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Total Synthesis and Stereochemical Revision of (+)-Aeruginosin 298-A

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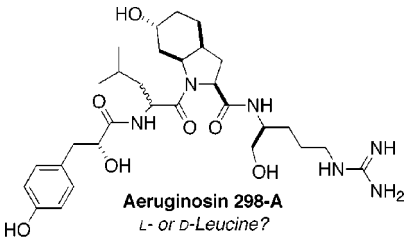
ABSTRACT



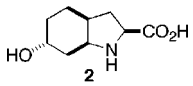
Aeruginosin 298-A
L- or D-Leucine?

Novel routes toward both enantiomers of the bicyclic proline surrogate 2-carboxy-6-hydroxyoctahydroindole, i.e., Choi, were developed on the basis of the oxidative cyclization of L-tyrosine. Synthesis of the proposed sequence of (+)-aeruginosin 298-A did not provide the natural product. Incorporation of a D-leucine residue, in contrast, led to the total synthesis of this thrombin inhibitor.

The discovery of novel anticoagulant agents for the treatment of thrombosis continues to receive significant attention.[1] Thrombin, a key enzyme in the blood coagulation cascade, catalyzes the conversion of fibrinogen to fibrin which then polymerizes to form a haemostatic plug.[2] Current therapeutic agents, such as heparins and coumarins, require careful monitoring of the patient to avoid excessive haemorrhage and are not orally active.[3]

In 1994, Murakami and co-workers isolated the thrombin inhibitor (IC_{50} of 0.5 μM) aeruginosin 298-A (**1**) from the blue-green freshwater algae *Microcystis aeruginosa*.[4] At the time, only the leucine stereochemistry was assigned by chiral GC analysis of the acid hydrolysate. The configurations of the hydroxyphenyllactic acid and argininol fragments were later established by Marfey analysis.[5]

In 1998, Tulinsky and co-workers reported an X-ray crystallographic structure of the ternary complex of **1** bound to hirugen-thrombin.[6] Surprisingly, the binding mode closely resembled that of D-Phe-Pro-Arg chloromethyl ketone with the L-Leu residue occupying the D-S3 subsite. Their work also confirmed the absolute stereochemistry of the novel hydroindole core **2** (L-Choi). Conformationally restricted



2

proline derivatives such as **2** project peptide chains into defined regions of space, promoting specific turns in peptide folding and conferring a bioactive conformation.[7] Furthermore, the hydroxyl group in **2** can participate in hydrogen bonding and increase water solubility or can be functionalized (e.g., as a sulfate) as found for certain aeruginosins.

Our approach toward **2** highlights the utility of our tyrosine oxidation−rearrangement methodology to prepare function-

(1) Vacca, J. *Annual Reports in Medicinal Chem*istry; Bristol, J. A., Ed.; Academic Press: San Diego, 1998; Vol. 33, pp 81−90.
(2) Buchanan, M. R.; Brister, S. J.; Ofosu, F. A. *Thrombin: Its Key Role in Thromogenesis: Implications For Its Inhibition Clinically*; CRC Press: Boca Raton, FL, 1995.
(3) Das, J.; Kimball, S. D. *Bioorg. Med. Chem. Lett.* **1995**, *3*, 999−1007.
(4) Murakami, M.; Okita, Y.; Matsuda, H.; Okino, T.; Yamaguchi, K. *Tetrahedron Lett.* **1994**, *35*, 3129−3132. Aeruginosin 298-A exhibits modest selectivity against trypsin (IC_{50} of 1.7 μM) but does not inhibit papain, elastase, chymotrypsin, or plasmin. One dozen aeruginosins have since been isolated with thrombin IC_{50} values ranging from 0.04 to 15 μM, all sharing the same hydroindole core **2**.[5]
(5) Ishida, K.; Okita, Y.; Matsuda, H.; Okino, T.; Murakami, M. *Tetrahedron* **1999**, *55*, 10971−10988.
(6) Steiner, J. L. R.; Murakami, M.; Tulinsky, A. *J. Am. Chem. Soc.* **1998**, *120*, 597−598.
(7) See, for example: Zhang, R.; Brownewell, F.; Madalengoita, J. S. *J. Am. Chem. Soc.* **1998**, *120*, 3894−3902. Blanco, M. J.; Paleo M. R.; Penide, C.; Sardina, F. J. *J. Org. Chem.* **1999**, *64*, 8786−8793. Tam, J. P.; Miao, Z. *J. Am. Chem. Soc.* **1999**, *121*, 9013−9022.

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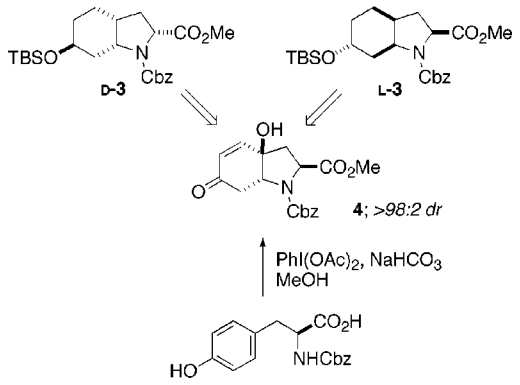
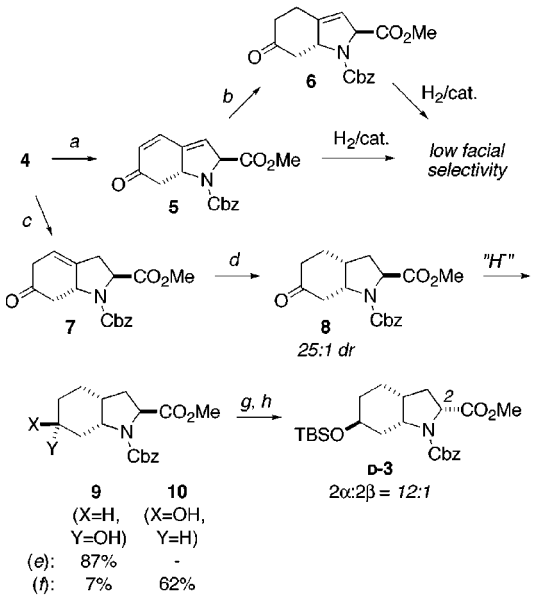


Figure 1. Diastereoselective cyclooxidation of Cbz-L-tyrosine: an entry to the aeruginosin core segment.

alized hydroindole amino acids (Figure 1).[8] Oxidation of L-Cbz-tyrosine with $PhI(OAc)_2$ followed by exposure to basic methanol furnishes **4** in >98:2 diastereoselectivity. Recent efforts have demonstrated the versatility of **4** for the synthesis of various natural products.[9]

We had hoped to access the *cis*-fused ring system in **2** by dehydration of the tertiary alcohol of **4**, followed by hydrogenation (Scheme 1). Treatment of **4** with $POCl_3$ in pyridine gave the kinetic elimination product **5**. Unfortunately, catalytic hydrogenation of this diene with various catalysts (Pt/C, Pd/C, PtO_2, $Rh(PPh_3)_3Cl$) and solvents (MeOH, EtOH, THF, AcOH/EtOH) proceeded with little

Scheme 1. Initial Approach to the Choi Core[a]



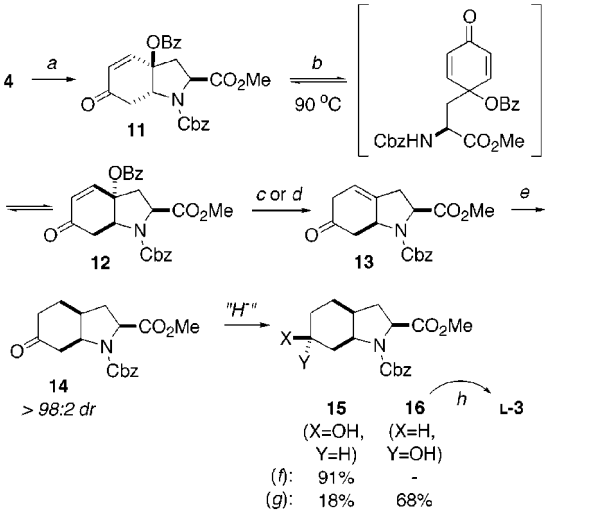
[a] Reagents and conditions: (a) $POCl_3$, pyr., 89%; (b) H_2, 5% Rh/Al_2O_3, MeOH, quant.; (c) Ms_2O, DMAP, pyr., CH_2Cl_2, −30 °C to 0 °C; activated Zn dust, AcOH/THF, 94%; (d) H_2, 1% PtO_2, 10% AcOH/EtOH, 0 °C, 92%; (e) $NaBH_4$, MeOH, 0 °C; (f) L-Selectride, THF, −78 °C; (g) TBSOTf, pyr., CH_2Cl_2, 94%; (h) $LiNEt_2$, 10% HMPA/THF, −78 °C; *t*-BuOH; 69%.

facial selectivity. Dienone **5** can be selectively reduced to **6**; however, further reduction again proceeded with little selectivity. Substituent effects may control which face of an olefin is adsorbed on the metal catalyst.[10] Accordingly, we speculated that varying the distance between the olefin and the ester could alter the selectivity for *cis*-hydrogenation. Elimination of the mesylate derived from **4** with activated zinc[11] gave **7** in 94% yield. In contrast to **6**, catalytic hydrogenation of the β,γ-enone **7** proceeded with excellent (25:1) facial selectivity leading to the *cis*-fused hydroindole **8** in 92% yield.[12]

The ketone was reduced to either the equatorial alcohol (**9**; 87%) with $NaBH_4$ or the axial alcohol with L-Selectride (**10**; 69%, 9:1).[13] Stereochemical assignments were made on the basis of comparison with the natural product and related work.[9] TBS-protection of **10** followed by epimerization of the hindered methyl ester with $LiNEt_2$[14] in 10% HMPA/THF afforded the fully protected unnatural amino acid D-**3** with up to 12:1 selectivity at C(2).

The synthesis of natural aeruginosin 298-A by this route would require starting with costly D-tyrosine. Consequently we considered an alternative pathway (Scheme 2) to the natural L-Choi configuration L-**3**.

Scheme 2. Synthesis of the L-Choi Core[a]



[a] Reagents and conditions: (a) Bz_2O, DMAP, pyr., CH_2Cl_2, 50 °C, 90%; (b) $NaHCO_3$, DMSO, 90 °C; **12** (78%); (c) activated Zn dust, AcOH/THF, 65 °C, 75%; (d) SmI_2, AcOH/THF, 65 °C, 5 min, 63%; (e) H_2, 5% PtO_2, 10% AcOH/EtOH, 0 °C, 95%; (f) $NaBH_4$, MeOH, 0 °C; (g) L-Selectride, THF, −78 °C; (h) TBSOTf, imidazole, CH_2Cl_2, 87%.

The *syn*-diastereomer of **4** was accessed through a thermodynamic equilibration of the benzoyl-protected[15]

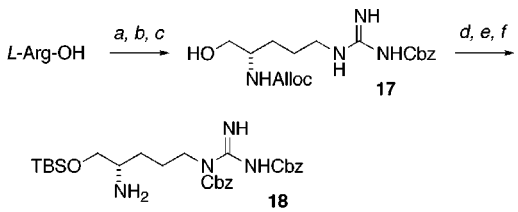
(8) Wipf, P.; Kim, Y. *Tetrahedron Lett.* **1992**, *33*, 5477−5480.
(9) Wipf, P.; Kim, Y.; Goldstein, D. M. *J. Am. Chem. Soc.* **1995**, *117*, 11106−11112. Goldstein, D. M.; Wipf, P. *Tetrahedron Lett.* **1996**, *37*, 739−742. Wipf, P.; Li, W. *J. Org. Chem.* **1999**, *64*, 4576−4577. Wipf, P.; Mareska, D. A. *Tetrahedron Lett.* **2000**, *41*, 4723−4727.
(10) Rylander, P. N. *Hydrogenation Methods*; Academic Press: London, 1985; pp 29−52.
(11) Knochel, P.; Yeh, M. C. P.; Berk, S. C.; Talbert, J. *J. Org. Chem.* **1988**, *53*, 2392−2394.

alcohol **11** to **12** via a retro-Michael−Michael addition sequence at 90 °C in basic DMSO.[16] While a modest 1.6:1 ratio of **12**:**11** was obtained after 1 h, chromatographic separation and recycling of recovered **11** provided an overall yield of 78% for **12**. MM2-level calculations suggest that diastereomer **12** is preferred by 0.3 kcal/mol.

Benzoate **12** required elevated temperatures (65 °C) to provide **13** in good yield using either zinc or SmI_2.[17] Interestingly, SmI_2 reduction of **12** at room temperature rendered only the saturated ketobenzoate. Hydrogenation followed by L-Selectride reduction (86%, 3.8:1) and TBS-protection afforded the L-enantiomer of **3**. The minor reduction product **15** could be recycled back to **14** by Ley oxidation (83%).[18] Finally the alcohol was protected as a TBS silyl ether (87%).[19]

With an efficient synthesis of the core accomplished, we turned to the preparation of the argininol (Argol) and hydroxyphenyl lactic acid (Hpla) fragments. The former was synthesized in six steps from L-arginine (Scheme 3). Selective

Scheme 3. Synthesis of the Argol Fragment[a]

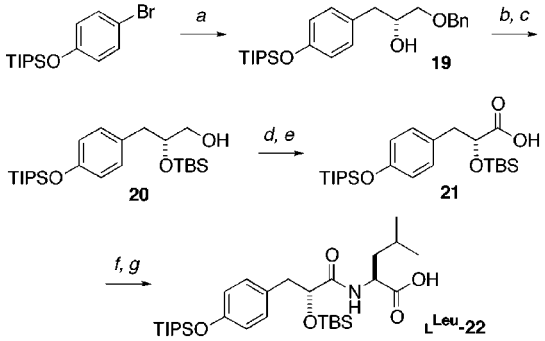


[a] Reagents and conditions: (a) AllocCl, aq. NaOH, 79%; (b) CbzCl, aq. NaOH, THF, 63%; (c) IBCF, NMM, DMF, −20 °C; $NaBH_4$, H_2O, 72%; (d) TBSOTf, imidazole, CH_2Cl_2, 81%; (e) CbzCl, DMAP, K_2CO_3, DMF, 86%; (f) Bu_3SnH, $Pd(PPh_3)_4$, AcOH, THF, 88%.

Alloc and Cbz protection followed by in situ $NaBH_4$ reduction of the mixed anhydride[20] formed with IBCF gave primary alcohol **17** (72%). Standard protective group manipulations provided segment **18**.

The key step in the synthesis of the Hpla fragment was a BF_3·OEt_2-catalyzed organocuprate addition to (*R*)-benzylglycidol (**I**), providing adduct **19** in 83% yield (Scheme 4).

Scheme 4. Synthesis of the D-Hpla-L-Leu Segment[a]

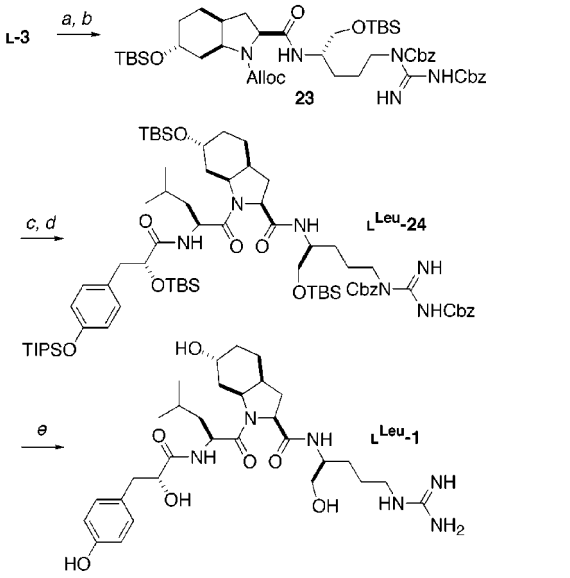


[a] Reagents and conditions: (a) *t*-BuLi, CuBr·SMe_2, THF, −78 °C to −45 °C; **I**, BF_3·OEt_2, −45 °C to −20 °C, 83%; (b) TBSOTf, imidazole, CH_2Cl_2; (c) H_2, 10% Pd/C, EtOAc, 92%; (d) DMP, CH_2Cl_2, 84%; (e) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH/H_2O, 93%; (f) L-Leu-OBn, DEPC, *i*Pr_2NEt, CH_2Cl_2, 73%; (g) H_2, Pd/C, EtOH, 93%.

Standard protective group manipulations followed by Dess−Martin[21] and $NaClO_2$ oxidations led to Hpla **21**. Finally, DEPC-mediated coupling to L-Leu-OBn and hydrogenolysis provided the desired segment $^{L\text{Leu}}$-**22**.

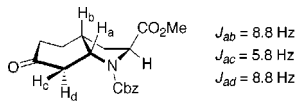
For the assembly of the tetrapeptide and subsequent deprotection, the Cbz group of L-**3** was exchanged for an Alloc group (Scheme 5). Subsequent saponification and pentafluorophenyl ester-mediated coupling to argol **18** provided dipeptide **23** in 69% yield. Alloc deprotection followed by DEPBT[22]-mediated segment coupling to $^{L\text{Leu}}$-**22** proceeded in 63% yield to give $^{L\text{Leu}}$-**24**. The tetrapeptide

Scheme 5. Synthesis of the Proposed Structure of Aeruginosin 298-A[a]



[a] Reagents and conditions: (a) H_2, Pd/C, EtOH; AllocCl, pyr., 91%; (b) LiOH, THF/H_2O, 40 °C; **18**, *i*Pr_2NEt, FDPP, CH_2Cl_2, 69%; (c) Bu_3SnH, $Pd(PPh_3)_4$, AcOH, CH_2Cl_2; (d) $^{L\text{Leu}}$-**22**, DEPBT, *i*Pr_2NEt, CH_2Cl_2, 63%; (e) HF (aq); H_2, Pd/C, EtOH, 42%.

(12) The relative stereochemistry of **8** was confirmed by comparison of the observed ^{1}H NMR coupling constants (shown below) to those calculated from an AM-1 minimized conformation (calculated coupling constants: J_{ab} = 9.3, J_{ac} = 4.9, J_{ad} = 11.3 Hz).



(13) Mitsunobu reaction of **9** with BzOH gave the axial benzoate in modest yield (67%) along with elimination product.

(14) Use of bulkier bases (e.g., LDA/HMPA) did not provide the epimer but instead a complex mixture, including the Claisen condensation dimer.

(15) Heating the unprotected alcohol **4** in basic DMSO led to rapid decomposition.

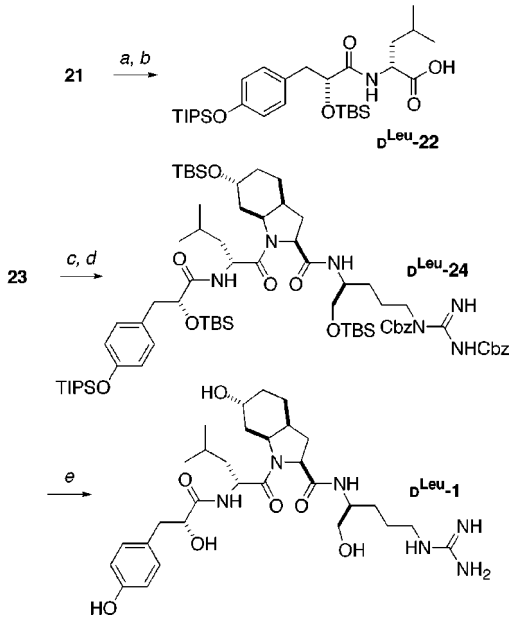
(16) Diastereomers **11** and **12** can be differentiated by their H(C-2) ^{1}H NMR coupling constants; J^{11} = 9.1, 2.4 Hz (dd); J^{12} = 8.5 Hz (t).

(17) Molander, G. A. *Org. React.* **1994**, *46*, 211−367.

was treated with HF(aq) for 2 h, neutralized with NaOH(aq), and extracted into CH_2Cl_2/EtOAc. Finally, hydrogenolysis cleaved the Cbz protective groups (42%; two steps). However, this product was spectroscopically distinctively different from natural aeruginosin 298-A; apparently it represented a diastereomer.[23]

Upon reinspection of the configuration of other members of the aeruginosin family, we began to suspect that the stereochemical assignment of the leucine residue might be incorrect.[24] Thus, we also prepared the D-leucine analogue of aeruginosin 298-A using the same strategy (Scheme 6).

Scheme 6. Synthesis of D-Leu-aeruginosin 298-A[a]



[a] Reagents and conditions: (a) NH_3Cl-D-Leu-OBn, DEPC, *i*Pr_2NEt, CH_2Cl_2, 68%; (b) H_2, Pd/C, EtOH, 95%; (c) Bu_3SnH, $Pd(PPh_3)_4$, AcOH, CH_2Cl_2; (d) D^{Leu}-**22**, DEPBT, *i*Pr_2NEt, CH_2Cl_2, 59%; (e) HF (aq); H_2, Pd/C, EtOH, 34%.

Indeed, the ^1H and ^{13}C NMR data of D^{Leu}-**1** matched exactly those reported for aeruginosin 298-A.[25,26]

While at this stage we cannot yet explain the discrepancy between our assignment and the X-ray structure of (+)-aeruginosin 298-A, we are confident that the revised structure D^{Leu}-**1**, which matches all available spectroscopic data of the natural product, is indeed representative of the actual stereochemistry of (+)-aeruginosin 298-A. A direct comparison between synthetic and natural samples is unfortunately not possible, since the natural product is no longer available.

In conclusion, a concise synthesis of the novel bicyclic amino acid **2** from L-tyrosine has been developed that can readily be adapted to the preparation of analogues and peptidomimetic scaffolds. The total synthesis of (+)-aeruginosin 298-A demonstrates the potentially biomimetic incorporation of an oxidative cyclization product of L-tyrosine into the highly functionalized backbone of this potent thrombin inhibitor. Key steps of the total synthesis include the efficient construction of other nonproteinogenic building blocks in aeruginosin 298-A as well as an extensive optimization of coupling strategies.

The preparation of L^{Leu}- as well as D^{Leu}-aeruginosin 298-A compels one to a reassignment of the configuration of the natural product. As a consequence, the binding mode of aeruginosin 298-A to thrombin is as expected,[27] with a D-leucine occupying the D-S3 binding site of the enzyme. This structural reassignment also explains the similarity[28] between the binding modes of 298-A and 98-B[29] (98-B has a D-*allo*isoleucine residue at P3) to serine proteases.[30]

Acknowledgment. This work has been supported by the National Institutes of Health (AI/GM-33506) and by a graduate fellowship to J.M. from FCAR (Québec). We thank Dr. H. Takahashi for preliminary experiments on the thermodynamic equilibration of **11** to **12**, Professor Murakami for providing ^1H and ^{13}C spectra of the natural product, and Professor Tulinsky for helpful discussions regarding the X-ray structural assignment.

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

OL006759X

(18) Griffith, W. P.; Ley, S. V. *Aldrichimica Acta* **1990**, *23*, 13−19.

(19) Cbz-Choi(TBS)-OMe (L-**3**) was converted into Ac-Choi-OMe, which by ^1H and ^{13}C NMR and $[\alpha]_D$ is identical to that prepared by Bonjoch et al. (Bonjoch, J.; Catena, J.; Isabal, E.; Lopez-Canet, M.; Valls, N. *Tetrahedron: Asymmetry* **1996**, *7*, 1899−1902).

(20) Rodriguez, M.; Llinares, M.; Doulut, S.; Heitz, A.; Martinez, J. *Tetrahedron Lett.* **1991**, *7*, 923−926. Fresh $NaBH_4$ was required to suppress lactam formation.

(21) Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277−7287. Ireland, R. E.; Liu, L. *J. Org. Chem.* **1993**, *58*, 2899.

(22) DEPBT: 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one. Li, H.; Jiang, X.; Ye, Y.; Fan, C.; Romoff, T.; Goodman, M. *Org. Lett.* **1999**, *1*, 91.

(23) An alternative synthetic strategy whereby the Hpla-Leu side chain was attached first gave identical material.

(24) The ^1H NMR data for 298-A (L-Leu) closely parallel those of 298-B-major, 89-A, 89-B, and desulfated, dimethyl acetal-derivatized 89-A and 89-B (all have D-Leu) but are very different from 298-B-minor (L-Leu); see Supporting Information.

(25) Marfey analysis of the acid hydrolysate of both L^{Leu}- and D^{Leu}-**1** confirmed that the leucine residues were indeed in the L- and D-form, respectively (procedure: Adamson, J. G.; Hoang, T.; Crivici, A.; Lajoie, G. A. *Anal. Biochem.* **1992**, *202*, 210−214; see Supporting Information).

(26) $[\alpha]_D$: L^{Leu}-**1** −18 (0.25, H_2O), D^{Leu}-**1** +16 (0.17, H_2O), lit. +22 (0.36, H_2O).

(27) Based on the well-established binding mode of D-Phe-Pro-Arg chloromethyl ketone (Bode, W.; Turk, D.; Karshikov, A. *Protein Sci.* **1992**, *1*, 426−471).

(28) The P1−P4 sequences of both 298-A and 98-B occupy the same binding sites: the D-*allo*-Ile and D-Hpla of 98-B hydrogen bond to Gly216 and Gly219, respectively; the Leu and D-Hpla of 298-A also bind to Gly216 and Gly219, respectively.

(29) Sandler, B.; Murakami, M.; Clardy, J. *J. Am. Chem. Soc.* **1998**, *120*, 595−596.

(30) After submission of this manuscript, another synthesis of aeruginosin 298-A was published that is in agreement with our stereochemical conclusions: Valls, N.; Lopez-Canet, M.; Vallribera, M.; Bonjoch, J. *J. Am. Chem. Soc. ASAP*, Nov. 1, 2000 Web publication date.