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Total Synthesis of Aeruginosin 98B

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Supporting Information Placeholder

ABSTRACT: The first total synthesis of aeruginosin 98B (**1**) was accomplished. The key step includes a highly diastereoselective Pd-catalyzed intramolecular asymmetric allylic alkylation (AAA) reaction of a diastereomeric mixture of allylic carbonates, which is enabled by the use of racemic phosphine ligand **L1**.

Aeruginosin 98B (**1**) is a tetrapeptide with an unnatural amino acid, isolated from a blue green algae *Microcystis aeruginosa* by Murakami and co-workers (Figure 1).¹ Its structure was determined by 2D-NMR analysis, and the absolute configurations of the seven stereogenic centers were determined by X-ray crystallographic analysis of its cocrystal with trypsin.² Aeruginosin 298A (**2**), another member of aeruginosin family, was synthesized by several groups.³ Oscillarin (**3**) was also isolated by Boehringer Mannheim GmbH.⁴ Recently, Hanessian and co-workers reported a total synthesis of aeruginosin 205B (**4**),⁵ which has a unique sulfonic sugar in the aeruginosin family.

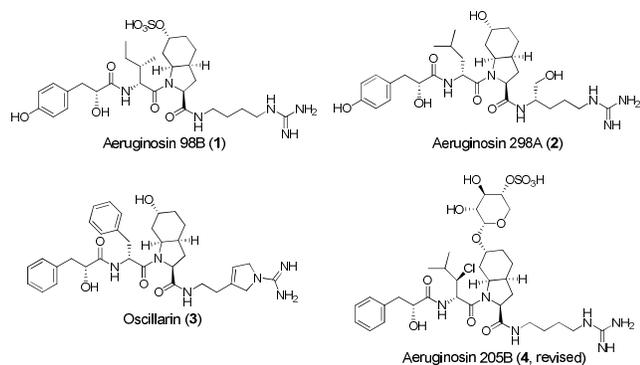


Figure 1. Structure of Aeruginosin 98B (1) and related compounds.

Aeruginosin family has gathered attention because of their promising biological activities. Aeruginosins exhibit inhibitory activities against serine proteases, especially thrombin and trypsin, and their activity profiles can be explained by a high degree of pharmacophoric and structural homology within the family.⁵ This array of structural and functional features is responsible for their high affinity to the catalytic binding pocket of trypsin, thrombin, and other serine proteases involved in the blood coagulation cascade. Aeruginosin 98B (**1**) was shown to

inhibit the serine proteases thrombin, plasmin, and trypsin with IC_{50} values of 10.0, 7.0 and 0.6 $\mu\text{g/mL}$, respectively.¹ The trypsin selectivity could be attributed to its unique 2-carboxy-6-hydroxyoctahydroindole sulfate (Choi sulfate) structure. The sulfonic acid group could interact with Tyr96 of trypsin through a H_2O -mediated hydrogen bonding.² However, other aeruginosins, aeruginosin 298A (**2**)⁶ and oscillarin (**3**)⁷ for example, are known to interact with Asp102 of thrombin. The negative interaction between the sulfonyl group and Asp102 may explain the IC_{50} value of aeruginosin 98B, which is 33 times smaller than that of aeruginosin 298. For these reasons, SAR studies of aeruginosin family were continued vigorously.^{3c, 8} Herein, we describe the first total synthesis of aeruginosin 98B.

A major goal in developing a new strategy is to enable facile structural diversity to examine structure-function relationships. Aeruginosin 98B can be dissected into four, suitably protected building blocks: 4-hydroxyphenyl lactic acid derivative (Hpla, **5**), bis-protected agmatine (**6**), 2-carboxyl-6-hydroxyoctahydroindole core (Choi, **7**), and *D*-*allo*-isoleucine (**8**) (Figure 2). It was further reasoned that the octahydroindole subunit could be prepared in a highly diastereoselective fashion by employing an intramolecular Pd-catalyzed AAA reaction⁹ on allylic carbonate **9a** and **9b**. Since the palladium-catalyzed allylic alkylation reaction is a stereospecific double inversion process, it is only necessary to control the relative stereochemistry between the protected hydroxyl and carbonate functional groups during the preparation of the AAA precursors **9a** and/or **9b**. An important component of developing a route was to avoid the use of tyrosine as a precursor to both Hpla and Choi fragments as was commonly done in the synthesis of the other aeruginosins, in order to allow more facile modification of those subunits.

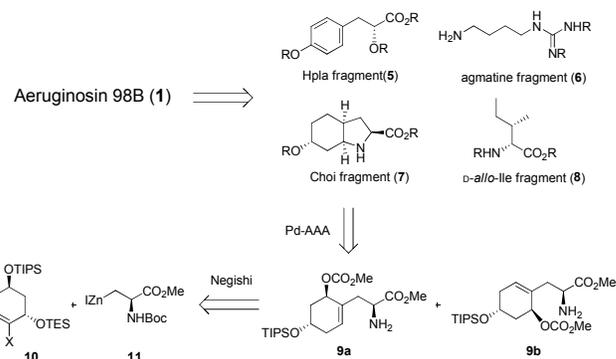
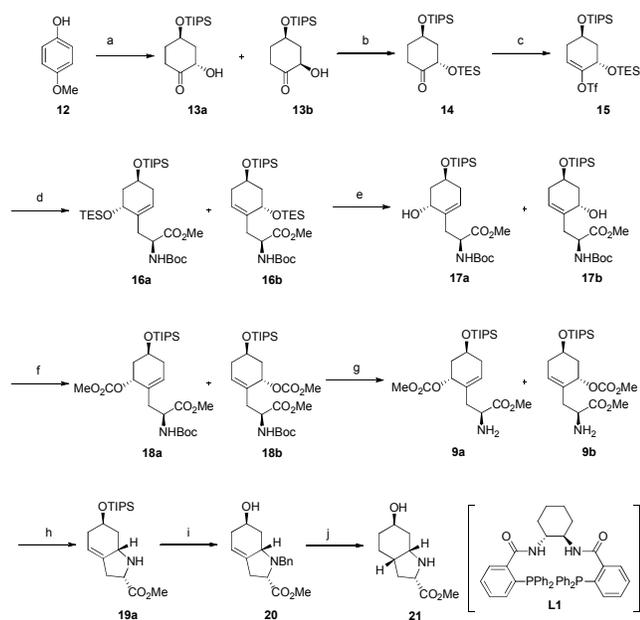


Figure 2. Retrosynthetic strategy of Aeruginosin 98B (1).

The allyl carbonates **9a** and **9b** were prepared from 4-methoxyphenol (**12**). Birch reduction¹¹ followed by TIPS silylation of the resultant alcohol provided methyl enol ether in 97% overall yield. Dihydroxylation of methyl enol ether provided a 2:1 mixture of α -hydroxyketones **13a** and **13b**. The direct hydroxylation of the TIPS ether of 4-hydroxycyclohexanone with MoOPH gave **13a** selectively in 72% yield. The *anti*-diastereomer **13a** was treated with TESCO under standard conditions to afford bis-silyl ether **14** in 97% yield. The enolate of ketone **14** was then allowed to react with Comins reagent¹² to furnish vinyl triflate **15** in 88% yield. Gratifyingly, compound **15** could be efficiently coupled with alkylzinc reagent **11**¹³ to give **16a** and **16b** as a 1:1 diastereomeric mixture. Amino esters **16a** and **16b** were treated with HF-pyridine complex to remove the TES protecting group selectively. The resulting allylic alcohols were easily separable by flash column chromatography and thus each diastereomer could be transformed separately toward the desired heterocycle. Treating **17a** and **17b** with methyl chloroformate and catalytic DMAP in CH₂Cl₂ furnished allylic carbonates **18a** and **18b** in 98% yield. Subsequent removal of the Boc protecting group under standard conditions, gave aminoesters **9a** and **9b** in 93% and 97% yield, respectively.

Scheme 1. Synthesis of Choi fragment.^a



^a(a) i) Li, NH₃, EtOH, -78°C, ii) TIPSOTf, 2, 6-lutidine, CH₂Cl₂, 0°C, iii) OsO₄, NMO, THF/H₂O=3/1, 0°C, 96%, **13a/13b**=2/1. (b) TESCO, imidazole, DMAP, CH₂Cl₂, r.t., 97%. (c) LHMDs, Comins reagent, -78°C to -40°C, 88%. (d) *N*-Boc-3-iodoalanine methyl ester, Zn, TMSiCl, Pd(PPh₃)₄, LiCl, DMA, 60°C, 87%. (e) HF-pyridine, CH₂Cl₂, 0°C, 93%. (f) Methyl chloroformate, pyridine, DMAP, CH₂Cl₂, 0°C, 98%. (g) TFA, CH₂Cl₂, -10°C, 93-97%. (h) [(η³-C₃H₅)PdCl]₂, racemic **L1**, TFA, THF, 60°C, 96%, **19a/19b**=11/1. (i) BnBr, Et₃N, CH₃CN, r.t., then TBAF, 89%. (j) 5% Pd/C, H₂, MeOH, r.t., 92%.

With the key intermediates **9a** and **9b** in hand, the diastereoselectivity of the Pd catalyzed allylic alkylation was exam-

ined. Using dppp as ligand with [(η³-C₃H₅)PdCl]₂ in THF at 60°C, no reaction was obtained (Table 1, entry 1).¹⁴ On the other hand, using the (*R,R*)-chiral ligand **L1**, a 4.4:1 ratio of **19a:19b** in a combined yield of 70% (Table 1, entry 2) was obtained. Surprisingly, switching to the (*S,S*) enantiomer of **L1**, a 3.8:1 ratio still favoring **19a** was obtained in 63% yield (Table 1, entry 3). Gratifyingly, switching to the racemic mixture of **L1** as ligand, this ratio favoring **19a** jumped to 11:1 in a 96% combined yield (Table 1, entry 4). Thus, a new role has been established for this family of ligands.

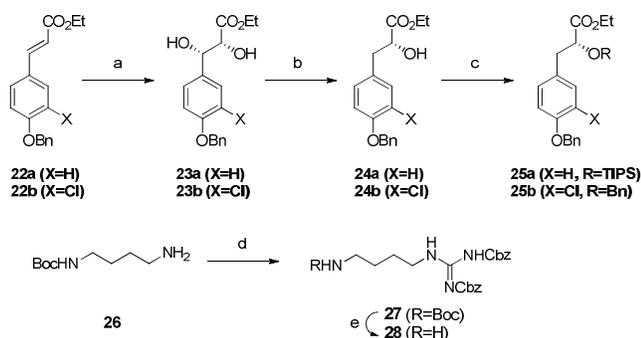
Completion of the synthesis of the Choi subunit required diastereoselective reduction of the olefin. Since the simple amino group proved unstable to TBAF conditions, this amino group was benzylated and subsequently treated with TBAF in the same pot to give alcohol **20** in 89% yield whose hydrogenation then directly led to amino alcohol **21** in 92% yield. The relative stereochemistry of **21** was assigned on the basis of nOe analysis (see Supporting Information). More importantly, the spectroscopic properties of its TFA salt exactly matched those previously reported by Bonjoch and co-workers.^{3a}

Table 1. Asymmetric allylic alkylation of diastereomeric mixture 9a and 9b.

entry	Ligand	19a (%)	19b (%)	Recovery (%)
1	dppp	0	0	71
2	(<i>R,R</i>)- L1	57	13	0
3	(<i>S,S</i>)- L1	50	13	trace
4	racemic L1	88	8	0

The synthetic routes to Hpla and agmatine fragments are shown in Scheme 2. The cinnamic ester **22a** was subjected to Sharpless asymmetric dihydroxylation to provide diol **23a**.¹⁵ The product was shown to have >99%ee by chiral HPLC analysis. Removal of the benzylic hydroxyl group was effected using triethylsilane and TFA in CH₂Cl₂ to afford α -hydroxyester **24a**. The hydroxyl group of **24a** was protected by TIPS by treatment with TIPSCl and imidazole. The same route starting from cinnamate **22b** (X=Cl) allows access to the hydroxylated ester **25b**, the intermediate required for the synthesis of aeruginosin 98A. Agmatine fragment **28**¹⁶ was prepared from *N*-Boc-1, 4-diaminobutane **26**, according to Rich and co-workers.¹⁷ Treatment of **26** with *N*, *N'*-bis(benzyloxycarbonyl)-1*H*-pyrazole-1-carboxamide in THF smoothly provided **27** in 65% yield. The Boc group was removed under standard conditions in 95% yield. *D*-*allo*-Ile methyl ester (**29**) was prepared by an esterification of *D*-*allo*-Ile, which was obtained by an optical resolution of *DL*-*allo*-Ile.¹⁸ Analytical data of **29** matched with that previously reported.¹⁹

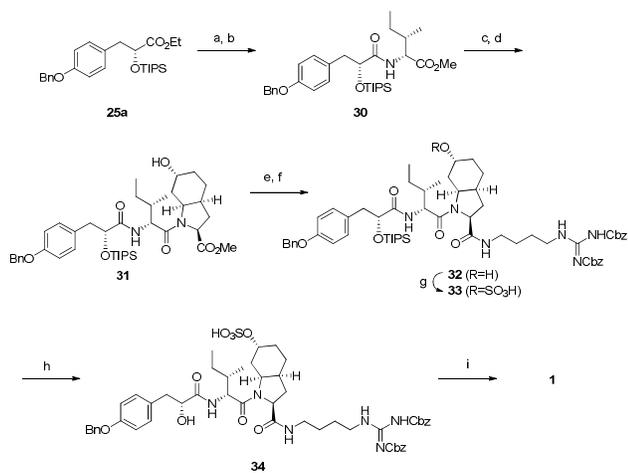
Scheme 2. Synthesis of other fragments.^a



^a(a) AD-mix α , MeSO₂NH₂, *t*-BuOH/H₂O=1/1, 0°C, **23a**; 86%, >99%ee, **23b**; 91%, 98%ee. (b) Et₃SiH, TFA, CH₂Cl₂, r.t., **24a**; 80%, **24b**; 82%. (c) **25a**; TIPSCl, imidazole, DMAP, CH₂Cl₂, r.t., 79%, **25b**; BnBr, Ag₂O, TBAI, PhH, reflux, >63%. (d) *N,N*-bis(benzyloxycarbonyl)-1*H*-pyrazole-1-carboxamide, THF, r.t., 65%. (e) TFA, CH₂Cl₂, 0°C, 95%.

Ethyl ester **25a** was hydrolyzed with 1N LiOH aq, and the resulting carboxylic acid was coupled with *D*-*allo*-Ile methyl ester (**29**) to afford dipeptide **30** in 73% yield (Scheme 3). Subsequent hydrolysis of the ester of **30** and the following peptide coupling with Choi fragment **21** provided tripeptide **31** in 69% yield. The tetrapeptide **32** was prepared in the same manner as the synthesis of tripeptide **31**, using agmatine fragment **28**. For the introduction of the sulfonic acid group, sulfur trioxide pyridine complex was used to afford sulfonic acid **33** in 75% yield. Removal of TIPS group was accomplished by treatment with HF-pyridine complex in acetonitrile to provide access to the precursor **34**. The removal of Bn and Cbz groups provided aeruginosin 98B (**1**) in quantitative yield. The crude material was purified by reverse phase HPLC to afford pure aeruginosin 98B (**1**) in 98% yield as a colorless solid. The ¹H- and ¹³C-NMR spectra matched those of the natural product. Surprisingly, the optical rotation of synthetic **1** was higher (-11.24, *c* 0.25, H₂O) than the natural product (-5.24, *c* 0.25, H₂O).

Scheme 3. Total synthesis of Aeruginosin 98B.^a



^a(a) 1N LiOH aq, THF/MeOH=3/2, r.t. (b) *D*-*allo*-Isoleucine methyl ester (**29**), TBTU, Et₃N, DMF, r.t. 73% in 2 steps. (c) 1N LiOH aq, THF/MeOH=3/2, r.t. (d) **21**, WSC, HOBt, Et₃N, DMF, 0°C-r.t., 69% in 2 steps. (e) 1N LiOH aq, THF/MeOH=3/2, r.t. (f)

28, WSC, HOBt, Et₃N, DMF, 0°C-r.t., 60% in 2 steps. (g) SO₃-pyridine, pyridine, 40°C, 75%. (h) HF-pyridine, CH₃CN, 0°C-r.t., 57%, 91%brsm. (i) Pd(OH)₂, H₂, MeOH, r.t., 98%.

In summary, we achieved the first total synthesis of aeruginosin 98B in eight steps from the four fragments (Choi, *D*-*allo*-Ile, Hpla and agmatine). After the construction of aeruginosin core, sulfonic acid was directly introduced by treatment with SO₃-pyridine complex. In the preparation of Choi fragment, a Pd-catalyzed intramolecular AAA reaction of a diastereomeric mixture of allyl carbonates **9a** and **9b** using racemic ligand **L1** provided hexahydroindole derivative **19a** in high diastereo- and enantioselectivity wherein the asymmetry derived from 3-iodo-*S*-alanine. Curiously, higher reactivity of the racemic mixture of the chiral ligands suggests that each enantiomer of the racemic ligand might have recognized the matched diastereomer of **9a** and **9b** imparting better kinetics for ionization but the substrate controls the diastereoselectivity. We are currently exploring the precise reaction mechanism that explains the observed diastereo- and enantioselectivity.

ASSOCIATED CONTENT

Supporting Information

Experimental procedures and analytical data for all new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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