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Syntheses of Both the Putative and Revised Structures of Aeruginosin EI461 Bearing a New Bicyclic α-Amino Acid

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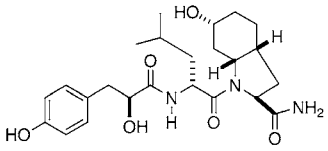
ABSTRACT



aeruginosin EI461 (revised)

L-Hpla-D-Leu-3a,7a-*diepi*-L-Choi-NH_2

The putative structure of the naturally occurring aquatic peptide aeruginosin EI461 has been prepared from D-tyrosine. A corrected structure for aeruginosin EI461 is proposed, and the structure is proven by synthesis, which was accomplished using the new α-amino acid (2*S*,3a*R*,6*R*,7a*R*)-6-hydroxy-2-carboxyoctahydroindole, prepared from L-tyrosine. Succesive couplings of the dipeptide D-Leu-3a,7a-*diepi*-L-Choi with L-Hpla and NH_4OH and a deprotection step gave aeruginosin EI461.

Aeruginosins are a class of protease inhibitors isolated from freshwater cyanobacterial blooms, which incorporate in their peptide backbone the bicyclic α-amino acid 2-carboxy-6-hydroxyoctahydroindole (L-Choi, Figure 1). The 14 initially reported members of this family of natural products[1] have the configuration 2*S*,3a*S*,6*R*,7a*S* in their azabicyclic core, involving an endo relationship with the substituent at C-2.[2] Recently, the aquatic peptide aeruginosin EI461 was isolated from the cyanobacterium *Microcystis aeruginosa* and structure **1** was proposed (Figure 2).[3] The aeruginosin EI461 structure attracted our attention[4,5] due to the fact that the configuration of the Choi was different from that found in all other aeruginosins previously described (Figure 1). In the

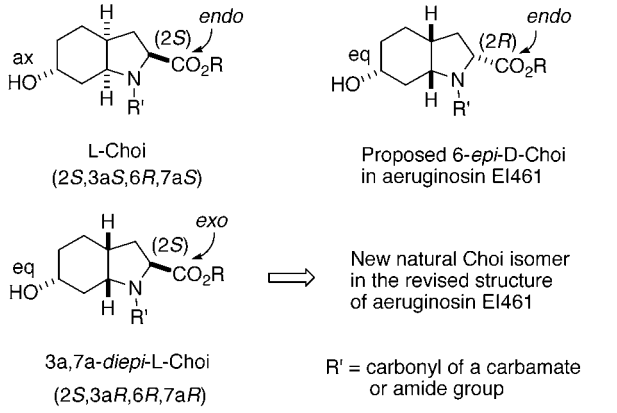


Figure 1. Structures of the Choi nucleus in aeruginosins.

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(1) (a) Ishida, K.; Okita, H.; Matsuda, H.; Okino, T.; Murakami, M. *Tetrahedron* **1999**, *55*, 10971−10988 and references therein. (b) Banker, R.; Carmeli, S. *Tetrahedron* **1999**, *55*, 10835−10844.

(2) For the synthesis and reassignment of aeruginosins 298-A and 298-B, see: Valls, N.; López-Canet, M.; Vallribera, M.; Bonjoch, J. *Chem. Eur. J.* **2001**, *7*, 3446−3460.

(3) Ploutno, A.; Shoshan, M.; Carmeli, S. *J. Nat. Prod.* **2002**, *65*, 973−978.

(4) For our previous work in the aeruginosin synthesis, see: (a) Valls, N.; López-Canet, M.; Vallribera, M.; Bonjoch, J. *J. Am. Chem. Soc.* **2000**, *122*, 11248−11249. (b) ref 2. (c) Valls, N.; Vallribera, M.; López-Canet, M.; Bonjoch, J. *J. Org. Chem.* **2002**, *67*, 4945−4950.

(5) For an alternative synthesis of the L-Choi and the aeruginosin 298-A, see: Wipf, P.; Methot, L.-L. *Org. Lett.* **2000**, *2*, 4213−4216.

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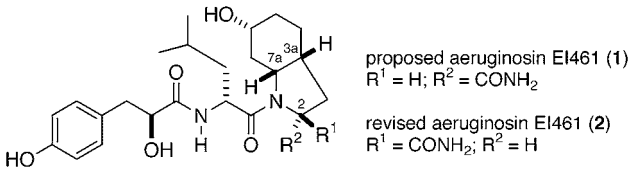


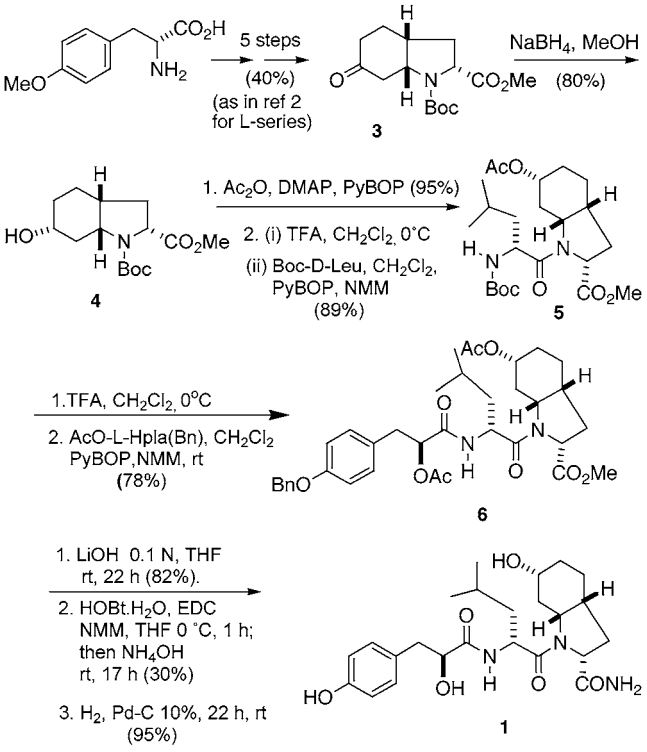
Figure 2. Originally assigned (**1**) and corrected (**2**) structures of aeruginosin EI461.

aeruginosin EI461, the hydroxyl group at C-6 in the new bicyclic α-amino acid is located equatorially (NMR data),[3] although this fact does not necessarily imply that the stereocenter at C-6 was configurationally different from that observed in the L-Choi in the other aeruginosins[6] since the octahydroindole ring is conformationally mobile.

Herein we describe the total synthesis of the proposed structure of aeruginosin EI461, and after revealing that the structure of the natural product has been assigned incorrectly, we suggest an alternative structure for this natural product and prove its correctness with a total synthesis.

As starting material for the synthesis of the proposed aeruginosin EI461 (**1**) shown in Figure 2, we used D-tyrosine, since the configuration of the Choi was 2*R*, and following the protocol developed by us in the L-series,[2,7] we obtained the azabicyclic ketone **3**. Reduction of this ketone with $NaBH_4$ gave stereoselectively alcohol **4** (Scheme 1). Interestingly, the hydroxyl group required protection before the following peptide-bond formation steps, which had not been necessary working in the axial series.[2] The O-acetylated derivative of the Choi **4**, after removal of the *N*-Boc group, was coupled with Boc-D-Leu to give in excellent yield the dipeptide **5**, which, in turn, was assembled with the L-Hpla fragment.[4c] The resulting peptide **6**, after simultaneous saponification of the three esters, was coupled with ammonium hydroxide to give, after a last debenzylation step, compound **1**, $[\alpha]_D$ +15.9 (*c* 0.5 MeOH), which has the proposed structure for aeruginosin EI461.

Scheme 1. Synthesis of Putative Aeruginosin EI461



We are sure that a better yield can be achieved in the last coupling en route to **1**, but our attention was diverted by the fact that the NMR spectra of **1** did not match those of aeruginosin EI461 (See Supporting Information). Most notably, in DMSO-d_6 the two methine protons H-2 and H-7a of the Choi unit both resonate at δ 4.23, whereas those of aeruginosin EI461 appear at δ 4.71 and 3.94, respectively. Also, the H-2 of D-Leu resonates at δ 4.57 in **1**, whereas it appears at δ 4.14 in the natural product. Moreover, significant differences in the ^{13}C NMR spectra of **1** and aeruginosin EI461 were observed for the Choi carbons (C-3, C-3a, C-7), which resonate in **1** at δ 30.6, 36.2, and 39.2, respectively, whereas those in the natural compound appear at δ 33.3, 32.6, and 36.1, respectively.

The multiplicity (doublet) of H-2 in the ^1H NMR spectrum of aeruginosin EI461, which differs from that of **1** (triplet), would be explained by structure **2** (Figure 2).[8] Structure **2** is also more consistent with the structures of previously isolated aeruginosins, which always have the configuration *S* at C-2.

Building on this point of view, we synthesized **2** to confirm the new structural assignment. First we prepared the new bicyclic α-amino acid 3a,7a-*diepi*-L-Choi (**10**). Starting from β-amino ketone **7**[9] to prepare the hydroxycarbamate **10**, we performed the required two-step sequence (change of the protective group and the reduction step) in two different ways (Scheme 2). When the debenzylative process with *tert*-butoxycarbonylation in situ was carried out from **7**, the conversion to ketone **8** was achieved in only 56% yield.[10] So, although the later reduction of ketone **8** was highly stereoselective, alcohol **10** being isolated with 90% yield, this protocol was discarded.

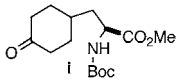
(6) Recently, the closely related marine natural product dysinosin A, incorporating a 5β-hydroxy-L-Choi core, has been isolated[6a] and synthesized.[6b] (a) Carroll, A. R.; Pierens, G. K.; Fechner, G.; Leone, P. A.; Ngo, A.; Simpson, M.; Hyde, E.; Hooper, J. N. A.; Boström, S.-L.; Musil, D.; Quinn, R. J. *J. Am. Chem. Soc.* **2002**, *124*, 13340−13341. (b) Hanessian, S.; Margarita, R.; Hall, A.; Johnstone, S.; Tremblay, M.; Parlanti, L. *J. Am. Chem. Soc.* **2002**, *124*, 13342−13343.

(7) For the first generation synthesis of Choi derivatives, see: Bonjoch, J.; Catena, J.; Isábal, E.; López-Canet, M.; Valls, N. *Tetrahedron: Asymmetry* **1996**, *7*, 1899−1902.

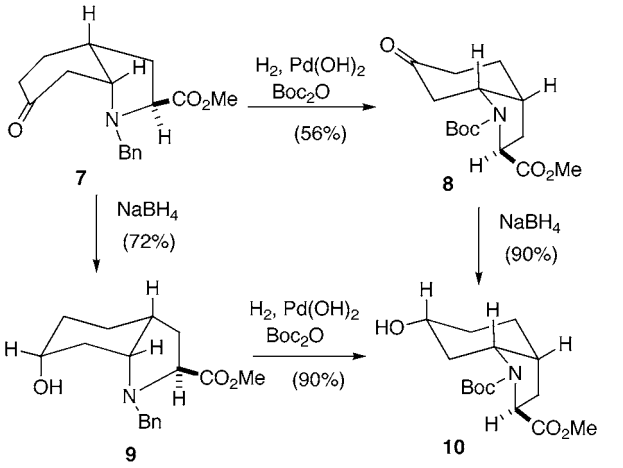
(8) For a discussion about the multiplicity of H-2 in Choi derivatives in their ^1H NMR spectra, see ref 2.

(9) Compound **7** was synthesized from *O*-methyl-L-tyrosine in three steps (33% yield) following our procedure described in ref 2, but using a 5 N solution of HCl in MeOH in the cyclization step to avoid the formation of minor quantities of the trans isomer.

(10) The reason for the moderate yield in the transformation (**7** → **8**) is the formation of the cyclohexanone **i**, due to the retro-Michael process of the starting β-amino ketone **7**, the product of which suffers hydrogenation in the reaction medium. This unwanted process did not occur during the formation of *N*-Boc derivative **3** in the *endo* series.



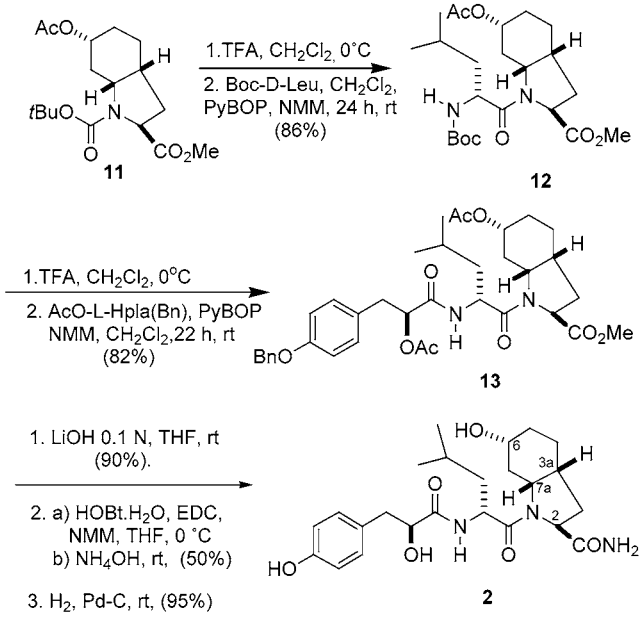
Scheme 2. Synthesis of 3a,7a-*diepi*-L-Choi, a New Natural α-Amino Acid



As expected, when the interconversion of the *N*-benzyl derivative to the *N*-Boc compound was carried out after the reduction step, the unwanted opening byprocess observed in **7** did not occur and the yield of the transformation (**9** → **10**) increased up to 90%. The previous reduction of ketone **7** was carried out with $NaBH_4$ to diastereoselectively afford the axial alcohol **9**.[11] This stereocontrol is due to the conformation of the cyclohexanone ring in **7**, which adopts a half-boat form that favors the hydride attack on the β-face.[12]

With the new α-amino acid **10** prepared, final assembly of **2** could begin (Scheme 3). It was also necessary in this exo series to protect the equatorial hydroxyl group before the coupling processes. We used the acetate group as the protecting group because it could be removed simultaneously with the other ester protecting groups during the saponification step, before the final coupling (**13** → **2**).

Scheme 3. Synthesis of Aeruginosin EI461



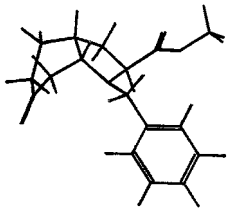
Removal of the Boc group from Choi derivative **11**, followed by coupling to Boc-D-Leu by using pyBOP, gave dipeptide **12**, which after removal of the Boc group and coupling with the L-Hpla derivative[4c] by using pyBOP gave compound **13**. From this L-Hpla-D-Leu-L-3a,7a-*diepi*-Choi derivative, a two-step sequence consisting of coupling with ammonium hydroxide by using PyBOP to give the amide and a deprotecting debenzylation gave aeruginosin EI461 (Scheme 3).

Compound **2** had NMR spectral data matching those reported for the isolated aeruginosin EI461,[13] which allowed us to conclude that the stereostructure of the natural product corresponds to that of **2**. Moreover, when samples of natural and synthetic aeruginosin EI461 (**2**) underwent acid hydrolysis and derivatization with Marfey's reagent,[14] the following HPLC analysis revealed an identical retention time for the *diepi*-L-Choi, L-Hpla, and D-Leu moieties in a coelution assay and consequently demonstrated that both compounds were identical.[15]

It is noteworthy that aeruginosin EI461 (**2**) in DMSO-d_6 solution appears as a 2:3 mixture of trans and cis rotamers around the 3a,7a-*diepi*Choi-Leu peptide bond (Figure 3) and hence is the unique member of this family of natural products in which the cis rotamer population is higher than that observed for the trans rotamer.[16] This fact is undoubtedly related to the exo relationship between the carboxamido group at C-2 and the carbocyclic ring in the Choi nucleus, since structure **1**, having an endo relationship in the Choi

(11) The reduction process of **7** using $NaBH_4$ at −78 °C gave **9** (72% yield), and its C-6 epimer was also formed in 11%.

(12) The preferred conformation of **7** was ascertained by NMR NOESY data and coupling constant analyses, and its twisted conformation (depicted below) was supported by molecular modeling studies (AM1 method). For details of the structure of compound **7**, see ref 2.



(13) The NOESY spectrum of **2** showed slight differences from the reported data.[3] We went over the ROESY spectrum of the natural product and found that the correlation between H-2(mj) and H-7a(mj) does exist in the spectrum. However, we found some correlations that contradict this correlation: H-2(mj) with H-3proS(mj), H-7a(mj) with H-3proR(mj), and H-7a(mj) with H-3a(mj). No correlation was found between H-2 and H-3proR. Therefore, we assume that the correlation between H-2(mj) and H-7a(mj), which of course was not observed in **2**, is due to a small impurity peak located at 4.72 ppm. The notation mj refers to the major rotamer observed; see Figure 3.

(14) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591−596.

(15) Optical rotation for synthetic **2**, $[\alpha]_D$ −28.4, was very different from the reported value for the natural aeruginosin EI461, $[\alpha]_D$ +5. Since the original sample of the natural product was slightly impure, its optical rotation was again measured in Tel Aviv from a purified aeruginosin EI461 used for Marfey's analysis (See Supporting Information). The sample was still not perfectly pure, and the quantity of material was low, 0.9 mg. The optical rotation measured was −11(±5) (*c* 0.0009, MeOH). Despite the remaining difference and considering all experimental data, we concluded that aeruginosin EI461 is levorotatory and that the real value of its optical rotation is that measured from the pure synthetic aeruginosin **2**.

(16) For the steric effects on the amide isomer equilibrium of prolyl peptides, see: (a) Beausoleil, E.; Lubell, W. D. *J. Am. Chem. Soc.* **1996**, *118*, 12092−12098. (b) Halab, L.; Lubell, W. D. *J. Am. Chem. Soc.* **2002**, *124*, 2474−2484.

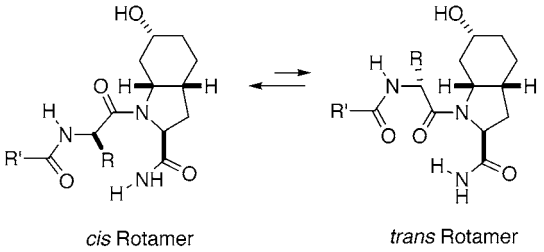


Figure 3. Amide isomer equilibrium of 3a,7a-*diepi*Choi-Leu peptide bond in the aeruginosin EI461 (**2**).

core (see Figure 1), in DMSO-d_6 solution appears as a 10:1 mixture of trans and cis rotamers around the same peptide bond.

In summary, the first synthesis of the revised structure of aeruginosin EI461 has been accomplished. This work has enabled the absolute configuration of the bicyclic core of aeruginosin EI461 to be established as 2*S*,3a*R*,6*R*,7a*R* and has led to the reporting of the new natural α-amino acid 3a,7a-*diepi*-L-Choi.

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Supporting Information Available: Experimental procedures and spectroscopic and analytical data for compounds **2** and **9**−**13** as well as NMR data comparative tables of **1** and aeruginosin EI461 (**2**). This material is available free of charge via the Internet at http://pubs.acs.org.

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