Natural Product Synthesis

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Total Synthesis of Bacilosarcins A and B**

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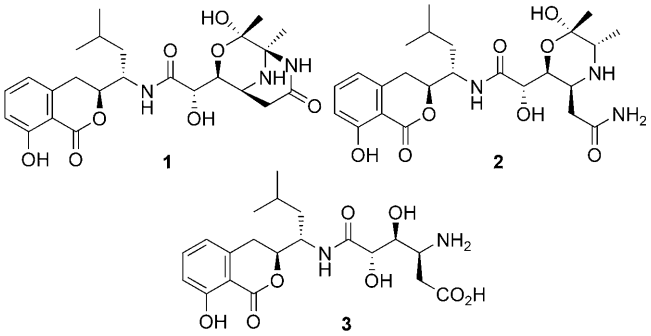
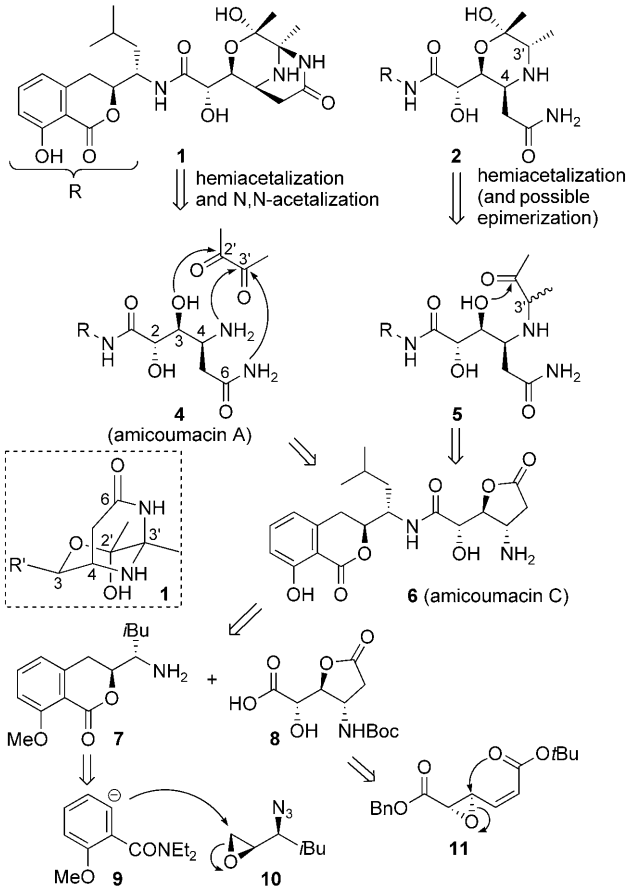
In the course of screening for plant growth regulators of microbial origin, Igarashi and co-workers discovered two novel herbicidal substances, bacilosarcins A and B, in the culture broth of the bacterium *Bacillus subtilis* TP-B0611 isolated from intestinal contents of the sardine *Sardinops melanostica*. Extensive spectroscopic analyses and chemical conversions allowed the elucidation of their structures as **1** and **2**, respectively (Scheme 1).[1] The growth inhibitory



Scheme 1. Structures of Bacilosarcins A (**1**), B (**2**), and AI-77-B (**3**).

activity of bacilosarcin A (**1**) was remarkably more potent against barnyard millets than that of herbimycin A (an ansamycin antibiotic with potent herbicidal activity),[2] while the herbicidal activity of **2** was weak. To date, the mode of action of **1** and **2** remains entirely unknown. From a structural viewpoint, both **1** and **2** belong to a small family of natural products that is characterized by a dihydroisocoumarin ring system linked to an unusual amino acid, as represented by AI-77-B (**3**; known as an antiulcerogenic substance produced by *Bacillus pumilus*).[3] At first glance, the molecular architectures of bacilosarcins A and B captured our interest, since the 3-oxa-6,9-diazabicyclo[3.3.1]nonane ring system incorporated in **1** was totally unprecedented both in natural and synthetic compounds, and the 2-hydroxymorpholine substructure contained in **2** was a very rare structural motif in natural products.[4] The structural novelty of these two natural products and the potent herbicidal activity of **1** prompted us to embark on the total synthesis of **1** and **2**, as well as structure–activity relationship studies to develop a new type of herbicide, which would also lead to the elucidation of their action mechanism. We describe herein the first total synthesis of bacilosarcins A (**1**) and B (**2**) together with a new efficient synthesis of AI-77-B (**3**).

Bacilosarcin A (**1**), which has the unusual heterobicyclic ring system, can be traced retrosynthetically to two simpler precursors, **4** (amicoumacin A)[5] and 2,3-butanedione, by dissecting the hemiacetal and N,N-acetal bonds of the bicyclic ring portion (Scheme 2). We expected that the formation of



Scheme 2. Retrosynthetic analysis of **1** and **2**.

the heterobicyclic ring system of **1**, in which the hydroxy group at the C3 position and the amino groups at the C4 and C6 positions of **4** participate, could take place preferentially by simply mixing **4** and 2,3-butanedione under appropriate acidic conditions, since molecular modeling studies showed

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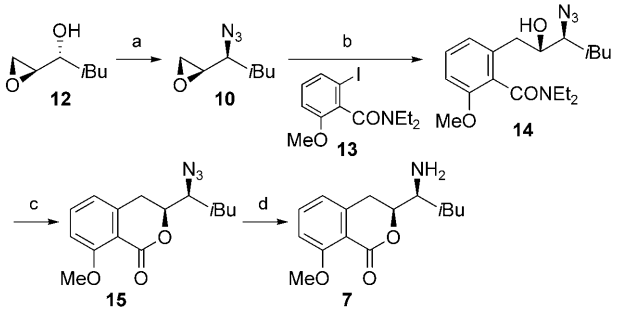
that any other mode of cyclization would lead to less stable bicyclic rings. The desired ring formation that leads to **1** should be favored by the thermodynamically preferred orientations of the substituents on the bicyclic ring of **1**—the axially oriented hydroxy group at the anomeric C2′ position as well as the three equatorially oriented alkyl groups at the C3, C2′, and C3′ positions (see the conformational diagram in Scheme 2). The cyclization precursor **4** could be obtained from **6** (amicoumacin C)[3d] by selective ammonolysis of its γ-lactone. On the other hand, the 2-hydroxymorpholine ring of **2** should be formed spontaneously from **5**, which could also be elaborated from **6** through N-alkylation of the amino group on the γ-lactone ring followed by ammonolysis of the resulting product. Since the stereochemical control at the nitrogen-bearing C3′ stereogenic center of **5** seemed to be difficult because of its proclivity for enolization, we decided to conduct the N-alkylation in a nonstereoselective manner. Instead we envisaged that the enolizability at the C3′ position of **5** could be exploited for the stereoconvergent formation of **2** by equilibration of the two epimers of **5**, since the formation of the 3′β-epimer of **2** seemed to be difficult because of severe 1,3-diaxial steric repulsion between its 3′β-methyl group and 4β-substituent. The common amide intermediate **6** was further divided into amine segment **7** and acid segment **8**. To date, various synthetic routes to **7** (or its nonmethylated derivative) and analogues of **8** have been developed by many research groups in their efforts toward the total synthesis of AI-77-B (**3**),[6–8] most of which successfully converted natural amino acids or sugars into the amine and acid segments. Any of these routes, however, seemed to leave room for improvement in reproducibility, selectivity, and conciseness. We planned to prepare **7** and **8** in a more efficient manner by utilizing the intermolecular epoxide ring opening of **10** with the aromatic nucleophile **9** and the intramolecular epoxide ring opening of **11**, respectively.

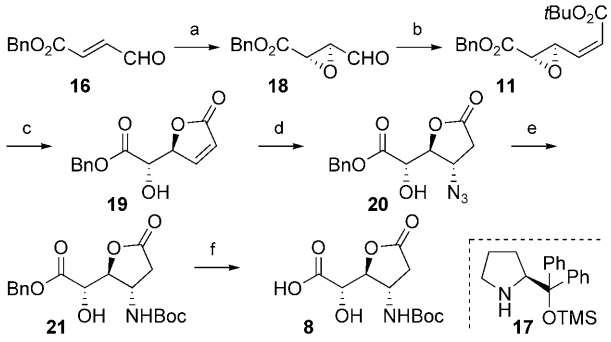
Our synthesis of the amine segment **7** began with the transformation of epoxy alcohol **12** into azido epoxide **10** with inversion of configuration (Scheme 3);[9] **12** in turn was readily produced in high yields (>90%) on multigram scales (>10 g) by the Sharpless kinetic resolution of 5-methyl-1-hexen-3-ol.[10] The epoxide ring of **10** was opened under the conditions reported by Ganem and co-workers with



Scheme 3. Preparation of amine segment **7**. Reagents and conditions: a) $(PhO)_2P(O)N_3$, DEAD, Ph_3P, THF, RT, 69%; b) *n*BuLi, BF_3OEt_2, toluene, −78°C, 48%; c) CSA, toluene, 100°C, 87%; d) H_2, 10% Pd/C, EtOH, RT, 94%. DEAD = diethyl azodicarboxylate, CSA = camphorsulfonic acid.

the lithium anion generated from **13**.[11,12] Treatment of the resulting azido alcohol **14** with acid afforded lactone **15** (96% *ee*, as determined by HPLC analysis using a chiral stationary phase). Finally, **15** was reduced to **7** by catalytic hydrogenation. Thus, our new synthesis of **7** was accomplished in 27% overall yield from **12** in only four highly reproducible steps.

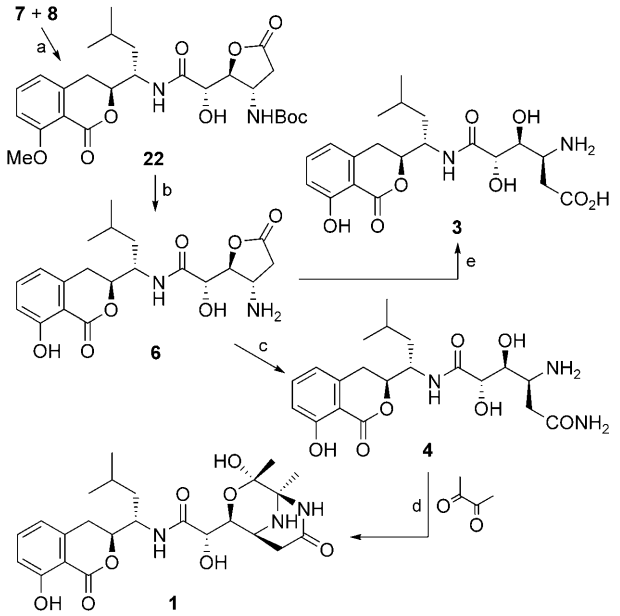
Scheme 4 shows our six-step synthesis of the acid segment **8** from the previously reported aldehyde **16**.[13] Asymmetric epoxidation of **16** under the organocatalytic conditions reported by Córdova and co-workers proceeded smoothly to give **18**.[14] Chain elongation of **18** by Ando's protocol



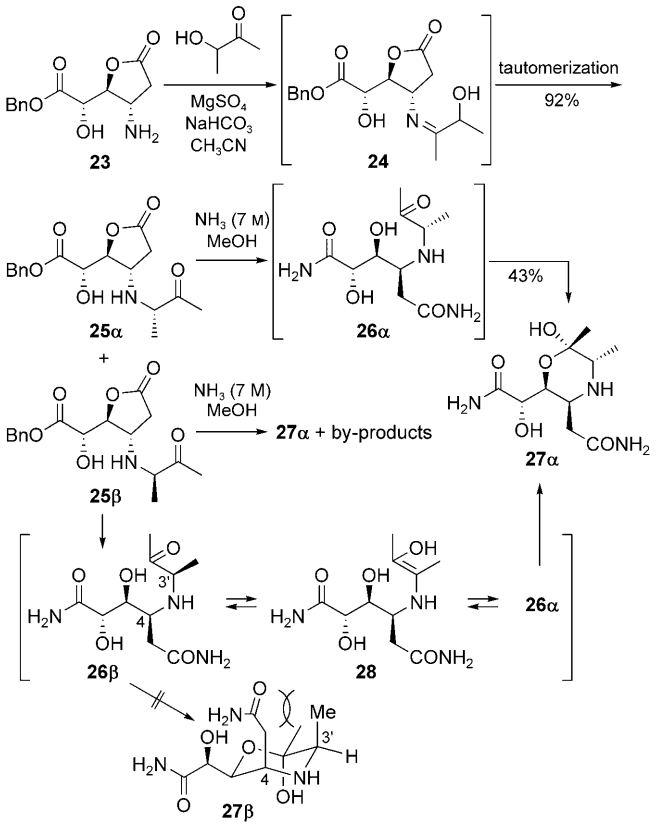
Scheme 4. Preparation of acid segment **8**. Reagents and conditions: a) aq H_2O_2, **17**, $CHCl_3$, RT, 91%; b) $(PhO)_2P(O)CH_2CO_2tBu$, NaH, THF, −78°C→RT, 69%; c) TFA, CH_2Cl_2, RT, 75%; d) NaN_3, aq AcOH, RT, 51% (71% based on recovered starting material); e) H_2, PtO_2, Boc_2O, EtOH, RT, 82%; f) H_2, Pd/C, EtOH, RT, quantitative. TFA = trifluoroacetic acid, Boc = *tert*-butoxycarbonyl.

afforded a 7:1 mixture of **11** and its *E* isomer.[15] Treatment of the mixture with TFA in CH_2Cl_2 brought about a smooth intramolecular epoxide ring-opening reaction to give hydroxy lactone **19**,[16] which was then reacted with sodium azide in aqueous acetic acid.[17] The resulting conjugate adduct, which was obtained in 51% yield (71% based on recovered starting material) as a 5:1 mixture of **20** and its epimer at the azide-bearing stereogenic center was subjected to catalytic hydrogenation in the presence of Boc_2O to furnish **21** (97% *ee*, as determined from the NMR spectra of the corresponding (*R*)- and (*S*)-MTPA esters (MTPA = α-methoxy-α-(trifluoromethyl)phenylacetyl). Finally, hydrogenolysis of the benzyl ether resulted in the quantitative formation of **8**. Thus, the synthesis of **8** was achieved in an overall yield of 20% from **16** in a concise six-step sequence.

Having succeeded in the efficient short-step syntheses of the amine segment **7** and the acid segment **8**, we turned our attention to their condensation and subsequent elaboration into bacilosarcin A (**1**; Scheme 5). Condensation of **7** and **8** proceeded smoothly to give amide **22**.[18] Unmasking of the methyl-protected phenolic hydroxy and Boc-protected amino groups of **22** were effected in one pot by treating **22** with BBr_3/CH_2Cl_2 in the presence of anisole to afford **6** (amicoumacin C, isolated as its hydrochloride salt by treatment of **6** with HCl/MeOH).[19] The hydrochloride salt of **6** was treated with methanolic ammonia to give **4** (amicoumacin A), which was then mixed with 2,3-butanedione under various sets of acidic



Scheme 5. Completion of the synthesis of bacilosarcin A (**1**). Reagents and conditions: a) EDC·HCl, DMAP, HOBt, CH_2Cl_2/DMF, −10 °C, 62 %; b) BBr_3, anisole, CH_2Cl_2, −78 to −5 °C, 73 %; c) NH_3(7 M)/MeOH, −5 °C; d) NH_4Cl, CH_3CN/MeOH/H_2O, RT, 57 % (2 steps); e) aq NaOH (0.02 M, pH 9) then aq HCl (0.02 M, pH 6.5), EtOH/H_2O, 90 %. EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, DMAP = 4-dimethylaminopyridine, HOBt = 1-hydroxybenzotriazole.

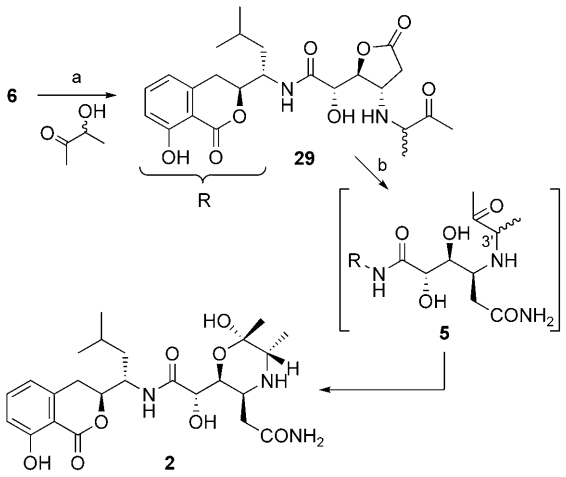


Scheme 6. N alkylation of model substrate **23** and morpholine ring formation from the resulting products, **25α** and **25β**.

conditions. Among the reaction conditions attempted, treatment of **4** with a catalytic amount of NH_4Cl in CH_3CN/MeOH containing a small amount of water gave the best result, and furnished the desired cyclization product **1** in 57 % yield from **6**·HCl. The spectral data of **1** were identical to those of the natural product.[1] Thus, the first total synthesis of bacilosarcin A was achieved in 5.1 % overall yield from **16** in 10 steps. The hydrochloride salt of **6** was also converted into AI-77-B (**3**) by following a reported procedure.[6] The new synthesis of AI-77-B was accomplished in 8.0 % overall yield from **16** in 9 steps.

For the synthesis of bacilosarcin B (**2**), we needed to install a 3-oxobut-2-yl substituent at the amino group of **6**. This N-alkylation reaction was examined by using model substrate **23**, which was obtained as its TFA salt by deprotection of **21** with TFA (Scheme 6). After several unsuccessful attempts (see the Supporting Information), we found that the N-alkylation could be achieved by simply treating **23**·TFA with 3-hydroxy-2-butanone in the presence of $MgSO_4$ and $NaHCO_3$ in CH_3CN, to give a 1:1 epimeric mixture of **25α** and **25β** in an excellent yield of 92 % after column chromatography. It is proposed that this reaction, known as the Amadori rearrangement,[20] proceeds via the imine intermediate **24**, followed by tautomerization to the two epimers. Fortunately, the epimers were found to be separable by careful preparative TLC, which provided us with the opportunity to investigate the aforementioned possibility that both epimers of **5** (Scheme 2) could be transformed stereoconvergently into the thermodynamically more stable stereoisomer **2** by epimerization at the C3′ position of **5**. Monitoring the reaction by TLC showed that when the less polar epimer **25α** was subjected to ammonolysis conditions, a clean reaction took place, from which **27α** was isolated in 43 % yield.[21] On the other hand, exposure of the more polar epimer **25β** to the same conditions gave a complex mixture in which, however, we could detect a considerable amount of **27α** by ^{1}H NMR spectroscopy. These results suggest that firstly, the less polar epimer, to which we assigned the structure **25α**, was first converted into **26α** under the ammonolysis conditions; this was then converted smoothly into **27α**, which possesses the equatorially oriented 3′α-methyl group; and secondly, in the case of the more polar epimer **25β**, the ring formation of ammonolysis product **26β** into **27β** was precluded because of the severe steric repulsion between the C4 carbamoylmethyl group and the axially oriented C3′ methyl substituent in **27β**. This repulsion resulted in the observed formation of **27α** by enolization of **26β** to **28** followed by tautomerization to **26α**.

With the encouraging results in the model studies in hand, we proceeded to the final stage of the total synthesis of bacilosarcin B (**2**; Scheme 7). When **6**·HCl was subjected to the Amadori rearrangement conditions, **29** was formed in 72 % yield.[22] Finally, treatment of **29** with NH_3/EtOH (2 M) furnished **2** in 39 % yield (isolated as its hydrochloride salt by treatment of **2** with HCl/MeOH) via intermediate **5**. In this case, we could not obtain any direct evidence for the



Scheme 7. Completion of the synthesis of bacilosarcin B (**2**). Reagents and conditions: a) $MgSO_4$ (5.0 equiv), $NaHCO_3$ (1.0 equiv), CH_3CN, 75 °C, 72%; b) NH_3(2 M)/EtOH, 39%.

stereoconvergent formation of **2** from both the 3′α and 3′β epimers of **5**, since the epimeric mixture **29** could not be separated. However, the detection of a trace amount of **4** (amicoumacin A, Scheme 5) in the crude product mixture by NMR spectroscopy was considered to indirectly indicate the intervention of equilibrium reactions (Scheme 6). This is because **4**, which lacks the 3-oxobut-2-yl substituent, would probably be generated by isomerization of **5** to the corresponding α-hydroxy imine intermediate and its hydrolysis with a trace amount of water contained in the reaction solvent. The spectral data of **2**·HCl were identical with those of natural bacilosarcin B.[23] The overall yield of **2** from **16** was 2.5% (10 steps).

In conclusion, the first total syntheses of bacilosarcins A (**1**) and B (**2**) that contain unusual heterocyclic ring systems in their molecular architectures have been accomplished by the thermodynamically controlled ring-forming reaction between cyclization precursor **4** (amicoumacin A) and 2,3-butanedione for **1**, and the nonstereoselective N-alkylation of **6** (amicoumacin C) with 3-hydroxy-2-butanone under Amadori rearrangement conditions coupled with stereoconvergent cyclization under equilibrium conditions for **2**. The common intermediates **7** and **8** were prepared by highly reproducible short-step sequences involving inter- and intramolecular epoxide ring-opening reactions, respectively. The improved accessibility to **7** and **8** enabled us to achieve a new efficient synthesis of AI-77-B (**3**), and will also facilitate the preparation of analogues of **1** and **2** for structure–activity relationship studies to develop new types of herbicides.

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[21] The moderate yield (43%) of the conversion of **25α** into **27α** despite the smooth transformation observed by TLC can be ascribed to the difficulty in the isolation of **27α** because of its extremely high polarity.
[22] The ^{1}H NMR spectrum of **29** indicated that a small degree of asymmetric induction took place in the Amadori reaction of **6**·HCl, which give **29** as a 3:2 epimeric mixture, instead of a 1:1 mixture, as in the case of the model compound **25**.
[23] The ^{1}H and ^{13}C NMR spectra (in CD_3OD) of **2**·HCl synthesized by us were identical to those of natural bacilosarcin B, while those of our synthetic sample of **2** (free amine) were not. We were informed of the possibility that the natural bacilosarcin B that was studied by NMR spectroscopy was not the free amine, but instead an ammonium salt formed from the free amine and KH_2SO_4, since the final purification of natural bacilosarcin B was performed by reverse-phase HPLC using MeCN/0.15% KH_2PO_4 (pH 3.5) as eluent. This possibility was supported by our observation that the ^{1}H NMR spectrum of a sample prepared by mixing the free amine **2** with aqueous KH_2PO_4 and then concentrating the resulting mixture showed very good agreement with that of natural bacilosarcin B as well as with that of **2**·HCl, but was significantly different from that of the free amine (see the Supporting Information).

