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Total Synthesis and Structural Revision of the Presumed Aeruginosins 205A and B

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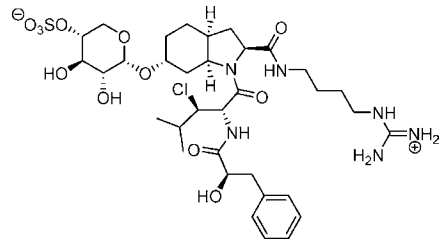
ABSTRACT



A stereoselective synthesis of enantiopure aeruginosin 205B aglycon confirms the presence of a (3*R*,2*S*)-3-chloroleucine amide residue and a (6*R*)-hydroxy (4a*R*,7a*S*)-octahydroindole-(2*S*)-2-carboxamide (Choi) subunit instead of a 6-chloro-substituted core (Ccoi). Enzyme inhibitory tests against thrombin revealed an IC_{50} of 0.31 μM. The total synthesis of the presumed aeruginosin 205B shows that the α-D-xylopyranosyl unit carries a sulfate group at C-4′ (and not at C-3′). Comparison of NMR data leads to the same revision of aeruginosin 205A.

In 1997, Murakami and co-workers[1] isolated the first glycopeptide aeruginosins designated as 205A and B, from the cyanobacterium *Oscillatoria agardhii*, collected from Lake Kasumigaura in Japan. Extensive spectroscopic studies based on previous reports on related aeruginosins,[2] suggested the presence of five subunits: Plas (phenyl lactic acid 2-*O*-sulfate), Hleu (3-hydroxy-leucine), Ccoi (2-carboxy-6-chloro-octahydroindole), Agma (agmatine), and Xyl (xylopyranose), assembled as a linear peptide array, and shown in expressions **1** and **2** (Figure 1). The absolute configurations of these aeruginosins were determined by acid hydrolysis and HPLC analysis of appropriate derivatives. The absolute stereochemistry of the Ccoi subunit remained undisclosed.

The (2*S*,3*R*)-stereochemistry of Hleu in aeruginosin 205B was assigned after derivatization with Marfey's reagent and comparison of HPLC retention times with the authentic three of the four possible diastereomers of the same compound. Since the retention time did not match, the stereochemistry was assigned by exclusion. In 2003, Toyooka and co-workers[3] reported a revision of the position of the sulfate group, switching it to the 3-position of the xylopyranose residue, as shown in expression (**3**), while maintaining the Ccoi subunit intact.

In spite of seemingly definitive evidence for the original and revised structures of **1** and **2** (or **3**), recent developments in the total synthesis of other members of this family of linear glycopeptides[2] have cast some doubt on the original structural and stereochemical assignments.

Valls, Bonjoch, and co-workers[4] reported the synthesis of the presumed Ccoi subunit in aeruginosins 205A (**1**) and B (**2**). Their results, based on comparison of ^{1}H and ^{13}C NMR resonances, cast doubt on the originally proposed structures.[1,3]

(1) Shin, H. J.; Matsuda, H.; Murakami, M.; Yamaguchi, K. *J. Org. Chem.* **1997**, *62*, 1810.

(2) For a recent review, see: Ersmark, K.; Del Valle, J. R.; Hanessian, S. *Angew. Chem., Int. Ed.* **2008**, *47*, 1202.

(3) Toyooka, N.; Nakazawa, A.; Himiyama, T.; Nemoto, H. *Heterocycles* **2003**, *59*, 75.

(4) Valls, N.; Vallribera, M.; Font-Bardia, M; Solans, X.; Bonjoch, J. *Tetrahedron: Asymmetry* **2003**, *14*, 1241..

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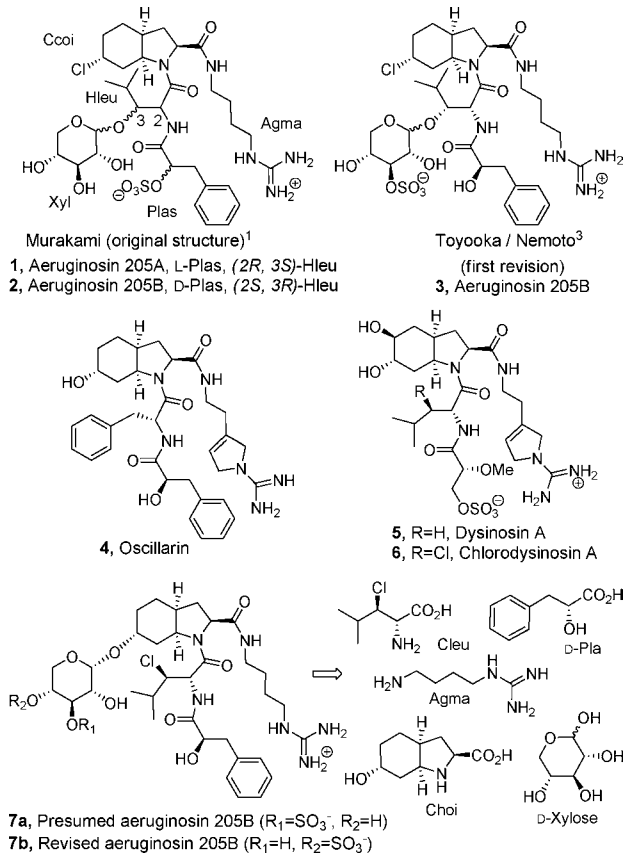


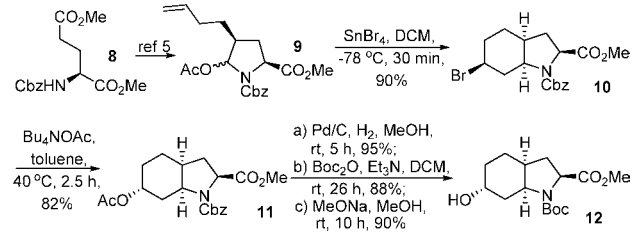
Figure 1. Originally proposed and first and second revised structures of aeruginosins 205A and B. Structures of oscillarin, dysinosin A, and chlorodysinosin A

Following our total synthesis and X-ray co-crystal structure determination of oscillarin **4**,[5] dysinosin A **5**,[6] and chlorodysinosin A **6**[7] with the enzyme thrombin, it also became apparent that aeruginosins 205A(**1**) and B (**2**) could contain a β-chloro-D-Leu residue rather than a 6-chloro-2-carboxyperhydroindole core unit as originally proposed by Murakami[1] and later revised by Toyooka, Nemoto, and their respective groups (Figure 1).[3]

We report herein a revision of the two originally postulated structures for aeruginosins 205A (**1**) and B (**2**), relying on definitive total syntheses of the aglycon and the presumed 205B (**3**). As a result, not only was the location of the chlorine atom incorrectly assigned as in Ccoi, but the sulfate group should reside at C-4′ of the α-D-xylopyranosyl glycosidic unit. From a biosynthetic perspective, it is interesting that aeruginosin 205B (**2**) possesses the same 6-hydroxy-2-carboxyperhydroindole (Choi) and D-phenyllactic acid (Pla) subunits as in oscillarin[5] but differs in the P_3 amino acid residue by incorporating a β-chloro-D-leucine (Cleu), as found in chlorodysinosin A (**6**).[7] The required subunits for the synthesis of the presumed aeruginosin 205B (**7a**) are shown in Figure 1. Due to the need to use orthogonally compatible protecting groups, the published protocols toward these subunits[5,7] had to be adapted to different *N*- and *O*-carbamates and ethers, respectively.

The synthesis of the Choi subunit was achieved starting with dimethyl *N*-Cbz-L-glutamate **8** as previously described[5] albeit with improved yields[8] (Scheme 1).

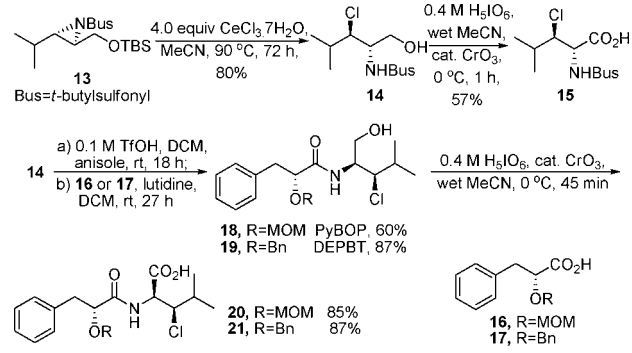
Scheme 1. Synthesis of *N*-Boc Choi (**12**)



Thus, treatment of **9** with $SnBr_4$ led to **10** in 90% yield. Displacement of the bromide with inversion of configuration afforded the acetate **11** which was converted to the *N*-Boc analogue **12** (*N*-Boc Choi).

The synthesis of the Cleu subunit was also improved starting from the aziridine **13** by a regioselective introduction of a chloride group with $CeCl_3$ to give **14** (Scheme 2).[7,9]

Scheme 2. Synthesis of (2*R*,3*S*)-3-Chloroleucinyl-*O*-benzyl-D-phenyllactic Acid (**21**)



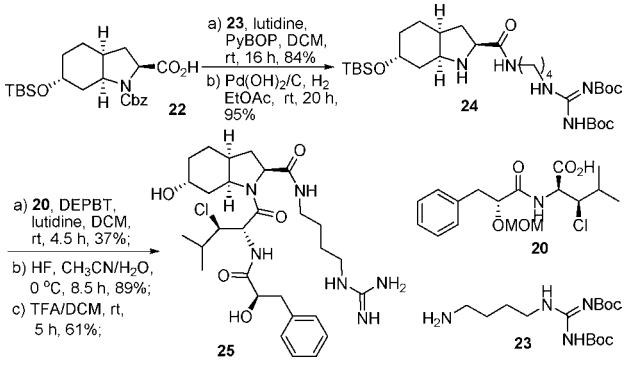
Deprotection followed by oxidation gave the new crystalline amino acid derivative **15**. Coupling of the amine derived from **14** with either the *O*-MOM or *O*-benzyl ether derivatives **16** and **17**, respectively, led to **18** and **19**. Finally, oxidation with H_5IO_6 in the presence of CrO_3 as a catalyst[10] gave the intended carboxylic acids **20** and **21**.

Our first objective was to assemble the aglycon portion of the presumed aeruginosin 205B (**7a**) and to test the

(5) Hanessian, S.; Tremblay, M.; Petersen, J. F. W. *J. Am. Chem. Soc.* **2004**, *126*, 6064.
(6) Hanessian, S.; Margarita, R.; Hall, A.; Johnstone, S.; Tremblay, M.; Parlanti, L *J. Am. Chem. Soc.* **2002**, *124*, 13342.
(7) Hanessian, S.; Del Valle, J. R.; Xue, Y.; Blomberg, N. *J. Am. Chem. Soc.* **2006**, *128*, 10491.
(8) See the Supporting Information.
(9) For another synthesis, see: Valls, N.; Borregán, M.; Bonjoch, J. *Tetrahedron Lett.* **2006**, *47*, 3701.
(10) Zhao, M.; Li, J.; Song, Z.; Desmond, R.; Tschaen, D., M.; Grabowski, E. J. J.; Reider, P. J *Tetrahedron Lett.* **1998**, *39*, 5323.

compatibility of specific protecting groups, cognizant of the possibility of β-elimination in the β-chloro-D-leu residue. The TBS ether **22** was coupled to 1-aminobutyl bis-*N*-Boc-guanidine **23** (bis-Boc agmatine), and the Cbz group was cleaved to give **24** (Scheme 3). Coupling with the *O*-MOM β-chloro-D-leu subunit **21** in the presence of DEPBT as a preferred reagent[7,11] proceeded uneventfully in spite of the modest yield. Sequential deprotection of the TBS and *O*-MOM groups with HF in aqueous MeCN, then the *N*-Boc groups with TFA, afforded the intended aglycon **25**. The ^{1}H and ^{13}C spectral characteristics matched those published for aeruginosin 205B (**2**)[1] except for the absence of resonances due to the sulfated D-xylopyranosyl moiety.[8] Thus, the reported values for the Pla and Cleu subunits were quasi-identical to those in the synthetic sample of 205B aglycon (**25**). The same was also true for the Choi subunit with allowance made for C-6, which carries a glycosidic moiety in the natural product. Compound **25** inhibited the enzyme thrombin at $IC_{50} = 0.31\ \mu M$.

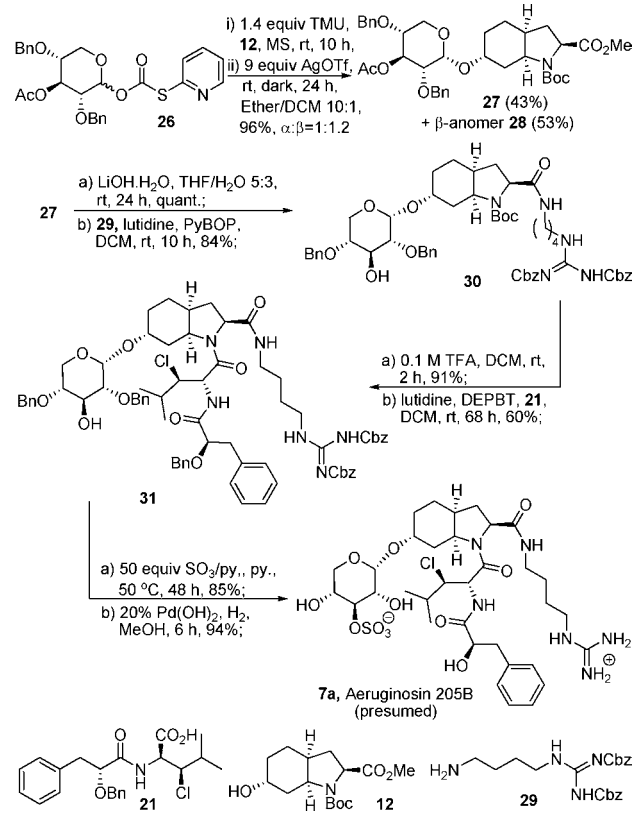
Scheme 3. Synthesis of Presumed Aeruginosin 205B (**7a**) Aglycone (**25**)



In order to definitively confirm our findings, we proceeded with the total synthesis of the presumed aeruginosin 205B (**7a**). This presented some logistic issues related to the choice of orthogonally compatible protecting groups as well as the order of subunit assembly due to the sensitivity of the *O*-sulfate group to protic acid and aqueous basic conditions. Additionally, α-glycosylation of the axially disposed C-6 hydroxyl group of the Choi subunit presented an additional challenge. While regioselective *O*-sulfation of suitably protected intermediates was not considered as a problem per se, the need for excess reagents and the final global deprotection to yield the intended target without loss of the chlorine atom through possible β-elimination or reduction, loomed as potential obstacles. Extensive model studies with preferentially *O*-protected methyl α-D-xylopyranosides, followed by *O*-sulfation and deprotection, led us to opt for a global deprotection of the assembled penultimate precursor under conditions of hydrogenolysis. Based on the Toyooka and Nemoto proposal,[3] we planned the synthesis with the intention of securing the 3-sulfate in the D-xylopyranosyl subunit of aeruginosin 205B (**7a**). Model studies indicated that the 2-thiopyridyl carbonate (TOPCAT) was a suitable method for anomeric activation.[12] The requisite glycosyl donor **26** was prepared in a straightforward manner.[8]

Treatment of a mixture of **12** and **26** in ether−DCM with AgOTf in the presence of tetramethylurea as an acid scavenger[12] led to the desired α-anomer **27** (43%), easily separable from the β-anomer **28** (53%) by column chromatography in 96% yield (Scheme 4). Many attempts to enrich the mixture in the α-anomer **27** were not successful. Nevertheless, we were pleased that glycoside synthesis of the axially oriented C-6 hydroxyl group in **12** had proceeded in such high yield and reasonable selectivity. Hydrolysis of the ester groups and amide coupling with the Agma amine led to **30**. Cleavage of the *N*-Boc group was followed by coupling with the Cleu-*O*-Bn-D-Pla acid subunit **21** to give **31** (Scheme 4).

Scheme 4. Synthesis of Presumed Aeruginosin 205B (**7a**)



At this point, all that remained was the sulfation of the D-xylopyranosyl unit, followed by global deprotection. Extensive model studies led us to use a large excess of SO_3−pyridine complex at 50 °C for 2 days. Nevertheless,

(11) For example, see: (a) Li, H.-T.; Jiang, X.-H.; Ye, Y.-H.; Fan, C.-X.; Romoff, T.; Goodman, M. *Org. Lett.* **1999**, *1*, 91. (b) Fan, C.-X.; Hao, X.-L.; Ye, Y.-H. *Synth. Commun.* **1996**, *26*, 1455.

(12) For example, see: (a) Lou, B.; Huynh, H. K.; Hanessian, S. In *Preparative Carbohydrate Chemistry*; Hanessian, S., Ed.; Dekker: New York, 1997; Chapter *19*, p 431. (b) Hanessian, S.; Mascitti, V.; Rogel, O. *J. Org. Chem.* **2002**, *67*, 3346.

this led to an 85% of the desired C-3′-sulfate. Hydrogenolysis in the presence of $Pd(OH)_2$ and hydrogen in MeOH, followed by purification by reversed-phase HPLC, afforded synthetic aeruginosin 205B (presumed **7a**) as the 3′-sulfate in the form of a colorless amorphous powder, $[\alpha]_D$ +39.0 (*c* 0.1, MeOH) [lit.[1] $[\alpha]_D$ +40.3 (*c* 0.1, MeOH)].

Following the same sequence, the β-anomer **28** was also converted to the unnatural β-anomer of aeruginosin 205B (**7a**) as the 3′-sulfate: $[\alpha]_D$ +13.0 (*c* 0.1, MeOH).[8] Comparison of ^{1}H and ^{13}C NMR reported data with those of synthetic **7a** revealed a disturbing discrepancy with regard to the position of the presumed 3′-sulfate on the D-xylopyranosyl unit.[8]

We therefore suspected that the sulfate group was located at C-4′. To prove this point we synthesized methyl α-D-xylopyranosides with sulfates at each of the hydroxyl groups.[8] Indeed, the reported ^{1}H and ^{13}C NMR data[1] for aeruginosin 205A (**1**) and B (**2**) showed unambiguous agreement with the presence of a C-4′-sulfate. Of the seven total syntheses of the 20 or so members of the aeruginosin family, five have required structural revision and/or confirmation.[5−7,13] The revised structure and absolute configuration of aeruginosin 205B (**7b**) can now be added to this list. The same conclusion can be made for aeruginosin 205A.

Acknowledgment. We thank NSERC and FCAR for financial assistance and AstraZeneca, Mölndal, Sweden, for biological tests and a grant. We thank Benoit Deschênes-Simard for the X-ray crystal structure determinations.

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

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(13) For example, see aeruginosin 298A: (a) Wipf, P.; Methot, J.-L. *Org. Lett.* **2000**, *2*, 4213. (b) Valls, N.; López-Canet, M.; Vallribera, M.; Bonjoch, J. *J. Am. Chem. Soc.* **2000**, *122*, 11248. (c) Valls, N.; López-Canet, M.; Vallribera, M.; Bonjoch, J. *Chem.—Eur. J.* **2001**, *7*, 3446. (d) Oshima, T.; Gnanadesikan, V.; Shibuguchi, T.; Fukuta, Y.; Nemoto, M.; Shibasaki, M. *J. Am. Chem. Soc.* **2003**, *125*, 11206. See also aeruginosin E1461: Valls, N.; Vallribera, M.; Carmeli, S.; Bonjoch, J. *Org. Lett.* **2003**, *5*, 447. See also refs 5−7.