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Total synthesis and chemical design of useful glycosidase inhibitors

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Abstract: The glycosidase inhibitors, cyclophellitol, nagstatin and gualamycin, which are microbial metabolites, and their analogs have been synthesized from carbohydrates to clarify their structure - activity relationships. The synthesis of cyclophellitols including the aziridine and thiirane analogs was mainly based on the stereospecific intramolecular [3+2]cycloaddition of a nitrile oxide to an olefin. Nagstatins including a variety of hydroxyl analogs were synthesized by inter- and intramolecular nucleophilic reaction of the imidazole moieties. Their glycosidase inhibiting activities were quite substrate-specific, indicating that the glycosidases recognize especially each carbons and configurations of the glycosidase inhibitors, and consequently, the inhibitors serve as antagonists of the corresponding glycopyranosides. Total synthesis of gualamycin was accomplished by glycosylation of a thio-phenol derivative of the disaccharide portion with a pyrrolidine-aglycone. The anti-mite activity of gualamycin was suggested to be due to its maltase inhibiting activity.

1. Introduction

In recent years, much attention has focused on the synthesis and development of glycosidase inhibitors because of an increasing awareness of the vital role played by carbohydrates in biological processes. The glycosidase inhibitors find utility as antiobesity drugs, antidiabetes, antifungals, insecticides, and antivirals, including substances active against the human immunodeficiency virus (HIV) and metastasis. Therefore, the chemical and biochemical studies on glycosidase inhibitors may enable us to understand the processes of intractable diseases such as diabetes mellitus, cancer and AIDS, and may also provide us therapeutic approaches to them. As part of an ongoing program to clarify the mode of action of glycosidase inhibitors, we have synthesized cyclophellitol, nagstatin and gualamycin, and their analogs having different configurations and functionalities.

2. Cyclophellitol

Cyclophellitol (**1**) is a novel β-D-glucosidase inhibitor isolated from culture filtrates of a mushroom, *Phellinus* sp., and structurally, is a fully oxygenated cyclohexane corresponding to a carba analogue of D-glucopyranose (ref. 1). Cyclophellitol (**1**) and its analogs (**2** - **7**) have been enantiospecifically synthesized from carbohydrates to clarify their mode of action in glycosidase inhibition in our laboratories (ref. 2 - 6). Recently, the elegant synthesis of **1** was reported by several groups (ref. 7). Our strategy for construction of these highly oxygenated compounds is an intramolecular cycloaddition of a nitrile oxide to an alkene (ref. 2 -4).

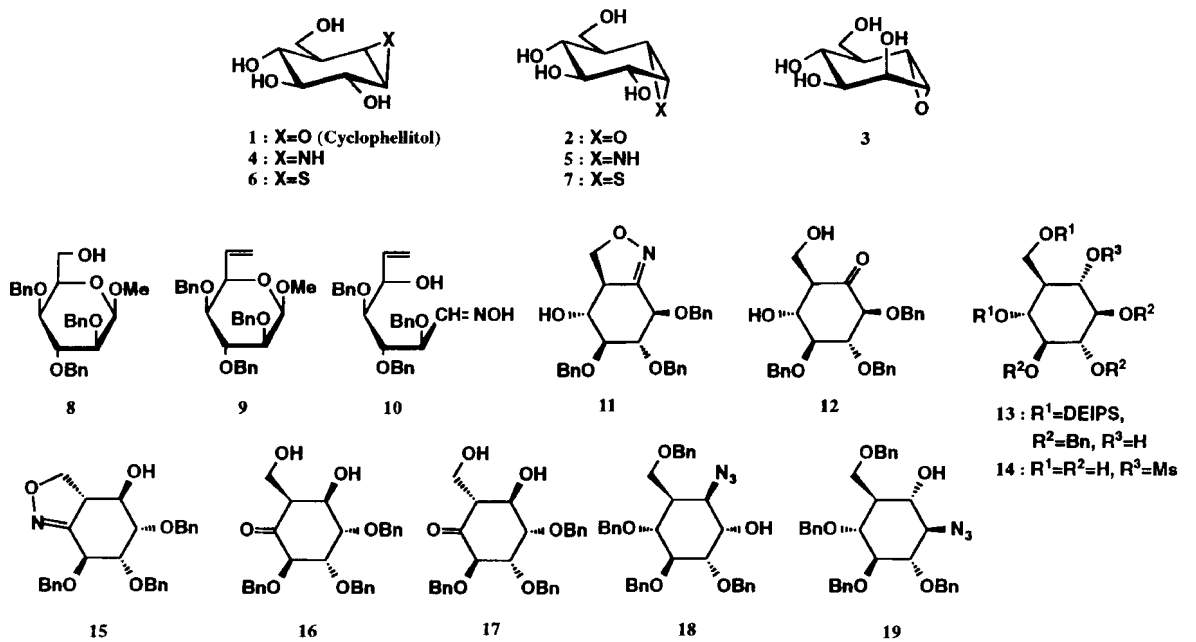
Swern oxidation of **8**, which was derived from L-glucose, afforded the unstable aldehyde, which was subjected to Wittig alkenation with salt-free methylidene-triphenylphosphorane to afford the alkene **9**. This was hydrolyzed with aqueous HCl in dioxane to an idopyranose derivative, which was treated with hydroxylamine hydrochloride in pyridine to give the oxime **10**. Intramolecular cycloaddition of **10** was realized by using NaOCl *via* the intermediary nitrile oxide to afford the isoxazoline **11** as a single product in 70% yield. The stereochemistry was confirmed by ^{1}H-NMR studies of compounds **11** - **14** and, finally the completion of the synthesis presented next.

The isoxazoline opening was achieved by treatment of **11** with H_2 and Raney Ni-W4 in aq. dioxane in the presence of AcOH to afford the keto-diol **12**. After silylation with diethylisopropylsilyl triflate, the resulting ketone was reduced with BH_3-Me_2S to afford the desired α-alcohol **13**. Diethylisopropylsilyl (DEIPS) group was developed in our laboratories and effectively used as an *O*-protecting group, because this silyl group was found to be readily removed under hydrogenolysis conditions using $Pd(OH)_2$ (ref. 8). Mesylation of **13** provided the labile mesylate, which was subjected to hydrogenolysis with $Pd(OH)_2$ in MeOH to give the deprotected **14**, followed by epoxidation with MeONa to give cyclophellitol (**1**).

In order to provide additional insight into the mode of action of cyclophellitol (**1**), the unnatural epoxide diastereomers (**2** - **3**) and heteroatom-containing analogs (**4** - **7**) were synthesized. From the fact that cyclophellitol (**1**) exhibits a very high β-D-glucosidase inhibiting activity, we have expected that 1,6-epicyclophellitol (**2**) and α-manno analog **3** inhibit α-D-glucosidase and α-D-mannosidase activities, respectively.

1,6-Epicyclophellitol (**2**) was similarly synthesized from methyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranoside through the isoxazoline **15**, which was subjected to acidic hydrogenolysis with Raney Ni-W4 to afford the desired keto-alcohol **16** with epimerization at the C-1 position.

The α-manno analog **3** was synthesized from **15** without epimerization at C-1 position. The hydrogenolysis of **15** was conducted using Raney Ni and $B(OH)_3$ to afford the keto-alcohol **17** in a quantitative yield, which was converted into **3** (ref. 4).



The aziridine analog **4** was synthesized from 1,6-epicyclophellitol (**2**). The tetra-*O*-benzyl derivative of **2** was treated with NaN_3 to afford a mixture of **18** and **19**, which was subjected to reduction with PPh_3 to give a single aziridine, followed by de-*O*-benzylation to give the β-aziridine analog **4** (ref. 4).

Similarly, the α-aziridine **5** was derived from cyclophellitol (**1**).

The thiirane analogs **6** and **7** were prepared from **2** and **1**, respectively, by treatment of their *O*-MPM derivatives with $Ph_3P{=}S$ and trifluoroacetic acid, followed by de-*O*-methoxybenzylation with DDQ (ref. 5).

The glycosidase inhibiting activities of cyclophellitol (**1**), 1,6-epicyclophellitol (**2**), and their analogs **3** - **7** were generally assayed according to the method reported by Saul *et al.* (ref. 1) and are shown in Table 1.

In dramatic contrast to natural cyclophellitol (**1**) which inhibited only β-D-glucosidase activity for 50% at 0.8 mg/ml, the *epi*-epoxide **2** exhibited the inhibiting activity only against α-D-glucosidase at IC_{50} 10 mg/ml. The α-manno analog **3** expectedly showed inhibitory activity against α-mannosidase of IC_{50} 19 mg/ml, and the β-aziridine analog **4** showed very high inhibitory activity against β-glucosidase of IC_{50} 0.22 mg/ml, while the α-aziridine **5** showed little α-glucosidase inhibiting activities. Remarkably, both thiirane analogs **6** and **7** showed no significant activities.

Structurally, cyclophellitol (**1**) and its aziridine analog **4** have *quasi*-equatorially oriented C1-O and C1-N bonds, which correspond to the equatorial C1-O bond of β-D-glucopyranosides, whereas *epi*-cyclophellitol (**2**) and α-manno analog **3** have *quasi*-axial C1-O bonds corresponding to the axial C1-O bond of α-D-glycopyranosides. Their glycosidase inhibiting activities emphasized that the α - and β-glycosidase recognized especially the C-1 positions and the residual portions as corresponding to those of α- and β-glycopyranosides. Consequently, these glycosidase inhibitors **1** - **4** serve as antagonists of the corresponding α- and β-D-glycopyranosides.

TABLE 1. Inhibitory activity of cyclophellitol (**1**), nagstatin (**20**) and their analogs (**2~4** and **21~25**) against glycosidases (IC_{50} : μg/ml)

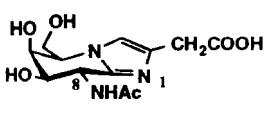
	Inhibitors									
Glycosidases	**1**	**2**	**3**	**4**	**20**	**21**	**22**	**23**	**24**	**25**
α-D-Glc[a]		10								
β-D-Glc[b]	0.8			0.22					0.14	
α-D-Man[c]			19							
β-D-Man[d]										0.023
β-D-Gal[e]							0.0016			
NAc-β-D-Glc[f]					0.004	0.0015		0.0017		

[a]Baker's yeast α-D-glucosidase; [b]Almond β-D-glucosidase; [c]Jack beans α-D-mannosidase;[d]Snail β-D-mannosidase; [e]Escherichia coli. β-D-galactosidase; [f]Bovine kidney N-acetyl-β-D-glucosaminidase.

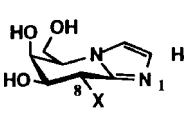
3. Nagstatin

Nagstatin (**20**) is an *N*-acetyl-β-D-glucosaminidase inhibitor isolated from fermentation broth of *Streptomyces amakusaensis* (ref. 9). In several diseases such as diabetes mellitus, leukemia and cancer, *N*-acetyl-β-D-glucosaminidase activity in serum has been reported to increase. Nagstatin (**20**) and a variety of its analogs (**21** - **25**) have been synthesized from carbohydrates through the inter- and intramolecular nucleophilic reactions with the imidazole moieties to clarify the structure - activity relationships (ref. 10 - 12). These compounds were expected to serve as antagonists of the corresponding β-glycopyranosides from the aforesaid findings.

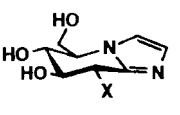
First of all, de-branched nagstatin (**21**) and its hydroxyl analog **22** were effectively synthesized from 2,3,5-tri-*O*-benzyl-L-ribofuranose. Reaction with lithiated *N*-tritylimidazole which was prepared from *N*-tritylimidazole and *n*-BuLi, gave the L-allo **26** and L-altro derivatives **27** in a ratio of 1 : 1, both of which were converted into **21** and **22** as follows. De-*N*-tritylation and the S_N2-type intramolecular cyclization of **26** were effectively realized in one-pot by reaction with $BnSO_2Cl$ in pyridine to give preferentially the 5-*O*-sulfonate followed by treatment with Ac_2O to give the desired acetate, which was de-*O*-acetylated to the nitrogenous D-talose analog **28**. The effective de-*N*-tritylation seemed to be affected by the producing pyridinium acetate. The inversion of the hydroxyl group in **28** using HN_3, *n*-Bu_3P and DEAD afforded the azido derivative **29**, which was subjected to hydrogenolysis with Pd-C and *N*-acetylation with Ac_2O in MeOH, leading to the *N*-acetyl-D-galactosamine analog **21**, which was corresponding to de-branched nagstatin.



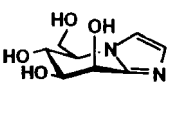
20 : Nagstatin



21 : X=NHAc
22 : X=OH



23 : X=NHAc
24 : X=OH



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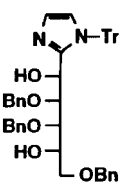

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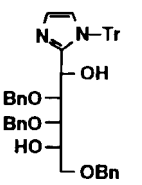

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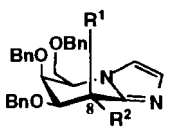
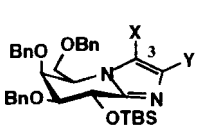
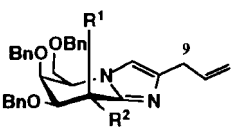


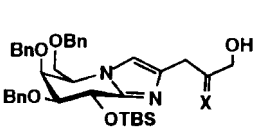
28 : R^1=OH, R^2=H
29 : R^1=H, R^2=N_3
30 : R^1=H, R^2=OH



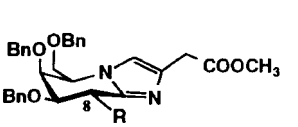
31 : X=Y=H
32 : X=Br, Y=H
33 : X=Y=Br
34 : X=H, Y=Br



35 : R^1=H, R^2=OTBS
40 : R^1=OTBS, R^2=H



36 : X=H, OH
37 : X=O



38 : R=OTBS
39 : R=N_3

Alternatively, **29** was prepared from the other isomer **27** through **30**. Reaction of **30** with HN_3, *n*-Bu_3P and DEAD gave **29** with retention of the C-8 configuration as expected. The S_N2 replacement of the C-2 equatorial group in carbohydrates,which is corresponding to C-8 in **29**, has been hardly known to occur because of the ring oxygen, the anomeric substituent and dipolar effects (ref. 10).

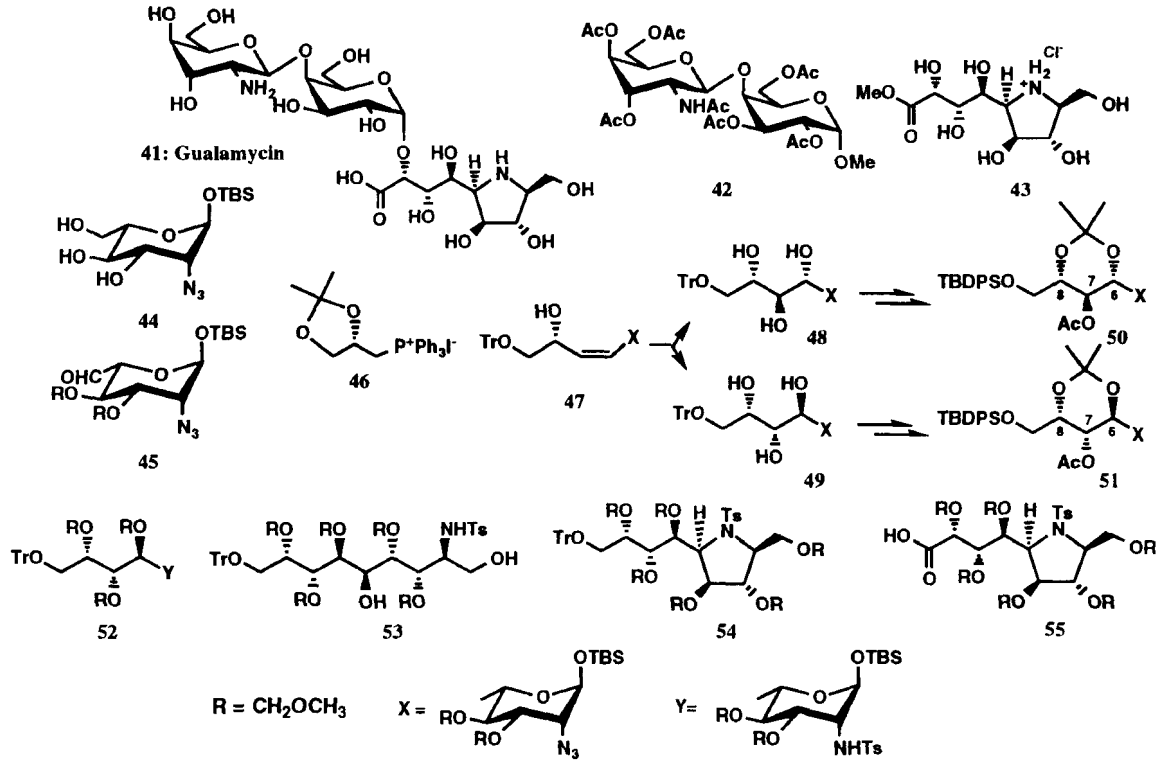
Hydrogenolysis of **30** afforded the nitrogenous D-galactose analog **22**.

Similarly, the enantiomeric *N*-acetyl-L-galactosamine analog **21'** and L-galactose analog **22'** were prepared from D-ribofuranose derivative by the same procedures as mentioned above.
Furthermore, nitrogenous *N*-acetyl-D-glucosamine, D-glucose and D-mannose analogs (**23, 24** and **25**) were efficiently prepared from L-xylofuranose derivative by the similar fashion as described above.
Now, the next step is set for the enantiospecific synthesis of nagstatin (**1**). The rational starting point is the aforesaid isomers **28** and **30** (ref. 12). The regioselective introduction of an allyl group on their C-2 positions was investigated under a variety of conditions. The C-2 position is generally known to be less reactive than the C-3. In fact, selective bromination of **31** gave the undesired C-3 bromo compound **32**. Accordingly, **31** was fully brominated with 2,4,4,6-tetrabromo-2,5-cyclohexadien-1-one to the dibromo compound **33**, the selective debromination of which was assayed. The best result was realized by regioselective lithiation with *t*-BuLi in THF followed by quenching with H_2O to give the desired monobromo compound **34**. The structure was confirmed by the ^{1}H-NMR NOE studies of the corresponding allyl derivative **35**.
Dihydroxylation with OsO_4 and NMO of **35** afforded **36**, which was oxidized by using modified Fetizon's conditions using Ag_2CO_3 to give the keto-alcohol **37**. Periodate oxidation followed by esterification with $TMSCHN_2$ provided the methyl ester **38**. Direct ozonolysis of **35**, or periodate oxidation of **36** caused concomitant oxidation at C-9 position.
Conversion of **38** to the azido compound **39** was carried out by de-*O*-silylation followed by treatment with HN_3, *n*-Bu_3P and DEAD. Expected retention of the configuration was observed at the C-8 position as described above. Hydrogenolysis of **39** followed by successive *N*-acetylation and saponification with aq. NaOH provided nagstatin (**20**), which was identical with the natural product in all respects including glycosidase inhibiting activities. The completion of the synthesis confirmed the absolute structure **20**.
In a similar manner, but with inversion of the configuration, the other C-8 *axial* isomer **28** was converted into the aforesaid azido compound **39** in a ten-step sequence through the allyl derivative **40**.
The glycosidase inhibiting activities were assayed as described above (ref. 11) and summarized in Table 1. *N*-Acetyl-D-galactosamine analog **21** exhibited the strong activity even against *N*-acetyl-β-D-glucosaminidase as similarly with nagstatin (**20**) and, consequently, was expected to inhibit *N*-acetyl-β-D-galactosaminidase, although this glycosidase was not available now. *N*-Acetyl-D-glucosamine analog **23** inhibited strongly *N*-acetyl-β-D-glucosaminidase activity and weakly β-D-glucosidase activity. The D-galacto, D-gluco and D-manno analogs (**22, 24** and **25**) showed very much stronger inhibiting activities against β-D-galactosidase, β-D-glucosidase and β-D-mannosidase, respectively, than against the corresponding α-D-glycosidases. Remarkably, the L-galactose analogs **21'** and **22'** showed no significant glycosidase inhibitory activities.
All analogs possess a *quasi*-equatorially oriented C8a-N1 bond, which corresponds to an equatorial C1-O bond of β-glycopyranosides, due to the fused imidazole ring. The configurations from C8a to C5 of the analogs parallel the alignment from C1 to C5 of the corresponding glycopyranosides. The strong β-D-glycosidase inhibiting activities of the analogs **21** - **25** indicated that the β-D-glycosidases including *N*-acetyl-β-D-glucosaminidase recognized especially their C-8a positions as the C-1 position of β-D-glycopyranosides. Furthermore, their substrate-specific activities emphasized that the analogs serve essentially as the antagonists of the corresponding stereochemically oriented β-D-glycopyranosides. These findings are similar with those of the aforesaid cyclophellitol (**1**) and its analogs.

4. Gualamycin

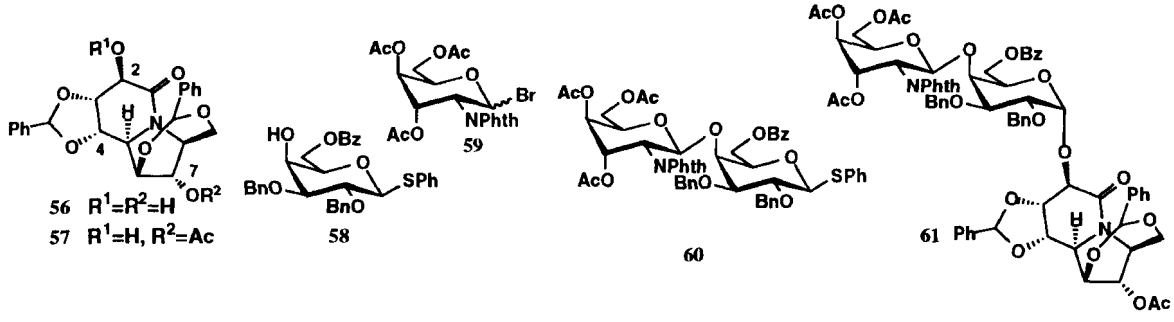
Gualamycin (**41**) is a novel water-soluble acaricide (anti-mite substance) isolated from the culture broth of *Streptomyces* sp. NK11687 (ref. 13). The absolute structure was mainly confirmed by enantiospecific syntheses of its amino-disaccharide and pyrrolidine-aglycone portions (**42** and **43**) in our laboratories (ref. 14 - 15). The structural complexity, as well as the goal of studying structure-activity relationships, prompted us to an exploration of the total synthesis, which was expected to confirm the absolute structure **41** and elucidate the origin of appearance of the acaricidal activity. The synthesis is mainly based on glycosylation of the glycosyl acceptor **57** with the donor **60** (ref. 16).
The pyrrolidine-containing aglycone unit **43** was synthesized from the azido sugar **44**, which was prepared by de-*O*-acetylation of *t*-butyldimethylsilyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-L-mannopyranoside. The azide **44** was selectively silylated with TBDMSCl and methoxymethylated to the fully protected product, which was selectively desilylated to give the alcohol. Swern oxidation gave the labile aldehyde **45**, which was treated with the Wittig reagent prepared from (4*S*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methyl-triphenylphosphonium iodide (**46**) and *n*-BuLi, successively followed by removal of the isopropylidene group and tritylation of the resulting primary alcohol to yield the *cis* olefin **47**. The *cis* dihydroxylation of **47** by OsO_4 gave two triols **48** and **49** in 34% and 46% yields, respectively. Their configurations were

determined by the ^{1}H-NMR studies of their corresponding isopropylidene derivatives **50** and **51** to show that **49** was the desired triol for the natural product. The triol **49** was methoxymethylated, followed by successive treatment with Ph_3P in PhMe and with aq. THF in refluxing to give the amino compound, which was tosylated to the product **52**. Desilylation with *n*-Bu_4NF and hydride reduction with $NaBH_4$ gave the alcohol **53**. This was selectively methoxymethylated and then submitted to the S_N2-type cyclization using Ph_3P and DEAD to give the pyrrolidine derivative **54**. After detritylation by hydrogenolysis, the forming alcohol was oxidized stepwise by Swern's conditions to give the aldehyde and by sodium chlorite oxidation with H_2NSO_3H to give the carboxylic acid **55**. De-*N*-tosylation by Birch's conditions with Li in liq. NH_3 followed by esterification with 5% HCl-MeOH gave the hydrochloride of the pyrrolidine-aglycone **43**, which was identical with the naturally derived sample in all respects.



The glycosyl acceptor, di-*O*-benzylidene derivative **57** was synthesized by a 5-step sequence from the aglycone **43**. Thus, treatment of **43** with Na_2CO_3 to give the δ-lactam whose *O*-benzylidenation with PhCHO and $ZnCl_2$ provided **56** in 54% overall yield. The two sets of two hydroxyl groups at the C-3 and 4 positions and at the C-6 and 9 positions were effectively protected by *O*-benzylidene groups as expected from the Dreiding model. The protection of the hydroxyl group at C-7 in **56** proceeded nonselectively under a variety of conditions, while the glycosylation of **56** gave unexpectedly the undesired 7-*O*-glycosyl derivative by using the glycosyl donor **60**. Accordingly, **56** was silylated with TMSCl and DIPEA, acetylated and desilylated to give the monoacetate **57** in 70% yield.

The glycosyl donor **60** was prepared from phenyl-1-thio-galactoside in five steps through **58**. The alcohol **58** was subjected to the reaction with the protected gulosaminyl bromide **59** in the presence of AgOTf and *s*-collidine to give the glycosyl donor **60**.



Coupling of **60** with the acceptor **57** was accomplished by using a modified Fraser-Reid's conditions using NIS and TfOH at -40°C for 1 hour to provide exclusively the desired α-glycoside **61**. Hydrogenolysis of **61** followed by treatment with 40% $MeNH_2$ in MeOH furnished the corresponding δ-lactam.

On the final stage, all attempts to open the δ-lactam to the imino acid **41** failed under alkaline conditions. Only the low yield of **41** was observed. However, the convenience of using acids to catalyze the process was especially appealing to us. Hydrolysis of the lactam was successfully conducted in 2M HCl at room temperature for 6 days to give the dihydrochloride of gualamycin (**41**) in 86% yield without further hydrolysis, which was identical with the natural product in all respects including acaricidal activities.
When the glycosidase inhibiting activities were assayed, gualamycin (**41**) was found to inhibit maltase activity at IC_{50} 25 mg/ml. This inhibiting activity seems to be the origin of appearance of the acaricidal (anti-mite) activity, suggesting that a mite could get maltose as a source of life.

5. Conclusions

The glycosidase inhibitors, cyclophellitol, nagstatin and gualamycin, which are microbial metabolites, and their analogs were effectively synthesized from carbohydrates to clarify their structure - activity relationships. As a result, new analogs having stronger activities than natural products were chemically designed and created. Their glycosidase inhibiting activities were quite substrate-specific, indicating that the α- and β-glycosidases recognize especially the C-1 positions and the residual portions as corresponding to those of α- and β-glycopyranosides, and consequently, the inhibitors serve as antagonists of the corresponding glycopyranosides. The anti-mite activity of gualamycin was suggested to be due to its maltase inhibiting activity.

Acknowledgment

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