学位申請論文

Development of New Methods for the Synthesis of Disaccharide Nucleosides and Cyclic Oligosaccharides Utilizing Thioglycosyl Donors

（チオ糖供与体を利用した

新規ジサッカライドヌクレオシドおよび

環状オリゴ糖合成法の開発）

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Contents

Abbreviation

A549	a human alveolar adenocarcinoma
Ac	acetyl
Ac_2O	acetic anhydride
AcOEt	ethyl acetate
Ade	adenine or adenosine
ADP	adenosine 5'-diphosphate
AgOTf	silver triflate
aq.	aqueous
Ar	aryl
ATR	attenuated total reflection
BAHA	tris(4-bromopheny-l)ammoniumyl hexachloroantimonate
$BF_3 \cdot OEt_2$	boron trifluoride diethyl ether complex
Bn	benzyl
Bu_4NBF_4	tetrabutylammonium tetrafluoroborate
Bu_4NOTf	tetrabutylammonium triflate
***n*-BuLi**	*n*-butyllithium
Bz	benzoyl
Ca.	circa (approximately)
CCDC	cambridge crystallographic data centre
5,10-CH_2-THF	5,10-methylenetetrahydrofolate
COSY	correlation spectroscopy
$Cu(OTf)_2$	copper triflate
CyD	cyclodextrin
Cyt	cytosine or cytidine

DA	dihedral angle
dF(CF$_3$)ppy	2-(2,4-didifluorophenyl)-5-trifluoromethylpyridyl
DHFU	5,6-dihydro-5-fluorouracil
DMAP	*N*,*N*-dimethyl-4-aminopyridine
DMEM	Dulbecco's Modified Eagle' Medium
DMSO	dimethyl sulfoxide
DMTST	dimethyl(methylthio)sulfonium triflate
DNA	deoxyribonucleic acid
DPD	dihydropyrimidine dehydrogenase
dtbbpy	4,4'-di-*tert*-butyl-2,2'-bipyridine
DTBMP	2,6-di-*tert*-butyl-4-methylpyridine
dTMP	2'-deoxythymidine 5'-monophosphate
dTTP	2'-deoxythymidine 5'-triphosphate
dUMP	2'-deoxyuridine 5'-monophosphate
dUTP	2'-deoxyuridine 5'-triphosphate
EC$_{50}$	half maximal effective concentration
EI	electron ionization
EPO	erythropoietin
ESI	electrospray ionization
Et	ethyl
EtCN	propionitrile
Et$_2$O	diethyl ether
EtOH	ethanol
eq.	equivalent
FAB	fast atom bombardment
FCS	fetal calf serum
FDG	2-deoxy-2-fluoro-D-glucose

FdUDP	5-fluoro-2'-deoxyuridine 5'-diphosphate
FdUMP	5-fluoro-2'-deoxyuridine 5'-monophosphate
FdUTP	5-fluoro-2'-deoxyuridine 5'-triphosphate
5-FU	5-fluorouricil
FUDP	5-fluorouridine 5'-diphosphate
FUdR	5-fluoro-2'-deoxyuridine
FUMP	5-fluorouridine 5'-monophosphate
FUR	5-fluorouridine
FUTP	5-fluorouridine 5'-triphosphate
Gal	galactose or galactose derivative
Glc	glucose or glucose derivative
GPC	gel permeation chromatography
Gua	guanine or guanosine
HeLa S3	a human cervical carcinoma
HFIP	hexafluoroisopropanol
HMBC	heteronuclear multiple bond coherence
HMQC	heteronuclear multiple quantum correlation
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
***i*Bu**	isobutyryl
IDPC	iodonium dicollidine perchlorate
Ins(1,4,5)P_3	D-*myo*-inositol 1,4,5-trisphosphate
IR	infrared
JASRI	japan synchrotron radiation research institute
LG	leaving group
Me	methyl
MeCN	acetonitrile

MEM	minimum essential medium
$MeNH_2$	methylamine
MeOH	methanol
MeOTf	methyl triflate
Man	mannose or mannose derivative
MPBT	*S*-(4-methoxyphenyl) benzenethiosulfinate
mRNA	messenger ribonucleic acid
MS	mass spectrometry or molecular sieve
MTT	3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolum bromide
NBS	*N*-bromosuccinimide
NIS	*N*-iodosuccinimide
NMPTC	*N*-(*p*-methylphenylthio)-ε-caprolactam
NMR	nuclear magnetic resonance
ODS	octadecyl silyl
OPRT	orotate phosphoribosyltransferase
ORTEP	Oak Ridge thermal ellipsoid plot
PBS	phosphate buffered saline
PET	positron emission tomography
PG	protecting group
Ph	phenyl
Ph.D.	doctor of philosophy
Pin	pinacolato
PRPP	phosphoribosyl pyrophosphate
PTLC	preparative thin-layer chromatography
quant.	quantitative
R	an arbitrary organic substituent
RNA	ribonucleic acid

RR	ribonucleotide reductase
rRNA	ribosomal ribonucleic acid
r.t.	room temperature
SDS	sodium dodecyl sulfate
SET	single electron transfer
sLe^X	sialyl Lewis-X
snRNA	small nuclear ribonucleic acid
TCCA	trichloroisocyanuric acid
TFA	trifluoroacetic acid
Tf_2O	triflic anhydride
THF	tetrahydrofuran
TIPS	triisopropylsilane
TK	thymidine kinase
TLC	thin-layer chromatography
TMEDA	*N*,*N*,*N'*,*N'*-tetramethylethylenediamine
TMG	*N*,*N*,*N*-trimethyl-D-glucosamine
TMS	trimethylsilyl or tetramethylsilane
TMSCl	trimethylsilyl chloride
TMSOTf	trimethylsilyl triflate
Tol	tolyl
***p*-TolSCl**	*p*-toluenesulfenyl chloride
TP	thymidine phosphorylase
$TrB(C_6F_5)_4$	tetrakis(pentafluorophenyl)borate
tRNA	transfer ribonucleic acid
TS	thymidylate synthase
TSP	3-(trimethylsilyl)propionic acid
UDG	uracil-DNA glycosylase

UK	uridine kinase
UP	uridine phosphorylase
Ura	uracil
Uri	uridine
UV	ultraviolet
VIS	visible

Chapter 1.

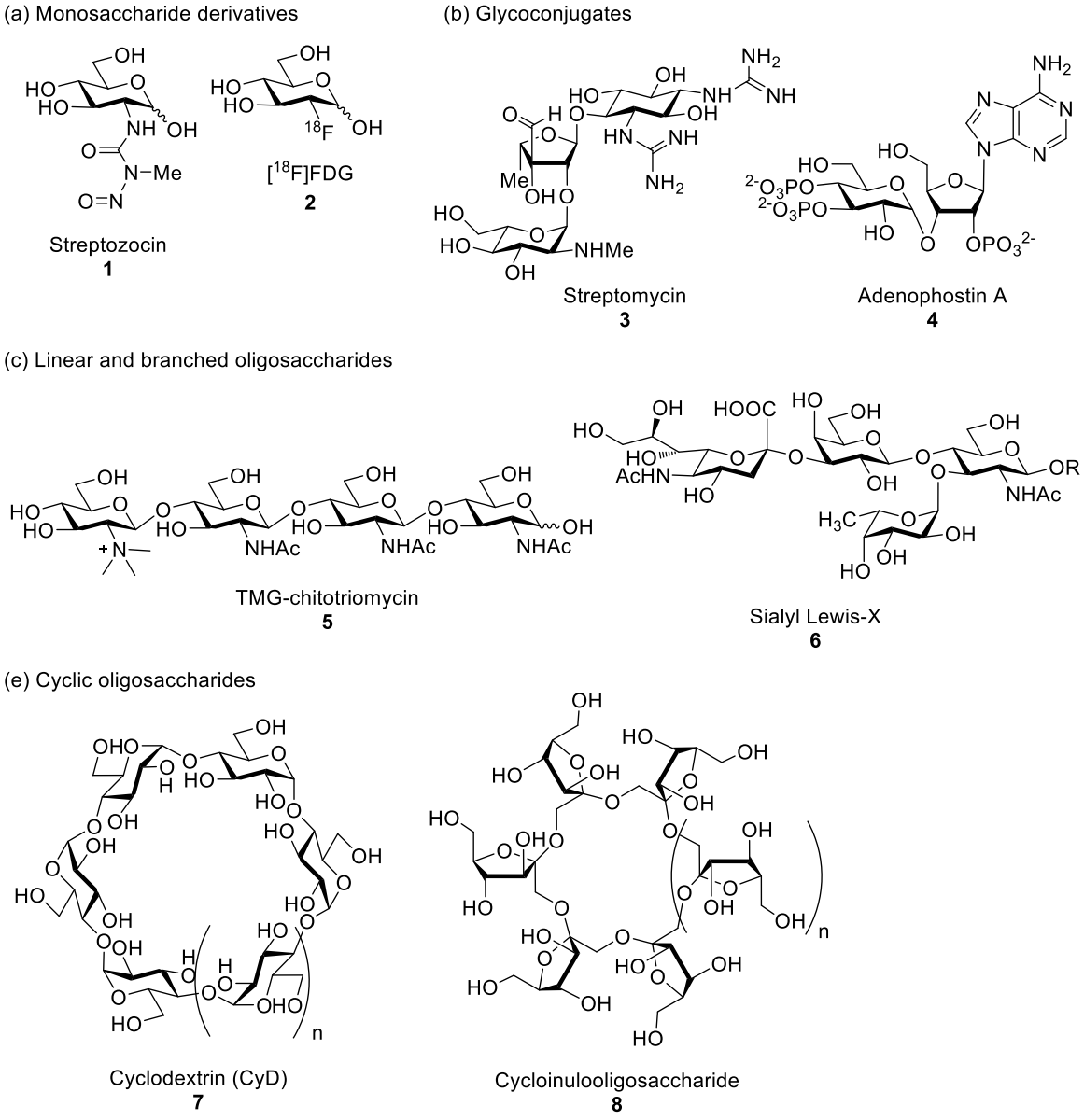
General Introduction

[1-1]: General introduction of carbohydrate derivatives

Carbohydrates are abundant biomolecules on earth as well as DNA, proteins and lipids and play important roles regarding molecular recognitions, biological reactions, homeostasis, and so on, in the life in nature [1]. For instance, the folding process of glycoproteins into the three-dimensional structure is important for them to work properly, and this process is regulated by molecular chaperones to recognize slightly different structure of a sugar chain [2]. Physiological activities of glycoproteins and glycolipids are also affected by the structure of sugar chains, and they are involved in various physiological phenomena such as cell-cell interactions, cancer metastasis and so on [3–5]. Recognition system of sugar chains is also applied to drug discovery. In the case of the preparation of erythropoietin (EPO) for the treatment of renal anemia, EPO analogues having more sialic acid residues in the molecule show a longer elimination half-life and higher hematopoietic activity than that before modification owing to suppression of metabolism for EPO in the liver [6]. Therefore, further development of new methods in glycobiology is highly desired.

Physiologically active and functional carbohydrate derivatives can be classified into the following four categories (Scheme 1-1). The first group includes monosaccharide derivatives such as streptozocin **1** [7] and 2-deoxy-2-(^{18}F)fluoro-D-glucose ([^{18}F]FDG) **2**, which are designed based on the theory that glucose uptake in the cancer cells is enhanced by higher expression of glucose transporters than that in the normal cells (Scheme 1-1a). Streptozocin **1** is glucosamine derivative having a *N*-methyl-*N*-nitrosourea moiety for methylation of DNA of the target cells and used for the treatment of pancreatic islet cell carcinoma as an antineoplastic agent that methylate DNA in the insulin-producing beta cells [8–9]. [^{18}F]FDG **2** is a glucose analog with the positron-emitting radionuclide fluorine-18 (^{18}F) used for the positron emission tomography (PET) as a medical imaging modality. [^{18}F]FDG **2** is transported into the cells through glucose transporters as similar to glucose and gets trapped there after

phosphorylation. Therefore, FDG-PET has been used for diagnosis, staging, and monitoring treatment of tumor tissues [10] .



Scheme 1-1. Examples of representative carbohydrate derivatives. (a) Monosaccharide derivatives; (b) glycoconjugates; (c) linear and branched oligosaccharides; and (d) cyclic oligo saccharides.

The second category is glycoconjugate including streptomycin **3** and adenophostin A **4** (Scheme 1-1b). These molecules have external sugar moieties linked to hydroxyl groups of the aglycone via an *O*-glycoside bond. Streptomycin **3** discovered from the metabolites of *streptomyces griseus* is an antibiotic used to treat bacterial infections to bind to the 30S subunit of the bacterial ribosome resulting in inhibition of a protein synthesis [11]. Adenophostin A **4** is one of disaccharide nucleosides and works as an agonist of D-*myo*-inositol 1,4,5-trisphosphate receptor ($Ins(1,4,5)P_3$ receptor) to release 100-fold higher Ca^{2+} from cerebellar microsomes than that of $Ins(1,4,5)P_3$ [12–13]. Chemical synthesis of these compounds requires to conduct glycosylation with glycosyl donors (this point will be further discribed below) to introduce sugar moieties in the aglycone [13–15].

The third group is linear and branched oligosaccharides that are composed of several monosaccharide moieties linked by *O*-glycosidic linkage (Scheme 1-1c). TMG-chitotriomycin **5** is linear tetrasaccharide which exhibited potent and selective inhibition against the β-*N*-acetylglucosaminidase of insects and fungi but no activity for humans and plants [16–17]. Meanwhile, sialyl Lewis-X (sLe^X) **6** is branched tetrasaccharide located on the surface of cells. In cancer cells, it is overexpressed and serve as ligands for selectins, cell adhesion molecules, to promote cancer metastasis. The reason why the expression of sLe^X **6** increase in cancer cells is that gene expressions responsible to the modifications of this molecule are suppressed by DNA methlation or histone deacetylation, which means incomplete synthesis of more complex sugar chains on normal cells [18]. Therefore, sLe^X **6** is utilized as the one of the tumor markers. It is ideal to supply theses oligosaccharides by chemical synthesis, because desired oligosaccharide obtained from natural source are often trace amount and it is difficult to isolate them from various contaminants.

The fourth category includes cyclic oligosaccharides such as cyclodextrins (CyDs) **7** and cycloinulooligosaccharides **8** (Scheme 1-1d). These molecules are

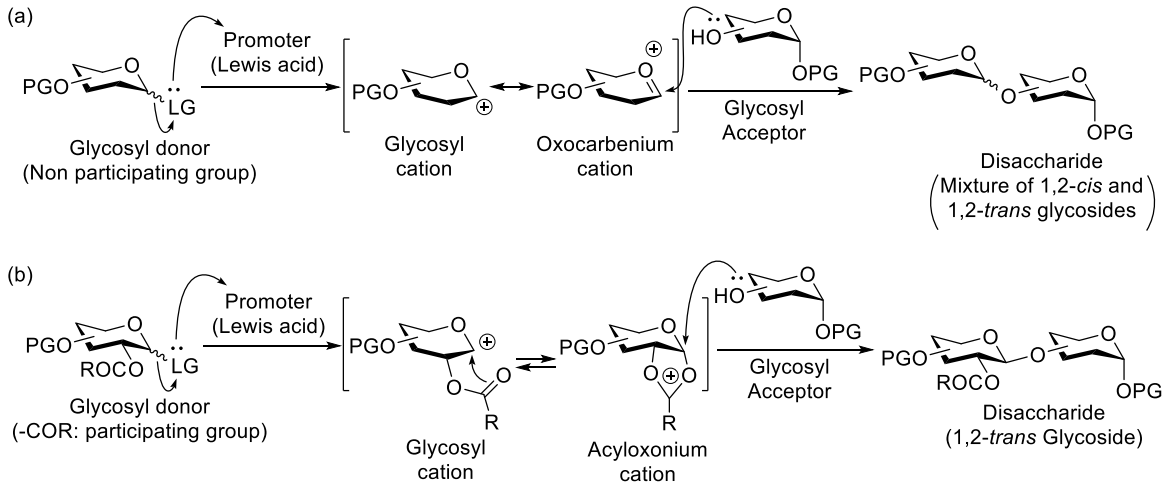
composed of a macrocyclic ring of monosaccharide subunits and have inner cavities to capture the various organic and/or inorganic guest molecules. For instance, CyDs **7** can make inclusion complexes with many kinds of organic molecules such as drugs and food ingredients to improve their solubility and stability in water [19,20]. On the other hand, it is reported that cycloinulooligosaccharides **8** capture the metal cations such as K^+, Rb^+, Cs^+, Ag^+, $Ba^{2+}$and Pb^{2+} [21]. The general method for the chemical synthesis of cyclic oligosaccharides is cyclization of linear oligosaccharides prepared from monosaccharides by stepwise synthesis. It requires tedious processes such as protecting group and/or leaving group manipulations, glycosylation reactions, and purifications after each synthetic step.

It is necessary to stably provide consistent quality and large amount of these carbohydrate derivatives for the research of those functions. Chemical synthesis is often useful for the large-scale synthesis of the structurally well-defined natural and artificial carbohydrate derivatives in high chemical yield. Thus, considerable efforts have been dedicated to develop the efficient chemical synthetic methods for the synthesis of desired carbohydrate derivatives [22–25].

[1-2]: General introduction of chemical glycosylation

In general *O*-glycosylation reactions, glycosyl donors having leaving group at anomeric position (*C*-1 position) are activated by promoters. The resulting oxocabenium cation intermediates react with glycosyl acceptors having a hydroxyl group via S_N1-like manner under the anhydrous reaction conditions. In other words, the anomeric carbon of intermediate is sp^2 hybridized, and nucleophilic attack of acceptor would be almost equally possible from either the top face (β-face) or bottom face (α-face) of the ring. Therefore, products are obtained as the mixture of 1,2-*cis* and 1,2-trans glycosides (Scheme 1-2a). The stereochemistry of the glycosylation products is dependent on various factors such as temperature, protecting groups of donors and

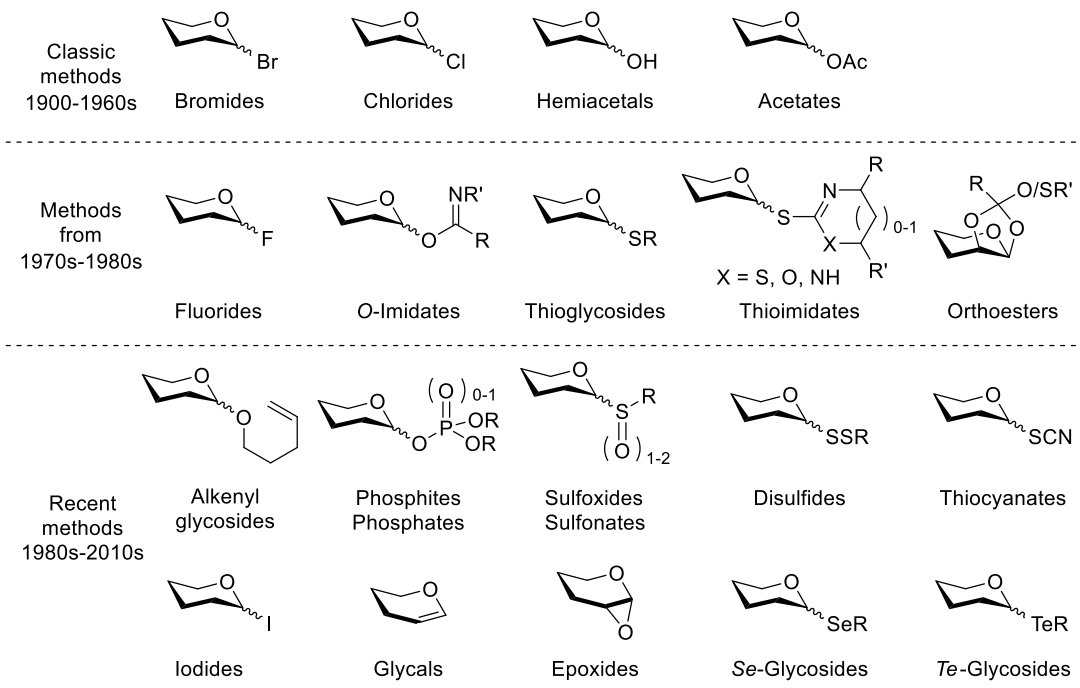
acceptors, solvents, promoters, steric hindrance and leaving groups [26]. The glycosylation utilizing a neighboring group participation is one of strategies widely used to control the stereochemistry of glycosylation reactions. A 1,2-*trans* glycosidic linkage can be selectively formed by anchimeric assistance of a neighboring participating group, generally an acyl protecting group. The glycosylation proceeds via acyloxonium cation resulted from the intramolecular stabilization of the glycosyl cation. In this reaction, the glycosyl acceptor attacks from the β-face of the sugar ring to permit stereoselective formation of the 1,2-*trans* glycoside (Scheme 1-2b) [27].



Scheme 1-2. The general mechanism of a glycosylation reaction. (a) Glycosylation using glycosyl donor without a participating group; and (b) glycosylation utilizing neighboring group participation. (PG = protecting group, LG = leaving group)

The most classical glycosylation reaction using glycosyl halides (glycosyl bromides and chlorides) as glycosyl donors was reported in 1901 by Koenigs and Knorr (Scheme 1-3) [28], which stimulated the research of the chemical glycosylation reactions and the development of many kinds of glycosyl donors and their activation methods. From 1970s to early 1980s, several types of glycosyl donors were developed: glycosyl fluorides by Mukaiyama *et al.* [29]; *O*-imidates by Sinay *et al*. [30] and Schmidt and Michel [31]; thioglycosides by Ferrier *et al*. [32], Nicolaou *et al*. [33], and Garegg *et al*.

[34]; thioimidates by Mukaiyama *et al*. [35], Hanessian *et al*. [36], and Woodward *et al*. [37]; and orthoester derivatives by Kochetkov *et al.* [38,39] . Moreover, various glycosyl donors have been developed from the end of 1980s to the present: alkenyl glycosides by Fraser-Reid *et al*. [40]; phosphites by Wong *et al*. [41]; sulfoxides by Kahne *et al*. [42] and so on [27]. In recent years, glycosyl fluorides, trichloroacetoimidates and thioglycosides have been widely used for the glycosylation to synthesize various carbohydrate derivatives [43–45].



Scheme 1-3. Classification of glycosyl donors

[1-3]: Aim and contents of thesis: the synthesis of disaccharide nucleosides and cyclic oligosaccharide using thioglycosides.

In this thesis, we report on the synthesis of disaccharide nucleosides and cyclic oligosaccharides using thioglycosides. Thioglycosyl donors are commonly used for the glycosylation reaction because of the four advantages: (i) applicability to glycosylation of a wide range of substrates due to mild activation conditions ; (ii) high reactivity under

specific promoter system which allows convergent, sequential, or one-pot glycosylation; (iii) high stability under various conditions to permit protecting group manipulations and derivatizations; and (iv) easy preparation and long shelf life. Activation method for the thioglycosides can be classified into 5 types as shown in Scheme 1-4 [46].

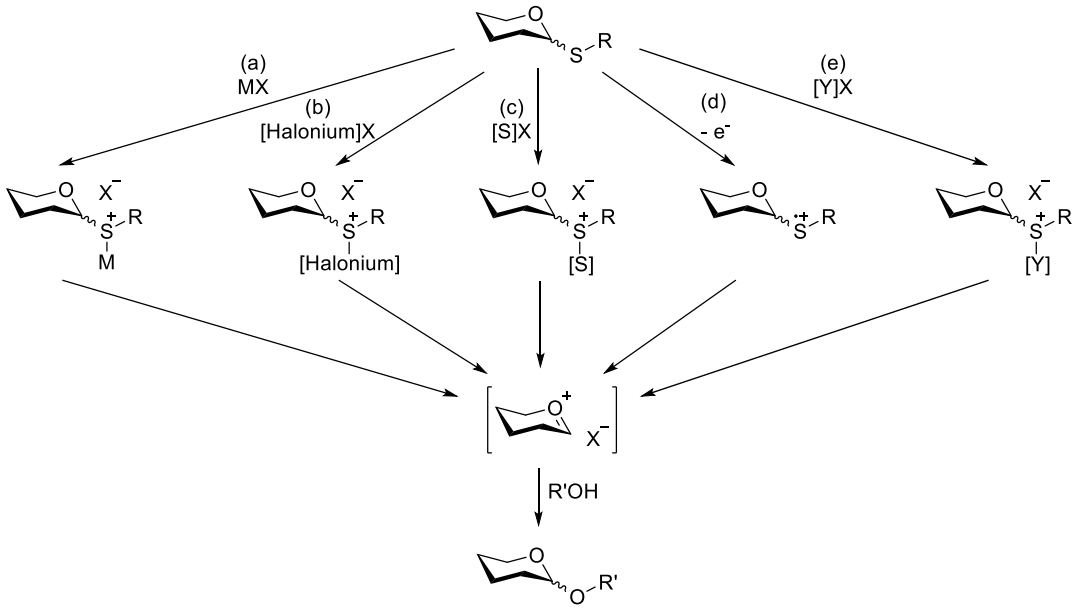
First, thiophilic metal salts such as $HgSO_4$ [32], $Cu(OTf)_2$[47], $AgPF_6$ [48] have been used in earlier years (Scheme 1-4a). However, the glycosylation using metal salts is limited only specific cases due to the toxicity of heavy metal and the formation of byproducts.

Second, halonium reagents, especially including bromonium or iodonium species can activate thioglycosides under milder conditions than that of the aforementioned metal salts (Scheme 1-4b). To date, a lot of *N*-Bromosuccinimide (NBS) or *N*-iodosuccinimide (NIS) based promoter systems have been developed and widely used [33,49–64]. Other activation methods for thioglycosides using halonium species include iodonium dicollidine perchlorate (IDPC) [65], iodosobenzene (PhIO)/triflic anhydride (Tf_2O) [66], trichloroisocyanuric acid (TCCA)/trimethylsilyl triflate (TMSOTf) [67] and so on.

The third groups is organosulfur reagents such as dimethyl(methylthio)sulfonium triflate (DMTST) [68], *p*-toluenesulfenyl chloride (*p*-TolSCl)/silver triflate (AgOTf) [69], *S*-(4-methoxyphenyl) benzenethiosulfinate (MPBT)/Tf_2O [70], *N*-(*p*-methylphenylthio)-*ε*-caprolactam (NMPTC)/TMSOTf [71], which are widely applied for the activation of thioglycosides (Scheme 1-4c).

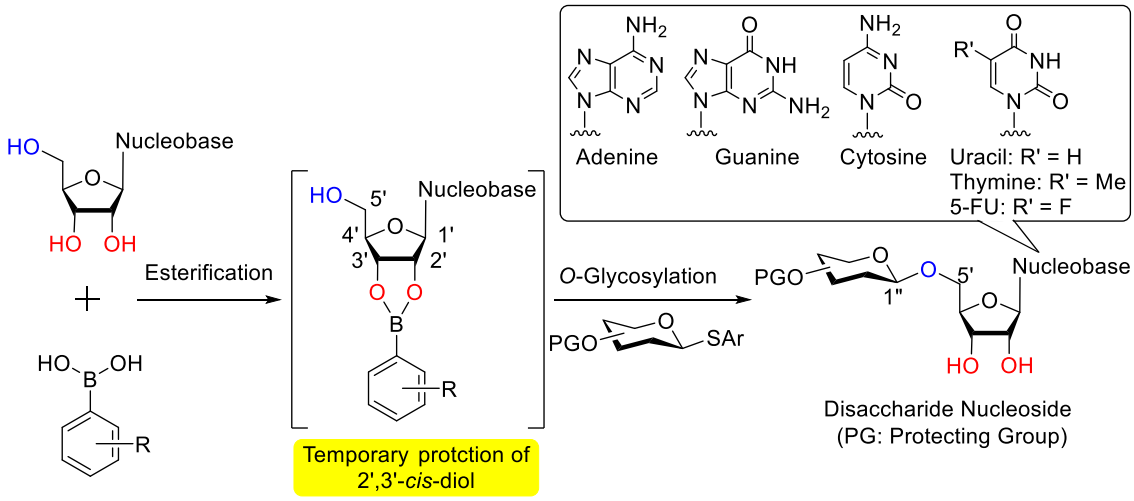
Electro-chemical oxidation utilizing single electron transfer (SET) also can activate thioglycosides, which can be categorized as the fourth method for the activation of thioglycosides (Scheme 1-4d) [72]. The glycosylations by SET are performed using electrode (anodic oxidation/Bu_4NBF_4, $LiClO_4$, Bu_4NOTf) [73–75], light ($[Ir\{dF(CF_3)ppy\}_2(dtbbpy)]PF_6$/$BrCCl_3$/HFIP) [76], and chemical reagents tris(4-bromopheny-l)ammoniumyl hexachloroantimonate (BAHA)) [77].

The fifth activation methods of thioglycosides classified into other categories include methyl triflate (MeOTf) [78], $NOBF_4$ [79], trityl tetrakis(pentafluorophenyl)borate ($TrB(C_6F_5)_4$)/$NaIO_4$ (Scheme 1-4e) [80] .



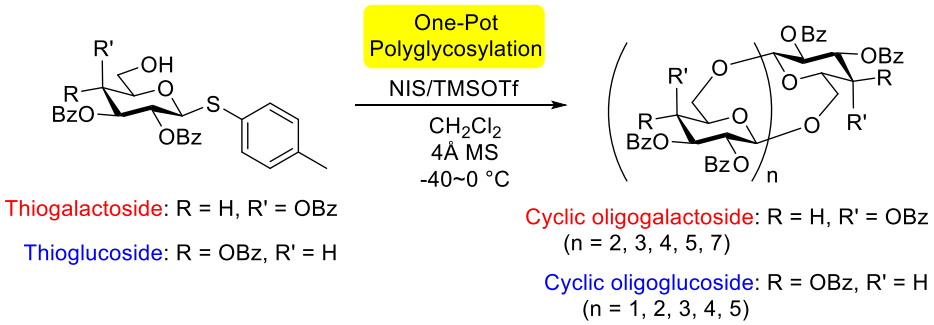
Scheme 1-4. Classification of promoters for the thioglycoside. Activated by (a) metal salts; (b) halonium reagents; (c) organosulfur reagents; (d) single electron transfer (SET); and (e) other reagents. (X^- = counter anion, [Y] = other activation species)

In Chapter 2, we report on the regioselective glycosylation of unprotected ribonucleosides via the temporary protection of 2',3'-*cis*-diol by a boronic ester for the synthesis of disaccharide nucleosides (**Scheme 1-5**). These results were published in *Molecules* and the experimental manipulations were published as video protocol in *Journal of Visualized Experiment* (*JoVE*). Moreover, anticancer activity of synthesized disaccharide nucleosides including 5-fluorouridine moiety was also examined.



Scheme 1-5. Synthesis of disaccharide nucleosides utilizing the temporary protection of the 2',3'-*cis*-diol of ribonucleosides by a boronic ester.

In Chapter 3, we describe the one-pot synthesis of cyclic oligosaccharides by the polyglycosylation of monothioglycosides such as thiogalactosides and thioglucosides (**Scheme 1-6**).



Scheme 1-6. Synthesis of cyclic oligosaccharides by the polyglycosylation of monothioglycosides.

Chapter 4 is the conclusion of this Ph.D. thesis with the prospects about these reactions for synthesis of carbohydrate derivatives and application for medicinal chemistry and other fields.

Chapter 2.

Regioselective Glycosylation of Ribonucleosides Utilizing Boronic Ester for Synthesis of Disaccharide Nucleosides

ボロン酸エステルを利用した

リボヌクレオシドの

位置選択的グリコシル化反応による

ジサッカライドヌクレオシドの合成

[2-1]: Introduction

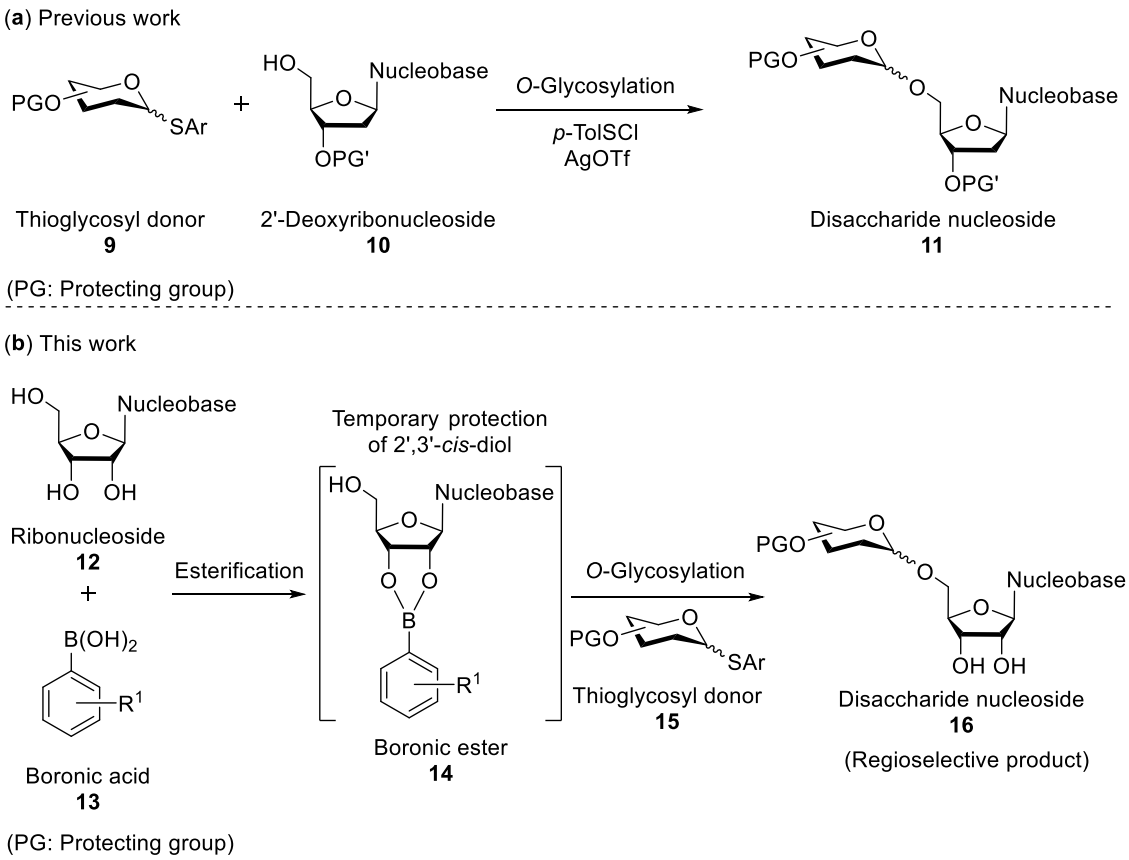
Disaccharide nucleosides, which contain an external sugar moiety linked to one of the hydroxyl groups of the nucleoside via an *O*-glycoside bond, constitute an important class of natural compounds [81–87]. They are found in biopolymers, such as tRNA and poly(ADP-ribose), as well as antibiotics and other biologically-active compounds [85,86,88–91]. Adenophostins [12,92–94], HF-7 [95], amicetin analogs [86,96], ezomycin [97] and some candidates for inhibitors of chitin synthase [98] are typical examples of disaccharide nucleosides that contain adenine, guanine, cytosine and uracil moieties, respectively. Therefore, disaccharide nucleosides and their analogs would be expected to be good drug candidates.

Several strategies for the synthesis of disaccharide nucleosides such as enzymatic *O*-glycosylation [99,100], chemical *N*-glycosylation [85,89,95,101–103] and chemical *O*-glycosylation [27,87,89,93,95,97,98,103,104–115] have been reported to date. Chemical *O*-glycosylation is often useful for the large-scale synthesis of the desired disaccharide nucleosides in higher chemical yields compared to chemical *N*-glycosylation. However, the neutralization of promoters, which are generally Lewis or Brønsted acids, by the nucleobase moieties would be a possible drawback. Moreover, it is reported that an excess amount of the glycosyl donor is required for glycosylation at the hydroxyl site to be complete, because it is likely that glycosylation preferentially proceeds on the nucleobase or other Lewis basic site [97,110,112,114]. Side reactions such as depurination (cleavage of the anomeric C–N bond of nucleosides), anomerization reaction and trans-purinylation have also been reported [113,116,117].

We previously reported on the synthesis of disaccharide nucleosides **11** by the direct *O*-glycosylation of 2'-deoxyribonucleoside **10** with the thioglycosyl donor **9** (PG: protecting group) (Scheme 2-1a) [118]. Among the glycosyl promoters tested, a combination of *p*-toluenesulfenyl chloride (*p*-TolSCl) and silver triflate (AgOTf) was found to give the corresponding products in moderate to high chemical yields. These

results prompted us to investigate the synthesis of disaccharide nucleosides via the *O*-glycosylation of ribonucleosides. The synthesis of disaccharide nucleosides using protected ribonucleosides as glycosyl acceptors, which requires tedious protecting group manipulations, has been reported in previous studies [87,89,93,95,97,98,103,110–115]. The development of direct and regioselective *O*-glycosylation using unprotected or temporarily-protected ribonucleosides would afford a more convenient synthetic route to prepare various biologically-active derivatives.

In this manuscript, we report on the *O*-glycosylation of unprotected ribonucleosides **12** at the 5'-hydroxyl group via the temporary protection of the 2',3'-*cis*-diol by a boronic ester **14**. It has been reported that boronic and borinic acids are capable of forming the cyclic esters with carbohydrate derivatives [119,120], and such derivatives have been utilized for regio- and/or stereo-selective alkylation, acylation, silylation and glycosylation [121–132]. In our strategy, the ribonucleoside **12** is treated with the boronic acid **13** to temporarily protect the 2',3'-*cis*-diol of **12** to prepare **14** in situ, which is then *O*-glycosylated at the 5'-hydroxyl group with the glycosyl donor **15** to afford the disaccharide nucleosides **16** in a regioselective manner (Scheme 2-1b) (in this manuscript, "disaccharide nucleosides" include the glycosylated deoxyribonucleosides and ribonucleosides, due to the generally-used terminology).



Scheme 2-1. (a) *O*-glycosylation of 2'-deoxyribonucleoside with a thioglycosyl donor using the *p*-toluenesulfenyl chloride (*p*-TolSCl)/promoter system; (b) regioselective *O*-glycosylation of ribonucleoside at the 5'-OH protection of 2',3'-*cis*-diol.

[2-2]: Results and Discussion

2-2-1. *O*-Glycosylation of nucleosides with thioglycosyl donors

We first examined the *O*-glycosylation of uridine **18** with the thiomannoside **17** [133] using 3.0 equivalents of *p*-TolSCl and 6.0 equivalents of AgOTf [69,134] against **18** (i.e., 2.0 equivalents of *p*-TolSCl and 4.0 equivalents of AgOTf against **17** according to our previous paper [118]). Thioglycosides are one of the most popular glycosyl donor due to their ease of preparation and modification, high stability and the many available activation methods [27,46,104–107]. After the glycosylation and crude purification, the

resulting compounds were acetylated to permit the desired products to be purified more easily.

The results for the glycosylation reactions are summarized in Table 2-1. In entry 1, the glycosylation of **18** with **17** without boronic acid derivatives gave a complex mixture. In entry 2, a mixture of **18** and phenylboronic acid **19a** was co-evaporated with pyridine and 1,4-dioxane followed by stirring in 1,4-dioxane under reflux conditions [122] to prepare the temporary 2',3'-*cis*-diol-protected intermediate **14** (in Scheme 2-1), to which **17** (corresponding to **15** in Scheme 2-1) was added. The glycosylation of **14** proceeded at its 5'-OH to afford **20** (corresponding to **16** in Scheme 2-1) in 41% (α/β = 1.6/1) in a regioselective manner. The formation of a 1",5'-glycosidic linkage of **20** was confirmed by comparing its ^{1}H NMR spectrum with that of the authentic sample prepared by another synthetic route (Scheme 2-2). The glycosylation of 2',3'-*O*-isopropylideneuridine **21**, which was prepared from **18** [135], with **17** in the presence of *p*-TolSCl, AgOTf, and 2,6-di-tert-butyl-4-methylpyridine (DTBMP) [136] followed by the cleavage of isopropylidene protecting group and acetylation gave the authentic sample **20** in 20% for four steps from **18** to **20** (excluding the steps required for the preparation of **17**).

In entry 3 of Table 2-1, a mixture of **17**, **18** and **19a** was co-evaporated with pyridine and 1,4-dioxane, and the resulting mixture was treated with promoters to give **20** in a yield nearly similar to that for entry 2. In the following entries 4–15, therefore, glycosylation reactions were conducted using a procedure similar to that used in entry 3 for easy manipulation.

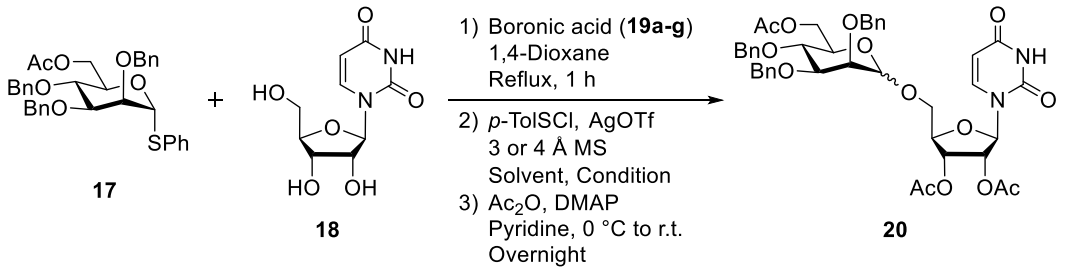
The electrostatic effect of the substituents of the boronic acid was studied in entries 4–8. Electron-deficient arylboronic acids such as 4-(trifluoromethyl)phenylboronic acid **19c** and 2,4-difluorophenylboronic acid **19d** resulted in higher chemical yields of **20** than that of 4-methoxyphenylboronic acid **19b**, possibly due to the higher stability of boronic ester intermediate prepared from electron-deficient arylboronic acid [137]. However, the use of 4-nitrophenylboronic acid **19e**, which has also an electron-

withdrawing group, resulted in a low chemical yield of **20** on account of the low solubility of boronic ester intermediate in acetonitrile. In entry 8, alkylboronic acid (cyclopentylboronic acid **19f**) was used instead of arylboronic acid, which resulted in a lower chemical yield of **20** than in that of arylboronic acids.

The solvent effect and reaction temperature were also examined in entries 9–13. It is well known that glycosylation in an ether-type solvent such as Et_2O, THF and 1,4-dioxane enhances α-stereoselectivity [138,139]. As shown in entry 9, 1,4-dioxane improved the α-stereoselectivity of the reaction, while the chemical yield was unsatisfactory. In entries 10 and 11, CH_2Cl_2 gave a small or negligible amount of **20**, due to the low solubility of the substrates at -20 and -40 °C. Glycosylations using EtCN gave **20** in higher chemical yields (entry 12, 13) than those for 1,4-dioxane (entry 9) and MeCN (entry 5) with the similar stereoselectivity of that in MeCN (entry 5), and the lower temperature afforded slightly higher chemical yield (entry 13).

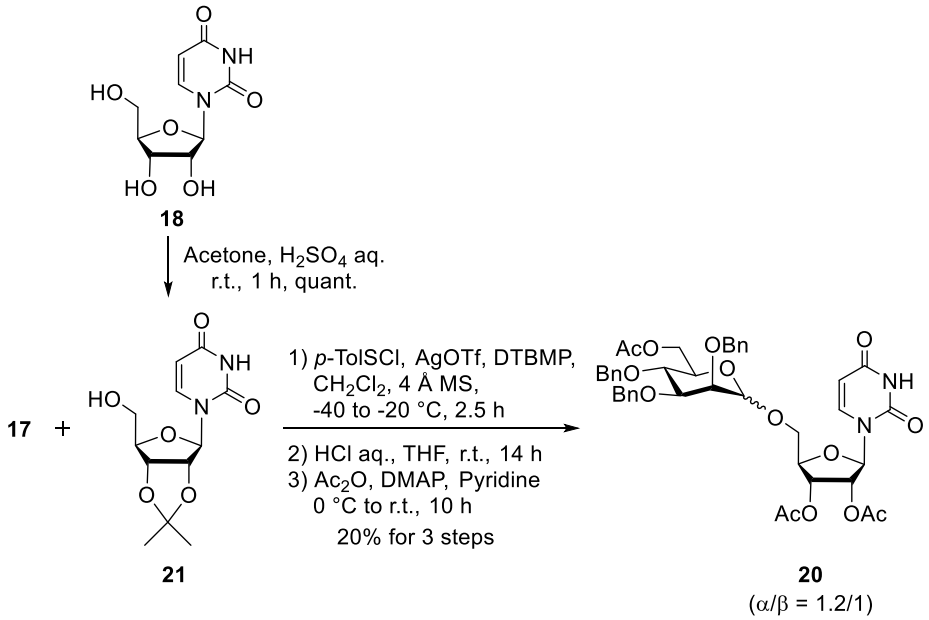
In entry 14, glycosylation using lower equivalents of promoters (1.8 equivalents of *p*-TolSCl and 3.6 equivalents of AgOTf against **18**) than those in entry 13 gave slightly lower chemical yield. Therefore, 3.0 equivalents and 6.0 equivalents of *p*-TolSCl and AgOTf were used in the following *O*-glycosylations to complete the reactions. In entry 15, phenylboronic acid having a C6 alkyl chain **19g** was used to improve the solubility of the boronic ester, albeit the chemical yield was not improved.

Table 2-1. *O*-Glycosylation of uridine **18** with the thiomannoside **17** in the absence and presence of boronic acid.



Entry	Boronic acid [b]	Solvent	Condition	Yield (for 3 Steps) [c]
1 [a]	-	MeCN	−20 °C, 1.5 h	<16% (complex mixture)
2 [a,d]	$PhB(OH)_2$ (**19a**)	MeCN	−20 °C, 1.5 h	41% (α/β = 1.6/1)
3 [a,e]	**19a**	MeCN	−20 °C, 1.5 h	45% (α/β = 1.6/1)
4 [a,e]	$4\text{-}MeOC_6H_4B(OH)_2$ (**19b**)	MeCN	−20 °C, 1.5 h	39% (α/β = 1.8/1)
5 [a,e]	$4\text{-}CF_3C_6H_4B(OH)_2$ (**19c**)	MeCN	−20 °C, 1.5 h	51% (α/β = 1.8/1)
6 [a,e]	$2,4\text{-}F_2C_6H_4B(OH)_2$ (**19d**)	MeCN	−20 °C, 1.5 h	46% (α/β = 1.8/1)
7 [a,e]	$4\text{-}NO_2C_6H_4B(OH)_2$ (**19e**)	MeCN	−20 °C, 1.5 h	24% (α/β = 1.6/1)
8 [a,e]	Cyclopentylboronic acid (**19f**)	MeCN	−20 °C, 1.5 h	8% (α/β = 1.7/1)
9 [a,e]	**19c**	1,4-Dioxane	r.t., 1.5 h	27% (α/β = 3.3/1)
10 [a,e]	**19c**	CH_2Cl_2	−20 °C, 1.5 h	5% (α/β = 2.1/1)
11 [a,e]	**19c**	CH_2Cl_2	−40 °C, 1.5 h	Trace
12 [a,e]	**19c**	EtCN	−20 °C, 1.5 h	55% (α/β = 1.7/1)
13 [a,e]	**19c**	EtCN	−40 °C, 1.5 h	61% (α/β = 1.6/1)
14 [e,f]	**19c**	EtCN	−40 °C, 1.5 h	57% (α/β = 1.5/1)
15 [a,e]	$4\text{-}CH_3(CH_2)_5C_6H_4B(OH)_2$ (**19g**)	EtCN	−40 °C, 1.5 h	30% (α/β = 1.6/1)

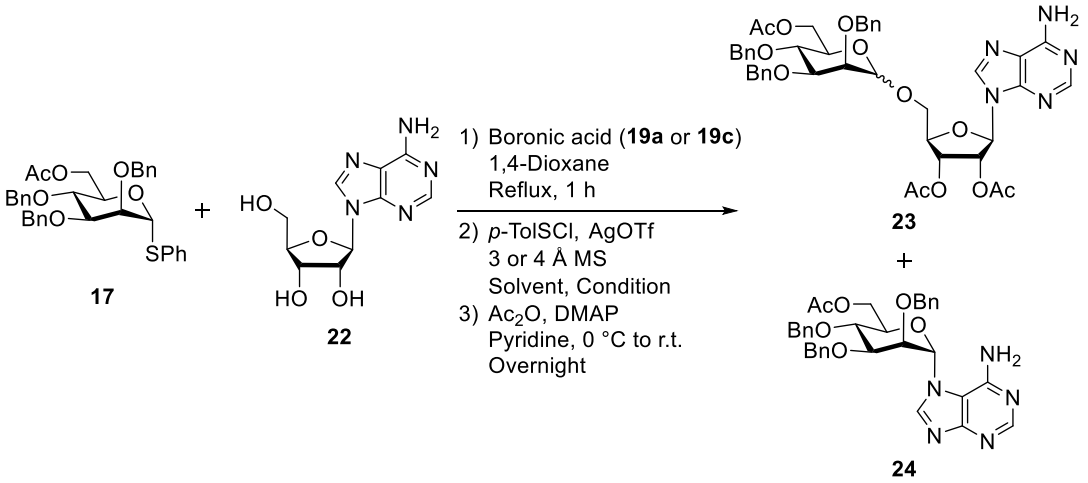
[a] Glycosylation reactions were carried out in the presence of 1.5 equivalents of **17**, 3.0 equivalents of *p*-TolSCl and 6.0 equivalents of AgOTf against **18**. Acetylation reactions were carried out in the presence of *ca.* 10 equivalents of Ac_2O and catalytic amount of DMAP. [b] Stoichiometry of **19** was 1.5 equivalents against **18**. [c] The α/β ratio was determined by ^1H NMR. [d] A mixture of **18** and **19a** was co-evaporated with pyridine and 1,4-dioxane, and then, a solution of **17** in MeCN was added. [e] A mixture of **17**, **18** and **19** was co-evaporated with pyridine and 1,4-dioxane and treated with promoters. [f] Glycosylation reactions were carried out in the presence of 1.5 equivalents of **17**, 1.8 equivalents of *p*-TolSCl and 3.6 equivalents of AgOTf against **18** as followed by acetylation with Ac_2O (*ca.* 10 equivalents) and DMAP (catalytic amount).



Scheme 2-2. Synthesis of disaccharide nucleoside **20** as authentic samples.

The *O*-glycosylation of adenosine **22** with **17** was examined next. As shown in entry 1 of Table 2-2, *O*-glycosylation in the absence of boronic acid derivatives gave a complex mixture, as in the case of uridine (entry 1 in Table 2-1). In entries 2 and 3, in which $PhB(OH)_2$ **19a** and $4\text{-}CF_3C_6H_4B(OH)_2$ **19c** were used, **23** was produced, but the yields were lower (14% and 11%, respectively) than those of **18** in entries 3 and 13 of Table 2-1, which can be attributed to the trans-purinylation of **22** and/or **23** (*N*-mannosyl adenine **24** was isolated in 6–27%) [113].

Table 2-2. *O*-Glycosylation of adenosine **22** with thiomannoside **17** in the absence and presence of boronic acid.



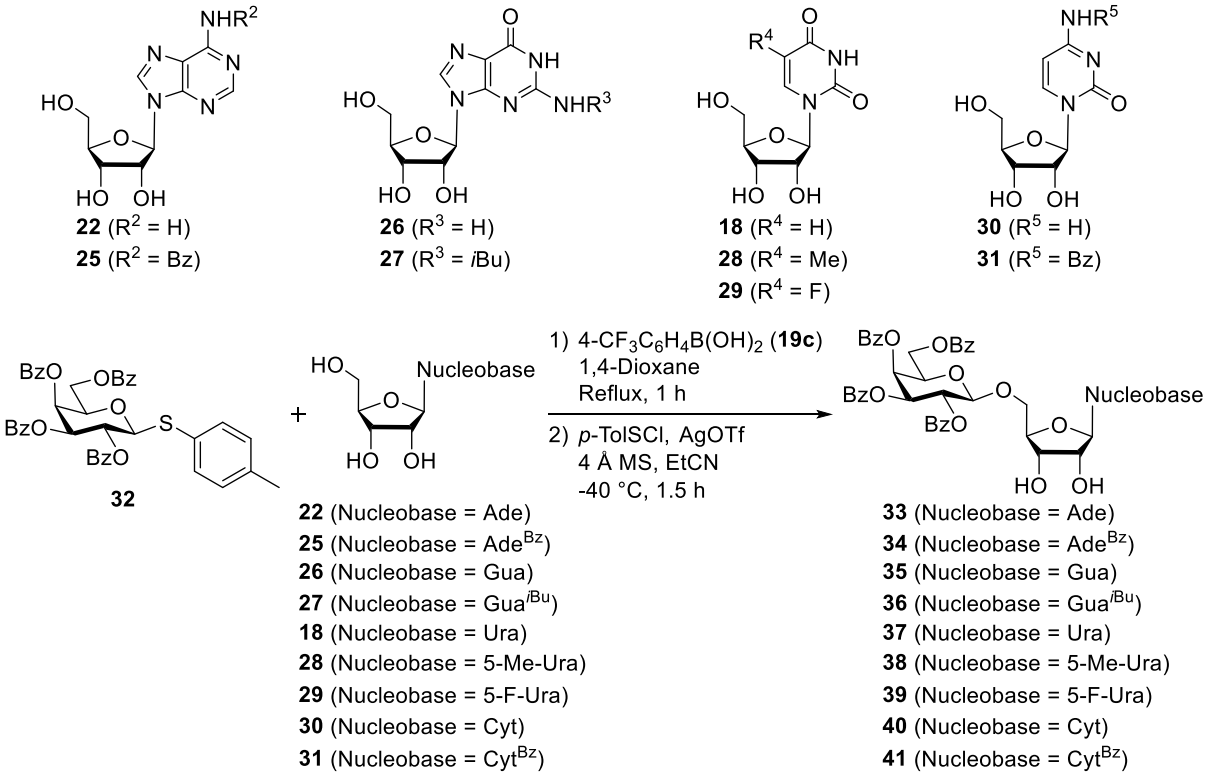
Entry [a]	Boronic acid [b]	Solvent	Condition	Yield of **23** (for 3 Steps) [c]	Yield of **24** (for 3 Steps)
1	-	MeCN	−20 °C, 1.5 h	<10% (complex mixture)	not isolated
2 [d]	$PhB(OH)_2$ (**19a**)	MeCN	−20 °C, 1.5 h	14% (α/β = 1/1.0)	6%
3 [d]	$4\text{-}CF_3C_6H_4B(OH)_2$ (**19c**)	EtCN	−40 °C, 1.5 h	11% (α/β = 1/1.2)	27%

[a] Glycosylation reactions were carried out in the presence of 1.5 equivalents of **17**, 3.0 equivalents of *p*-TolSCl and 6.0 equivalents of AgOTf against **22**. Acetylation reactions were carried out in the presence of *ca.* 10 equivalents of Ac_2O and the catalytic amount of DMAP. [b] Stoichiometry of **19** was 1.5 equivalents against **22**. [c] The α/β ratio was determined by ^1H NMR. [d] A mixture of **17**, **22** and **19** was co-evaporated with pyridine and 1,4-dioxane and treated with promoters.

We attempted the *O*-glycosylations of various nucleosides **18**, **22** and **25**–**31** with the thiogalactoside **32** [140], in which the hydroxyl groups were protected by benzoyl groups to achieve β-selective *O*-glycosylation by neighboring group participation at the O2 benzoyl group (Table 2-3). The formation of a β-1",5'-glycosidic linkage between the galactose moiety and ribonucleoside in the products **33**–**41** was confirmed by NNR measurements (^1H NMR, ^{13}C NMR, ^1H-^1H COSY, ^1H-^{13}C HMQC and ^1H-^{13}C HMBC). As listed in Table 3, the reaction of the unprotected and *N*-protected adenosine, **22** and

25 [141], afforded the desired products β-**33** and β-**34** in 42% and 30%, respectively (entries 1 and 2). Note that the use of unprotected adenosine **22** gave a better yield than that for the protected **25**, phenomena similar to the *O*-glycosylation of 2'-deoxyadenosine reported by us in a previous study (Scheme 2-1a) [118]. It should also be noted that the reaction of **22** with **32** (entry 1) gave negligible amounts of *N*-galactosyl adenine as a byproduct unlike the use of the mannosyl donor **17** in an *O*-glycosylation reaction (entries 2 and 3 in Table 2-2). In entries 3 and 4, the reaction of the unprotected and *N*-protected guanosine, **26** and **27** [142], afforded the corresponding products β-**35** and β-**36** in 12% and 44%, respectively. The higher yield of β-**36** is possibly due to better solubility of the boronic ester intermediate prepared from the *N*-protected **27** in EtCN than that from the unprotected **26**. In entry 5, the *O*-glycosylation of uridine **18** with **32** gave the desired product β-**37** in 42% yield, and the electrophilic substitution reaction at the 5-position of the uracil moiety of **18** and/or β-**37** with the *p*-toluenesulfenyl cation was observed (*ca.* 15%) [143]. In entries 6 and 7, the *O*-glycosylation of 5-metyluridine **28** and 5-fluorouridine **29** afforded the corresponding products β-**38** and β-**39** in 53% and 61%, respectively. In entries 8 and 9, the reaction of unprotected and *N*-protected cytidine, **30** and **31** [144], afforded β-**40** and β-**41** in 55% and 40%, respectively. It should be noted that the use of the unprotected **30** gave β-**40** in slightly higher yield than β-**41** from the protected **31**.

Table 2-3. *O*-Glycosylation of nucleosides **18**, **22** and **25**–**31** with the thiogalactoside **32**.

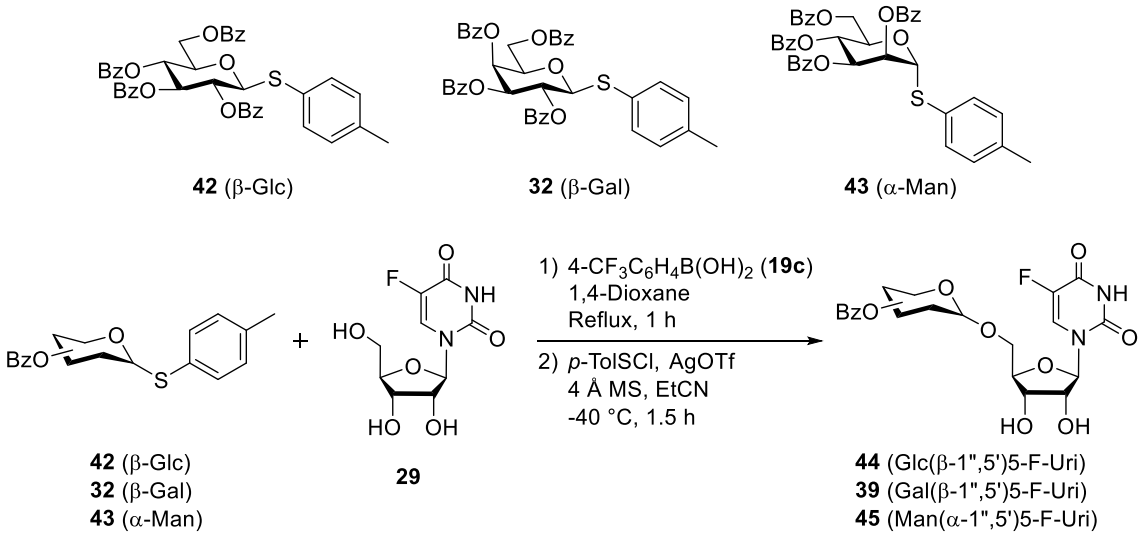


Entry [a]	Acceptor	Product	Yield (for 2 Steps)
1	**22** (Ade)	β-**33**	42%
2	**25** (Ade^{Bz})	β-**34**	30%
3	**26** (Gua)	β-**35**	12%
4	**27** (Gua^{iBu})	β-**36**	44%
5	**18** (Uri)	β-**37**	42% (*ca.* 15%: nucleobase = 5-STol-Uri)
6	**28** (5-Me-Uri)	β-**38**	53%
7	**29** (5-F-Uri)	β-**39**	61%
8	**30** (Cyt)	β-**40**	55%
9	**31** (Cyt^{Bz})	β-**41**	40%

[a] Glycosylation reactions were carried out in the presence of 1.5 equivalents of **32**, 3.0 equivalents of *p*-TolSCl and 6.0 equivalents of AgOTf against the acceptor (**18**, **22** or **25**–**31**). Stoichiometry of **19c** was 1.5 equivalents against acceptor (**18**, **22** or **25**–**31**). A mixture of **32**, acceptor (**18**, **22**, or **25**–**31**) and **19c** was co-evaporated with pyridine and 1,4-dioxane and treated with promoters.

The *O*-glycosylation of 5-fluorouridine **29** with glucosyl, galactosyl and mannosyl donors was also examined. As summarized in Table 2-4, the glucosyl donor **42** [145] and the galactosyl donor **32** afforded the corresponding products β-**44** and β-**39** in reasonably acceptable yields, while the use of the mannosyl donor **43** [146] gave a mixture.

Table 2-4. *O*-Glycosylation of 5-fluorouridine with thioglycosides **32**, **42** and **43**.

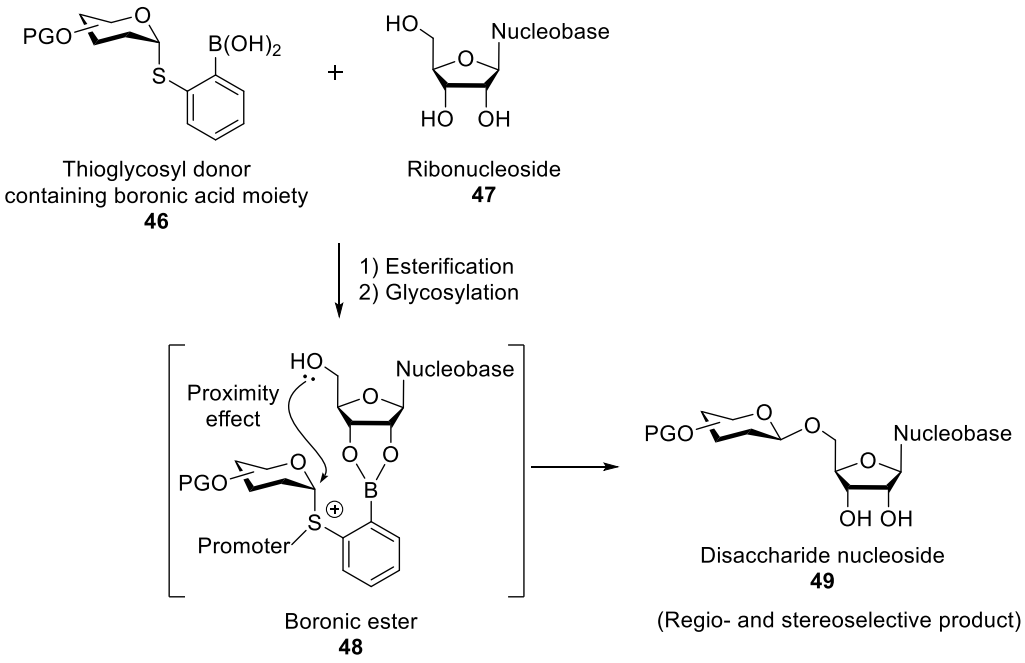


Entry [a]	Donor	Product	Yield (for 2 Steps)
1	**42** (β-Glc)	β-**44**	54%
2 [b]	**32** (β-Gal)	β-**39**	61%
3	**43** (α-Man)	α-**45**	<39% (mixture)

[a] Glycosylation reactions were carried out in the presence of 1.5 equivalents of donor (**32**, **42** or **43**), 3.0 equivalents of *p*-TolSCl and 6.0 equivalents of AgOTf against **29**. Stoichiometry of **19c** was 1.5 equivalents against **29**. A mixture of donor (**32**, **42** or **43**), **29** and **19c** was co-evaporated with pyridine and 1,4-dioxane and treated with promoters.
[b] Taken from entry 7 of Table 2-3 in this manuscript.

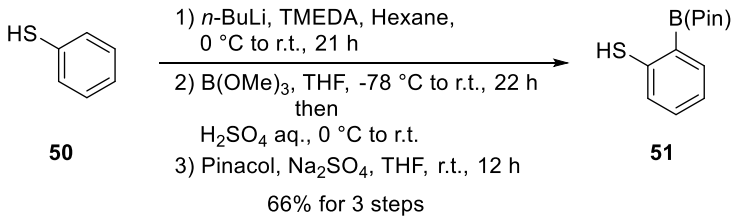
2-2-2. *O*-Glycosylation of nucleosides with thioglycosyl donors containing the boronic acid moiety on the leaving group

Shen and co-workers recently reported on the 1,2-*cis* glycosylation of some simple alcohols using glucosyl donors containing a boronic acid moiety on the leaving group, which is referred to leaving group-based aglycon delivery [126]. These results prompted us to examine the use of the thioglycosyl donor **46** containing a boronic acid moiety on the leaving group, which was expected to form a boronic ester with the 2',3'-*cis*-diol of ribonucleoside **47** to give the intermediate **48** (Scheme 2-3). It was expected that the *O*-glycosylation of **48** would produce **49** in a pseudo-intramolecular manner.



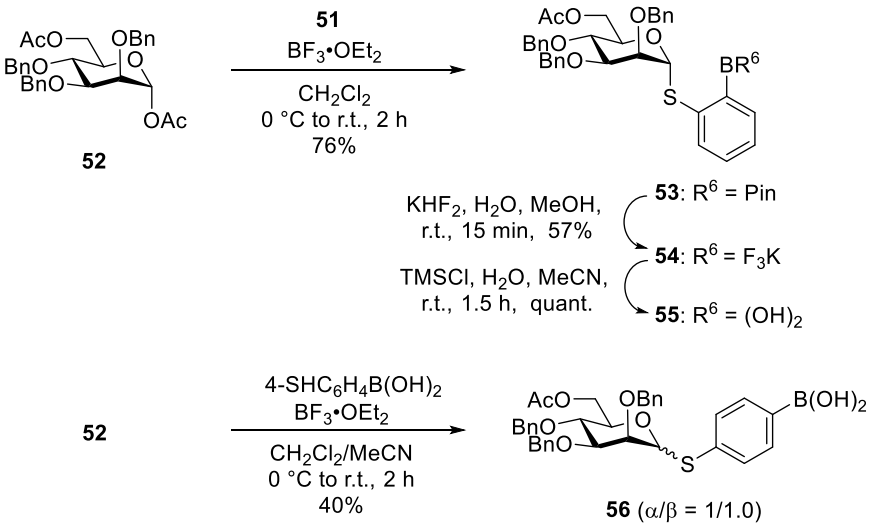
Scheme 2-3. Regio- and stereo-selective *O*-glycosylation of the ribonucleoside utilizing the glycosyl donor containing a boronic acid.

For the synthesis of glycosyl donors containing a boronic acid on the leaving group, the 2-mercaptophenylboronic acid pinacol ester **51** was synthesized from thiophenol **50** using *n*-BuLi-TMEDA complex and trimethyl borate followed by the protection of the boronic acid with pinacol (Scheme 2-4) [147–148].



Scheme 2-4. Synthesis of 2-mercaptophenylboronic acid pinacol ester **51**.

The *S*-glycosylation of **52** [149] with **51** in the presence of $BF_3 \cdot OEt_2$ gave thioglycoside **53**, in which the pinacol group was converted to **55** via **54** as shown in Scheme 2-5 [150]. The thioglycoside **56** containing a boronic acid on the 4 (para) position was also prepared by the reaction of 4-mercaptophenylboronic acid with **52**.

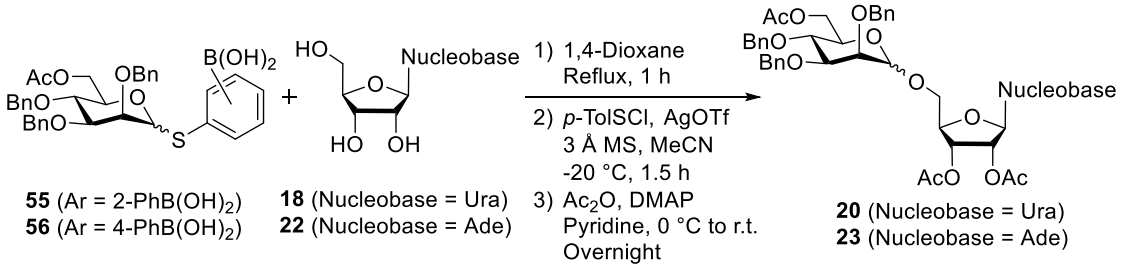


Scheme 2-5. Synthesis of glycosyl donors containing a boronic acid on the leaving group.

In Table 2-5, the results for the *O*-glycosylation of uridine **18** and adenosine **22** with the glycosyl donors **55** and **56** are summarized. In entries 1 and 2, the reactions of **18** and **22** with **55** afforded the corresponding products **20** and **23** in 44% (α/β = 1.9/1) and 16% (α/β = 1.3/1), respectively. In entries 3 and 4, **56** gave the almost the same chemical yields and stereoselectivities as those in Entries 1 and 2. These results are

similar to entry 3 in Table 2-1 and entry 2 in Table 2-2, in which **18** or **22** was reacted with **17** and phenylboronic acid **19a** under the same conditions in Table 2-5, indicating that the introduction of a boronic acid on the thiophenyl leaving group in our reactions has a negligible effect on the overall reaction.

Table 2-5. *O*-Glycosylation of uridine **18** and adenosine **22** with thioglycosides **55** and **56**.

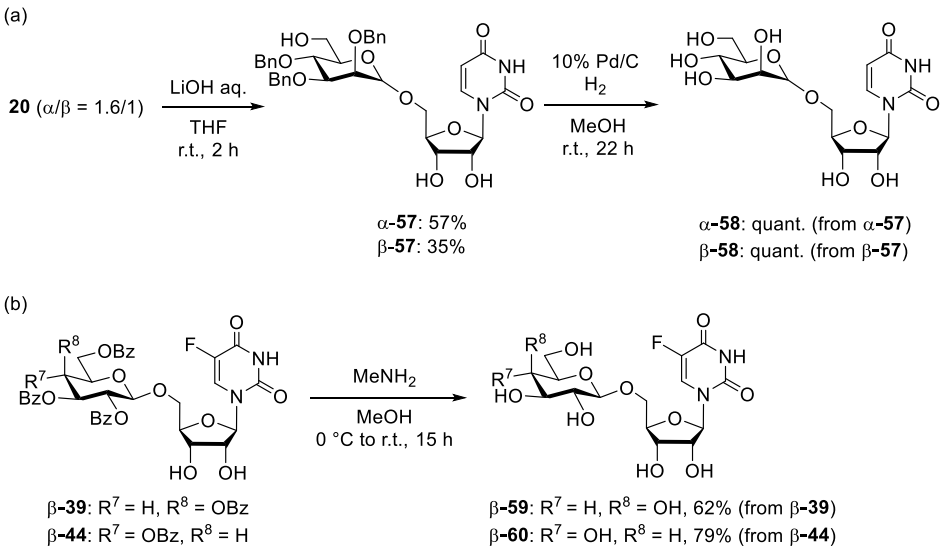


Entry [a]	Donor	Acceptor	Product	Yield (for 3 Steps) [b]
1	**55** (Ar = 2-$C_6H_4B(OH)_2$) (α form)	**18** (Nucleobase = Ura)	**20**	44% (α/β = 1.9/1)
2	**55** (Ar = 2-$C_6H_4B(OH)_2$) (α form)	**22** (Nucleobase = Ade)	**23**	16% (α/β = 1.3/1)
3	**56** (Ar = 4-$C_6H_4B(OH)_2$) (α/β = 1/1.0)	**18** (Nucleobase = Ura)	**20**	36% (α/β = 2.1/1)
4	**56** (Ar = 4-$C_6H_4B(OH)_2$) (α/β = 1/1.0)	**22** (Nucleobase = Ade)	**23**	14% (α/β = 1.1/1)

[a] Glycosylation reactions were carried out in the presence of 1.5 equivalents of donor (**55** or **56**), 3.0 equivalents of *p*-TolSCl and 6.0 equivalents of AgOTf against the acceptor (**18** or **22**). A mixture of donor (**55** or **56**) and acceptor (**18** or **22**) was co-evaporated with pyridine and 1,4-dioxane and treated with promoters. Acetylation reactions were carried out in the presence of *ca.* 10 equivalents of Ac_2O and a catalytic amount of DMAP.
[b] The α/β ratio was determined by ^1H NMR.

2-2-3. Deprotection of the glycosylation products

The deprotection of the glycosylation product **20** (α/β = 1.6/1) involved a treatment with aqueous LiOH to afford α-**57** and β-**57**, which were separated by silica gel column chromatography. The deprotection of the benzyl groups of α-**57** and β-**57** under traditional reaction conditions (10% Pd/C with H_2 gas) gave α-**58** and β-**58**, respectively (Scheme 2-6a) [151]. The deprotection of β-**39** and β-**44** by treatment with $MeNH_2$ [152] afforded corresponding β-**59** [153] and β-**60** in 62% and 79%, respectively (Scheme 2-6b).

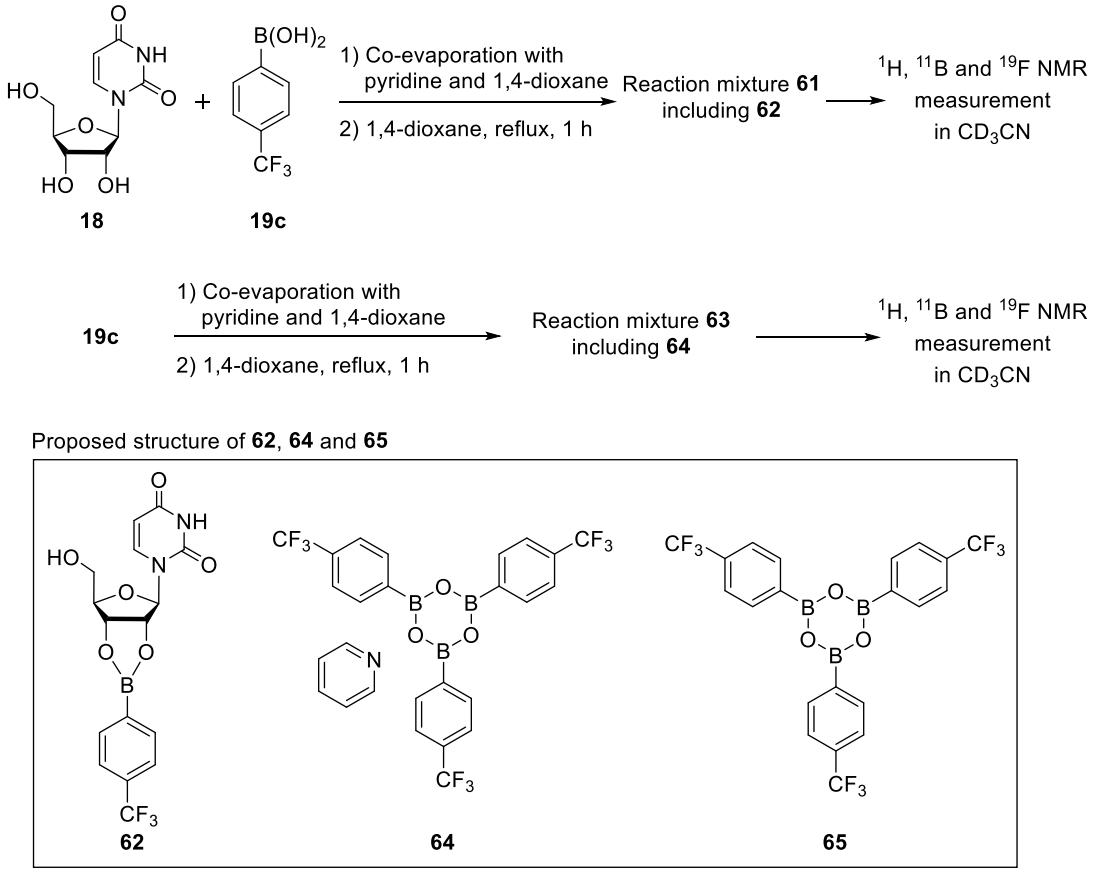


Scheme 2-6. Deprotection of **20** (a) and β-**39** and β-**44** (b).

2-2-4. Interaction of uridine and 4-(trifluoromethyl)phenylboronic acid studied by NMR spectroscopy

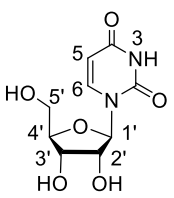
The temporary protection of the 2'3'-*cis*-diol of ribonucleoside with a boronic acid was checked by NMR spectroscopy. The ^{1}H, ^{11}B and ^{19}F NMR measurements of uridine **18**, 4-(trifluoromethyl)phenylboronic acid **19c** and a mixture of **18** and **19c** were undertaken in CD_3CN (Scheme 2-7 and Figure 2-1). For the preparation of the third sample, a mixture

of **18** and **19c** was azeotroped with pyridine and 1,4-dioxane, followed by stirring in 1,4-dioxane under the reflux conditions for 1 h. For comparison, **19c** was azeotroped in a similar manner, and the ^{11}B and ^{19}F NMR spectra of the resulting mixture were obtained (Scheme 2-7). As shown in Figures 2-1a and 1b, the peaks for the 2' and 3' hydroxyl groups disappeared, and the 2' and 3' proton signals were shifted considerably upfield upon the addition of **19c**. In Figures 2-1c–e, it was assumed that the peaks at 21 ppm, 28 ppm and 32 ppm correspond to a 2,4,6-tris[4-(trifluorometyl)phenyl]boroxine pyridine complex, the proposed structure of which is **64** (some NMR spectra of boroxine pyridine complexes were reported [154–156]), **19c** and/or 2,4,6-tris[4-(trifluorometyl)phenyl]boroxine **65** and the desired boronic ester **62**, respectively. In Figures 2-1f–h, we assumed that the peaks at -63.3 ppm, -63.2 ppm and -62.8 ppm correspond to **62**, **19c** and/or **65** and **64**, respectively.

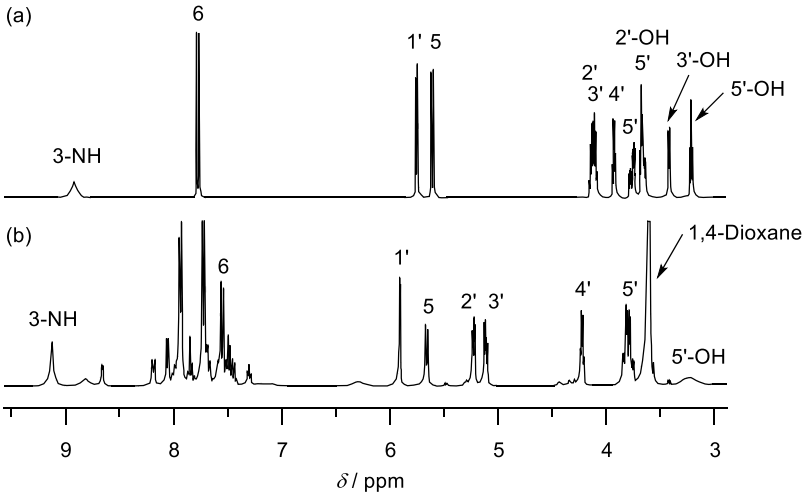


Scheme 2-7. Preparation of reaction mixture **61** and **63**.

^{1}H NMR spectra

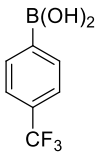


18

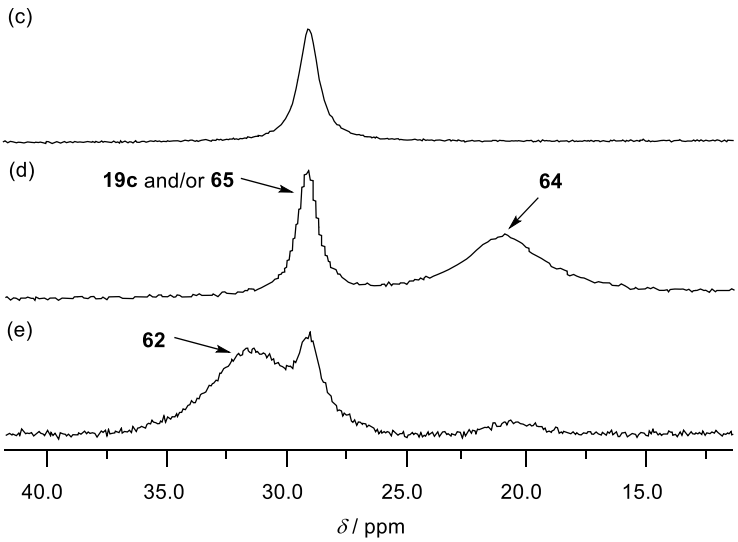


Reaction mixture **61**

^{11}B NMR spectra



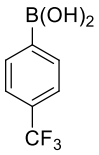
19c



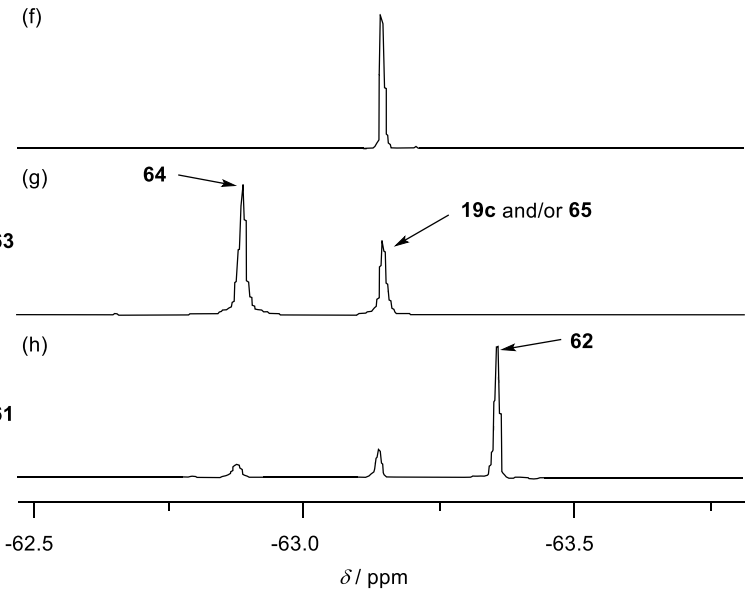
Reaction mixture **63**

Reaction mixture **61**

^{19}F NMR spectra



19c



Reaction mixture **63**

Reaction mixture **61**

Figure 2-1. NMR study of the cyclic boronic ester intermediate **62** prepared from uridine **18** and 4-(trifluoromethyl)phenylboronic acid **19c** by ^{1}H, ^{11}B, and ^{19}F NMR measurements (CD_3CN at 25 °C). (a) ^{1}H NMR of **18**; (b) ^{1}H NMR of a mixture **61**; (c) ^{11}B NMR of **19c**; (d) ^{11}B NMR of a mixture **63**; (e) ^{11}B NMR of a mixture **61**; (f) ^{19}F NMR of **19c**; (g) ^{19}F NMR of a mixture **63**; and (h) ^{19}F NMR of a mixture **61**.

2-2-5. Evaluation of cytotoxicity of disaccharide nucleoside

It is known that 5-Fluorouracil (5-FU) **66** which is categorized fluoropyrimidine-based antimetabolite has anticancer activity. The anticancer effect of 5-FU **66** is exhibited by inhibition of thymidylate synthase (TS) and incorporation of its metabolites into DNA and RNA (Figure 2-2) [157]. More than 80% of administrated 5-FU **66** is firstly converted to 5,6-dihydro-5-fluorouracil (DHFU) by dihydropyrimidine dehydrogenase (DPD) in liver. 5-FU **66** taken up to cancer cells is eventually metabolized to three active metabolites namely 5-fluoro-2'-deoxyuridine 5'-monophosphate (FdUMP), 5-fluoro-2'-deoxyuridine 5'-triphosphate (FdUTP) and 5-fluorouridine 5'-triphosphate (FUTP). The main pathway to activate 5-FU **66** starts from the conversion of it to 5-fluorouridine 5'-monophosphate (FUMP) by orotate phosphoribosyltransferase (OPRT) with phosphoribosyl pyrophosphate (PRPP) or sequential reaction depend on uridine phosphorylase (UP) and uridine kinase (UK) via 5-fluorouridine (FUR) **29**. FUMP is then phosphorylated to 5-fluorouridine 5'-diphosphate (FUDP) followed by either further phosphorylation to be the FUTP or reduction to be a 5-fluoro-2'-deoxyuridine 5'-diphosphate (FdUDP) by ribonucleotide reductase (RR). The FUTP is incorporated into RNA resulted in inhibition of the processing of pre-rRNA, post-transcriptional modification of tRNA, activity of snRNA/protein complexes, and splicing of pre-mRNA. The FdUDP is converted to FdUTP or FdUMP by phosphorylation or dephosphorylation. As an alternative pathway, conversion of 5-FU **66** to FdUMP via 5-fluoro-2'-deoxyuridine (FUdR) **67** proceed in the presence of thymidine phosphorylase (TP) and thymidine kinase (TK). The FdUMP

exerts anticancer activity by inhibition of thymidylate synthase (TS) which catalyze the methylation of 2'-deoxyuridine 5'-monophosphate (dUMP) to 2'-deoxythymidine 5'-monophosphate (dTMP) with the 5,10-methylenetetrahydrofolate (5,10-CH_2-THF). FdUMP can bind to the nucleotide-binding site of TS to form ternary complex with the TS and 5,10-CH_2-THF, resulted in block of the dUMP binding to TS and inhibition of dTMP synthesis. Consequently, depletion of dTMP induces the lack of 2'-deoxythymidine 5'-triphosphate (dTTP) and inhibits DNA synthesis. Moreover, accumulated dUMP is phosphorylated to 2'-deoxyuridine 5'-triphosphate (dUTP) and incorporated into DNA with the FdUTP. The repair of DNA including uracil and 5-FU **66** by uracil-DNA glycosylase (UDG) is ineffectual when dUTP and FdUTP are much more than dTTP, which enhance the incorporation of dUTP and FdUTP and repeat the cycles of misincorporation, excision and repair to induce DNA break and cell death [157].

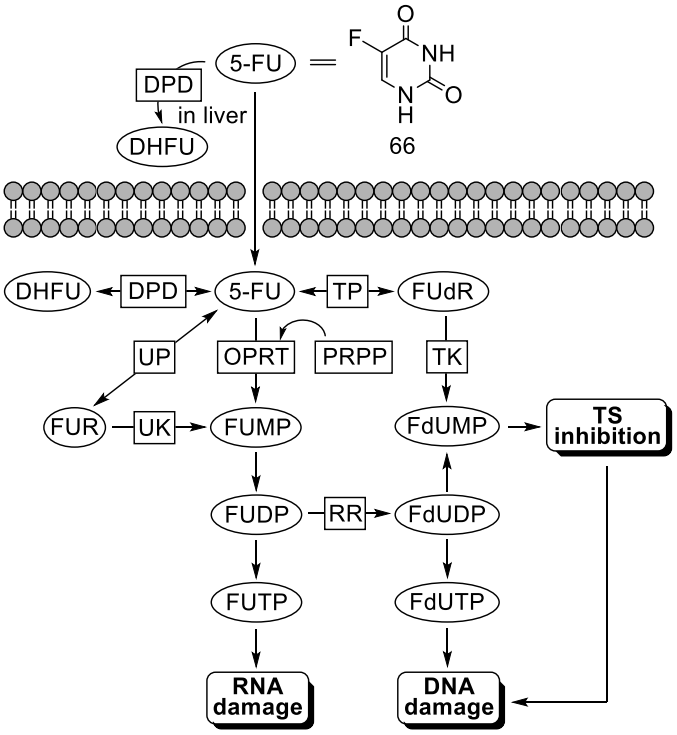
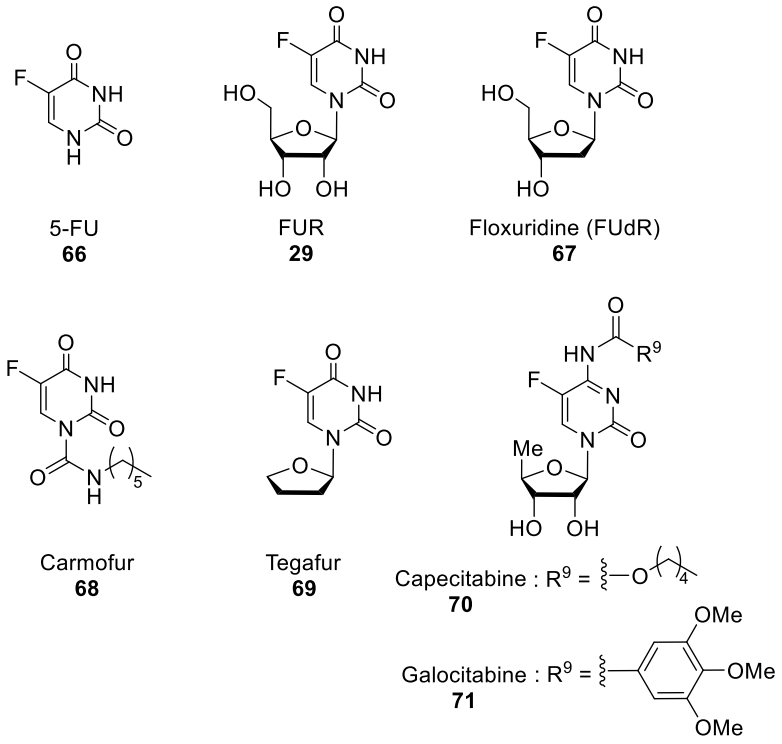


Figure 2-2. Metabolism of 5-FU and targets of 5-FU metabolites. Abbreviations of each compound, metabolite and enzyme are as follows: 5-Fluorouracil (5-FU) **66**; 5,6-dihydro-5-fluorouracil (DHFU); 5-fluorouridine (FUR) **29**; 5-fluorouridine 5'-

monophosphate (FUMP); 5-fluorouridine 5'-diphosphate (FUDP); 5-fluorouridine 5'-triphosphate (FUTP); 5-fluoro-2'-deoxyuridine (FUdR) **67**; 5-fluoro-2'-deoxyuridine 5'-monophosphate (FdUMP); 5-fluoro-2'-deoxyuridine 5'-diphosphate (FdUDP); 5-fluoro-2'-deoxyuridine 5'-triphosphate (FdUTP); dihydropyrimidine dehydrogenase (DPD); uridine phosphorylase (UP); phosphoribosyl pyrophosphate (PRPP); orotate phosphoribosyltransferase (OPRT); uridine kinase (UK); thymidine phosphorylase (TP); thymidine kinase (TK); ribonucleotide reductase (RR); thymidylate synthase (TS).

5-FU analogs are known to exhibit anticancer activity by catabolism to 5-FU **66**. Although 5-FU-related agents including 5-FU **66**, FUR **29**, Floxuridine (FUdr) **67** are a powerful anticancer agent for many types of cancer cells, its serious side effects have become critical problem. To date, various 5-FU derivatives namely, carmofur **68**, tegafur **69**, capecitabine **70** and galocitabine **71** have been developed to reduce side effects and improve selectivity to target cells or tissues (Scheme 2-8) [158,159].



Scheme 2-8. Representative examples of 5-FU-related agents.

We expected that synthesized disaccharide nucleosides β–**59** and β–**60** which have FUR moiety linked to galactose or glucose unit via *O*-glicosidic linkage could improve the selectivity for cancer cells because the activities of several glycosidases such as β-glucosidase and β-galactosidase in tumor tissues are higher than that of normal tissues [160–165]. In our strategy, β–**59** and β–**60** could mask the toxicity of FUR **29** by the external sugar moiety followed by release of **29** by enzymatic hydrolysis in tumor tissue to exert anticancer effect.

Evaluation of cytotoxicity of β-**59** and β-**60** against A549 (a human alveolar adenocarcinoma) and HeLa S3 (a human cervical carcinoma) cells was conducted by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolum bromide (MTT) assays in comparison with 5-FU **66** and FUR **29**. The A549 and HeLa S3 cells (1×10^4 /well) were incubated in 10% fetal calf serum (FCS)/Dulbecco's Modified Eagle' Medium (DMEM) for A549 cells or Minimum Essential Medium (MEM) for HeLa S3 cells containing β-**59**, β-**60**, **66** and **29** (0~250 μM) at 37 ºC for 48 hr, and then treated with MTT reagent to measure cell viability. As shown in Figure 2-3, 5-FU derivatives, especially FUR **29** showed the cytotoxicity against both A549 and HeLa S3 cells whose half maximal effective concentration (EC_{50}) values were 15.6~31.3 μM against both type of cells, while EC_{50} values of β–**59** and β–**60** were higher (>250 μM) than that of FUR **29**. These results suggest that the activity of FUR **29** is masked by introduction of sugar moiety at the 5'-position and release of FUR **29** from β–**59** and β–**60** by enzymatic hydrolysis is not enough regarding these cancer cell lines. The mechanistic study, synthesis of other disaccharide nucleosides and evaluation of those anticancer activities are now underway.

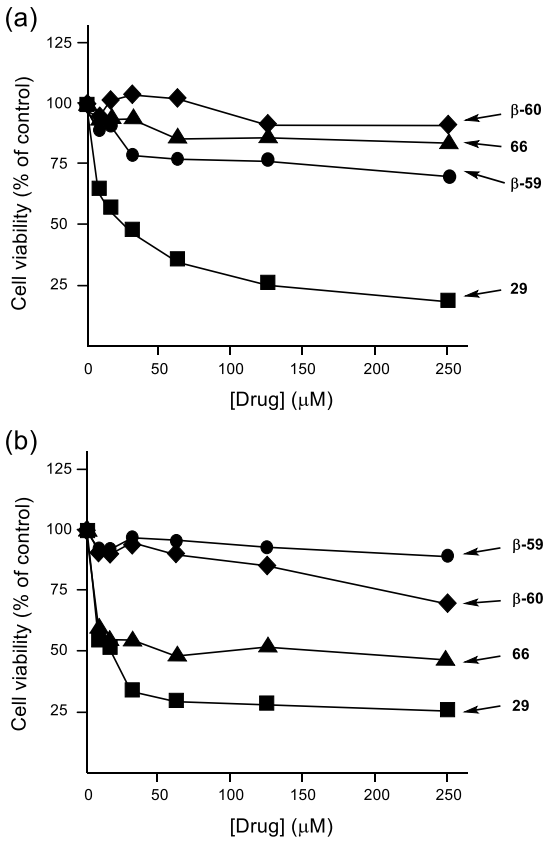


Figure 2-3. Results of MTT assay with 5'-*O*-*β*-galactopyranosyl-5-fluorouridine β-**59** (●), 5'-*O*-*β*-glucopyranosyl-5-fluorouridine β-**60**(◆), 5-fluorouracil (5-FU) **66** (▲) and 5-fluorouridine (FUR) **29** (■) against A549 (a), and HeLa S3 (b). Cell viability of A549 and HeLa S3 cells (% of control: 0 μM of agent) in the presence of β-**59**, β-**60, 66** and **29** (0~250 μM) for 48 hr.

[2-3]: Conclusions

We report herein on the synthesis of disaccharide nucleosides utilizing the temporary protection of the 2',3'-*cis*-diol of ribonucleosides by a boronic ester. The glycosylation of the uridine **18**, which is temporarily protected by a boronic acid, with the thioglycoside **17** using a *p*-TolSCl/AgOTf promoter system followed by acetylation gave the disaccharide nucleoside **20** containing a 1",5'-glycosidic linkage in reasonable chemical yield. This synthetic method was applied to the glycosylation of protected or unprotected adenosine, guanosine, uridine or cytidine, **18**, **22**, **25**–**31**, with the galactosyl donor **32** to afford the desired products in moderate chemical yields. *O*-Glycosylations of 5-fluorouridine (FUR) **29** with the glucosyl donor **42**, the galactosyl donor **32** and the mannosyl donor **43** were also conducted. The introduction of a boronic acid on the phenylthio leaving group had only a negligible effect on the reactivity and stereoselectivity of the system. The protocols of this glycosylation reaction were published as a video presentation in *Journal of Visualized Experiment* (*JoVE*). The deprotection of compounds **20**, β-**39** and β-**44** was also demonstrated to give the corresponding deprotected compounds α-**58** and β-**58** from **20**, β-**59** from β-**39,** and β-**60** from β-**44**, respectively.

^{1}H, ^{11}B and ^{19}F NMR measurements of a mixture of uridine **18** and 4-(trifluoromethyl)phenylboronic acid **19c** suggest that the 2' and 3' hydroxyl groups of **18** react with **19c** to form the cyclic boronic ester intermediate **62**, as expected, resulting in selective *O*-glycosylation of the ribonucleoside acceptors at the 5'-position.

Finally, we evaluated the anticancer activity of galactose-FUR conjugate β-**59** and glucose-FUR conjugate β-**60** against A549 and HeLa S3 cells by MTT assay. The results showed that β-**59** and β-**60** have lower cytotoxicity than FUR **29**, which suggest the external sugar moiety could mask the toxicity of **29**.

These results afford important and useful information regarding the concise and short-step synthesis of various biologically-active disaccharide nucleoside derivatives via

the *O*-glycosylation of temporarily-protected nucleosides and related compounds. Moreover, β-**59**, β-**60** and its analogs represent potentially new drug candidates because 5-fluorouridine and 5-fluorouracil have been reported to have anticancer, antivirus and antibacterial activities [103,153,166–172].

Chapter 3.

One-Pot Synthesis of Cyclic Oligosaccharides by the Polyglycosylation of Monothioglycosides

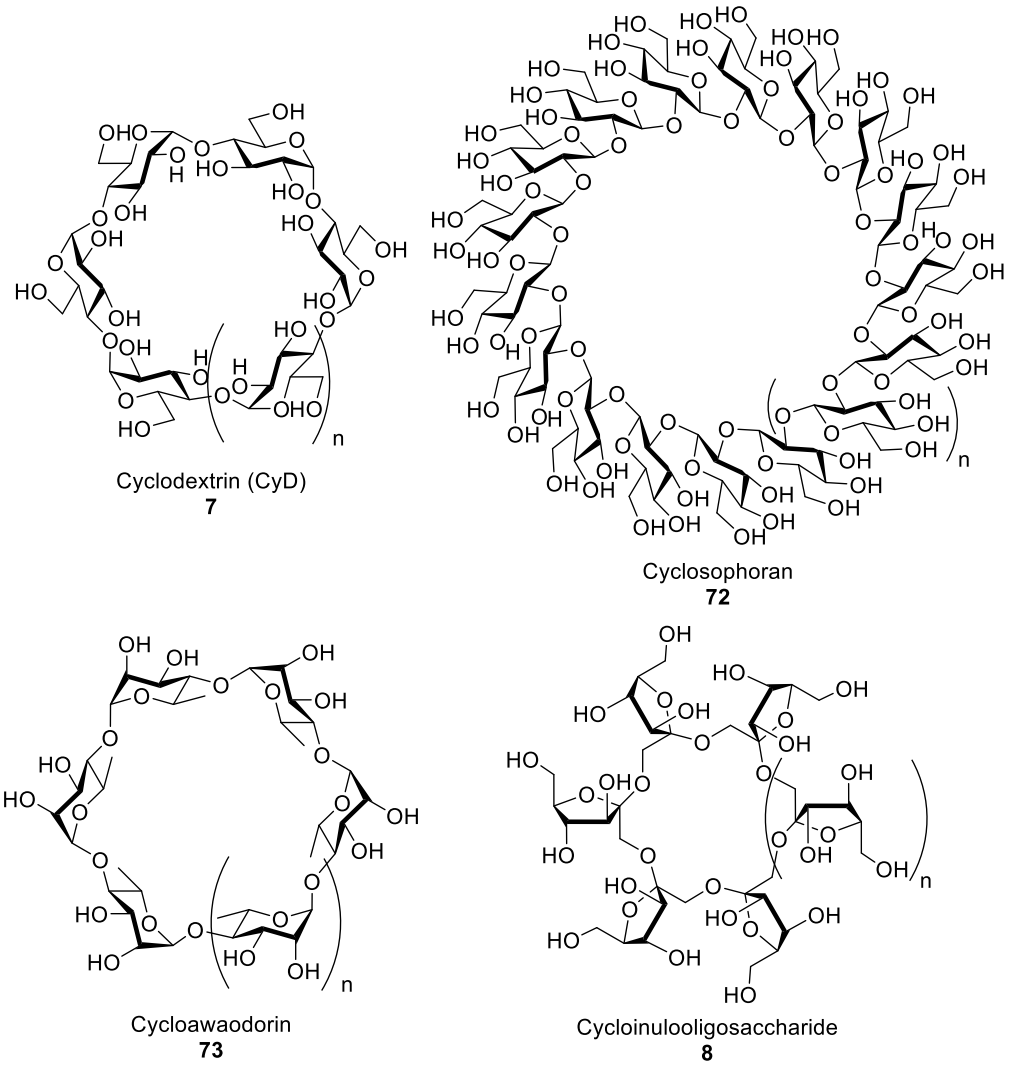
モノチオ糖のポリグリコシル化反応による

環状オリゴ糖のワンポット合成

[3-1]: Introduction

Cyclic oligosaccharides are ring-shaped molecules which consist of carbohydrate oligomers. The first paper regarding cyclic oligosaccharides, which are now recognized as cyclodextrins **7** (cyclo(1→4)-α-D-glucooligosaccharides) (CyDs) was reported by Villiers in 1891, and Schardinger isolated cyclodextrins followed by elucidation of their structure by Freudenberg in early the1900s [173,174] (Scheme 3-1). In recent studies, Yamada and co-workers achieved a synthesis of the smallest cyclodextrins, namely CyD3 and CyD4 [175]. CyDs have a unique ability to capture various molecules within their hydrophobic cavities, resulting in the formation of inclusion complexes [176]. Due to these properties and their low toxicity, CyDs are used extensively in pharmaceutical science [19], in analytical chemistry [177], in supramolecular chemistry [178], as well as in foods [20] and cosmetics [179].

Other CyD analogs such as cyclosophorans **72** (cyclo(1→2)-β-D-glucooligosaccharides) [180]; cycloawaodorins **73** (cyclo(1→4)-α-L-rhamnooligosaccharides) [181]; cycloinulooligosaccharides (cyclo(2→1)-β-D-fructooligosaccharides) **8** [182] and others [25] have also been reported (Scheme 3-1). Although it is well known that these cyclic oligosaccharides form inclusion complexes with organic molecules or metal cations [21,183,184], they are able to recognize different molecules depending on the size and the shape of their inner cavities as compared to those of CyDs. Therefore, the design and synthesis of novel CyD analogues with new properties and functions would be highly desirable.

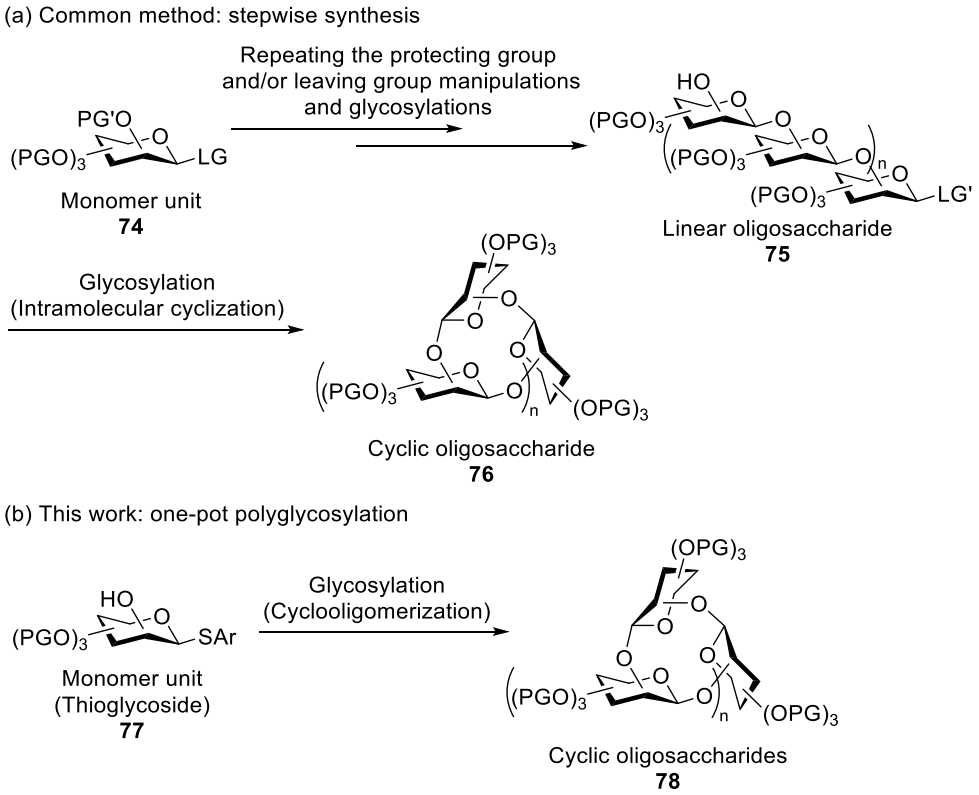


Scheme 3-1. Typical examples of cyclic oligosaccharides.

The major synthetic methods of cyclic oligosaccharides can be classified into the following four categories: (i) isolation from bacterial products [185–187]; (ii) enzymatic synthesis [188–191]; (iii) chemical synthesis [175,181,25,192–204]; and (iv) chemoenzymatic synthesis [205–207]. Chemical synthesis has advantages in the large-scale synthesis of natural and artificial cyclic oligosaccharides and can be categorized into two approaches. The first method is the stepwise preparation of linear oligosaccharides **75** from monomer units **74** followed by their cyclization to afford cyclic oligosaccharides **76** (Scheme 3-2a). For instance, the cyclic oligosaccharides

including D-glucose moieties [175,193,195,198], D-glucosamine moieties [198,203,204], 4-deoxy-4-*C*-methyl-D-glucose moieties [201,202] and L-rhamnose moieties [181] were synthesized from the corresponding linear oligosaccharides, which contain a glycosyl bromide, glycosyl fluoride or thioglycoside unit at the reducing end. The second method is the one-pot polyglycosylation (polycondensation) [208] of sugar units that function as both glycosyl donors and glycosyl acceptors such as monosaccharide units (e.g. 1,2-*O*-(1-cyano)ethylidene derivatives [194] and thioglycosides [200]) and disaccharide units (e.g. glycosyl bromides [192], 1,2-*O*-(1-cyano)ethylidene derivatives [197], glycosyl acetates [196] and thioglycosides [199]).

Although the first approach has been the most commonly employed method for the construction of cyclic oligosaccharides, it requires tedious processes such as protecting group and/or leaving group manipulations, glycosylation reactions, and purifications after each synthetic step. In order to extend the second approach, we attempted to develop the efficient method for the construction of cyclic oligosaccharides from simple monosaccharides. This manuscript reports on the synthesis of cyclic oligosaccharides **78** by the glycosylation of the thioglycosides **77** in which functions of a glycosyl donor and a glycosyl acceptor are contained in the same molecule (Scheme 3-2b).



Scheme 3-2. Chemical synthesis of cyclic oligosaccharides. (a) Stepwise synthesis of cyclic oligosaccharides; (b) one-pot polyglycosylation to afford cyclic oligosaccharides (this work). (PG = protecting group, LG = leaving group, Ar = aryl group)

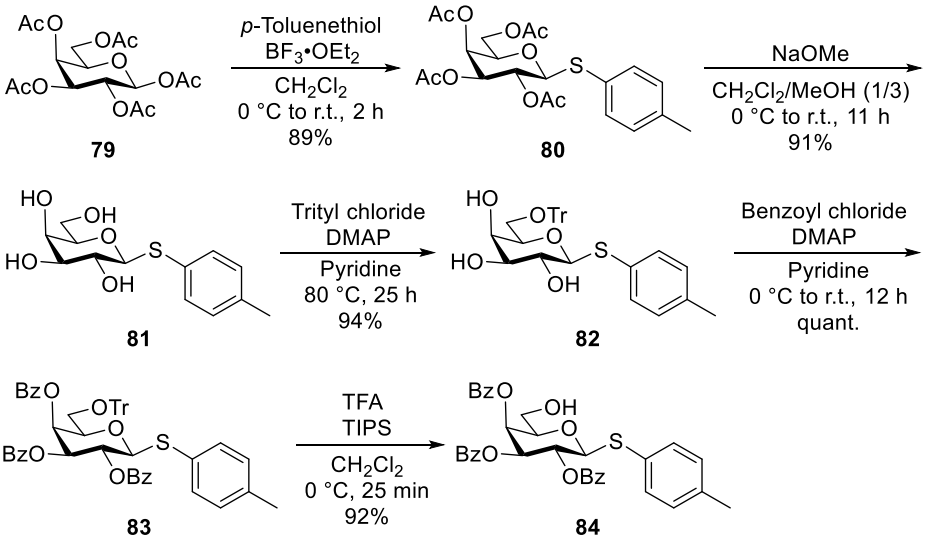
[3-2]: Result and discussion

3.2.1. Polyglycosylation of monothiogalactosides for synthesis of cyclic oligosaccharides

In conducting the one-pot polyglycosylation of monosaccharides, we focused on the use of thioglycosides as substrates due to their ease of preparation and derivatization, long shelf life and high reactivity in the presence of specific promoters [27,46,104–107]. Kartha and co-workers recently reported on the one-pot synthesis of cyclic oligosaccharides obtained as a mixture of stereoisomers from armed monothioglycosides in which the hydroxy groups were protected by benzyl and trityl groups [200].

These results prompted us to examine the synthesis of cyclic oligosaccharides using benzoylated monothioglycosides, which would permit the stereochemistry of the products

to be controlled. We prepared the thiogalactoside **84** [209] for use in a one-pot polyglycosylation as shown in Scheme 3-3. The *S*-glycosylation of penta-*O*-acetyl-β-galactose **79** with *p*-toluenethiol and cleavage of acetyl groups gave thiogalactoside **81**. The hydroxy groups of **81** were protected by trityl and benzoyl groups followed by cleavage of trityl group gave **84**.

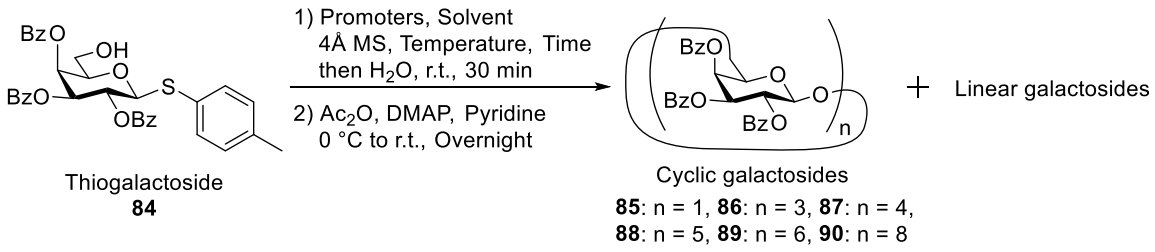


Scheme 3-3. Preparation of **84**.

We examined the effects of glycosylation promoters, reaction solvents, the concentration of **84** and reaction temperature on the nature of the glycosylation products (Table 3-1). The *O*-glycosylations of 2,3,4-tri-*O*-benzoyl-1-tolylthio-β-D-galactoside **84** (corresponding to **77** in Scheme 3-2b) were conducted under specific conditions as listed in Table 3-1 and quenched with water. Note that the crude reaction mixture was roughly purified by silica gel column chromatography and then treated with acetic anhydride to acetylate linear saccharides and other byproducts for the easy purification of the cyclic oligogalactosides (it was difficult to separate and purify linear saccharides for their characterization). As summarized in Table 3-1, a series of typical promoters such as *N*-iodosuccinimide (NIS)/trimethylsilyl triflate (TMSOTf) [50], *p*-

toluenesulfenyl chloride (*p*-TolSCl)/silver triflate (AgOTf) [69,118,210,211] and methyl triflate (MeOTf) [78] were examined. The reaction using NIS/TMSOTf afforded the cyclic oligogalactosides **86**–**90**, whereas the use of a combination of *p*-TolSCl and AgOTf gave negligible amounts of cyclic oligosaccharides (entries 1 and 2 of Table 3-1). The use of MeOTf furnished a mixture of linear and cyclic saccharides (entry 3). In order to examine the effect of halonium species, the glycosylation reaction was conducted in the presence of *N*-bromosuccinimide (NBS)/TMSOTf [61], as shown in entry 4, in which the isolated yields of cyclic oligogalactosides **86**–**90** were slightly lower than those in entry 1 (NIS/TMSOTf). Figure 3-1a summarizes the chemical yield of cyclic monosaccharide (1,6-anhydrogalactose) **85** and cyclic oligogalactosides **86**–**90**, showing that the polyglycosylation of **84** with NIS/TMSOTf and NBS/TMSOTf gives cyclic trimer **86**, cyclic tetramer **87** and cyclic pentamer **88** in higher chemical yields than other cyclic galactosides. The use of NIS/AgOTf [51] was examined to evaluate the effect of silver cations, resulting in the formation of the cyclic trisaccharide **86** in higher chemical yield than those of cyclic tetra~octagalactosides **87**–**90** (entry 5). The use of *N*-(*p*-methylphenylthio)-ε-caprolactam (NMPTC) [71], an organosulfur reagent, and TMSOTf afforded only negligible amounts of cyclic oligosaccharides (entry 6). The above results suggest that using a combination of NIS and TMSOTf is preferred compared to other promoter systems.

Table 3-1. Examination of reaction conditions for synthesis of cyclic oligogalactosides **86**–**90** by the one-pot polyglycosylation of **84**



Entry	Promoters (eq.)	Solvent	Concentration of **84**	Temperature	Time	Yields (%)						
						85	**86**	**87**	**88**	**89**	**90**	Linear saccharides
1	NIS (3.0) TMSOTf (1.5)	CH_2Cl_2	0.01 M	-40 °C	2.5 h	3	13	23	18	5	5	<9
2	*p*-TolSCl (2.0) AgOTf (3.0)	CH_2Cl_2	0.01 M	-40 °C	2.5 h	-	-	-	-	-	-	<52
3	MeOTf (16)	CH_2Cl_2	0.01 M	0 °C to r.t.	48 h	14	<13	<10	<6	-	-	<23
4	NBS (3.0) TMSOTf (1.5)	CH_2Cl_2	0.01 M	-40 °C	2.5 h	<2	12	18	17	8	4	<22
5	NIS (3.0) AgOTf (1.5)	CH_2Cl_2	0.01 M	-40 to 0 °C	5 h	15	19	13	11	5	2	<13
6	NMPTC (2.0) TMSOTf (2.3)	CH_2Cl_2	0.01 M	-40 °C to r.t.	24 h	-	-	-	-	-	-	<15
7	NIS (3.0) TMSOTf (1.5)	EtCN	0.01 M	-40 °C	2.5 h	-	-	<9	<7	-	-	<42
8	NIS (3.0) TMSOTf (1.5)	THF	0.01 M	-40 °C	2.5 h	No reaction						
9	NIS (3.0) TMSOTf (1.5)	Toluene	0.01 M	-40 °C	2.5 h	-	-	-	-	-	-	<5
10	NIS (3.0) TMSOTf (1.5)	Toluene	0.01 M	-40 to 0 °C	3.5 h	-	6	<3	<7	-	-	<18
11	NIS (3.0) TMSOTf (1.5)	CH_2Cl_2	0.001 M	-40 °C	2.5 h	-	15	28	7	5	3	<18
12	NIS (3.0) TMSOTf (1.5)	CH_2Cl_2	0.1 M	-40 °C	2.5 h	-	3	17	21	5	6	<4
13	NIS (3.0) TMSOTf (1.5)	CH_2Cl_2	0.01 M	-78 °C	2.5 h	-	-	-	-	-	-	<56
14	NIS (3.0) TMSOTf (1.5)	CH_2Cl_2	0.01 M	0 °C	2.5 h	-	21	22	13	6	3	-
15[a]	NIS (3.0) TMSOTf (1.5)	CH_2Cl_2	0.01 M	-40 °C	2.5 h	2	13	22	18	5	5	<12
16[b]	NIS (3.0) TMSOTf (1.5)	CH_2Cl_2	0.1 M	-40 °C	2.5 h	-	6	11	14	4	3	-

[a] Glycosylation was carried out in the presence of 3.0 eq. of $LiClO_4$ against **84**.

[b] Products were isolated without acetylation (second step).

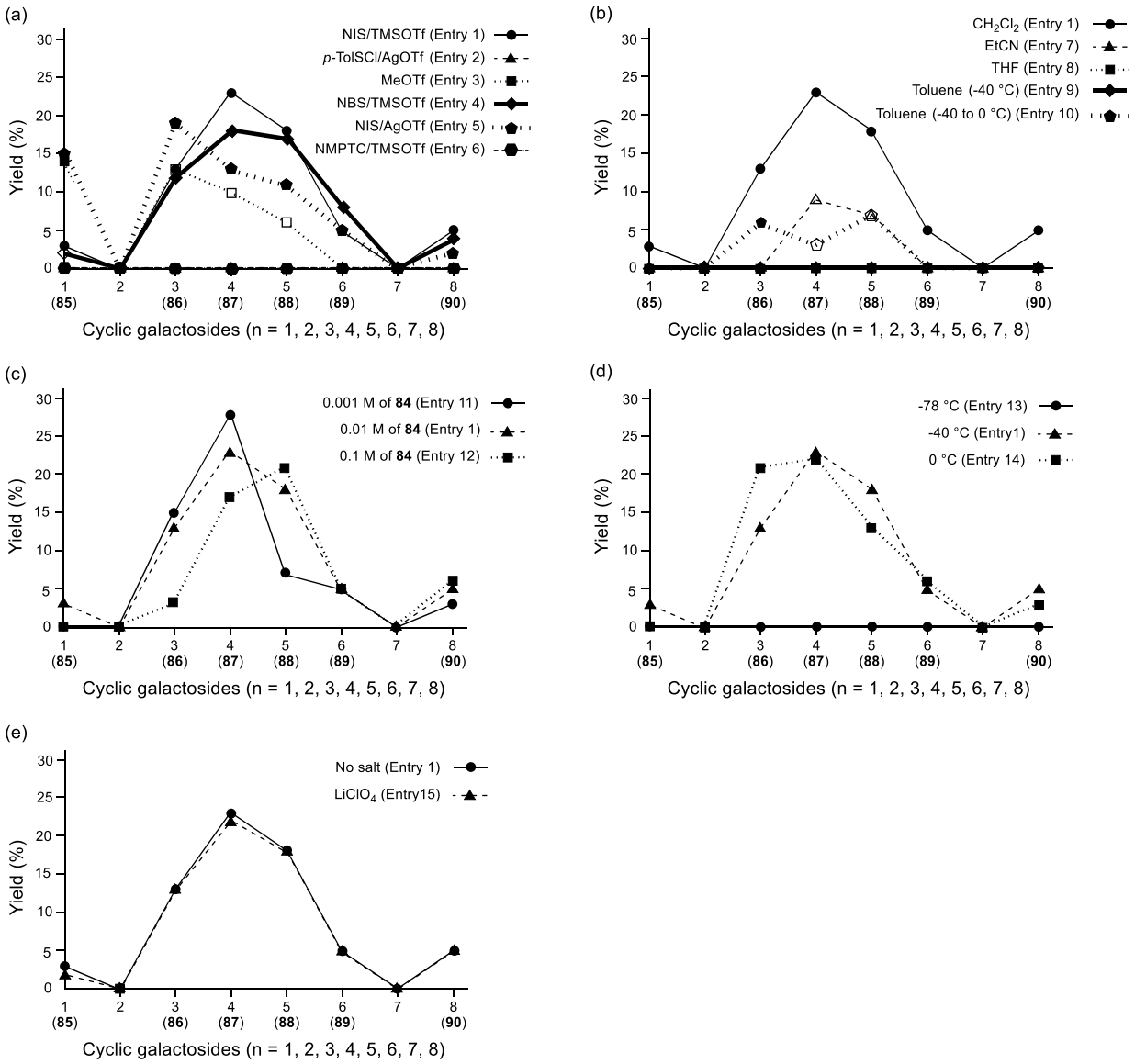


Figure 3-1. Relationship between reaction conditions and isolated chemical yields of cyclic oligogalactosides. Open symbols denote the chemical yield, which include very little amounts of impurities. The bold numbers under the "n" in each graph indicate the compound number of cyclic galactosides (**85**–**90** shown in Table 3-1). (a) Effect of promoters; (b) solvent effect; (c) effect of the concentration of **84**; (d) effect of reaction temperature; and (e) effect of salts.

The effect of the solvent on the synthesis of cyclic oligosaccharides was studied (entries 1 and 7–10 in Table 3-1 and Figure 3-1b). The use of propionitrile gave small amounts of the cyclic tetragalactoside **87** and cyclic pentagalactoside **88** including unidentified products (entry 7). As shown in entries 8 and 9, the glycosylations in THF and toluene at -40 °C afforded negligible amounts of cyclic oligosaccharides. The use of toluene at -40 °C to 0 °C gave the cyclic oligogalactosides **86**–**88** in lower chemical yields (entry 10) compared to dichloromethane in entry 1, suggesting that dichloromethane is better than the other solvents.

The effect of the concentration of reactants on the polyglycosylation of **84** was also examined (entries 1, 11 and 12 in Table 3-1 and Figure 3-1c). Figure 3-1c suggests that the chemical yields of larger cyclic oligogalactoside were increased when higher concentrations of **84** were used, possibly because higher concentration conditions accelerate the intermolecular glycosylation to give a longer linear oligosaccharide intermediate, followed by intramolecular cyclization.

The effect of reaction temperature on the glycosylation products was also investigated (entries 1, 13 and 14 in Table 3-1 and Figure 3-1d). The reaction at -78 °C gave linear saccharides instead of cyclic saccharides (entry 13), possibly because the activation energy for the cyclization is high. A comparison of the reactions at -40 °C and 0 °C suggested that, when the reaction was carried out at a lower temperature, a larger cyclic oligosaccharide is produced in slightly higher yields (entries 1 vs. 14 in Table 3-1 and Figure 3-1d).

The presence of $LiClO_4$ in the reaction was examined, in order to observe the effect of the presence of a salt in controlling the selectivity of the ring size of cyclic oligogalactosides due to the interaction between oxygen atoms and lithium cations. However, the result (entry 15 in Table 3-1) was almost same as that shown in entry 1 (Figure 3-1e). The reaction without acetylation process was also checked and the same cyclic oligogalactosides **86**–**90** were produced as in entry 16.

The structures of cyclic oligosaccharides **86**–**90** were determined by NMR spectroscopy and mass spectrometry. The assignments of the ^{1}H NMR spectra of **86**–**90** (Figure 3-2) suggested that these molecules have highly symmetrical structures because the spectra showed simple peak signals corresponding to the repeating monomer units. The coupling constants associated with the anomeric proton and the proton at the 2-position ($^{3}J_{H1-H2}$) of **86**–**90** were 7.2 to 8.0 Hz, which are reasonable values compared with common β-galactosides. The formation of 1,6-glycosidic linkages between the galactose units and/or ester bonds between benzoyl groups and hydroxy groups at the 2- ,3- and 4-positions were confirmed by the measurement of ^{1}H–^{13}C HMBC.

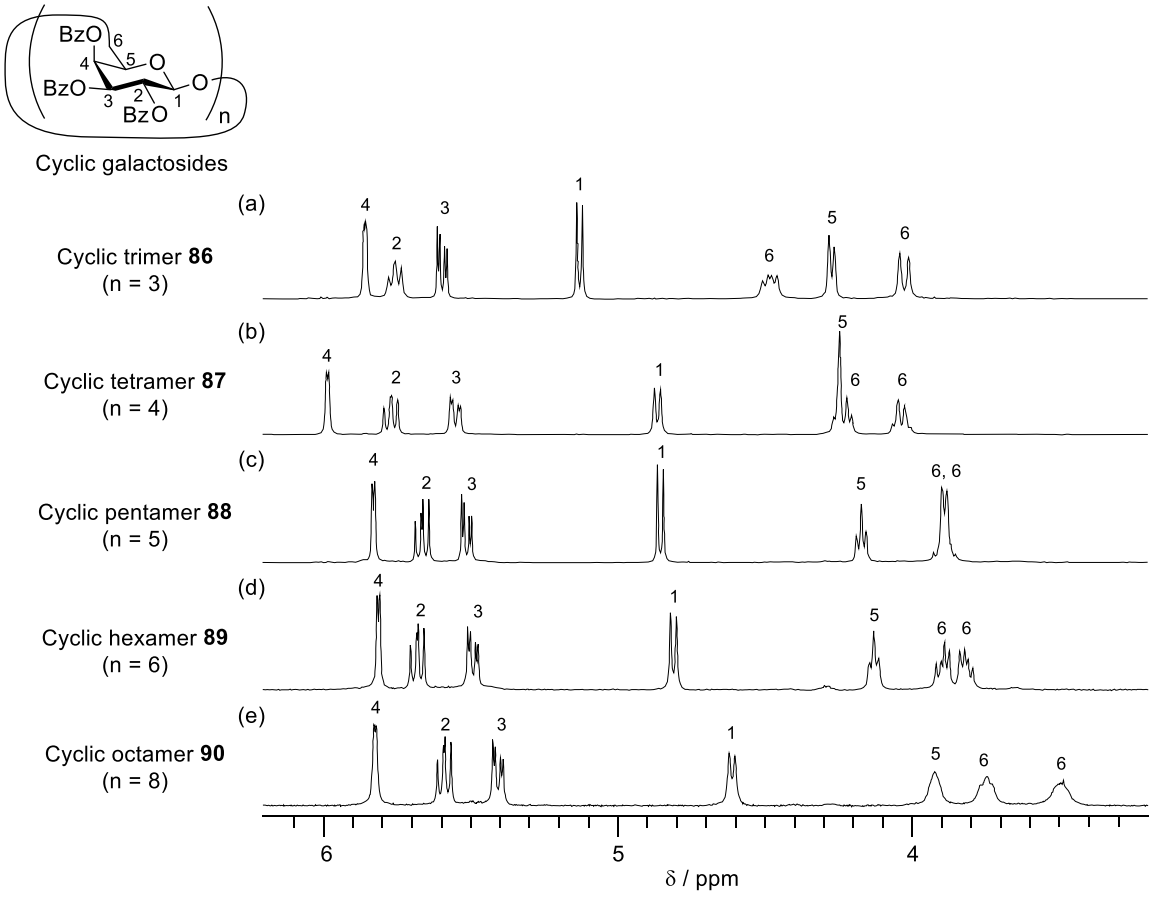


Figure 3-2. The ^{1}H NMR spectra of cyclic oligogalactosides in $CDCl_3$ at 400 MHz. (a) cyclic trisaccharide **86**; (b) cyclic tetrasaccharide **87**; (c) cyclic pentasaccharide **88**; (d) cyclic hexasaccharide **89**; and (e) cyclic octasaccharide **90**.

The number of galactose units constituting **86**–**90** was determined by ESI-MS (Figure 3-3). The peaks of $[M+Na]^+$ ions were observed for the cyclic tri-, tetra-, and pentagalactosides, corresponding to **86**, **87**, and **88** respectively. The cyclic hexagalactoside **89** and the octagalactoside **90** were detected as 2 peaks corresponding to $[M+Na]^+$ and $[M+2Na]^{2+}$ ions for **89** and $[M+2Na]^{2+}$ and $[M+3Na]^{3+}$ ions for **90**. The above results indicate that **86**–**90** are cyclic oligosaccharide with repeating D-galactopyranosyl moieties linked by β-(1→6) glycosidic bonds.

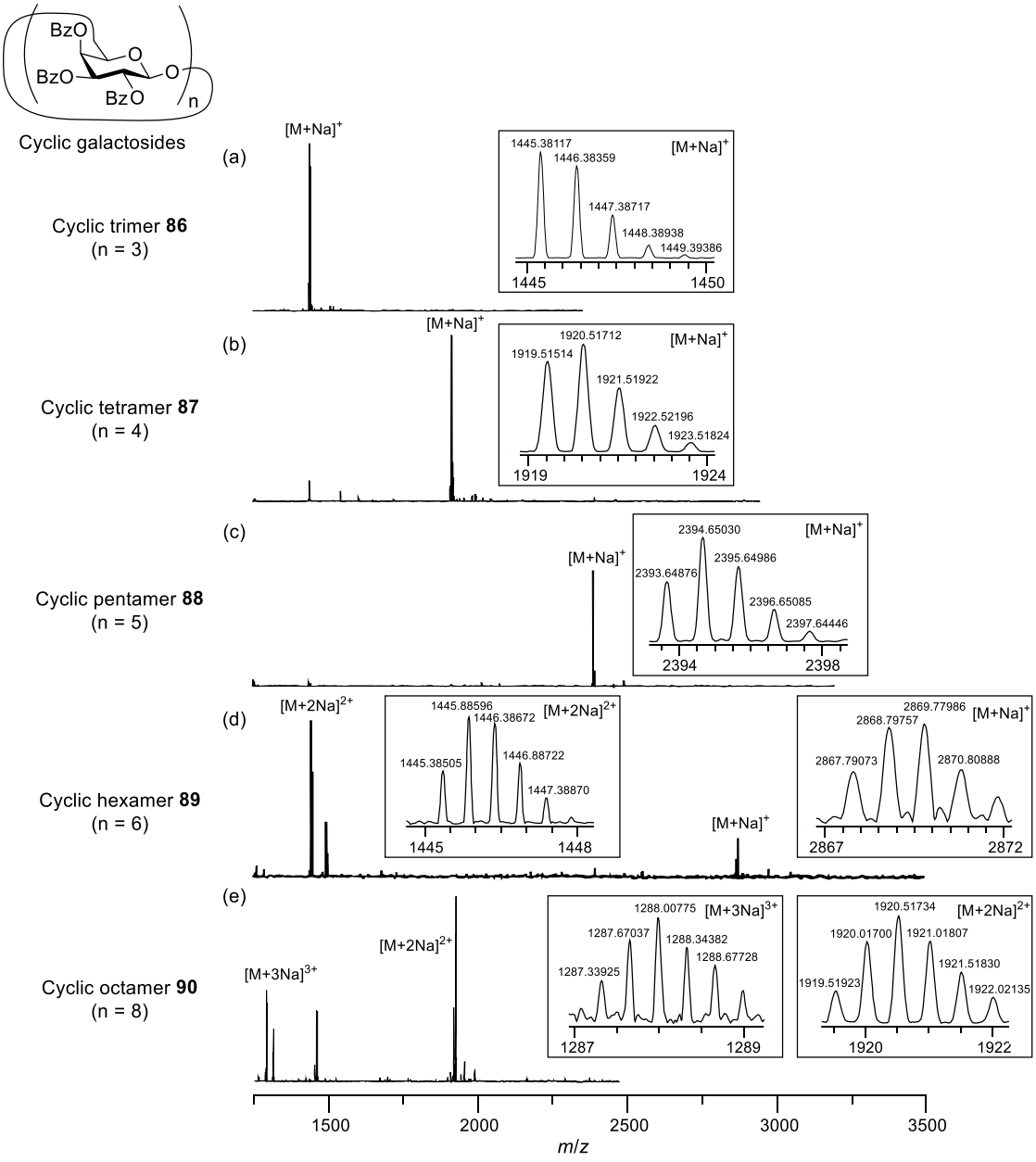


Figure 3-3. The ESI-MS spectra of cyclic oligogalactosides **86**–**90**. (a) cyclic trisaccharide **86**; (b) cyclic tetrasaccharide **87**; (c) cyclic pentasaccharide **88**; (d) cyclic hexasaccharide **89**; and (e) cyclic octasaccharide **90**.

We were able to obtain good crystals of the cyclic tetragalactoside **87** that were suitable for a single crystal X-ray structure analysis by recrystallization from toluene. The ORTEP drawing of **87** and its typical crystallographic parameters are described in Figure 3-4 and Table 3-2.

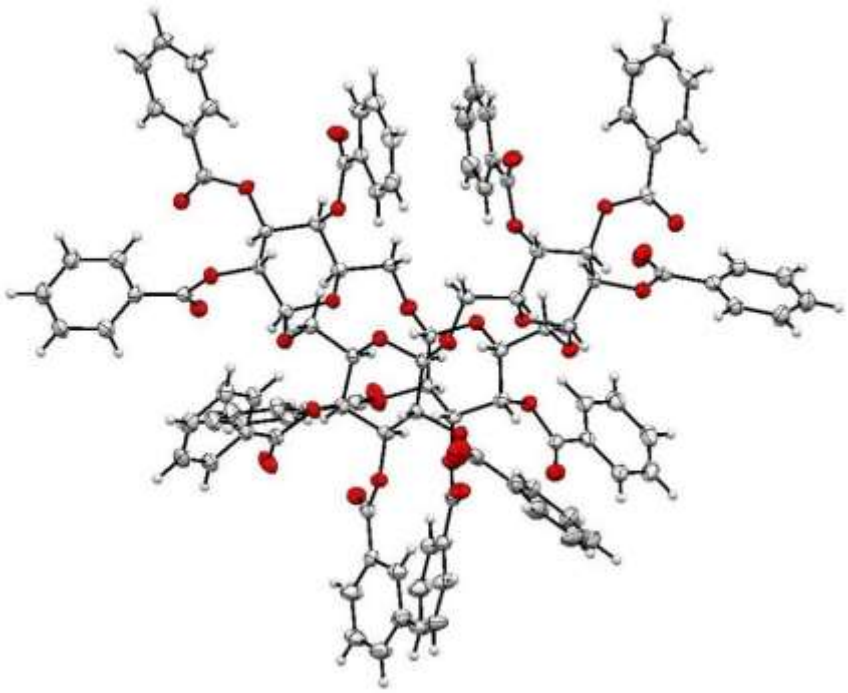


Figure 3-4. ORTEP drawing of single crystal structure of **87** (50% probability ellipsoids). For clearly, toluene included in the crystal was omitted.

Table 3-2. Crystal data and structure refinement for **87** (CCDC deposit number: 1961094).

Empirical formula	$C_{115}H_{96}O_{32}$
Formula weight	1989.91
Temperature	173 K
Wavelength	0.41270 Å
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	a = 14.66800 Å
	b = 14.85100 Å
	c = 46.25600 Å
Volume	10076 $Å^3$
Z	4
Density (calculated)	1.312 Mg/m^3
Absorption coefficient	0.039 mm^{-1}
F(000)	4168.0
Crystal size	0.05 x 0.04 x 0.02 mm^3
Theta range for data collection	2.21 to 23.81°
Index ranges	-19<=h<=18 -19<=k<=19, -60<=l<=60
Reflections collected	218186
Independent reflections	89729 [R(int) = 0.1247]
Completeness to theta = 23.81°	97 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.5888 and 1.000
Refinement method	Full-matrix least-square on F^2
Goodness-of-fit on F^2	0.932
Final R indices [I>2sigma(I)]	R_1 = 0.0523, wR_2 = 0.0905
R indices (all data)	R_1 = 0.0946, wR_2 = 0.0799

The stick and stick-CPK models (atoms constituting the inner cavity are presented by CPK model and the other atoms are stick model) of **87** shown in Figure 3-5, revealed its structure with the repeating β-(1→6) glycosidic linkage and a small rectangular-like inner cavity, whose diameter is 2.4~3.3 Å.

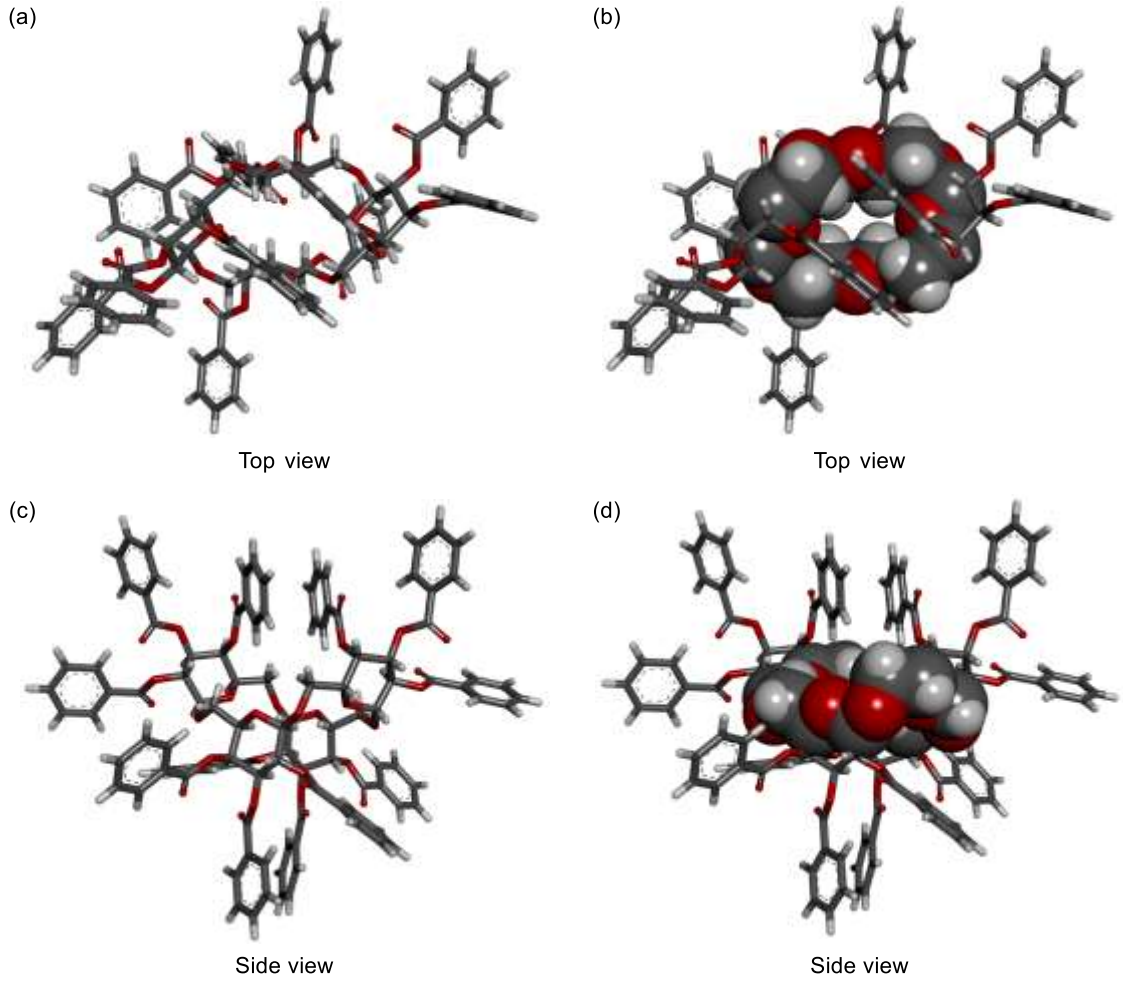
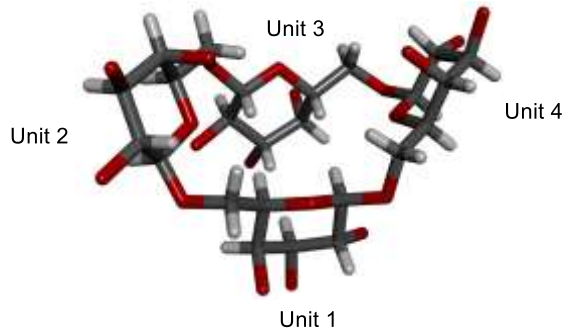


Figure 3-5. Crystal structure of **87**. (a) Top view of a stick model; (b) top view of a stick-CPK model, in which only the scaffold forming inner cavity is presented in the CPK model; (c) side view of the stick model; (d) side view of the stick-CPK model.

The 6-membered pyranose ring of **87** adopts a nearly $^4C_1$ (chair) conformation, as suggested by the polar coordinate values, meridian angle (ϕ), azimuthal angle (θ) and radius (Q), which were calculated by a Cremer-Pople puckering parameter analysis in Table 3-3 [212–215].

Table 3-3. Cremer-Pople puckering parameter analysis of **87**. (a) Stick drawing of **87**, in which benzoyl groups are removed for clearly; (b) Calculated polar coordinate values and meridian angle (ϕ), azimuthal angle (θ) and radius (Q) correspond to those in conformational globe in Table 3-3(c); (c) Conformational globe of a 6-membered pyranose ring structure, as described by the value of ϕ, θ and Q. The conformations of each galactose unit 1~4 are plotted with red circle (unit 1), blue triangle (unit 2), green square (unit 3), and pink diamond (unit 4), respectively.

(a)



(b)

Galactose unit	ϕ (°)	θ (°)	Q (Å)
1	2.766	4.696	0.574
2	275.391	7.114	0.574
3	19.583	4.900	0.579
4	291.34	4.481	0.570
Average	147.27	5.298	0.574
Deviation	136.342	1.059	0.003

(c)

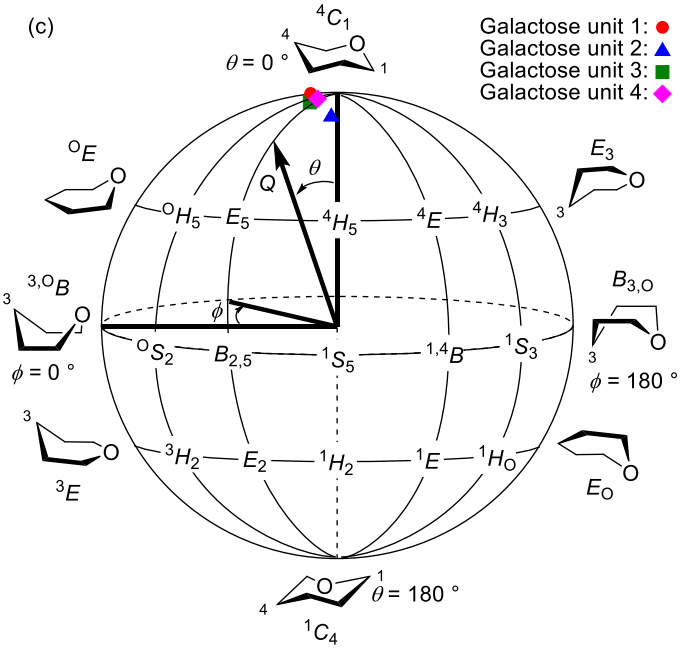
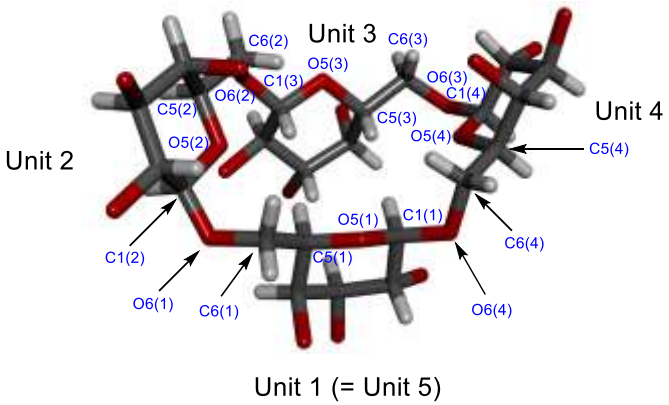


Table 3-4 summarizes representative dihedral angles of galactose units 1~4 of **87** ($DA_n(x)$, x = number of galactose unit in **87**, n = 1~3), suggesting that two pairs of two opposite galactose units in **87** adopt two different conformation with respect to the linkages between adjacent galactose units. Namely, $DA_1(1)$ and $DA_1(3)$ values are *ca.* 174~176 °, indicating the conformation of the O5(1 or 3)–C5(1 or 3) bond and C6(1 or 3)–O6(1 or 3) bond adopt *s-trans* (antiperiplanar) conformation. On the other hand, $DA_1(2)$ and $DA_1(4)$ values are *ca.* 68~71 ° that correspond to *s-cis* (synclinal) conformation. $DA_2(x)$ values of C5(x)–C6(x)–O6(x)–C1(x+1) of all the galactose units are *ca.* -108~-99 °, which means their *s-trans* (anticlinal) conformation with respect to C5(x)–C6(x) bond and O6(x)–C1(x+1) bond. The $DA_3(1)$ and $DA_3(3)$ values for C6(x)–O6(x)–C1(x+1)–O5(x+1) are *ca.* 50~57 ° and $DA_3(2)$ and $DA_3(4)$ values (note that C1(5) and O5(5) mean C1(1) and O5(1), respectively) are *ca.* -86~-74 ° that correspond to *s-cis* (synclinal) conformation with respect to C6–O6 bond and C1–O5 bond (in the adjacent galactose unit) in both cases.

Table 3-4. Representative dihedral angles ($DA_n(x)$) regarding the linkage between galactose units consisting **87**. The benzoyl groups are removed for clearly.

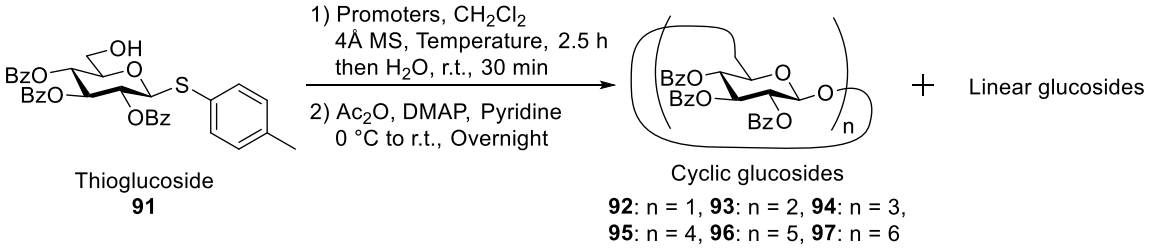


Dihedral angles of specific galactose (x) ($DA_n(x)$)	Angle (°)			
	Galactose unit 1–2 (x = 1)	Galactose unit 2–3 (x = 2)	Galactose unit 3–4 (x = 3)	Galactose unit 4–1 (x = 4)
$DA_1(x)$: O5(x)–C5(x)–C6(x)–O6(x)	176.4	70.5	173.9	67.9
$DA_2(x)$: C5(x)–C6(x)–O6(x)–C1(x+1)	-107.5	-99.0	-100.1	-101.7
$DA_3(x)$: C6(x)–O6(x)–C1(x+1)–O5(x+1)	50.3	-86.4	57.3	-74.1

We next carried out the glycosylation of thioglucoside **91** [216] to produce the cyclic oligoglucosides (Table 3-5). The use of NIS/TMSOTf in the glycosylation of **91** gave the cyclic monoglucoside (1,6-anhydroglucose) **92** in 18% yield and cyclic diglucoside **93** in 10% yield, respectively (entry 1). This result was different from the case of the thiogalactoside **84** (entry 1 in Table 3-1), in which cyclic tri- to octagalactosides **86**–**90** was obtained, possibly due to the lower reactivity of the thioglucoside **91** than the thiogalactoside **84** [217]. In entry 2, the use of a combination of *p*-TolSCl and AgOTf as promoter system gave negligible amounts of cyclic oligosaccharides (1,6-anhydroglucose **92** and linear glucosides including some impurities were major products), similar the results for entry 2 in Table 3-1. The reaction in higher concentration of **91** afforded cyclic oligoglucosides with larger ring sizes such as the

cyclic trisaccharide **94**, the tetrasaccharide **95**, the pentasaccharide **96** and the hexasaccharide **97** (entry 3). The high reaction temperature (0 °C) for this glycosylation afforded the 1,6-anhydroglucose **92** in higher yield (entry 4) than that of entry 3. The reaction without acetylation process was also examined in entry 5 and afforded the same products **92**–**97** as those of entries 3 and 4. It should be noted that cyclic diglucoside **93** was obtained in 4~14% from **91** (entry 1, 3, 4 and 5 in Table 3-5), while cyclic digalactoside was negligibly obtained from **84** (Table 1), possibly due to the steric hindrance of the benzoyl groups on the axial 4-OH group of thiogalactoside.

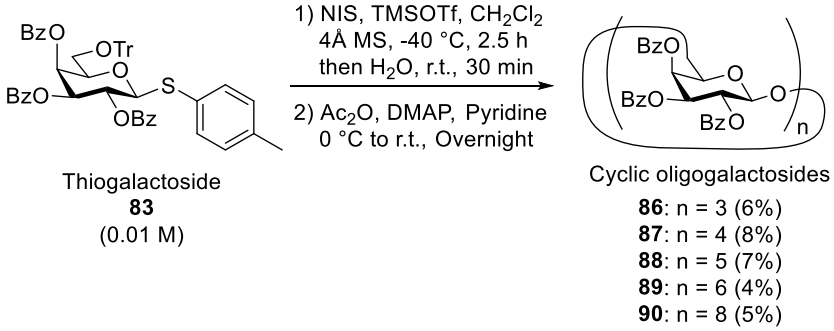
Table 3-5. Polyglycosylation of thioglucoside **91** for the synthesis of cyclic oligoglucosides **93**–**97**.



Entry	Promoters (eq.)	Concentration of **91**	Temperature	Yields (%)						
				92	**93**	**94**	**95**	**96**	**97**	Linear saccharides
1	NIS (3.0) TMSOTf (1.5)	0.01 M	-40 °C	18	10	-	-	-	-	<38
2	*p*-TolSCl (2.0) AgOTf (3.0)	0.01 M	-40 °C	25	-	-	-	-	-	<18
3	NIS (3.0) TMSOTf (1.5)	0.1 M	-40 °C	15	7	16	22	5	3	-
4	NIS (3.0) TMSOTf (1.5)	0.1 M	0 °C	32	4	14	11	3	2	-
5[a]	NIS (3.0) TMSOTf (1.5)	0.1 M	-40 °C	8	14	21	23	6	4	-

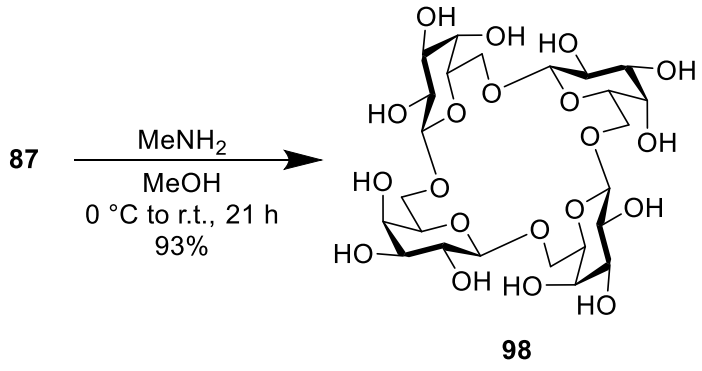
[a] Products were isolated without acetylation (second step).

The glycosylation of the thiogalactoside **83** (Scheme 3-4), in which the hydroxy group at the 6-position was protected by trityl group was also conducted, because the use of substrates with a trityl group for the glycosylation has been reported [194,197,200]. As shown in Scheme 3-4, the glycosylation of **83** gave the cyclic oligogalactosides **86**–**90**, similar to the products obtained by the glycosylation of thiogalactoside **84** despite the fact that the chemical yields were lower than that using **84** in entry 1 of Table 3-1.



Scheme 3-4. Glycosylation of thiogalactoside **83** to afford the cyclic oligosaccharides **86**–**90**.

The deprotection of the cyclic tetragalactoside **87** was carried out by treatment with $MeNH_2$ to afford **98** in 93% yield (Scheme 3-5).



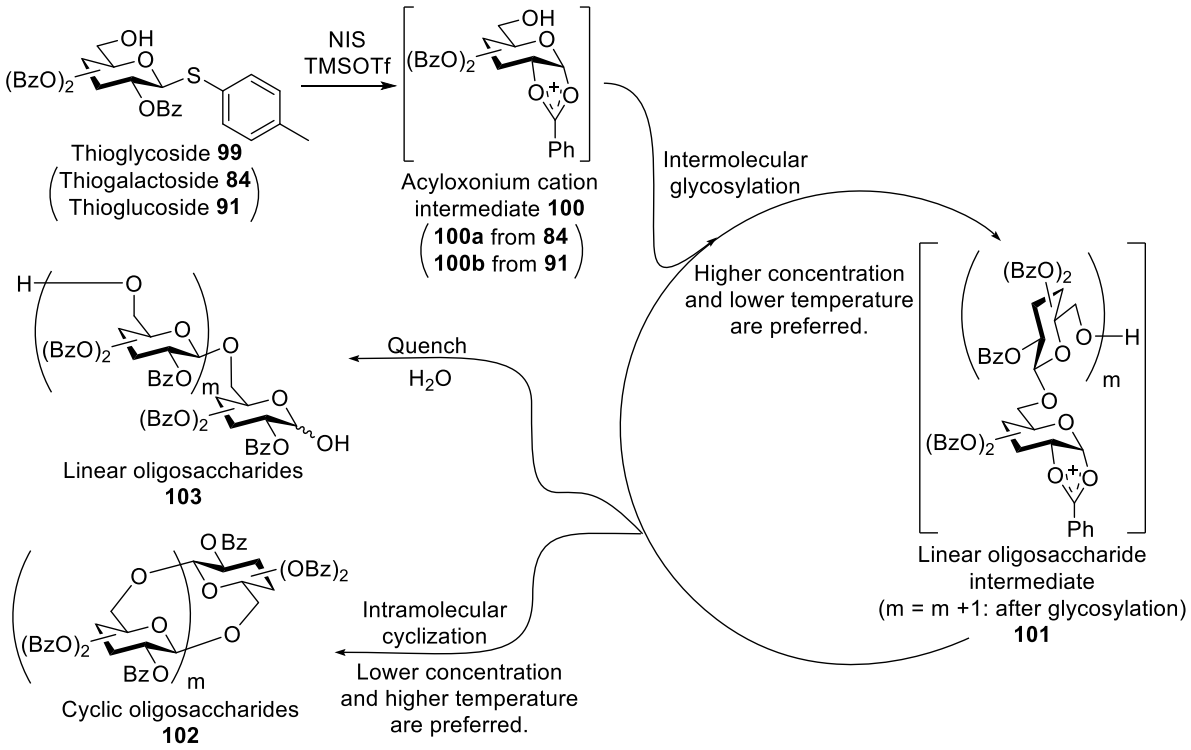
Scheme 3-5. Deprotection of **87**.

Finally, a possible reaction mechanism for the polyglycosylation of monothioglycosides in the presence of NIS and TMSOTf is proposed as displayed in Scheme 3-6, based on the aforementioned experimental results. The reaction is initiated by the activation of thioglycoside **99** (actually, thiogalactoside **84** and thioglucoside **91**) by NIS/TMSOTf to generate the acyloxonium cation species **100**, which bifunctionally react as a glycosyl donor and acceptor. The intermolecular glycosylation of **100** gives the linear oligosaccharide intermediate **101** (m = 1) and this step is repeated to elongate sugar chain (m = 2, 3, 4, …). It is assumed that the conversion of **100** to **101** prefers higher concentrations and lower temperature (e.g. -78 °C) and linear oligosaccharides **103** are obtained after the quench with H_2O due to insufficient activation energy for the intramolecular cyclization.

On the other hand, it is considered that the intramolecular cyclization of **101** to give cyclic oligosaccharides **102** requires relatively high temperature (-40 °C~0 °C). The reason why the glycosylation at 0 °C gives smaller cyclic oligosaccharides **102** than that at -40 °C (entry 1 vs entry 14 in Table 3-1 and entry 3 vs entry 4 in Table 3-5) is that shorter linear oligosaccharide intermediate **101** undergoes intramolecular cyclization at higher temperature. The formation of larger cyclic oligogalactosides is preferred at higher concentrations of **99** (entry 1 vs entry 12 in Table 3-1 and entry 1 vs entry 3 in Table 3-5) due to the acceleration of intermolecular glycosylation from **100** to **101** followed by the intramolecular cyclization. Therefore, we conclude that the balance between concentrations of the substrates and reaction temperature is important for the efficient synthesis of cyclic oligosaccharides **102**.

It should be noted that the total chemical yields of cyclic tri~octagalactosides from **84** and those of cyclic di~hexaglucosides from **91** are almost same at [thioglycoside] = 0.1 M and -40 °C (entry 12 in Table 3-1 vs entry 3 in Table 3-5). Comparison of entry 1 in Table 3-1 and entry 1 in Table 3-5 indicates that the total chemical yields of cyclic di~hexaglucosides by the polyglycosylation of **91** are much lower than those of tri~octaglucosides from **84** at lower concentration (0.01 M). These facts imply that the intermediate **100b** generated from thioglucoside **91** is less reactive than **100a** generated

from thiogalactoside **84** due to the difference of reactivity between glucosyl donors and galactosyl donors, as generally accepted [217]. The improvement of chemical yields and selectivity of the cyclic and/or linear oligosaccharides by the adjustment of reaction conditions and/or the use of template molecules (for cyclization) based on this proposed mechanism is now in progress.



Scheme 3-6. Possible mechanism for polyglycosylation of monothioglycosides using NIS/TMSOTf.

[3-3]: Conclusion

We report herein on the one-pot synthesis of cyclic oligosaccharides via the polyglycosylation of simple and readily available monothioglycosides such as thiogalactoside **84** and thioglucoside **91**. Among the glycosylation promoters and solvents tested, the use of a combination of NIS and TMSOTf in CH_2Cl_2 in the glycosylation of **84** was found to give the cyclic oligogalactosides **86**–**90** in better chemical yields than when other promoter systems and solvents were used. A study of the effect of the concentration of reactants and reaction temperature suggested that higher concentrations and lower temperatures (-40 °C vs. 0 °C) afforded slightly larger cyclic oligogalactosides. The measurement of NMR and mass spectroscopy confirmed the formation of β-(1→6) glycosidic linkage and the number of the galactose units. The crystal structure of **87** also supported the construction of β-(1→6) glycosidic linkage and revealed the $^4C_1$-conformation of pyranose ring and the small inner cavity having inner diameter of 2.4~3.3 Å, by a single crystal X-ray structure analysis. This method also can be applied to the glycosylation of the thioglucoside **91** to give the corresponding cyclic oligoglucosides **93**–**97**. The deprotection of cyclic tetragalactoside **87** was also carried out to give the deprotected cyclic tetragalactoside **98**. The inclusion of some organic and/or inorganic compounds in these cyclic oligogalactosides and cyclic oligoglucosides composed of β-(1→6) glycosidic linkages are currently underway.

Chapter 4.

Closing Remarks

In this thesis, we developed new methods for the synthesis of disaccharide nucleosides (Chapter 2) and cyclic Oligosaccharides (Chapter 3) utilizing thioglycosyl donors.

In Chapter 2, we report on the synthesis of disaccharide nucleosides utilizing the temporary protection of the 2',3'-*cis*-diol of ribonucleosides by a boronic ester. It is known that disaccharide nucleosides have a wide range of biological activities and antibacterial, antiviral and antitumor functions. Therefore, disaccharide nucleosides and their analogs would be expected to be good drug candidates. In this work, we synthesized disaccharide nucleosides utilizing the temporary protection of the 2',3'-*cis*-diol of ribonucleosides, such as adenosine, guanosine, uridine, 5-metyluridine, 5-fluorouridine and cytidine, by a cyclic boronic ester. The temporary protection of the above ribonucleosides permits the regioselective glycosylation of the 5'-hydroxyl group with thioglycosides using a combination of *p*-toluenesulfenyl chloride and silver triflate. The examination of several boronic acids and solvent suggested that glycosylation reaction using 4-(trifluoromethyl)phenylboronic acid and propionitrile gave a disaccharide nucleoside in highest chemical yield. The formation of a cyclic boronic ester prepared from uridine and 4-(trifluoromethyl)phenylboronic acid was observed by ^{1}H, ^{11}B and ^{19}F NMR spectroscopy. The video presentation of these experimental manipulations was also published in *Journal of Visualized Experiment* (*JoVE*). The evaluation of anticancer activity of disaccharide nucleosides including 5-fluorouridine against A549 and HeLaS3 cells suggested that cytotoxicity of FUR could be masked by introduction of external sugar moiety at 5'-position.

In Chapter 3, we report on the one-pot synthesis of cyclic oligosaccharides by the polyglycosylation of monothioglycosides. The synthesis of cyclic oligosaccharides by commonly used stepwise synthetic method (preparation of linear oligosaccharide followed by cyclization of them) requires tedious processes such as protection/deprotection of hydroxy group, glycosylations, and purifications after each

synthetic step. Therefore, the development of new synthetic methods for cyclic oligosaccharides from simple and readily available glycosyl donors would be highly desirable. In this work, we carried out the polyglycosylation of monothioglycosides to afford cyclic oligosaccharides. Among the glycosylation promoters and solvents tested for the glycosylation of thiogalactoside, which have a hydroxy group at the 6-position, a combination of *N*-iodosuccinimide and trimethylsilyl triflate in dichloromethane was found to afford cyclic tri~pentagalactosides containing repeated β-(1→6) glycosidic linkage as major products, as proven by X-ray single crystal structure analysis of the cyclic tetramer. The effect of reaction temperature and concentrations on the glycosylation products was also investigated. These reaction conditions were also applied to the glycosylation of thioglucosides to obtain the corresponding cyclic oligoglucosides.

We believe that these findings not only afford important and useful information on the concise and short-step synthesis of various natural and artificial compounds as well as carbohydrate derivatives, but also medicinal chemistry, food chemistry, host-guest chemistry and other research fields.

Chapter 5.

Experimental Section

General Information

All reagents and solvents were purchased at the highest commercial quality and were used without further purification. Anhydrous CH_2Cl_2 was distilled from calcium hydride. Anhydrous MeCN and EtCN were prepared by distillation from calcium hydride and successive distillation from phosphorus (V) oxide. Anhydrous THF and 1,4-dioxane were prepared by distillation from sodium and benzophenone. Anhydrous toluene and pyridine were purchased. All aqueous solutions were prepared using deionized water.

^{1}H (300 and 400 MHz), ^{11}B (128 MHz), ^{13}C (75 and 100 MHz), and ^{19}F (376 MHz) NMR spectra were recorded on a JEOL Always 300 (JEOL, Tokyo, Japan), a JEOL Lamda 400 (JEOL, Tokyo, Japan) and a JNM-ECZ400S (JEOL, Tokyo, Japan) spectrometer. Two-dimensional NMR spectra (correlation spectroscopy (COSY), heteronuclear multiple quantum correlation (HMQC) and heteronuclear multiple bond coherence (HMBC)) were recorded on a JEOL Lamda 400 (JEOL, Tokyo, Japan) and a JNM-ECZ400S (JEOL, Tokyo, Japan) spectrometer. Tetramethylsilane (TMS) was used as an internal reference (0 ppm) for ^{1}H NMR measurements in $CDCl_3$, CD_3OD, CD_3CN, acetone-d_6 and DMSO-d_6. 3-(Trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium (TSP) was used as an internal reference (0 ppm) for ^{1}H NMR measurements in D_2O. 1,4-Dioxane was used as an internal reference (67.19 ppm) for ^{13}C NMR measurements in D_2O. ^{11}B and ^{19}F NMR spectra were measured in a quartz NMR tube. The boron trifluoride-diethyl ether complex ($BF_3 \cdot OEt_2$) in $CDCl_3$ was used as an external reference (0 ppm) for ^{11}B NMR, and trifluoroacetic acid (TFA) in $CDCl^3$ was used as an external reference (−76.5 ppm) for ^{19}F NMR. IR spectra were recorded on a Perkin-Elmer FTIR Spectrum 100 (ATR) (PerkinElmer, Massachusetts, USA). MS measurements were performed on a JEOL JMS-700 (JEOL, Tokyo, Japan) and Varian 910-MS (Varian Medical Systems, California, USA) spectrometer. Elemental analyses were performed on a Perkin-Elmer CHN 2400 analyzer (PerkinElmer, Massachusetts, USA). Optical

rotations were determined with a JASCO P-1030 digital polarimeter (JASCO, Tokyo, Japan) in 50-mm cells using the D line of sodium (589 nm). Thin-layer chromatography (TLC) and preparative thin-layer chromatography (PTLC) were performed using Merck Silica gel 60 F254 plate (Merck KGaA, Darmstadt, Germany). Silica gel column chromatography was performed using Fuji Silysia Chemical FL-100D (Fuji Silysia Chemical, Aichi, Japan). HPLC experiments were carried out using a system consisting of a PU-2089 Plus intelligent HPLC pump (JASCO, Tokyo, Japan), a UV-2075 Plus intelligent UV-visible detector (JASCO, Tokyo, Japan), a Rheodine injector (Model No. 7125) and a Chromatopak C-R8A (Shimadzu, Kyōto, Japan). For preparative HPLC, a SenshuPak Pegasil ODS column (Senshu Scientific Co., Ltd., Tokyo, Japan) (20ϕ x 250 mm, No. 0509271H) was used. Gel permeation chromatography (GPC) experiments were carried out using a system consisting of a PUMP P-50 (Japan Analytical Industry Co., Ltd., Tokyo, Japan), a UV/VIS DETECTOR S-3740 (Soma, Tokyo, Japan), a Manual Sample Injector 7725i (Rheodyne, Bensheim, Germany) and an MDL-101 1 PEN RECORDER (Japan Analytical Industry Co., Ltd., Tokyo, Japan), equipped with two GPC columns, JAIGEL-1H and JAIGEL-2H (Japan Analytical Industry Co., Ltd., Tokyo, Japan) (20ϕ x 600 mm, No. A605201 and A605204).

Experimental procedures in Chapter 2

2',3'-Di-*O*-acetyl-5'-*O*-(6"-*O*-acetyl-2",3",4"-tri-*O*-benzyl-α/β-D-mannopyranosyl)uridine (20)

Procedure for entry 13 in Table 2-1

A mixture of **17** (28.4 mg, 48.6 μmol), **18** (7.9 mg, 32.4 μmol) and **19c** (9.3 mg, 49.0 μmol) was co-evaporated with anhydrous pyridine (three times) and anhydrous 1,4-dioxane (three times) and dissolved in anhydrous 1,4-dioxane (320 μL). This reaction mixture was stirred under reflux conditions for 1 hr and concentrated under reduced pressure. The resulting mixture was stirred with activated 4 Å molecular sieves (64 mg)

in anhydrous EtCN (640 μL) at room temperature for 30 min and then cooled to −40 °C, to which *p*-TolSCl (12.8 μL, 96.8 μmol) and AgOTf (49.9 mg, 194 μmol) were added at the same temperature. After stirring for 1.5 hr at −40 °C, the reaction mixture was quenched with saturated aqueous $NaHCO_3$, diluted with $CHCl_3$ and filtered through Celite. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The remaining residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 1/0–50/1) to give 5'-*O*-(6"-*O*-acetyl-2",3",4"-tri-*O*-benzyl-α/β-D-mannopyranosyl)uridine including a small amount of byproducts as a colorless syrup (15.2 mg). To the resulting crude compound in anhydrous pyridine (200 μL), Ac_2O (20.4 μL, 21.6 μmol, 10.0 equiv. based on the crude compound) and DMAP (catalytic amount) were added at 0 °C. The reaction mixture was stirred at the same temperature for 30 min and then allowed to warm to room temperature. After stirring overnight, the reaction mixture was diluted with $CHCl_3$, washed with 1 M aqueous HCl, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 1/0–90/1) to give **20** as a colorless amorphous solid (15.8 mg, 61% yield for 3 steps, α/β = 1.6/1): ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ = 8.56 (s, 0.6H), 8.29 (s, 0.4H), 7.89 (d, J = 8.1 Hz, 0.4H), 7.41–7.19 (m, 15.6H), 6.29 (d, J = 7.2 Hz, 0.4H), 6.15–6.05 (m, 0.6H), 5.55 (dd, J = 5.1, 1.2 Hz, 0.4H), 5.39 (dd, J = 8.1, 1.8 Hz, 0.6H), 5.33–5.23 (m, 2H), 5.01–4.86 (m, 2H), 4.80–4.55 (m, 4.6H), 4.46 (s, 0.4H), 4.39–4.21 (m, 3H), 4.13 (dd, J = 10.5, 1.8 Hz, 0.4H), 4.05 (d, J = 2.7 Hz, 0.4H), 3.99–3.84 (m, 2.2H), 3.84–3.68 (m, 1.6H), 3.68–3.57 (m, 1H), 3.44 (dt, J = 9.9, 6.9 Hz, 0.4H), 2.15 (s, 1.2H), 2.12 (s, 1.8H), 2.09 (s, 1.2H), 2.09 (s, 1.8H), 2.06 (s, 1.8H), 2.00 (s, 1.2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 170.9, 170.8, 170.1, 169.8, 169.7, 169.6, 162.8, 162.6, 150.8, 150.4, 141.0, 138.8, 138.2, 137.9, 137.8, 137.7, 128.5, 128.5, 128.4, 128.4, 128.1, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 103.3, 103.2, 100.0 ($C_{1''}$, $^1J_{CH}$ = 153.6 Hz, β form), 98.5 ($C_{1''}$, $^1J_{CH}$ = 171.0 Hz, α form), 86.3, 85.2, 82.8, 82.1, 81.4,

80.1, 77.3, 75.2, 75.2, 75.0, 74.8, 74.7, 74.3, 73.9, 73.7, 73.5, 72.9, 72.8, 72.5, 72.2, 71.9, 71.2, 71.0, 68.6, 66.8, 63.3, 63.2, 20.9, 20.9, 20.7, 20.6, 20.4 ppm; IR (ATR): ν = 3200, 3065, 3032, 2930, 2877, 1742, 1691, 1498, 1455, 1373, 1310, 1231, 1073, 1042, 1029, 925, 901, 811, 737, 697, 635, 597 cm^{-1}; HRMS (FAB+): *m/z* calcd. for $[M+H]^+$, $C_{42}H_{47}N_2O_{14}$, 803.3027; found, 803.3028.

Procedure for Scheme 2-1

A mixture of **17** (30.0 mg, 51.3 μmol), **21** (29.2 mg, 102 μmol) and activated 4 Å molecular sieves (150 mg) was stirred in anhydrous CH_2Cl_2 (1.5 mL) for 30 min, then cooled to -40 °C, to which DTBMP (31.6 mg, 154 μmol), AgOTf (39.5 mg, 154 μmol) and *p*-TolSCl (17.0 μL, 129 μmol) were added at the same temperature. The reaction mixture was stirred at the same temperature for 1 hr and then allowed to warm to -20 °C. After stirring for 1.5 hr, the reaction mixture was quenched with saturated aqueous $NaHCO_3$, diluted with $CHCl_3$, and filtered through Celite. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 1/0 to 90/1) followed by GPC ($CHCl_3$) to give the product as a colorless syrup (12.0 mg). To a solution of resulting compound in THF (300 μL), 2M aqueous HCl (300 μL) was added at room temperature. After stirring at the same temperature for 14 hr, the reaction mixture was neutralized with saturated aqueous $NaHCO_3$, extracted with $CHCl_3$, washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 1/0 to 70/1) to give a product (7.7 mg). To a solution of this product in pyridine (200 μL), Ac_2O (13.8 μL, 146 μmol) and DMAP (catalytic amount) were added at 0 °C. The reaction mixture was stirred at the same temperature for 30 min and allowed to warm to room temperature. After stirring for 10 hr, the reaction mixrture was diluted with $CHCl_3$, washed with 1M aqueous HCl, saturated

aqueous $NaHCO_3$, and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 1/0 to 90/1) to give **20** as a colorless amorphous solid (8.1 mg, 20% yield for 3 steps, α/β = 1.2/1).

2',3'-Di-*O*-acetyl-5'-*O*-(6"-*O*-acetyl-2",3",4"-tri-*O*-benzyl-α/β-D-mannopyranosyl)adenosine (23) and 7-*N*-(6'-*O*-acetyl-2',3',4'-tri-*O*-benzyl-α-D-mannopyranosyl)adenine (24) (2ntry 2 in Table 2)

A mixture of **17** (28.4 mg, 48.6 μmol), **22** (8.6 mg, 32.2 μmol) and **19a** (5.9 mg, 48.4 μmol) was co-evaporated with anhydrous pyridine (three times) and anhydrous 1,4-dioxane (three times) and dissolved in anhydrous 1,4-dioxane (320 μL). This reaction mixture was stirred under reflux conditions for 1 hr and concentrated under reduced pressure. The resulting mixture was stirred with activated 3 Å molecular sieves (64 mg) in anhydrous MeCN (640 μL) at room temperature for 30 min and then cooled to −20 °C, to which *p*-TolSCl (12.8 μL, 96.8 μmol) and AgOTf (49.9 mg, 194 μmol) were added at the same temperature. After stirring for 1.5 hr at −20 °C, the reaction mixture was quenched with saturated aqueous $NaHCO_3$, diluted with $CHCl_3$ and filtered through Celite. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 1/0–10/1) to give 5'-*O*-(6"-*O*-acetyl-2",3",4"-tri-*O*-benzyl-α/β-D-mannopyranosyl)adenosine including a small amount of byproducts as a colorless syrup (6.3 mg). To the resulting crude compound in anhydrous pyridine (200 μL), Ac_2O (8.0 μL, 84.9 μmol, 10.0 equiv. based on the crude compound) and DMAP (catalytic amount) were added at 0 °C. The reaction mixture was stirred at the same temperature for 30 min and then allowed to warm to room temperature. After stirring overnight, the reaction mixture was diluted with $CHCl_3$, washed with aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated

under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 1/0–5/1) to give **23** as a colorless amorphous solid (3.8 mg, 14% yield for 3 steps, α/β = 1/1.0) and **24** as a colorless syrup (1.1 mg, 6% yield for 3 steps): **23** (α/β = 1/1.0); ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ = 8.36 (s, 1H), 8.35 (s, 0.5H), 7.93 (s, 0.5H), 7.42–7.27 (m, 13.5H), 7.16 (t, J = 2.7 Hz, 1.5H), 6.31 (d, J = 6.0 Hz, 0.5H), 6.20 (d, J = 5.7 Hz,0.5H), 5.92 (t, J = 5.7 Hz, 0.5H), 5.78–5.67 (m, 1H), 5.67–5.52 (m, 2.5H), 4.97–4.83 (m, 3H), 4.77–4.45 (m, 5H), 4.45–4.16 (m, 3H), 4.05–3.82 (m, 3H), 3.82–3.62 (m, 1H), 3.58–3.41 (m, 1H), 2.15 (s, 1.5H), 2.13 (s, 1.5H), 2.07 (s, 1.5H), 2.06 (s, 1.5H), 2.03 (s, 1.5H), 2.01 (s, 1.5H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 171.1, 170.9, 169.9, 169.6, 169.4, 169.3, 155.5, 155.4, 153.4, 153.1, 150.0, 150.0, 139.7, 138.7, 138.4, 138.2, 138.1, 138.0, 137.9, 128.4, 128.4, 128.4, 128.4, 128.4, 128.2, 128.1, 127.9, 127.9, 127.8, 127.7, 127.7, 127.6, 127.6, 127.3, 120.1, 119.8, 100.9 ($C_{1''}$, $^1J_{CH}$ = 156.9 Hz, β form), 98.6 ($C_{1''}$, $^1J_{CH}$ = 168.5 Hz, α form), 85.7, 85.2, 82.3, 82.2, 81.4, 80.2, 75.3, 75.1, 75.0, 74.5, 74.4, 74.3, 74.1, 73.9, 73.1, 73.0, 72.4, 71.7, 71.1, 70.7, 69.3, 66.6, 63.5, 63.4, 21.0, 20.9, 20.7, 20.6, 20.4, 20.4 ppm; IR (ATR): ν = 3332, 3171, 3066, 3032, 2927, 2875, 1742, 1635, 1595, 1498, 1473, 1455, 1424, 1366, 1332, 1293, 1234, 1213, 1071, 1042, 1027, 903, 825, 799, 736, 697, 667, 649, 602 cm^{-1}; HRMS (FAB+): m/z calcd. for $[M+H]^+$, $C_{43}H_{48}N_5O_{12}$, 826.3299; found, 826.3294; **24**; ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ = 8.44 (s, 1H), 7.90 (s, 1H), 7.41–7.28 (m, 10H), 7.23–7.19 (m, 1H), 7.18–7.08 (m, 2H), 6.86–6.74 (m, 2H), 5.88 (s, 2H), 5.61 (s, 1H), 4.97 (d, J = 10.8 Hz, 1H), 4.77 (s, 2H), 4.70 (d, J = 6.3 Hz, 1H), 4.66 (d, J = 6.6 Hz, 1H), 4.45 (dd, J = 12.0, 3.3 Hz, 1H), 4.26 (dd, J = 12.0, 2.4 Hz, 1H), 4.25 (d, J = 11.1 Hz, 1H), 4.11 (t, J = 9.6 Hz, 1H), 3.96 (s, 1H), 3.88 (dd, J = 9.3, 2.7 Hz, 1H), 3.76 (dt, J = 9.6, 3.0 Hz, 1H), 1.98 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ = 170.0, 160.7, 153.2, 151.6, 143.0, 137.3, 135.9, 128.7, 128.6, 128.5, 128.5, 128.4, 128.3, 128.3, 128.2, 127.7, 111.8, 85.7, 82.8, 76.7, 76.1, 75.4, 75.1, 73.0, 72.5, 62.2, 20.7 ppm; IR (ATR): ν = 3449, 3371, 3167, 3089, 3064, 3031, 2927, 2873, 1742, 1627, 1587, 1551, 1497, 1475, 1455, 1425, 1389,

1365, 1340, 1296, 1228, 1094, 1019, 966, 909, 887, 825, 736, 695, 602 cm^{-1}; HRMS (FAB+): *m*/*z* calcd. for $[M+H]^+$, $C_{34}H_{36}N_5O_6$, 610.2666; found, 610.2668; $[\alpha]_D^{25} = -20.6$ ° (*c* = 1.0, $CHCl_3$).

5'-*O*-(2",3",4",6"-Tetra-*O*-benzoyl-β-D-galactopyranosyl)adenosine (β-33)

A mixture of **32** (80.4 mg, 114 μmol), **22** (20.4 mg, 76.3 μmol) and **19c** (21.7 mg, 114 μmol) was co-evaporated with anhydrous pyridine (three times) and anhydrous 1,4-dioxane (three times) and dissolved in anhydrous 1,4-dioxane (760 μL). This reaction mixture was stirred under reflux conditions for 1 hr and concentrated under reduced pressure. The resulting mixture was stirred with activated 4 Å molecular sieves (150 mg) in anhydrous EtCN (1.50 mL) at room temperature for 30 min and then cooled to −40 °C, to which *p*-TolSCl (30.3 μL, 229 μmol) and AgOTf (117.6 mg, 458 μmol) were added at the same temperature. After stirring for 1.5 hr at −40 °C, the reaction mixture was quenched with saturated aqueous $NaHCO_3$, diluted with $CHCl_3$ and filtered through Celite. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 1/0–30/1) to give β-**33** as a colorless solid (27.4 mg, 42% yield): ^1H NMR (400 MHz, $CDCl_3$, TMS): δ = 8.46 (s, 1H), 8.07 (dd, *J* = 7.6, 2.0 Hz, 2H), 8.02–7.98 (m, 3H), 7.97–7.93 (m, 2H), 7.83–7.79 (m, 2H), 7.57–7.51 (m, 1H), 7.49 (t, *J* = 7.6 Hz, 1H), 7.45–7.32 (m, 8H), 7.20 (t, *J* = 8.0 Hz, 2H), 6.47 (brs, 2H), 6.13 (d, *J* = 6.4 Hz, 1H), 6.04 (d, *J* = 3.2 Hz, 1H), 5.90 (dd, *J* = 10.4, 8.0 Hz, 1H), 5.73 (dd, *J* = 10.4, 3.2 Hz, 1H), 4.92 (d, *J* = 8.0 Hz, 1H), 4.70 (dd, *J* = 11.2, 6.4 Hz, 1H), 4.63 (t, *J* = 5.6 Hz, 1H), 4.47–4.33 (m, 4H), 4.20 (d, *J* = 4.8 Hz, 1H), 3.77 (d, *J* = 8.4 Hz, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 166.1, 166.1, 165.6, 165.5, 155.3, 152.0, 148.8, 139.0, 133.8, 133.5, 133.4, 133.4, 130.0, 129.8, 129.8, 129.7, 129.3, 128.7, 128.7, 128.6, 128.5, 128.4, 119.0, 101.5, 88.3, 83.9, 76.3, 72.5, 71.6, 71.2, 70.1, 70.0, 68.0, 61.8 ppm; IR (ATR): ν = 3345, 3203, 3070, 2929,

1721, 1639, 1602, 1585, 1475, 1452, 1421, 1316, 1259, 1177, 1092, 1066, 1025, 1002, 938, 906, 857, 799, 753, 705, 685, 649, 617 cm^{-1}; HRMS (FAB+): *m/z* calcd. for $[M+H]^+$, $C_{44}H_{40}N_5O_{13}$, 846.2623; found, 846.2626; Anal. Calcd. for $C_{44}H_{39}N_5O_{13}\cdot 1.5H_2O$: C, 60.55; H, 4.85; N, 8.02; found: C, 60.47; H, 4.61; N, 7.98; $[\alpha]_D^{25}$ = +13.9 ° (*c* = 1.0, $CHCl_3$).

6-*N*-Benzoyl-5'-*O*-(2",3",4",6"-tetra-*O*-benzoyl-β-D-galactopyranosyl)adenosine (β-34)

O-Glycosylation using **32** (80.5 mg, 115 μmol), **25** (28.4 mg, 76.5 μmol), **19c** (21.8 mg, 115 μmol), anhydrous 1,4-dioxane (760 μL), *p*-TolSCl (30.3 μL, 229 μmol), AgOTf (117.8 mg, 458 μmol), 4 Å molecular sieves (150 mg) and anhydrous EtCN (1.50 mL) was conducted according to the procedure used for the synthesis of β-**33**. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 1/0–50/1) to give β-**34** as a colorless solid (21.9 mg, 30% yield for 2 steps): ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ = 9.21 (brs, 1H), 8.66 (s, 1H), 8.57 (s, 1H), 8.17-8.08 (m, 2H), 7.90–7.81 (m, 4H), 7.90–7.81 (m, 2H), 7.76 (d, *J* = 7.5 Hz, 2H), 7.62–7.36 (m, 11H), 7.30 (t, *J* = 7.8 Hz, 2H), 7.22 (t, *J* = 7.8 Hz, 2H), 6.15 (d, *J* = 5.1 Hz, 1H), 6.01 (d, *J* = 3.0 Hz, 1H), 5.78 (dd, *J* = 10.2, 7.5 Hz, 1H), 5.65 (dd, *J* = 10.5, 3.3 Hz, 1H), 5.49 (brs, 1H), 4.88 (d, *J* = 7.8 Hz, 1H), 4.76–4.59 (m, 2H), 4.43 (dd, *J* = 11.1, 6.3 Hz, 1H), 4.38–4.21 (m, 4H), 3.81 (dd, *J* = 10.5, 2.7 Hz, 1H), 3.51 (s, 1H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ = 166.1, 165.5, 165.5, 164.6, 152.2, 151.0, 149.4, 141.7, 133.7, 133.5, 133.4, 132.8, 130.2, 129.8, 129.6, 129.3, 128.9, 128.8, 128.8, 128.7, 128.6, 128.5, 128.3, 127.9, 122.8, 101.5, 89.4, 84.2, 75.8, 71.8, 71.6, 71.2, 69.9, 69.3, 68.0, 61.9 ppm; IR (ATR): ν = 3336, 3066, 2938, 1721, 1612, 1603, 1584, 1510, 1489, 1452, 1406, 1316, 1250, 1177, 1092, 1066, 1025, 1002, 938, 901, 858, 824, 798, 755, 704, 685, 644, 616 cm^{-1}; HRMS (FAB+): *m/z* calcd. for $[M+H]^+$, $C_{51}H_{44}N_5O_{14}$, 950.2885; found, 950.2885; Anal. Calcd. for $C_{51}H_{43}N_5O_{14}\cdot 1.5H_2O$: C, 62.70; H, 4.75; N, 7.17; found: C, 62.80; H, 4.57; N, 7.22; $[\alpha]_D^{25}$ = +4.88 ° (*c* = 1.0, $CHCl_3$).

5'-*O*-(2",3",4",6"-Tetra-*O*-benzoyl-β-D-galactopyranosyl)guanosine (β-35)

O-Glycosylation using **32** (80.5 mg, 115 μmol), **26** (21.6 mg, 76.3 μmol), **19c** (21.8 mg, 115 μmol), anhydrous 1,4-dioxane (760 μL), *p*-TolSCl (30.3 μL, 229 μmol), AgOTf (117.6 mg, 458 μmol), 4 Å molecular sieves (150 mg) and anhydrous EtCN (1.50 mL) was conducted according to the procedure used for the synthesis of β-**33**. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 1/0–8/1) to give β-**35** as a colorless solid (8.1 mg, 12% yield for 2 steps): ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ = 10.68 (s, 1H), 8.11-8.07 (m, 2H), 8.05 (s, 1H), 7.94 (d, J = 8.4 Hz, 2H), 7.85 (d, J = 8.4 Hz, 2H), 7.76–7.69 (m, 3H), 7.69–7.63 (m, 3H), 7.62–7.48 (m, 4H), 7.43 (t, J = 7.6 Hz, 2H), 7.35 (t, J = 7.6 Hz, 2H), 6.51 (s, 2H), 5.91 (d, J = 3.2 Hz, 1H), 5.86 (dd, J = 10.4, 3.2 Hz, 1H), 5.70 (d, J = 6.0 Hz, 1H), 5.60 (t, J = 10.0 Hz, 1H), 5.39 (d, J = 6.4 Hz, 1H), 5.22 (d, J = 7.6 Hz, 1H), 5.19 (d, J = 3.6 Hz, 1H), 4.69 (t, J = 6.4 Hz, 1H), 4.52 (dd, J = 11.2, 2.8 Hz, 1H), 4.42 (dd, J = 11.2, 6.8 Hz, 1H), 4.37 (dd, J = 11.2, 6.0 Hz, 1H), 4.10 (d, J = 8.8, 1H), 4.03 (d, J = 2.8 Hz, 1H), 3.92 (d, J = 2.8 Hz, 1H), 3.82 (dd, J = 10.8, 4.0 Hz, 1H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6, TMS): δ = 165.1, 165.1, 165.1, 164.4, 156.8, 153.6, 151.4, 135.1, 133.8, 133.7, 133.5, 133.5, 129.4, 129.2, 129.1, 129.0, 129.0, 128.8, 128.7, 128.7, 128.7, 128.6, 128.6, 128.4, 116.6, 99.9, 86.4, 83.1, 73.8, 71.2, 70.7, 70.0, 69.6, 68.4, 61.7 ppm; IR (ATR): ν = 3332, 3128, 3065, 2935, 1724, 1673, 1638, 1602, 1584, 1572, 1538, 1491, 1452, 1350, 1316, 1261, 1177, 1092, 1067, 1025, 1002, 938, 904, 857, 801, 781, 755, 706, 686, 638, 617 cm^{-1}; HRMS (FAB+): *m/z* calcd. for $[M+H]^+$, $C_{44}H_{40}N_5O_{14}$, 862.2572; found, 862.2573; Anal. Calcd. for $C_{44}H_{39}N_5O_{14}\cdot 1.5H_2O$: C, 59.46; H, 4.76; N, 7.88; found: C, 59.52; H, 4.62; N, 7.87; $[\alpha]_D^{24}$ = +11.3 ° (*c* = 1.0, DMSO).

2-*N*-Isobutyryl-5'-*O*-(2",3",4",6"-tetra-*O*-benzoyl-β-D-galactopyranosyl) guanosine (β-36)

Glycosylation using **32** (80.5 mg, 115 μmol), **27** (27.0 mg, 76.4 μmol), **19c** (21.8 mg, 115 μmol), anhydrous 1,4-dioxane (760 μL), *p*-TolSCl (30.3 μL, 229 μmol), AgOTf (117.8 mg, 458 μmol), 4 Å molecular sieves (150 mg) and anhydrous EtCN (1.50 mL) was conducted according to the procedure used for the synthesis of β-**33**. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 1/0–20/1) to give β-**36** as a colorless solid (31.4 mg, 44% yield for 2 steps): ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ = 12.11 (s, 1H), 10.33 (s, 1H), 8.09–7.90 (m, 6H), 7.88 (s, 1H), 7.79–7.67 (m, 2H), 7.58–7.31 (m, 10H), 7.24 (t, *J* = 7.8 Hz, 2H), 6.20 (brs, 1H), 6.00 (d, *J* = 3.3 Hz, 1H), 5.79 (dd, *J* = 10.5, 7.5 Hz, 1H), 5.72–5.60 (m, 2H), 4.98 (d, *J* = 5.1 Hz, 1H), 4.84 (d, *J* = 8.1 Hz, 1H), 4.67 (dd, *J* = 10.5, 5.1 Hz, 1H), 4.42–4.24 (m, 3H), 4.16 (d, *J* = 2.7 Hz, 1H), 4.05 (brs, 2H), 3.72 (d, *J* = 8.1 Hz, 1H), 2.62–2.49 (m, 1H), 1.13 (d, *J* = 6.9 Hz, 3H), 0.94 (d, *J* = 6.6 Hz, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ = 179.6, 166.5, 166.0, 165.5, 165.4, 155.6, 148.7, 147.8, 139.4, 133.8, 133.4, 129.9, 129.7, 129.6, 129.2, 128.8, 128.6, 128.5, 128.3, 120.6, 101.8, 89.4, 83.7, 72.5, 71.4, 71.2, 70.1, 69.4, 67.9, 61.6, 36.1, 18.8, 18.5 ppm; IR (ATR): ν = 3201, 3067, 2974, 2936, 1720, 1677, 1602, 1560, 1475, 1452, 1404, 1376, 1350, 1316, 1258, 1178, 1156, 1092, 1066, 1026, 1002, 949, 908, 856, 802, 784, 752, 706, 687, 642, 617 cm^{-1}; HRMS (FAB+): *m/z* calcd. for $[M + H]^+$, $C_{48}H_{46}N_5O_{15}$, 932.2990; found, 932.2990; Anal. Calcd. for $C_{48}H_{45}N_5O_{15}\cdot 1.5H_2O$: C, 60.12; H, 5.05; N, 7.30; found: C, 60.29; H, 4.86; N, 7.34; $[\alpha]_D^{25}$ = +25.9 ° (*c* = 1.0, $CHCl_3$).

5'-*O*-(2",3",4",6"-Tetra-*O*-benzoyl-β-D-galactopyranosyl)uridine (β-37)

O-Glycosylation using **32** (80.4 mg, 114 μmol), **18** (18.6 mg, 76.2 μmol), **19c** (21.7 mg, 114 μmol), anhydrous 1,4-dioxane (760 μL), *p*-TolSCl (30.3 μL, 229 μmol), AgOTf (117.6 mg, 458 μmol), 4 Å molecular sieves (150 mg) and anhydrous EtCN (1.50 mL) was conducted according to the procedure used for the synthesis of β-**33**. The

residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 1/0–40/1) to give β-**37** as a colorless solid (26.1 mg, 42% yield for 2 steps): ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 9.91 (s, 1H), 8.10–8.05 (m, 2H), 8.04–7.99 (m, 2H), 7.95–7.86 (m, 3H), 7.79–7.75 (m, 2H), 7.63 (t, J = 7.6 Hz, 1H), 7.59–7.49 (m, 3H), 7.50–7.38 (m, 4H), 7.33 (t, J = 7.6 Hz, 2H), 7.23 (t, J = 7.6 Hz, 2H), 6.02 (d, J = 2.8 Hz, 1H), 5.92–5.84 (m, 2H), 5.77 (dd, J = 10.4, 8.0 Hz, 1H), 5.67 (dd, J = 10.8, 3.6 Hz, 1H), 5.03 (d, J = 4.0 Hz, 1H), 4.91 (d, J = 7.6 Hz, 1H), 4.71 (dd, J = 10.8, 6.0 Hz, 1H), 4.49–4.39 (m, 3H), 4.23 (d, J = 4.4 Hz, 1H), 4.13–4.02 (m, 2H), 3.79 (d, J = 10.0 Hz, 1H), 3.39 (d, J = 5.6 Hz, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 166.1, 165.5, 165.5, 165.5, 163.6, 151.3, 140.2, 133.9, 133.6, 133.4, 129.8, 129.8, 129.7, 129.3, 128.9, 128.7, 128.6, 128.6, 128.5, 128.4, 102.6, 101.5, 90.6, 83.4, 75.2, 71.7, 71.2, 70.0, 69.8, 68.2, 68.1, 61.9 ppm; IR (ATR): ν = 3356, 3069, 2972, 1720, 1687, 1602, 1585, 1492, 1452, 1383, 1316, 1261, 1178, 1093, 1067, 1027, 1002, 907, 858, 806, 763, 706, 686, 617 cm^{-1}; HRMS (FAB+): *m/z* calcd. for $[M+H]^+$, $C_{43}H_{39}N_2O_{15}$, 823.2350; found, 823.2352; Anal. Calcd. for $C_{43}H_{38}N_2O_{15}\cdot H_2O$: C, 61.43; H, 4.80; N, 3.33; found: C, 61.45; H, 4.70; N, 3.38; $[\alpha]_D^{25}$ = +50.7 ° (c = 1.0, $CHCl_3$).

5-Metyl-5'-*O*-(2",3",4",6"-tetra-*O*-benzoyl-β-D-galactopyranosyl)uridine (β-38)

O-Glycosylation using **32** (80.5 mg, 115 μmol), **28** (19.7 mg, 76.3 μmol), **19c** (21.8 mg, 115 μmol), anhydrous 1,4-dioxane (760 μL), *p*-TolSCl (30.3 μL, 229 μmol), AgOTf (117.6 mg, 458 μmol), 4 Å molecular sieves (150 mg) and anhydrous EtCN (1.50 mL) was conducted according to the procedure used for the synthesis of β-**33**. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 1/0–40/1) to give β-**38** as a colorless solid (33.8 mg, 53% yield for 2 steps): ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 10.04 (s, 1H), 8.09–8.05 (m, 2H), 8.04–8.00 (m, 2H), 7.97–7.93 (m, 2H), 7.79–7.74 (m, 2H), 7.67 (s, 1H), 7.61–7.52 (m, 2H), 7.50–7.37 (m, 6H), 7.34 (t, J = 7.6 Hz, 2H), 7.20 (t, J = 7.6 Hz, 2H), 6.03 (d, J = 3.6 Hz, 1H), 5.88 (d, J = 4.4 Hz, 1H),

5.81 (dd, J = 10.4, 7.6 Hz, 1H), 5.71 (dd, J = 10.4, 3.2 Hz, 1H), 5.08 (s, 1H), 4.90 (d, J = 7.6 Hz, 1H), 4.70 (dd, J = 11.2, 6.4 Hz, 1H), 4.50–4.37 (m, 3H), 4.21 (d, J = 4.4 Hz, 1H), 4.09 (dd, J = 10.0, 4.4 Hz, 1H), 4.01 (dd, J = 10.0, 4.8 Hz, 1H), 3.77 (d, J = 9.2 Hz, 1H), 3.41 (d, J = 4.8 Hz, 1H), 2.06 (s, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 166.1, 165.6, 165.6, 165.5, 164.2, 151.3, 136.1, 133.8, 133.6, 133.4, 129.9, 129.8, 129.7, 129.7, 129.3, 128.9, 128.7, 128.6, 128.5, 128.5, 128.3, 111.3, 102.2, 89.8, 83.3, 74.6, 71.8, 71.2, 70.2, 69.8, 69.3, 68.1, 61.9, 12.8 ppm; IR (ATR): ν = 3385, 3067, 2930, 1720, 1686, 1602, 1585, 1492, 1468, 1452, 1386, 1349, 1316, 1259, 1177, 1092, 1066, 1025, 1002, 937, 909, 858, 802, 793, 755, 705, 685, 616 cm^{-1}; HRMS (FAB+): *m/z* calcd. for $[M+H]^+$, $C_{44}H_{41}N_2O_{15}$, 837.2507; found, 837.2510; Anal. Calcd. for $C_{44}H_{40}N_2O_{15}\cdot H_2O$: C, 61.82; H, 4.95; N, 3.28; found: C, 61.70; H, 4.85; N, 3.30; $[\alpha]_D^{25}$ = +28.1 ° (c = 1.0, $CHCl_3$).

5-Fluoro-5'-*O*-(2",3",4",6"-tetra-*O*-benzoyl-β-D-galactopyranosyl)uridine (β-39)

O-Glycosylation using **32** (80.4 mg, 114 μmol), **29** (20.0 mg, 76.3 μmol), **19c** (21.7 mg, 114 μmol), anhydrous 1,4-dioxane (760 μL), *p*-TolSCl (30.3 μL, 229 μmol), AgOTf (117.6 mg, 458 μmol), 4 Å molecular sieves (150 mg) and anhydrous EtCN (1.50 mL) was conducted according to the procedure used for the synthesis of β-**33**. The residue was purified by silica gel column chromatography ($CHCl_3$ then AcOEt/$CHCl_3$ = 1/1) to give β-**39** as a colorless solid (38.8 mg, 61% yield for 2 steps): ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ = 9.81 (brs, 1H), 8.10 (t, J = 7.2 Hz, 3H), 8.04–7.99 (m, 2H), 7.94–7.89 (m, 2H), 7.78–7.73 (m, 2H), 7.64–7.48 (m, 4H), 7.47–7.37 (m, 4H), 7.33 (t, J = 7.5 Hz, 2H), 7.21 (t, J = 7.2 Hz, 2H), 6.03 (d, J = 3.0 Hz, 1H), 5.88 (d, J = 3.9 Hz, 1H), 5.78 (dd, J = 10.2, 7.5 Hz, 1H), 5.71 (dd, J = 10.2, 3.3 Hz, 1H), 4.86 (d, J = 7.2 Hz, 1H), 4.72 (dd, J = 11.1, 6.3 Hz, 1H), 4.51 (brs, 1H), 4.52–4.33 (m, 3H), 4.27 (d, J = 3.3 Hz, 1H), 4.19 (t, J = 4.8 Hz, 1H), 4.02 (s, 1H), 3.74 (d, J = 9.6 Hz, 1H), 3.32 (brs, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 166.1, 165.8, 165.7, 165.5, 157.0 (d, $^2J_{CF}$ = 26.4 Hz), 149.9, 140.8 (d, $^1J_{CF}$ = 237.0 Hz), 133.9, 133.7, 133.4, 130.0, 129.8, 129.7, 129.3,

128.8, 128.7, 128.6, 128.5, 128.4, 124.9 (d, $^{2}J_{CF}$ = 35.5 Hz), 101.7, 90.5, 84.0, 75.3, 71.8, 71.0, 70.9, 69.9, 69.0, 67.9, 61.8 ppm; ^{19}F NMR (376 MHz, $CDCl_3$, TFA): δ = −164.57 (s) ppm; IR (ATR): ν = 3447, 3074, 2941, 1715, 1602, 1585, 1493,1452, 1351, 1317, 1258, 1178, 1092, 1066, 1026, 1002, 936, 894, 858, 800, 753, 706, 687, 617 cm^{-1}; HRMS (FAB+): *m/z* calcd. for $[M+Na]^+$, $C_{43}H_{37}FN_2O_{15}Na$, 863.2076; found, 863.2072; Anal. Calcd. for $C_{43}H_{37}FN_2O_{15}\cdot H_2O$: C, 60.14; H, 4.58; N, 3.26; found: C, 60.02; H, 4.41; N, 3.32; $[\alpha]_D^{25}$ = +37.2 ° (*c* = 1.0, $CHCl_3$).

5'-*O*-(2",3",4",6"-Tetra-*O*-benzoyl-β-D-galactopyranosyl)cytidine (β-40)

O-Glycosylation using **32** (80.4 mg, 114 μmol), **30** (18.5 mg, 76.1 μmol), **19c** (21.7 mg, 114 μmol), anhydrous 1,4-dioxane (760 μL), *p*-TolSCl (30.3 μL, 229 μmol), AgOTf (117.6 mg, 458 μmol), 4 Å molecular sieves (150 mg) and anhydrous EtCN (1.50 mL) was conducted according to the procedure used for the synthesis of β-**33**. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 1/0–10/1) to give β-**40** as a colorless solid (34.1 mg, 55% yield for 2 steps): ^{1}H NMR (300 MHz, acetone-d_6, TMS): δ = 8.13-8.08 (m, 2H), 8.06–8.01 (m, 2H), 7.97–7.92 (m, 2H), 7.88 (d, *J* = 7.5 Hz, 1H), 7.76 (dd, *J* = 8.4, 1.1 Hz, 2H), 7.73-7.65 (m, 1H), 7.65-7.56 (m, 3H), 7.56-7.44 (m, 4H), 7.39 (t, *J* = 7.2 Hz, 2H), 7.32 (t, *J* = 7.5 Hz, 2H), 6.90 (brs, 2H), 6.10 (dd, *J* = 3.0, 0.9 Hz, 1H), 5.97 (d, *J* = 7.8 Hz, 1H), 5.90 (d, *J* = 7.8 Hz, 1H), 5.87–5.77 (m, 2H), 5.33 (d, *J* = 7.8 Hz, 1H), 4.78 (t, *J* = 6.3 Hz, 1H), 4.70 (dd, *J* = 10.8, 6.0 Hz, 1H), 4.54 (dd, *J* = 10.8, 6.6 Hz, 1H), 4.38 (dd, *J* = 11.1, 1.8 Hz, 1H), 4.19–4.12 (m, 1H), 4.06 (t, *J* = 4.5 Hz, 1H), 4.02–3.93 (m, 2H) ppm; ^{13}C NMR (100 MHz, acetone-d_6, TMS): δ = 166.9, 166.4, 166.3, 166.2, 165.8, 157.0, 142.1, 134.6, 134.3, 134.3, 134.2, 130.7, 130.5, 130.4, 130.3, 130.2, 130.2, 130.1, 129.8, 129.5, 129.4, 129.3, 102.2, 95.4, 91.6, 84.0, 76.4, 72.8, 72.0, 71.1, 70.9, 69.8, 69.7, 62.8 ppm; IR (ATR): ν = 3350, 3208, 3072, 2935, 1723, 1642, 1602, 1529, 1486, 1452, 1349, 1316, 1259, 1178, 1092, 1065, 1025, 1002, 940, 909, 857, 788, 753, 705, 685, 616 cm^{-1}; HRMS (FAB+): *m/z* calcd. for $[M+H]^+$, $C_{43}H_{40}N_3O_{14}$,

822.2510; found, 822.2507; Anal. Calcd. for $C_{43}H_{39}N_3O_{14}\cdot 1.5H_2O$: C, 60.85; H, 4.99; N, 4.95; found: C, 60.87; H, 4.72; N, 4.97; $[\alpha]_D^{25}$ = +62.4 ° (*c* = 1.0, $CHCl_3$).

4-*N*-Benzoyl-5'-*O*-(2",3",4",6"-tetra-*O*-benzoyl-β-D-galactopyranosyl)cytidine (β-41)

O-glycosylation using **32** (80.6 mg, 115 μmol), **31** (26.6 mg, 76.6 μmol), **19c** (21.8 mg, 115 μmol), anhydrous 1,4-dioxane (760 μL), *p*-TolSCl (30.3 μL, 229 μmol), AgOTf (117.8 mg, 458 μmol), 4 Å molecular sieves (150 mg) and anhydrous EtCN (1.50 mL) was conducted according to the procedure used for the synthesis of β-**33**. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 1/0–50/1) to give β-**41** as a colorless solid (28.0 mg, 40% yield for 2 steps): ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 8.93 (brs, 1H), 8.28 (d, *J* = 7.6 Hz, 1H), 8.06–8.01 (m, 4H), 7.91 (dd, *J* = 8.4, 1.6 Hz, 2H), 7.87 (d, *J* = 7.2 Hz, 2H), 7.73 (dd, *J* = 8.0, 1.6 Hz, 2H), 7.68 (brs, 1H), 7.60–7.53 (m, 2H), 7.53–7.37 (m, 9H), 7.32 (t, *J* = 8.0 Hz, 2H), 7.19 (t, *J* = 8.0 Hz, 2H), 6.03 (d, *J* = 3.2 Hz, 1H), 5.86 (d, *J* = 3.6 Hz, 1H), 5.76 (dd, *J* = 10.4, 8.0 Hz, 1H), 5.69 (dd, *J* = 10.4, 3.6 Hz, 1H), 5.54 (brs, 1H), 4.92 (d, *J* = 7.6 Hz, 1H), 4.78 (dd, *J* = 11.6, 6.4 Hz, 1H), 4.48 (dd, *J* = 11.2, 6.4 Hz, 1H), 4.43–4.35 (m, 3H), 4.14 (t, *J* = 4.4 Hz, 1H), 4.10 (d, *J* = 3.6 Hz, 1H), 3.81 (dd, *J* = 11.6, 2.4 Hz, 1H), 3.66 (brs, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 166.1, 165.6, 165.5, 165.3, 162.6, 144.7, 133.6, 133.4, 133.4, 133.1, 132.9, 129.9, 129.8, 129.8, 129.7, 129.4, 128.9, 128.8, 128.6, 128.5, 128.4, 128.3, 127.7, 101.7, 97.1, 93.1, 84.8, 76.4, 71.8, 71.3, 71.2, 69.6, 68.7, 68.1, 61.9 ppm; IR (ATR): ν = 3320, 3066, 2930, 1724, 1645, 1603, 1556, 1481, 1452, 1379, 1315, 1248, 1177, 1092, 1066, 1025, 1002, 938, 899, 859, 802, 787, 756, 704, 685, 616 cm^{-1}; HRMS (FAB+): *m/z* calcd. for $[M+H]^+$, $C_{50}H_{44}N_3O_{15}$, 926.2772; found, 926.2773; Anal. Calcd. for $C_{50}H_{43}N_3O_{15}\cdot H_2O$: C, 63.62; H, 4.81; N, 4.45; found: C, 63.34; H, 4.71; N, 4.56; $[\alpha]_D^{25}$ = +46.6 ° (*c* = 1.0, $CHCl_3$).

5-Fluoro-5'-*O*-(2",3",4",6"-tetra-*O*-benzoyl-β-D-glucopyranosyl)uridine (β-44)

O-Glycosylation using **42** (80.4 mg, 114 μmol), **29** (20.0 mg, 76.2 μmol), **19c** (21.7 mg, 114 μmol), anhydrous 1,4-dioxane (760 μL), *p*-TolSCl (30.3 μL, 229 μmol), AgOTf (117.6 mg, 458 μmol), 4 Å molecular sieves (150 mg) and anhydrous EtCN (1.50 mL) was conducted according to the procedure used for the synthesis of β-**33**. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 1/0–30/1) to give β-**44** as a colorless solid (34.5 mg, 54% yield for 2 steps): ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ = 9.66 (brs, 1H), 8.09 (d, J = 6.6 Hz, 1H), 8.04-7.98 (m, 2H), 7.91 (d, J = 8.1 Hz, 4H), 7.87–7.82 (m, 2H), 7.56–7.24 (m, 12H), 5.97 (t, J = 9.9 Hz, 1H), 5.82 (d, J = 3.0 Hz, 1H), 5.69 (t, J = 9.9 Hz, 1H), 5.48 (dd, J = 10.2, 7.8 Hz, 1H), 4.90 (d, J = 8.1 Hz, 1H), 4.70 (dd, J = 12.0, 3.0 Hz, 1H), 4.55 (brs, 1H), 4.52 (dd, J = 12.0, 4.8 Hz, 1H), 4.33 (dd, J = 10.8, 2.1 Hz, 1H), 4.28–4.15 (m, 3H), 4.06 (s, 1H), 3.76 (d, J = 9.6 Hz, 1H), 3.31 (s, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 166.2, 165.8, 165.5, 165.0, 157.1 (d, $^2J_{CF}$ = 26.4 Hz), 149.8, 140.7 (d, $^1J_{CF}$ = 236.2 Hz), 133.7, 133.5, 133.4, 133.3, 129.9, 129.8, 129.7, 129.4, 128.7, 128.6, 128.5, 128.4, 128.4, 124.8 (d, $^2J_{CF}$ = 34.6 Hz), 100.8, 90.6, 83.9, 75.4, 72.7, 72.3, 71.9, 70.6, 69.6, 68.0, 62.8 ppm; ^{19}F NMR (376 MHz, $CDCl_3$, TFA): δ = −165.00 (s) ppm; IR (ATR): ν = 3426, 3072, 2953, 1716, 1602, 1585, 1493,1452, 1369, 1317, 1260, 1178, 1091, 1068, 1027, 1003, 936, 895, 855, 800, 758, 708, 687, 618 cm^{-1}; HRMS (FAB+): *m/z* calcd. for $[M+H]^+$, $C_{43}H_{38}FN_2O_{15}$, 841.2256; found, 841.2261; Anal. Calcd. for $C_{43}H_{37}FN_2O_{15}\cdot 1.5H_2O$: C, 59.52; H, 4.65; N, 3.23; found: C, 59.51; H, 4.47; N, 3.26; $[\alpha]_D^{25}$ = +8.39 ° (*c* = 1.0, $CHCl_3$).

2-Mercaptophenylboronic acid pinacol ester (51)

A mixture of thiophenol **50** (750 μL, 7.33 mmol) and TMEDA (2.4 mL, 16.1 mmol) were stirred at 0 °C, to which 1.6M *n*-BuLi in hexane (13.8 mL, 27.0 mmol) was added at the same temperature. The reaction mixture was stirred for 3 hr at 0 °C and was allowed to warm to room temperature. After stirring for 18 hr, the reaction mixture

became a white suspension. The white precipitate was collected by centrifugation, washed with anhydrous hexanes and dissolved in anhydrous THF (7.0 mL). The solution was cooled to -78 °C, to which $B(OMe)_3$ (1.1 mL, 9.53 mmol) was added. The reaction mixture was stirred at the same temperature for 3 hr and then allowed to warm to room temperature. After stirring for 19 hr, the reaction mixture was quenched with 10% aqueous H_2SO_4 in an ice bath and extracted with $CHCl_3$, washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dissolved in anhydrous THF (20.0 mL) at room temperture, to which Na_2SO_4 (3.00 g, 21.1 mmol) and pinacol (1.30 g, 11.0 mmol) were added at the same temperature. The reaction mixture was stirred at the same temperature. After stirring for 12 hr, the reaction mixture was filtered and concentrated under reduce pressure, the residue was diluted with $CHCl_3$, washed with H_2O, and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexanes/AcOEt = 100/1) to give **51** as a colorless liquid (1.18 g, 68% yield for 3 steps): ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ = 7.75 (dd, J = 7.2, 1.2 Hz, 1H), 7.25 (td, J = 6.8, 1.6 Hz, 1H), 7.22 (dd, J = 7.2, 1.2 Hz, 1H), 7.08 (td, J = 7.2, 1.6 Hz, 1H), 5.20 (s, 1H), 1.37 (s, 12H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 140.5, 137.2, 131.3, 128.5, 123.9, 84.2, 24.9 ppm; ^{11}B NMR (128 MHz, $CDCl_3$, $BF_3{\cdot}OEt_2$): δ = 26.13 (brs) ppm; IR (ATR): ν = 3063, 2979, 2932, 2569, 1589, 1559, 1476, 1427, 1381, 1373, 1343, 1315, 1266, 1214, 1141, 1127, 1102, 1049, 1038, 963, 856, 830, 755, 735, 709, 670, 654, 580 cm^{-1}; HRMS (EI+): *m/z* calcd. for $[M]^+$, $C_{12}H_{17}{}^{10}BO_2S$, 235.1079; found, 235.1083.

2-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (53)

To a solution of **52** (1.10 g, 2.06 mmol) and **51** (729 mg, 3.09 mmol) in anhydrous CH_2Cl_2 (21.0 mL), $BF_3{\cdot}OEt_2$ (776 μL, 6.18 mmol) was added at 0 °C. The

reaction mixture was stirred at the same temperature for 30 min and allowed to warm to room temperature. After stirring for 2 hr, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ and diluted with AcOEt. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexanses/AcOEt = 20/1 to 8/1) to give **53** as a colorless syrup (1.11 g, 76% yield): ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ = 7.62 (dd, J = 7.5, 1.8 Hz, 1H), 7.50 (d, J = 7.2 Hz, 1H), 7.40-7.21 (m, 17H), 5.74 (d, J = 1.5 Hz, 1H), 4.95 (d, J = 11.1 Hz, 1H), 4.74 (d, J = 12.0 Hz, 1H), 4.68-4.56 (m, 4H), 4.41-4.24 (m, 3H), 4.05-3.94 (m, 3H), 2.00 (s, 3H), 1.33 (s, 12H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 170.8, 139.0, 138.2, 138.1, 138.1, 135.3, 132.2, 130.9, 128.4, 128.4, 128.3, 128.0, 127.8, 127.7, 127.5, 126.7, 85.2, 84.1, 80.2, 76.6, 75.2, 74.7, 72.0, 71.7, 70.9, 63.5, 24.9, 24.7, 20.8 ppm; ^{11}B NMR (128 MHz, $CDCl_3$, $BF_3{\cdot}OEt_2$): δ = 26.34 (brs) ppm; IR (ATR): ν = 3063, 3031, 2978, 2932, 2869, 1739, 1586, 1497, 1455, 1429, 1380, 1371, 1348, 1315, 1238, 1144, 1101, 1043, 1028, 962, 910, 857, 834, 735, 696, 669, 658, 604, 580 cm^{-1}; HRMS (FAB+): *m/z* calcd. for $[M+Na]^+$, $C_{41}H_{47}{}^{10}BO_8SNa$, 732.3019; found, 732.3018; $[\alpha]_D^{23}$ = +84.6 ° (*c* = 1.0, $CHCl_3$).

Potassium [2-(6-*O*-Acetyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)thiophenyl] trifluoroborate (54)

To a solution of **53** (165 mg, 0.232 mmol) in MeOH (1.0 mL), 7.0 M aqueous KHF_2 (331 μL, 2.32 mmol) was added at room temperature. After stirring at the same temperature for 15 min, the reaction mixture was concentrated under reduced pressure, diluted with hot acetone and filtered through Celite. The solution was concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/acetone = 5/1 to 1/1) to give **54** as a colorless syrup (91.3 mg, 57% yield): ^{1}H NMR (300 MHz, acetone-d_6, TMS): δ = 7.64 (dd, J = 6.6, 1.8 Hz, 1H), 7.46-7.24 (m, 16H), 7.11-7.01 (m,2H), 5.84 (s, 1H), 4.95 (d, J = 11.4 Hz, 1H), 4.76 (d, J = 12.3 Hz, 1H),

4.71-4.60 (m, 3H), 4.57 (d, J = 11.7Hz, 1H), 4.45-4.36 (m, 1H), 4.33-4.22 (m, 3H), 3.96 (dd, J = 9.3, 3.0 Hz, 1H), 3.91 (d, J = 9.6 Hz, 1H), 1.98 (s, 3H) ppm; ^{13}C NMR (100 MHz, aceton-d_6, TMS): δ = 170.9, 139.6, 139.6, 139.5, 138.0, 133.4, 128.9, 128.9, 128.8, 128.8, 128.6, 128.6, 128.5, 128.4, 128.4, 128.2, 128.1, 128.0, 127.9, 127.3, 126.7, 86.1, 81.2, 77.2, 75.6, 75.2, 71.9, 71.5, 71.4, 64.1, 55.2, 31.9, 20.7 ppm; ^{11}B NMR (128 MHz, acetone-d_6, $BF_3{\cdot}OEt_2$): δ = 4.20 (brs) ppm; ^{19}F NMR (376 MHz, aceton-d_6, TFA): δ = −140.99 (s) ppm; IR (ATR): ν = 3475, 3032, 2873, 1737, 1585, 1559, 1497, 1455, 1428, 1367, 1238, 1191, 1091, 1073, 1023, 947, 735, 696, 671, 606 cm^{-1}; HRMS (ESI−): *m/z* calcd. for $[M-K]^-$, $C_{35}H_{35}{}^{10}BO_6F_3S^-$, 650.2241; found, 650.2238; $[\alpha]_D^{22}$ = +72.3 ° (c = 1.0, acetone).

2-Boronophenyl 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (55)

To a solution of **54** (105 mg, 0.152 mmol) in MeCN (1.5 mL), H_2O (10.9 μL, 0.605 mmol) and TMSCl (76.8 μL, 0.605 mmol) were added at room temperature. After stirring at the same temperature for 1.5 hr, the reaction mixture was diluted with H_2O, extracted with $CHCl_3$, washed brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexanes/AcOEt = 3/1) to give **55** as a colorless syrup (95.1 mg, 99%.): ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ = 8.10-7.76 (m, 1H), 7.46-7.27 (m, 18H), 5.82 (s, 2H), 5.33 (d, J = 1.8 Hz, 1H), 4.92 (d, J = 11.1 Hz, 1H), 4.69-4.52 (m, 5H), 4.33 (d, J = 3.6 Hz, 2H), 4.27-4.18 (m, 1H), 3.98-3.81 (m, 3H), 2.03 (s, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 170.7, 137.9, 137.7, 137.4, 137.0, 136.4, 135.5, 130.8, 128.4, 128.4, 128.4, 128.0, 127.9, 127.9, 127.8, 87.9, 79.4, 75.9, 75.1, 74.3, 72.0, 71.8, 71.8, 63.2, 58.7, 20.7, 17.1 ppm; ^{11}B NMR (128 MHz, $CDCl_3$, $BF_3{\cdot}OEt_2$): δ = 29.53 (brs) ppm; IR (ATR): ν = 3414, 3064, 3031, 2871, 1740, 1585, 1559, 1497, 1455, 1432, 1368, 1338, 1313, 1239, 1090, 1026, 910, 866, 846, 738, 696, 665, 646, 605 cm^{-1}; HRMS (ESI−): calcd for

$[M+Cl]^-$, $C_{35}H_{37}{}^{10}BO_8S^{35}Cl^-$, 662.2033; found, 662.2031; $[\alpha]_D^{24}$ = +84.5 ° (*c* = 1.0, $CHCl_3$).

4-Boronophenyl 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-1-thio-α/β-D-mannopyranoside (56)

S-Glycosylation using **52** (400 mg, 0.748 mmol), 4-mercaptophenylboronic acid (231 mg, 1.50 mmol), $BF_3{\cdot}OEt_2$ (500 μL, 3.74 mmol), anhydrous CH_2Cl_2 (4.5 mL), and anhydrous MeCN (3.0 mL) was conducted according to the procedure used for the synthesis of **53**. The residue was purified by silica gel column chromatography (hexanses/AcOEt = 4/1 to 2/1) to give **56** as a colorless syrup (186.6 mg, 40% yield, α/β = 1/1.0): ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ = 8.09 (d, *J* = 8.1 Hz, 1H), 7.65 (d, *J* = 8.1 Hz, 0.5H), 7.54 (d, *J* = 7.8 Hz, 1H), 7.44 (d, *J* = 8.1 Hz, 0.5H), 7.68-7.27 (m, 16H), 5.73 (d, *J* = 0.9 Hz, 0.5H), 5.65 (d, *J* = 1.5 Hz, 0.5H), 4.95 (dd, *J* = 11.1, 3.6 Hz, 1H), 4.80-4.54 (m, 6H), 4.45-4.21 (m, 4H), 4.00 (m, 2H), 3.88 (td, *J* = 9.0, 3.0 Hz, 1H), 2.04 (s, 1.5H), 2.02 (s, 1.5H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ = 171.0, 170.9, 140.2, 138.0, 137.9, 137.7, 137.1, 136.0, 134.4, 133.9, 130.0, 130.0, 129.4, 128.8, 128.5, 128.5, 128.4, 128.2, 128.2, 128.1, 127.9, 127.9, 127.9, 127.8, 127.6, 85.0, 84.7, 80.2, 80.1, 77.2, 76.1, 76.0, 75.2, 74.5, 74.4, 72.2, 72.1, 72.0, 71.2, 71.0, 63.4, 63.3, 58.8, 58.5, 23.3, 20.9, 20.9, 18.4, 17.2 ppm; ^{11}B NMR (128 MHz, $CDCl_3$, $BF_3{\cdot}OEt_2$): δ = 29.75 (brs) ppm; IR (ATR): ν = 3443, 3065, 3031, 2871, 1739, 1658, 1593, 1548, 1497, 1455, 1396, 1365, 1342, 1317, 1240, 1087, 1041, 1016, 910, 829, 734, 696, 666, 645, 630, 604 cm^{-1}; HRMS (ESI−): *m/z* calcd. for $[M+Cl]^-$, $C_{35}H_{37}{}^{10}BO_8S^{35}Cl^-$, 662.2033; found, 662.2036.

***O*-Glycosylation using glycosyl donors containing boronic acid on leaving group (Entries 1–4 in Table 2-5)**

A mixture of **55** or **56** (30.2 mg, 48.0 μmol) and **18** (7.8 mg, 31.9 μmol) or **22** (8.6 mg, 32.2 μmol) was co-evaporated with anhydrous pyridine (three times) and anhydrous 1,4-dioxane (three times) and dissolved in anhydrous 1,4-dioxane (320 μL).

This reaction mixture was stirred under reflux conditions for 1 hr and concentrated under reduced pressure. *O*-glycosylation and acetylation were conducted according to the procedure used for the synthesis of **20** or **23** using *p*-TolSCl (12.7 μL, 96.1 μmol), AgOTf (49.5 mg, 193 μmol), 3 Å molecular sieves (64 mg), anhydrous MeCN (640 μL), anhydrous pyridine (200 μL), Ac_2O (10.0 equiv. based on the crude compound) and DMAP (catalytic amount).

5'-*O*-(2",3",4"-Tri-*O*-benzyl-α/β-D-mannopyranosyl)uridine (57)

To a solution of **20** (25.2 mg, 31.4 μmol, α/β = 1.6/1) in THF (400 μL), 1 M aqueous LiOH was added at room temperature. After stirring for 2 hr, the reaction mixture was neutralized with 0.1 M aqueous HCl, extracted with $CHCl_3$, washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 20/1) to give α-**57** as a colorless solid (12.1 mg, 57% yield) and β-**57** as a colorless solid (7.5 mg, 35% yield): α-**57**; ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ = 10.11 (s, 1H), 7.39 (d, J = 8.4 Hz, 1H), 7.36–7.17 (m, 15H), 5.74 (d, J = 2.1 Hz, 1H), 5.43 (d, J = 8.4 Hz, 1H), 4.91 (s, 1H), 4.87 (t, J = 6.3 Hz, 2H), 4.75 (d, J = 12.0 Hz, 1H), 4.67 (d, J = 9.0 Hz, 1H), 4.64–4.51 (m, 3H), 4.20 (s, 1H), 4.13 (s, 1H), 4.02 (s, 2H), 3.90–3.74 (m, 4H), 3.73–3.56 (m, 4H), 3.00 (s, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 163.7, 151.1, 139.7, 138.0, 137.8, 128.5, 128.4, 128.4, 128.3, 127.9, 127.8, 127.7, 127.6, 102.3, 98.3 ($C_{1"}$, $^1J_{CH}$ = 169.4 Hz), 90.1, 82.6, 79.7, 75.1, 75.0, 74.8, 74.7, 73.1, 73.0, 72.4, 69.9, 66.6, 62.3 ppm; IR (ATR): ν = 3384, 3064, 3032, 2924, 2879, 1683, 1497, 1455, 1389, 1364, 1321, 1269, 1210, 1068, 1027, 909, 864, 845, 810, 735, 697 cm^{-1}; HRMS (FAB+): *m/z* calcd. for $[M+H]^+$, $C_{36}H_{41}N_2O_{11}$, 677.2710; found, 677.2709; Anal. Calcd. for $C_{36}H_{40}N_2O_{11}\cdot H_2O$: C, 62.24; H, 6.09; N, 4.03; found: C, 62.36; H, 6.01; N, 4.13; $[\alpha]_D^{25}$ = +42.7 ° (c = 0.2, $CHCl_3$); β-**57**; ^{1}H NMR (300 MHz, CD_3OD, TMS): δ = 7.96 (d, J = 8.1 Hz, 1H), 7.41–7.20 (m, 15H), 5.96 (d, J = 5.4 Hz, 1H), 5.34 (d, J = 8.1 Hz, 1H), 4.92–4.84 (m, 2H),

4.75–4.55 (m, 5H), 4.28 (t, J = 5.7 Hz, 1H), 4.22 (dd, J = 5.1, 3.3 Hz, 1H), 4.18–4.10 (m, 2H), 4.08 (d, J = 2.1 Hz, 1H), 3.86–3.73 (m, 3H), 3.67 (dd, J = 9.6, 2.7 Hz, 1H), 3.60 (dd, J = 11.7, 6.3 Hz, 1H), 3.35–3.26 (m, 1H) ppm; ^{13}C NMR (100 MHz, CD_3OD, TMS): δ = 166.1, 152.6, 143.4, 139.8, 139.7, 129.7, 129.4, 129.3, 129.3, 128.9, 128.8, 128.7, 103.0, 101.8 ($C_{1''}$, $^1J_{CH}$ = 156.1 Hz), 89.9, 85.2, 84.1, 77.9, 76.9, 76.2, 76.0, 75.8, 75.6, 73.0, 72.2, 70.0, 62.8 ppm; IR (ATR): ν = 3387, 3063, 3032, 2926, 2874, 1673, 1498, 1456, 1401, 1364, 1316, 1274, 1249, 1211, 1179, 1072, 1027, 906, 866, 811, 786, 736, 696 cm^{-1}; HRMS (FAB+): *m/z* calcd. for $[M+H]^+$, $C_{36}H_{41}N_2O_{11}$, 677.2710; found, 677.2709; Anal. Calcd. for $C_{36}H_{40}N_2O_{11}\cdot H_2O$: C, 62.24; H, 6.09; N, 4.03; found: C, 62.29; H, 5.86; N, 4.20; $[\alpha]_D^{23}$ = −67.6 ° (c = 0.5, CH_3OH).

5'-*O*-(α/β-D-Mannopyranosyl)uridine (58)

A mixture of α-**57** (19.2 mg, 28.4 μmol), 10% Pd/C (19.0 mg) in MeOH (540 μL) was vigorously stirred for 22 hr at room temperature under a H_2 atmosphere. The mixture was filtered through Celite with MeOH and H_2O, and then, the filtrate was concentrated under reduced pressure to give α-**58** as a colorless solid (11.4 mg, 99% yield): α-**58**; ^{1}H NMR (300 MHz, D_2O, TSP): δ = 7.89 (d, J = 8.1 Hz, 1H), 5.96–5.87 (m, 2H), 4.96 (s, 1H), 4.33 (s, 3H), 4.06–3.96 (m, 2H), 3.90 (t, J = 10.5 Hz, 2H), 3.57–3.84 (m, 4H) ppm; ^{13}C NMR (75 MHz, D_2O, 1,4-dioxane): δ = 166.8, 152.1, 142.0, 102.5, 100.3, 90.4, 82.9, 74.6, 73.6, 71.2, 70.6, 69.8, 67.2, 66.3, 61.5 ppm; IR (ATR): ν = 3289, 2935, 2502, 1666, 1466, 1397, 1273, 1199, 1129, 1104, 1050, 1025, 912, 868, 810, 801, 765, 720, 676, 622 cm^{-1}; HRMS (FAB+): *m/z* calcd. for $[M+Na]^+$, $C_{15}H_{22}N_2O_{11}Na$, 429.1121; found, 429.1118; Anal. Calcd. for $C_{15}H_{22}N_2O_{11}\cdot 2.75H_2O$: C, 39.52; H, 6.08; N, 6.14; found: C, 39.58; H, 5.93; N, 5.81; $[\alpha]_D^{24}$ = +29.3 ° (c = 0.8, H_2O).

Cleavage of benzyl groups using β-**57** (17.8 mg, 26.3 μmol), 10% Pd/C (18.0 mg) and MeOH (500 μL) was conducted according to the procedure for synthesis of α-**58** to give the β-**58** as a colorless solid (10.5 mg, 98% yield): β-**58**; ^{1}H NMR (300 MHz,

D_2O, TSP): δ = 8.05 (d, J = 8.1 Hz, 1H), 5.96 (d, J = 4.2 Hz, 1H), 5.89 (d, J = 8.1 Hz, 1H), 4.74 (s, 1H), 4.49–4.12 (m, 4H), 4.05 (s, 1H), 3.95 (d, J = 12.3 Hz, 1H), 3.88 (d, J = 11.7 Hz, 1H), 3.75 (dd, J = 11.7, 6.6 Hz, 1H), 3.70–3.52 (m, 2H), 3.40 (t, J = 6.6 Hz, 1H) ppm; ^{13}C NMR (75 MHz, D_2O, 1,4-dioxane): δ = 167.8, 153.0, 142.7, 103.0, 100.9, 89.7, 83.6, 76.9, 74.4, 73.5, 70.9, 70.4, 69.0, 67.5, 61.7 ppm; IR (ATR): ν = 3288, 2933, 2503, 1670, 1510, 1465, 1390, 1266, 1133, 1053, 1023, 879, 815, 790, 764, 714, 632, 616 cm^{-1}; HRMS (FAB+): *m/z* calcd. for $[M+Na]^+$, $C_{15}H_{22}N_2O_{11}Na$, 429.1121; found, 429.1119; Anal. Calcd. for $C_{15}H_{22}N_2O_{11}\cdot 2.6H_2O$: C, 39.76; H, 6.05; N, 6.18; found: C, 40.15; H, 6.00; N, 5.80; $[\alpha]_D^{25}$ = −10.6 ° (c = 0.8, H_2O).

5-Fluoro-5'-*O*-(β-D-galactopyranosyl)uridine (β-59)

A mixture of β-**39** (25.2 mg, 30.0 μmol) and 10 M $MeNH_2$ in MeOH (2.0 mL) was stirred at 0 °C for 2 hr and then allowed to warm to room temperature. After stirring for 13 hr, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in H_2O, and the *N*-methylbenzamide was removed by successive washing of the aqueous phase with CH_2Cl_2. The aqueous layer was concentrated under reduced pressure. The residue was purified by preparative HPLC (H_2O (0.1%TFA)) to give β-**59** as a colorless amorphous solid (7.9 mg, 62% yield): ^{1}H NMR (300 MHz, D_2O, TSP): δ = 8.18 (d, J = 6.6 Hz, 1H), 5.94 (d, J = 1.8 Hz, 1H), 4.51 (d, J = 7.2 Hz, 1H), 4.48–4.24 (m, 4H), 3.98–3.86 (m, 2H), 3.86–3.77 (m, 2H), 3.77–3.69 (m, 1H), 3.69–3.58 (m, 2H) ppm; ^{13}C NMR (100 MHz, D_2O, 1,4-dioxane): δ = 160.1 (d, $^2J_{CF}$ = 26.4 Hz), 150.8, 141.4 (d, $^1J_{CF}$ = 232.1 Hz), 126.3 (d, $^2J_{CF}$ = 38.1 Hz), 103.7, 89.9, 83.6, 75.9, 74.4, 73.4, 71.5, 69.9, 69.2, 68.9, 61.6 ppm; ^{19}F NMR (376 MHz, D_2O, TFA): δ = −166.73 (s) ppm; IR (ATR): ν = 3357, 3075, 2935, 2827, 1661, 1477, 1398, 1365, 1258, 1202, 1035, 952, 921, 890, 843, 793, 750, 722, 697 cm^{-1}; HRMS (FAB+): *m/z* calcd. for $[M+Na]^+$, $C_{15}H_{21}FN_2O_{11}Na$, 447.1027; found, 447.1030; $[\alpha]_D^{25}$ = +17.6 ° (c = 0.3, H_2O).

5-Fluoro-5'-*O*-(β-D-glucopyranosyl)uridine (β-60)

Cleavage of benzoyl groups using β-**44** (12.5 mg, 14.8 μmol) and 10 M $MeNH_2$ in MeOH (2.0 mL) was conducted according to the procedure for synthesis of β-**59** to give the β-**60** as a colorless amorphous solid (5.9 mg, 79% yield): β-**60**; ^{1}H NMR (400 MHz, D_2O, TSP): δ = 8.26 (d, J = 6.8 Hz, 1H), 5.94 (dd, J = 3.4, 1.6 Hz, 1H), 4.57 (d, J = 7.6 Hz, 1H), 4.42–4.35 (m, 2H), 4.34–4.26 (m, 2H), 3.98–3.90 (m, 2H), 3.71 (dd, J = 12.4, 5.6 Hz,1H), 3.57–3.46 (m, 2H), 3.37 (dd, J = 18.4, 9.6 Hz, 2H) ppm; ^{13}C NMR (150 MHz, D_2O, 1,4-dioxane): δ = 163.5, 160.0 (d, $^2J_{CF}$ = 26.0 Hz), 150.8, 141.3 (d, $^1J_{CF}$ = 232.3 Hz), 126.4 (d, $^2J_{CF}$ = 34.8 Hz), 102.7, 89.9, 83.7, 76.6, 76.4, 74.6, 74.0, 70.4, 70.0, 68.5, 61.6 ppm; ^{19}F NMR (376 MHz, D_2O, TFA): δ = −167.12 (s) ppm; IR (ATR): ν = 3346, 3077, 2976, 2927, 1665, 1474, 1428, 1398, 1363, 1257, 1198, 1100, 1071, 1036, 950, 919, 840, 798, 751, 721, 699 cm^{-1}; HRMS (FAB+): *m/z* calcd. for $[M+Na]^+$, $C_{15}H_{21}FN_2O_{11}Na$, 447.1027; found, 447.1028; $[\alpha]_D^{23}$ = +13.8 ° (*c* = 0.2, H_2O).

^{1}H, ^{11}B and ^{19}F NMR measurements of mixtures of uridine (18) and boronic acid (19c) (Figure 2-3)

A mixture of **18** (34.3 mg, 140 μmol) and **19c** (40.0 mg, 211 μmol) was co-evaporated with anhydrous pyridine (three times) and anhydrous 1,4-dioxane (three times). The resulting residue was dissolved in anhydrous 1,4-dioxane (1.40 mL) and then stirred under reflux conditions for 1 hr. The reaction mixture (140 μL) was separated and concentrated under reduced pressure. The residue **61** dissolved in CD_3CN (640 μL) was measured by a ^{1}H, ^{11}B and ^{19}F NMR spectrometers. **19c** was treated under the same conditions as were used to prepare **63** for the ^{11}B and ^{19}F NMR measurements.

Cell cultures

HeLa S3 cells were cultured in Minimum Essential Medium (MEM) (D-glucose: 1.0 g/L) with 10% heat-inactivated fetal calf serum (FCS), L-glutamine,

penicillin/streptomycin in a humidified 5% CO_2 incubator at 37 ºC. A549 cells were grown in Dulbecco's Modified Eagle' Medium (DMEM) (D-glucose: 1.0 g/L) supplemented with 10% FCS, L-glutamine, and penicillin/streptomycin under 5% CO_2 at 37 ºC.

Evaluation of cytotoxicity (MTT assay) [218]

HeLa S3 and A549 cells (1×10^5 cells/mL) were incubated in 10% FCS MEM (D-glucose: 1.0 g/L) or DMEM (D-glucose: 1.0 g/L) containing solution of β-**59**, β-**60**, 5-fluorouracil **66** (Tokyo Chemical Industry, Japan) and 5-fluorouridine **29** (Tokyo Chemical Industry, Japan) (0~250 μM) under 5% CO_2 at 37 ºC for 48 hr on 96 well plates (BD Falcon), 0.5% MTT (3-(4,5-dimthyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide) reagent in PBS (10 μL) was then added to the cells. After incubation for 4 hr with MTT reagent, a formazan lysis solution (10% SDS in 0.01 N aqueous HCl) (100 μL) was added, and the resulting solution was incubated under the same conditions overnight. The absorbance at λ = 570 nm was then measured with a microplate reader (Bio-Rad).

Experimental procedures in Chapter 3

4-Metylphenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (80)

To a solution of penta-*O*-acetyl-β-D-galactopyranose **79** (2.50 g, 6.40 mmol) and *p*-toluenethiol (1.8 g, 14.5 mmol) in CH_2Cl_2 (60 mL), $BF_3{\cdot}OEt_2$ (2.41 mL, 19.2 mmol) was added at 0 °C. The reaction mixture was stirred at 0 °C for 30 min and then allowed to warm to room temperature. After stirring for 1.5 hr, the reaction was quenched with saturated aqueous $NaHCO_3$ and the resulting solution was diluted with $CHCl_3$. The solution was washed with saturated aqueous $NaHCO_3$. The aqueous layer was extracted with $CHCl_3$. The combined organic layer was then washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by precipitation from hexanes/AcOEt and filtered to afford **80** as a colorless solid

(2.60 g, 89% yield). Spectroscopic data for the product were identical to previously reported data [219].

4-Metylphenyl 1-thio-β-D-galactopyranoside (81)

To a solution of compound **80** (2.50 g, 5.50 mmol) in CH_2Cl_2/MeOH (1/3) (40 ml), sodium methoxide (26.0 mg, 0.48 mmol) was added at 0 °C. The reaction mixture was stirred at 0 °C for 30 min and allowed to warm to room temperature. After stirring for 10.5 hr, the reaction was neutralized with DOWEX™ 50WX2 (H^+ form), filtered and concentrated. The residue was purified by precipitation from Et_2O/MeOH and filtered to afford **81** as a colorless solid (1.44 g, 91% yield). Spectroscopic data for the product were identical to previously reported data [220].

4-Metylphenyl 6-*O*-trityl-1-thio-β-D-galactopyranoside (82)

To a solution of compound **81** (1.10 g, 3.84 mmol) in pyridine (4.3 ml), 4-dimethylaminopyridine (264 mg, 2.16 mmol) and trityl chloride (2.17 g, 7.79 mmol) were added. The reaction mixture was stirred at 80 °C for 19 hr. Trityl chloride (845 mg, 3.03 mmol) and pyridine (2.0 mL) were then added and the reaction mixture was stirred for 6 hr to complete the reaction. The reaction mixture was co-evaporated with toluene to remove pyridine. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 100/1 –20/1) to afford **82** as a colorless solid (1.91 g, 94% yield): ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 7.52–7.941 (m, 8H, Ar), 7.32–7.26 (m, 5H, Ar), 7.25–7.19 (m, 4H, Ar), 7.08–7.03 (m, 2H, Ar), 4.44 (d, J = 9.6 Hz, 1H, H-1), 3.90 (s, 1H, H-4), 3.63 (t, J = 9.6 Hz, 1H, H-2), 3.55–3.44 (m, 3H, H-3, H-5, H-6), 3.33 (brs, 1H, OH), 3.33 (dd, J = 9.4, 2.7 Hz, 1H, H-6), 3.09 (brs, 1H, OH), 2.83 (brs, 1H, OH), 2.31 (s, 3H, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 143.6 (Ar), 138.1 (Ar), 132.9 (Ar), 129.8 (Ar), 128.6 (Ar), 127.9 (Ar), 127.1 (Ar), 88.8 (C-1), 87.0 (*C*Ph_3), 77.6 (C-5), 74.8 (C-3), 69.9 (C-2), 69.6 (C-4), 63.5 (C-6), 21.2 (CH_3) ppm; IR (ATR): ν = 3375, 3056,

3022, 2921, 2874, 1596, 1490, 1447, 1398, 1364, 1279, 1217, 1184, 1147, 1059, 1029, 984, 899, 870, 831, 807, 745, 702, 631 cm^{-1}; HRMS (FAB+): *m*/*z* calcd. for $[M+Na]^+$, $C_{32}H_{32}O_5SNa$, 551.1868; found, 551.1868; $[\alpha]_D^{24} = -8.1$ º (*c* = 1.0, $CHCl_3$).

4-Metylphenyl 2,3,4-tri-*O*-benzoyl-6-*O*-trityl-1-thio-β-D-galactopyranoside (83)

To a solution of compound **82** (1.00 g, 1.89 mmol) in pyridine (6.7 ml), 4-dimethylaminopyridine (24.6 mg, 0.20 mmol) and benzoyl chloride (1.17 mL, 10.1 mmol) were added at 0 °C. The reaction mixture was stirred at 0 °C for 30 min and allowed to warm to room temperature. After stirring for 11.5 hr, the reaction was quenched with H_2O and stirred for 10 hr. The reaction mixture was diluted with AcOEt and washed with 1 M aqueous HCl to remove pyridine and DMAP. The aqueous layer was extracted with AcOEt. The combined organic layer was then washed with saturated aqueous $NaHCO_3$ to remove benzoic acid resulted from hydrolysis of benzoyl chloride, followed by washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford **83** as an amorphous solid (1.67 g, quant). The compound **83** was used for a next reaction without further purification: ^1H NMR (400 MHz, $CDCl_3$, TMS): δ = 7.99–7.92 (m, 2H, Ar), 7.79–7.71 (m, 4H, Ar), 7.62–7.55 (m, 1H, Ar), 7.54–7.48 (m, 1H, Ar), 7.47–7.33 (m, 13H, Ar), 7.25–7.07 (m, 13H, Ar), 5.97 (d, *J* = 2.8 Hz, 1H, H-4), 5.60 (t, *J* = 9.6 Hz, 1H, H-2), 5.53 (dd, *J* = 9.6, 2.8 Hz, 1H, H-3), 4.89 (d, *J* = 9.6 Hz, 1H, H-1), 4.00 (t, *J* = 6.4 Hz, 1H, H-5), 3.52 (dd, *J* = 9.2, 6.0 Hz, 1H, H-6), 3.26 (dd, *J* = 9.6, 7.2 Hz, 1H, H-6), 2.38 (s, 3H, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 165.5 (C=O), 165.1 (C=O), 165.1 (C=O), 143.4 (Ar), 138.5 (Ar), 134.2 (Ar), 133.2 (Ar), 133.1 (Ar), 133.1 (Ar), 129.9 (Ar), 129.8 (Ar), 129.6 (Ar), 129.4 (Ar), 129.3 (Ar), 129.0 (Ar), 128.6 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 127.8 (Ar), 127.6 (Ar), 127.0 (Ar), 87.0 (*C*Ph_3), 86.1 (C-1), 76.5 (C-5), 73.3 (C-3), 68.4 (C-4), 68.0 (C-2), 61.4 (C-6), 21.4 (CH_3) ppm; IR (ATR): ν = 3060, 3032, 2937, 2871, 1786, 1726, 1600, 1584, 1490, 1449, 1314, 1278, 1258, 1212, 1176, 1141, 1090, 1067, 1025, 996, 900, 806, 763,

746, 702, 631 cm^{-1}; HRMS (FAB+): *m*/*z* calcd. for $[M+Na]^+$, $C_{53}H_{44}O_8SNa$, 863.2655; found, 863.2656; $[\alpha]_D^{24}$ = +85.2 º (*c* = 1.0, $CHCl_3$).

4-Metylphenyl 2,3,4-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside (84)

To a solution of **83** (506 mg, 0.60 mmol) and triisopropylsilane (620 μL, 3.01 mmol) in CH_2Cl_2 (10.5 mL), trifluoroacetic acid (340 μL, 4.44 mmol) was added at 0 °C. After stirring for 25 min at the same temperature, the reaction was quenched with saturated aqueous $NaHCO_3$ and the resulting solution was diluted with $CHCl_3$. The solution was washed with saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexanes/AcOEt = 5/1 –3/1) to afford **84** as a colorless amorphous solid (332 mg, 92% yield): ^1H NMR (400 MHz, $CDCl_3$, TMS): δ = 8.04–7.95 (m, 2H, Ar), 7.94–7.87 (m, 2H, Ar), 7.82–7.73 (m, 2H, Ar), 7.65–7.58 (m, 1H, Ar), 7.56–7.50 (m, 1H, Ar), 7.48–7.36 (m, 7H, Ar), 7.25–7.19 (m, 2H, Ar), 7.17–7.11 (m, 2H, Ar), 5.83 (d, *J* = 3.2 Hz, 1H, H-4), 5.77 (t, *J* = 9.6 Hz, 1H, H-2), 5.57 (dd, *J* = 10.0, 3.2 Hz, 1H, H-3), 4.96 (d, *J* = 10.0 Hz, 1H, H-1), 4.08 (t, *J* = 6.4 Hz, 1H, H-5), 3.90–3.79 (m, 1H, H-6), 3.66–3.56 (m, 1H, H-6), 2.60 (t, *J* = 7.2 Hz, 1H, OH) 2.38 (s, 3H, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ= 166.5 (C=O), 165.5 (C=O), 165.2 (C=O), 138.8 (Ar), 134.5 (Ar), 133.8 (Ar), 133.3 (Ar), 133.3 (Ar), 130.1 (Ar), 129.8 (Ar), 129.8 (Ar), 129.7 (Ar), 129.3 (Ar), 128.7 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 127.0 (Ar), 85.8 (C-1), 77.8 (C-5), 73.2 (C-3), 69.0 (C-4), 68.0 (C-2), 60.8 (C-6), 21.4 (CH_3) ppm; IR (ATR): ν = 3503, 3063, 3033, 2941, 2867, 1722, 1601, 1584, 1492, 1450, 1314, 1278, 1258, 1177, 1091, 1066, 1025, 1001, 936, 908, 877, 856, 805, 752, 703, 684 cm^{-1}; HRMS (FAB+): *m*/*z* calcd. for $[M+H]^+$, $C_{34}H_{31}O_8S$, 599.1740; found, 599.1739; $[\alpha]_D^{24}$ = +121.6 º (*c* = 1.0, $CHCl_3$). Spectroscopic data for the product were identical to previously reported data [221].

General procedure of polyglycosylation of monothioglycosides

Glycosylation of thiogalactoside 84 (entry 1 in Table 3-1)

A mixture of thiogalactoside **84** (24.0 mg, 0.040 mmol), activated 4 Å molecular sieves (400 mg) and anhydrous CH_2Cl_2 (4.0 mL) was stirred at room temperature for 30 min and then cooled to −40 °C, to which NIS (27.0 mg, 0.12 mmol) and TMSOTf (11.0 μL, 0.061 mmol) were added at the same temperature. After stirring for 2.5 hr at −40 °C, H_2O (1.0 mL) was added to the reaction mixture followed by stirring for 30 min at room temperature. The reaction mixture was diluted with $CHCl_3$ and filtered through a Celite pad. The organic layer was washed with 10w/v% aqueous $Na_2S_2O_3$, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The remaining residue was purified by silica gel column chromatography (hexanes/AcOEt = 10/1–1/1) to give a crude compound as a colorless solid (21.0 mg). To the solution of this crude compound in anhydrous pyridine (400 μL), Ac_2O (50.0 μL, 0.53 mmol) and DMAP (0.3 mg, 0.0025 mmol) were added at 0 °C. The reaction mixture was stirred at the same temperature for 30 min and then allowed to warm to room temperature. After stirring overnight, the reaction mixture was diluted with $CHCl_3$, washed with 1 M aqueous HCl, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by GPC (solvent: $CHCl_3$, flow rate: 3.6 mL/min, retention time (t_r): fraction 1: 36~47 min including cyclic tri~octa galactosides, fraction 2: 47~51 min including 1,6-anhydrogalactose) followed by PTLC for the GPC fraction 1 (hexanes/AcOEt = 2/1 for 5 times, the compounds were extracted with $CHCl_3$) to isolate 1,6-anhydrogalactose **85** (0.6 mg, 3% yield, R_f = 0.87 (hexanes/AcOEt = 2/1 once)), cyclic trigalactoside **86** (2.4 mg, 13% yield, R_f = 0.73 (hexanes/AcOEt = 2/1 for 5 times)), cyclic tetragalactoside **87** (4.3 mg, 23% yield, R_f = 0.58 (hexanes/AcOEt = 2/1 for 5 times)), cyclic pentagalactoside **88** (3.4 mg, 18% yield, R_f = 0.42 (hexanes/AcOEt = 2/1 for 5 times)), cyclic hexagalactoside

89 (1.0 mg, 5% yield, R_f = 0.29 (hexanes/AcOEt = 2/1 for 5 times)) and cyclic octagalactoside **90** (0.9 mg, 5% yield, R_f = 0.11 (hexanes/AcOEt = 2/1 for 5 times)).

Glycosylation of thioglucoside 91 (entry 3 in Table 3-5)

A mixture of thioglucoside **91** (60.0 mg, 0.10 mmol), activated 4 Å molecular sieves (100 mg) and anhydrous CH_2Cl_2 (1.0 mL) was stirred at room temperature for 30 min and then cooled to −40 °C, to which NIS (67.8 mg, 0.30 mmol) and TMSOTf (27.0 μL, 0.15 mmol) were added at the same temperature. After stirring for 2.5 hr at −40 °C, H_2O (1.0 mL) was added to the reaction mixture followed by stirring for 30 min at room temperature. The reaction mixture was diluted with $CHCl_3$ and filtered through a Celite pad. The organic layer was washed with 10w/v% aqueous $Na_2S_2O_3$, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The remaining residue was purified by silica gel column chromatography (hexanes/AcOEt = 10/1–1/1) to give a crude compound as a colorless syrup (63.3 mg). To the solution of this crude compound in anhydrous pyridine (1.0 mL), Ac_2O (189 μL, 2.00 mmol) and DMAP (1.2 mg, 0.0098 mmol) were added at 0 °C. The reaction mixture was stirred at the same temperature for 30 min and then allowed to warm to room temperature. After stirring overnight, the reaction mixture was diluted with $CHCl_3$, washed with 1 M aqueous HCl, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by GPC (solvent: $CHCl_3$, flow rate: 4.6 mL/min, t_r: fraction 1: 30~36 min including cyclic tri~hexa glucosides, fraction 2: 36~42 min including 1,6-anhydroglucose and cyclic diglucoside) followed by PTLC for GPC fraction 1 and 2 (hexanes/AcOEt = 3/2 for 5 times, then toluene/AcOEt = 5/1 twice regarding the GPC fraction 1 and hexanes/AcOEt = 3/1 twice regarding the GPC fraction 2, compounds were extracted with $CHCl_3$) to isolate cyclic 1,6-anhydroglucose **92** (7.2 mg, 15% yield, R_f = 0.40 (hexanes/AcOEt = 3/1 twice)), cyclic diglucoside **93** (3.2 mg, 7% yield, R_f = 0.20 (hexanes/AcOEt = 3/1 twice)), cyclic

triglucoside **94** (7.8 mg, 16% yield, R_f = 0.34 (toluene/AcOEt = 5/1 twice)), cyclic tetraglucoside **95** (10.3 mg, 22% yield, R_f = 0.57 (toluene/AcOEt = 5/1 twice)), cyclic pentaglucoside **96** (2.2 mg, 5% yield, R_f = 0.60 (toluene/AcOEt = 5/1 twice)), cyclic hexaglucoside **97** (1.5 mg, 3% yield, R_f = 0.51 (toluene/AcOEt = 5/1 twice)).

1,6-Anhydro-2,3,4- tri-*O*-benzoyl-β-D-galactopyranose (85)

Colorless syrup: spectroscopic data for the product were identical to previously reported data [222].

Cyclotris-(1→6)-(2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl) (86)

Colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 8.01–7.96 (m, 6H, Ar), 7.95–7.90 (m, 6H, Ar), 7.79–7.72 (m, 6H, Ar), 7.56–7.50 (m, 3H, Ar), 7.49–7.37 (m, 12H, Ar), 7.29–7.14 (m, 12H, Ar), 5.85 (dd, J = 3.2, 1.6 Hz, 3H, H-4), 5.75 (t, J = 9.2 Hz, 3H, H-2), 5.59 (dd, J = 10.0, 3.2 Hz, 3H, H-3), 5.12 (d, J = 7.2 Hz, 3H, H-1), 4.48 (dd, J = 12.0, 7.6 Hz, 3H, H-6), 4.27 (d, J = 7.6 Hz, 3H, H-5), 4.03 (d, J = 12.0 Hz, 3H, H-6) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 165.7 (C=O), 165.4 (C=O), 165.2 (C=O), 133.4 (Ar), 129.8 (Ar), 129.2 (Ar), 128.8 (Ar), 128.7 (Ar), 128.6 (Ar), 128.5 (Ar), 128.3 (Ar), 99.4 (C-1), 73.8 (C-5), 71.3 (C-3), 69.3 (C-4), 69.0 (C-2), 67.8 (C-6) ppm; IR (ATR): ν = 3064, 2935, 2876, 1723, 1601, 1584, 1492, 1451, 1315, 1255, 1176, 1089, 1066, 1025, 1001, 936, 854, 801, 753, 703, 685, 616 cm^{-1}; HRMS (ESI+): m/z calcd. for $[M+Na]^+$, $C_{81}H_{66}O_{24}Na$, 1445.3836; found, 1445.3812; $[\alpha]_D^{24}$ = +132.0 º (c = 1.0, $CHCl_3$).

Cyclotetrakis-(1→6)-(2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl) (87)

Colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 7.98–7.90 (m, 8H, Ar), 7.88–7.81 (m, 8H, Ar), 7.70–7.63 (m, 8H, Ar), 7.52–7.34 (m, 20H, Ar), 7.33–7.37–7.27 (m, 8H, Ar), 7.23–7.15 (m, 8H, Ar), 5.97 (d, J = 2.8 Hz, 4H, H-4), 5.76 (dd, J = 10.2, 8.0 Hz, 4H, H-2), 5.54 (dd, J = 10.4, 3.2 Hz, 4H, H-3), 4.86 (d, J = 8.0 Hz, 4H, H-1), 4.30–

4.18 (m, 8H, H-5, H-6), 4.04 (dd, J = 15.8, 7.6 Hz, 4H, H-6) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 165.3 (C=O), 165.3 (C=O), 165.2 (C=O), 133.4 (Ar), 133.3 (Ar), 133.1 (Ar), 129.8 (Ar), 129.7 (Ar), 129.2 (Ar), 128.9 (Ar), 128.8 (Ar), 128.5 (Ar), 128.1 (Ar), 101.2 (C-1), 74.8 (C-5), 72.1 (C-3), 68.5 (C-2), 68.3 (C-4), 65.9 (C-6) ppm; IR (ATR): ν = 3065, 2938, 2878, 1721, 1602, 1585, 1492, 1451, 1315, 1250, 1176, 1088, 1064, 1025, 1002, 936, 855, 801, 753, 701, 685, 616 cm^{-1}; HRMS (ESI+): m/z calcd. for $[M+Na]^+$, $C_{108}H_{88}O_{32}Na$, 1919.5151; found, 1919.5151; $[\alpha]_D^{24}$ = +177.4 º (c = 1.0, $CHCl_3$).

Cyclopentakis-(1→6)-(2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl) (88)

Colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 7.99–7.92 (m, 10H, Ar), 7.87–7.81 (m, 10H, Ar), 7.76–7.71 (m, 10H, Ar), 7.59–7.53 (m, 5H, Ar), 7.49–7.29 (m, 30H, Ar), 7.25–7.19 (m, 10H, Ar), 5.82 (d, J = 3.2 Hz, 5H, H-4), 5.66 (dd, J = 10.4, 8.0 Hz, 5H, H-2), 5.51 (dd, J = 10.2, 3.6 Hz, 5H, H-3), 4.85 (d, J = 8.0 Hz, 5H, H-1), 4.17 (t, J = 6.4 Hz, 5H, H-5), 3.94–3.84 (m, 10H, H-6) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 165.4 (C=O), 165.2 (C=O), 165.1 (C=O), 133.5 (Ar), 133.2 (Ar), 133.1 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7 (Ar), 129.2 (Ar), 128.9 (Ar), 128.6 (Ar), 128.3 (Ar), 128.2 (Ar), 100.5 (C-1), 72.7 (C-5), 71.6 (C-3), 69.2 (C-2), 67.9 (C-4), 64.8 (C-6) ppm; IR (ATR): ν = 3065, 2939, 2883, 1722, 1602, 1585, 1492, 1451, 1315, 1251, 1176, 1089, 1065, 1025, 1002, 935, 855, 801, 760, 703, 685, 616 cm^{-1}; HRMS (ESI+): m/z calcd. for $[M+Na]^+$, $C_{135}H_{110}O_{40}Na$, 2393.6466; found, 2393.6488; $[\alpha]_D^{25}$ = +118.1 º (c = 1.0, $CHCl_3$).

Cyclohexakis-(1→6)-(2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl) (89)

Colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 8.00–7.95 (m, 12H, Ar), 7.94–7.88 (m, 12H, Ar), 7.78–7.72 (m, 12H, Ar), 7.62–7.56 (m, 6H, Ar), 7.49–7.31 (m, 36H, Ar), 7.25–7.19 (m, 12H, Ar), 5.80 (d, J = 3.2 Hz, 6H, H-4), 5.67 (dd, J = 10.4, 8.0 Hz, 6H, H-2), 5.48 (dd, J = 10.4, 3.2 Hz, 6H, H-3), 4.81 (d, J = 8.0 Hz, 6H, H-1), 4.13 (t, J = 6.4 Hz, 6H, H-5), 3.90 (dd, J = 10.8, 6.0 Hz, 6H, H-6), 3.82 (dd, J = 10.8, 6.4 Hz, 6H,

H-6) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 165.3 (C=O), 165.3 (C=O), 165.0 (C=O), 133.7 (Ar), 133.2 (Ar), 133.1 (Ar), 130.0 (Ar), 129.8 (Ar), 129.8 (Ar), 129.3 (Ar), 129.1 (Ar), 129.0 (Ar), 128.5 (Ar), 128.4 (Ar), 128.2 (Ar), 101.5 (C-1), 72.9 (C-5), 71.6 (C-3), 69.5 (C-2), 67.9 (C-4), 66.7 (C-6) ppm; IR (ATR): ν = 3067, 2931, 2863, 1721, 1603, 1586, 1492, 1452, 1315, 1251, 1176, 1090, 1065, 1025, 1002, 935, 853, 801, 759, 703, 686, 617 cm^{-1}; HRMS (ESI+): *m*/*z* calcd. for $[M+2Na]^{2+}$, $C_{162}H_{132}O_{48}Na_2$, 1445.3836; found, 1445.3851; $[\alpha]_D^{26}$ = +122.6 º (*c* = 0.2, $CHCl_3$).

Cyclooctakis-(1→6)-(2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl) (90)

Colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 7.95–7.86 (m, 32H, Ar), 7.76–7.70 (m, 16H, Ar), 7.48–7.38 (m, 24H, Ar), 7.35–7.27 (m, 32H, Ar), 7.23–7.15 (m, 16H, Ar), 5.82 (d, *J* = 3.2 Hz, 8H, H-4), 5.59 (dd, *J* = 10.4, 8.0 Hz, 8H, H-2), 5.41 (dd, *J* = 10.2, 3.2 Hz, 8H, H-3), 4.62 (d, *J* = 7.6 Hz, 8H, H-1), 4.00–3.88 (m, 8H, H-5), 3.83–3.71 (m, 8H, H-6), 3.60-3.48 (m, 8H, H-6) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 165.3 (C=O), 165.3 (C=O), 165.1 (C=O), 133.2 (Ar), 130.0 (Ar), 129.8 (Ar), 129.3 (Ar), 129.3 (Ar), 128.9 (Ar), 128.4 (Ar), 128.2 (Ar), 101.2 (C-1), 72.6 (C-5), 71.7 (C-3), 69.5 (C-2), 67.9 (C-4), 66.7 (C-6) ppm; IR (ATR): ν = 3066, 2925, 2853, 1720, 1602, 1585, 1492, 1451, 1315, 1254, 1176, 1089, 1065, 1025, 1002, 935, 853, 801, 749, 703, 686, 617 cm^{-1}; HRMS (ESI+): *m*/*z* calcd. for $[M+2Na]^{2+}$, $C_{216}H_{176}O_{64}Na_2$, 1919.5151; found, 1919.5192; $[\alpha]_D^{26}$ = +79.5 º (*c* = 0.2, $CHCl_3$).

1,6-Anhydro-2,3,4- tri-*O*-benzoyl-β-D-glucopyranose (92)

Colorless solid: spectroscopic data for the product were identical to previously reported data [223].

Cyclobis-(1→6)-(2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl) (93)

Colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 8.26–8.19 (m, 4H, Ar), 8.13–8.08 (m, 4H, Ar), 8.07–8.02 (m, 4H, Ar), 7.62–7.56 (m, 2H, Ar), 7.55–7.49 (m, 4H, Ar), 7.49–7.44 (m, 4H, Ar), 7.44–7.37 (m, 8H, Ar), 6.80 (dd, J = 10.6, 9.6 Hz, 2H, H-4), 5.76 (dd, J = 9.2, 2.8 Hz, 2H, H-3), 5.43 (dd, J = 2.6, 1.6 Hz, 2H, H-2), 5.05 (d, J = 1.2 Hz, 2H, H-1), 4.31–4.20 (m, 4H, H-5, H-6), 3.87 (dd, J = 12.8, 1.2 Hz, 2H, H-6) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 166.1 (C=O), 165.2 (C=O), 164.2 (C=O), 133.5 (Ar), 133.1 (Ar), 133.1 (Ar), 130.5 (Ar), 130.0 (Ar), 129.9 (Ar), 129.6 (Ar), 129.4 (Ar), 129.3 (Ar), 128.5 (Ar), 128.3 (Ar), 128.3 (Ar), 100.3 (C-1), 75.6 (C-2), 74.0 (C-3), 73.4 (C-5), 71.5 (C-6), 66.9 (C-4) ppm; IR (ATR): ν = 3065, 2926, 2856, 1720, 1601, 1584, 1491, 1451, 1363, 1315, 1246, 1176, 1090, 1067, 1025, 976, 939, 843, 801, 704, 686, 616 cm^{-1}; HRMS (FAB+): m/z calcd. for $[M+H]^+$, $C_{54}H_{45}O_{16}$, 949.2702; found, 949.2704; $[\alpha]_D^{24}$ = +130.2 º (c = 0.3, $CHCl_3$).

Cyclotris-(1→6)-(2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl) (94)

Colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 7.91–7.82 (m, 18H, Ar), 7.53–7.41 (m, 9H, Ar), 7.36–7.27 (m, 18H, Ar), 5.87 (t, J = 9.6 Hz, 3H, H-3), 5.56 (t, J = 9.6 Hz, 3H, H-4), 5.51 (dd, J = 9.2, 7.2 Hz, 3H, H-2), 5.01 (d, J = 6.8 Hz, 3H, H-1), 4.32 (dd, J = 12.4, 6.8 Hz, 3H, H-6), 4.13 (t, J = 6.8 Hz, 3H, H-5), 3.94 (d, J = 11.2 Hz, 3H, H-6) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 165.9 (C=O), 165.0 (C=O), 164.9 (C=O), 133.2 (Ar), 133.2 (Ar), 133.0 (Ar), 130.1 (Ar), 129.9 (Ar), 129.8 (Ar), 129.8 (Ar), 129.8 (Ar), 129.4 (Ar), 129.1 (Ar), 128.9 (Ar), 128.3 (Ar), 128.3 (Ar), 100.7 (C-1), 74.2 (C-5), 72.4 (C-2), 72.4 (C-3), 69.1 (C-4), 68.7 (C-6) ppm; IR (ATR): ν = 3064, 2925, 2854, 1725, 1601, 1584, 1492, 1451, 1367, 1315, 1248, 1176, 1089, 1066, 1025, 976, 937, 851, 800, 704, 686, 617 cm^{-1}; HRMS (ESI+): m/z calcd. for $[M+Na]^+$, $C_{81}H_{66}O_{24}Na$, 1445.3836; found, 1445.3827; $[\alpha]_D^{25}$ = +8.6 º (c = 0.3, $CHCl_3$).

Cyclotetrakis-(1→6)-(2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl) (95)

Colorless solid: spectroscopic data for the product were identical to previously reported data [198]. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 7.91–7.78 (m, 24H, Ar), 7.49–7.39 (m, 12H, Ar), 7.35–7.27 (m, 24H, Ar), 5.85 (t, J = 9.6 Hz, 4H, H-3), 5.65–5.57 (m, 8H, H-2, H-4), 5.02 (brs, 4H, H-1), 4.25 (brs, 4H, H-5), 4.17–4.02 (m, 8H, H-6) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 165.9 (C=O), 165.0 (C=O), 164.8 (C=O), 133.2 (Ar), 133.0 (Ar), 129.9 (Ar), 129.8 (Ar), 129.8 (Ar), 129.5 (Ar), 129.1 (Ar), 128.9 (Ar), 128.3 (Ar), 101.0 (C-1), 74.4 (C-5), 73.1 (C-3), 71.4 (C-2), 69.1 (C-4), 67.5 (C-6) ppm; IR (ATR): ν = 3064, 2939, 2884, 1723, 1601, 1584, 1492, 1451, 1366, 1315, 1247, 1176, 1088, 1065, 1025, 975, 936, 852, 800, 704, 685, 616 cm^{-1}; HRMS (ESI+): *m*/*z* calcd. for $[M+Na]^+$, $C_{108}H_{88}O_{32}Na$, 1919.5151; found, 1919.5147; $[\alpha]_D^{24}$ = +11.3 º (*c* = 1.0, $CHCl_3$).

Cyclopentakis-(1→6)-(2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl) (96)

Colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 7.90–7.80 (m, 30H, Ar), 7.50–7.39 (m, 15H, Ar), 7.36–7.27 (m, 18H, Ar), 7.26–7.20 (m, 12H, Ar), 5.84 (t, J = 9.6 Hz, 5H, H-3), 5.54–5.45 (m, 10H, H-2, H-4), 4.87 (d, J = 7.6 Hz, 5H, H-1), 4.23–4.13 (m, 10H, H-5, H-6), 3.98 (dd, J = 11.0, 6.4 Hz, 5H, H-6) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 165.8 (C=O), 164.9 (C=O), 164.8 (C=O), 133.2 (Ar), 133.1 (Ar), 132.9 (Ar), 129.9 (Ar), 129.6 (Ar), 129.3 (Ar), 129.0 (Ar), 128.3 (Ar), 128.2 (Ar), 100.9 (C-1), 74.0 (C-5), 72.6 (C-3), 71.3 (C-2), 69.4 (C-4), 67.7 (C-6) ppm; IR (ATR): ν = 3063, 2925, 2854, 1726, 1601, 1584, 1491, 1451, 1364, 1315, 1257, 1177, 1090, 1066, 1025, 975, 938, 853, 800, 705, 686, 616 cm^{-1}; HRMS (ESI+): *m*/*z* calcd. for $[M+2Na]^{2+}$, $C_{135}H_{110}O_{40}Na_2$, 1208.3179; found, 1208.3173; $[\alpha]_D^{24}$ = −7.9 º (*c* = 0.2, $CHCl_3$).

Cyclohexakis-(1→6)-(2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl) (97)

Colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 7.95–7.86 (m, 24H, Ar), 7.80–7.74 (m, 12H, Ar), 7.47–7.40 (m, 6H, Ar), 7.40–7.32 (m, 12H, Ar), 7.32–7.27 (m,

12H, Ar), 7.24–7.20 (m, 12H, Ar), 7.19–7.13 (m, 12H, Ar), 5.85 (t, J = 9.6 Hz, 6H, H-3), 5.51 (t, J = 8.8 Hz, 6H, H-2), 5.46 (t, J = 9.2 Hz, 6H, H-4), 4.90 (d, J = 7.6 Hz, 6H, H-1), 4.11–4.03 (m, 6H, H-6), 4.02–3.94 (m, 12H, H-5, H-6) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 165.8 (C=O), 165.0 (C=O), 164.7 (C=O), 133.0 (Ar), 133.0 (Ar), 132.8 (Ar), 130.0 (Ar), 129.9 (Ar), 129.8 (Ar), 129.6 (Ar), 129.2 (Ar), 129.1 (Ar), 128.3 (Ar), 128.2 (Ar), 128.1 (Ar), 100.6 (C-1), 73.7 (C-5), 73.1 (C-3), 71.7 (C-2), 69.7 (C-4), 66.8 (C-6) ppm; IR (ATR): ν = 3063, 2922, 2851, 1723, 1601, 1584, 1491, 1450, 1367, 1315, 1247, 1176, 1089, 1065, 1025, 975, 936, 852, 801, 704, 685, 616 cm^{-1}; HRMS (ESI+): m/z calcd. for $[M+2Na]^{2+}$, $C_{162}H_{132}O_{48}Na_2$, 1445.3836; found, 1445.3853; $[\alpha]_D^{24}$ = +11.4 º (c = 0.4, $CHCl_3$).

Cyclotetrakis-(1→6)-(β-D-galactopyranosyl) (98)

A mixture of **87** (30.0 mg, 0.016 mmol) and 9.8 M $MeNH_2$ in MeOH (3.0 mL) was stirred at 0 °C for 2 hr and then allowed to warm to room temperature. After stirring for 19 h, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in H_2O, and the *N*-methylbenzamide was removed by successive washing of the aqueous phase with $CHCl_3$. The aqueous layer was concentrated under reduced pressure to obtain colorless solid. The residue was purified by precipitation from EtOH/H_2O and filtered to give **98** as a colorless solid (9.5 mg, 93% yield): ^{1}H NMR (400 MHz, D_2O, TSP): δ = 4.52 (d, J = 7.6 Hz, 4H, H-1), 4.09 (dd, J = 13.0, 8.4 Hz, 4H, H-6), 4.02–3.95 (m, 8H, H-5, H-6), 3.93 (d, J = 3.6 Hz, 4H, H-4), 3.67 (dd, J = 9.8, 3.6 Hz, 4H, H-3), 3.56 (dd, J = 10.0, 8.0 Hz, 4H, H-2) ppm; ^{13}C NMR (100 MHz, D_2O, 1,4-Dioxane): δ = 104.0 (C-1), 74.5 (C-5), 73.3 (C-3), 71.3 (C-2), 70.4 (C-6), 69.5 (C-4) ppm; IR (ATR): ν = 3339, 2893, 2512, 1646, 1376, 1286, 1220, 1137, 1062, 1022, 943, 919, 894, 873, 774, 701 cm^{-1}; HRMS (FAB+): m/z calcd. for $[M+Na]^+$, $C_{24}H_{40}O_{20}Na$, 671.2011; found, 671.2012; $[\alpha]_D^{25}$ = +30.3 º (c = 0.3, H_2O).

Synchrotron single crystal X-ray diffraction study of 87

The cyclic tetragalactoside **87** was recrystallized from toluene to produce crystals suitable for single-crystal X-ray analysis. The synchrotron X-ray diffraction study for **87** was carried out at the BL02B1 beam-line in SPring-8 with the approval of the Japan Synchrotron Radiation Research Institute (JASRI) with a diffractometer equipped with a Rigaku Mercury2CCD detector. A data set was obtained by merging two data sets measured with 1.0 degrees oscillation (ω = 0 - 180°) for 3.0 second radiation with 60.5 mm detector distance, ϕ = 0 and 180°, and χ = 45°, and 2θ = 0°. The collected diffraction data were processed with the *RAPID AUTO* software program. The structure was solved by charge flipping method and refined by full-matrix least-squares on F^2 using the SHELX program suite. Geometrical restraints, i.e. AFIX, MERG, OMIT were used in the refinements. All non-hydrogen atoms were refined anisotropically in the structure. All non-hydrogen atoms were refined anisotropically in the structure. The crystal data in this manuscript can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. Crystal data and structure refinement for **87** (CCDC deposit number: 1961094) was described in supplementary data.

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Chapter 6.

References and Notes

References and Notes

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List of Publications

主論文を構成する論文

1. Synthesis of Disaccharide Nucleosides Utilizing the Temporary Protection of the 2',3'-*cis*-Diol of Ribonucleosides by a Boronic Ester
   Hidehisa Someya, Taiki Itoh and Shin Aoki
   molecules, Vol.22, Issue.10, ID.1650 (2017 年 10 月)
   DOI: 10.3390/molecules22101650

2. Regioselective *O*-Glycosylation of Nucleosides *via* the Temporary 2',3'-Diol Protection by a Boronic Ester for the Synthesis of Disaccharide Nucleosides
   Hidehisa Someya, Taiki Itoh, Mebae Kato and Shin Aoki
   Journal of Visualized Experiments, Issue.137, ID.e57897 (video and the protocol) (2018 年 7 月)
   DOI: 10.3791/57897

3. One-pot synthesis of cyclic oligosaccharides by the polyglycosylation of monothioglycosides
   Hidehisa Someya, Takehito Seki, Gota Ishigami, Taiki Itoh, Yutaka Saga, Yasuyuki Yamada and Shin Aoki
   Carbohydrate Research, Vol.487, ID.107888
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参考論文

なし