Ph. D. Thesis (2014)

Design, synthesis and binding study of the amino saccharide derivatives as a ligand for the hairpin RNAs

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Chapter 1 Introduction

1-1. Expression of genetic information and their regulation

DNA has an important role to play in holding the genetic code of the many viruses and all of the cells of living matters. The completion of decoding the sequence of the human genome composed of ”about 3 billion bases” was declared in 2003, and common interest is shifting to the research on the practical use of the genetic code of the human genome recently. Some genetic code may give us the information to cure specific disease**.** Chemical genetics also has been started aiming at developing novel medicine.

The genetic code is stored in the arrangement of DNA that consists of four kinds of nucleobases, adenine, guanine, cytosine and thymine. DNA is not directly translated into protein. At first, the genetic information in the DNA is transcribed into RNA. After an appropriate splicing, a mature RNA is translated into a polypeptide. The scheme of this gene expression called central dogma, is precisely regulated by various mechanisms. Especially, the interaction of the RNA and the protein regulates a lot of function in the cell, such as transcription, RNA splicing, and translation.

Although most intracellular DNAs fold as double helical conformation, the single strand RNA can have a precisely fold tertiary structure like a protein. Since the single strand RNA have also complementary short sequence, RNA is folded up by the base pairing into secondary structure like a hairpin loop, or bulge and an internal loop[1] (Figure 1-1). These special structure parts are often used as a protein-binding site. Design and synthesis of the molecules that recognizes and binds to these specific parts of the RNA might regulate specific function in the cell.[2, 3]

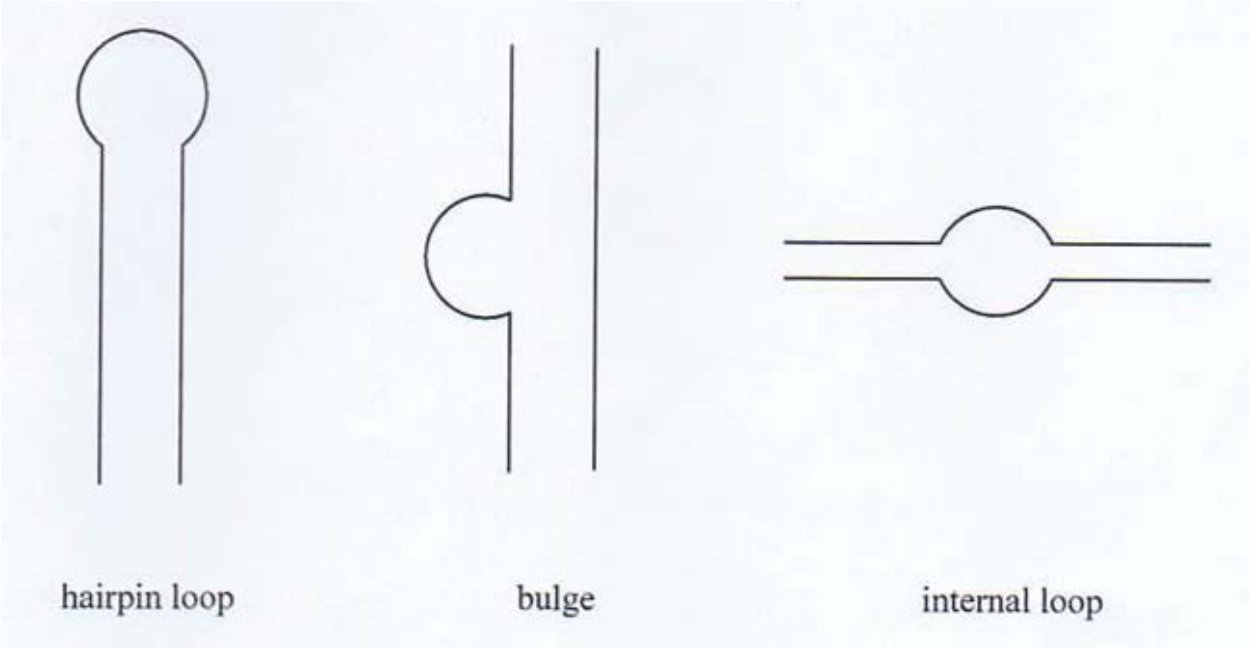

Figure 1-1 The various secondary structure of RNA

The ribozyme which catalyzes, intron splicing for example, the reaction in the living organism in the absence of protein was discovered,[4] and the expression control factor for the response of the metabolic substance in the cell called a riboswitch was found.[5-7] And it was reported by them that expression control of a genetic code is performed by these expression control factors.[4-7] Although artificial gene silencing or restriction used to mostly depended on the antisense technology. Recent discovery, RNAi and riboswitch for example, have been uncovering the other roles of RNA as a gene regulator. However, since genetic code expression was performed through RNA from DNA in fact, in this study, I have determined the binding affinity the synthetic compounds with various RNAs, and discovery of the molecules that recognizes intricate structures of RNA specifically. Discovery of the small molecules which bind to specific RNA might enable to regulate genetic expression, also, that may develop novel gene therapy as well (Figure 1-2).

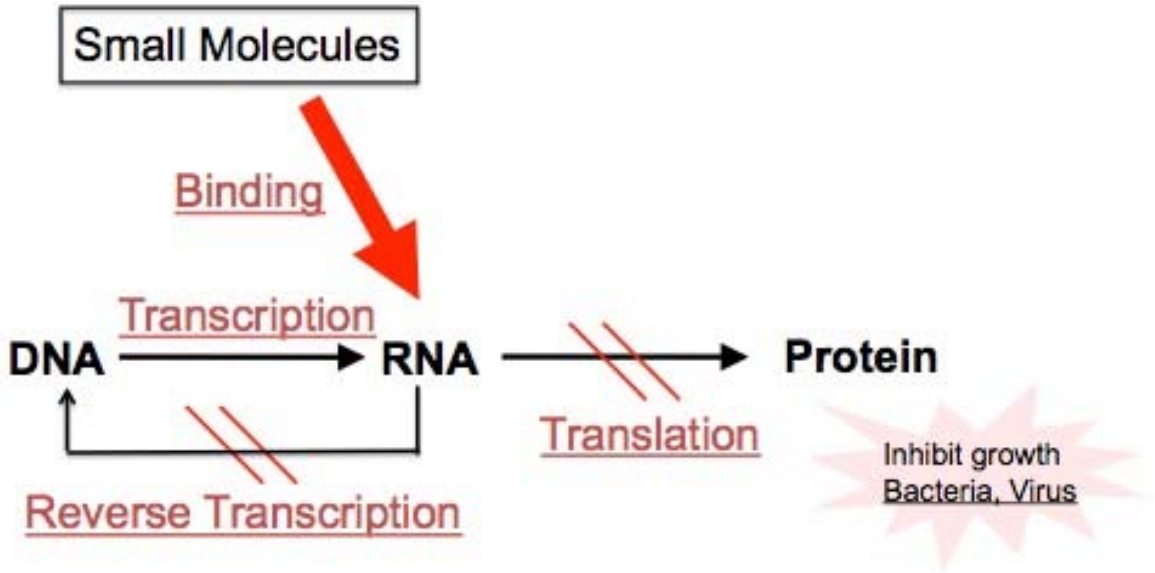

Figure 1-2　Expression of genetic information and their regulation

1-2. Human immunodeficiency virus (HIV) and their RNA structure

HIV (human immunodeficiency virus) is the retrovirus that causes AIDS (acquired immunodeficiency syndrome). HIV is the envelope type of viruses. Its diameter is 110 nm, an artillery shell type core including reverse transcriptase and two RNA genomes made of about 9,500 bases[8] (Figure 1-3).

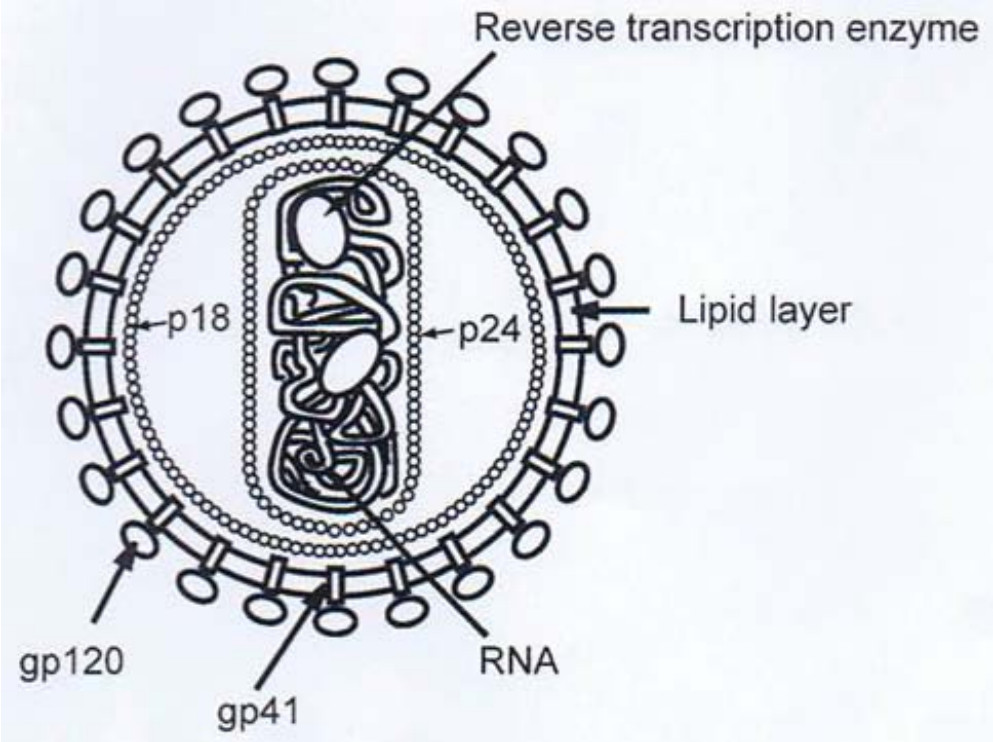

Figure 1-3 Structure of HIV particle

Viral DNA consists of gag (group specific antigen) gene which encodes basic core proteins, pol (polymerase) gene which encodes reverse transcriptase, env (envelope) gene which encodes coat protein, furthermore, LTR (long terminal repeat) which exists in the both ends of 5' and 3’ of a viral RNA, the gene cluster considered to be related to proliferation of the large number of virus containing Tat (trans-activator) and Rev

(regulatory protein of viral expression) [9, 10] (Figure 1-4).

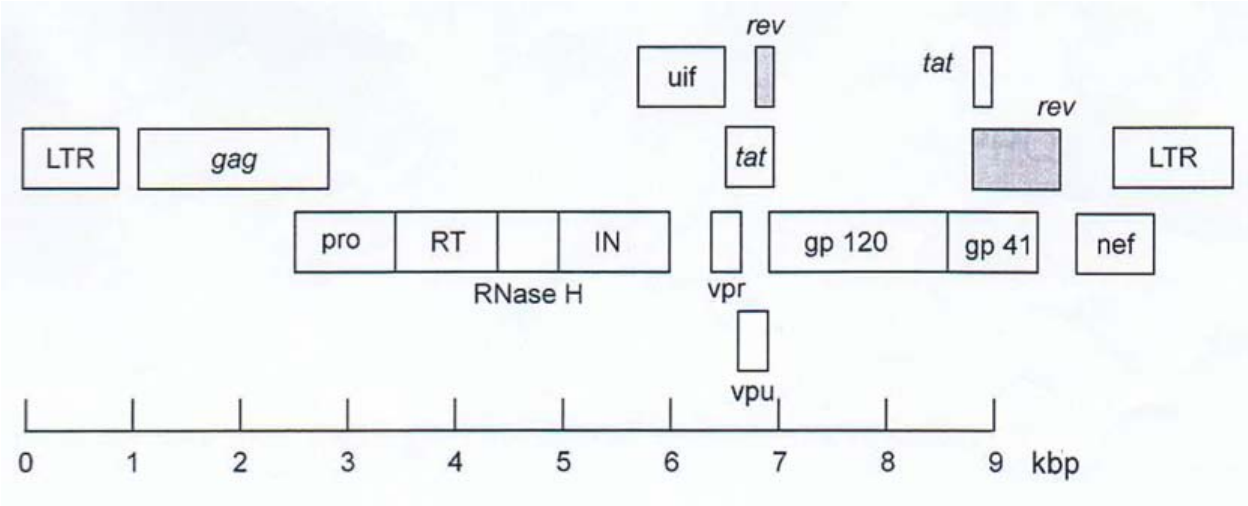

Figure 1-4 Structure of HIV particle

The proliferation mechanism of HIV is roughly classified into the adsorption and fusion for host cells, the reverse transcription of virus RNA, integration to the DNA of the host cell and production of a viral particle. At first, gp120 encoded by the env gene of the virus interact with both the glycoprotein molecule (CD4) which are the main virus receptors in a host cell membrane and chemokine receptor (CXCR4), and enters into a cell (Figure 1-5). After entry into a host cell, reverse transcription is carried out to the DNA by its own reverse transcriptase, and this is integrated into the host DNA by suitable expression of the LTR gene region that has an important role in the process of the transcription initiation and the reverse transcription.[9]

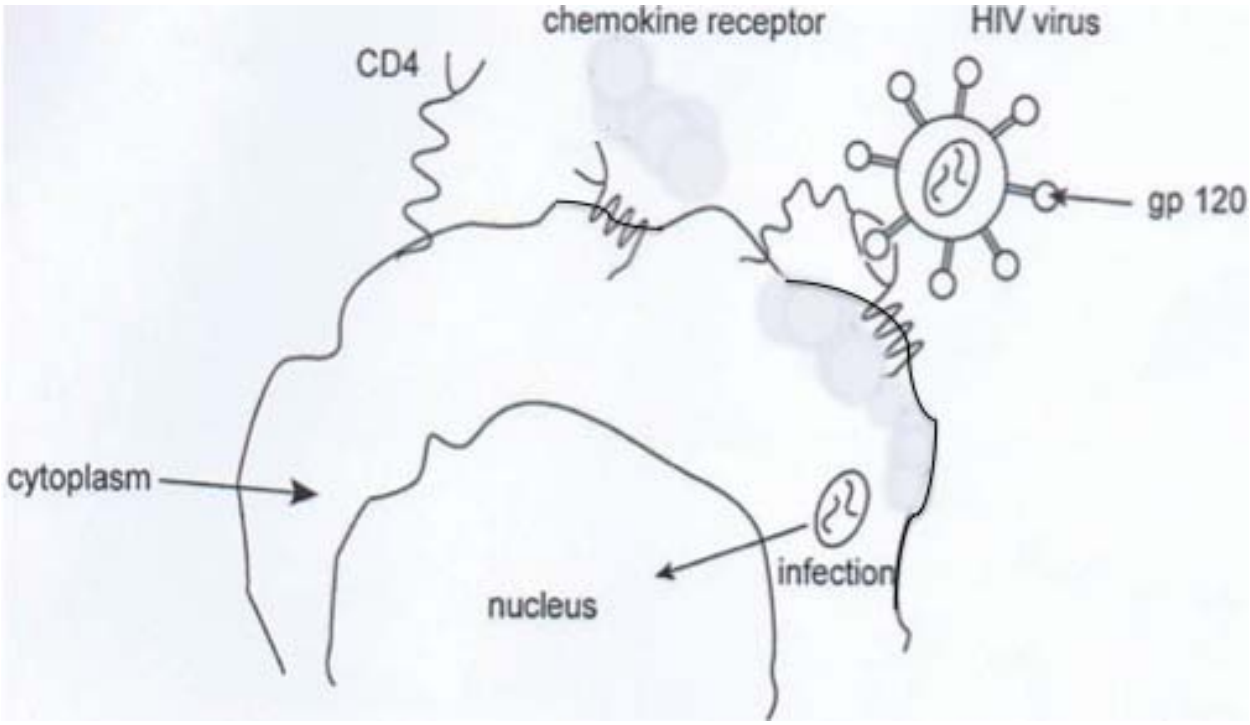

Figure 1-5 Infection of HIV virus

LTR has a TAR (trans-activation response element) region with a special structure, it binds to the Tat protein (composition from 86 residues) specifically which is an activating transcription factor produced by translation of an early stage, and expression of HIV is promoted even hundreds times by this activating transfer.[10-13] The RRE (rev responsive element) which is the base sequence having the higher order structure in the env region of virus RNA recognizes specifically and binds to the Rev (regulatory protein of virion expression) protein which is an activation transcription factor produced by translation of an early stage as well as Tat protein[14-19] (Figure 1-6) (Figure 1-7). In this study, I have pay attention to discover the compound of which bind to TAR RNA or RRE RNA specifically in order to inhibit multiplication of the virus.

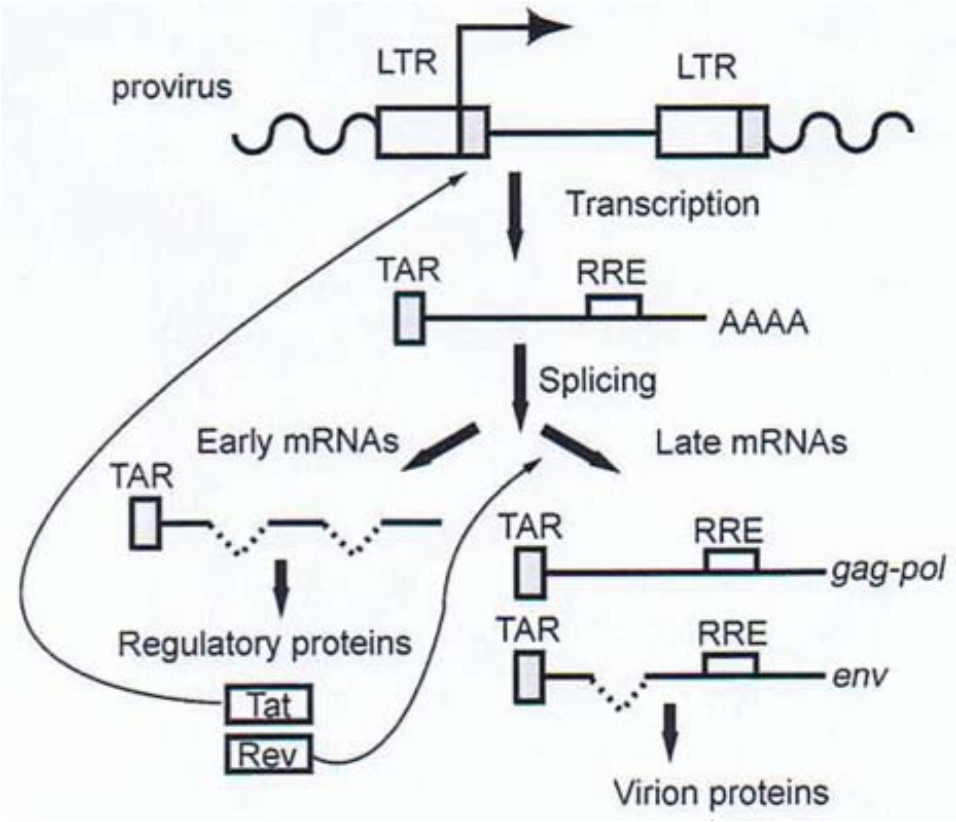

Figure 1-6　Schematic illustration of the regulatory system of transcription (HIV)

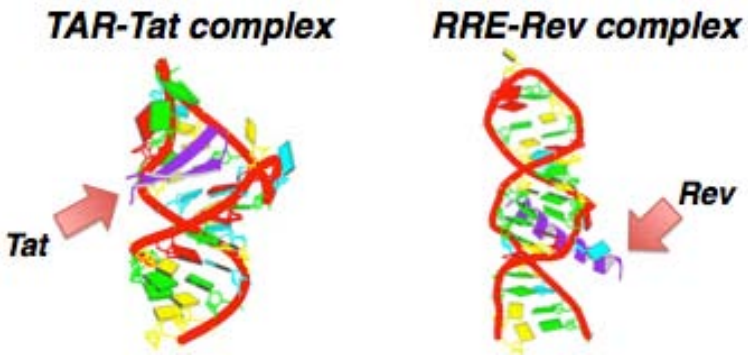

Figure 1-7　TAR-Tat complex and RRE-Rev complex

As I have discussed, it is becoming clear that various mechanisms work, and are adjusted in order to HIV virus multiplication. On the other hand, the study about the treatment approach of AIDS is also advanced by a number of researchers. There are

representative examples of the treatment approach for AIDS. Development of the drug such as (1) the vaccine to kill the virus or the cell infected the virus, (2) the inhibitor which prevented the invasion to the virus and (3) the inhibitor of the reverse transcriptase have been reported. Among these, reverse transcriptase inhibitor AZT has been developed as the first anti-HIV drug in 1987.[20] Furthermore, another reverse transcriptase inhibitor (ddC, ddI) have been also reported.[20] Thus, it is thought that reverse transcriptase inhibitor is the most important method as chemotherapy to an HIV. However, it is a problem that these drugs have strong side effects and the appearance of the resistant virus. In this research, I tried to search the novel reverse transcriptase inhibitor specifically binding to TAR RNA or RRE RNA in order to inhibit this multiplication mechanism.

1-3. Amino sugar derivatives as a binding molecule toward the RNA "Aminoglycoside antibiotics"

As described 1-1 and 1-2, it is expect that the discovery of small molecule that specifically bind to a specific RNA could be the novel type of medicine. Since compounds with low molecular weight, such as an aminoglycoside has an excellence in the permeability across a cell membrane or nuclear membrane compared to ones with high molecular weight, such as a protein. It is expected that the compound with low molecular weight is effective in medical treatment that related to gene expression.

Although design of sequence-specific DNA-binding molecules have been established,[21] due to its intricate structure, that for RNA has not been established. However, a few examples for the design of the RNA-binding molecules have been reported. One of the strategies is employing polycyclic aromatic stacking molecule, such as ethidium or acridine, as a starting material. The other is employing the RNA-binding natural compounds, such as aminoglycoside antibiotics. Aminoglycosides have been studied not only as an antibacterial reagent but also as a scaffold to construct an RNA binding molecule.[22-25]

There are two major aims to construct an RNA-binding molecule starting from the aminoglycoside. One aim is how to enhance the binding affinities of the aminoglycoside toward RNA. The other is how to acquire the binding specificity. Due to positive charge of the aminoglycoside antibiotics, the driving force of the binding interaction depends on electrostatic interaction. That kind of interaction may abolish the binding specificity. For example, neomycin, kanamycin, paromomycin and their derivatives with similar chemical structures (Figure 1-8) can bind to the A-site of 16S rRNA in the 30S subunit of the bacterial ribosome, resulting in interference or blockage of protein biosynthesis in bacterial infections.[26-33] Also, they inhibit the binding of TAR-Tat and RRE-Rev.[34-38] Furthermore, we can easily find that some of the aminoglycosides have neamine unit as

their consensus structure (Figure 1-8). We knew that neamine inhibits RRE-Rev and TAR-Tat interaction with micromolar of dissociation constant.[39]

Other than, aminoglycosides bind to several kinds of RNA fragment with micromolar level of dissociation constants. Nevertheless, it is thought that an appropriate modification to the aminoglycoside may improve not only binding affinity but also binding specificity of the aminoglycoside toward some specific RNA fragment.[40-44]

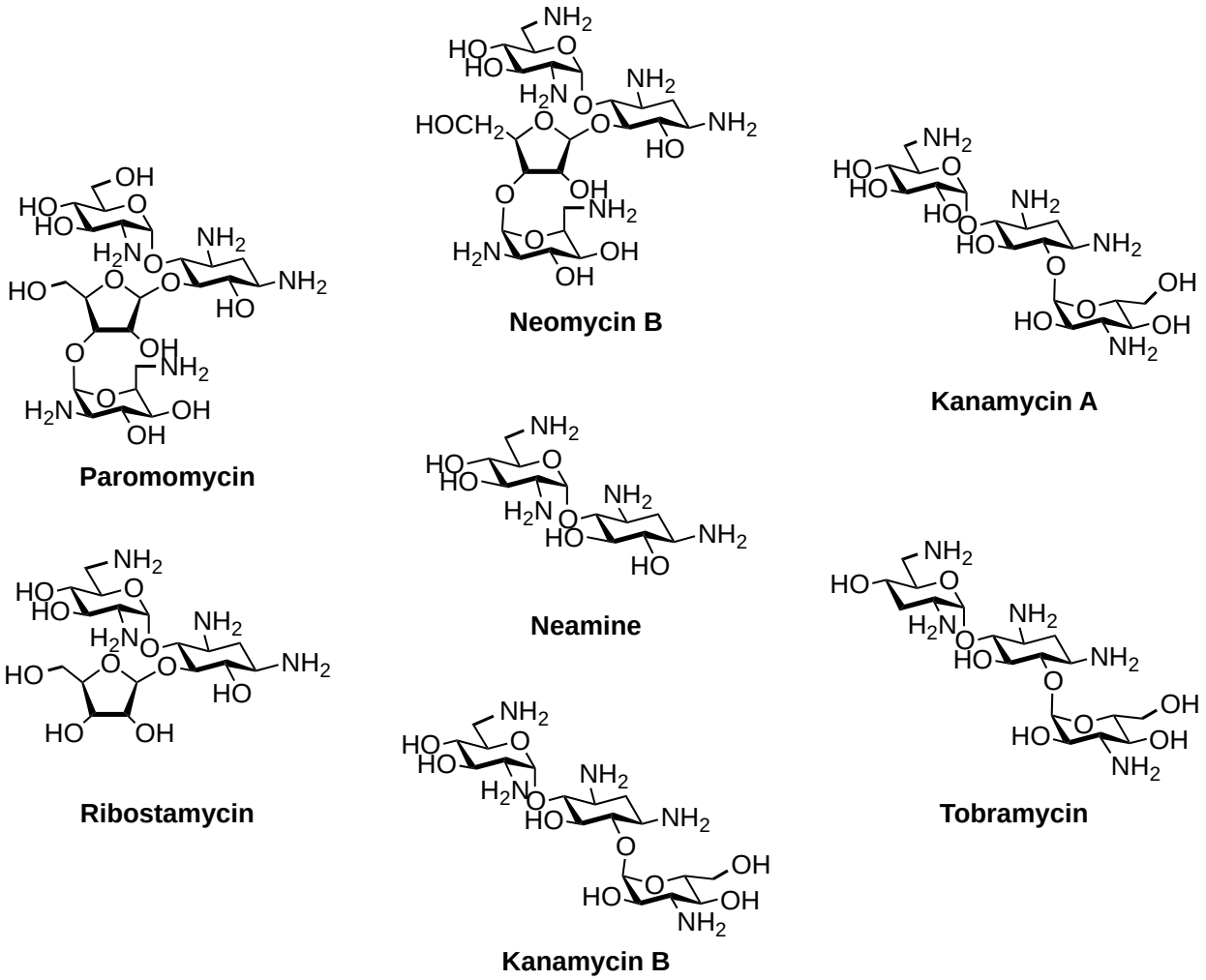

Figure 1-8 Aminoglycoside antibiotics

1-4. The Recent studies for the construction of RNA-binding molecules based on amino saccharide as a scaffold

Aminoglycoside antibiotics bind to several kinds of RNA fragments with micromolar level of dissociation constants. Although aminoglycosides are known as an antibacterial reagent, the existence of the pathogens having aminoglycoside-resistance has triggered the synthetic study of non-natural type of aminoglycoside derivatives.[45-47] One of the recent focuses is directed to the discovery of potentially neamine-like small molecules with synthetic strategy. Due to multiple roles of RNAs, the discovery of small molecules that bind to the RNA specifically may provide novel RNA-targeted drugs. There are several reports which showed appropriate modifications[48-50] (Figure 1-9) or dimerization[51-52] (Figure 1-10) of neamine enhances its binding toward RNA. Under these circumstances, I have been attempted neamine as minimum skeleton that bind to RNA, and employed as a scaffold to construct the novel RNA-binding reagent. In addition, several groups also have reported the syntheses of small molecules by mimicking neamine using carbohydrate[53] (Figure 1-11) However, most of these small molecules reported so far have indicated only a modestly enhanced and selectivity for the RNA.

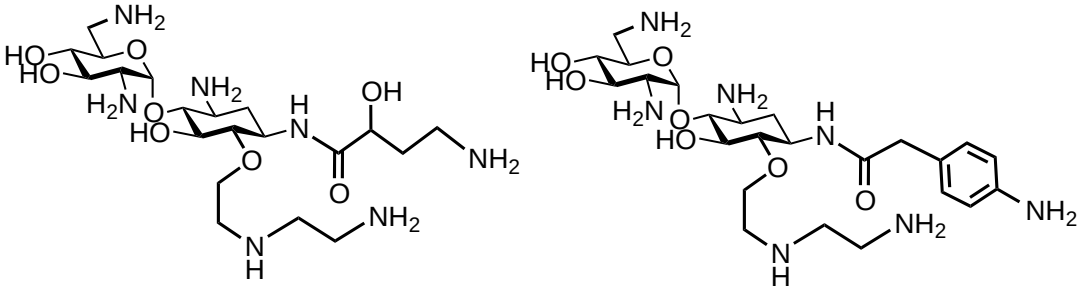

Figure 1-9 The designed the modified neamine

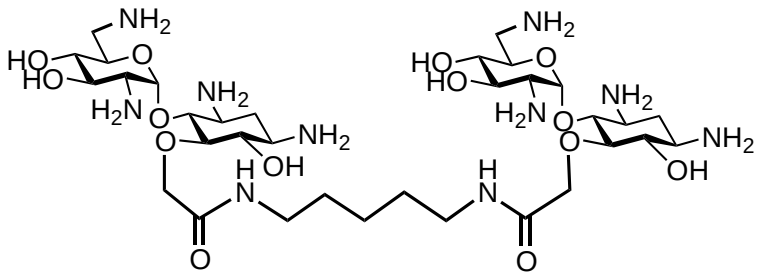

Figure 1-10　An example of designed neamine

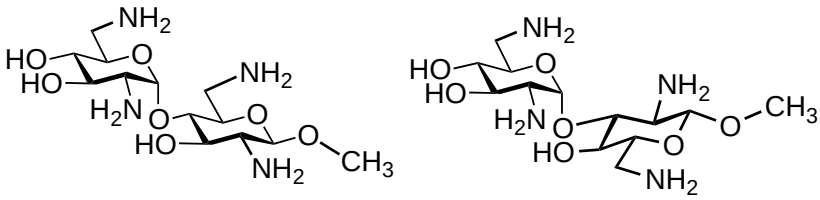

Figure 1-11　The designed neamine mimics

1-5. Strategies of this work

In this study, I have chosen two RNAs derived from HIV-1 as the targets and designed the compound so that specifically bind to the RNAs in order to inhibit the binding of the corresponding viral proteins.

As described in 1-3 and 1-4, a number of research groups have been attempted to employ neamine as a minimum skeleton that binds to RNA and as a scaffold to construct an RNA-binding reagent. However, most of the small molecules reported so far have indicated only a modest affinity and selectivity for the target RNA. Then I have designed non-reducing amino disaccharides (trehalosamine) and aromatic group-modified amino saccharide (aromatic aminoglycoside) as novel neamine mimics (Figure 1-12 Study 1), and nucleobase modified neamines with a lysine as a linker (NbKε-neamine) as novel modified neamines (Figure 1-12 Study 2).

The compounds that have the structure of non-reducing disaccharides have a number of excellent functions and they have played the important role in the life. In this study, I have attempt to the non-reducing disaccharide as a candidate of a novel scaffold that displaces neamine.

Aromatic aminoglycoside has an aromatic aglycone structure which replaces the 2-deoxystreptamine unit of neamine with 2,4-diaminophenol, and the aromatic group is expected to increase the RNA-binding affinities by π-π stacking effects.

Most of aminoglycosides which is capable of binds to the A-site of 16S rRNA with modest binding affinity have 6'-amino group.[54] However modified neamine at 6'-amino group bound to either TAR or RRE RNA.[55-57] Therefore, I have decided to focuse on the modification at the 6'-amino group of neamine. When the nucleobase was introduced at α-amino group of lysine or an arginine as a linker the binding affinity was not depend on the nucleobase but depend on the amino acid as a linker.[1] An appropriate distance between the nucleobase and the neamine might clarify the binding specificity derived

from the difference of nucelobase.

I have chosen TAR and RRE RNA as the targets and designed trehalosamine aromatic aminoglycoside and NbKε-neamine. Then, I evaluate inhibition for TAR-Tat and RRE-Rev.

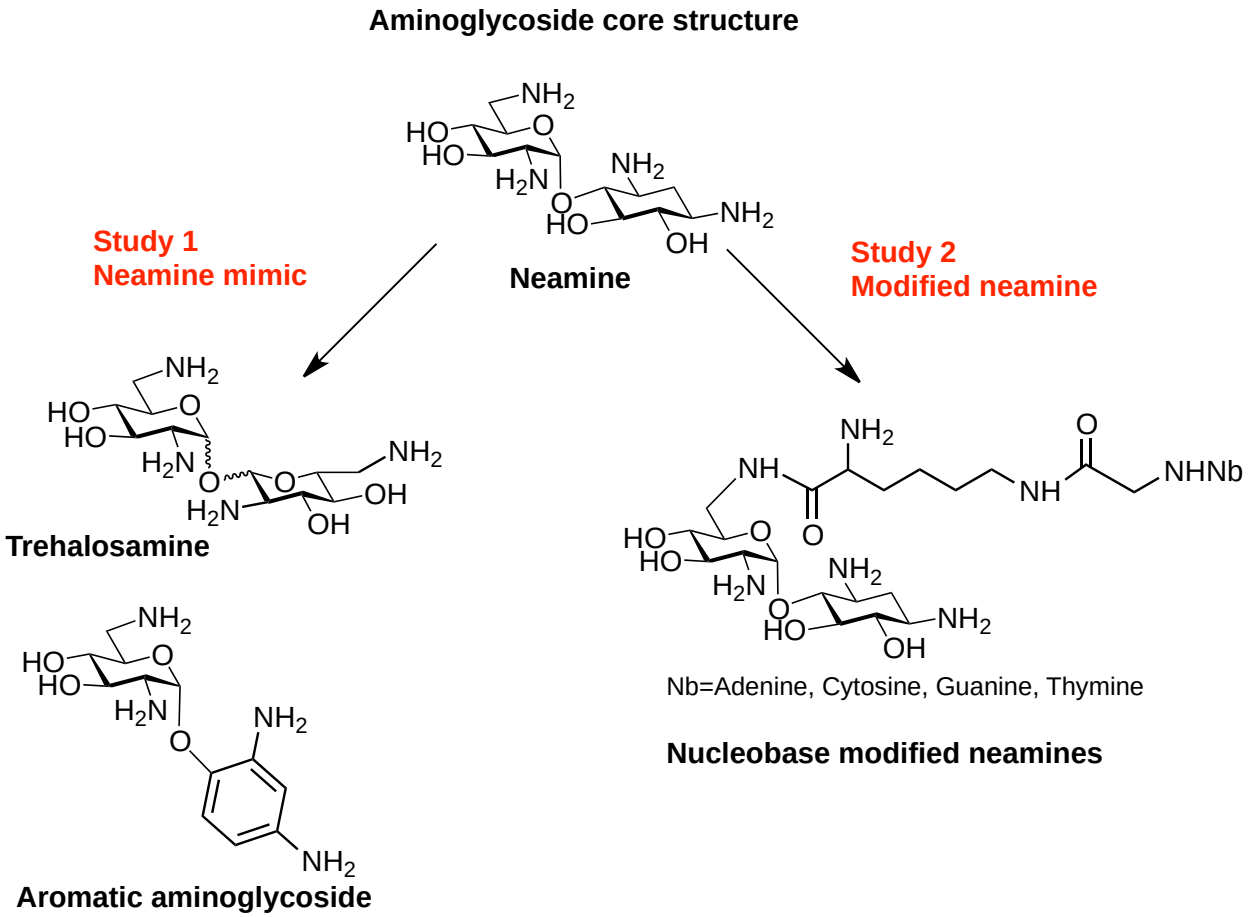

Figure 1-12　Designed novel amino saccharides (neamine derivatives)

In chapter 2, I describe the development of the useful glycosylation method to construction of non-reducing disaccharide. In chapter 2-2-1, I examined the condensation method of 1-hydroxy sugars with several alcohols in the presence of $Bi(OTf)_3$. In chapter 2-2-2, I describe the synthesis of the various non-reducing

disaccharide compositions by the efficient dehydrative glycosylation reaction which used $Bi(OTf)_3$. In chapter 2-2-2-1, I examined the optimal conditions for carrying out the self-condensation of these, using glucose, mannose, galactose, 2-*N_3*-glucose, 2-*N_3*-6-*OAc*-glucose and arabinose as 1-hydroxy sugars. In chapter 2-2-2-2, I describe about the stereospecificity of non-reducing disaccharide formation.

In chapter 3, I describe about the synthetic approach to trehalosamine and aromatic aminoglycoside as the neamine mimics. In chapter 3–2–1, I describe about the synthesis of novel non-reducing amino disaccharides (trehalosamine). In chapter 3–2–2, I describe about the synthesis of aromatic modified amino saccharide (aromatic aminoglycoside).

In chapter 4, I describe the synthetic approach to nucleobase modified neamines with a lysine as a linker, nucleobase substituted on the ε-amino group of the amino acid (NbKε-namines) as the novel small compound expected to bind to the hairpin RNA specifically.

In chapter 5, I describe the evaluation of the “Trehalosamine” and “NbKε-neamine” as inhibitors of TAR-Tat and RRE-Rev. In chapter 5–2–1, I described the evaluation of the “Trehalosamine” and “NbKε-neamine” as inhibitors of TAR-Tat. In chapter 5–2–2, I describe the evaluation of the “NbKε-neamine” as inhibitors of RRE-Rev.

In chapter 6, finally I describe about the summary of this research.

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Chapter 2 The development of novel dehydrative glycosidation to modify the neamine scaffold

2-1. Introduction

In this chapter, I focused on the structure of non-reducing disaccharides that would be novel neamine analogs, and describe the development of the useful glycosylation method to construction of non-reducing disaccharides.

2-1-1. Structure of non-reducing disaccharides and their functions

The disaccharides of which condensed two mono saccharides at the anomeric hydroxyl groups are called non-reducing disaccharides. The trehalose composed of two glucose molecules is a typical example (Fig. 2-1).

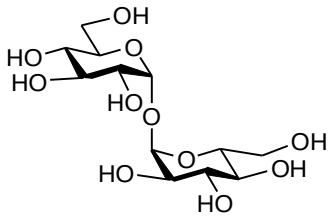


Figure 2-1 Structure of non-reducing disaccharides (trehalose)

Trehalose is broadly used in the fields of medical treatment, foods, and cosmetics, and its functions attract attention. Trehalose have three kinds of structural isomers, αα-conformation (trehalose), αβ-conformation (neotrehalose) and ββ-confor –mation (isotrehalose). Most naturally-occurring trehalose is αα-conformation. αα-Trehalose is metabolized into glucose by trehalase that is the catabolic enzyme. And trehalase is distributed over the internal organs of many organism species, such as a small intestine and the kidney.

Trehalose is widely distributed over insects, mushrooms, shrimps, mold, yeast, red alga and the lichen. Especially in case of the plants, trehalose not only exists as the main saccharide, but it has an effect as an antifreeze agent, it is known that they have cold resistance. Furthermore, trehalose is used in various fields, such as cosmetics as a moisturizer, improving keeping of food and maintaining the freshness of vegetables or fruits. In this way, it is clear that trehalose have very attractive behaviors, such as the stabilization of the protein, the cytoprotective action from freeze and dryness and an effect as the moisturizer.[1,2] Additionally, the preventive medicine of neurodegenerating diseases including Alzheimer's disease and a possibility that osteoporosis could be controlled were suggested to trehalose recently, and the derivatives of treharose as medical supplies are increasing.[3,4]

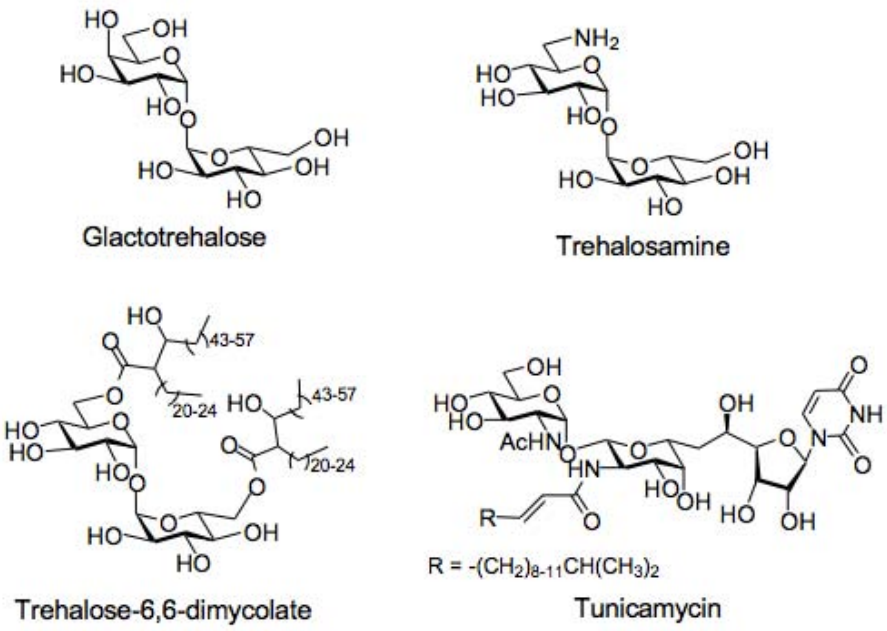


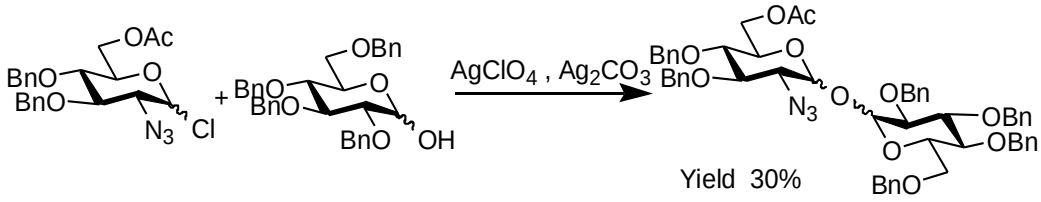
Figure 2-2 Naturally-occurring non-reducing disaccharides

It has been reported that the natural products having the non-reducing disaccharides unit have antibacterial and antiviral activity. Galactotrehalose is an analog of trehalose formed by replacing one of the glucose units by galactose. It is reported that galactotrehalose works as a ligand of the Shiga toxin produced by the Escherichia coli.[5] Trehalosamine is the other analog of trehalose in which the hydroxyl group at C'6-position is replaced to the amino group, and it is known that it is active against mycobacterium tuberculosis.[6] Trehalose-6,6'-dimycolate has a trehalose as its skeleton to which the 6-hydroxyl groups are modified with lipids, it has antitumor activity and antimycobacterium tuberculosis activity.[7] Tunicamycin which consists of *N*-acetylglucosamine and *N*-acylglactosamine derivative, has been reported as an antibiotic having the *N*-glycosylation inhibition (Fig. 2-2).[8,9]

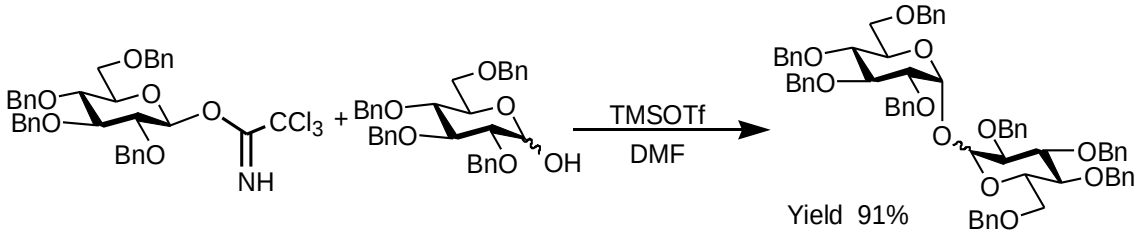
Thus, the compounds that have non-reducing disaccharides structure have a number of useful functions and they play the important role for the life. In this study, I paid attention to this non-reducing disaccharide as a candidate of another scaffold which might replaceable with neamine. I describe in this chapter the development of the convenient synthetic route for the non-reducing disaccharides.

2-1-2. Conventional method of the glycosylation for the synthesis of non-reducing disaccharides

Some synthetic methods of the non-reducing disaccharide have already reported.[10,11] However, a classical Koenig-Knorr method using Br or Cl as leaving group has only reported.[10] Recently, it is reported that the yield was improved when trichloroacetimidate was used as a leaving group.[11] However this method has serious problem. This method requires excessive amount of the activating reagent for leading intermediate such as glycosyl haride or glycosyl imidate before glycosylation reaction Since the glycosylation reaction is depends on the combination of the donor and the acceptor of the monosaccharide, the yield is relatively low (Scheme 2-1, 2-2). Although biochemical feasibility of non-reducing disaccharide was not clarified until recent years, the development of synthetic methodology had not well established.

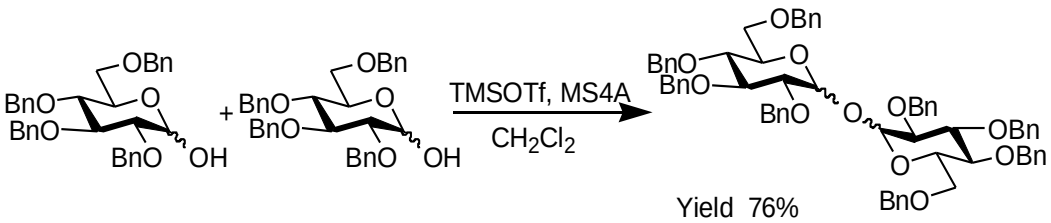


Scheme 2-1 Koenig-Knorr method



Scheme 2-2 Imidate method

Also the synthetic technique of non-reducing disaccharides without introducing a leaving group by dehydrative glycosidation of the two aldose sugars which have the anomer hydroxyl group (1-hydroxy sugar) was reported in recent years. Most of the synthetic methods of non-reducing disaccharide consist of two steps for introducing an appropriate leaving group, it is thought that dehydrative glycosylation without introducing a leaving group is the most convenient method in the synthesis of the symmetric non-reducing disaccharides that built from the same sugar. Furthermore, since the stability of 1-hydroxy sugar is high, it is an advantage for manufacture of a monosaccharide as a donor to be also easy. However, there are few examples that report the synthesis of non-reducing disaccharide using the dehydrative glycosylation. Due to the difficulty of the activation of the hydroxyl group at anomeric carbon is not easy. It has been reported the example using strong acid as catalysts, such as TMSOTf or Tf_2O in surplus (Scheme 2-3).[12,13] Therefore, if the reactivity of various 1-hydroxy sugars in dehydrative glycosylation can be raised, it might be applicable to the synthesis of the non-reducing disaccharide.



Scheme 2-3 The dehydrative glycosylation using powerful acid catalysts

As mentioned above, it is worth study the development of the way of the efficient dehydrative glycosylation which activates 1-hydroxy sugar directly, and to make it apply to non-reducing disaccharide.

2-1-3. Conventional dehydrative glycosylation methods

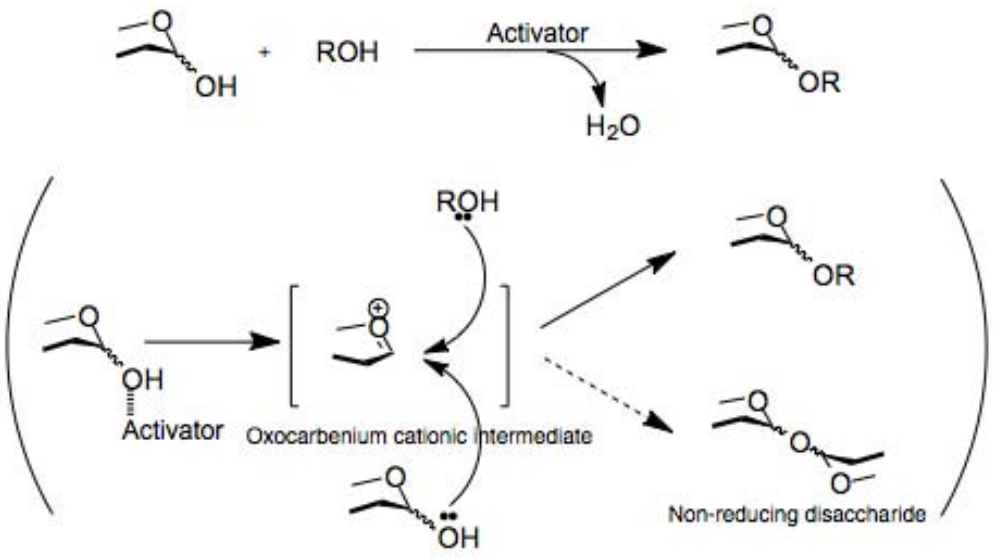
As described in Chapter 2-1-2, in order to attain effective synthesis of various non-reducing disaccharide, the development of the novel method for dehydrative glycosylation that directly activates 1-hydroxy sugar is required.

A glycosylation reaction transforms the donor of the sugar which has a suitable functional group for the anomeric position of the saccharide into an oxocarbenium cationic intermediate by an activator, and makes glycoside form, when the alcohol which is an acceptor saccharide attacks this. It is known that most of the glycosylation reaction is considered to proceed via S_N1 reaction, and the reactivity greatly depend on the combination of a leaving group, the activator, the solvent, and the protecting group. Especially the combination of a leaving group and an activator is very critical.

When 1-hydroxy sugar was used for a sugar donor, the ability of leaving-group of an anomeric hydroxyl group is low, and transformation to an oxocarbenium cationic intermediate is not easy. However, recruitment of an appropriate activating reagent might solve this problem. The reagent activates an anomeric hydroxyl group effectively and turn it as leaving group.

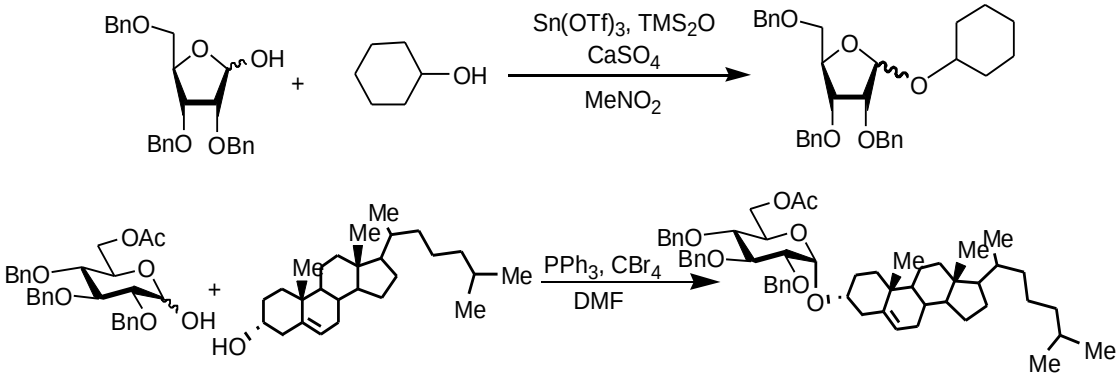
Moreover, the dehydrative glycosylation reaction using 1-hydroxy sugar is considered as the most efficient synthetic method of non-reducing disaccharide because 1-hydroxy sugar works also as a sugar receptor.

On the other hand, the condensation reaction of a 1-hydroxy sugar and common alcohols, it is necessary to avoid condensation reaction of the 1-hydroxy monosaccharide by itself (Scheme 2-4).



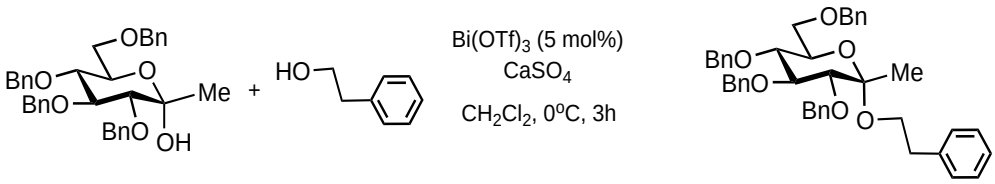
Scheme 2-4　The dehydrative glycosylation reaction

Recently, several groups have reported the dehydrative glycosylation using the tetramethylsilylation[14] and bromination[15,16] of an anomeric hydroxyl group during the glycosylation reaction. However, more convenient method for the synthesis of non-reducing disaccharide is demanded (Scheme 2-5).



Scheme 2-5　Conventional dehydrative glycosylation reactions

Our previous work showed that the *O*-ketopyranosidation[17] via the dehydrative condensation of artificial ketopyranoses, i.e., 1-*C*-alkylated sugar derivatives, were obtained successfully when catalytic bismuth(III) triflate ($Bi(OTf)_3$) as the activator was used (Scheme 2-6).[18]

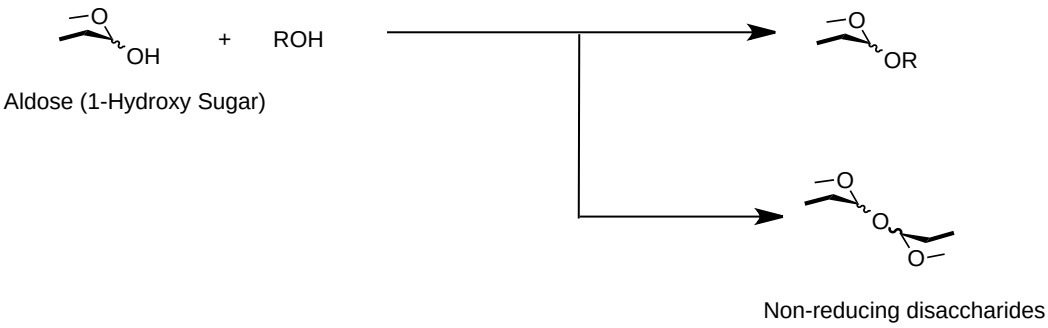


Scheme 2-6 Dehydrative glycosylation using 1-*C*-alkylated sugar derivatives

This finding made me try to synthesize dehydrative *O*-aldosylation by dehydrative glycosidation using $Bi(OTf)_3$ as an activator.[19] Then I have studied the direct synthesis of disaccharide with various *O*-glycosidic linkages start from 1-hydroxy sugars.

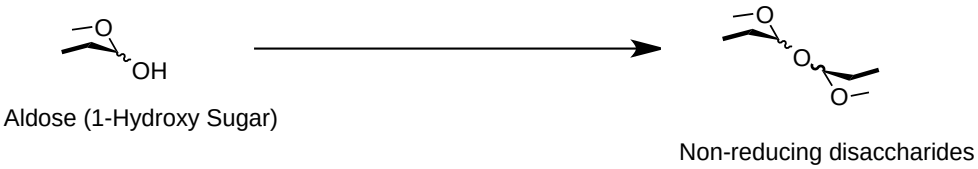
2-1-4. Strategies to establish novel ways to the synthesis of non-reducing disaccharides

As an aim to establish novel method for the synthesis of non-reducing disaccharides, we focused on $Bi(OTf)_3$ as one of the useful activator in the dehydrative glycosylation. At first chapter 2-2-1, I developed the method in condensation for 1-hydroxy sugars with several alcohols. I have studied in detail about the conditions giving non-reducing disaccharides was obtained as a by-product while clarifying the reaction characteristic of 1-hydroxy sugars (Scheme 2-7).



Scheme 2-7 Dehydrative glycosylation using Aldose(1-Hydroxy sugar)

I have studied the synthesis of the various non-reducing disaccharides by the efficient dehydrative glycosylation reaction which used $Bi(OTf)_3$ (Chapter 2-2-2-1). Then I had optimized the conditions for carrying out the self-condensation of 1-hydroxy sugars, glucose, mannose, galactose, 2-N_3-glucose, 2-N_3-6-*OAc*-glucose and arabinose (Scheme 2-8). Finally, we have examined the stereoselectivity of non-reducing disaccharides composition (Chapter 2-2-2-2).



Aldose (1-Hydroxy Sugar)

Non-reducing disaccharides

Scheme 2-8　The synthetic strategy of non-reducing disaccharides

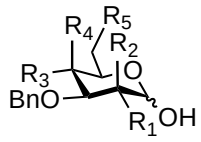
2-2. Results and Discussion

2-2-1. The development of novel dehydrative glycosidation

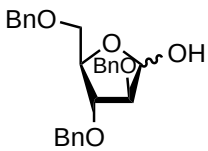
"The various reactions for 1-hydroxy sugars and alcohols"

In this chapter, we developed the condensation reaction of 1-hydroxy sugars with several alcohols in the presence of $Bi(OTf)_3$. Also I investigated in detail about the conditions that gave non-reducing disaccharides as by-products while clarifying the reaction characteristic of 1-hydroxy sugars.[20,21]

The 1-hydroxy sugars and alcohols as glycosyl acceptors used in this study are shown in Figure 2-3 and 2-4. The typical *O*-glycosides and 1,1'-disaccharides produced are shown in Figure 2-5 and 2-6.

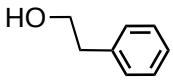


1: R_1=OBn, R_2=H, R_3=OBn, R_4=H, R_5= OBn
2: R_1=H, R_2=OBn, R_3=OBn, R_4=H, R_5= OBn
3: R_1=OBn, R_2=H, R_3=H, R_4=OBn, R_5= OBn
4: R_1=N_3, R_2=H, R_3=OBn, R_4=H, R_5= OBn
5: R_1=N_3, R_2=H, R_3=OBn, R_4=H, R_5= OAc



6

Figure 2-3 1-hydroxy sugars used



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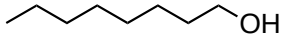

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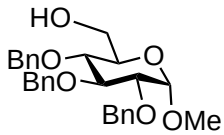

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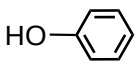

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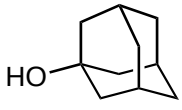

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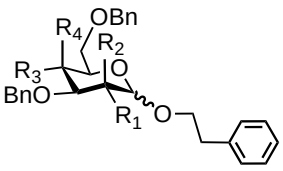
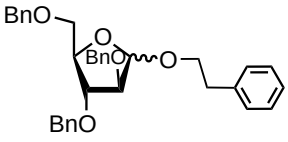
Figure 2-4 Alcohols used



12: R_1=OBn, R_2=H, R_3=OBn, R_4=H
13: R_1=H, R_2=OBn, R_3=OBn, R_4=H
14: R_1=OBn, R_2=H, R_3=H, R_4=OBn
15: R_1=N_3, R_2=H, R_3=OBn, R_4=H



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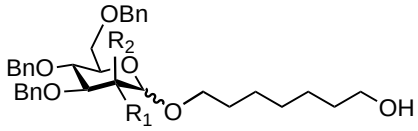
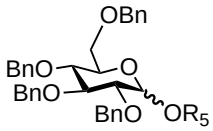
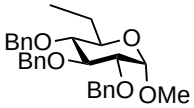
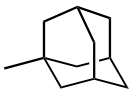


17: R_1=OBn, R_2=H
18: R_1=H, R_2=OBn



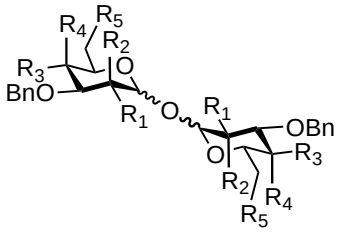
19: R_5= 

20: R_5= 

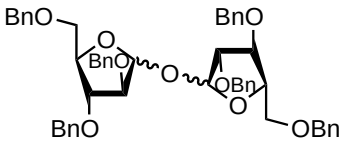
21: R_5= 

22: R_5= Bn

Figure 2-5 Typical *O*-glycosides produced



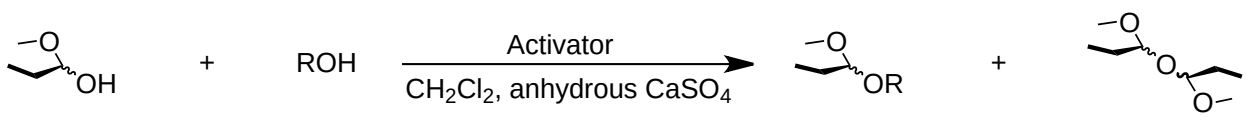
23: R_1=OBn, R_2=H, R_3=OBn, R_4=H, R_5= OBn
24: R_1=H, R_2=OBn, R_3=OBn, R_4=H, R_5= OBn
25: R_1=OBn, R_2=H, R_3=H, R_4=OBn, R_5= OBn
26: R_1=N_3, R_2=H, R_3=OBn, R_4=H, R_5= OBn
27: R_1=N_3, R_2=H, R_3=OBn, R_4=H, R_5= OAc



28

Figure 2-6 1-1'-Disaccharides produced

I first investigated the glycosidation of 2,3,4,6-tetra-*O*-benzyl-**D**-glucopyranose (**1**) with an equimolar amount of 2-phenylethyl alcohol (**7**). The amount of $Bi(OTf)_3$ was varied in CH_2Cl_2 for 2 h at room temperature in the presence of anhydrous calcium sulfate ($CaSO_4$) as a drying reagent. These reaction conditions were similar to those previously reported.[22] The reaction using 5 mol% $Bi(OTf)_3$ afforded the corresponding glycoside (**12**) in only 35% yield. The use of 10 mol% $Bi(OTf)_3$ effectively promoted the glycosidation to produce **12** in a respectable yield of 85% with an α/β ratio of 71/29. It is noteworthy that under these reaction conditions the production of 1,1'-disaccharide (**23**) as a by-product decreased only to 7% as a yield. The use of 20 mol% $Bi(OTf)_3$ slightly reduced the yield of **12** to 68% with an increase in the yield of **23** as a by-product to 13%. When the reaction temperature was raised to reflux, the glycosidation using only 5 mol% of $Bi(OTf)_3$ in CH_2Cl_2 for 15 min easily proceeded to give the product **12** in a good yield of 87% (α/β = 69/31) with the formation of **23** as a by-product in only 9% yield. A similar reaction using a mixed CH_2Cl_2/CH_3CN (v/v = 1/2) solvent at reflux temperature gave **12** in 72% yield (α/β = 34/66). The effect of other metal triflates was examined. Similar reaction conditions employing 5 mol% scandium(III) triflate ($Sc(OTf)_3$) and ytterbium(III) triflate ($Yb(OTf)_3$) afforded **12** in 82% yield (α/β = 65/35) and 14% yield (α/β = 44/56), respectively. $Sc(OTf)_3$ was fairly effective in dehydrative glycosidation. Thus, a reaction using only 5 mol% $Bi(OTf)_3$ at reflux temperature in CH_2Cl_2 for 15 min effectively promoted the glycosidation between **1** and **7** (1 equivalent) to produce the desired **12** with only a slight production of **23** (Scheme 2-9, Table 2-1; Entry **1-7**).



1-4,6 **7-11** **12-21** **23-26,28**

Scheme 2-9 The formation of *O*-glycosides by the reaction of 1-4,6 with 7-11

Table 2-1 The formation of *O*-glycosides by the reaction of 1-4,6 with 7-11

Entry[a]	1-Hydroxy sugar	Alcohol	Activator (mol%)	Glycoside		1,1'-Disaccharide	
				Product	Yield / % (α / β)[b]	Product	Yield / %
1[c]	**1**	**7**	$Bi(OTf)_3$ (5)	**12**	35 (57 / 43)	**23**	4
2[c]	**1**	**7**	$Bi(OTf)_3$ (10)	**12**	85 (71 / 29)	**23**	7
3[d]	**1**	**7**	$Bi(OTf)_3$ (20)	**12**	68 (57 / 43)	**23**	13
4	**1**	**7**	$Bi(OTf)_3$ (5)	**12**	87 (69 / 31)	**23**	9
5[e]	**1**	**7**	$Bi(OTf)_3$ (5)	**12**	72 (34 / 66)	**23**	17
6	**1**	**7**	$Sc(OTf)_3$ (5)	**12**	82 (65 / 35)	**23**	11
7[f]	**1**	**7**	$Yb(OTf)_3$ (5)	**12**	14 (44 / 56)	**23**	4
8	**1**	**8**	$Bi(OTf)_3$ (5)	**17**	85 (62 / 38)	**23**	9
9	**1**	**9**	$Bi(OTf)_3$ (5)	**19**	52 (75 / 25)	**23**	11
10[g]	**1**	**10**	$Bi(OTf)_3$ (5)	**20**	40 (80 / 20)	**23**	21
11	**1**	**11**	$Bi(OTf)_3$ (5)	**21**	58 (65 / 35)	**23**	17
12	**2**	**7**	$Bi(OTf)_3$ (5)	**13**	73 (100 / 0)	**24**	16
13	**2**	**8**	$Bi(OTf)_3$ (5)	**18**	81 (91 / 9)	**24**	16
14	**3**	**7**	$Bi(OTf)_3$ (5)	**14**	70 (77 / 23)	**25**	10
15	**4**	**7**	$Bi(OTf)_3$ (5)	**15**	51 (52 / 48)	**26**	15
16	**6**	**7**	$Bi(OTf)_3$ (5)	**16**	81 (85 / 15)	**28**	5

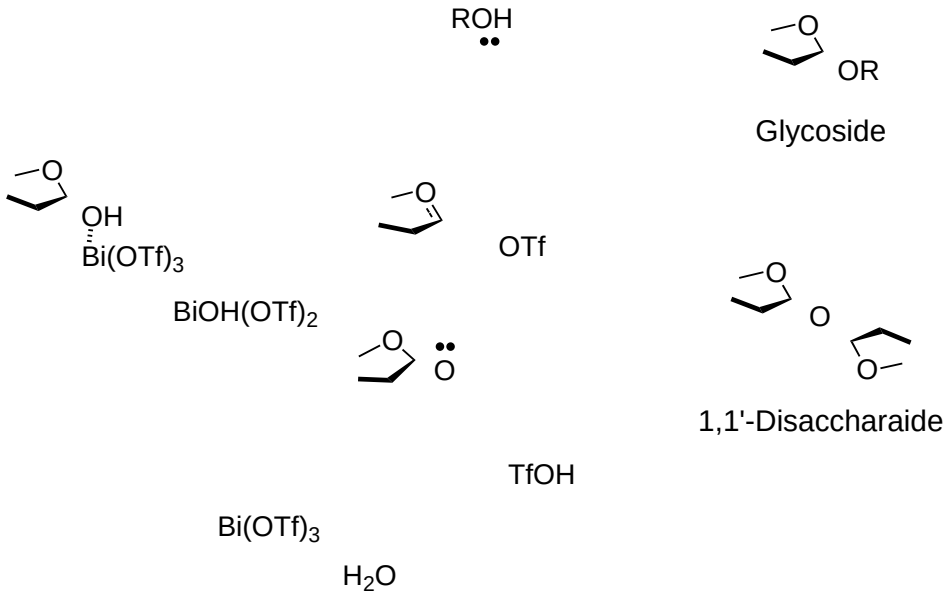
[a]Reaction conditions: molar ratio of donor : acceptor = 1 : 1; solvent = CH_2Cl_2; reflux temperature; reaction time = 15 min.
[b]All the α/β ratios were determined by NMR. [c]Reaction conditions: room temperature; reaction time = 12 h.
[d]Reaction conditions: room temperature; reaction time = 2 h. [e]The mixed solvent (CH_2Cl_2/CH_3CN = 1/2) was used.
[f]Reaction time = 40 min. [g]Compound **22** was obtained in 31% yield as a byproduct.

Next, I examined the glycosidation of **1** with various alcohols (1 equivalent) using 5 mol% $Bi(OTf)_3$ at reflux temperature in CH_2Cl_2 for 15 min. A reaction using *n*-octanol (**8**) produced the glycoside **17** in a good yield of 85% (α/β = 62/38) with the production of **23** in only 9% yield. The glycosylation of **1** to methyl 2,3,4-tri-*O*-benzyl-α-**D**-glucopyranoside (**9**) afforded **19** in a moderate yield of 52% (α/β = 75/25) with the production of **23** in 11% yield. Even reactions using less reactive acceptors such as phenol (**10**) and 1-adamantanol (**11**) produced the corresponding glycosides **20** and **21** in 40% (α/β =

80/20) and 58% (α/β = 65/35) yields with the production of **23** in 21% and 17% yields, respectively. In addition, during the reaction using **10**, the benzyl glucoside **22** was obtained as another byproduct in 31% yield. It seemed that the benzyl alcohol was formed along with the degradation of **1** under the reaction conditions using **10**. The glycosidation of **1** with some primary alcohols afforded the desired glycosides in good yields. In contrast, for the reactions using less reactive alcohols such a benzyl alcohol, for example, the yields of the desired glycosides decreased due to increase in the yield of **23** and the appearance of a new byproduct **22** (Scheme2-9, Table 2-1; Entry 8-11).

Similar to **1**, 2,3,4,6-tetra-*O*-benzyl-**D**-mannopyranose (**2**), 2,3,4,6-tetra-*O*-benzyl-**D**-gal-actopyranose (**3**), 2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-**D**-glucopyranose (**4**) and 2,3,5-tri-*O*-benzyl-**D**-arabinofuranose (**6**) were used as 1-hydroxy sugars. The reaction of **2** with **7** or **8** under the reaction conditions using 5 mol% $Bi(OTf)_3$ at reflux temperature for 15 min afforded the corresponding glycosides with some stereoselectivity, **13** or **18** in 73% yield (α only) or 81% yield (α/β = 91/9) and the production of the 1,1'-disaccharide **24** in 16% yield in both cases. Similar reaction conditions using **3**, **4**, or **6** with **7** gave the glycosides **14**, **15** or **16** in 70% yield (α/β = 77/23), 51% yield (α/β = 52/48) or 81% yield (α/β = 85/15) with the production of the 1,1'-disaccharide **25**, **26** or **28** in 10%, 15% or 5% yield, respectively (Scheme2-9, Table 2-1; Entry 12-16).

This reaction is a competitive reaction of **7-11** with **1-4,6** which are also an acceptor, since **7-11** have nucleophilicity stronger than **1-4**,**6**, it is thought that glycosides (**12-21**) ware obtained preferentially. Thus, since nucleophilicity of sugar alcohols like **1-4,6** is low, it was suggested that examination of the further conditions is required in order to compound non-reducing disaccharide. The postulated reaction mechanism is shown in Scheme 2-10.



Scheme 2-10　The mechanism of this dehydrative glycosylation

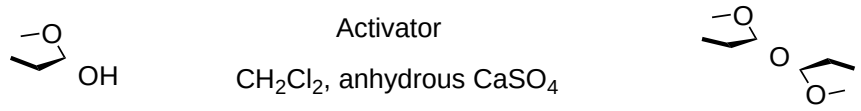
As I have discussed in this chapter, I have successfully developed the condensation reaction of 1-hydroxy sugars with several alcohols. The reactions of several benzylated 1-hydroxy sugars with certain primary alcohols using only 5 mol% $Bi(OTf)_3$ at reflux temperature in dichloromethane for 15 min successfully afforded the desired *O*-glycosides in good yields. These reaction conditions restrict the generation of undesired 1,1'-disaccharides. When bulky alcohol with low reactivity was used, generation of many kind of non-reducing disaccharide was observed. It is expected that non-reducing disaccharide is produced in good yield in the absence of alchohol. Moreover, when glucose, galactose, and arabinose were used, α-structure was preferentially acquired according to the influence of an anomeric effect. In addition, when mannose was used, it was shown clearly that glycoside denotes α-stereoselectivity as a result of the steric hindrance of the transition state.

2-2-2. The development for the synthesis of non-reducing disaccharides by dehydrative glycosidation

2-2-2-1. The synthesis of symmetric non-reducing disaccharides from the 1-hydroxy sugars

In chapter 2-2-2-1, I have described about the synthesis of the various non-reducing disaccharides by the efficient dehydrative glycosylation reaction using $Bi(OTf)_3$. I examined the other optimal conditions for carrying out the self-condensation of these, using glucose, mannose, galactose, 2-*N_3*-glucose, 2-*N_3*-6-*OAc*-glucose and arabinose as 1-hydroxy sugars.[21]

We investigated the formation of the symmetrical 1,1'-disaccharide **23** by the self-condensation of **1** in detail. The reactions using 5 mol% and 10 mol% $Bi(OTf)_3$ at reflux temperature for 15 min promoted the self-condensation of **1** to produce **23** in 77% and 71% yields. At the same time, however, the benzylglucoside **22** was obtained as a by-product in 19% and 14% yields, respectively. The reaction using 5 mol% $Bi(OTf)_3$ at room temperature for 15 h afforded **23** in only 12% yield without the production of **22**. The use of 10 mol% $Bi(OTf)_3$ at room temperature increased the yield of **23** to 85% with an $\alpha\alpha/\alpha\beta/\beta\beta$ isomer ratio of 53/33/14. The use of 50 mol% $Bi(OTf)_3$ decreased the yield of **23** to 59% and increased the yield of **22** to 35%. Larger amount of $Bi(OTf)_3$ seemed to promote the degradation of **1** (Scheme 2-11, Table 2-2; Entry 1-6).



1-6 **23-28**

Scheme 2-11 The formation of 1,1'-disaccharides by the self-condensation of **1-6**

Table 2-2 The Formation of 1,1'-disaccharides by the self-condensation of 1-6

Entry[a]	1-Hydroxy sugar	Activator (mol%)	1,1'-Disaccharide	
			Product	Yield / % (αα / αβ / ββ)[b]
1[c]	**1**	$Bi(OTf)_3$ (5)	**23**	77
2[d]	**1**	$Bi(OTf)_3$ (10)	**23**	71
3	**1**	$Bi(OTf)_3$ (5)	**23**	12
4	**1**	$Bi(OTf)_3$ (10)	**23**	85 (53 / 33 / 14)
5	**1**	$Bi(OTf)_3$ (20)	**23**	84 (65 / 29 / 6)
6[e]	**1**	$Bi(OTf)_3$ (50)	**23**	59
7	**1**	$Sc(OTf)_3$ (20)	**23**	67 (55 / 32 / 13)
8	**1**	$Yb(OTf)_3$ (20)	**23**	–
9[f]	**2**	$Bi(OTf)_3$ (10)	**24**	85 (100 / 0 / 0)
10[g]	**3**	$Bi(OTf)_3$ (10)	**25**	70 (71 / 29 / 0)
11	**4**	$Bi(OTf)_3$ (20)	**26**	–
12	**4**	$Bi(OTf)_3$ (50)	**26**	23
13	**4**	$Bi(OTf)_3$ (100)	**26**	53 (35 / 32 / 33)
14	**5**	$Bi(OTf)_3$ (50)	**27**	17
15	**5**	$Bi(OTf)_3$ (100)	**27**	24 (14 / 44 / 42)
16[h]	**6**	$Bi(OTf)_3$ (10)	**28**	93 (85 / 15 / 0)

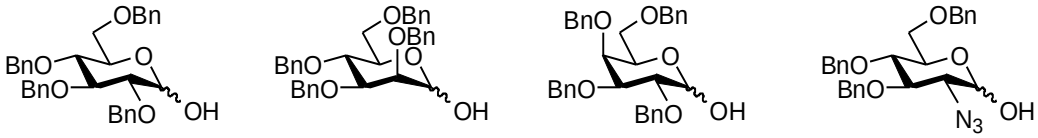
[a]Reaction conditions: solvent = CH_2Cl_2; room temperature; reaction time = 1 d. [b]All the isomer ratios were determined by NMR.
[c]Reaction conditions: reflux temperature; reaction time = 10 min; compound **22** was obtained in 19% yield as a byproduct.
[d]Reaction conditions: reflux temperature; reaction time = 10 min; compound **22** was obtained in 14% yield as a byproduct.
[e]Compound **22** was obtained in 35% yield. [f]Reaction conditions: room temperature; reaction time = 1 h.
[g]Reaction conditions: 0 °C; reaction time = 1 d. [h]Reaction conditions: 0 °C; reaction time = 3.5 h.

We examined the effect of other metal triflates using $Sc(OTf)_3$ and $Yb(OTf)_3$. While $Sc(OTf)_3$ was a fairly effective activator for the production of **23** in 67% yield, $Yb(OTf)_3$ was completely ineffective. This result roughly corresponded to that of entries 6 and 7 in Table 2-1. Subsequently, the synthesis of various symmetrical 1,1'-disaccharides **24**, **25**, **26, 27** and **28** by the self-condensation of the 1-hydroxy sugars **2**-**6** was examined. Similar reaction conditions using 10 mol% $Bi(OTf)_3$ in CH_2Cl_2 at room temperature for 3

to 15 h successfully gave **24**, **25** and **28** in good yields of 85% (αα only), 70% (αα/αβ/ββ= 71/29/0) and 93% (αα/αβ/ββ= 85/15/0) from **2**, **3** and **6**, respectively. However, the same reaction conditions were not applicable to self-condensation of **4**. The reaction conditions using 100 mol% $Bi(OTf)_3$ increased the yield of **26** up to 53% (αα/αβ/ββ= 35/32/33). In addition, when we use 6-*O*-Acetyl-2-azido-3,4-di -*O*-benzyl-2-deoxy-**D**-glucopyranose (**5**) which is introduced acetyl group at 6th hydroxyl group by acetolysis reaction[23], in the presence of 100 mol% $Bi(OTf)_3$, the yield of **27** increased to 24% (αα/αβ/ββ= 14/44/42). The reason reduced reactivity is explained as the difficult desorption of the anomeric hydroxyl group, since the electron density of the pyranose ring is decreased by the azido group and an acetyl group. Although reactivity of the glycosidation decreased, the convenient formulation of the non-reducing disaccharide which introduced azido groups, such as **26** and **27** were successfully acquired (Scheme 2-11, Table 2-2; Entry 7-16).

2-2-2-2. Discussions for stereoselectivity in the synthesis of non-reducing disaccharides

In chapter 2-2-2-2, we considered the stereospecificity of non-reducing disaccharide formation.



Glc α/β = 92/8 Man α/β = 81/19 Gal α/β = 54/46 $GlcN_3$ α/β = 58/42

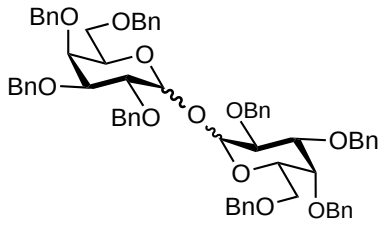
1 **2** **3** **4**

Figure 2-7 The αβ abundance ratio of various aldopyranoses 30 minutes after dissolving in CD_2Cl_2 solvent

In order to study the stereospecificity of a dehydrative glycosydation reaction, the anomeric effect of 1-hydroxy sugar shoud be considered. I decided to examine αβ ratio of various 1-hydroxy sugars. The αβ ratio of 1-hydroxy sugar which estimated by ^{1}H-NMR 30 minutes after the starting material were dissolved in CD_2Cl_2. The results were summarized in Figure 2-7. Glucose, mannose, galactose, and 2-*N_3*-glucose were converted to the corresponding non-reducing disaccharides in the αβ ratios of 92/8, 81/19, 54/46, and 58/42, respectively.

In the synthesis of non-reducing disaccharides using 1-hydroxy sugar(**1**), the αβ ratio depend on the stereospecificity of the glycosylation reaction, then α-configuration of the compound was acquired preferentially. The reaction using **4** showed similarly αβ ratio of the product-**4**, and αα/αβ/ββ ratio was approximately 1/1/1. Also, the reaction using **2**, since **2** has a benzyl group at the C2-position in the false axial side in a transition state, and it becomes the steric hindrance of the β side, the steric structure of the sugar donor is controlled to α conformation. Moreover, it is also considered that the steric structure of

the sugar acceptor was restricted to α structure, in order to tend to take 1,2-trans conformation since **2** has a benzyl group of the 2-position in the false axial side. When the reaction was start from **3,** αα/αβ/ββ ratio of the non-reducing disaccharide was obtained about 71/29/0 (Figure 2-8). The stereospecificity of **3** is differrent from the abundance ratio shown in Figure 2-7, it is thought that αβ ratio of **3** is approximately 7/3, since anomerization is promoted in the presence of $Bi(OTf)_3$. And it is expected that this αβ ratio of **3** was derived from the stereospecificity of non-reducing disaccharide.



αα / αβ / ββ = 71 / 29 / 0

Figure 2-8 The αβ abundance ratio of **25**

There are following three possible reason why ββ conformation of non-reducing disaccharide derived from 3 was not found. (1) Since it is the anomeric hydroxyl group of β conformation is easily removed, (2) due to the benzyl group of the 4-position is in the axial side, and it is surmised that (3) most of **3** which works as a acceptor of the saccharide is α-conformation (Figure 2-9).

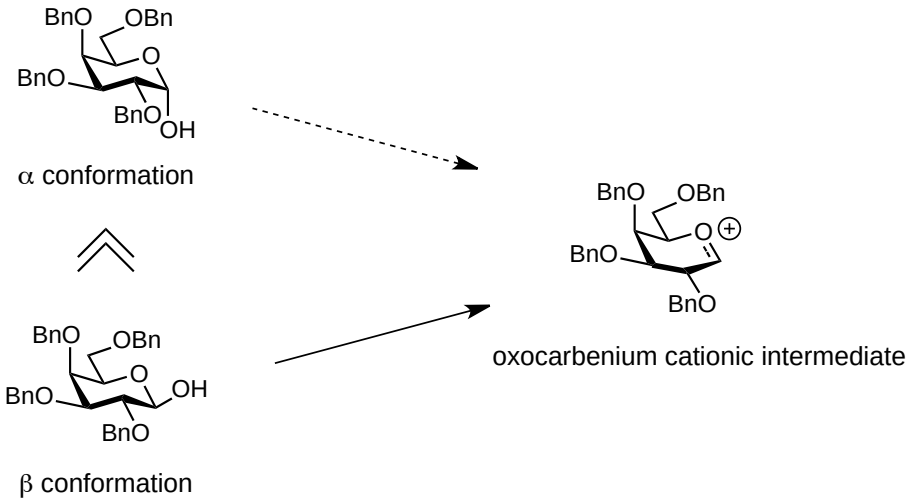


Figure 2-9 The stereospecificity of **3** as a sugar acceptor

2-3. Conclusion

In chapter 2, I focused on the structure of non-reducing disaccharides that could be a novel neamine mimic, and describe the development of the useful method for the glycosylation to construct non-reducing disaccharide.

In chapter 2-2-1, I developed the condensation reaction of 1-hydroxy sugars with several alcohols (**7-11**) in the presence of $Bi(OTf)_3$. The reactions of several benzylated 1-hydroxy sugars (**1-4, 6**) with certain primary alcohols (**7-8**) using only 5 mol% $Bi(OTf)_3$ at reflux temperature in CH_2Cl_2 for 15 min successfully afforded the desired *O*-glycosides in good yields. These reaction conditions restricted the production of undesired 1,1'-disaccharides. When bulky alcohol (**9-11**) with low reactivity was used, several kind of non-reducing disaccharide was observed. In the absence of alcohols, it is expected that non-reducing disaccharide is produced in good yield. Moreover, when glucose, galactose, and arabinose were used, α-structure was preferentially acquired from the influence of an anomeric effect. In addition, when mannose was used, it was shown clearly that glycosilation reaction obtained α-stereoselectivity derived from the steric hindrance of the transition state.

In chapter 2-2-2, we considered the synthesis of the various non-reducing disaccharides by the efficient dehydrative glycosylation reaction which used $Bi(OTf)_3$. In chapter 2-2-2-1, we examined the optimal conditions for carrying out the self-condensation of these, using glucose, mannose, galactose, 2-N_3-glucose, 2-N_3-6-*OAc*-glucose and arabinose as 1-hydroxy sugars. The yield of self-condensation with 1-hydroxy sugars was promoted by 10-100 mol% $Bi(OTf)_3$ at room temperature in CH_2Cl_2 for 18 h and produced various 1,1'-disaccharides. Then we successfully established convenient formulation of the non-reducing disaccharide which introduced azido groups, such as **26** and **29** which has convertible structure to neamine derivatives. In chapter 2-2-2-2, we considered the stereospecificity of non-reducing disaccharide

formation.

As described above, I have established the novel dehydrative glycosydation which is capable of building a 1,1'-disaccharide structure efficiently.

2-4. Experimental Section

^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectra were recorded on a JEOL ECA-600 spectrometer in $CDCl_3$ using TMS as an internal standard. Optical rotations were recorded on a JASCO DIP-360 digital polarimeter. HRMS were obtained on a Mariner spectrometer (PerSeptive Biosystems Inc.). Preparative TLC was performed using Merck silica gel 60GF254. Column chromatography was conducted using silica gel 60 N (40~50 μm, Kanto Chemical Co., INC.). $Bi(OTf)_3$ was purchased from Sigma-Aldrich. All anhydrous solvents were purified according to standard methods.

2-Phenylethyl 2,3,4,6-Tetra-*O*-benzyl-D-glucopyranoside(12)

To a stirred suspension of $Bi(OTf)_3$ (5.4 mg, 0.0082 mmol) and **7** (20.3 mg, 0.17 mmol) in CH_2Cl_2 (3 mL) was added **1** (89.2 mg, 0.17 mmol) in the presence of anhydrous $CaSO_4$ (ca. 100 mg) under an Ar atmosphere. The resulting mixture was stirred at reflux temperature for 15 min. The reaction was then quenched by the addition of a saturated aqueous $NaHCO_3$ solution (5 mL). The reaction mixture was extracted with EtOAc, and the organic layer was washed with water and a saturated aqueous NaCl solution. After the organic layer was dried over Na_2SO_4, the solvent was evaporated under reduced pressure. The crude product was purified using a preparative silica gel TLC (PTLC; EtOAc/hexane = 1/3) to give the desired glycoside **12** (92.4 mg, 87%, α mixture of a and β anomers) as a colorless oil and 1,1'-disaccharide **23** (8.3 mg, 9%) as a colorless oil.

^{1}H NMR δ 2.92-2.99 (m, $CH_2CH_2Ph\alpha\beta$), 3.99 (dd, J = 8.2 Hz, J = 8.9 Hz, H-3α), 4.19-4.23 (m, $CH_aH_bCH_2Ph\beta$), 4.41 (d, J = 7.6 Hz, H-1β), 4.77 (d, J = 3.4 Hz, H-1α)

^{13}C NMR δ 35.9, 36.2, 68.4, 68.7, 68.9, 70.1, 70.6, 73.1, 73.36, 73.41,74.6, 74.80, 74.82, 74.9, 75.59, 75.60, 77.6, 77.8, 80.0, 81.9, 82.2, 84.6, 96.8 (C-1α), 103.6 (C-1β)

HRMS (ESI, α/β mixture) *m/z* calcd for 667.3030 ($C_{42}H_{44}O_6+Na^+$), found 667.2993.

Octyl 2,3,4,6-Tetra-*O*-benzyl-D-glucopyranoside (17)

The same procedure used for the preparation of **12** using $Bi(OTf)_3$ (5.1 mg, 0.0078 mmol), **8** (20.2 mg, 0.16 mmol), and **1** (84.1 mg, 0.16 mmol) in CH_2Cl_2 (3 mL) gave the desired glycoside **17** (PTLC; EtOAc/hexane = 1/3, 85.6 mg, 85%, a mixture of α and β anomers) as a colorless oil and **23** (7.1 mg, 9%).

^{1}H NMR δ 0.86-0.89 (m, CH_3), 1.27-1.70 (m, (CH_2)$_6$), 3.40-3.47 (m, H-2β, H-5β, CH_aH$_b$(CH$_2$)$_6$CH$_3$α), 3.51-3.69 (m, H-2α, H-4α, H$_a$-6α, CH$_a$$H_b$(CH$_2$)$_6CH_3$α, H-3β, H-4β, H$_a$-6β, C$H_aH_b$(CH$_2$)$_6CH_3$β), 3.72 (dd, *J* = 3.4 Hz, *J* = 10.3 Hz, H$_b$-6α), 3.73-3.75 (m, H$_b$-6β), 3.77-3.80 (m, H-5α), 3.95-3.99 (m, CH$_a$$H_b$ (CH$_2$)$_6$CH$_3$β), 3.99 (dd, *J* = 8.9 Hz, *J* = 9.6 Hz, H-3α), 4.39 (d, *J* = 7.6 Hz, H-1β), 4.76 (d, *J* = 4.1 Hz, H-1α)

^{13}C NMR δ 14.1, 22.6, 26.11, 26.14, 29.18, 29.21, 29.3, 29.3, 29.4, 29.7, 31.8, 31.8, 68.2, 68.5, 69.0, 70.0, 70.1, 73.1, 73.4, 73.4, 74.7, 74.8, 74.9, 75.0, 75.6, 75.6, 77.7, 77.9, 80.1, 82.1, 82.2, 84.7, 96.8 (C-1α), 103.6 (C-1β)

HRMS (ESI, α/β mixture) *m/z* calcd for 675.3656 ($C_{42}H_{52}O_6$+Na^+), found 675.3680.

Methyl 6-*O*-(2,3,4,6-Tetra-*O*-benzyl-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-gluco -pyranoside (19)

The same procedure used for the preparation of **12** using $Bi(OTf)_3$ (5.0 mg, 0.0076 mmol), **9** (70.8 mg, 0.15 mmol), and **1** (82.4 mg, 0.15 mmol) in CH_2Cl_2 (3 mL) gave the desired glycoside **19** (PTLC; EtOAc/hexane = 1/3, 77.7 mg, 52%, a mixture of α and β anomers) as a colorless oil and **23** (9.2 mg, 11%).

^{1}H NMR δ 3.25 (s, CH_3β), 3.35 (s, CH_3 α), 3.43 (dd, *J* = 3.4 Hz, *J* = 8.9 Hz, H-2'α), 3.48-3.57 (m, H-2α, H-2'β), 3.94-4.01 (m, H-3'α, H-3α), 4.35 (d, *J* = 8.2 Hz, H-1'β), 4.50-4.62 (m, H-1α, H-1 β), 4.98 (d, *J* = 4.1 Hz, H-1'α)

^{13}C NMR δ 97.2 (C-1' αα), 97.9 (C-1αα), 98.0 (C-1βα), 103.7 (C-1' βα)

HRMS (ESI, α/β mixture) *m/z* calcd for 1009.4497 ($C_{62}H_{66}O_{11}$+Na^+), found 1009.4479.

Phenyl 2,3,4,6-Tetra-*O*-benzyl-D-glucopyranoside (20)

The same procedure used for the preparation of **12** using $Bi(OTf)_3$ (5.4 mg, 0.0082 mmol), **10** (15.6 mg, 0.16 mmol), and **1** (89.0 mg, 0.16 mmol) in CH_2Cl_2 (3 mL) gave **20** (PTLC; toluene/EtOAc = 10/1, 40.3 mg, 40%, a mixture of α and β anomers) as a colorless oil, **23** (18.5 mg, 21%) as a colorless oil and **22** (32.2 mg, 31%) as a colorless oil.

^{1}H NMR δ 3.56 (dd, *J* = 2.1 Hz, *J* = 10.3 Hz, H_a-6α), 3.60-3.80 (m, H-2α, H_b-6α, H-2β, H-3β, H-4β, H-5β, H_{ab}-6β, H_b-6β), 3.79 (dd, *J* = 8.9 Hz, *J* = 10.3 Hz, H-4α), 3.87-3.89 (m, H-5α), 4.21 (dd, *J* = 8.9 Hz, *J* = 9.4 Hz, H-3α), 5.01 (d, *J* = 7.6 Hz, H-1β), 5.48 (d, *J* = 3.4 Hz, H-1α)

^{13}C NMR δ 68.2, 68.8, 70.8, 73.3, 73.4, 73.5, 75.02, 75.05, 75.11, 75.77, 75.78, 77.4, 77.7, 79.7, 82.0, 84.7, 95.4 (C-1α), 101.6 (C-1β)

HRMS (ESI, α/β mixture) *m/z* calcd for 639.2717 ($C_{40}H_{40}O_6$+Na^+), found 639.2693.

1-Adamantyl 2,3,4,6-Tetra-*O*-benzyl-D-glucopyranoside (21)

The same procedure used for the preparation of **12** using $Bi(OTf)_3$ (5.6 mg, 0.0085 mmol), **11** (27.1 mg, 0.18 mmol), and **1** (89.9 mg, 0.17 mmol) in CH_2Cl_2 (3 mL) gave the desired glycoside **21** (PTLC; EtOAc/hexane = 1/3, 65.2 mg, 58%, a mixture of α and β anomers) as a colorless oil and **23** (15.0 mg, 17%).

^{1}H NMR δ 1.56-2.17 (30H, m, 1-adamantyl), 3.42-3.50 (m, H-2β, H-4β, H-5β), 3.53 (dd, *J* = 4.1 Hz, *J* = 9.6 Hz, H-2α), 3.60-3.67 (m, H_a-6α, H-4α, H_a-6β, H-3β), 3.73 (dd, *J* = 2.1 Hz, *J* = 11.0 Hz, H_b-6β), 3.76 (dd, *J* = 3.4 Hz, *J* = 10.3 Hz, H_b-6α), 4.00-4.03 (m, H-5α), 4.01 (dd, *J* = 8.9 Hz, *J* = 9.6 Hz, H-3α), 4.70 (d, *J* = 7.6 Hz, H-1β), 5.28 (d, *J* = 3.4 Hz, H-1α)

^{13}C NMR δ 30.6, 30.7, 36.3, 36.3, 42.4, 42.8, 68.8, 69.5, 69.6, 72.8, 73.3, 73.4, 74.51, 74.54, 74.9, 75.1, 75.3, 75.5, 75.7, 78.1, 78.2, 80.1, 82.1, 82.3, 85.1, 89.8 (C-1α), 96.2 (C-1β)

HRMS (ESI, α/β mixture) *m/z* calcd for 697.3500 ($C_{44}H_{50}O_6$+Na^+), found 697.3538.

2-Phenylethyl 2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranoside (13) (Table 1, Entry 13)

The same procedure used for the preparation of **12** using $Bi(OTf)_3$ (5.7 mg, 0.0087 mmol), **7** (21.3 mg, 0.17 mmol), and **2** (94.1 mg, 0.17 mmol) in CH_2Cl_2 (3 mL) gave the desired glycoside **13** (PTLC; EtOAc/hexane = 1/3, 82.1 mg, 73%) as a colorless oil and **24** (14.7 mg, 16%) as a colorless oil. $[\alpha]_D^{22}$+30.5° (*c* 4.1, $CHCl_3$)

^{1}H NMR δ 2.82 (2H, t, *J* = 6.9 Hz, CH_2CH_2Ph), 3.57-3.63 (2H, m, $CH_aH_bCH_2$Ph, H-5), 3.67 (1H, dd, *J* = 2.1 Hz, *J* = 11.0 Hz, H_a-6), 3.71-3.74 (2H, m, H-2, H_b-6), 3.84-3.88 (2H, m, $CH_aH_bCH_2$Ph, H-3), 3.95(1H, t, *J* = 9.6 Hz, H-4), 4.84 (1H, d, *J* = 2.1 Hz, H-1)

^{13}C NMR δ 36.0, 68.1, 69.3, 71.8, 72.1, 72.5, 73.3, 74.7, 74.9, 74.9, 80.0, 97.7 (C-1, $J_{C1\text{-}H1}$ = 168.3 Hz)

HRMS (ESI) *m/z* calcd for 667.3030 ($C_{42}H_{44}O_6$+Na^+), found 667.3073.

Octyl 2,3,4,6-Tetra-*O*-benzyl-D-mannopyranoside (18)

The same procedure used for the preparation of **12** using $Bi(OTf)_3$ (5.3 mg, 0.0081 mmol), **8** (21.1 mg, 0.16 mmol), and **2** (87.3 mg, 0.16 mmol) in CH_2Cl_2 (3 mL) gave the desired glycoside **18** (PTLC; EtOAc/hexane = 1/3, 84.9 mg, 81%, a mixture of α and β anomers) as a colorless oil and **24** (13.5 mg, 16%).

^{1}H NMR δ 0.88 (t, *J* = 6.9 Hz, CH_3α), 1.26-1.52 (m, $CH_2(CH_2)_6CH_3$), 3.35 (ddd, *J* = 2.8 Hz, *J* = 6.9 Hz, *J* = 9.6 Hz, $CH_aH_b(CH_2)_6CH_3$α), 3.65 (ddd, *J* = 2.8 Hz, *J* = 6.9 Hz, *J* = 9.6 Hz, $CH_aH_b(CH_2)_6CH_3$α), 3.72-3.80 (m, H-2α, H-5α, H_a-6α, H_b-6α), 3.91 (dd, *J* = 3.4 Hz, *J* = 9.6 Hz, H-3α), 3.99 (t, *J* = 9.6 Hz, H-4α), 4.37 (d, *J* = 0.7 Hz, H-1β), 4.86 (d, *J* = 1.4 Hz, H-1α)

^{13}C NMR δ 14.1, 14.5, 22.61, 22.63, 26.08, 26.12, 29.16, 29.24, 29.3, 29.4, 29.7, 31.8, 67.6, 69.3, 69.7, 70.0, 71.3, 71.7, 72.1, 72.5, 73.3, 73.4, 73.5, 73.6, 74.8, 74.9, 75.0, 75.1, 75.9, 80.3, 82.3, 97.8 (C-1α, J_{C1-H1} = 165.7 Hz), 101.7 (C-1β, J_{C1-H1} = 152.6 Hz)
HRMS (ESI, α/β mixture) *m/z* calcd for 675.3656 ($C_{42}H_{52}O_6$+Na^+), found 675.3663.

2-Phenylethyl 2,3,4,6-Tetra-*O*-benzyl-D-galacutopyranoside (14)

The same procedure used for the preparation of **12** using $Bi(OTf)_3$ (4.6 mg, 0.007 mmol), **7** (17.1 mg, 0.14 mmol), and **3** (75.9 mg, 0.14 mmol) in CH_2Cl_2 (3 mL) gave the desired glycoside **14** (PTLC; EtOAc/hexane = 1/3, 63.6 mg, 70%, a mixture of α and β anomers) as a colorless oil and **25** (7.6 mg, 10%) as a colorless oil.

^{1}H NMR δ 2.91-2.97 (m, CH_2CH_2Ph), 3.92 (dd, *J* = 2.8 Hz, *J* = 9.6 Hz, H-3α), 4.03 (dd, *J* = 3.4 Hz, *J* = 9.6 Hz, H-2α), 4.15-4.19 (m, $CH_aH_bCH_2$Phβ), 4.37 (d, *J* = 7.6 Hz, H-1β), 4.83 (d, *J* = 3.4 Hz, H-1α)

^{13}C NMR δ 36.0, 36.2, 68.6, 68.8, 68.9, 69.2, 70.5, 73.0, 73.1, 73.2, 73.30, 73.36, 73.39, 73.5, 74.4, 74.7, 75.0, 75.1, 76.5, 78.8, 79.5, 82.1, 97.4 (C-1α), 103.9 (C-1β)
HRMS (ESI, α/β mixture) *m/z* calcd for 667.3030 ($C_{42}H_{44}O_6$+Na^+), found 667.3011.

2-Phenylethyl 2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranoside (15)

The same procedure used for the preparation of **12** using $Bi(OTf)_3$ (6.8 mg, 0.01 mmol), **7** (24 μL, 0.2 mmol), and **4** (95.2 mg, 0.2 mmol) in CH_2Cl_2 (3 mL) gave the desired glycoside **15** (PTLC; EtOAc/hexane = 1/3, 59.5 mg, 51%, a mixture of α and β anomers) as a colorless oil and **26** (31.2 mg, 33%) as a colorless oil.

^{1}H NMR δ 2.92-2.99 (4H, m, CH_2CH_2Ph), 3.34 (dd, *J* = 3.4 Hz, *J* = 10.3 Hz, H-2α), 3.84-3.88 (m, $CH_aH_bCH_2$Phα), 3.95 (t, *J* = 9.6 Hz, H-3α), 4.12-4.16 (m, $CH_aH_bCH_2$Phβ), 4.27 (d, *J* = 7.6 Hz, H-1β), 4.93 (d, *J* = 3.4 Hz, H-1α)

^{13}C NMR δ 36.0, 36.2, 63.3, 66.4, 68.2, 68.6, 68.9, 70.6, 70.9, 73.4, 73.5, 74.8, 75.0, 75.3, 75.5, 77.7, 78.2, 80.1, 83.1, 97.6 (C-1α), 102.1 (C-1β)

HRMS (ESI, α/β mixture) *m/z* calcd for 602.2625 ($C_{35}H_{37}O_5N_3$+Na^+), found 602.2668.

2-Phenylethyl 2,3,5-Tri-*O*-benzyl-D-arabinofuranoside (16)

The same procedure used for the preparation of **12** using $Bi(OTf)_3$ (7.9 mg, 0.012 mmol), **7** (29.3 mg, 0.24 mmol), and **6** (101.2 mg, 0.24 mmol) in CH_2Cl_2 (3 mL) gave the desired glycoside **16** (PTLC; EtOAc/hexane = 1/3, 102.6 mg, 81%, a mixture of α and β anomers) as a colorless oil and **28** (4.5 mg, 5%) as a colorless oil.

^{1}H NMR δ 2.86 (t, *J* = 6.9 Hz, CH_2CH_2Phβ), 2.90 (t, *J* = 6.9 Hz, CH_2CH_2Phα), 3.42 (d, *J* = 6.2 Hz, H_a-5β, H_b-5β), 3.57 (dd, *J* = 5.5 Hz, *J* = 11.0 Hz, H_a-5α), 3.57 (ddd, *J* = 2.8 Hz, *J* = 6.9 Hz, *J* = 10.3 Hz, $CH_aH_bCH_2$Phβ), 3.61-3.66 (m, $CH_aH_bCH_2$Phα, H_b-5α), 3.87 (ddd, *J* = 2.8 Hz, *J* = 6.9 Hz, *J* = 9.6 Hz, $CH_aH_bCH_2$Phβ), 3.91 (dd, *J* = 2.8 Hz, *J* = 6.9 Hz, H-3α), 3.97 (ddd, *J* = 2.8 Hz, *J* = 6.9 Hz, *J* = 9.6 Hz, $CH_aH_bCH_2$Phα), 4.00 (d, *J* = 2.1 Hz, H-2α), 4.03 (dd, *J* = 4.1, 6.9 Hz, H-2β), 4.07-4.11 (m, H-3β, H-4β), 4.13-4.16 (m, H-4α), 4.86 (d, *J* = 4.1 Hz, H-1β), 5.02 (s, H-1α)

^{13}C NMR δ 36.0, 36.1, 68.3, 68.4, 69.6, 71.8, 72.0, 72.25, 72.28, 72.5, 73.2, 73.3, 80.2, 80.4, 83.3, 83.4, 84.2, 88.3, 100.4 (C-1β), 106.2 (C-1α)

HRMS (ESI, α/β mixture) *m/z* calcd for 547.2455 ($C_{34}H_{36}O_5$+Na^+), found 547.2446. A part of α anomer was isolated. α Anomer: $[\alpha]_D^{25}$ +46.6° (*c* 4.1, $CHCl_3$).

2,3,4,6-Tetra-*O*-benzyl-D-glucopyranosyl 2,3,4,6-Tetra-*O*-benzyl-D-glucopyrano-side (23)

To a stirred solution of $Bi(OTf)_3$ (8.3 mg, 0.013 mmol) in CH_2Cl_2 (3 mL) was added **1** (68.4 mg, 0.13 mmol) in the presence of anhydrous $CaSO_4$ (ca. 100 mg) under an Ar atmosphere. The resulting mixture was stirred at room temperature for 1 d. The same procedure used for the preparation of **12** was followed. The crude product was purified using a preparative silica gel TLC (EtOAc/hexane = 1/3) to provide the desired

1,1'-disaccharide **23** (57.0 mg, 85%, a mixture of αα, αβ, and ββ isomers) as a colorless oil.

^{1}H NMR δ 3.36-3.38 (m, H_a-6αα), 3.48-3.54 (m, H_b-6αα, H-2αβ-β, H-5ββ), 3.53 (dd, *J* = 7.6 Hz, *J* = 8.2 Hz, H-2ββ), 3.57-3.65 (2H, m, H-2αα, H-2αβ-α), 3.68 (1H, t, *J* = 9.6 Hz, H-4αα), 3.76 (dd, *J* = 7.6 Hz, *J* = 10.3 Hz, H-4αβ-α), 4.02 (1H, dd, *J* = 8.9 Hz, *J* = 9.6 Hz, H-3αα-α), 4.09 (1H, t, *J* ^{20}= 9.6 Hz, H-3αβ-α), 4.15-4.17 (1H, m, H-5αα-α), 4.58 (1H, d, *J* = 7.6 Hz, H-1αβ-β), 4.90 (d, *J* = 8.2 Hz, H-1ββ), 5.16 (1H, d, *J* = 3.4 Hz, H-1αβ-α), 5.23 (1H, d, *J* = 3.4 Hz, H-1αα-α)

^{13}C NMR δ 68.1, 68.9, 70.6, 73.5, 74.6, 74.97, 75.03, 75.6, 77.6, 77.7, 79.3, 81.7, 81.9, 82.2, 84.6, 94.4 (C-1αα), 99.3 (C-1ββ), 99.4 (C-1αβ-α), 104.1 (C-1αβ-β)

HRMS (ESI, isomer mixture) *m/z* calcd for 1085.4810 ($C_{68}H_{70}O_{11}$+Na^+), found 1085.4798.

A part of ββ isomer was isolated. ββ Isomer: [α] D^{20} +15.9° (*c* 0.42, $CHCl_3$).

2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl 2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyra-noside (24)

A similar procedure as employed for the preparation of **23** by stirring both $Bi(OTf)_3$ (12.5 mg, 0.019 mmol) and **2** (103.2 mg, 0.19 mmol) in CH_3Cl_3 (3 mL) at rt for 1 h gave the desired 1,1'-disaccharide **24** (PTLC; EtOAc/hexane = 1/3, 86.0 mg, 85%) as a colorless oil. [α] D^{25} +30.2° (*c* 0.88, $CHCl_3$)

^{1}H NMR δ 3.53-3.56 (2H, m, H-5), 3.60 (2H, dd, *J* = 2.1 Hz, *J* = 3.4 Hz, H-2), 3.63 (2H, dd, *J* = 2.1 Hz, *J* = 11.0 Hz, H_a-6), 3.69-3.72 (4H, m, H_b-6, H-3), 3.97 (2H, dd, *J* = 9.6 Hz, *J* = 10.3 Hz, H-4), 5.20 (2H, d, *J* = 2.1 Hz, H-1)

^{13}C NMR δ 69.0, 72.1, 72.4, 72.6, 73.5, 74.0, 74.6, 75.3, 79.5, 93.3 (C-1, $J_{C1\text{-}H1}$= 167.5 Hz)

HRMS (ESI) *m/z* calcd for 1085.4810 ($C_{68}H_{70}O_{11}$+Na^+), found 1085.4823.

2,3,4,6-Tetra-*O*-benzyl-D-galactopyranosyl 2,3,4,6-Tetra-*O*-benzyl-D-galactopyrano-side (25)

A similar procedure as employed for the preparation of **23** by stirring both $Bi(OTf)_3$ (6.4 mg, 0.0098 mmol) and **3** (52.9 mg, 0.098 mmol) in CH_2Cl_2 (3 mL) at 0 ^oC for 1 d gave the desired 1,1'-disaccharide **25** (PTLC; EtOAc/hexane = 1/3, 36.3 mg, 70%, a mixture of αα and αβ isomers) as a colorless oil.

^1H NMR δ 4.54 (1H, d, *J* = 7.6 Hz, H-1αβ-β), 5.20 (1H, d, *J* = 3.4 Hz, H-1αβ-α), 5.28 (2H, d, *J* = 4.1 Hz, H-1αα)

^{13}C NMR δ 93.5 (C-1αα), 99.8 (C-1αβ-α), 103.7 (C-1αβ-β)

HRMS (ESI, isomer mixture) *m/z* calcd for 1085.4810 ($C_{68}H_{70}O_{11}$+Na^+), found 1085.4816.

A part of αα isomer was isolated. αα Isomer: $[\alpha]_D^{21}$ +74.5° (*c* 0.81, $CHCl_3$).

2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranosyl 2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranoside (26)

A similar procedure as employed for the preparation of **23** by stirring both $Bi(OTf)_3$ (142.5 mg, 0.22 mmol) and **4** (103.8 mg, 0.22 mmol) in CH_2Cl_2 (3 mL) at rt for 1 d gave the desired 1,1'-disaccharide **26** (PTLC; EtOAc/hexane = 1/3, 53.8 mg, 53%, a mixture of αα, αβ, and ββ isomers) as a colorless oil.

^1H NMR δ 4.33 (1H, d, *J* = 7.56 Hz, H-1αβ-β), 4.72 (1H, d, *J* = 8.25 Hz, H-1ββ), 5.17 (1H, d, *J* = 3.44 Hz, H-1αβ-α), 5.21 (1H, d, *J* = 3.44 Hz, H-1αα)

^{13}C NMR δ 95.4 (C-1αα), 97.6 (C-1αβ-α), 101.3 (C-1αβ-β), 103.5 (C-1ββ)

HRMS (ESI, isomer mixture) *m/z* calcd for 955.4001 ($C_{54}H_{56}O_9N_6$+Na^+), found 955.4026.

2,3,5-Tri-*O*-benzyl-D-arabinofuranosyl 2,3,5-Tri-*O*-benzyl-D-arabinofuranoside (28)

A similar procedure as employed for the preparation of **23** by stirring both $Bi(OTf)_3$ (15.8 mg, 0.024 mmol) and **6** (101.2 mg, 0.24 mmol) in CH_2Cl_2 (3 mL) at 0 ^oC for 3.5 h

gave the desired 1,1'-disaccharide **28** αα (PTLC; EtOAc/hexane = 1/2, 78.2 mg, 79%) as a colorless oil and **28** αβ (13.7 mg, 14%) as a colorless oil.

αα Isomer: $[\alpha]_D^{22}$ +54.9° (*c* 3.9, $CHCl_3$)

^{1}H NMR δ 3.61-3.64 (2H, m, H_a-5), 3.65-3.68 (2H, m, H_b-5), 3.94-3.96 (2H, m, H-3), 4.10-4.11 (2H, m, H-2), 4.24-4.27 (2H, m, H-4), 5.50 (2H, s, H-1)

^{13}C NMR δ 69.7, 71.8, 72.1, 73.4, 81.2, 83.7, 87.9, 102.1 (C-1).

HRMS (ESI) *m/z* calcd for 845.3660 ($C_{52}H_{54}O_9$+Na^+), found 845.3686.

αβ Isomer: $[\alpha]_D^{25}$ +18.5° (*c* 0.69, $CHCl_3$);

^{1}H NMR δ 3.56-3.63 (3H, m, H_a-5α, H_b-5α, H_a-5β), 3.67 (1H, dd, *J* = 6.2 Hz, *J* = 10.3 Hz, H_b-5β), 3.99 (1H, dd, *J* = 2.8 Hz, *J* = 6.2 Hz, H-3α), 4.10-4.15 (4H, m, H-2α, H-2β, H-3β, H-4β), 4.30-4.32 (1H, m, H-4α), 5.18 (1H, d, *J* = 3.4 Hz, H-1β), 5.20 (1H, s, H-1α)

^{13}C NMR δ 69.5 (C-5α), 71.7 (*C*H_2Ph), 72.1 (*C*H_2Ph), 72.2 (*C*H_2Ph), 72.6 (*C*H_2Ph), 72.7 (C-5β), 73.1 (*C*H_2Ph), 73.3 (*C*H_2Ph), 80.8 (C-4β), 81.4 (C-4α), 83.58 (C-3α), 83.62 (C-2β or C-3β), 84.56 (C-2β or C-3β), 87.8 (C-2α), 99.5 (C-1β), 106.1 (C-1α)

HRMS (ESI) *m/z* calcd for 845.3660 ($C_{52}H_{54}O_9$+Na^+), found 845.3659.

6-*O*-Acetyl-2-Azido-3,4-di-*O*-benzyl-2-deoxy-D-glucopyranosyl-6'-*O*-acetyl-2'-azido -3',4'-di-*O*-benzyl-2'-deoxy-D-glucopyranoside (27)

^{1}H NMR (600 MHz, $CDCl_3$): δ 2.02-2.05 (18H, m, COC*H*$_3$), 4.40 (1H, d, *J* = 8.25 Hz, H-1αβ-β), 4.69 (1H, d, *J* = 7.6 Hz, H-1ββ), 5.11 (1H, d, *J* = 3.4 Hz, H-1αα), 5.17 (1H, d, *J* = 4.1 Hz, H-1αβ-α).

^{13}C NMR (150 MHz, $CDCl_3$): δ 20.8 (CO*C*H_3), 93.4 (C-1αα), 97.4 (C-1ββ), 99.3 (C-1αβ-α), 101.5 (C-1αβ-β), 170.6 (*C*OCH$_3$).

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22. Anhydrous calcium sulfate was used as a scavenger for water. We previously investigated the glycosidation catalyzed by a Lewis acid in presence of several anhydrous salts.

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23. Y. Cao, Y. Okada, and H. Yamada, *Carbohydr. Res*., 2006, 341, 2219.

Chapter 3 Design and synthesis of novel amino saccharide mimicking neamine backbone

"The development of synthetic approach to non-reducing amino disaccharides (Trehalosamine) and aromatic modified amino saccharide (Aromatic aminoglycoside)."

3-1. Introduction

Some aminoglycosides, have a consensus structure, i.e., 4-*O*-(2,6-diamino-2,6-dideoxy -α-**D**-glucopyranosyl)-2-deoxystreptamine. This compound is named neamine. Neamine is common structural unit as aminoglycoside antibiotics. The discovery of the pathogens having aminoglycoside-resistant has triggered the synthetic study of non-natural type of novel aminoglycoside derivatives.[1] One of the recent focuses is directed to the synthetic exploration of neamine-like small molecules.[2] Due to diverse role of RNA, the development of small molecules which bind to the specific RNA might becoming a contemporary demand that provide novel drugs which is capable of combatting serious diseases that we are facing. A few groups have reported the synthesis of small molecules by mimicking neamine using carbohydrate[3] and heterocyclic compounds.[4] However, most of the small molecules reported so far have indicated only a modest affinity and selectivity for RNA. Then I have designed non-reducing amino disaccharides (Trehalosamine) and aromatic modified amino saccharide (Aromatic aminoglycoside) as a novel neamine mimic as shown in Figure 3-1.

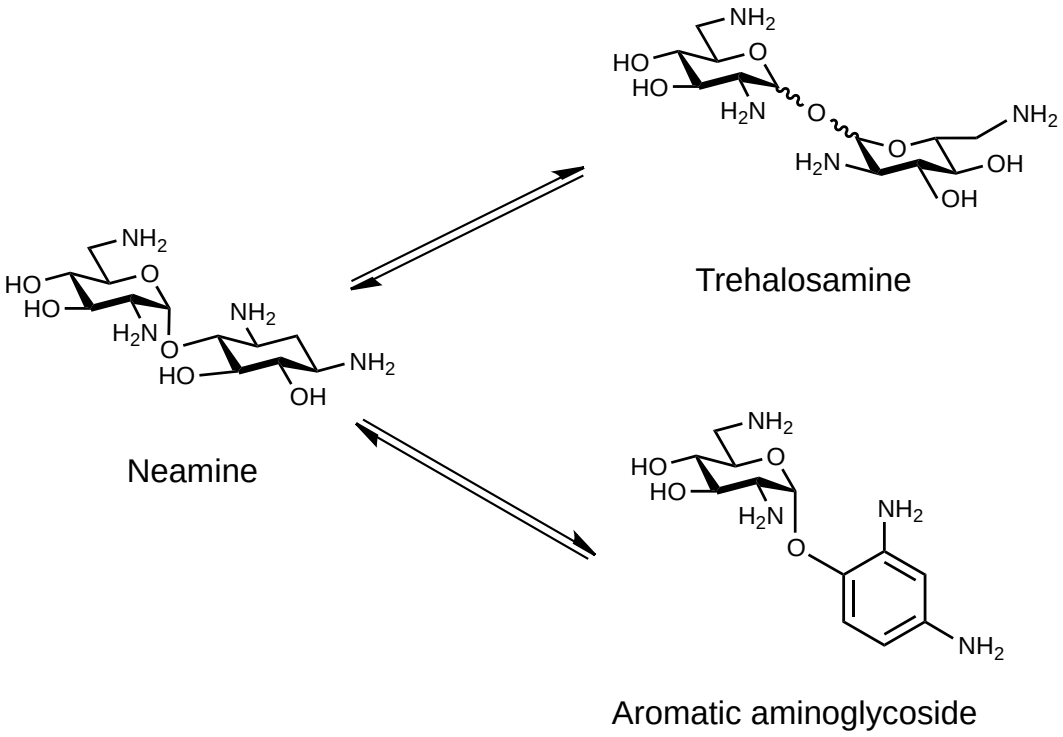


Figure 3-1　Trehalosamine and aromatic aminoglycoside as novel neamine mimics

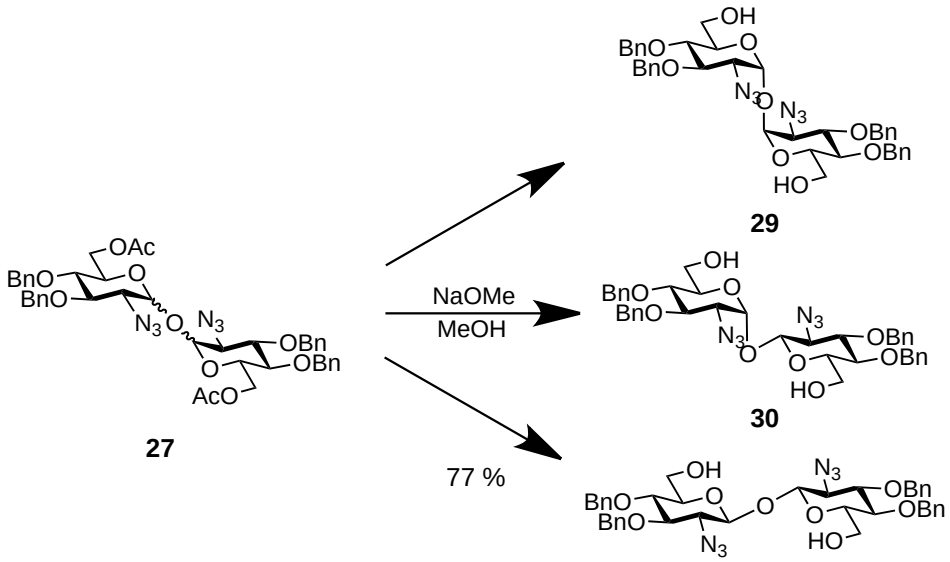
In this chapter, I describe the synthetic approach to trehalosamine (3-2-1) and aromatic aminoglycoside (3-2-2).

3-2. Results and Discussion

3-2-1. The synthesis of novel non-reducing amino disaccharides (trehalosamine)

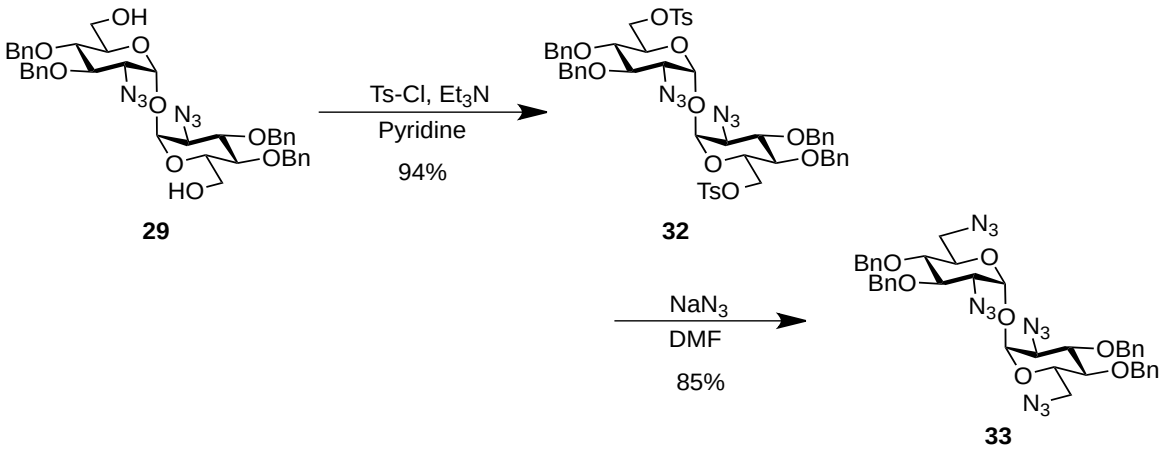
In chapter 3–2–1, I describe about the synthesis of novel non-reducing amino disaccharides (trehalosamine) as a neamine mimic. I have suggested the introduction of the azido group to the 6-position hydroxyl group of 6-*O*-Acetyl-2-azido-3,4-di-*O*-benzyl -2-deoxy-D-glucopy-ranosyl-6'-*O*-acetyl-2'-azido-3',4'-di-*O*-benzyl-2'-deoxy-D-glucopyra noside (**27**) and a way of removing protective groups.

The acetyl group of the 6-position of **27** was deacetylated in the MeOH using NaOMe, purified by thin-layer chromatography, and it gave the trehalosamine derivatives as three structural isomers (αα(**29**), αβ(**30**), ββ(**31**)) which the C6-position is free (Scheme 3-1).



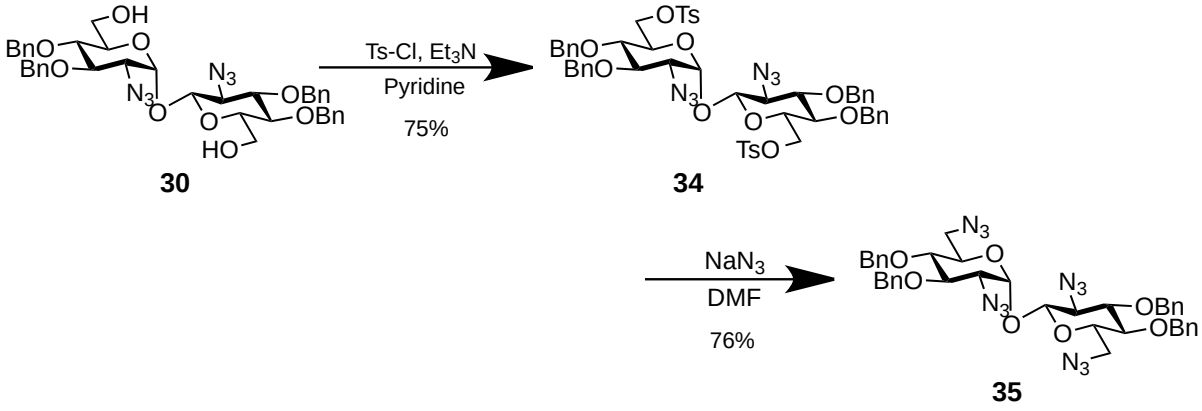
Scheme 3-1 Deacetylation of **27**

Then C6-position hydroxyl group of **29** is tosylated at 94% in a pyridine using Ts-Cl in the presence of Et_3N, and the non-reducing disaccharide which has azido groups in the C6-position (**33**) was obtained at 85% using NaN_3 (Scheme 3-2).



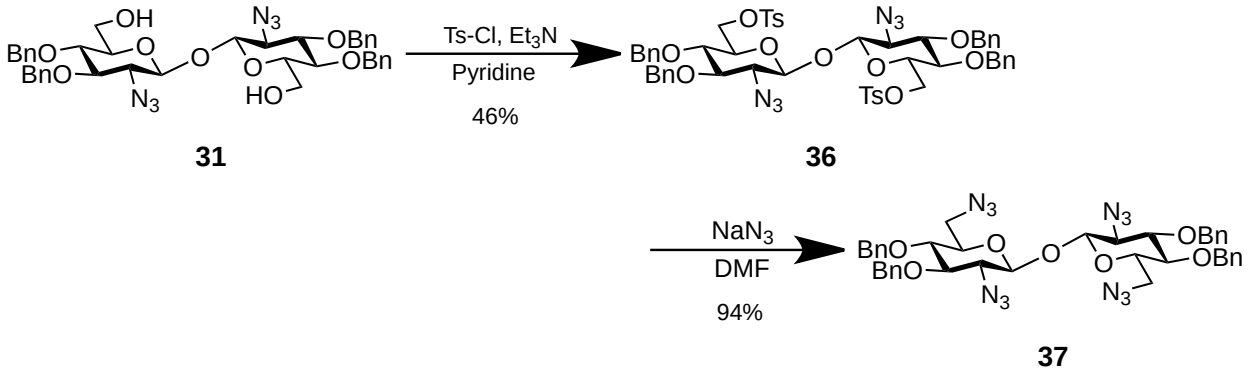
Scheme 3-2　Introduction of the azido group to the C6-position hydroxyl groups of **29**

Same as described above, C6-position hydroxyl group of **30** is tosylated at 75%, and the non-reducing disaccharide which has azido groups in the C6-position (**35**) was obtained at 76% (Scheme 3-3).



Scheme 3-3　Introduction of the azido group to the C6-position hydroxyl groups of **30**

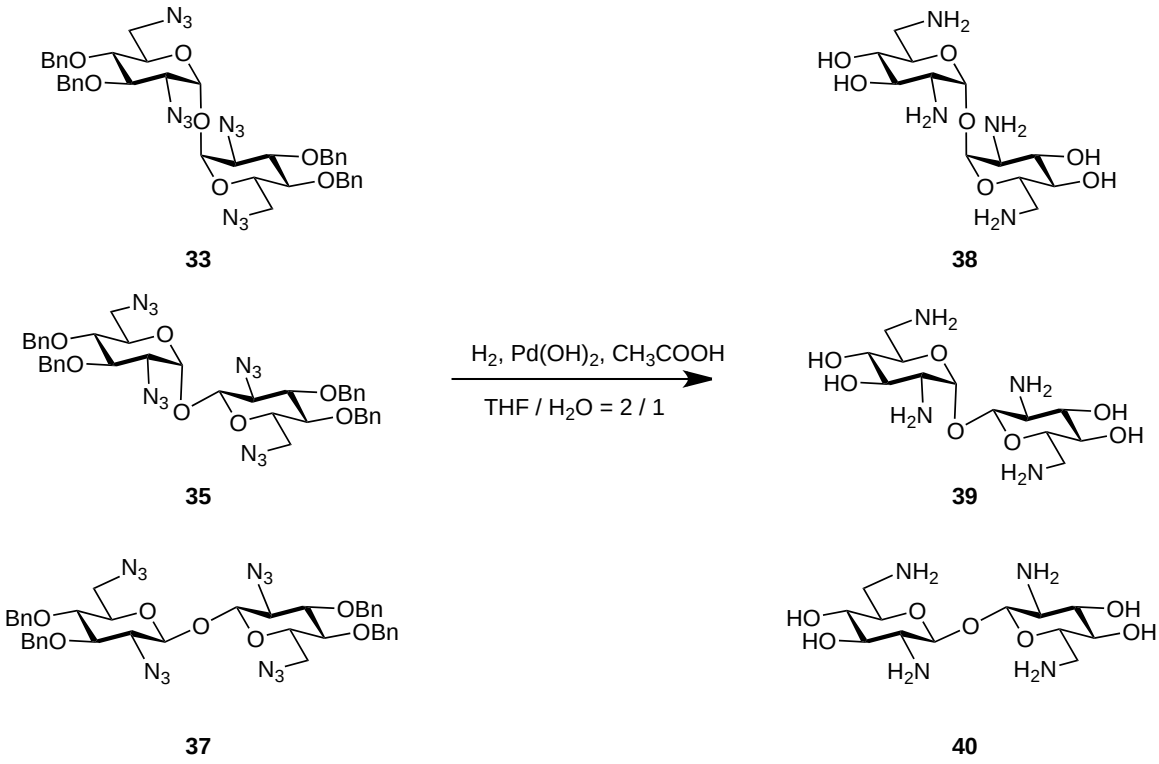
The compound **37** was synthesized with same manner as **33** and **35**. Similarly, 6-position hydroxyl group of **31** is tosylated at 46%, and the non-reducing disaccharide which has azido groups in the C6-position (**37**) was obtained at 94% (Scheme 3-4). At this time, it is thought that the yield of tosylation improved by examining reaction conditions, since the compound in which only one Ts basis was introduced was observed.



31 **36** **37**

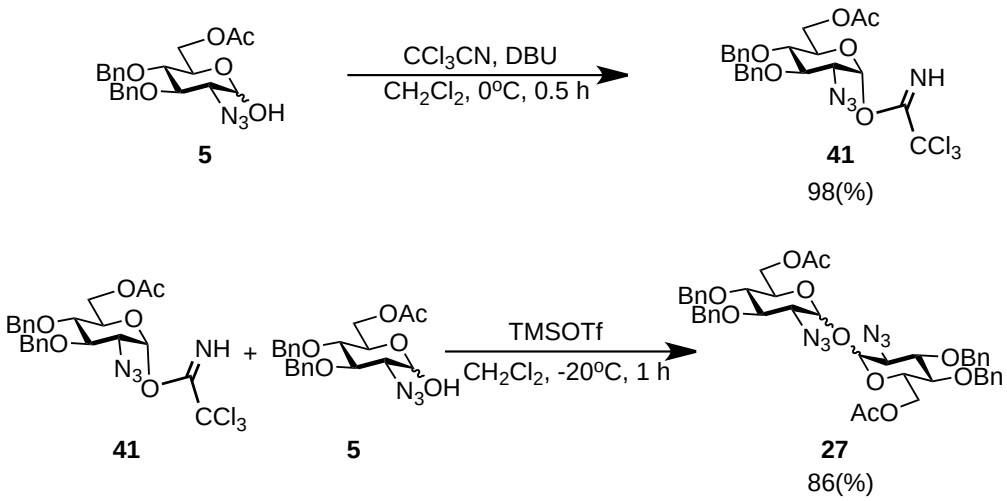
Scheme 3-4 Introduction of the azido group to the 6-position hydroxyl group of **31**

The azido groups and the benzyl groups of **33, 35, 37** were removed in the THF/H_2O using $Pd(OH)_2$ and CH_3COOH, and it obtained the trehalosamine as three structural isomers ($\alpha\alpha$(**38**), $\alpha\beta$(**39**), $\beta\beta$(**40**)) which have amino groups at the C2 and C6-positions (Scheme 3-5). Then the three non-reducing disaccharides introduced four amino groups were successfully obtained as neamine mimics.



Scheme 3-5　Consideration of deprotection

The yield of non-reducing disaccharide (**27**), synthesized with dehydrative glycosidation (described in Chap. 2) was low (Table 2-2 Entry 14 (17%), 15 (24%)). And so, when imidate was employed as a leaving group, the yield of compound **27** was obviously improved. Glycosyl imidate (**41**) was prepared in 98% yield from **5** using CCl_3CN and DBU. When the glycosylation of **41** with **5** was carried out using TMSOTf in CH_2Cl_2 at -20 ^oC for 1h, the desired non-reducing disaccharide **27** was obtained in 89% yield (Scheme 3-6). This reaction is not convenient compared with a dehydrative glycosydation, since it requires two steps. However, the yield was obviously improved, and it is thought that this method is a useful for the low reactive 1-hydroxy sugars.

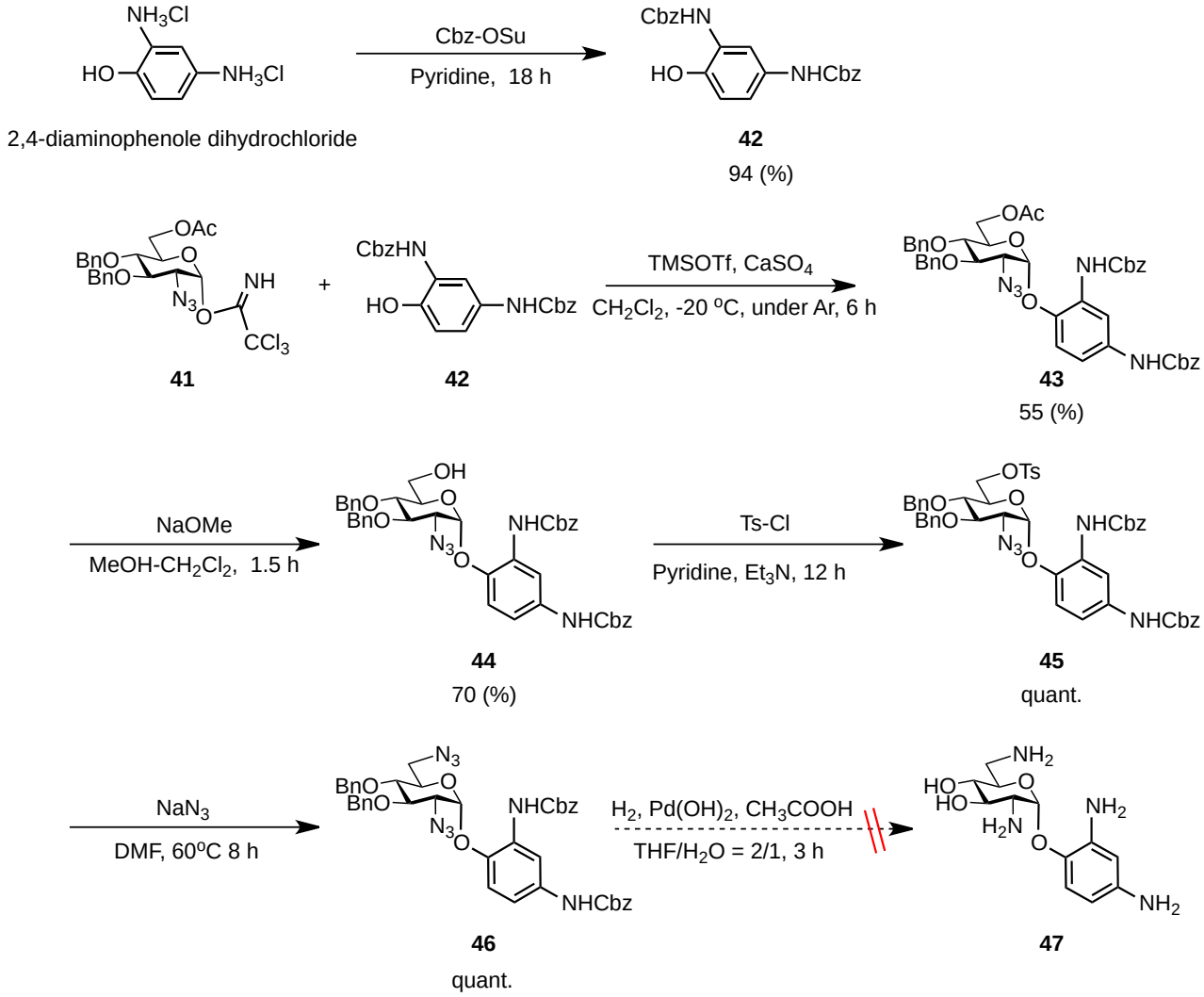


Scheme 3-6　The synthesis of non-reducing disaccharide using the imidate method

As describe above, I studied about introduction of the azido group and deprotection. And, the synthesis of novel non-reducing amino disaccharides (trehalosamine) as a neamine mimic was successfully achieved. In addition, the imidate method was also examined and the yield of glycosylation has been improved. Therefore, I succeeded in the composition of the novel small compound which is able to bind to hairpin RNA specifically. Then, I described the results of the binding affinity with RNAs in chapter 5-2-1.

3-2-2. The synthesis of novel aromatic modified amino saccharide (aromatic aminoglycoside)

In chapter 3–2–2, I describe about the synthesis of aromatic modified amino saccharide (Aromatic aminoglycoside) as a neamine mimic. Aromatic aminoglycoside (**47**) has an aromatic aglycone structure which replaces the 2-deoxystreptamine unit of neamine with 2,4-diaminophenol, and it is expected to have aromatic π-π stacking effects to increase the RNA-binding affinities.[5] There is only one report that described about the synthesis of an aromatic glycoside having more than one amino group in the monosaccharide moiety.[6] The synthetic route for the preparation of **47** is shown in Scheme 3-7. One of the major hurdle for synthesizing was the formation of the α-glycosidic linkage. I decided to make the linkage by the glycosylation procedure using **41** and **42.** Compound **42** was the glycosyl acceptor and **41** was the glycosyl donor. Compound **42** was prepared in 94% yield from 2,4-diaminophenol dihydrochloride using *N*-(benzyloxycarbonyloxy) succinimide in pyridine. When the glycosylation reaction of **41** with **42** was carried out using TMSOTf in CH_2Cl_2 at -20 °C for 6 h, the desired glycoside **43** was successfully obtained in 55% yield, and the glycosidic linkage of **43** was formed with an α-stereoselectivity. The α-stereoselectivity during the glycosidation would be explained by the effect of the acetyloxy group at C-6 position of **41**. Deprotection of the C-6 position acetyl group of **43** was performed using NaOMe in MeOH to afford **44** in 70% yield. The introduction of a tosyl group into the C-6 position of **44** was carried out using TsCl in pyridine to provide **45** with quantitative yield. The following reaction of **45** with sodium azide in DMF at 60 °C quantitatively gave **46**. However, aromatic aminoglycoside (**47**) could not be obtained by the hydrogenation of **46** which was the protected precursor of **47**. It is necessary to carry out more detailed examination about the conditions of of removal of the protection group.



Scheme 3-7　The synthesis of aromatic aminoglycoside

In conclusion, compound **46** which the precursor of the aromatic aminoglycoside (**47**) was successfully synthesized from *N*-acetyl-**D**-glucosamine and 1,3-diaminophenol based on the technique of the glycosylation. However, aromatic aminoglycoside (**47**), initially desired compound, could not be obtained by the hydrogenation of **46**. It is necessary to make a consideration of more detailed examination about the conditions of deprotection.

3-3. Conclusion

In this chapter, I described the synthetic approach to trehalosamine (3-2-1) and aromatic aminoglycoside (3-2-2) as the neamine mimics.

In chapter 3–2–1, I described about the synthesis of novel non-reducing amino disaccharides (trehalosamine). I studied about introduction of the azido group to the 6-position hydroxyl group of 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-D-glucopy -ranosyl-6'-*O*-acetyl-2'-azido-3',4'-di-*O*-benzyl-2'-deoxy-D-glucopyranoside (**27**) and the way of removing the protection group. And, I succeeded the synthesis of trehalosamine as three structural isomers ($\alpha\alpha$(**38**), $\alpha\beta$(**39**), $\beta\beta$(**40**)) which the 2,6-position is NH_2 in good yield. In addition, the synthetic route via imidate was also examined then yield of glycosylation has been improved.

In chapter 3–2–2, I describe about the synthesis of aromatic substituted amino saccharide (aromatic aminoglycoside). The precursor **46** of the aromatic aminoglycoside (**47**) was successfully synthesized from *N*-acetyl-D-glucosamine and 1,3-diaminophenol based on the glycosylation technique. However, aromatic aminoglycoside (**47**) could not be obtained by the hydrogenation.

Therefore, I describe the binding affinity and binding specificity of trehalosamine (three structural isomers $\alpha\alpha$(**38**), $\alpha\beta$(**39**), $\beta\beta$(**40**)) toward some specific RNA fragment.

3-4. Experimental Section

^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectra were recorded on a JEOL ECA-600 spectrometer in $CDCl_3$ using TMS as an internal standard. Optical rotations were recorded on a JASCO DIP-360 digital polarimeter. HRMS were obtained on a Mariner spectrometer (PerSeptive Biosystems Inc.). Preparative TLC was performed using Merck silica gel 60GF254. Column chromatography was conducted using silica gel 60 N (40~50 μm, Kanto Chemical Co., INC.). $Bi(OTf)_3$ was purchased from Sigma-Aldrich. All anhydrous solvents were purified according to standard methods.

2-Azido-3,4-di-*O*-benzyl-2-deoxy-6-hydroxy-α-D-glucopyranosyl-2'-azido-3',4'-di-*O*-benzyl-2'-deoxy-6'-hydroxy-α-D-glucopyranoside (29)

^{1}H NMR (600 MHz, $CDCl_3$): δ 3.49 (2H, dd, *J* = 4.1, 10.3 Hz, H-2), 3.68 (2H, dd, *J* = 8.9, 9.6 Hz, H-4), 3.75-3.82 (4H, m, H-6a and H-6b), 3.94 (2H, dt, *J* = 2.8, 9.6 Hz, H-5), 4.04 (2H, t, *J* = 9.6 Hz H-3), 5.11 (2H, d, *J* = 3.44 Hz H-1).

^{13}C NMR (150 MHz, $CDCl_3$): δ 61.3 (C-6), 63.2 (C-2), 72.3 (C-5), 77.5 (C-4), 80.2 (C-3), 93.2 (C-1).

$[\alpha]_D^{28.5}$ = +91.3°（C = 1.545, $CHCl_3$）

HRMS(ESI): *m/z* calcd for $C_{40}H_{44}N_6O_9Na^+$: 775.3062, found: 775.3047

2-Azido-3,4-di-*O*-benzyl-2-deoxy-6-hydroxy-α-D-glucopyranosyl-2'-azido-3',4'-di-*O*-benzyl-2'-deoxy-6'-hydroxy-β-D-glucopyranoside (30)

^{1}H NMR (600 MHz, $CDCl_3$): δ 3.37-3.42 (4H, m, H-2α, H-4α, H-4β, and H-5β), 3.44 (1H, d, H-2β), 3.48-3.51 (1H, m, H-3β), 3.53-3.59 (2H, m, H-6α-a and H-6α-b), 3.79 (1H, d, *J* = 12.4 Hz, H-6β-b), 3.83 (1H, d, J = 11.7 Hz, H-6β-a), 4.03 (1H, dd, J = 8.9, 9.6 Hz, H-3α), 4.11-4.14 (1H, m, H-5α), 4.44 (1H, d, J = 7.6 Hz, H-1β), 5.14 (1H, d, J = 3.4 Hz, H-1α).

^{13}C NMR (150 MHz, $CDCl_3$): δ 61.9 (C-6β), 62.3 (C-6α), 63.4 (C-2α), 66.8 (C-2β), 72.4 (C-5α), 76.4, 77.5, 78.8 (C-4α or C-4β or C-5β), 79.9 (C-3α), 83.1 (C-3β), 99.4 (C-1α), 102.1 (C-1β).

$[\alpha]_D^{28.5}$ = +22.1°（C = 4.345, $CHCl_3$）

HRMS(ESI): *m/z* calcd for $C_{40}H_{44}N_6O_9Na^+$: 775.3062, found: 775.3090

2-Azido-3,4-di-*O*-benzyl-2-deoxy-6-hydroxy-β-D-glucopyranosyl-2'-azido-3',4'-di-*O*-benzyl-2'-deoxy-6'-hydroxy-β-D-glucopyranoside (31)

^{1}H NMR (600 MHz, $CDCl_3$): δ 3.37 (2H, td, *J* = 2.9, 3.3, 9.1 Hz, H-4), 3.45-3.49 (4H, m, H-2 and H-5), 3.64 (2H, dt, *J* = 2.9, 9.3 Hz, H-3), 3.72-3.75 (2H, m, H-6a), 3.86-3.88 (2H, d, *J* = 12.0 Hz, H-6b), 4.71 (2H, dd, *J* = 2.2, 5.3 Hz, H-1).

^{13}C NMR (150 MHz, $CDCl_3$): δ 61.5 (C-6), 65.7 (C-2), 75.6 (C-4), 77.0 (C-3), 82.6 (C-5), 98.2 (C-1).

$[\alpha]_D^{28.0}$ = -10.9°（C = 1.835, $CHCl_3$）

HRMS(ESI): *m/z* calcd for $C_{40}H_{44}N_6O_9Na^+$: 775.3062, found: 775.3066

2-Azido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-tosyl-α-D-glucopyranosyl-2'-azido-3',4'-di-*O*-benzyl-2'-deoxy-6'-*O*-tosyl-α-D-glucopyranoside (32)

^{1}H NMR (600 MHz, $CDCl_3$): δ 2.41 (6H, s, S(OO)PhC*H*$_3$), 3.43 (2H, ddd, *J* = 3.4, 4.1, 10.3 Hz, H-2), 3.57 (2H, dd, *J* = 8.9, 10.3 Hz, H-4), 3.89 (2H, t, *J* = 9.6 Hz, H-3), 4.03-4.05 (2H, m, H-5), 4.03-4.05 (2H, m, H-5), 4.15 (2H, d, *J* = 10.3 Hz, H-6a), 4.29 (2H, dd, *J* = 3.4, 7.6 Hz, H-6b), 4.89 (2H, d, *J* = 3.4 Hz, H-1).

^{13}C NMR (150 MHz, $CDCl_3$): δ 63.0 (C-2), 68.0 (C-6), 70.0 (C-5), 77.3 (C-4), 80.2 (C-3), 93.1 (C-1).

$[\alpha]_D^{22.0}$ = +45.6°（C = 1.820, $CHCl_3$）

HRMS(ESI): *m/z* calcd for $C_{54}H_{56}N_6O_{13}S_2Na^+$: 1083.3239, found: 1099.3237

2-Azido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-tosyl-α-D-glucopyranosyl-2'-azido-3',4'-di-*O*-benzyl-2'-deoxy-6'-*O*-tosyl-β-D-glucopyranoside (34)

^{1}H NMR (600 MHz, $CDCl_3$): δ 2.36 (12H, s, S(OO)PhC*H*$_3$), 3.34-3.37 (2H, m, H-2α and H-2β), 3.40-3.42 (3H, m, H-5β, H-4β, and H-3β), 3.64 (1H, dd, *J* = 8.9, 10.3 Hz, H-4α), 3.94 (1H, dd, *J* = 4.1, 11.0 Hz, H-6α-a), 3.98 (1H, dd, J = 8.9, 10.3 Hz, H-3α), 4.12-4.14 (2H, m, H-5α and H-6β-α), 4.19 (1H, dd, *J* = 2.1, 11.0 Hz, H-6α-b), 4.29 (1H, dd, *J* = 3.4, 11.0 Hz, H-6β-b), 4.33 (1H, d, *J* = 8.2 Hz, H-1β), 5.08 (1H, d, *J* = 3.4 Hz, H-1α).

^{13}C NMR (150 MHz, $CDCl_3$): δ 21.6 (S(OO)Ph*C*H$_3$), 63.0 (C-2α), 66.4 (C-2β), 68.0 (C-6α), 68.1 (C-6β), 70.0 (C-5α), 73.0 (C-4β or C-5β), 76.4 (C-4β or C-5β), 77.0 (C-4α), 80.0 (C-3α), 82.9 (C-3β), 99.2 (C-1α), 101.0 (C-1β).

$[\alpha]_D^{28.0}$ ＝ +22.9°（C = 0.700, $CHCl_3$）

HRMS(ESI): *m/z* calcd for $C_{54}H_{56}N_6O_{13}S_2Na^+$: 1083.3239, found: 1099.3209

2-Azido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-tosyl-β-D-glucopyranosyl-2'-azido-3',4'-di-*O*-benzyl-2'-deoxy-6'-*O*-tosyl-β-D-glucopyranoside (36)

^{1}H NMR (600 MHz, $CDCl_3$): δ 2.42 (6H, s, S(OO)PhC*H*$_3$), 3.42-3.47 (8H, m, H-2, H-3, H-4, and H-5), 4.11 (2H, ddd, *J* = 2.1, 2.8, 11.0 Hz, H-6a), 4.20 (2H, d, *J* = 11.0 Hz, H-6b), 4.63 (2H, dd, *J* = 3.4, 4.1 Hz, H-1).

^{13}C NMR (150 MHz, $CDCl_3$): δ 21.7 (S(OO)Ph*C*H$_3$), 65.6 (C-2), 68.0 (C-6), 73.1 (C-4), 76.5 (C-3), 82.7 (C-5), 96.8 (C-1).

$[\alpha]_D^{28.5}$ ＝ -10.8°（C = 1.115, $CHCl_3$）

HRMS(ESI): *m/z* calcd for $C_{54}H_{56}N_6O_{13}S_2Na^+$: 1083.3239, found: 1099.3286

2,6-Diazido-3,4-di-*O*-benzyl-2,6-dideoxy-α-D-glucopyranosyl-2'6'-diazido-3',4'-di-*O*-benzyl-2'6'-dideoxy-α-D-glucopyranoside (33)

^{1}H NMR (600 MHz, $CDCl_3$): δ 3.37 (2H, dd, *J* = 4.8, 13.1 Hz, H-6a), 3.52 (2H, dd, *J* = 2.1,

13.1 Hz, H-6b), 3.51-3.60 (4H, m, H-2 and H-4), 3.98 (2H, dd, *J* = 8.9, 10.3 Hz, H-3), 4.10-4.13 (2H, m, H-5), 5.16 (2H, d, *J* = 3.44 Hz H-1).

^{13}C NMR (150 MHz, $CDCl_3$): δ 50.9 (C-6), 63.3 (C-2), 71.3 (C-5), 78.6 (C-4), 80.3 (C-3), 93.4 (C-1).

$[\alpha]_D^{22.0}$ = +100.4°（C = 1.125, $CHCl_3$）

HRMS(ESI): *m/z* calcd for $C_{40}H_{42}N_{12}O_7Na^+$: 825.3192, found: 825.3224

2,6-Diazido-3,4-di-*O*-benzyl-2,6-dideoxy-α-D-glucopyranosyl-2'6'-diazido-3',4'-di-*O*-benzyl-2'6'-dideoxy-β-D-glucopyranoside (35)

^{1}H NMR (600 MHz, $CDCl_3$): δ 3.30 (1H, dd, *J* = 4.8, 13.1 Hz, H-6α-b), 3.36 (1H, dd, *J* = 4.8, 13.7 Hz, H-6α-a), 3.45-3.50 (5H, m, H-2α, H-2β, H-3β, H-4β, and H-6β-a), 3.54-3.59 (3H, m, H-4α, H-5β, and H-6β-b), 4.03 (1H, dd, *J* = 8.9, 10.3 Hz, H-3α), 4.16-4.19 (1H, m, H-5α), 4.46 (1H, dd, *J* = 2.8, 5.5 Hz, H-1β), 5.18 (1H, d, *J* = 3.4 Hz, H-1α).

^{13}C NMR (150 MHz, $CDCl_3$): δ 50.8 (C-6α), 50.9 (C-6β), 63.4 (C-2α), 66.7 (C-2β), 71.7 (C-5α), 74.7 (C-4β), 77.5, 78.5 (C-4α or C-5β), 79.9 (C-3α), 82.9 (C-3β), 99.1 (C-1α), 101.8 (C-1β).

$[\alpha]_D^{27.0}$ = +76.4°（C = 2.290, $CHCl_3$）

HRMS(ESI): *m/z* calcd for $C_{40}H_{42}N_{12}O_7Na^+$: 825.3192, found: 825.3227

2,6-Diazido-3,4-di-*O*-benzyl-2,6-dideoxy-β-D-glucopyranosyl-2'6'-diazido-3',4'-di-*O*-benzyl-2'6'-dideoxy-β-D-glucopyranoside (37)

^{1}H NMR (600 MHz, $CDCl_3$): δ 3.30-3.37 (4H, m, H-6a and H-6b), 3.45-3.49 (4H, m, H-3, and H-4), 3.51-3.58 (4H, m, H-2 and H-5), 4.79 (2H, d, *J* = 8.3 Hz, H-1).

^{13}C NMR (150 MHz, $CDCl_3$): δ 50.9 (C-6), 65.8 (C-2), 75.4 (C-5), 77.9 (C-4), 82.8 (C-3), 96.8 (C-1).

$[\alpha]_D^{28.0}$ = -18.8°（C = 0.640, $CHCl_3$）

HRMS(ESI): *m/z* calcd for $C_{40}H_{42}N_{12}O_7Na^+$: 825.3192, found: 825.3150

6-*O*-Acetyl-2-azido-3,4-di-*O*-benzyl-1-*O*-(2,2,2-trichloroacetimidoyl)-2-deoxy-α-D-glucopyranose (41)

To a stirred solution of 6-*O*-Acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranose (325 mg, 0.76 mmol) and in CH_2Cl_2 (2 mL) was added 2,2,2-trichloroacetonitrile (380 µL, 3.8 mmol) and 1,8-diazabicyclo[5.4.0]undeca-7-ene (18 µL, 0.11 mmol) at 0 °C under Ar atmosphere. The above solution was stirred at 0 °C for 1 h, then concentrated and purified using a flash silica gel column chromatography (1:5 AcOEt-hexane) to give **41** (426 mg, 98% yield) as a colorless oil.

^{1}H NMR ($CDCl_3$): δ 2.01 (3H, s, CH_3), 3.68 (1H, t, *J* = 9.6 Hz, H-4), 3.69 (1H, dd, *J* = 3.4 Hz, *J* = 9.6 Hz, H-2), 4.05-4.08 (1H, m, H-5), 4.06 (1H, t, *J* = 9.6 Hz, H-3), 4.24 (1H, dd, *J* = 4.1 Hz, *J* = 12.4 Hz, H-6a), 4.30 (1H, dd, *J* = 2.1 Hz, *J* = 12.4 Hz, H-6b), 4.60 (1H, d, *J* = 11.0 Hz, CH_2Ph), 4.88 (1H, d, *J* = 11.0 Hz, CH_2Ph), 4.94 (2H, s, CH_2Ph), 6.41 (1H, d, *J* = 3.4 Hz, H-1), 7.26-7.41 (10H, m, Ph), 8.74 (1H, s, NH)

^{13}C NMR ($CDCl_3$): δ 20.7 (CH_3), 62.2 (C-6), 63.1 (C-2), 71.7 (C-5), 75.3 (CH_2Ph), 75.6 (CH_2Ph), 77.3 (C-4), 80.2 (C-3), 90.8 (CCl_3), 94.5 (C-1), 128.1-137.4 (Ph), 160.7 (OC(NH)), 170.5 (C=O)

HRMS (ESI): m/z calcd for $C_{24}H_{25}N_4O_6$•Na^+: 593.0732, found: 593.0721.

2,4-Bis(benzyloxycarbonylamido)phenol (42)

To a stirred solution of 2,4-diaminophenol dihydrochloride (107 mg, 0.54 mmol) in pyridine (5 mL) was added *N*-(benzyloxycarbonyloxy)succinimide (402 mg, 1.6 mmol). After stirring for 18 h, a 30% citric acid solution (5 mL) was added to the reaction mixture. The resulting mixture was extracted with CH_2Cl_2 (5 mL), and the organic layer was washed with water and a sat. NaCl solution. After the organic layer was dried over anhydrous Na_2SO_4, the solvent was filtered and evaporated under reduced pressure.

The crude product was purified using a preparative silica gel TLC (1:7 AcOEt-benzene) to give **42** (200 mg, 94% yield) as a white solid. mp 172-174 °C

^{1}H NMR (DMSO): δ 6.51 (2H, s, CH_2), 6.55 (2H, s, CH_2), 8.14-9.25 (13H, m, Ph)

^{13}C NMR (DMSO): δ 66.9 (CH_2), 67.4 (CH_2), 112.6-143.7 (Ph), 154.9 (C=O), 155.6 (C=O).

Dibenzyl 4-(6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyloxy) -1,3-phenylenedicarbamate (43)

To a stirred solution of **42** (173 mg, 0.44 mmol) and **41** (167 mg, 0.29 mmol) in CH_2Cl_2 (4 mL) was added TMSOTf (53 μL, 0.29 mmol) in the presence of anhydrous $CaSO_4$ (ca. 100 mg) at -20 °C under Ar atmosphere. After the reaction mixture was stirred for 6 h, the reaction was then quenched by addition of a sat. $NaHCO_3$ solution (5 mL). The reaction mixture was extracted with CH_2Cl_2 (5 mL), and the organic layer was washed with water and a sat. NaCl solution. After the organic layer was dried over anhydrous Na_2SO_4, the solvent was filtered and evaporated under reduced pressure. The crude product was purified using a preparative silica gel TLC (1:2 AcOEt-hexane) to give **43** (129.5 mg, 55% yield) as a colorless oil.

$[\alpha]_D^{25.0}$ = +43° (*c* 2.4, $CHCl_3$)

^{1}H NMR ($CDCl_3$): δ 2.16 (3H, s, CH_3), 3.61 (1H, dd, *J* = 8.9 Hz, *J* = 9.6 Hz, H-4), 3.69 (1H, dd, *J* = 4.1 Hz, *J* = 9.6 Hz, H-2), 4.11 (1H, dd, *J* = 8.9 Hz, *J* = 9.6 Hz, H-3), 4.20-4.23 (1H, m, H-5), 4.26 (1H, dd, *J* = 5.5 Hz, *J* = 11.7 Hz, H-6a), 4.36 (1H, dd, *J* = 2.1 Hz, *J* = 11.7 Hz, H-6b), 5.01 (1H, d, *J* = 4.1 Hz, H-1), 6.95-8.09 (22H, m, Ph, H-5' or H-6'), 7.03 (1H, d, *J* = 8.2 Hz, H-5' or H-6')

^{13}C NMR ($CDCl_3$): δ 20.7 (CH_3), 62.6 (C-6), 64.1 (C-2), 66.9 (CH_2Ph), 70.3 (C-5), 75.1 (CH_2Ph), 75.8 (CH_2Ph), 77.8 (C-4), 81.0 (C-3), 99.6 (C-1), 120.1 (C-5' or C-6'), 127.9-137.2 (Ph, C-5' or C-6'), 153.2 (C=O), 163.6 (C=O), 170.6 (C=O)

HRMS (ESI): m/z calcd for $C_{44}H_{43}N_5O_{10}$•Na^+: 824.2902, found: 824.2869.

Dibenzyl 4-(2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyloxy)-1,3-pheny-lenedicarbamate (44)

To a solution of **43** (62 mg, 0.077 mmol) in MeOH (20 mL)-CH_2Cl_2 (0.5 mL) were added a 28% sodium methylate methanol solution (0.3 mL, 0.0016 mmol) at rt. After stirring 1.5 h, water (5 mL) was added to the reaction mixture. The resulting mixture was extracted with AcOEt (5 mL), and the organic layer was washed with water and brine. After the organic layer was dried over anhydrous Na_2SO_4, the organic solvent was filtered and evaporated under reduced pressure. The crude product was purified using a preparative silica gel TLC (1:1 AcOEt-hexane) to give **44** (41 mg, 70% yield) as a colorless oil.

$[\alpha]_D^{27.0}$ = +41° (*c* 1.5, $CHCl_3$)

^{1}H NMR ($CDCl_3$): δ 3.65 (1H, dd, *J* = 3.4 Hz, *J* = 9.6 Hz, H-2), 3.72 (1H, t, *J* = 9.6 Hz, H-4), 3.79 (1H, bd, *J* = 11.7 Hz, H-6a), 3.88 (1H, bd, *J* = 12.4 Hz, H-6b), 4.02-4.09 (1H, m, H-5), 4.11 (1H, t, *J* = 9.6 Hz, H-3), 4.99 (1H, d, *J* = 3.4 Hz, H-1), 6.95-8.09 (22H, m, Ph, H-5' or H-6'), 6.98 (1H, d, *J* = 8.2 Hz, H-5' or H-6')

^{13}C NMR ($CDCl_3$): δ 61.2 (C-6), 64.2 (C-2), 66.9 (CH_2Ph), 72.6 (C-5), 75.1 (CH_2Ph), 75.8 (CH_2Ph), 77.5 (C-4), 80.8 (C-3), 99.8 (C-1), 120.3 (C-5' or C-6'), 127.8-137.6 (Ph, C-5' or C-6'), 153.1 (C=O), 153.2 (C=O)

HRMS (ESI): m/z calcd for $C_{42}H_{41}N_5O_9$•Na^+: 782.2796, found: 782.2776.

Dibenzyl 4-(2-azido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-tosyl-α-D-glucopyranosyloxy)-1,3-phenylenedicarbamate (45)

To a solution of **44** (40 mg, 0.053 mmol) and TsCl (201 mg, 1.1 mmol) in pyridine (5 mL) was added Et_3N (147 μL, 1.1 mmol) at rt. After stirring for overnight, a solution of 30% citric acid (5 mL) was added to the reaction mixture. The resulting mixture was extracted with CH_2Cl_2 (5 mL). After the organic layer was dried over anhydrous Na_2SO_4, the

solvent was filtered and evaporated under reduced pressure. The crude product was purified using a preparative silica gel TLC (1:2 AcOEt-hexane) to give **45** (48 mg, quantitative yield) as a colorless oil.

$[\alpha]_D^{25.0}$ = +40° (*c* 1.2, $CHCl_3$)

^{1}H NMR ($CDCl_3$): δ 2.40 (3H, s, CH_3), 3.61 (1H, dd, *J* = 3.4 Hz, *J* = 9.6 Hz, H-2), 3.64 (1H, t, *J* = 8.9 Hz, H-4), 4.05 (1H, t, *J* = 9.6 Hz, H-3), 4.10-4.14 (1H, m, H-5), 4.24 (1H, bd, *J* = 10.3 Hz, H-6a), 4.29 (1H, dd, *J* = 4.1 Hz, *J* = 11.0 Hz, H-6b), 4.94 (1H, d, *J* = 3.4 Hz, H-1), 6.67-8.02 (24H, m, Ph, H-5' or H-6'), 6.86 (1H, d, *J* = 8.9 Hz, H-5' or H-6') 7.77 (2H, d, *J* = 7.6 Hz, $SO_2C_6H_4$)

^{13}C NMR ($CDCl_3$): δ 21.6 (CH_3), 63.9 (C-2), 66.9 (CH_2Ph), 67.7 (C-6 and CH_2Ph), 70.1 (C-5), 75.1 (CH_2Ph), 75.8 (CH_2Ph), 77.2 (C-4), 80.7 (C-3), 99.6 (C-1), 119.9 (C-5' or C-6'), 127.8-137.2 (Ph, C-5' or C-6'), 152.7 (C=O), 153.1 (C=O)

HRMS (ESI): m/z calcd for $C_{49}H_{47}N_5O_{11}$•Na^+: 936.2885, found: 936.2877.

Dibenzyl 4-(2,6-azido-3,4-di-*O*-benzyl-2,6-dideoxy-α-D-glucopyranosyloxy)-1,3-phenylenedicarbamate (46)

To a solution of **45** (45 mg, 0.049 mmol) in DMF (5 mL) was added NaN_3 (16 mg, 0.24 mmol). After stirring at 60 °C for 8 h, water (5 mL) was added to the reaction mixture. The resulting mixture was extracted with EtOAc (5 mL). After the organic layer was dried over anhydrous Na_2SO_4, the solvent was filtered and evaporated under reduced pressure. The crude product was purified using a preparative silica gel TLC (1:3 AcOEt-hexane) to give **46** (39 mg, quantitative yield) as a colorless oil.

$[\alpha]_D^{25.0}$ = +52° (*c* 0.91, $CHCl_3$)

^{1}H NMR ($CDCl_3$): δ 3.40 (1H, dd, *J* = 5.5 Hz, *J* = 13.1 Hz, H-6a), 3.57 (1H, dd, *J* = 2.7 Hz, *J* = 13.1 Hz, H-6b), 3.64 (1H, dd, *J* = 8.9 Hz, *J* = 9.6 Hz, H-4), 3.70 (1H, dd, *J* = 3.4 Hz, *J* = 9.6 Hz, H-2), 4.09 (1H, t, *J* = 9.6 Hz, H-3), 4.13-4.15 (1H, m, H-5), 5.05 (1H, d, *J* = 3.4 Hz, H-1), 6.69-8.05 (22H, m, Ph, H-5' or H-6'), 7.04 (1H, d, *J* = 8.2 Hz, H-5' or H-6')

^{13}C NMR ($CDCl_3$): δ 51.0 (C-6), 64.1 (C-2), 66.9 (CH_2Ph), 71.7 (C-5), 75.2 (CH_2Ph), 75.9 (CH_2Ph), 78.4 (C-4), 80.7 (C-3), 99.7 (C-1), 120.0 (C-5' or C-6'), 127.7-137.4 (Ph, C-5' or C-6'), 153.1 (C=O), 153.2 (C=O)

HRMS (ESI): m/z calcd for $C_{42}H_{40}N_8O_8 \bullet Na^+$: 807.2861, found: 807.2892.

3-5. Reference and Notes

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Chapter 4　Design and synthesis of novel nucleobase modified neamines

4-1.　Introduction

4-1-1. Nucleobase modified neamines with a lysine or an arginine as a linker, nucleobase substituted on the α-amino group of the amino acid.

On the binding of aminoglycoside antibiotics (Figure 1-8) to the A-site of 16S rRNA, it was reported that 6'-amino group of the compound is necessary.[1] However modified neamine at 6'-amino group bound to either TAR or RRE RNA.[2,3,4] Therefore, our group have been focusing on the modification at the 6'-amino group of neamine. Then, we have chosen TAR and RRE RNA as the targets and designed compounds that having an adenine (Ab), a cytosine (Cb), a guanine (Gb) or a thymine (Tb) at the 6'-amino group with L-lysine (NbKα-neamine) or L-arginine (NbRα-neamine) as the linker (Figure 4-1).

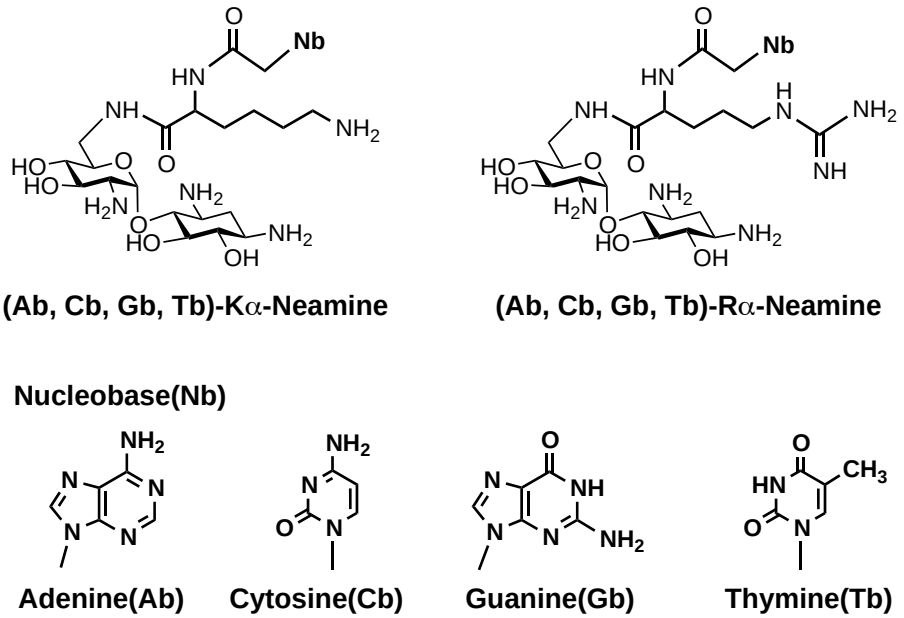


Figure 4-1　Nucleobase modified neamines with a lysine or an arginine as a linker, nucleobase substituted on the α-amino group of the amino acid.

For the evaluation of the NbKα-neamine and NbRα-neamine as the binding inhibitor for TAR RNA, fluorescently labeled Tat peptide, FTatRhd[5] was employed as the binding tracer (the detailed method for the evaluation of the binding is described in **5-1-2**). The dissociation constants (Kd) of those neamine derivatives are summarized in Table 1. No matter what kind of nucleobase, modified neamines with lysine as a linker have 1.7-1.9 μM as dissociation constants. These Kd values were 10 times smaller than that of lysine-modified neamine (K-neamine, Kd = 19 μM) and 16 times smaller than that of neamine. These results suggest that the nucleobase moiety in the neamine derivatives can not interact with specific base in the RNA when the nucleobase was introduced at the α-amino group of the linker amino acid. The Kd of arginine-modified neamine (R-neamine) was 0.60 μM. On the other hand, nucleobase-modified neamines with arginine as a linker have 0.25-0.54 μM as Kd. These results were very close to that of R-neamine, and 100 times smaller than that of neamine itself (Table 4-1).[2]

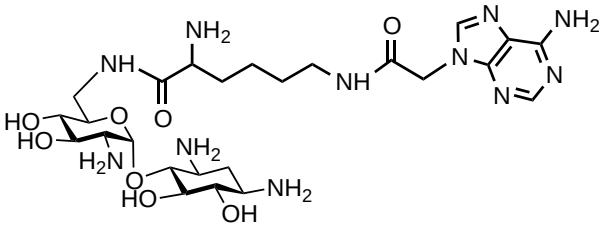
Table 4-1 Dissociation constants(Kd) of the neamine, K-neamine, NbKα-neamine R-neamine and NbRα-neamine for TAR RNA

Compounds	Kd (μM)
Neamine	28
K-Neamine	19
AbKα-Neamine	1.8
CbKα-Neamine	1.9
GbKα-Neamine	1.8
TbKα-Neamine	1.7
R-Neamine	0.60
AbRα-Neamine	0.29
CbRα-Neamine	0.29
GbRα-Neamine	0.25
TbRα-Neamine	0.54

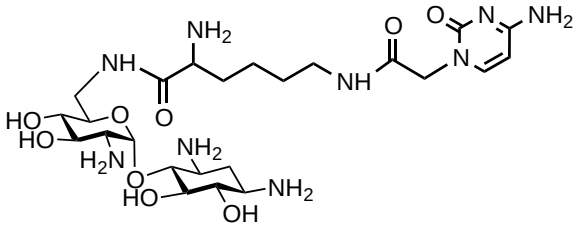
As described above, It was revealed that the binding affinities of neither NbKα-neamine nor NbRα-neamine for TAR depended on the nucleobases. Therefore, in this study, I have decided that the nucleobase is introduced at the ε-amino group of the L-lysine (NbKε-neamine).

4-1-2. Nucleobase modified neamines with a lysine as a linker, nucleobase substituted on the ε-amino group of the amino acid.

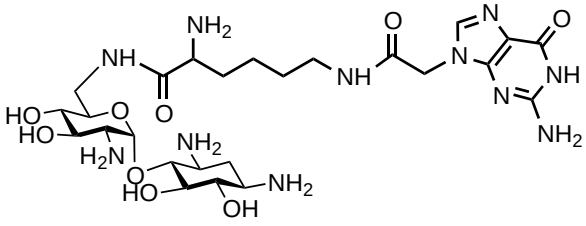
Nucleobase modified neamine at the C6-amino group of the neamine with lysine as a linker via conjugation at the ε-amino group of the lysine (NbKε-neamines) were designed which have an adenine (Ab), a cytosine (Cb), a guanine (Gb) or a thymine (Tb) (Figure 4-2). An appropriate distance between the nucleobase and the neamine might make difference of binding affinity that depend on the nucleobase.



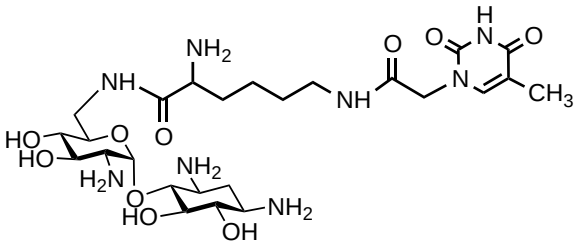
AbKε-Neamine



CbKε-Neamine



GbKε-Neamine



TbKε-Neamine

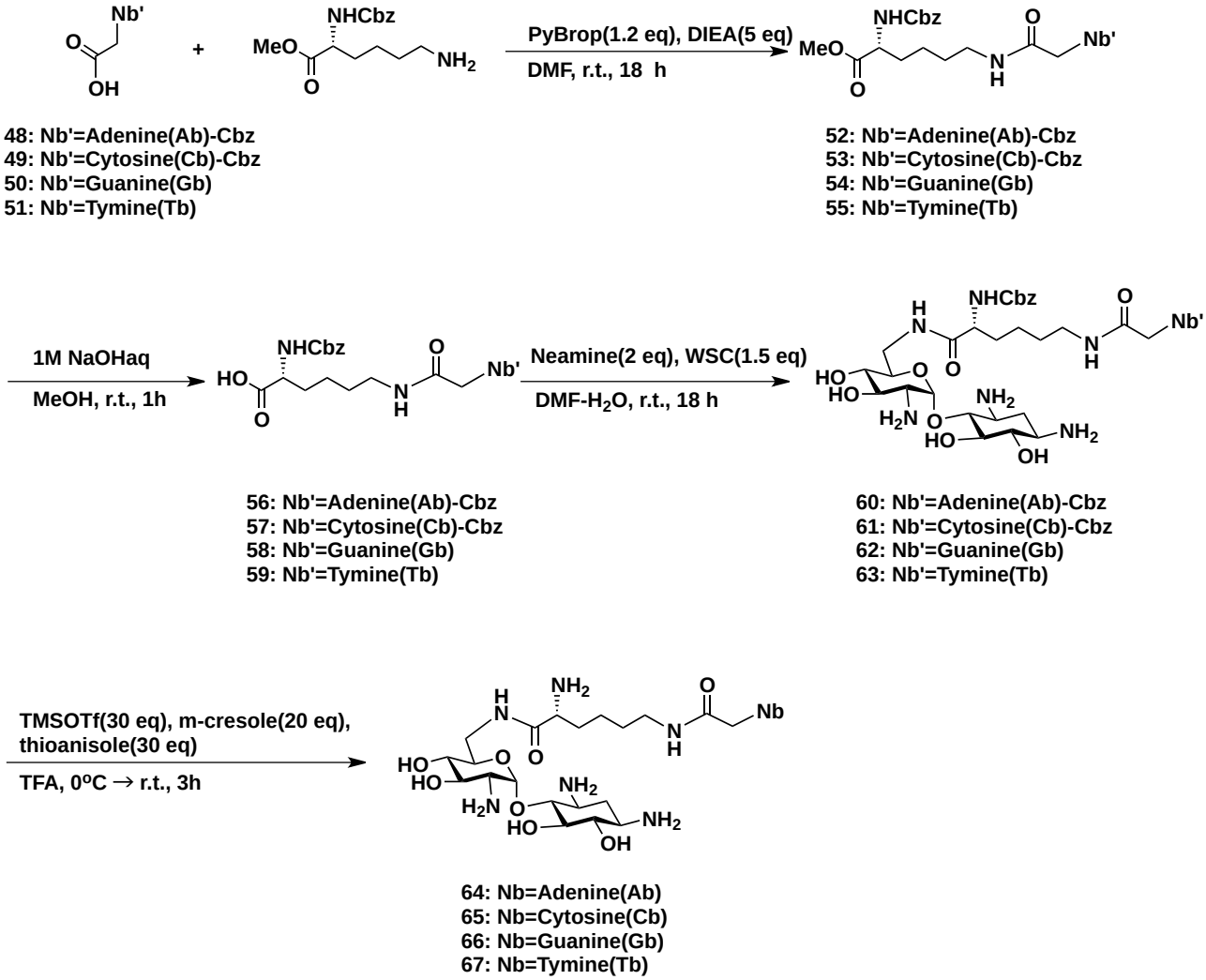
Figure 4-2 Nucleobase modified neamines with a lysine as a linker, nucleobase substituted on the ε-amino group of the amino acid.

4-2. Results and Discussion

Synthesis of adenine, guanine, cytosine and thymine-Kε-neamine

In chapter 4–2, I describe about the synthesis of adenine(Ab), guanine(Gb), cytosine(Cb) and thymine(Tb)-Kε-Neamine as a novel nucleobase modified neamine. The nucleobases conjugated acetic acid (N^6-Cbz-adenine-9-yl)-, (N^4-Cbz-cytosine-1-yl)-, and thymine-1-yl-acetic acid (**48**, **49**, and **50**, respectively) were prepared by a previously reported method.[6] These purine or pyrimidine derivatives (**48-51**) were introduced to the ε-amino group of lysine via amide bond using PyBrOP as a coupling reagent and in the presence of DIEA in DMF at r.t. for 18 hours, the desired compounds (**52-55)** were successfully obtained in 55-82% yield. After deprotection of the α-carbonyl group, nucleobase conjugated lysine unit (**56-59**) was introduced to the 6'-amino group of the neamine by the condensation reaction using WSC as a coupling reagent in DMF-H_2O at room temperature for 18 hours, the desired glycosides (**60-63**) were successfully obtained in 5-25% yield. Finally, after all the protective groups were removed, the compounds were purified by reversed phase HPLC and characterized by MALDI-TOFMS and ^{1}H NMR (Scheme 4-1).

Then the four novel nucleobase modified neamine with L-lysine as the linker (NbKε-neamine, **64-67**) were successfully obtained as the novel small compound expected to bind to the hairpin RNA specifically.



Scheme 4-1　Synthetic route of nucleobase modified neamines (NbKε-neamines)

4-3. Conclusion

In this chapter, I described the synthetic approach to nucleobase modified neamines with a lysine as a linker, nucleobase substituted on the ε-amino group of the amino acid (NbKε-namines).

NbKε-neamines having an adenine (Ab), a cytosine (Cb), a guanine (Gb) or a thymine (Tb) at the 6'-amino group with L-lysine as the linker were designed. The nucleobase was introduced at the ε-amino group of the L-lysine, due to the binding affinities for TAR were no difference when the nucleobase was conjugated to the α-amino group of lysine or an arginine as a linker. An appropriate distance between the nucleobase and the neamine might clarify the binding specificity derived from the difference of nucelobases. Then the four novel nucleobase modified neamine with L-lysine as the linker (NbKε-neamine, Ab(**64**), Cb(**65**), Gb(**66**), Tb(**67**)) were successfully obtained as the novel small compound expected to bind to the hairpin RNA specifically.

Then, I will describe about the binding affinity and binding specificity of four NbKε-neamine, ((Ab(**64**), Cb(**65**), Gb(**66**), Tb(**67**)) toward RNA fragments derived from HIV-1 activator region.

4-4. Experimental Section

Amino acids were purchased either from Novabiochem or Kokusan Chemical Co., Ltd. All other chemicals were from Wako Pure Chemicals. HPLC was carried out with HITACHI L7100 pump and L7400 UV detector on YMC-Pack Pro C18 RS column. ^{1}H NMR spectra were recorded with a JEOL JNM-LA300 or JNM-ECS400 spectrometer. Mass spectra were recorded with Shimadzu Axima CFR plus.

Neamine

Neamine was obtained by the hydrolysis of neomycin[7] and the counter ion chlorine was removed by ion exchange chromatography with Sephadex QAE A-25

Nucleobase-conjugated amino acids

For the conjugation of the nucleobase unit with lysine, an acetic acid unit was introduced to the nucleobase unit. This unit was then conjugated to the amino acid by the con- densation reaction. Nucleobase-acetic acid derivatives (N^6-Cbz-adenine-9-yl)-, (N^4-Cbz-cytosine-1-yl)-, guanine-1-yl-acetic acid, and thymine-1-yl-acetic acid (**48**, **49**, **50**, and **51**, respectively), were prepared according to the previously reported method.[8]

Methyl-$^{\alpha}$*N*-(benzyloxycarbonyl)-$^{\varepsilon}$*N*-[[6-*N*-(benzyloxy carbonyl)-adenine-9-yl]acetyl] lysinate (Cbz-Lys(Cbz(Cbz-Lys(Cbz-Ab -OMe)))) (52)

To a stirred suspension of **48** (0.101 g, 0.309 mmol) in DMF (3.0 ml) was added Cbz-Lys-OMe HCl (0.0771 g, 0.233 mmol). After cooling to 0 °C, to this suspension was added a solution of PyBrOP (0.174 g, 0.373 mmol) in DMF (1.0 ml) and DIEA (0.160 ml, 0.904 mmol) in small portion. After stirring for 2 h in an ice bath, the reaction mixture was stirred for another 19 h at room temperature. The reaction mixture was poured into cold water (60 ml) and stored in a refrigerator overnight. The precipitate was collected on the

filter, washed with water, and dried in vacuum. The desired product **52** was obtained as a white powder (0.0989 g, 0.164 mmol, yield 70.0%).

*R*f = 0.80 ($CHCl_3$/MeOH = 3/2)

^{1}H NMR (in d_6-DMSO, 300 MHz) δ 10.7 (br s, 1H), 8.59 (s, 1H), 8.37 (s, 1H), 8.35 (t, 1H), 7.73 (d, 1H), 7.75-7.29 (m, 10H), 5.22 (s, 2H), 5.04 (s, 2H), 4.92 (s, 2H), 4.01 (q, 1H), 3.63 (s, 3H), 3.07 (q, 2H), 1.64 (m, 2H), 1.39 (m, 4H).

Methyl $^{\alpha}$*N*-(benzyloxycarbonyl)-$^{\varepsilon}$*N*-[[4-*N*-(benzyloxycar -bonyl)-cytosine-1-yl] acetyl]lysinate (Cbz-Lys(Cbz-Cb) -OMe) (53)

To a stirred suspension of **49** (0.240 g, 0.791 mmol) in DMF (7.8 ml) was added Cbz-Lys-OMe HCl (0.175 g, 0.527 mmol). After cooling to 0 °C, to this suspension was added a solution of PyBrOP (0.282 g, 0.633 mmol) in DMF (7.8 ml) and DIEA (0.360 ml, 2.10 mmol) in small portion. After stirring for 2 h in an ice bath, the reaction mixture was stirred for another 22 h in room temperature. The reaction mixture was poured into cold water (100 ml) and stored in a refrigerator overnight. The precipitate was collected on the filter, washed with water, and dried in vacuum. The desired product **53** was obtained as a white powder (0.250 g, 0.431 mmol, yield 81.7%).

*R*f = 0.81 ($CHCl_3$/MeOH = 3/2)

^{1}H-NMR (in d_6-DMSO, 300 MHz) δ 10.7 (s, 1H), 8.16 (t, 1H), 7.96 (d, 1H), 7.73 (d, 1H), 7.40 (m, 5H), 7.36 (m, 5H), 6.99 (d, 1H), 5.19 (s, 2H), 5.04 (s, 2H), 4.41 (s, 2H), 4.00 (q, 1H), 3.63 (s, 3H), 3.05 (q, 2H), 1.62 (m, 2H), 1.36 (m, 4H).

Methyl $^{\alpha}$*N*-(benzyloxycarbonyl)-$^{\varepsilon}$*N*-[(guanine-9-yl)acetyl]lysinate (Cbz-Lys(Gb)-OMe) (54)

To a stirred suspension of **50** (0.101 g, 0.483 mmol) in DMF (3.0 ml) was added Cbz-Lys-OMe HCl (0.143 g, 0.432 mmol). After cooling to 0 °C, to this suspension was added a solution of PyBrOP (0.286 g, 0.612 mmol) in DMF (1.0 ml) and DIEA (0.270 ml,

1.53 mmol) in small portion. After stirring for 2 h in an ice bath, the reaction mixture was stirred for another 22 h in room temperature. The reaction mixture was poured into cold water (60 ml) and stored in a refrigerator overnight. The precipitate was collected on the filter, washed with water, and dried in vacuum. The desired product **54** was obtained as a white powder (0.105 g, 0.216 mmol, yield 50.0%).

*R*f = 0.75 ($CHCl_3$/MeOH = 3/2)

^{1}H NMR (in d_6-DMSO, 300 MHz) δ 10.6 (br s, 1H), 8.22 (t, 1H), 8.04 (s, 1H), 7.74 (d, 1H), 7.37-7.31 (m, 5H), 6.90 (br s, 2H), 5.03 (s, 2H), 4.72 (s, 2H), 3.99 (m, 1H), 3.63 (s, 3H), 3.05 (q, 2H), 1.64 (m, 2H), 1.36 (m, 4H).

Methyl $^{\alpha}$*N*-(benzyloxycarbonyl)-$^{\varepsilon}$*N*-[(thymine-1-yl) acetyl]lysinate (Cbz-Lys(Tb)-OMe) (55)

To a stirred suspension of **51** (0.116 g, 0.631 mmol) in DMF (3.0 ml) was added Cbz-Lys-OMe HCl (0.140 g, 0.422 mmol). After cooling to 0 °C, to this suspension was added a solution of PyBrOP (0.225 g, 0.505 mmol) in DMF (1.0 ml) and DIEA (0.290 ml, 1.69 mmol) in small portion. After stirring for 2 h in an ice bath, the reaction mixture was stirred for another 22 h in room temperature. The reaction mixture was poured into cold water (50 ml) and stored in a refrigerator overnight. The precipitate was collected on the filter, washed with water, and dried in vacuum. The desired product **55** was obtained as a white powder (0.127 g, 0.276 mmol, yield 65.2%).

*R*f = 0.71 ($CHCl_3$/MeOH = 3/2)

^{1}H-NMR (in d_6-DMSO, 300 MHz) δ 11.3 (s, 1H), 8.10 (t, 1H), 7.73 (d, 1H), 7.41 (s, 1H), 7.34 (m, 5H), 5.03 (s, 2H), 4.24 (s, 2H), 4.00 (q, 1H), 3.63 (s, 3H), 3.03 (d, 2H), 1.74 (s, 3H), 1.62 (m, 2H), 1.36 (m, 4H).

$^{\alpha}$*N*-(benzyloxycarbonyl)-$^{\varepsilon}$*N*-[[6-*N*-(benzyloxycarbonyl)adenine-9-yl]acetyl]lysine (Cbz-Lys(Cbz-Ab)-OH) (56)

Compound **52** (0.095 g, 0.157 mmol) was dissolved in MeOH (5.0 ml). And 1M NaOH aq. (2.0 ml) was added to this solution and stirred for 0.5 h. This reaction mixture was concentrated to 20% of original volume and 0.25M HCl aq. was dropped, and it adjusted to pH 3 and stored in a refrigerator overnight. Resultant precipitate was collected by centrifugation (8000 r.p.m., 15 min.) and dried in vacuum. The desired product **56** was obtained as a white powder (0.0562g, 0.0953 mmol, yield 61.0%).

*R*f = 0.50 ($CHCl_3$/MeOH/NH_3(aq) = 2/2/1).

$^{\alpha}$*N*-(benzyloxycarbonyl)-$^{\varepsilon}$*N*-[[4-*N*-(benzyloxycarbonyl)-cytosine-1-yl]acetyl]lysine (Cbz-Lys(Cbz-Cb)-OH) (57)

Compound **53** (0.164 g, 0.282 mmol) was dissolved in MeOH (7.0 ml). And 1M NaOH aq. (2.0 ml) was added to this solution and stirred for 1.0 h. This reaction mixture was concentrated to 20% of original volume and 0.25M HCl aq. was dropped, and it adjusted to pH 3 and stored in a refrigerator overnight. Resultant precipitate was collected by centrifugation (8000 r.p.m., 15 min.) and dried in vacuum. The desired product **57** was obtained as a white powder (0.0715g, 0.126 mmol, yield 44.8%).

*R*f = 0.46 ($CHCl_3$/MeOH/NH_3(aq) = 2/2/1).

$^{\alpha}$*N*-(benzyloxycarbonyl)-$^{\varepsilon}$*N*-[(guanine-9-yl)acetyl]lysine (Cbz-Lys(Gb)-OH) (58)

Compound **54** (0.0920 g, 0.189 mmol) was dissolved in MeOH(3.0 ml). And 1M NaOH aq. (1.5 ml) was added to this solution and stirred for 2.0 h. This reaction mixture was concentrated to 20% of original volume and 0.25M HCl aq. was dropped, and it adjusted to pH 3 and stored in a refrigerator overnight. Resultant precipitate was collected by centrifugation (8000 r.p.m., 15 min.) and dried in vacuum. The desired product **58** was obtained as a white powder (0.0729g, 0.155 mmol, yield 82.0%).

*R*f = 0.30 ($CHCl_3$/MeOH/NH_3(aq) = 2/2/1).

$^{\alpha}$*N*- (benzyloxycarbonyl)-$^{\varepsilon}$*N*-[(thymine-1-yl)acetyl] lysine (Cbz-Lys(Tb)-OH) (59)

Compound **55** (0.123 g, 0.266 mmol) was dissolved in MeOH (5.0 ml). And 1M NaOH aq. (1.8 ml) was added to this solution and stirred for 1.0 h. This reaction mixture was concentrated to 20% of original volume and 0.25M HCl aq. was dropped, and it adjusted to pH 3 and stored in a refrigerator overnight. Resultant precipitate was collected by centrifugation (8000 r.p.m., 15 min.) and dried in vacuum. The desired product **59** was obtained as a white powder (0.102 g, 0.229 mmol, yield 85.9%).

*R*f = 0.60 ($CHCl_3$/ MeOH /NH_3(aq) = 2/2/1).

6'-*N*-[$^{\alpha}$*N*-(benzyloxycarbonyl)-$^{\varepsilon}$*N*-[[6-*N*-(benzyloxycarbonyl)adenine-9-yl]acetyl] lysinyl]neamine (Cbz-Lys (Cbz-Ab)-neamine) (60)

A solution of **56** (0.0530 g, 0.0899 mmol) in DMF (1.0 ml) was cooled to 0 °C. WSC HCl (0.0189 g, 0.0986 mmol) was added to this solution and stirred for 3 h. This solution was added to a solution of neamine (0.0610 g, 0.189 mmol) in DMF (0.5 ml) and water (1.0 ml), and stirred for 2 h at 0 °C and another 18 h at room temperature. The reaction mixture was evaporated. The crude product was dissolved in 10% acetic acid aqueous solution, purified by reversed-phase HPLC (YMC-Pack Pro C18 RS, Φ10 mm x 250 mm). The desired product **60** was obtained as a colorless oil (0.0036 g, 0.00403 mmol, yield 4.5%).

*R*f = 0.30 ($CHCl_3$/MeOH/ NH_3(aq) = 5/3/1),

^{1}H NMR (in D_2O, 300 MHz) δ 8.53 (s, 1H), 8.37 (s, 1H), 7.43-7.13 (m, 10H), 5.53 (d, 1H), 5.26 (s, 2H), 5.01 (s, 4H), 3.93 (m, 1H), 3.70-3.18 (m, majority), 2.36 (m, 1H), 1.60 (m, 2H), 1.43 (m, 2H), 1.26 (m, 2H).

6'-*N*-[$^{\alpha}$*N*-(benzyloxycarbonyl)-$^{\varepsilon}$*N*-[[4-*N*-(benzyloxycarbonyl)cytosine-1-yl]acetyl]lys -inyl]neamine (Cbz-Lys(Cbz -C_b)-Neamine) (61)

A solution of **57** (0.0848 g, 0.150 mmol) in DMF (1.0 ml) was cooled to 0 °C. WSC HCl (0.0431 g, 0.0225 mmol) was added to this solution and stirred for 2 h. This solution was added to a solution of neamine (0.0965 g, 0.300 mmol) in DMF (0.5 ml) and water (1.0 ml), and stirred for 2 h at 0 °C and another 22 h at room temperature. The reaction mixture was evaporated. The crude product was dissolved in 10% acetic acid aqueous solution, purified by reversed-phase HPLC (GL Sciences Inc. Intersil ODS-3, Φ10 mm x 230 mm). The desired product **61** was obtained as a colorless oil (0.0111 g, 0.0125 mmol, yield 8.3%).

*R*f = 0.39 ($CHCl_3$/MeOH/NH_3(aq) = 2/2/1)

^{1}H NMR (in D_2O, 300 MHz) δ 7.60 (d, 1H), 7.27-7.24 (m, 10H), 6.00 (d, 1H), 5.52 (d-t, 1H), 4.98 (d, 2H), 4.38 (s, 2H), 3.90 (m, 1H), 3.72-3.04 (m, majority), 2.33 (m, 1H), 1.75-1.51 (m, 2H), 1.37 (m, 2H), 1.23 (m, 2H), 1.01 (t, 1H).

6'-*N*-[$^{\alpha}$*N*-(benzyloxycarbonyl)-$^{\varepsilon}$*N*-[(guanine-9-yl)acetyl]lysinyl]neamine (Cbz-Lys(Gb)-Neamine) (62)

A solution of **58** (0.0652 g, 0.138 mmol) in DMF (1.0 ml) was cooled to 0 °C. WSC HCl (0.0319 g, 0.154 mmol) was added to this solution and stirred for 2 h. This solution was added to a solution of neamine (0.0833 g, 0.256 mmol) in DMF (0.33 ml) and water (0.66 ml), and stirred for 2 h at 0 °C and another 19 h at room temperature. The reaction mixture was evaporated. The crude product was dissolved in 10% acetic acid aqueous solution, purified by reversed-phase HPLC (YMC-Pack Pro C18 RS, Φ10 mm x 250 mm). The desired product **62** was obtained as a colorless oil (0.0146 g, 0.0188 mmol, yield 13.6%).

*R*f = 0.43 ($CHCl_3$/MeOH/ NH_3(aq) = 2/2/1)

^{1}H NMR (in D_2O, 300 MHz) δ 8.66 (s, 1H), 7.82 (s, 2H), 7.24-7.17 (m, 5H), 5.58 (d, 1H),

4.93 (d, 2H), 3.82-3.16 (m, majority), 2.37 (m, 1H), 1.60 (m, 2H), 1.45 (m, 2H), 1.30 (m, 2H).

6'-*N*-[[$^{\alpha}$*N*-(benzyloxycarbonyl)]-$^{\varepsilon}$*N*-](thymine-1-yl)acetyl]lysinyl]neamine (Cbz-Lys(Tb)-Neamine) (63)

A solution of **59** (0.109 g, 0.245 mmol) in DMF (1.4 ml) was cooled to 0 °C. WSC HCl (0.0583 g, 0.304 mmol) was added to this solution and stirred for 2 h. This solution was added to a solution of neamine (0.127 g, 0.393 mmol) in DMF (0.7 ml) and water (1.4 ml), and stirred for 2 h at 0 °C and another 22 h at room temperature. The reaction mixture was evaporated. The crude product was dissolved in 10% acetic acid aqueous solution, purified by reversed-phase HPLC (GL Sciences Inc. Intersil ODS-3, Φ10 mm x 230 mm). The desired product **63** was obtained as a colorless oil (0.0461 g, 0.0614 mmol, yield 25.0%).

*R*f = 0.38 ($CHCl_3$/MeOH/NH_3(aq) = 2/2/1)

^{1}H NMR (in D_2O, 300 MHz) δ 7.26-7.21 (m, 6H), 5.52 (d, 1H), 4.98 (d, 2H), 4.25 (s, 2H), 3.92-3.64 (m, 3H), 3.52-3.07 (m, 8H), 2.33 (m, 1H), 1.69 (s, 3H), 1.57 (m, 2H), 1.36 (m, 2H), 1.20 (m, 2H).

6'-*N*-[[$^{\varepsilon}$*N*-(adenine-9-yl)acetyl]lysinyl]neamine (Ab-(D,L)Kε-Neamine) (64)

A solution of **60** (0.0036 g, 0.00403 mmol) in TFA (0.5 ml) were added *m*-cresol (0.067 ml, 0.64 mmol), thioanisole (0.113 ml, 0.966 mmol), and trimethylsilyl trifluoromethanesulfonate (TMSOTf, 0.187 ml, 0.969 mmol) and stirred for 0.5 h at 0 °C, 2 h at room temperature. The reaction mixture was concentrated to around 0.3 ml, and cold Et_2O (6.0 ml) was added to this solution. The resultant precipitate was collected by centrifugation (6000 r.p.m., 10 min.) and dried in vacuum. The crude product was obtained as a white powder and purified by reversed-phase HPLC (YMC-Pack Pro C18 RS Φ10 mm x 250 mm). The desired product **64** was obtained as a white powder (0.0007

g, 0.00117 mmol, 29.0%).

*R*f = 0.025 ($CHCl_3$/MeOH/NH_3(aq) = 5/3/1)

UV (H_2O) λmax (ε) 260 (2996) nm

^{1}H NMR (in D_2O, 400 MHz) *δ* 8.19-8.09 (m, 8H), 5.47 (d, 2H), 5.03-5.01 (m, 8H), 4.85 (dd 1H), 4.68-4.64 (m, majority), 4.15-4.11 (m, 3H), 3.95 (dd, 2H), 3.73-2.98 (m, majority), 2.86-2.81 (m, 8H), 2.34-2.22 (m, 3H), 1/70-1.49 (m, majority), 1.38-1.24 (m, 8H)

^{13}C NMR (in D_2O, 100 MHz) *δ* 174.8, 174.7, 169.4, 169.0, 163.8, 163.4, 149.9, 149.6, 148.6, 148.5, 144.9, 144.8, 118.6, 118.6, 118.3, 115.4, 97.3, 97.1, 79.4, 79.1, 75.5, 75.4, 75.3, 75.2, 73.0, 72.3, 71.7, 70.7, 69.1, 69.0, 55.9, 54.9, 54.3, 54.2, 50.2, 49.1, 48.8, 48.7, 46.3, 40.6, 39.7, 39.7, 31.0, 30.7, 28.8, 28.7, 26.9, 26.8, 22.8, 22.7

MALDI-TOF MS found $M+H^+$, $M+Na^+$, $M+K^+$ (calcd $M+H^+$, $M+Na^+$, $M+K^+$) 626.0139, 647.9873, 663.9427 (626.3369, 648.3188, 664.2928)

6'-*N*-[[$^{\varepsilon}$*N*-(cytosine-1-yl)acetyl]lysinyl]neamine (Cb-(D,L)Kε-Neamine) (65)

A solution of **61** (0.0111 g, 0.0125 mmol) in TFA (0.5 ml) were added *m*-cresol (0.080 ml, 0.764 mmol), thioanisole (0.088 ml, 0.753 mmol), and trimethylsilyl trifluoromethane-sulfonate (TMSOTf, 0.145 ml, 0.750 mmol) and stirred for 0.5 h at 0 °C, 3 h at room temperature. The reaction mixture was concentrated to around 0.3 ml, and cold Et_2O (5.0 ml) was added to this solution. The resultant precipitate was collected by centrifugation (6000 r.p.m., 10 min.) and dried in vacuum. The crude product was obtained as a white powder and purified by reversed-phase HPLC (GL Sciences Inc. Intersil ODS-3, Φ10 mm x 230 mm). The desired product **65** was obtained as a white powder (0.0059 g, 0.00972 mmol, 77.6%).

*R*f = 0.13 ($CHCl_3$/MeOH/NH_3 (aq) = 2/2/1)

UV (H_2O) λmax (ε) 272 (1477) nm

^{1}H NMR (in D_2O, 400 MHz) *δ* 7.43-7.39 (m, 3H), 5.89-5.86 (m, 3H), 5.54 (d, 1H), 5.35 (d, 1H), 4.79-4.60 (m, majority), 4.53-4.51 (m, 7H), 4.43-4.30 (m, 4H), 4.12-4.08 (m, 2H),

3.98 (m, 1H), 3.73-3.12 (m, majority), 2.83-2.79 (m, 7H), 2.30-2.26 (m, 2H), 1.73-1.47 (m, majority), 1.38-1.16 (m, 8H)

^{13}C NMR (in D_2O, 100 MHz) δ 170.5, 170.4, 163.8, 163.4, 148.3, 148.1, 118.3, 115.4, 97.5, 97.0, 96.5, 96.3, 79.6, 79.2, 75.5, 75.4, 73.0, 72.5, 71.4, 71.4, 71.3, 71.2, 70.6, 70.5, 69.2, 69.1, 55.3, 54.8, 54.3, 54.2, 52.7, 52.6, 50.2, 50.1, 49.2, 48.9, 39.7, 39.6, 30.8, 30.6, 28.8, 28.7, 26.8, 26.7, 26.7. 26.6, 22.7, 22.6

MALDI-TOF MS found $M+H^+$, $M+Na^+$, $M+K^+$ (calcd $M+H^+$, $M+Na^+$, $M+K^+$) 602.5131, 624.4747, 640.4361 (602.3257, 624.3076, 640.2815)

6'-*N*-[[$^{\varepsilon}$*N*-(guanine-9-yl)acetyl]lysinyl]neamine (Gb-(D,L)Kε-Neamine) (66)

A solution of **62** (0.0146 g, 0.0188 mmol) in TFA (0.5 ml) were added *m*-cresol (0.0601 ml, 0.573 mmol), thioanisole (0.067 ml, 0.573 mmol), and trimethylsilyl trifluoromethanesulfonate (TMSOTf, 0.110 ml, 0.570 mmol) and stirred for 0.5 h at 0 °C, 2.5 h at room tempera- ture. The reaction mixture was concentrated to around 0.3 ml, and cold Et_2O (10.0 ml) was added to this solution. The resultant precipitate was collected by centrifugation (6000 rpm, 10 min.) and dried in vacuum. The crude product was obtained as a white powder and purified by reversed-phase HPLC (YMC-Pack Pro C18 RS Φ10 mm x 250 mm). The desired product **66** was obtained as a white powder (0.0075 g, 0.0120 mmol, 63.8%).

*R*f = 0.038 ($CHCl_3$/MeOH/NH_3 (aq) = 2/2/1)

UV (H_2O) λmax (ε) 252 (2886) nm, λmax (ε) 270 (2094) nm.

^{1}H NMR (in D_2O, 400 MHz) δ 7.63-7.54 (m, 6H), 5.26 (d, 1H), 4.71-4.49 (m, majority), 3.96-3.92 (m, 2H), 3.75 (m, 1H), 3.59-2.82 (m, majority), 2.75-2.70 (m, majority), 2.10-2.05 (m, 4H), 1.62-1.12 (m, majority)

^{13}C NMR (in D_2O, 100 MHz) δ 174.7, 174.4, 169.9, 169.5, 159.6, 159.5, 154.7, 154.6, 152.6, 152.5, 141.0, 140.9, 121.7, 118.6, 116.2, 115.4, 97.5, 97.0, 75.5, 75.3, 73.0, 72.9,

72.6, 72.5, 71.9, 70.9, 70.2, 69.7, 69.1, 69.0, 56.3, 55.1, 54.4, 54.3, 54.2, 50.1, 49.2, 48.7, 46.3, 46.0, 41.4, 40.1, 39.7, 39.6, 30.5, 30.4, 28.9, 28.8, 26.9, 26.8, 22.9, 22.8.

MALDI-TOF MS found $M+H^+$, $M+Na^+$, $M+K^+$ (calcd $M+H^+$, $M+ Na^+$, $M+K^+$) 641.7694, 663.7467, 679.7185 (642.3318, 664.3137, 680.2877).

6'-*N*-[[$^{\varepsilon}$*N*-(thymine-1-yl)acetyl]lysinyl]neamine (Tb-(L)Kε-Neamine) (67)

A solution of **63** (0.0461 g, 0.0614 mmol) in TFA (1.0 ml) were added m-cresol (0.0126 ml, 0.120 mmol), thioanisole (0.211 ml, 1.80 mmol), and trimethylsilyl trifluoromethane-sulfonate (TMSOTf, 0.349 ml, 1.80 mmol) and stirred for 0.5 h at 0 °C, 2 h at room temperature. The reaction mixture was concentrated to around 0.6 ml, and cold Et_2O (10.0 ml) was added to this solution. The resultant precipitate was collected by centrifugation (6000 r.p.m., 10 min.) and dried in vacuum. The crude product was obtained as a white powder and purified by reversed-phase HPLC (GL Sciences Inc. Intersil ODS-3, Φ10 mm x 230 mm). The desired product **67** was obtained as a white powder (0.0205 g, 0.0332 mmol, 54.1%).

*R*f = 0.29 ($CHCl_3$/MeOH/NH_3 (aq) = 2/2/1)

UV (H_2O) λmax (ε) 268 (2579) nm

^{1}H NMR (in D_2O, 400 MHz) *δ* 7.29-7.22 (m, 5H), 5.44 (d, 3H), 5.28 (d, 1H), 4.72-4.61 (m, majority), 4.39-4.29 (m, 9H), 4.13-4.09 (m, 3H), 3.99 (m, 1H), 3.70-3.04 (m, majority), 2.81-2.78 (m, 9H), 2.22-2.17 (m, 4H), 1.76-1.21 (m, majority)

^{13}C NMR (in D_2O, 100 MHz) *δ* 174.8, 170.0, 167.6, 163.4, 152.8, 143.7, 118.3, 115.4, 111.6, 97.3, 75.6, 73.3, 72.3, 70.7, 69.5, 54.6, 54.4, 51.1, 50.4, 49.1, 39.7, 30.9, 26.8, 22.7, 11.8

MALDI-TOF MS found $M+H^+$, $M+Na^+$, $M+K^+$ (calcd $M+H^+$, $M+Na^+$, $M+K^+$) 616.6538, 638.6233, 654.5807 (617.3253, 639.3073, 655.2812).

4-5. Reference and Notes

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Chapter 5 Binding of nucleobase modified neamine to TAR and RRE RNA derived from HIV-1

5-1. Introduction

5-1-1. Target hairpin RNAs (HIV-1 TAR RNA and HIV-1 RRERNA)

The aim of this study is the discovery of the molecule that eventually inhibits the replication of an HIV. One of conventional drug that combat HIV are protease inhibitor. However, It was known that HIV acquired resistance for the protease inhibitors. On the replication of HIV, two viral proteins (Tat and Rev) are known as activators that bind to the activator region of the viral RNA (HIV-1 TAR RNA and HIV-1 RRE RNA), then amplify the replication of HIV. On this circumstance, I have decided to discover for the compound which binds to HIV-1 TAR RNA or HIV-1 RRE RNA specifically in order to inhibit the binding of viral peptide with the RNA (Figure 5-1)[1,2]. Then I have chosen TAR and RRE RNA as the targets and designed trehalosamine and NbKε-neamine as a scaffold. And, I evaluate about inhibition of TAR-Tat and RRE-Rev.

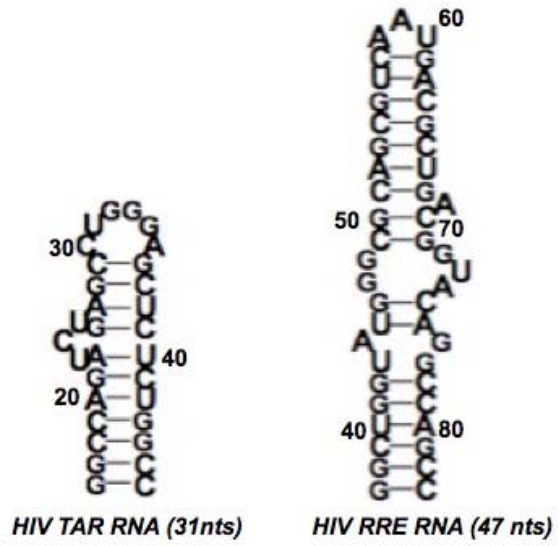

Figure 5-1 HIV-1 TAR RNA(31 nts) and HIV-1RRE RNA(47 nts)

5-1-2. The binding study for the evaluation of nucleobase-modified neamines as the inhibitor for either TAR-Tat or RRE-Rev.

In this chapter, I describe the evaluation of the trehalosamine and NbKε-neamine as inhibitors of TAR-Tat and RRE-Rev. The evaluation method adopted the spectroscopic method using fluorescence peptide as a tracer, and it aimed at clarifying detailed relation between the steric structure of the molecule and RNA binding capacity.

The evaluation methods for the binding of neamine derivatives to TAR RNA

For the evaluation of the neamine derivatives as the binding inhibitor for TAR RNA, fluorescently labeled Tat peptide, FTatRhd[3] was employed as the binding tracer (Figure 5-2). Upon the binding, Tat peptide was bended by TAR RNA, as a result two fluorescent dyes were placed closer proximity for FRET emission, then fluorescence emission increases with increasing concentration of TAR RNA. On the other hand, FRET emission (fluorescence intensity of FtatRhd around 577nm) was decreased with increasing concentration of neamine derivatives. Dissociation constants of the neamine derivatives (drags) were determined by the curve-fitting analysis with the Eq.(1) of 1:1 stoichiometry (Refer to **5-4. Experimental Section**).[4]

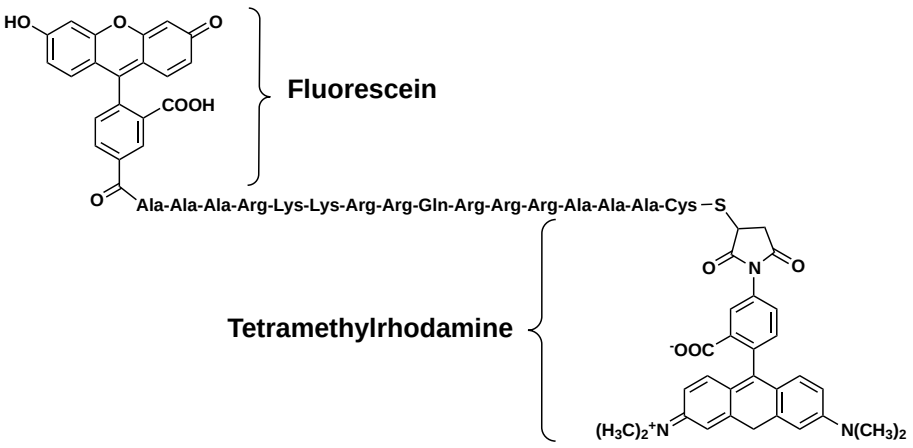

Figure 5-2　Amino acid sequence of the fluorescent Tat peptide (FTatRhod)

The evaluation methods for the binding of neamine derivatives to RRE RNA

For the binding study of the neamine derivatives with RRE RNA, fluorescein-labeled Rev peptide, FLRev[2], was employed as the binding tracer (Figure 5-3). On the binding of FLRev with RRE, increasing fluorescence anisotropy of FLRev around 520 nm was observed. When the neamine derivatives compete with the probe, the fluorescent anisotropy should decrease. On the other hand, when the neamine derivatives bound not with FLRev but with RRE-FLRev complex, fluorescence anisotropy was more increased. Dissociation constants of the neamine derivatives were determined by the curve-fitting analysis with the Eq.(2) of 1:1 stoichiometry (Refer to **5-4. Experimental Section**).[5]

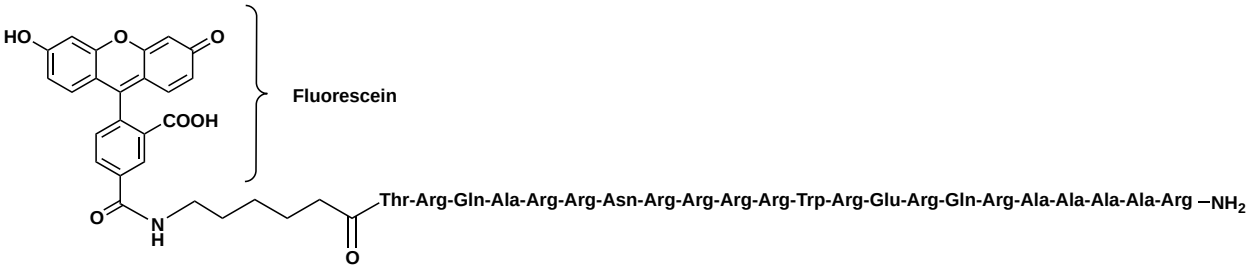

Figure 5-3　Amino acid sequence of the fluorescent Rev peptide (FLRev)

5-2. Results and Discussion

5-2-1. Binding of neamine derivatives toward the HIV-1 TAR RNA

For the evaluation of the trehalosamine as the binding inhibitor for TAR RNA, fluorescently labeled Tat peptide, FTatRhd[3] was employed as the binding tracer. In the complex of TAR-Tat, Tat peptide forms β-turm, as a result, two fluorescent dyes were placed closer proximity for FRET emission, therefore fluorescence emission increases with increasing concentration of TAR RNA. On the other hand, if αα, αβ and ββ-trehalosamine bind to the TAR RNA, FRET emission was decreased. However, FRET emission has not changed much with increasing concentration of αα, αβ and ββ-trehalosamine (0 - 800 μM) (Figure 5-4). These results denoted that αα, αβ, ββ-trehalosamine did not bind to the TAR RNA, although neamine bound the RNA with considerable affinity (Figure 5-5). Since the part of the sugar backbone of neamine has similar structure as trehalosamine, it was suggested that the unit of deoxystreptamine in a neamine is greatly related to affect the binding toward TAR RNA. These results have shown that the binding affinity toward the RNA varies greatly depend on the slight difference in the structure of the disaccharide and the position of amino groups. Therefore, it is thought that the new design of novel small compounds with low molecular weight requires more precise examinations.

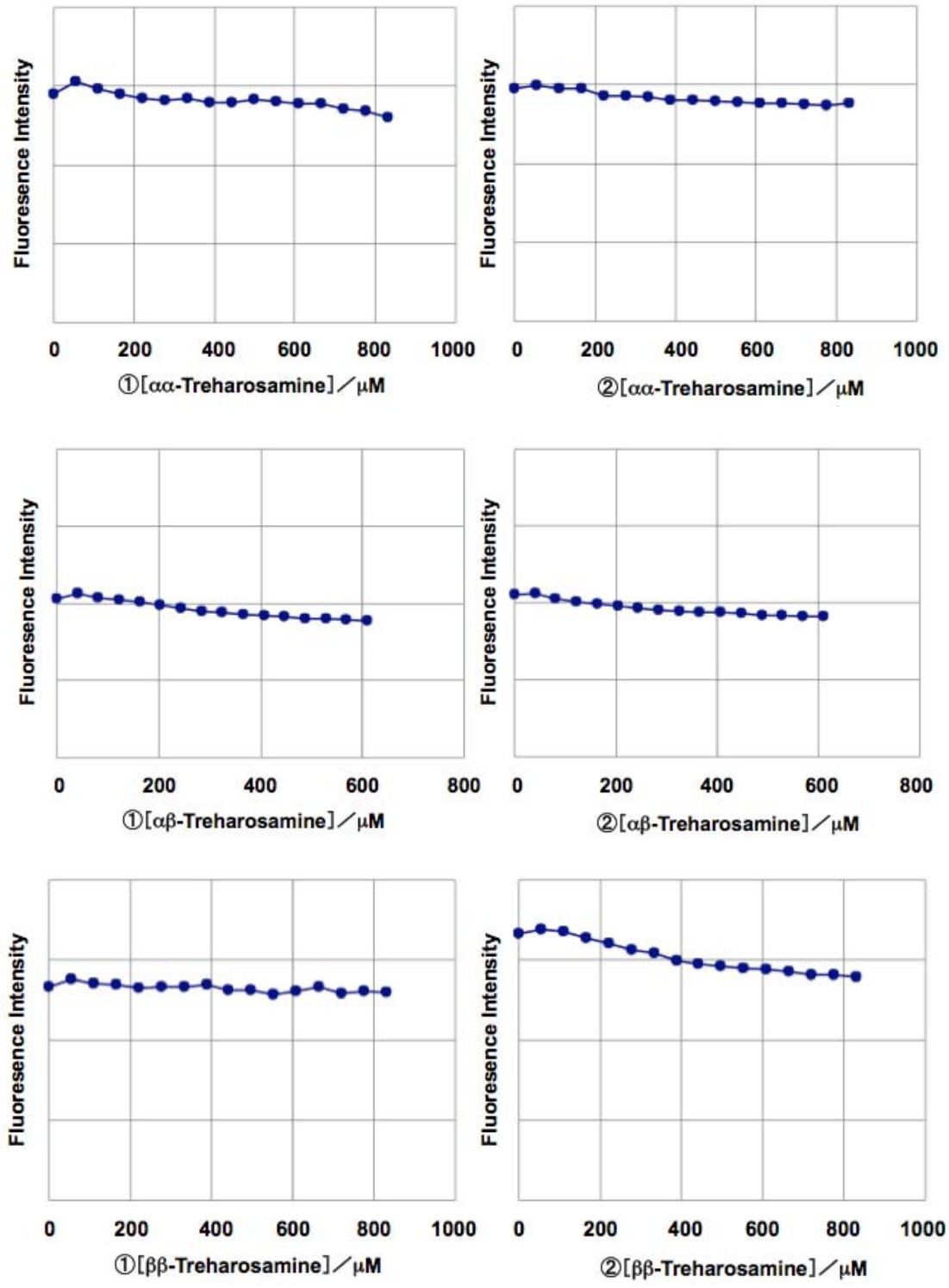

Figure 5-4　Fluorescence titration curves as a function of the concentration of αα, αβ and ββ-trehalosamine. The concentrations of TAR RNA and FtatRhd were fixed at 800 and 100 nM, respectively.

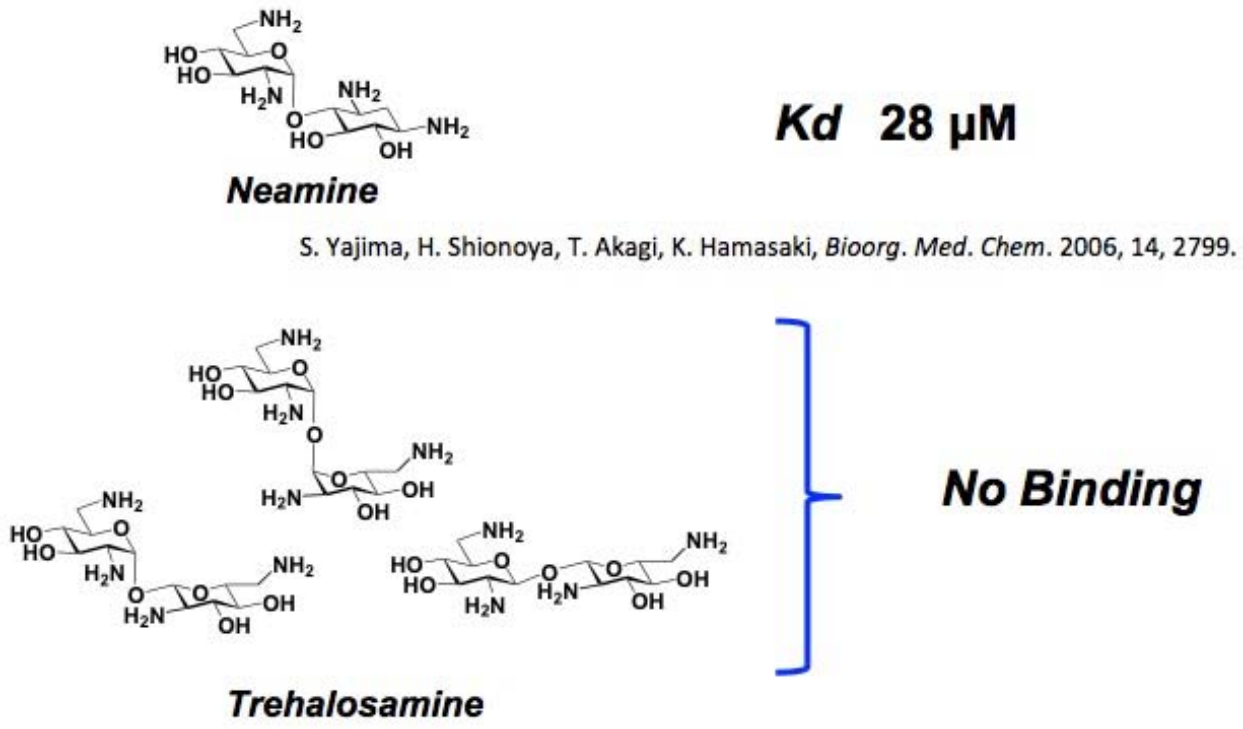

Figure 5-5　Dissociation constants (Kd) of the neamine derivatives for TAR RNA

In the binding experiment of NbKε-neamine with TAR RNA, fluorescently labeled Tat peptide, FTatRhd[3] was employed as the binding tracer. Upon the binding with TAR, Tat peptide forms β-turn, as a result, two fluorescent dyes were placed closer proximity for FRET emission, therefore fluorescence emission increases with increasing concentration of TAR RNA. On the other hand, FRET emission was decreased with increasing concentration of NbKε-neamine (Figure 5-6, 5-7 Spectra and plots).[6] The data points of each binding experiment were fitted finely with the theoretical curve with 1:1 stoichiometry. Then dissociation constants between NbKε-neamines and TAR RNA were determined and summarized in Table 5-1. The dissociation constant of neamine with TAR RNA was 28 μM. That of Ab, Cb, Gb and Tb-Kε-neamine were 0.74, 1.4, 0.24 and 0.86 μM, respectively. The modification of the neamine with the nucleobase enhanced binding affinity 20 to 100 times smaller than native neamine. Among four NbKε-neamines, Gb-Kε-neamine has shown the highest affinity for the binding to TAR RNA. The adenine and the guanine in the NbKε-neamines have potential to interact with the uracil and cytosine in the tri bulge of TAR RNA. Even though the buldge does not have any adenine, the binding of Tb-Kε-neamine was comparable to that of Ab-Kε-neamine. Despite Watson-Crick type base pairing of A22-U40 would be predicted from the secondary structure of TAR RNA for A22-U40 of which locating at the lower stem of the tri bulge, hydrogen bonds of this base pairings are relaxed due to twist across the tri base bulge, C24 stack on U23 and U23 stack on A22.[2,5,7] This structural feature of TAR RNA may make the thymine of Tb-Kε-neamine interact with the A22. Only the cytosine of Cb-Kε-neamine has no possible to interact with the bases around the bulge. That is the reason why CbKe-neamine does not bind as the other three(Figure 5-8).

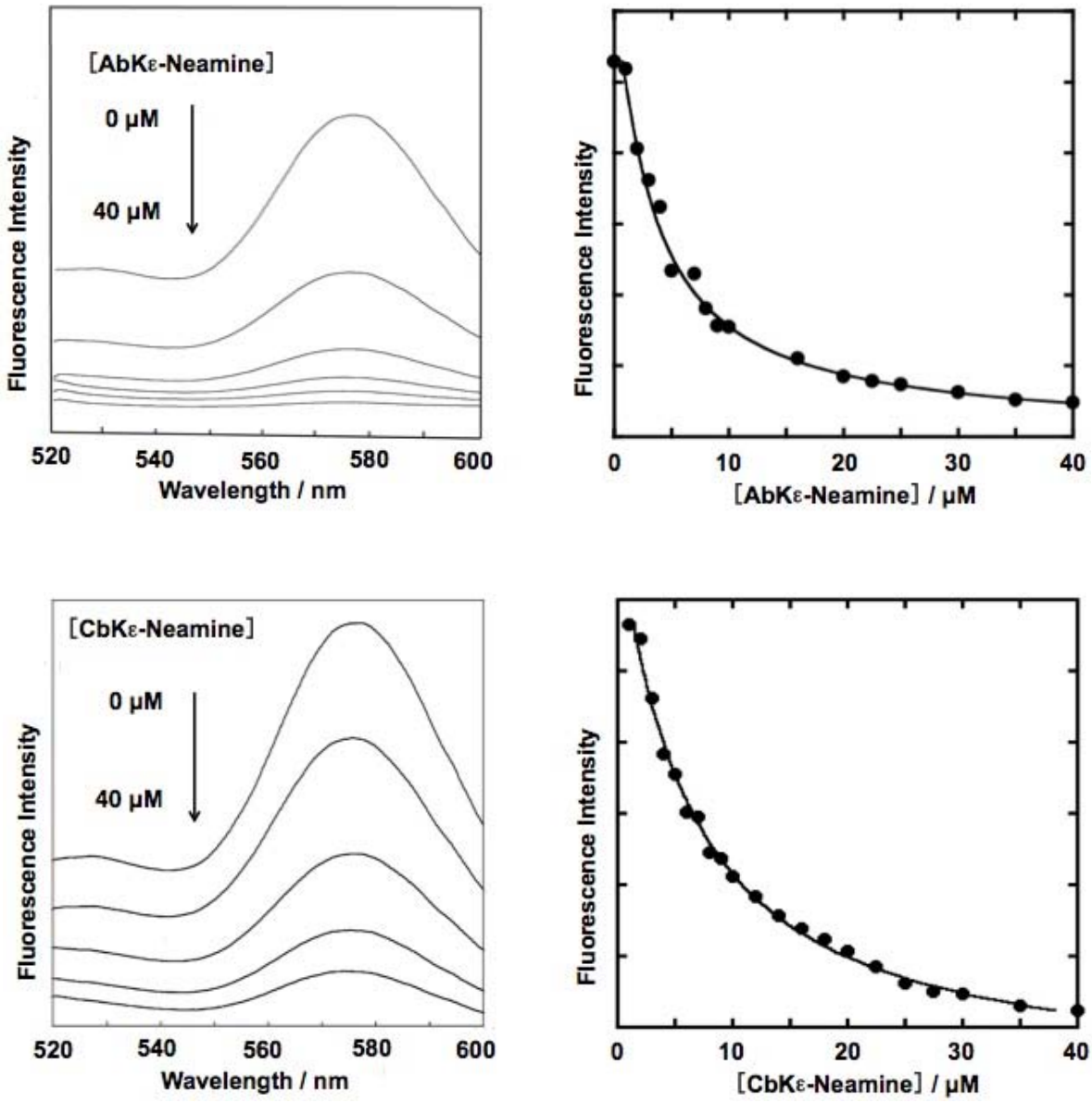

Figure 5-6 Fluorescence spectra of FtatRhd in the absence and in the presence of various concentrations of Ab, CbKε-Neamine (left) and fluorescence titration curves as a function of the concentration of Ab,CbKε-Neamine(right).
The concentrations of TAR RNA and FtatRhd were fixed at 800 and 100 nM respectively.

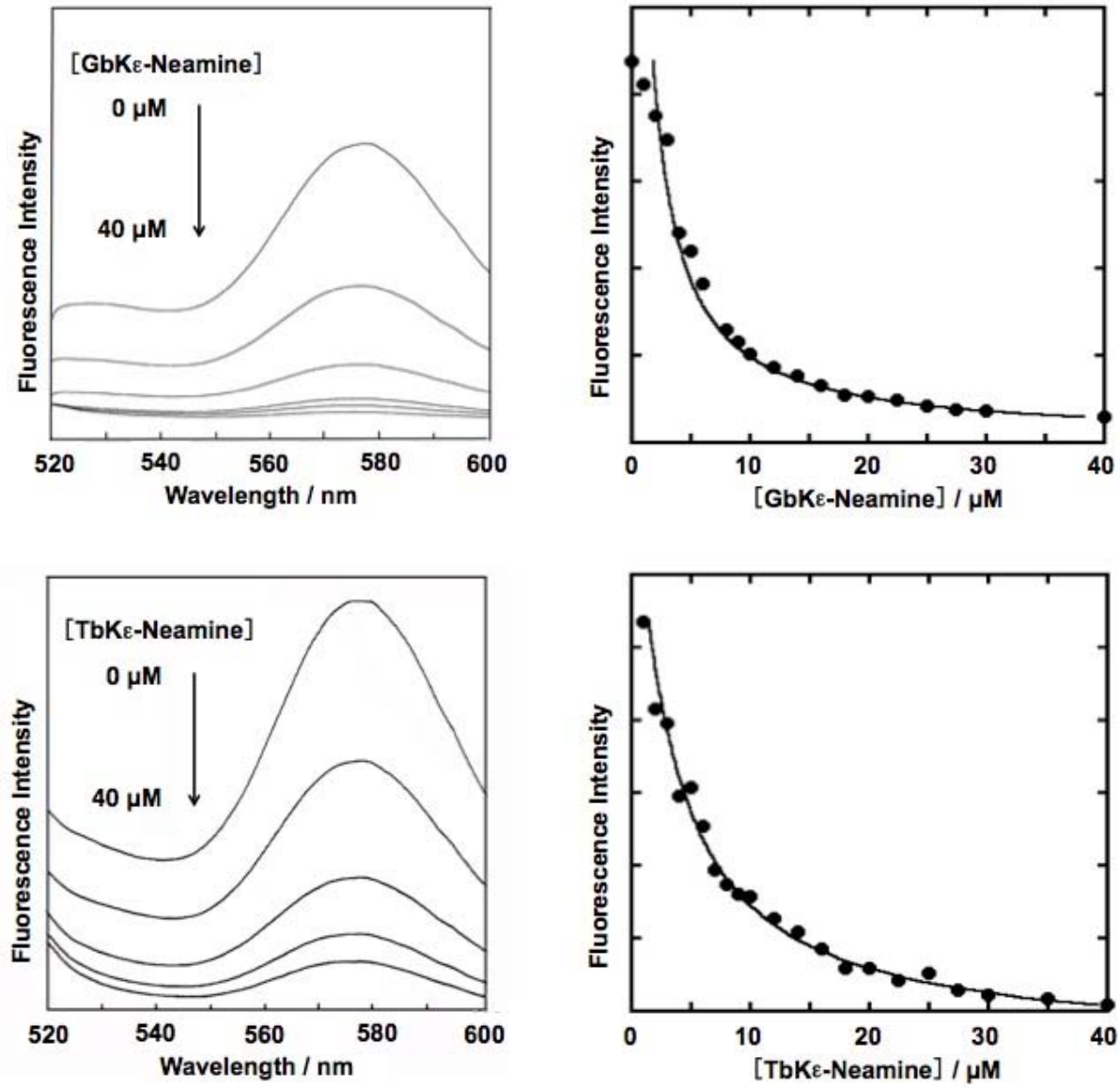

Figure 5-7　Fluorescence spectra of FtatRhd in the absence and in the presence of various concentrations of Gb, TbKε-Neamine (left) and fluorescence titration curves as a function of the concentration of Gb,TbKε-Neamine(right).
The concentrations of TAR RNA and FtatRhd were fixed at 800 and 100 nM respectively.

Table 5-1　Dissociation constants(Kd) of the neamine derivatives for TAR RNA

Compounds	Kd (μM)
Neamine	28 [a]
K-Neamine	19 [a]
AbKε-Neamine	0.74
CbKε-Neamine	1.4
GbKε-Neamine	0.24
TbKε-Neamine	0.86

[a] Ref. 1

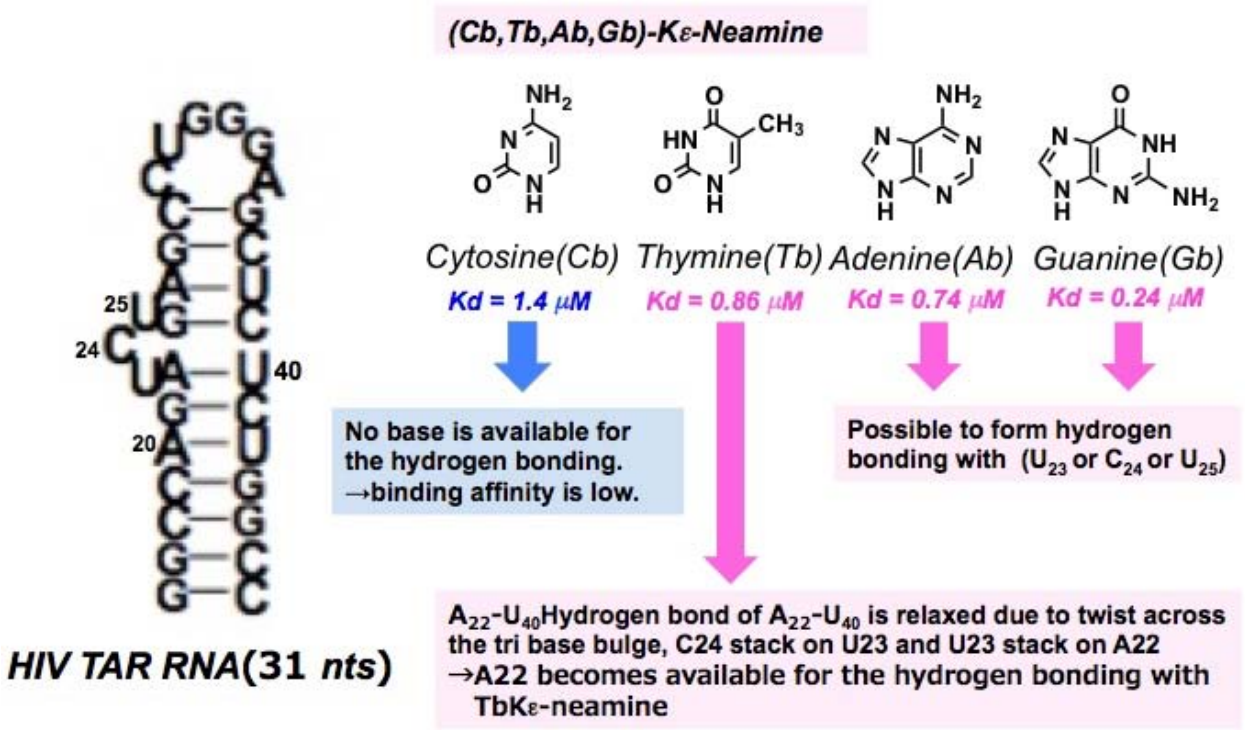

Figure 5-8　Possible explanation for the difference of the binding affinity of NbKε-neamine with TAR RNA.

5-2-2. Binding of neamine derivatives toward the HIV-1 RRE RNA

For the binding study of NbKε-neamines with RRE RNA, fluorescein labeled Rev peptide, FLRev[2], was used as the probe. On the binding of FLRev with RRE, increasing fluorescence anisotropy was observed. When the compound competes with the probe, the fluorescent anisotropy should decrease. However, neamine and l-lysine modified neamine (K-neamine) and all four NbKε-neamines keep increased fluorescence anisotropy of RRE-FLRev complex with the increasing concentration of neamine and its derivatives (Figure 5-9). On the other hand, in the absence of RRE, neamine and its derivatives did not increase the fluorescent anisotropy of FLRev. These results indicate that NbKε-neamine bound not with FLRev but with RRE-FLRev complex. Multiple binding site of RRE was suggested for neomycin B.[8] The primary binding site for neomycin B is independent from the that for Rev. Similar to neomycin B. Due to tight binding of RRE-FLRev complex, neamine and its derivatives do not compete with FLRev, they might bind the independent binding site from that of FLRev (Figure 5-10). The binding magnitude of the neamine derivative were determined as the dissociation constants from the increasing fluorescence anisotropy of FLRev-RRE and summarized in Table 5-2. Although the modification of neamine with lysine did not enhance its binding (neamine; 5.4 μM, K-neamine; Kd = 5.7 μM), NbKε-neamine obviously enhanced its binding toward RRE-FLRev (NbKε-neamine; 0.53-0.64 μM). However, NbKε-neamines were not effective as the binding inhibitor for RRE-Rev.

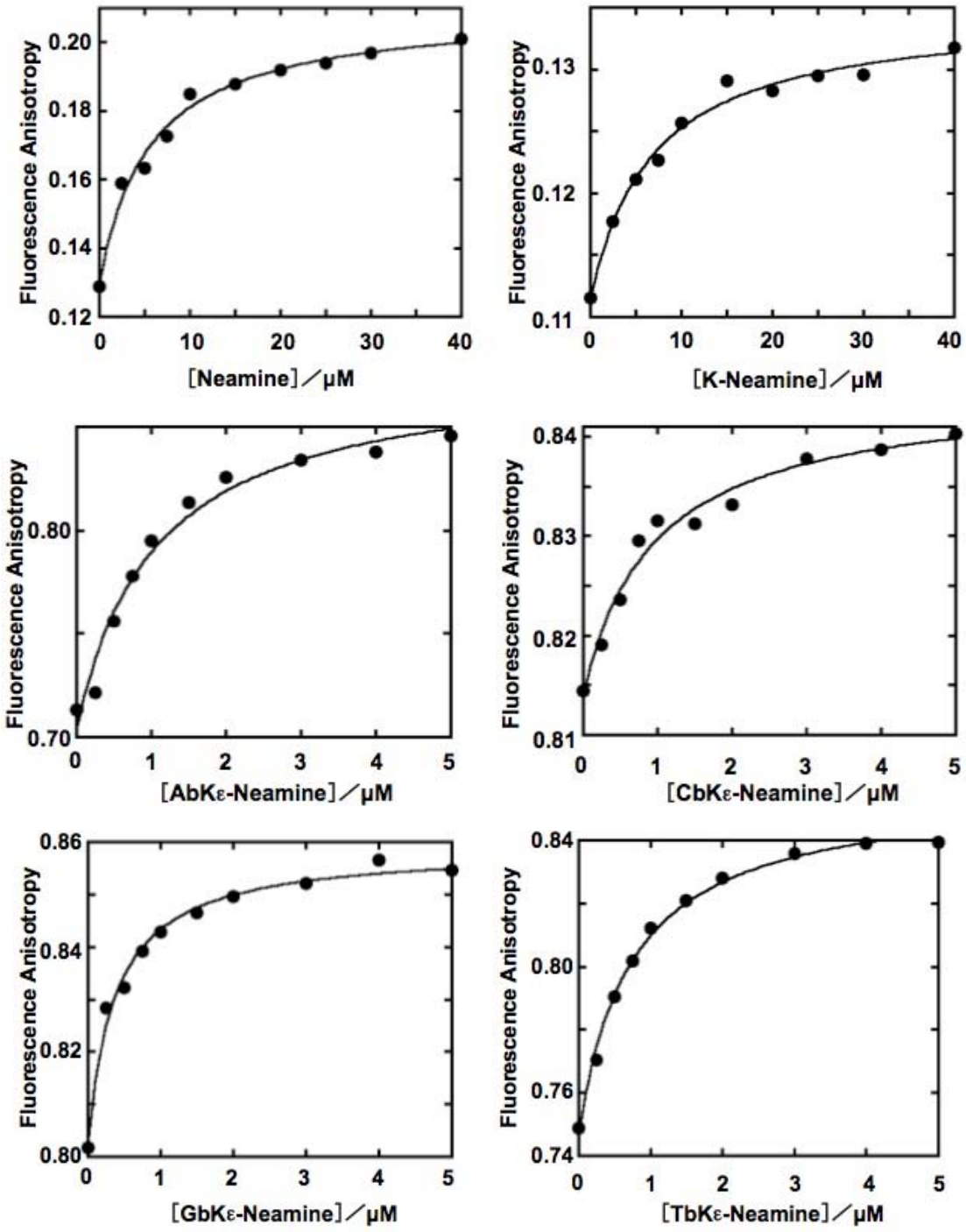

Figure 5-9　Fluorescence titration curve as a function of the increasing concentration of Neamine, K-Neamine, NbKε-Neamine. The concentrations of both RRE RNA and FLRev were fixed at 10 nM.

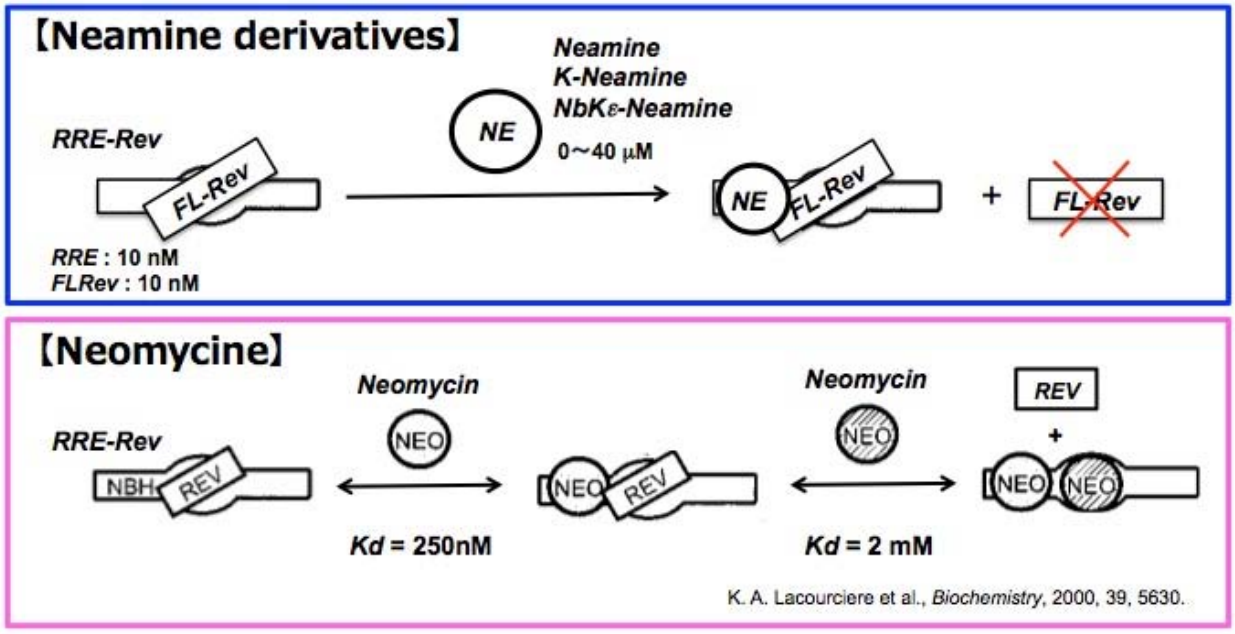

Figure 5-10　The presumed binding site of neamine derivatives

Table 5-2. Dissociation constants of the neamine derivatives for RRE RNA

Compounds	Kd (μM)
Neamine	5.6
K-Neamine	6.9
AbKε-Neamine	0.57
CbKε-Neamine	0.64
GbKε-Neamine	0.53
TbKε-Neamine	0.57

5-3. Conclusion

In this chapter, I described the evaluation of the trehalosamine and NbKε-neamine as inhibitors of TAR-Tat and RRE-Rev. The spectroscopic method using fluorescent peptide as a tracer, and it aimed at clarifying detailed binding behavior of neamine derivatives with the RNAs.

In chapter 5–2–1, I described the evaluation of the trehalosamine and NbKε-neamine as inhibitors of TAR-Tat. Despite neamine bind to TAR RNA with the dissociation constant (Kd) of 28 μM, αα, αβ, ββ-trehalosamine did not bind to the TAR RNA, even if treharosamin is structurally resemble to neamine. It is considered that the unit of deoxystreptamine of in neamine is greatly related to the binding with TAR RNA. This study made it clear that the slight difference in the structure of the disaccharide and the position of the amino group strongly affect the binding affinities with the specific RNA fragment. NbKε-neamine bound TAR RNA competing with Tat. Among four of NbKε-neamines, GbKε-neamine denotes the highest binding with TAR, AbKε-neamine and TbKε-neamine follow that of GbKε-neamine. These three bases are able to interact with the nucleobases around the tri base of bulge in TAR RNA.

In chapter 5–2–2, I described the evaluation of the NbKε-neamine as inhibitors of RRE-Rev. NbKε-neamines bind to RRE with relatively high affinity, however due to independent binding site of RRE for aminoglycosides other than for Rev peptide, NbKε-neamines could not be an inhibitor for Rev, one of the activator of HIV-1. All the NbKε-neamines enhanced its binding affinities for RRE about 10 times higher than that of neamine itself. But that was not depends on the difference of nucleobase of the compound except for GbKε-neamine having slightly better affinity than the others. An appropriate modification with the nucleobase on the neamine enhanced it binding affinity for the RNA. NbKε-neamines act as competitive inhibitor for TAR-Tat but not for RRE-Rev, and that inhibitory capacity was depend on the nucleobase of NbKε-neamine.

5-4. Experimental Section

Steady state fluorescence spectroscopy was performed at 25^{o}C on a HITACHI F3000 or F2500 fluorescence spectrophotometer.

Evaluation as binding inhibitor for TAR-Tat

Fluorescent Tat peptide, FtatRhd[3], was employed as a tracer to monitor the inhibition for TAR-Tat.[4] On the addition of the drugs, fluorescence intensity of FtatRhd around 577nm was decreased. Dissociation constants of the neamine derivatives were determined by the curve-fitting analysis with the Eq.(1) of 1:1 stoichiometry.

$$[\text{neamine derivatives}]_0 = (K_D (i_\infty - i) / K_d (i - i_0) + 1) \times ([\text{TAR-FtatRhd}]_0 - K_d (i - i_0) / (i_\infty - i) - [\text{FtatRhd}]_0 (i - i_0) / (i_\infty - i_0)) \quad (1)$$

The parameters, i and i_0 of Eq.(1) are fluorescence intensities of FtatRhd in the presence and absence of the TAR-RNA, respectively, and i_∞ is the fluorescence intensity in the presence of infinite concentration of the neamine derivatives. [neamine derivatives]$_0$ is the initial concentration of the neamine derivatives, K_D and K_d are the dissociation constants of TAR, neamine derivatives and TAR-FtatRhd complex, respectively. The concentrations of [FtatRhd]$_0$ and [TAR-FtatRhd]$_0$ were fixed at 100 and 800 nM, respectively. The concentrations [neamine derivatives] were varied from 0 to 40 μM. Fluorescence intensity of FtatRhd was measured at 580 nm for each concentration of neamine derivatives.

Evaluation as binding inhibitor for RRE-Rev

Fluorescent Rev peptide, FLRev[2], was employed as a tracer to monitor the inhibition for RRE-Rev.[5] On the addition of the neamine derivatives, fluorescence anisotropy of FLRev

around 520 nm was more increased. Dissociation constants of the neamine derivatives were determined by the curve-fitting analysis with the Eq.(2) of 1:1 stoichiometry.

$$i = io + \Delta i \left([\text{FLRev}]_0 + [\text{neamine derivatives}]_0 + K_d - (([\text{FLRev}]_0 + [\text{neamine derivatives}]_0 + K_d)^2 - 4\,[\text{FLRev}]_0 \times [\text{neamine derivatives}]_0)^{0.5} \right) / 2 \quad (2)$$

The parameters, i and i_0 of Eq. (2) are fluorescence anisotropies of FLRev in the presence and absence of the RRE-RNA, respectively, and Δi is the amount of fluorescence anisotropy change by unit concentration change of the neamine derivatives. $[\text{neamine derivatives}]_0$ is the initial concentration of the neamine derivatives, K_d is the dissociation constants of RRE-Neamine complex. The concentrations, $[\text{FLRev}]_0$ and [RRE] were fixed at 10 nM. The concentrations of neamine derivatives were varied from 0 to 5 μM. Fluorescence anisotropy of FLRev was measured at 520 nm for each concentration of neamine derivatives.

5-5. Reference and Notes

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Chapter 6　Summary

Although design of sequence-specific DNA-binding molecules have been established, due to its intricate structure, that for RNA has not been established yet. However, a few examples for the design of the RNA-binding molecules have been reported. One strategy is based on the polycyclic stacking molecule as a starting material, such as ethidium or acridine, the other is employing RNA-binding natural compounds as the scaffold, such as aminoglycoside antibiotics. There are two major aims to construct an RNA-binding molecule starting from the aminoglycoside. One aim is how to enhance the binding affinities of the aminoglycoside toward RNA. The other is how to acquire the binding specificity. Due to positive charge of the aminoglycoside antibiotics, the driving force of the binding interaction depends on electrostatic interaction. That kind of interaction may abolish binding specificity.

On the other hand, it has been known that replication of the human immunodeficiency virus (HIV) genome is regulated by virally encoded regulatory proteins, trans-activator protein (Tat) and retroviral protein (Rev), those two proteins bind to the specific region of the viral hairpin RNAs, trance-activator responsive region (TAR) and Rev responsive element (RRE), respectively. The molecule that compete with Tat and Rev protein may act as an inhibitor for above two regulations.

In this study, I have been trying to discover the novel compound specifically binds to HIV-1 RNA in order to inhibit the multiplication of an HIV, eventualy. As described in 1-3 and 1-4, a number of groups attempted neamine as a minimum skeleton that bind RNA and employed as a scaffold to construct an RNA binding reagent. However, most of the small molecules reported so far have indicated only a modest affinity and selectivity for RNA. Then I have chosen TAR and RRE RNA as the targets and designed trehalosamine, aromatic aminoglycoside and NbKε-neamine. And, I evaluate those

neamine mimics and derivatives about inhibition of TAR-Tat and RRE-Rev.

In chapter 2, I focused on structure of non-reducing disaccharides that is one of the novel neamine analogs, and describe the development of the useful glycosylation method to construction of non-reducing disaccharide. In chapter 2-2-1, we developed the condensation method of 1-hydroxy sugars with several alcohols in the presence of $Bi(OTf)_3$. The reactions of several benzylated 1-hydroxy sugars with certain primary alcohols using only 5 mol% $Bi(OTf)_3$ at reflux temperature in dichloromethane for 15 min successfully afforded the desired *O*-glycosides in good yields. In chapter 2-2-2, we described the synthesis of the various non-reducing disaccharides composition by the efficient dehydrative glycosylation reaction which used $Bi(OTf)_3$. In chapter 2-2-2-1, we examined the optimal conditions for carrying out the self-condensation of these, using glucose, mannose, galactose, 2-*N_3*-glucose, 2-*N_3*-6-*OAc*-glucose and arabinose as 1-hydroxy sugars. Self-condensation with 1-hydroxy sugars was promoted by 10-100 mol% $Bi(OTf)_3$ at room temperature in dichloromethane for 1d and produced various 1,1'-disaccharides. In chapter 2-2-2-2, I described the stereospecificity of non-reducing disaccharide formation. Therefore, I have established the novel dehydrative glycosydation which can build a 1,1'-disaccharide structure efficiently.

In chapter 3, I described the synthetic approach to trehalosamine and aromatic aminoglycoside as the neamine mimics. In chapter 3–2–1, I described about the synthesis of novel non-reducing amino disaccharides (trehalosamine). I succeeded the synthesis of trehalosamine as three structural isomers ($\alpha\alpha$, $\alpha\beta$, $\beta\beta$) which the 2,6-position is NH_2 in good yield. In chapter 3–2–2, I described about the synthesis of aromatic modified amino saccharide (aromatic aminoglycoside). The precursor of the aromatic aminoglycoside was synthesized from *N*-acetyl-**D**-glucosamine and 1,3-diaminophenol based on the glycosylation technique. However, the final removal of the protection groups was failed.

In chapter 4, I described the synthetic approach to nucleobase modified neamines. Lysine was employed as a linker which connect a nucleobse and a neamine. The nucleo base was connected at the ε-amino group of a lysine as that a placing the nucleobase with an appropriate distance from the scaffold of neamine (NbKε-namines). The four novel nucleobase modified neamine with L-lysine as the linker (NbKε-neamine) were successfully obtained as the novel small compound expected to bind TAR or RRE RNA specifically.

In chapter 5, I described the evaluation of the trehalosamine and NbKε-neamine as inhibitors of TAR-Tat and RRE-Rev. In chapter 5–2–1, I described the evaluation of the trehalosamine and NbKε-neamine as inhibitors of TAR-Tat. It was not observed the binding of αα, αβ, ββ-trehalosamine did not bind with the TAR RNA, although neamine had bind to the RNA. It became clear that the RNA-binding affinities vary greatly according to the slight difference in the structure and the position of amino groups. NbKε-neamine bound to TAR RNA as a competitor for Tat. Among four of NbKε-neamines, GbKε-neamine denotes the highest binding to TAR, AbKε-neamine and TbKε-neamine followed that of GbKε-neamine. These three bases are potentially able to interact with the nucleobases around the tri base of bulge in TAR RNA. In chapter 5–2–2, I described the evaluation of the “NbKε-neamine” as inhibitors of RRE-Rev. NbKε-neamines bind RRE with relatively high affinity. However the binding site for NbKε-neamine is independent from that of Rev. As a result, NbKε-neamine is not able to competitive with Rev. All the NbKε-neamines enhanced its binding affinities for RRE about 10 times higher than that of neamine itself. But there is no difference that depend on the nucleobase. An appropriate modification with the nucleobase on the neamine enhanced it binding affinity for the RNA. NbKε-neamines act as competitive inhibitor for TAR-Tat but not for RRE-Rev, and that inhibition magnitude was depend on the nucleobase of NbKε-neamine.

NbKε-neamine could be a candidate of potential inhibitor for TAR-Tat. It is expected

that the results obtained in this study will be useful for the discovery of the compound specifically bind to HIV-1 RNA in order to inhibit the multiplication mechanism of a HIV.

Publications related to this thesis

● Article

1) T. Yamanoi, **R. Inoue**, S. Matsuda and K. Hamasaki, *Lett*. *in Org*. *Chem*. **2008**, 5, 30

" Bismuth(III) triflate-catalyzed dehydrative glycosidation using 1-hydroxy sugars "

2) T. Yamanoi, **R. Inoue**, S. Matsuda, K. Iwao, Y. Oda, A. Yoshida and K. Hamasaki, *Heterocycles* **2009**, 77, 445

" Bismuth(III) triflate-catalyzed dehydrative glycosidation using 1-hydroxy sugars "

3) **R. Inoue**, S. Matsuda, Y. Oda, H. Ooyama, A. Yoshida, K. Hamasaki and T. Yamanoi, *Heterocycles* **2012**, 84, 1335

" A synthetic approach to aromatic aminoglycoside as a neamine mimic "

4) **R. Inoue**, K. Watanabe, T. Katou, Y. Ikezawa and K. Hamasaki, *Bioorg. Med. Chem.* **2015**, in press.

" Nucleobase modified neamines with L-lysine as a linker, synthesis and evaluation as potential inhibitors for HIV-1 TAR-Tat "

● Article (reference)

1) T. Yamanoi, S. Matsuda, I. Yamazaki, **R. Inoue**, K. Hamasaki, M. Watanabe, *Heterocycles* **2006**, 68, 673

2) T. Yamanoi, **R. Inoue**, S. Matsuda, K. Katsuraya, K. Hamasaki, *Tetrahedron: Asymmetry* **2006**, 17, 2914

3) **R. Inoue**, S. Matsuda, A. Yoshida, T. Yamanoi, K. Katsuraya, K. Hamasaki, *Glycoconjugate Journal* **2007**, 24, 339

4) T. Yamanoi, **R. Inoue**, Y. Oda and K. Hamasaki, *Molbank* **2010**, M671

5) T. Yamanoi, **R. Inoue**, Y. Oda, *Molbank* **2012**, M761

●International conference presentation（only first author）

1) XIX International Symposium on Glycoconjugates (2007)

R. Inoue, S. Matsuda, A. Yoshida, T. Yamanoi, K. Katsuraya, K. Hamasaki

"Synthesis of non-reducing disaccharides by the bismuth (III) triflate-catalyzed glycosidation"

2) The 2nd International Symposium on Fluorous Technologies (2007)

T. Yamanoi, **R. Inoue**, Y. Oda, A. Yoshida, K. Hamasaki

"Fluorous Lewis acid-catalyzed glycosidations"

3) The 40th International Symposium on Nucleic Acids Chemistry (2013)

R. Inoue, K. Watanabe, T. Katou, Y. Ikezawa and K. Hamasaki

"Nucleobase modified neamines with L-lysine as a linker, their binding toward hairpin RNAs"

4) The 41th International Symposium on Nucleic Acids Chemistry (2014)

R. Inoue, H. Matsumoto, K. Watanabe, T. Katou, Y. Ikezawa and K. Hamasaki

"Design, synthesis and binding study of the amino saccharide derivatives as a potential inhibitor for the RNA-protein interactions"

●Conference presentation（only first author）

1) 日本化学会第86回春季年会　2006年3月20日

山ノ井孝、〇**井上亮**、松田翔、濱崎啓太

「機能性トレハロース誘導体の合成研究」

2) 第26回日本糖質学会年会　2006年8月24日

〇**井上亮**、山ノ井孝、濱崎啓太

「ビスマス(III)トリフレートを用いた脱水縮合型グリコシル化反応によるトレハロース誘導体の合成」

3) 第32回反応と合成の進歩シンポジウム　2006年12月5日

〇**井上亮**、山ノ井孝、濱崎啓太

「ビスマス(III)トリフレートを用いた脱水縮合型グリコシル化反応の開発と非還元性二糖合成への応用」

4) 日本化学会第87回春季年会　2007年3月21日

〇**井上亮**、山ノ井孝、濱崎啓太

「ビスマス(III)トリフレートを用いた新規なグリコシル化反応と非還元性二糖合成への応用」

5) 第27回日本糖質学会年会　2007年8月1日

〇**井上亮**、山ノ井孝、濱崎啓太

「ネアミン構造をミミックする非還元性二糖の合成研究」

6) 第54回有機合成化学協会関東支部シンポジウム　2007年12月1日

〇**井上亮**、山ノ井孝、濱崎啓太

「脱水縮合型グリコシル化反応を基盤とした非還元性二糖の合成研究」

7) 日本化学会第88回春季年会 2008年3月27日

〇**井上亮**、山ノ井孝、濱崎啓太

「グリコシル化法によるネアミン類縁体の合成研究」

●Award

1）芝浦工業大学 大学院工学研究科 応用化学専攻 有元賞（2008）

Acknowledgments

I would like to express my sincerest thanks to Prof. Keita Hamasaki for his valuable suggestions, precise advice, extensive discussion, and continuous encouragement through this work. I could finish my graduate study under the direction of Prof. Hamasaki.

I wish to thank Prof. Takashi Yamanoi (Prof. School of Pharmaceutical Science, Josai University) for his variable suggestions, precise advice, extensive discussion. When I was a student of the master course, Prof. Yamanoi taught me the all of the synthesis technology and the attitude as a researcher.

I also really appreciate to Dr. Yoshiki Oda, Dr. Sho Matsuda and Dr. Akihiro Yoshida Mr. Kazuya Iwao for much precise advice of research techniques and extensive discussion.

Also I would like to express my thanks to Prof. Kaname Katsuraya (Prof. School of Home Economics, Wayo Women's University) for his extensive discussion and continuous encouragement through this work.

I have to thank the graduating students of the laboratory, Mr. Kentarou Watanabe, Mr. Toyofusa Katou, Mr. Yasunori Ikezawa, Mr. Kazuki Akasaka for their much advice and encouragement.

I wish to express my thanks to Dr. Tomomichi Okano, Dr. Norio Tobori, Mr. Kousuke Tanaka, Mr. Takayuki Hasegawa, Dr. Atsushi Naito, Mr. Ryo Hyoudou, Dr. Taku Ogura, Dr. Atsunori Morigaki (Lion Corporation) for their much advice and encouragement.

Finally, I would like to express my thanks to my parents and my wife Atsuko for giving a lot of support both in financial and mental.

Ryo Inoue