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Total Synthesis of Aeruginosin 98B

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Supporting Information

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

Aeruginosin 98B (**1**) (Figure 1) is a tetrapeptide with an unnatural amino acid that was isolated from a blue-green

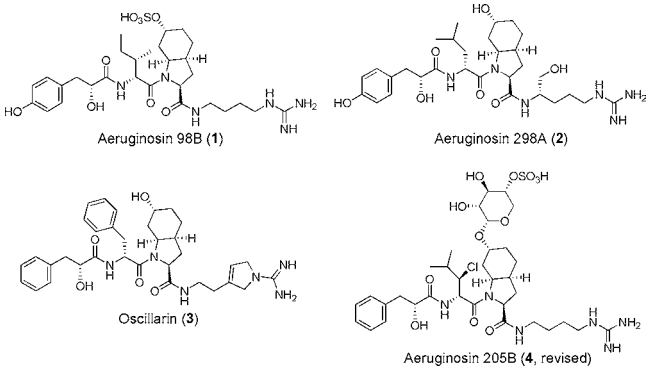

Figure 1. Structure of aeruginosin 98B (**1**) and related compounds.

algae, *Microcystis aeruginosa*, by Murakami and co-workers.[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 contains a sulfated sugar that is unique in the aeruginosin family.

The members of the aeruginosin family have 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 was shown to inhibit the serine proteases thrombin, plasmin, and trypsin with IC_{50} values of 10.0, 7.0, and 0.6 μg/mL, respectively.[1] The trypsin selectivity could be attributed to its unique 2-carboxy-6-hydroxyoctahydroindole sulfate (Choi sulfate) structure. The hydrogen sulfate group could interact with Tyr96 of trypsin through H_2O-mediated hydrogen bonding.[2] However, other aeruginosins, such as aeruginosin 298A[6] and oscillarin,[7] are known to interact with Asp102 of thrombin. The negative interaction between the hydrogen sulfate group and Asp102 may explain the IC_{50} value for aeruginosin 98B, which is 33 times smaller than that for aeruginosin 298A. For these reasons, structure–activity relationship studies of the 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: a 4-hydroxyphenyl lactic acid (Hpla) derivative (**5**), a bisprotected agmatine (**6**), the 2-carboxyl-6-hydroxyoctahydroindole (Choi) core (**7**), and D-*allo*-isoleucine (**8**) (Figure 2). It was further reasoned that the Choi subunit could

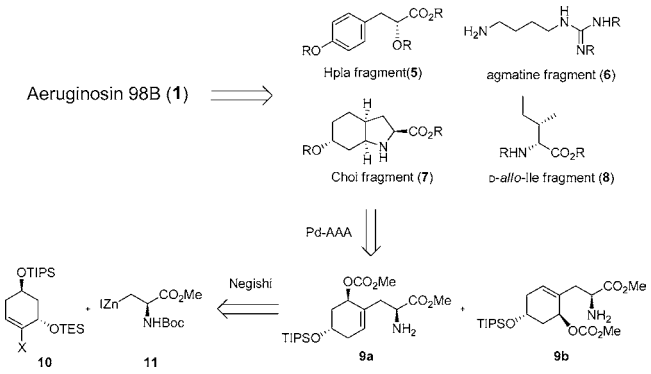

Figure 2. Retrosynthetic strategy for aeruginosin 98B (**1**).

be prepared in a highly diastereoselective fashion by employing an intramolecular Pd-catalyzed asymmetric allylic alkylation (AAA) reaction[9] on allylic carbonates **9a** and **9b**. Since the palladium-catalyzed AAA reaction is a stereospecific double-inversion process, it is necessary to control only 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 of both the 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.

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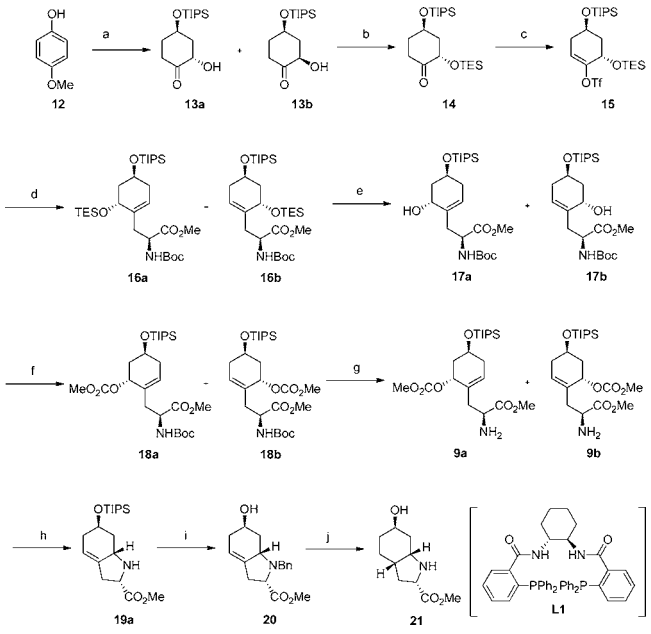
The allyl carbonates **9a** and **9b** were prepared from 4-methoxyphenol (**12**) (Scheme 1). Birch reduction[11] followed by triisopropylsilyl (TIPS) protection of the resultant alcohol provided the corresponding methyl enol ether in 97% overall yield. Dihydroxylation of the methyl enol ether provided a 2:1 mixture of α-hydroxyketones **13a** and **13b**. Direct hydroxylation of the TIPS ether of 4-hydroxycyclohexanone with MoOPH gave the anti diastereomer **13a** selectively in 72% yield. **13a** was treated with triethylsilyl chloride (TESCl) under standard conditions to afford bis-silyl ether **14** in 97% yield. The enolate of ketone **14** was then allowed to react with Comins reagent[12] 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, the two diastereomers could be transformed separately toward the desired heterocycle. Treating **17a** and **17b** with methyl chloroformate and catalytic 4-dimethylaminopyridine (DMAP) in CH_2Cl_2 furnished allylic carbonates **18a** and **18b** in 98% yield. Subsequent removal of the Boc protecting group under standard conditions gave amino esters **9a** and **9b** in 93% and 97% yield, respectively.

With the key intermediates **9a** and **9b** in hand, the diastereoselectivity of the Pd-catalyzed allylic alkylation was examined. When 1,3-bis(diphenylphosphino)propane (dppp) was used as the ligand with $[(\eta^3\text{-}C_3H_5)PdCl]_2$ in tetrahydrofuran (THF) at 60 °C, no reaction was obtained (Table 1, entry 1).[14] On the other hand, using the chiral ligand (*R*,*R*)-**L1** afforded **19a** and **19b** in a 4.4:1 ratio with a combined yield of 70% (entry 2). Surprisingly, with (*S*,*S*)-**L1**, a 3.8:1 ratio still favoring **19a** was obtained in 63% yield (entry 3). Gratifyingly, when a racemic mixture of **L1** was used as the ligand, this ratio favoring **19a** jumped to 11:1 with a 96% combined yield (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 to be unstable under TBAF conditions, this amino group was benzylated, and the resulting compound was subsequently treated with TBAF in the same pot to give alcohol **20** in 89% yield. Hydrogenation of **20** then directly led to amino alcohol **21** in 92% yield. The relative stereochemistry of **21** was assigned on the basis of nuclear Overhauser effect (NOE) analysis (see the Supporting Information). More importantly, the spectroscopic properties of its trifluoroacetic acid (TFA) salt exactly matched those previously reported by Bonjoch and co-workers.[3a]

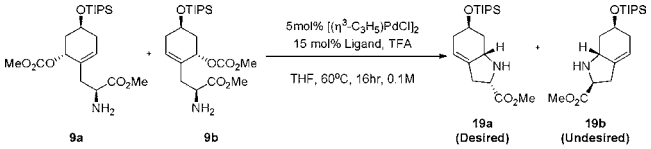
The synthetic routes to the Hpla and agmatine fragments are shown in Scheme 2. Cinnamic ester **22a** was subjected to Sharpless asymmetric dihydroxylation to provide diol **23a**.[15] The product was shown to have >99% ee by chiral HPLC

Scheme 1. Synthesis of the Choi Fragment[a]



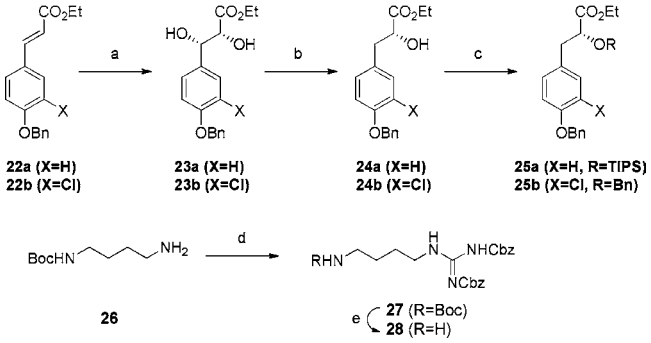
[a](a) (i) Li, NH_3, EtOH, −78 °C; (ii) TIPSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C; (iii) OsO_4, NMO, 3:1 THF/H_2O, 0 °C; 96%, **13a**/**13b** = 2:1. (b) TESCl, imidazole, DMAP, CH_2Cl_2, rt, 97%. (c) LHMDS, Comins reagent, −78 to −40 °C, 88%. (d) *N*-Boc-3-iodoalanine methyl ester, Zn, TMSCl, $Pd(PPh_3)_4$, LiCl, DMA, 60 °C, 87%. (e) HF–pyridine, CH_2Cl_2, 0 °C, 93%. (f) Methyl chloroformate, pyridine, DMAP, CH_2Cl_2, 0 °C, 98%. (g) TFA, CH_2Cl_2, −10 °C, 93–97%. (h) $[(\eta^3\text{-}C_3H_5)PdCl]_2$, racemic **L1**, TFA, THF, 60 °C; 96%, **19a**/**19b** = 11:1. (i) BnBr, Et_3N, CH_3CN, rt, then TBAF, 89%. (j) 5% Pd/C, H_2, MeOH, rt, 92%.

Table 1. Asymmetric Allylic Alkylation of the Diastereomeric Mixture 9a and 9b



		yield (%)		
entry	ligand	**19a**	**19b**	% recovery
1	dppp	0	0	71
2	(*R*,*R*)-**L1**	57	13	0
3	(*S*,*S*)-**L1**	50	13	trace
4	*rac*-**L1**	88	8	0

Scheme 2. Synthesis of Other Fragments[a]



[a](a) AD-mix α, $MeSO_2NH_2$, 1:1 *t*-BuOH/H_2O, 0 °C; **23a**, 86%, >99% ee; **23b**, 91%, 98% ee. (b) Et_3SiH, TFA, CH_2Cl_2, rt; **24a**, 80%; **24b**, 82%. (c) **25a**: TIPSCl, imidazole, DMAP, CH_2Cl_2, rt, 79%; **25b**: BnBr, Ag_2O, TBAI, PhH, reflux, >63%. (d) *N*,*N*′-bis(benzyloxycarbonyl)-1*H*-pyrazole-1-carboxamidine, THF, rt, 65%. (e) TFA, CH_2Cl_2, 0 °C, 95%.

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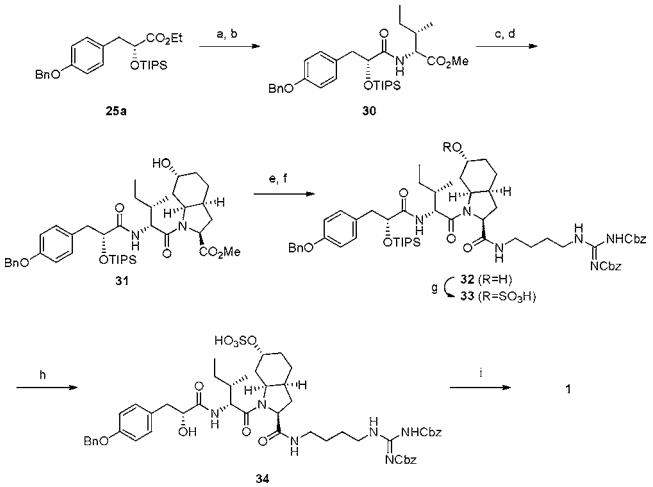
analysis. Removal of the benzylic hydroxyl group was effected using triethylsilane and TFA in CH_2Cl_2 to afford α-hydroxy ester **24a**. The hydroxyl group of **24a** was TIPS-protected by treatment with TIPSCl and imidazole. The same route starting from cinnamate **22b** (X = Cl) provided access to 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 the method of Rich and co-workers.[17] Treatment of **26** with *N*,*N*′-bis-(benzyloxycarbonyl)-1*H*-pyrazole-1-carboxamidine 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 optical resolution of DL-*allo*-Ile.[18] Analytical data for **29** matched that previously reported.[19]

Ethyl ester **25a** was hydrolyzed with 1 N LiOH(aq), and the resulting carboxylic acid was coupled with **29** to afford dipeptide **30** in 73% yield (Scheme 3). Subsequent hydrolysis

Scheme 3. Total Synthesis of Aeruginosin 98B[a]



[a](a) 1 N LiOH(aq), 3:2 THF/MeOH, rt. (b) **29**, TBTU, Et_3N, DMF, rt; 73% over two steps. (c) 1 N LiOH(aq), 3:2 THF/MeOH, rt. (d) **21**, WSC, HOBt, Et_3N, DMF, 0 °C to rt; 69% over two steps. (e) 1 N LiOH(aq), 3:2 THF/MeOH, rt. (f) **28**, WSC, HOBt, Et_3N, DMF, 0 °C to rt; 60% over two steps. (g) SO_3–pyridine, pyridine, 40 °C, 75%. (h) HF–pyridine, CH_3CN, 0 °C to rt, 57%, 91% brsm. (i) $Pd(OH)_2$, H_2, MeOH, rt, 98%.

of the ester of **30** followed by peptide coupling with Choi fragment **21** provided tripeptide **31** in 69% yield. Tetrapeptide **32** was prepared in the same manner as **31** using agmatine fragment **28**. For the introduction of the hydrogen sulfate group, sulfur trioxide–pyridine complex was used to afford hydrogen sulfate **33** in 75% yield. Removal of the TIPS group was accomplished by treatment with HF–pyridine complex in acetonitrile to provide access to precursor **34**. Removal of the Bn and Cbz groups provided aeruginosin 98B (**1**) in quantitive yield. The crude material was purified by reversed-phase HPLC to afford pure **1** in 98% yield as a colorless solid. The ^1H and ^{13}C NMR spectra matched those of the natural product. Surprisingly, the optical rotation of synthetic **1** was higher (−11.24, *c* 0.25, H_2O) than that of the natural product (−5.24, *c* 0.25, H_2O).

In summary, we achieved the first total synthesis of aeruginosin 98B in eight steps from four fragments (Choi, D-*allo*-Ile, Hpla, and agmatine). After the construction of the aeruginosin core, sulfonic acid was directly introduced by treatment with SO_3–pyridine complex. In the preparation of the 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, the higher reactivity of the racemic mixture of the chiral ligands suggested that each enantiomer of the racemic ligand might have recognized the matched diastereomer of **9a** or **9b**, imparting better kinetics for ionization; however, the substrate controlled the diastereoselectivity of cyclization. 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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Notes

The authors declare no competing financial interest.

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