Total Synthesis and Reassignment of Configuration of Aeruginosin 298-A

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Aeruginosin 298-A (**1**) is a peptidic active-site protease inhibitor containing nonstandard amino acids produced by a blue-green alga, reported for the first time in 1994,[1] whose structural elucidation was not effected until 1998.[2] At that time, the X-ray crystallographic structure of the ternary complex of 298-A bound to hirugen−thrombin was reported, revealing several unexpected interactions that may be useful and of importance for structure-based drug design.

The structure **1a** depicted in Figure 1 was assigned to aeruginosin 298-A in which the core ring consisting of a 2-carboxy-6-hydroxyoctahydroindole (choi) is unprecedented among natural or synthetic products. Until now, fourteen members of the aeruginosin family have been identified,[3,4] all of which share the aforementioned new bicyclic α-amino acid choi or a closely related derivative.[5]

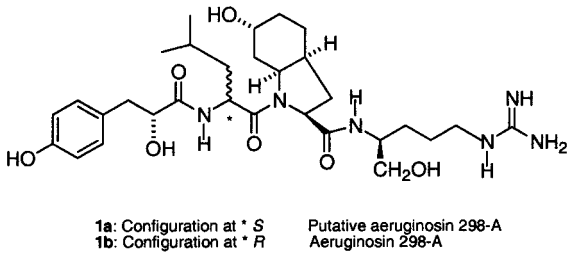


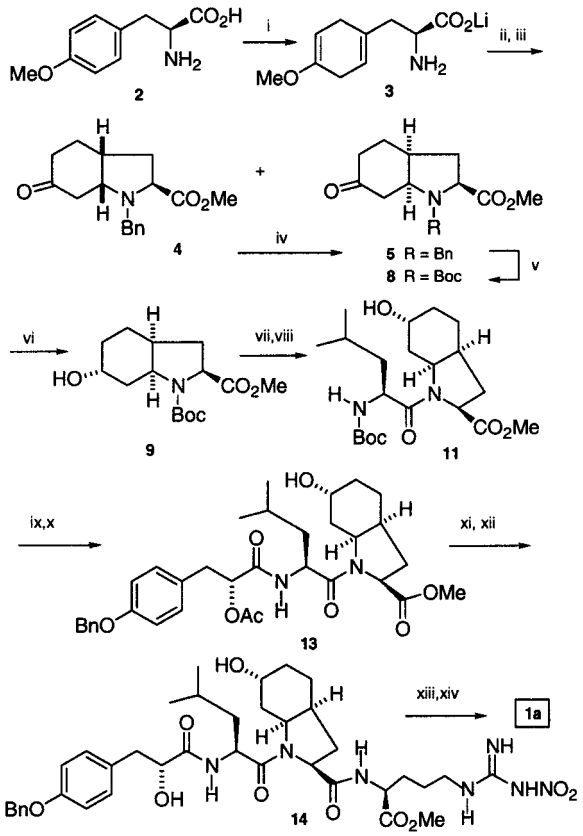
Figure 1. Configuration at **S* putative aeruginosin 298-A (**1a**) and configuration at **R* Aeruginosin 298-A (**1b**).

In this paper, we described the first total synthesis of aeruginosin 298-A and in turn clarify its configuration, since we also report that structure **1a** does not correspond to natural aeruginosin 298-A. We give synthetic evidence that compound **1b**, incorporating a D-Leu (not L-Leu), corresponds to the natural structure. The strategy we have developed for assembling the four units of the peptide involves the construction of an appropriately functionalized and stereochemically pure octahydroindole (choi core) and the coupling with the other fragments.

Initially, we synthesized the putative aeruginosin 298-A (**1a**). The starting material for our synthesis was the protected L-tyrosine **2**, which was submitted to the Birch reduction conditions followed by treatment of the resulting dihydroanisole **3** with a methanol solution of hydrogen chloride to give a mixture of octahydroindol-6-ones,[6] which were benzylated to furnish a diastereomeric mixture of exo and endo isomers in a 1.8:1 ratio. After chromatographic separation of both isomers, the exo isomer **4** was transformed to the desired isomer endo **5** by warming in methanol containing hydrogen chloride, taking advantage of the β-amino ketone moiety. The equilibration process took place in more than 90% of extension in favor of the endo isomer. After these operations octahydroindolone **5** with the adequate relationship between its three stereocenters was available in multigrams amounts, the overall yield for these four initial steps being 44%.

To achieve the functionalization and stereochemistry adequate at C-6, ketone **5** was subjected to reduction under several conditions, but in all cases the undesired isomer **6** was the major product irrespective of the hydride used (i.e. $NaBH_4$ or LS-selectride). This fact is probably due to the conformation of

Scheme 1. Synthesis of the Putative Aeruginosin 298-A (**1a**)[a]



[a] Reagents and conditions: (i) Li, NH_3, THF-*t*BuOH (2.5:1), −78 °C; (ii) MeOH−7.5 N HCl, 35 °C, 48 h; (iii) BnBr, $NaHCO_3$, EtOH, 70 °C, 6 h; (iv) MeOH−8 N HCl, 65 °C, 17 h (44% from **2**), (v), H_2, $Pd(OH)_2$ 20%, Boc_2O, EtOAc, room temperature, 48 h, 79%, (vi) LS-selectride (1 M in THF, 1.3 equiv), THF, −78 °C, 53% for isomer **9**, (vii) TFA, CH_2Cl_2, 0 °C, 45 min; (viii) Boc-L-Leu, BOP, NMM, CH_2Cl_2, 0 °C, 30 min, then room temperature, 22 h, 43% from **9**; (ix) TFA, CH_2Cl_2, 0 °C, 45 min; (x) **12**, BOP, NMM, CH_2Cl_2, 0 °C, 30 min, then room temperature, 18 h, 60% from **11**; (xi) 0.1 N LiOH, THF, room temperature, 20 h, 92%; (xii) L-Arg(NO_2)OMe.HCl, BOP, NMM, DMF, 0 °C, 30 min, then room temperature, 24 h 46%; (xiii) $LiBH_4$ (2 M in THF), THF, room temperature, 3 h, 50%; (xiv) H_2, Pd−C 10%, EtOAc−MeOH, 6 N HCl, 1 atm, room temperature, 8 h, 70%.

(1) Murakami, M.; Okita, Y.; Matsuda, H.; Okino, T.; Yamaguchi, K. *Tetrahedron Lett.* **1994**, *35*, 3129.

(2) Rios Steiner, J. L.; Murakami, M.; Tulinsky, A. *J. Am. Chem. Soc.* **1998**, *120*, 597.

(3) (a) Aeruginosins 98-A and B: Murakami, M.; Ishida, K.; Okino, T.; Okita, Y.; Matsuda, H.; Yamaguchi, K. *Tetrahedron Lett.* **1995**, *36*, 2785. Sandler, B.; Murakami, M.; Clardy, J. *J. Am. Chem. Soc.* **1998**, *120*, 596. (b) Aeruginosins 102-A and B: Matsuda, H.; Okino, T.; Murakami, M.; Yamaguchi, K. *Tetrahedron* **1996**, *52*, 14501 (c) Aeruginosin 103-A: Kodani, S.; Ishida, K.; Murakami, M. *J. Nat. Prod.* **1998**, *61*, 1046. (d) Aeuginosins 89-A and B, 98-C, 101 and 298-B: Ishida, K.; Okita, H.; Matsuda, H.; Okino, T.; Murakami, M. *Tetrahedron* **1999**, *55*, 10971.

(4) Microcin SF608, isolated in the Red Sea, has the same structural pattern: Banker, R.; Carmeli, S. *Tetrahedron* **1999**, *55*, 10835.

(5) Aeruginosins 205-A and 205-B show a 6-chloro substituent in the octahydroindole ring: Shin, H. J.; Matsuda, H.; Murakami, M.; Yamaguchi, K. *J. Org. Chem.* **1997**, *62*, 1810.

(6) For the use of tyrosine compounds as building blocks for the synthesis of octahydroindolone derivatives, see: (a) Bonjoch, J.; Catena, J.; Isábal, E.; López-Canet, M.; Valls, N. *Tetrahedron: Asymmetry* **1996**, *7*, 1899. (b) Bonjoch, J.; Catena, J.; Terricabras, D.; Fernàndez, J.-C.; López-Canet, M.; Valls, N. *Tetrahedron: Asymmetry* **1997**, *8*, 3143.

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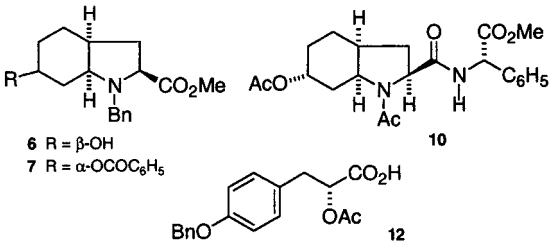
cyclohexanone ring in **5**, which adopts a half-boat form that precludes the attack from the β-face. Although we have explored a route involving a Mitsunobu process that converts alcohol **6**[7] into α-benzoate **7**, from the synthetic standpoint, the best results were obtained when we transformed the *N*-benzyl derivative **5** into the corresponding *N*-Boc derivative **8** and the resulting ketone was reduced with LS-selectride to afford α-alcohol **9** and its C-6 epimer in a ratio of 8:1. The change of the stereochemical course in the reduction of ketones **5** and **8** lies in that both show a different conformational behavior.[8] The *N*-Boc derivative **8** adopts a chair conformation for the cyclohexanone ring, which allows the preferential equatorial attack of the bulky hydride reagent, thus achieving the configuration of the natural target in the choi nucleus. The identity of our synthetic choi with the natural one, isolated by Murakami from aeruginosins by acid hydrolysis, was confirmed by conversion of **9** into **10** and comparison of its ^{1}H NMR data (500 MHz) with that described for the same compound prepared from the natural source by bisacetylation and coupling with (*S*)-methoxyphenylglycine.[3d]

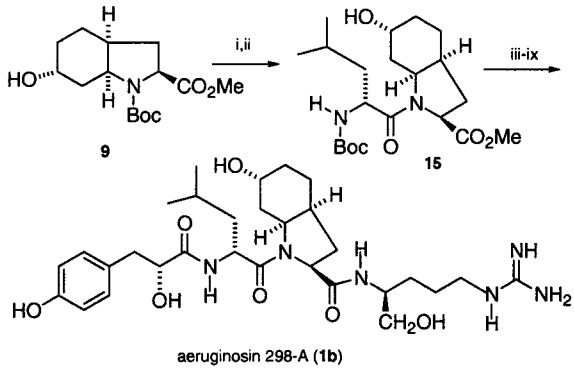


Deprotection of *N*-Boc derivative **9** followed by coupling with Boc-L-Leu using BOP as the condensating agent furnished the dipeptide **11** in 53% yield (2 steps), it being noteworthy that the C-6 hydroxyl group in choi does not need protection due to its low reactivity. The peptide chain was further elongated by coupling with (*R*)-O-benzylphenyllactic (hpla moiety) protected as its acetate **12**, which was synthesized from 4-hydroxyphenylpyruvic acid by enantioselective reduction employing (+)-B-chlorodiisopinocampheylborane [(+)-DIP-Cl],[9] followed by benzylation of the phenol group and acetylation of the α-hydroxy acid generated. After saponification of methyl ester in compound **13**, which simultaneously induces deprotection of the acetate group of the hpla fragment, coupling to the desired tetraunit system was achieved by treatment of tripeptide with arginine (N^G-NO_2) methyl ester in DMF medium and using BOP.

$LiBH_4$ reduction of **14** (1.75 mmol for each mmol of ester) induces the transformation of the methyl ester to the hydroxymethyl group in the arginine moiety. Finally, treatment of the resulting compound with palladium on charcoal in acid medium caused the cleavage of the benzyl group and the deprotection of the guanidine moiety to give the target **1a**. Purification by reversed-phase HPLC provided pure **1a** (a positive Sakaguchi test) in 63% yield. However, the ^{1}H and ^{13}C NMR spectra of our synthetic aeruginosin 298-A (**1a**) unexpectedly showed some different chemical shifts[10] from those reported in the literature for the natural product although the patterns of the peaks were quite similar.[11]

After careful analyses of NMR data of **1a**, as well as other aeruginosins and microcin incorporating D- and L-amino acids linked to the choi nucleus, we suspected that the proposed configuration for aeruginosin 298-A was incorrect and should be revised. Building on this point of view, stereoselective synthesis of **1b**, an aeruginosin incorporating a D-Leu instead of an L-Leu, was carried out.

Scheme 2. Synthesis of Aeruginosin 298-A (**1b**)[a]



[a] Reagents and conditions: (i) TFA, CH_2Cl_2, 0 °C, 45 min; (ii) Boc-D-Leu, BOP, NMM, CH_2Cl_2, 0 °C, 30 min, then room temperature, 22 h, 73% from **9**; (iii) TFA, CH_2Cl_2, 0 °C, 45 min; (iv) **12**, BOP, NMM, CH_2Cl_2, 0 °C, 30 min, then room temperature, 18 h, 60%; (v) 0.1 N LiOH, THF, room temperature, 20 h, 85%; vi, L-Arg(NO_2)OMe·HCl, PyBOP, NMM, DMF, 0 °C, 30 min, then room temperature, 22 h 90%; (vii) $LiBH_4$ (2 M in THF), THF, room temperature, 3 h, 53%; (viii) H_2, Pd−C 10%, 1:1 EtOAc−MeOH, 6 N HCl (2 drops), 1 atm, room temperature, 8 h, 95%.

Working as described for the synthesis of **1a** but coupling choi derivative **9** with Boc-D-Leu and following the sequence depicted in Scheme 2 we synthesized **1b**, which showed NMR spectral data matching those reported by Murakami, when he isolated the aeruginosin 298-A. Thus, we concluded that the stereostructure of the natural product corresponds to that of **1b**.

In summary, the first synthetic entry to aeruginosins has been achieved, after developing an efficient synthesis of the new α-amino acid choi. The synthesis of the putative aeruginosin 298-A (**1a**) and the aeruginosin 298-A itself (**1b**) clarifies the real structure of this natural peptide.[12]

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Supporting Information Available: NMR data of compounds **1a**, **1b**, **5**, **8**, **9**, **11**, and **15** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org..

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(7) The reduction process of **5** using $NaBH_4$−$CeCl_3$ at −23 °C gave **6** (80% yield) and its C-6 epimer in a 7.5:1 ratio.

(8) The different preferred conformation of **5** and **8** was ascertained by NOESY and coupling constant analyses and was supported by molecular modeling studies (AM1 method). The downfield C-4 chemical shift (δ 26.6) is diagnostic for the half-boat conformation of **5** (δ 23.7 for **8**).

(9) Wang, Z.; La, B.; Fortunak, J. M.; Meng, X.-J.; Kabalka, G. W. *Tetrahedron Lett.* **1998**, *39*, 5501.

(10) The most significant differences in the NMR data of **1a** as compared with that reported for the natural compound[1] correspond to the signals attributable (COSY, HSQC) to H-7a (δ 4.4) and H-7eq (δ 1.83) of choi and the methyl groups of Leu (δ 0.84 and 0.85), as well as to C-7 of choi (δ 34.4) and C-1 of Leu (δ 170.5). For detailed NMR data of **1a** and **1b**, see Supporting Information.

(11) Unfortunately, a sample of natural aeruginosin 298-A was unavailable for chromatographic comparisons.

(12) Interestingly, this fact shows that all aeruginosins[1,3,5] have the D(*R*) configuration for the α-amino acid attached to the perhydroindole nitrogen, while the related microcin SF608[4] is the only compound with the L configuration for the α-amino acid linked to choi.