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Asymmetric Total Synthesis of (+)-K01-0509 B: Determination of Absolute Configuration

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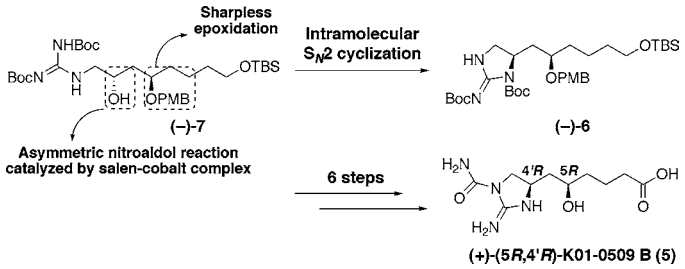
ABSTRACT



K01-0509 B is a novel natural product which contains a carbamoylated cyclic guanidine. Our asymmetric total synthesis features a Sharpless asymmetric epoxidation and a stereocontrolled construction of the cyclic guanidine via an asymmetric nitroaldol reaction, followed by intramolecular S_N2 cyclization. These reactions allowed the cyclic guanidine and the adjacent hydroxy group to be assembled, facilitating the asymmetric total synthesis and determination of the absolute stereochemistry of K01-0509 B.

K01-0509 B (**1**), a novel natural compound comprised of a carbamoylated cyclic guanidine, an adjacent hydroxy group, and a carboxylic acid, was isolated from the culture broth of *Streptomyces* sp. K01-0509 during a search for selective inhibitors of type III secretion system.[1] The type III secretion system is used by many Gram-negative pathogens, including enteropathogenic *Escherichia coli* (EPEC), enterohemoragic *E. coli* (EHEC), *Pseudomonas aeruginosa*, *Salmonella* spp., and *Shigella* spp.[2] to deliver their effector proteins into the host cell during the infection process.[3]

Consequently, a potent, selective inhibitor of type III secretion systems could prove to be a novel anti-infective drug. During recent screening of microbial resources for candidate compounds, we discovered three potent, selective inhibitors as typified by guadinomines A (**2**), B (**3**), and C (**4**) (Figure 1), which are also novel cyclic guanidine natural products, all derived from the same culture broth.[4,5] The planar structures of **1** and guadinomines were elucidated by detailed analysis of their 1-D and 2-D NMR spectra, but the relative and absolute configuration remained undefined due to insufficient quantities of the natural products. Moreover, the evidence for the connected positions of these carbamoyl groups were inconclusive because of the weak signals in the C−H long-range coupled NMR spectrum (HMBC) between

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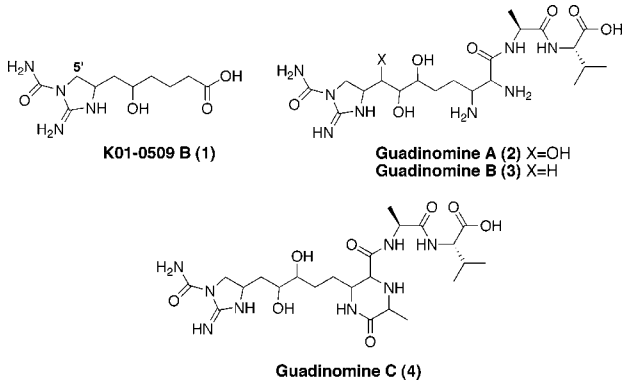


Figure 1. Structure of K01-0509 B and guadinomines.

carbamoyl carbon and H-5′ in **1** as well as guadinomines A (**2**), B (**3**), and C (**4**).

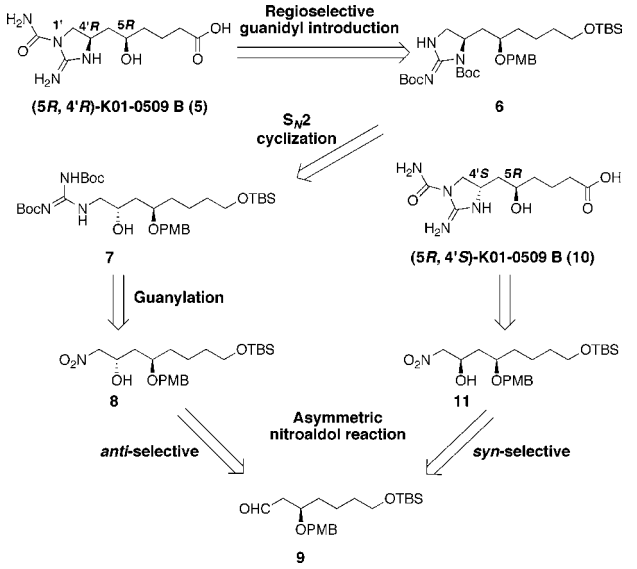
Although there are three natural products (enduracidin,[6] minosaminomycin,[7] and an amino acid derivative from the marine ascidian[8]) which contain a 5-membered cyclic guanidine (2-iminoimidazolidine), the structure is quite rare in natural products. Moreover, although the antimicrobacterial activity of such compounds is known, no other biological activity has been reported. Consequently, we undertook the total synthesis of guadinomines.

Although K01-0509 B (**1**) itself has no bioactivity against the type III secretion system, we regarded the compound as a key starting point. We expected that K01-0509 B (**1**) was a biosynthetic intermediate en route to guadinomines. Thus, the relative and absolute configuration of K01-0509 B (**1**) became of some importance for determining relative and absolute configurations and for refining the possible stereoisomers for a total synthesis of the guadinomines.

For determination of the relative and absolute stereochemistry of **1**, our approach was to devise an efficient synthesis that relied on readily available chiral material derived from a highly reliable asymmetric synthesis. Our strategy was designed to provide rapid access to all possible diastereomers to allow comparison of spectral data between synthetic and natural compounds.

Our synthetic strategy is shown in Scheme 1. We set up the absolute configuration of the hydroxy group at the C-5 position (*R*) and planned a divergent synthetic route to the two diastereomers (5*R*,4′*R*) and (5*R*,4′*S*). The regioselective introduction of the carbamoyl group into the 1′-nitrogen of (5*R*,4′*R*)-**5** was configured in the final phase. Thus, the synthesis of (5*R*,4′*R*)-**5** was planned from 1′-nitrogen unprotected cyclic guanidine **6** by oxidation and introduction

Scheme 1. Retrosynthetic Analysis of K01-0509 B[a]

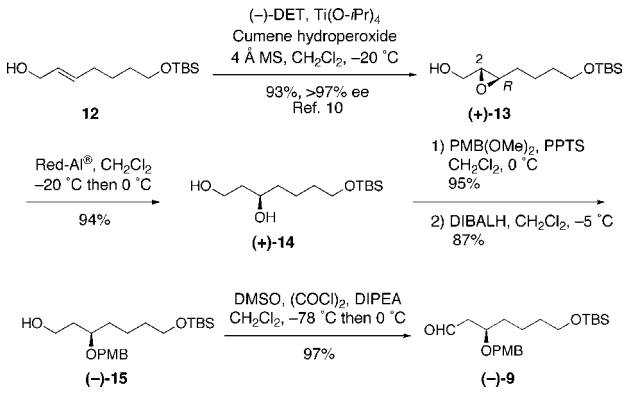


[a] PMB = *p*-methoxybenzyl.

of carbamoyl group, followed by full deprotection. The stereocontrolled construction of the cyclic guanidine, which is the key procedure of this total synthesis, uses intramolecular S_N2 cyclization of guanidine **7** and *anti*-selective asymmetric nitroaldol reaction of chiral aldehyde **9**. The *syn*-selective asymmetric nitroaldol reaction of **9** allowed us to synthesize the *syn*-nitroaldol **11** for the precursor of another diastereomer, (5*R*,4′*S*)-**10**.

An enantioselective synthesis of aldehyde (−)-**9** is outlined in Scheme 2. Sharpless asymmetric epoxidation of allyl

Scheme 2. Synthesis of Aldehyde (−)-**9**[a]



[a] (−)-DET = (−)-diethyl D-tartrate, DIPEA = *N*,*N*-diisopropylethylamine.

alcohol **12**[9] provided the epoxy alcohol (+)-**13** in 93% yield and 97% ee.[10] Reductive opening of the epoxide with Red

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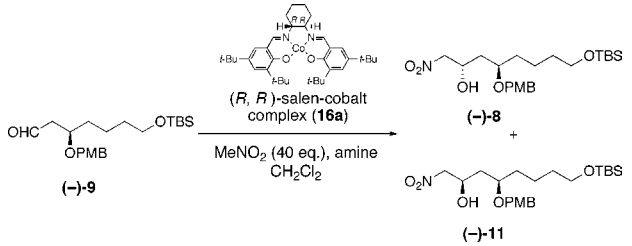
Al proceeded at the C-2 position with excellent regioselectivity and furnished the 1,3-diol (+)-**14**; the primary alcohol (−)-**15** was then synthesized by chemoselective protection of secondary alcohol through the acetalization and reductive cleavage of the resulting acetal ring. Subsequent Swern oxidation of (−)-**15** afforded aldehyde (−)-**9** in 97% yield. After the precursor was obtained for the nitroaldol reaction, we examined the asymmetric nitroaldol reaction of aldehyde (−)-**9**. Initially, we used Trost's asymmetric catalyst,[12] which involves a dinuclear zinc complex center with a chiral semi-azacrown ligand for this asymmetric nitroaldol reaction. Unfortunately, the attempts to use this catalyst were unsuccessful. The stereoselectivity was very high (up to 92% de), but the yield (55%) of desired product was unsatisfactory.

We subsequently applied Yamada's conditions[13] for this step on account of its mildness and convenience. Furthermore, both enantiomers of the catalysts (**16a**, **16b**) are available commercially and more easily at hand. The results of our asymmetric nitroaldol reaction toward the aldehyde (−)-**9** are shown in Table 1.

Table 1. Investigation of Asymmetric Nitroaldol Reaction of (−)-**9** Using Salen Cobalt Complexes ((*R*,*R*) = **16a**, (*S*,*S*) = **16b**)



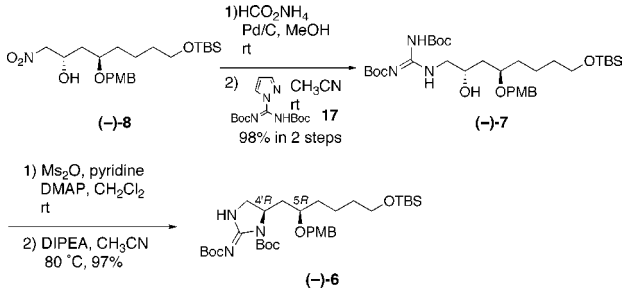
entry[a]	cat. (equiv)	bases (equiv)	time (h)	yield[e] (%)	selectivity[k] (% de)
1[b]	**16a** (0.02)	DIPEA (1.0)	63	**8**:[f] 22[g]	46
2	**16a** (0.02)	DIPEA (1.0)	62	**8**: 31[h]	78
3[c]	**16a** (0.02)	DIPEA (1.0)	129	**8**: 71[i]	34
4	**16a** (0.02)	DBU (1.0)	0.75	**8**: 94	0
5	**16a** (0.1)	DIPEA (1.0)	129	**8**: 29[j]	98
6	**16a** (0.02)	DIPEA (3.0)	97	**8**: 73	81
7	**16a** (0.1)	DIPEA (2.5)	60	**8**: 89	97
8[d]	**16b** (0.1)	DIPEA (2.5)	69	**11**: 92	78

[a] Unless otherwise noted, all reactions were run at −40 °C. [b] Carried out at −78 °C. [c] Carried out at −20 °C. [d] (*S*,*S*)-salen cobalt complex (**16b**) was used. [e] Isolated yields. [f] The absolute stereochemistry was determined by analysis of the Mosher's ester of compound **7**.[14] [g] 65% of **9** was recovered. [h] 39% of **9** was recovered. [i] 17% of **9** was recovered. [j] 15% of **9** was recovered. [k] Diastereomeric excesses were determined by HPLC using Chiralcel OD and hexane/2-propanol (99/1) as eluent.

We carried out the reaction at various temperatures under the standard conditions[12b] reported by Yamada et al. At −78 and −20 °C, the selectivity was very low (entries 1 and 3). When we effected the reaction at −40 °C, the diastereoselectivity of the *anti*-product (−)-**8** was up to 78% de, but the yield was still low (entry 2). For improvement in yield, we examined the type of base. By using DBU as a base, the reaction afforded the product (−)-**8** in 94% yield, with no selectivity (entry 4). Varying the equivalents of catalyst and DIPEA (entries 5−7), revealed that the best conditions were as in entry 7, which requires 0.1 equiv of catalyst, 2.5 equiv of DIPEA; this afforded a high yield (89%) of product with excellent selectivity (97% de). However, in the case of application of (*S*,*S*)-salen cobalt complex (**16b**), we obtained the corresponding *syn*-diastereomer (−)-**11** in high yield (92%) with good selectivity (78% de) (entry 8).

The construction of the cyclic guanidine part is shown in Scheme 3. Introduction of guanidyl group was achieved

Scheme 3. Synthesis of Cyclic Guanidine (−)-**6**[a]



[a] Ms_2O = methanesulfonic anhydride.

through reduction of the nitro group to the primary amine with Pd and ammonium formate, followed by guanylation with the reagent **17**.[15] After the preparation of guanidine compound (−)-**7**, we chose to investigate the S_N2 cyclization procedure for construction of 5-membered cyclic guanidine. Treatment of guanidine compound (−)-**7** with Ms_2O, pyridine, and DMAP in CH_2Cl_2 provided the corresponding mesylate. Then exposure of the mesylate to tertiary amine and heat allowed us to obtain the desired cyclic guanidine (−)-**6** in 97% yield with inversion of the C-4′ stereocenter.

To deliver the (5*R*,4′*R*)-K01-0509 B (**5**) from (−)-**6**, introduction of the carbamoyl function and oxidation to carboxylic acid remained. Deprotection of the TBS group with TBAF, followed by oxidation of the resulting primary alcohol, furnished the aldehyde (−)-**18** in excellent yield (Scheme 4). With this aldehyde, we introduced the carbamoyl function on the guanidyl nitrogen. Further oxidation by treatment with $NaClO_2$ under standard conditions (NaH_2PO_4, 2-methyl-2-butene) gave the carboxylic acid (−)-**19**. Removal of the PMP group using CAN in a mixture (1:1) of

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(14) The synthesis of Mosher's ester of **6** and the results of the analysis are described in the Supporting Information.

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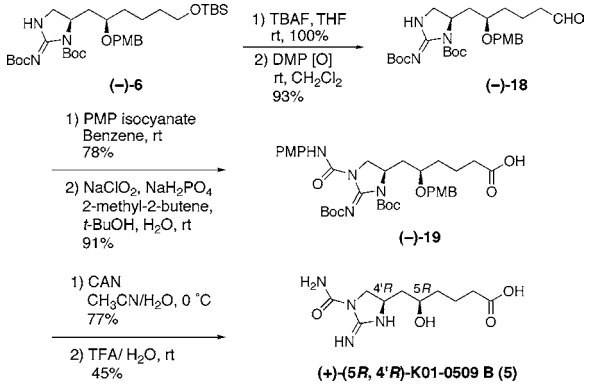
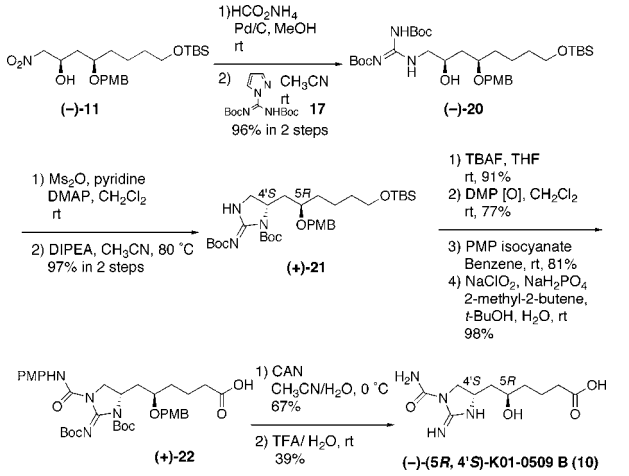
Scheme 4. Synthesis of (+)-(5*R*,4′*R*)-K01-0509 B (**5**)[a]



[a] PMP = *p*-methoxyphenyl. DMP [O] = Dess−Martin periodinane.

Scheme 5. Synthesis of (−)-(5*R*,4′*S*)-K01-0509 B (**10**)



CH_3CN and H_2O at 0 °C,[16] followed by deprotection of three protective groups in aqueous TFA, produced the desired compound. After purification by HPLC, we obtained the final compound (+)-(5*R*,4′*R*)-K01-0509 B (**5**).

To obtain the *anti*-diastereomer (5*R*,4′*S*)-K01-0509 B (**10**), we started synthesis from the *syn*-nitroaldol product (−)-**11** (Scheme 5). The guanylation process involved reduction and nucleophilic addition to reagent **17**, affording (−)-**20** in 96% yield. S_N2 cyclization via the *O*-mesylate provided (5*R*,4′*S*)-cyclic guanidine (+)-**21**. Introduction of the carbamoyl function and formation of carboxylic acid was carried out in the same way as for the synthesis of (5*R*,4′*R*)-isomer (−)-**19**. Total deprotection of compound (+)-**22** gave the *anti*-diastereomer (−)-(5*R*,4′*S*)-K01-0509 B (**10**).

We compared the spectral data of the two diastereomers (+)-**5** and (−)-**10** with natural (+)-**1**. (+)-(5*R*,4′*R*)-K01-0509 B (**5**) was completely identical to the natural product in all respects including the ^1H NMR (300 MHz, in D_2O), ^{13}C NMR (75 MHz, in D_2O), HPLC,[17] HRMS, IR, optical rotation (synthetic: (+)-(5*R*,4′*R*)-K01-0509 B (**5**), $[\alpha]^{26}_D$ +32.4, *c* 0.16, MeOH; natural: K01-0509 B (+)-**1**, $[\alpha]^{25}_D$ +31.9, *c* 0.10, MeOH). On the other hand, (−)-(5*R*,4′*S*)-K01-0509 B (**10**) was not identical to the natural material in all respects and was more labile than the natural compound (+)-**1**. Therefore, the synthetic compound **5** corresponds to natural K01-0509 B.

In conclusion, we have achieved asymmetric total synthesis of (+)-K01-0509 B and its diastereomer. As a result of these syntheses, we have elucidated the relative and absolute configuration of (+)-K01-0509 B and confirmed the connected position of the carbamoyl group. We expect that the stereochemistry of K01-0509 B will provide insights and guidance for the future total synthesis and determination of the absolute stereochemistry of the closely related guadinomines.

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Supporting Information Available: Experimental procedures and spectral data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL062282Z

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(17) The ^1H NMR spectra and HPLC charts of synthetic compounds and natural K01-0509 B are given in the Supporting Information.