Biosynthetic Studies on the Cyclopentane Ring Formation of Allosamizoline, an Aminocyclitol Component of the Chitinase Inhibitor Allosamidin

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Allosamizoline (**1**) is an aminocyclitol component of allosamidin, a *Streptomyces* metabolite, and has a cyclopentane ring originated from D-glucosamine. Biosynthesis of the cyclopentane ring was studied by feeding experiments with a variety of deuterium-labeled glucosamine and glucose. In the feeding experiments with [3-^{2}H]- and [4-^{2}H]-D-glucosamine and [1-^{2}H]-D-glucose, deuterium was incorporated into C-3, C-4, and C-1 of **1**, respectively. On the other hand, feeding experiments with [5-^{2}H]- and [6,6-$^2H_2$]-D-glucosamine showed that deuterium on C-5 and one of the two deuterium atoms on C-6 of glucosamine were lost during the cyclopentane ring formation of **1**. In the feeding experiments with (6*R*)- and (6*S*)-[6-$^2H_1$]-D-glucose, the (6*R*)-deuterium of glucose was incorporated into the *proS* position on C-6 of **1**, but the (6*S*)-deuterium of glucose was not incorporated into **1**. These results suggested that an intermediate with a 6-aldehyde group is involved in the biosynthesis of the cyclopentane ring moiety of **1** and overall inversion of stereochemistry of the C-6 methylene group occurred by stereospecific oxidation and reduction on C-6 during the formation of **1**. The 6-aldehyde intermediate may play a key role in the biosynthetic step(s) of cyclization to form the cyclopentane ring and/or deoxygenation at C-5.

Allosamidin, a *Streptomyces* metabolite, is an inhibitor of family 18 chitinases and has a pseudotrisaccharide structure (Figure 1) consisting of two units of *N*-acetyl-D-allosamine and one unit of an aminocyclitol derivative, allosamizoline (**1**).[2] Allosamizoline has a cyclopentanoid structure, which is highly oxygenated and fused with a dimethylaminooxazoline ring. Our biosynthetic studies on allosamidin revealed that the carbon skeleton of each allosamine and the cyclopentane moiety of **1** is derived from D-glucose, and D-glucosamine is their close precursor.[3] Furthermore, we showed that the nitrogen atom on C-2 and the carbon skeleton of each moiety originate from an intact D-glucosamine molecule. A cyclopentanoid skeleton biosynthesized from carbohydrate is uncommon in natural products compared with a cyclohexanoid skeleton, but several compounds with important biological activities are known to have a cyclopentanoid skeleton

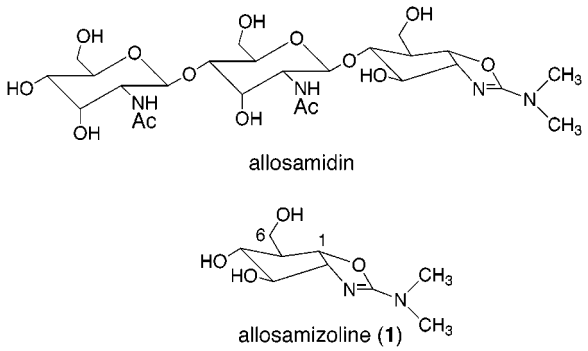

Figure 1. Structures of allosamidin and allosamizoline (**1**).

to which a hydroxymethyl group is attached.[4] Besides the cyclopentane ring of **1**, the cyclopentane rings of pactamycin,[5] bacteriohopane,[6] and aristeromycin[7] have been proved to be biosynthesized from glucose. In the cyclopentane ring formation of **1**, pactamycin and bacteriohopane, the hydroxymethyl carbon originates from C-6 of D-glucose, and C−C bond formation occurs between C-5 and C-1 of D-glucose. On the other hand, the C-1 carbon of D-glucose is the origin of the hydroxymethyl carbon in the case of the cyclopentane ring of aristeromycin, in which a C−C bond formation occurs between C-2 and C-6

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Table 1. Deuterium Incorporation into Allosamine and Allosamizoline Moieties of Allosamidin Derived from ^{2}H-Labeled Glucosamines

labeled glucosamine	increased % of monolabeled molecules[a]	
	in allosaminitol hexaacetate	in allosamizoline triacetate
3-^{2}H	12.5	14.6
4-^{2}H	10.9	8.4
5-^{2}H	10.9	*b*
6,6-$^2H_2$	2.7 (14.0[c])	3.7 (*b*,*c*)

[a] Calibrated from CI-MS spectra of labeled and nonlabeled samples. [b] Increase was not observed (<1.0%). [c] Increased % of dilabeled molecules.

of D-glucose. In the latter case, the mechanism of the cyclopentane ring formation has been studied by feeding experiments with specifically ^{3}H-labeled glucoses.[7] Since the mechanism in the former case had not yet been studied, we performed feeding experiments with specifically ^{2}H-labeled D-glucosamines to elucidate the mechanism of the cyclopentane ring formation of **1** and reported the results in a preliminary form.[8] We now report a detailed description of the experiments, as well as the results of further feeding experiments with specifically ^{2}H-labeled glucoses. Our results suggest that an intermediate with a 6-aldehyde group may play a key role in the biosynthesis of the cyclopentane ring moiety of **1**.

Streptomyces sp. AJ9463, a high producer of allosamidin, was used throughout this work. Cultivation of the strain and a feeding experiment with a labeled precursor were performed according to the methods previously described.[3] Since our previous feeding experiments with ^{14}C- or ^{13}C-labeled precursors showed that D-glucosamine was incorporated into each moiety of allosamine and **1** at much higher level than D-glucose, we first planned feeding experiments with specifically ^{2}H-labeled glucosamines. Four labeled glucosamines, [3-^{2}H]-, [4-^{2}H]-, [5-^{2}H]-, and [6,6-$^2H_2$]-D-glucosamine, were prepared, and feeding experiments with each of them were carried out. After cultivation of strain AJ9463 with the labeled glucosamine, labeled allosamidin was isolated and hydrolyzed to afford labeled D-allosamine and **1**. ^{2}H NMR spectra of the labeled samples were measured to evaluate incorporation of deuterium and to determine the position of the incorporated deuterium. The labeled allosamine and **1** were converted to the labeled allosaminitol hexaacetate and the triacetate of **1**, respectively, for CI-MS analysis to estimate the levels of deuterium incorporation, which are listed in Table 1. Since glucosamine is a common precursor of both **1** and allosamine, the comparison of deuterium incorporation into **1** with that into allosamine was very useful to evaluate if a deuterium loss from the labeled glucosamine or glucose specifically occurred during the biosynthesis of the cyclopentane ring of **1**.

In the feeding experiment with [4-^{2}H]-D-glucosamine, deuterium incorporation was observed at C-4 of each of allosamine and **1**. Deuterium incorporation was observed at C-3 of **1** and also at C-3 of allosamine in the experiment with [3-^{2}H]-D-glucosamine. This deuterium incorporation at C-3 of allosamine suggested that the epimerization of a hydroxyl group at C-3 occurred with retention of the deuterium on C-3 of glucosamine. Such a hydrogen

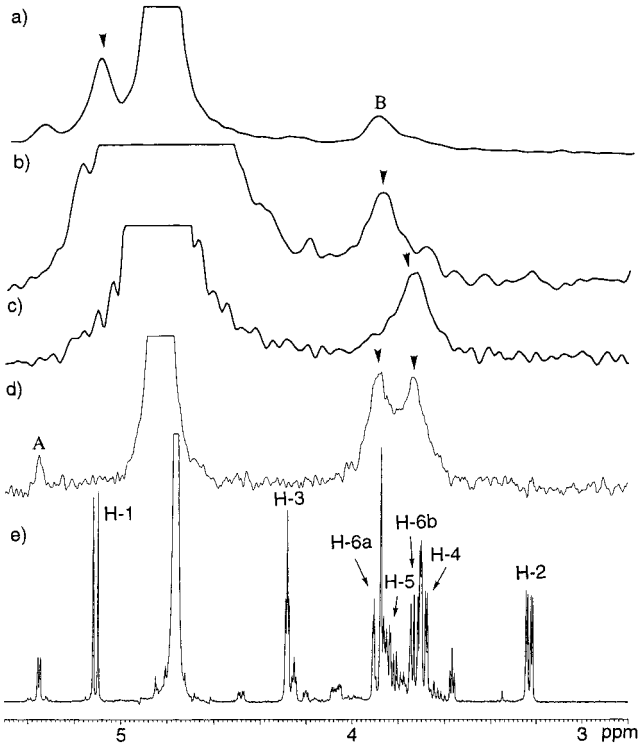

Figure 2. ^{2}H NMR spectra of allosamine derived from (a) [1-^{2}H]-glucose (76.65 MHz, 0.8 mg in 0.3 mL of H_2O, 2856 scans), (b) (6*S*)-[6-$^2H_1$]-D-glucose (76.65 MHz, 0.3 mg in 0.3 mL of H_2O, 9401 scans), (c) (6*R*)-[6-$^2H_1$]-D-glucose (76.65 MHz, 0.4 mg in 0.3 mL of H_2O, 14489 scans), and (d) [6,6-$^2H_2$]-D-glucosamine (61 MHz, 5 mg in 0.6 mL of H_2O). (e) ^{1}H NMR spectrum of natural allosamine (400 MHz, 10 mg in 0.6 mL of D_2O, 16 scans). Signals of the β-anomer are mainly observed. Acetone $\delta_{H(D)}$ 2.225 was used as a standard. (A) Ghost signal observed in all spectra recorded at 61 MHz in this study due to the measurement conditions. (B) Signal that may be observed as a result of the metabolism of [1-^{2}H]-D-glucose to (6*S*)-[6-$^2H_1$]-D-glucose and incorporation of it into allosamine moiety.

retention during a process of epimerization has been observed in, for example, the reaction of UDP-D-glucose-4-epimerase, which catalyzes a reversible reaction between UDP-glucose and UDP-galactose. In the catalytic process of the enzyme, C-4 of the hexose is oxidized by enzyme-bound NAD and subsequently reduced by stereospecific return back of the hydride from NADH, leading to retention of the hydrogen on C-4 during the epimerization at C-4.[9] In the feeding experiment with [5-^{2}H]-D-glucosamine, deuterium incorporation was observed at C-5 of allosamine, but no incorporation into **1** was detected. In the ^{2}H NMR spectrum of the labeled allosamine obtained from the feeding experiment with [6,6-$^2H_2$]-D-glucosamine, two deuterium signals, which have chemical shifts of 3.90 and 3.73 ppm corresponding to those of the methylene protons on C-6 of allosamine, were observed (Figure 2d). On the other hand, only one deuterium signal with a chemical shift of 3.88 ppm, which was the same value as that of one of the two protons (3.88 and 3.72 ppm) on C-6 of **1**, was observed in the spectrum

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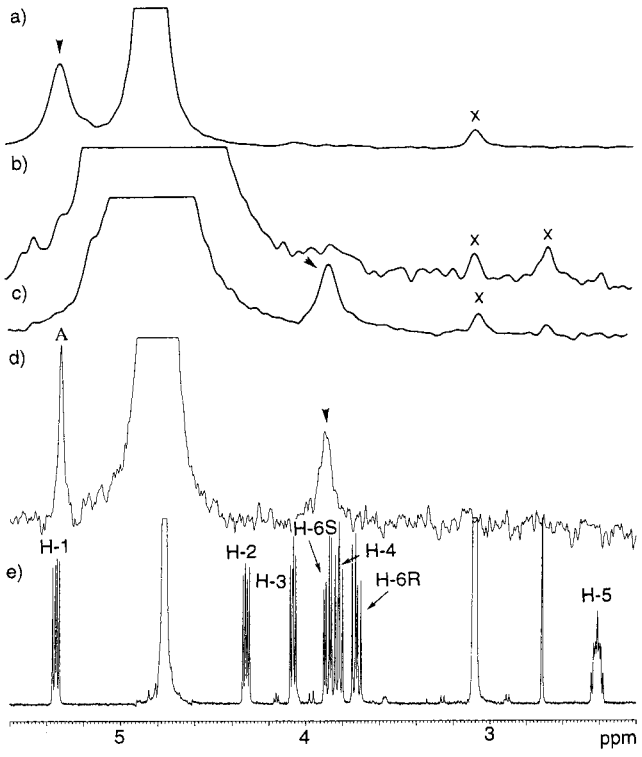

Figure 3. ^{2}H NMR spectra of **1** derived from (a) [1-^{2}H]-glucose (76.65 MHz, 0.8 mg in 0.3 mL of H_2O, 11049 scans), (b) (6*S*)-[6-$^2H_1$]-D-glucose (76.65 MHz, 0.17 mg in 0.3 mL of H_2O, 35006 scans), (c) (6*R*)-[6-$^2H_1$]-glucose (76.65 MHz, 0.22 mg in 0.3 mL of H_2O, 35005 scans), and (d) [6,6-$^2H_2$]-D-glucosamine (61 MHz, 3 mg in 0.6 mL of H_2O). (e) ^{1}H NMR spectrum of natural **1** (400 MHz, 10 mg in 0.6 mL of D_2O, 16 scans). Signals of the β-anomer are mainly observed. Acetone $\delta_{H(D)}$ 2.225 was used as a standard. (A) Ghost signal observed in all spectra recorded at 61 MHz in this study due to the measurement conditions. (X) Unidentified signal.

of labeled **1** (Figure 3d). Furthermore, the CI-MS spectrum of labeled allosaminitol hexaacetate indicated that the dilabeled molecules increased mainly, but an increase in only monolabeled molecules was observed in the spectrum of the triacetate of **1** (Table 1).

Next, feeding experiments with (6*R*)- and (6*S*)-[6-$^2H_1$]-D-glucose[10] were performed to investigate the fate of each of the methylene protons at C-6 during the biosynthesis of **1**. In the ^{2}H NMR spectrum of each of labeled allosamine and **1** derived from (6*R*)-[6-$^2H_1$]-glucose (Figures 2c and 3c), one deuterium signal was observed at 3.73 and 3.88 ppm, respectively. On the other hand, in the feeding experiment with (6*S*)-[6-$^2H_1$]-glucose, a deuterium signal was observed at 3.90 ppm in the spectrum of labeled allosamine (Figure 2b), but no deuterium signal was observed in that of labeled **1** (Figure 3b).

To determine the absolute stereochemistry of the proton at C-6 of **1** in which deuterium was incorporated in the above experiments with [6,6-$^2H_2$]-D-glucosamine and (6*R*)-[6-$^2H_1$]-glucose, we attempted to assign the absolute configurations of the methylene protons at C-6 of **1** by NMR. Since conformation analysis of the hydroxymethyl group of **1** was unsuccessful as a result of its free rotation, isopropylidene derivatives of **1** were prepared with expectation of the formation of the 4,6-*O*-isopropylidene of **1** (**2**). However, an isopropylidene

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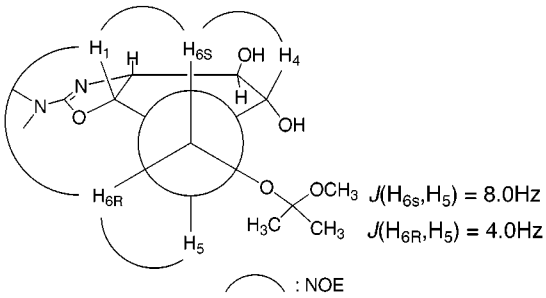


Figure 4. Conformation for C-5−C-6 of **3**.

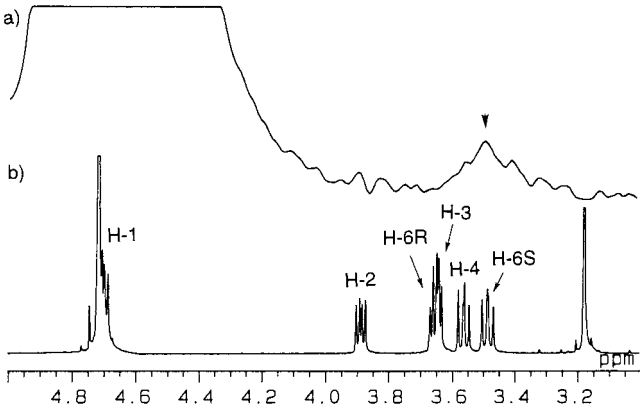
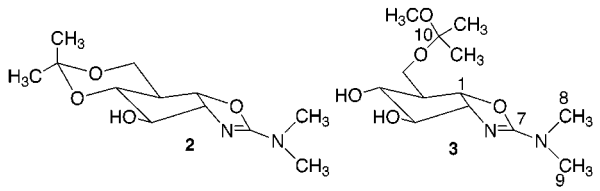


Figure 5. (a) ^{2}H NMR spectrum of **3** derived from (*R*)-[6-$^2H_1$]-D-glucose (76.65 MHz, 0.15 mg in 0.3 mL of 0.1 N NaOH, 22222 scans). (b) ^{1}H NMR spectrum of natural **3** (500 MHz, 1 mg in 0.3 mL of 0.1 N NaOD, 16 scans). Acetonitrile $\delta_{H(D)}$ 1.98 was used as a standard.

derivative (**3**) was mainly obtained by the reaction of **1** and dimethoxypropane under the presence of *p*-TsOH.



In the case of **3**, conformation for the methine and methylene system at C-5−C-6 could be determined by NOE and *J* values as shown in Figure 4. Therefore, the absolute configurations of the methylene protons with the chemical shifts of 3.49 and 3.66 ppm in the NMR spectrum of **3** were assigned to be *proS* and *proR*, respectively (Figure 5). Thus, labeled **1** derived from (6*R*)-[6-$^2H_1$]-glucose was converted to labeled **3**, and its ^{2}H NMR spectrum was measured. In the spectrum, the deuterium signal around 3.49 ppm was observed (Figure 5), suggesting that deuterium was incorporated into the *proS* position of C-6 of **1**. From this fact, the methylene protons at 3.88 and 3.72 ppm in the NMR spectrum of **1** could be assigned to be *proS* and *proR*, respectively (Figure 3).

Finally, a feeding study with [1-^{2}H]-D-glucose was carried out. In the experiment, deuterium incorporation was observed at C-1 of each of allosamine and **1** in their ^{2}H NMR spectra (Figures 2a and 3a).

Discussion

The loss of one of the two deuteriums on C-6 of glucosamine observed in the feeding experiment with

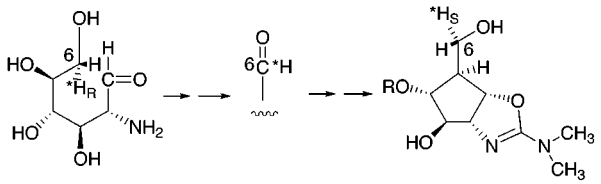


Figure 6. Stereochemistry at C-6 during the biosynthesis of the cyclopentanoid moiety of allosamidin.

[6,6-$^2H_2$]-D-glucosamine strongly suggested that an intermediate with a 6-aldehyde (or its enol equivalent) group is involved in the biosynthesis of the cyclopentane ring moiety of **1**. The reduction of the aldehyde group to the hydroxymethyl group of **1** may proceed stereospecifically, since only deuterium at the *proS* position remained on C-6 of labeled **1** in the experiment (Figure 3). The fact that deuterium incorporation was observed in labeled **1** derived from (6*R*)-[6-$^2H_1$]-D-glucose but not in **1** from (6*S*)-[6-$^2H_1$]-D-glucose indicates that a stereospecific oxidation of the hydroxymethyl group to the aldehyde group accompanying a loss of the *proS* proton is involved in the biosynthesis of the cyclopentane ring moiety of **1**. This stereospecific oxidation and reduction on C-6, shown in Figure 6, suggests that stereochemical inversion of the methylene proton at C-6 occurs during the cyclopentane ring formation of **1**. The lower level of deuterium incorporation into **1** (3.7% increase of monolabeled molecules) than that into allosamine (14.0% increase of dilabeled molecules) observed in the experiment with [6,6-$^2H_2$]-D-glucosamine might reflect some isotope effects involved in these reactions at C-6 or deuterium loss by a presumed side reaction such as interconversion of aldehyde and carboxylic acid at C-6.

The mechanisms of the formation of cyclohexane rings observed in the biosynthesis of *myo*-inositol 1-phosphate,[4] dehydroquinate,[4] or 2-deoxy-*scyllo*-inosose[11] are known well. By analogy to the biosynthetic mechanisms of these compounds, we may presume that the cyclization to form the cyclopentane ring of **1** proceeds via a 4-keto or 6-aldehyde (or their enol equivalents) glucosamine derivative, which would undergo an aldol condensation of C-5 with C-1. Therefore, three pathways to form the cyclopentane ring of **1** are possible, as shown in Figure 7. Pathways A and B are analogous mechanisms of cyclization in *myo*-inositol biosynthesis. On the other hand, pathway C is analogous to the mechanism of cyclization in the biosynthesis of dehydroquinate and 2-deoxy-*scyllo*-inosose.

The loss of deuterium on C-5 of glucosamine observed in the feeding experiment with [5-^{2}H]-D-glucosamine may rule out the possibility of pathway C, in which the deuterium should be retained at C-5 of **1**. This is supported by deuterium incorporation at C-4 of **1** observed in the feeding experiment of [4-^{2}H]-D-glucosamine, since the deuterium may be lost in the pathway C. In the pathways A and B, the C-4 or C-6 position of the glucosamine derivative is once oxidized to a carbonyl group, which facilitates C−C bond formation between C-5 and C-1 by an aldol condensation. After the formation of the cyclopentane ring with a tertiary hydroxyl group at C-5, reduction of the carbonyl group and deoxygenation at C-5 occur to create the cyclopentane ring skeleton of **1**.

There are two possible roles of a 6-aldehyde intermediate mentioned above in this biosynthetic mechanism of the cyclopentane ring of **1**. One is in the cyclization reaction and the other is that in the deoxygenation at C-5. When the intermediate is involved in the cyclization reaction, the cyclopentane ring of **1** is probably formed through pathway B. On the other hand, the deoxygenation via the intermediate may proceed according to the mechanism as shown in Figure 8 if we assume that it is analogous to the deoxygenation mechanism observed in the formation of deoxysugars biosynthesized from CDP-D-glucose.[12] Otherwise a dehydration from an intermediate with hydroxymethyl and tertiary hydroxyl groups at C-5 to produce a double bond between C-5 and C-6 may also afford a 6-aldehyde intermediate without a hydroxyl group at C-5. In pathway B, it may be possible that both of the two reactions involve 6-aldehyde intermediates, but only the deoxygenation reaction may involve a 6-aldehyde intermediate in the case of pathway A.

If the cyclization reaction involves an enzyme-bound NAD (NADP) for oxidation and subsequent reduction at C-4 or C-6 similarly to the case of *myo*-inositol 1-phosphate synthase,[13] deuterium loss from C-4 or C-6 of labeled glucosamine may not occur in the cyclization process. Therefore, retention of C-4 deuterium in the feeding experiment with [4-^{2}H]-D-glucosamine cannot rule out pathway A, and cyclization through the pathway B without loss of any deuteriums on C-6 of [6,6-$^2H_2$]-D-glucosamine is also possible. In such cases involving an enzyme-bound coenzyme, the loss of one of the two deuteriums on C-6 of glucosamine may be involved in the deoxygenation reaction.

The result of the feeding experiment with [1-^{2}H]-glucose suggests that a 1-oxo intermediate might not be involved in the deoxygenation step. Studies on enzyme systems involved in the biosynthesis of **1** are now in progress. An enzyme system that catalyzes the cyclization step would provide a convenient in vitro method to form a cyclopentanoid skeleton from a simple carbohydrate.

Experimental Section

Culture and Isolation of Allosamidin. One strain of *Streptomyces* sp. AJ9463 was used in this study.[3] Cultivation of the strain and isolation of allosamidin were performed according to the procedure described previously. In brief, spores of the strain were inoculated into Bennet medium (100 mL), which consisted of glucose (1%), peptone (0.2%), meat extract (0.1%), and yeast extract (0.1%) (pH 7.2) for preculture in a 500-mL Erlenmeyer flask, and the flask was incubated at 28 °C and 150 rpm on a rotary shaker for 48 h. This culture (2 mL) was transferred into the same medium (100 mL) for the main culture. After incubation for 120 h under the same conditions as those of preculture, the culture broth was filtered, and the mycelial cake obtained was extracted with 80% aqueous methanol. The extract was concentrated and adsorbed on a charcoal column. Allosamidin was eluted with 50% ethanol from the column, and the fraction was purified by HPLC (column, Capcell-Pak C_{18}, 4.6 mm × 250 mm, Shiseido; mobile phase, gradient elution of 0−50% CH_3CN in 10mM

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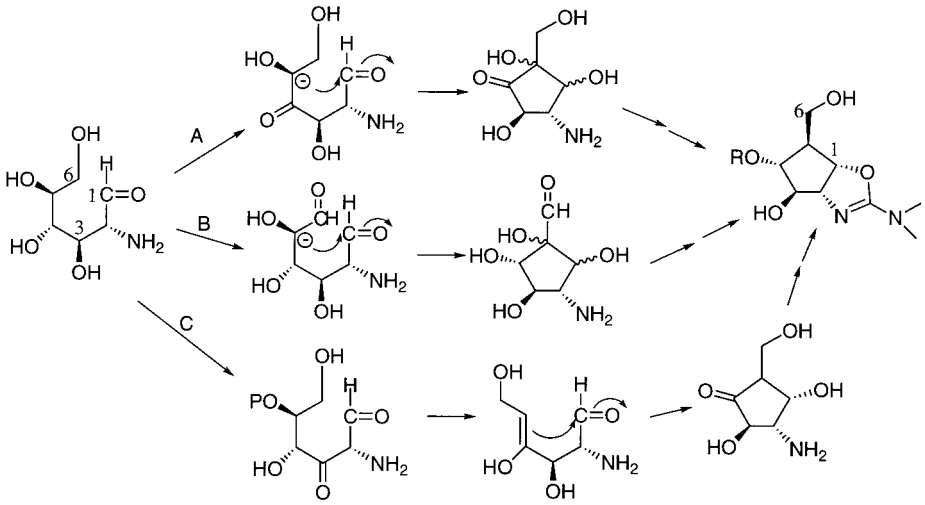

Figure 7. Plausible mechanism of cyclization of the cyclopentane ring of **1**. Glucosamine is used as a tentative substrate in the cyclization reaction.

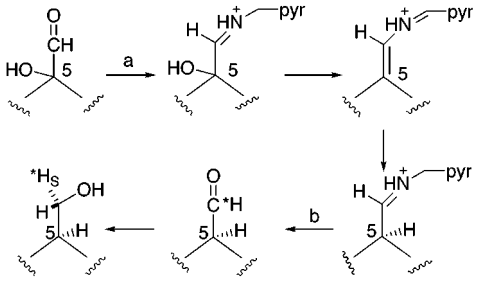

Figure 8. Plausible mechanism of deoxygenation at C-5 via 6-aldehyde intermediate. (a) Pyridoxamine 5′-phosphate. (b) H_2O.

$AcONH_4/NH_4OH$ pH 8.9 in 30 min; flow rate, 1 mL/min) to afford allosamidin.

Acid hydrolysis of allosamidin was performed with 4 N HCl at 100 °C for 4 h according to the procedure previously described.[2c] The hydrolysate obtained was purified by HPLC using the same conditions as used in the isolation of allosamidin to give D-allosamine and allosamizoline. Allosaminitol hexaacetate and allosamizoline triacetate were prepared by the method as previously described.[2c]

^{2}H-Labeled Glucosamines. [3-^{2}H]-D-Glucosamine and [4-^{2}H]-D-glucosamine were prepared from benzyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-*β*-D-glucopyranoside with $NaBH_4$ (98 atom % ^{2}H) according to the method of Bundle et al.[14] [3-^{2}H]-D-Glucosamine (98 atom % ^{2}H): FABMS (glycerol matrix) *m*/*z* 181 (M + H)$^+$; ^{1}H NMR (D_2O, 600 MHz) *δ* 3.51(0.65H, d, $J_{4,5}$ = 10 Hz, H-4 of α-anomer), 3.50(0.35H, d, $J_{4,5}$ = 10 Hz, H-4 of *β*-anomer), 3.32(0.65H, d, $J_{1,2}$ = 3.5 Hz, H-2 of α-anomer), 3.03(0.35H, d, $J_{1,2}$ = 8.5 Hz, H-2 of *β*-anomer). Other signals are the same as those of unlabeled glucosamine [^{1}H NMR (D_2O, 600 MHz) *δ* 5.47(0.65H, d, $J_{1,2}$ = 3.5 Hz, H-1 of α-anomer), 4.97(0.35H, d, $J_{1,2}$ = 8.5 Hz, H-1 of *β*-anomer), 3.94(0.35H, dd, $J_{5,6a}$ = 2 Hz, $J_{6a,6b}$ = 12 Hz, H-6a of *β*-anomer), 3.92(0.65H, ddd, H-5 of α-anomer), 3.92(0.65H, dd, $J_{3,4}$ = 10 Hz, $J_{2,3}$ = 10.5 Hz, H-3 of α-anomer), 3.88(0.65H, dd, $J_{5,6a}$ = 2 Hz, $J_{6a,6b}$ = 12 Hz, H-6a of α-anomer), 3.81(0.65H, dd, $J_{5,6b}$ = 5 Hz, $J_{6a,6b}$ = 12 Hz, H-6b of α-anomer), 3.78(0.35H, dd, $J_{5,6b}$ = 5 Hz, $J_{6a,6b}$ = 12 Hz, H-6b of *β*-anomer), 3.71(0.35H, dd, $J_{3,4}$ = 10 Hz, $J_{2,3}$ = 10.5 Hz, H-3 of *β*-anomer), 3.55(0.35H, ddd, H-5 of *β*-anomer), 3.51(0.65H, t, $J_{4,5}$ = $J_{3,4}$ = 10 Hz, H-4 of α-anomer), 3.50(0.35H, dd, $J_{4,5}$ = $J_{3,4}$ = 10 Hz, H-4 of *β*-anomer), 3.33(0.65H, dd, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 10.5 Hz, H-2 of α-anomer), 3.04(0.35H, dd, $J_{1,2}$ = 8.5 Hz, $J_{2,3}$ = 10.5 Hz, H-2 of *β*-anomer)]. [4-^{2}H]-D-Glucosamine (72 atom % ^{2}H): FABMS (glycerol matrix) *m*/*z* 181 (M + H)$^+$; ^{1}H NMR (D_2O, 600 MHz) *δ* 3.92(0.65H, d, $J_{2,3}$ = 10 Hz, H-3 of α-anomer), 3.92-(0.65H, H-5 of α-anomer), 3.71(0.35H, d, $J_{2,3}$ = 10 Hz, H-3 of *β*-anomer), 3.54(0.35H, dd, $J_{5,6a}$ = 2 Hz, $J_{5,6b}$ = 5 Hz, H-5 of *β*-anomer) Other signals are the same as those of unlabeled glucosamine.

[5-^{2}H]-D-Glucosamine and [6,6-$^2H_2$]-D-glucosamine were converted from [5-^{2}H]-D-glucose[15] and [6-$^2H_2$]-D-glucose,[16] respectively, by the method of Nishida et al.[17] [5-^{2}H]-D-glucosamine (70 atom % ^{2}H): FABMS (glycerol matrix) *m*/*z* 181 (M + H)$^+$; ^{1}H NMR (D_2O, 600 MHz) *δ* 3.93(0.35H, d, $J_{6a,6b}$ = 12 Hz, H-6a of *β*-anomer), 3.87(0.65H, d, $J_{6a,6b}$ = 12 Hz, H-6a of α-anomer), 3.80(0.65H, d, $J_{6a,6b}$ = 12 Hz, H-6b of α-anomer), 3.77(0.35H, d, $J_{6a,6b}$ = 12 Hz, H-6b of α-anomer), 3.50(0.65H, d, $J_{4,3}$ = 10 Hz, H-4 of α-anomer), 3.49(0.35H, d, $J_{4,3}$ = 10 Hz, H-4 of *β*-anomer). Other signals are the same as those of unlabeled glucosamine. [6,6-$^2H_2$]-D-Glucosamine: FABMS (glycerol matrix) *m*/*z* 182 (M + H)$^+$; ^{1}H NMR (D_2O, 600 MHz) *δ* 3.93(0.65H, d, $J_{5,4}$ = 10 Hz, H-5 of α-anomer), 3.55(0.35H, d, $J_{5,4}$ = 10 Hz, H-5 of *β*-anomer). Other signals are the same as those of unlabeled glucosamine.

Isopropylidene Derivative of 1 (3). 2,2-Dimethoxypropane (200 *μ*L) and a catalytic amount of anhydrous *p*-TsOH were added to a solution of **1** (2 mg) in dry DMF (200 *μ*L) at −20 °C, and the solution was stirred at −20 °C for 4 h. After adding aqueous 28% NH_4OH (200 *μ*L) and a solution of 10 mM $AcONH_4/NH_4OH$ (pH 8.9, 1 mL), the reaction solution was purified by HPLC (column, Capcell-Pak C_{18}, 10 mm × 250 mm, Shiseido; mobile phase, gradient elution of 0−50% CH_3CN in 10 mM $AcONH_4/NH_4OH$ pH 8.9 in 30 min; flow rate, 5 mL/min) to afford a solution of **3**. After lyophilizing the solution, the residue was dissolved in 0.1 N NaOH (300 *μ*L) because **3** was not stable in neutral or acidic condition. The yield of **3** was ca. 1.5 mg, which was estimated by HPLC analysis. In the same manner, labeled **3** (150 *μ*g) was obtained from labeled **1** (220 *μ*g) derived from (6*R*)-[6-$^2H_1$]-D-glucose. Data for **3**: ^{1}H NMR (0.1 N NaOD, 500 MHz) *δ* 4.70(1H, dd, *J* = 9.0 and 5.0 Hz, H-1), 3.89(1H, dd, *J* = 9.0 and 5.5 Hz, H-2), 3.66(1H, dd, *J* = 9.5 and 4.0 Hz, H-6R), 3.64(1H, dd, *J* = 7.5 and 5.5 Hz, H-3), 3.56(1H, dd, *J* = 9.5 and 7.5 Hz, H-4), 3.49(1H, dd, *J* = 9.5 and 8.5 Hz, H-6S), 3.18(3H, s, H-13), 2.80(6H, s, H-8 and 9), 2.07(1H, H-5), 1.33(6H, s, H-11 and 12); ^{13}C NMR (0.1 N NaOD, 125 MHz) *δ* 164.4(C-7), 102.2(C-10), 84.9(C-3), 83.8-(C-1), 75.8(C-4), 71.8(C-2), 61.2(C-6), 50.4(C-5), 49.1(C-13),

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37.8(C-8,9), 24.2(C-11,12); HMBC correlation H-6R to C-11, H-6S to C-11, H-13 to C-11, H-10 to C-11, H-12 to C-11.

Administration of Labeled Compounds to *Streptomyces* sp. AJ9463. The labeled glucosamine was dissolved in distilled water, and the solution was autoclaved before administration. The solution (1 mL) was added in one portion of each 500-mL flask containing the medium (100 mL) at the 36th hour of cultivation. In this manner, 12.0, 9.8, 6.5, and 11.0 mg of allosamidin was obtained from broths (21 × 100, 12 × 100,
10 × 100, and 19 × 100 mL) in feeding experiments in which each flask received [3-^{2}H]-D-glucosamine (25 mg), [4-^{2}H]-D-glucosamine (10 mg), [5-^{2}H]-D-glucosamine (12 mg), and [6,6-$^2H_2$]-D-glucosamine (10 mg).

In the feeding experiments with labeled glucose, the following replacement culture was used.[3] Fermentation was initiated under the same conditions as described above. After 38 h, the fermentation broth (100 mL) was transferred to a sterile centrifuge bottle, and the mixture was centrifuged at 6000*g* for 10 min. The supernatant was decanted, and the remaining mycelial pellet was resuspended in modified Bennet medium (100 mL) in which the glucose content was reduced to 0.2%. After replacement, fermentation was carried out in the usual manner. Under the replacement conditions, a mixture of (6*R*)-[6-$^2H_1$]-D-glucose (100 mg; 95 atom % ^{2}H, *R*:*S* = 9:1), (6*S*)-[6-$^2H_1$]-D-glucose (100 mg; 95 atom % ^{2}H, *S*:*R* = 9:1), or [1-^{2}H]-D-glucose (100 mg; 97 atom % ^{2}H; Sigma) and unlabeled D-glucose (100 mg) was added to each flask at the replacement time as a component of the medium. After cultivation and workup, 1.14, 0.71, and 4.0 mg of allosamidin was obtained from the broths (100 × 3, 100 × 3, and 100 × 5 mL) in the experiments with (6*R*)-[6-$^2H_1$]-, (6*S*)-[6-$^2H_1$]-, and [1-^{2}H]-D-glucose, respectively.

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Supporting Information Available: ^{2}H NMR spectra of allosamine and **1** derived from [3-^{2}H]-, [4-^{2}H]-, and [5-^{2}H]-D-glucosamine and ^{1}H, ^{13}C, HMBC, and NOESY spectra of **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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