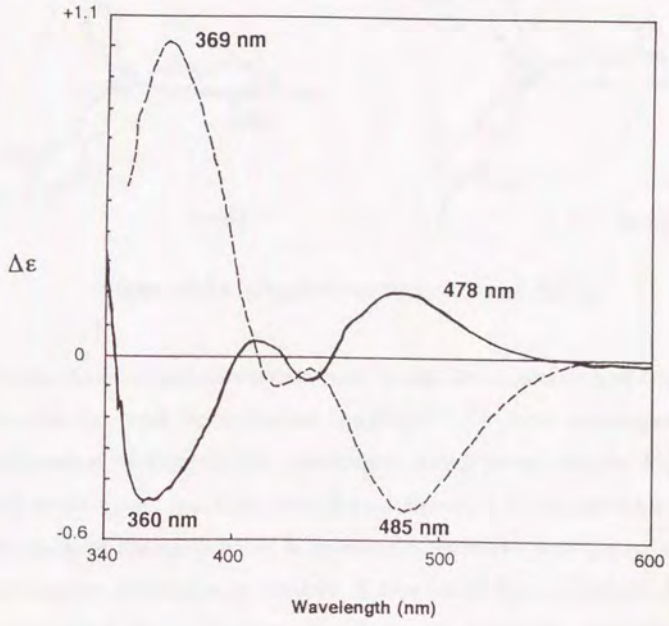


Figure 6-13. CD spectra of iron(III) complex of **27a,b** in aqueous solutions at pH 6.0: [Fe**27a**]=0.3 mM (-); [Fe**27b**]=0.6 mM (--).

The iron complex of **27a** showed a negative band at 360 nm and a positive band at 478 nm. This spectroscopic result indicates that the configuration of the iron complex of **27a** is a Λ–*cis* in an aqueous solution. On the other hand, the iron complex of **27b** showed the opposite CD pattern (λ(nm) 369 and 485), indicating a Δ-*cis* configuration as shown in Figure 6-14.

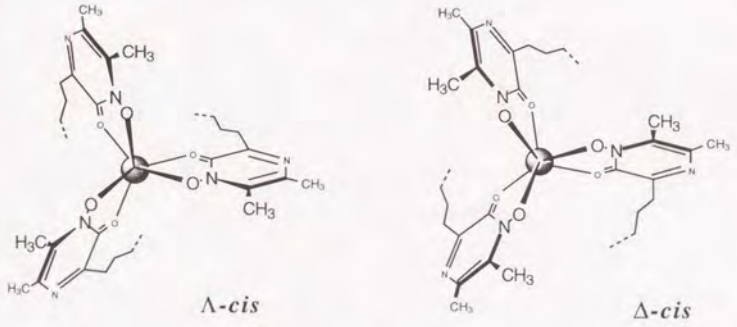


Figure 6-14. Absolute configurations of Fe/**27**.

Recently, Shanzer and co-workers have synthesized L-amino acid-containing enterobactin and ferrichrome analogs[21,22] and investigated the configuration of their Fe(III) complexes; strong intramolecular hydrogen-bonds in the ligand could constrain the conformation of the molecule and the orientation of the side chains to stabilize a particular configuration of the iron complex (enterobactin models: Δ type. ferrichrome models: Λ type.) As mentioned above, no particular hydrogen bond was observed in the ligands. However, it may be possible that a hydrogen bond is formed when the ligand forms a 1:1 complex. It is quite interesting that the difference in

amino acid residue of the ligands causes dramatic change in absolute configuration, although the mechanism remains unclear. The difference of efficiency in iron removal from transferrin between **27a** and **27b** may reflect their absolute configurations.