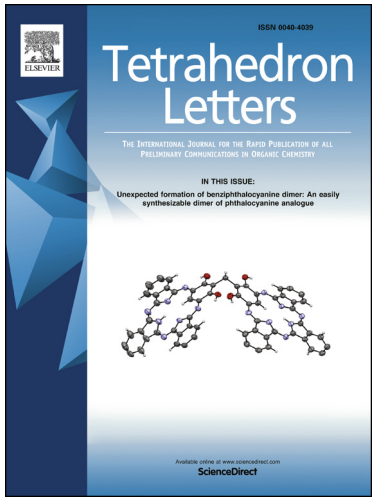

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Two-step Total Synthesis of an Anti-MRSA and Myosin-inhibiting Marine Natural Product Pentabromopseudilin via Suzuki-Miyaura Coupling of a MIDA Boronate Ester

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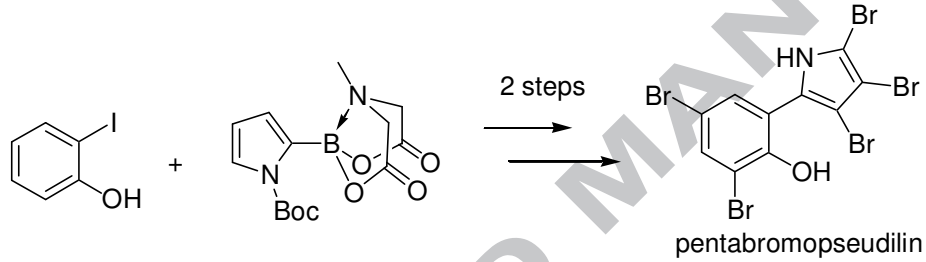
Abstract:
A marine natural product isolated from *Pseudoalteromonas sp.*, pentabromopseudilin, with promising anti-MRSA and myosin ATPase inhibition activities was synthesized in two steps using recently developed MIDA boronate Suzuki-Miyaura coupling technology. Additionally, bromination was shown to be necessary for the antimicrobial activity of pentabromopseudilin.

Graphical Abstract:



Keywords:
Suzuki-Miyaura;
Boronic acid MIDA ester;
MRSA;
Brominated pyrrole alkaloid

Introduction

Many medicinally important natural products contain a 2-substituted pyrrole unit, owing to their biosynthetic origin in proline.[1] As such, transition metal-catalyzed carbon-carbon coupling reactions to append pyrrole to another synthon would be a useful tool.[2] However, there have not been many cases where this coupling strategy was realized in the context of 2-aryl pyrrole natural product synthesis. As noted by Burke and co-workers, *N*-Boc-pyrrole-2-boronic acid (**1**) is a very labile substrate and often presents difficulties in Suzuki-Miyaura type couplings.[3] When compound **1** has been successfully used in a natural product synthesis, extensive experimentation to investigate different conditions and ligands was necessary.[4]

Since the introduction of the *N*-methyliminodiacetic acid (MIDA) boronic acid esters by Burke and co-workers, many such derivatives have become commercially available. However, only a handful of examples in natural product syntheses have emerged in the literature.[4] This may be partially attributed to the exquisite stability of MIDA boronate; in many attempted reactions, the boronate ester is too slow to carry out the coupling reaction with mild bases that are typically used for Suzuki-Miyaura coupling (SMC), such as K_2CO_3.[5]

A marine natural product isolated from *Pseudoalternomonas sp.*, pentabromopseudilin (**2**) possesses just such a difficult aryl-pyrrole connection.[6] It has been shown to possess impressive antimicrobial activity against MRSA at a level comparable to those of last resort drugs such as vancomycin (IC_{50} of 0.1 μM for **2** and 0.91 μM for vancomycin, respectively).[7] A more recent study has shown that it is a selective and potent myosin ATPase inhibitor (IC_{50} of 25 μM for class-2 myosins).[8] Naturally, compound **2** has attracted much attention from the organic synthesis community and a variety of unique and elegant syntheses have been described.[8,9] Moreover, the biosynthesis of **2** has recently been described in detail by Moore and co-workers.[10] However, all of the previous syntheses involve seven or more steps.[8,9] Herein, we report a two-step total synthesis of pentabromopseudilin (**2**) through the use of the MIDA boronic ester technology.

In all previous syntheses of **2**, the pyrrole ring was constructed *en route*, involving many steps to install the necessary functional groups for cyclization. In the example by Correia and co-workers, a pyrroline ring was installed *via* Heck coupling onto a protected phenol and subsequent oxidation was necessary to form the desired pyrrole.[9a] Inspired by Schmidt and co-workers unprotected halophenol SMC[11] and Weinreb and co-workers SMC of **1** with a decorated heterocycle in the synthesis of ageladine,[4a] a retrosynthetic dissection was made at the aryl-pyrrole carbon-carbon bond without a protecting group for the phenol.

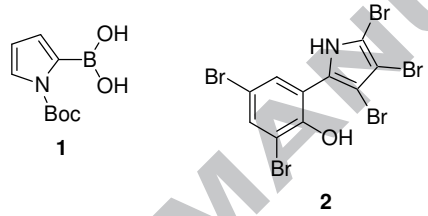
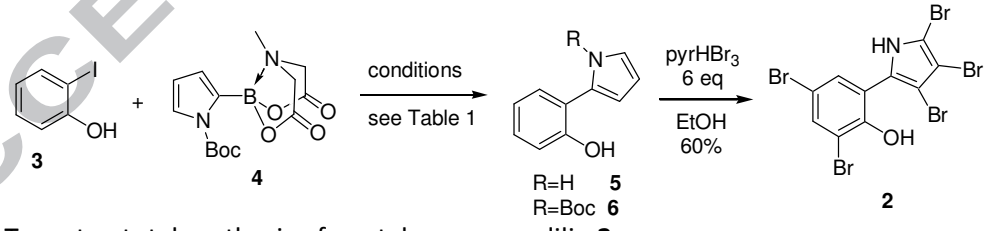


Figure 1. Labile boronic acid **1** and 2-aryl pyrrole marine natural product, pentabromopseudilin **2**.

Results and Discussion

When 2-iodophenol **3** and **1** were selected as the initial SMC partners with Pd/C as a heterogeneous catalyst only rapid decomposition and oxidative dimerization of **1** were observed. Attributing this to the instability of **1**, we turned our attention to the MIDA boronic ester substrate **4**. Firstly, the choice of an appropriate aqueous base was investigated by comparing K_2CO_3 and K_3PO_4. As previously reported,[3] the more basic K_3PO_4 was required for sufficiently rapid hydrolysis of the boronate ester, as indicated by the observation that **4** remained intact after many hours of reflux when K_2CO_3 was employed.



Scheme 1. Two-step total synthesis of pentabromospeudilin **2**.

Entry	Pd catalyst	Solvent	Base	Yield 5 (%)[b]
1	Pd/C, CyJohnPhos	THF/H_2O	K_3PO_4	0%
2	$PdCl_2$, CyJohnPhos	THF/H_2O	KOH	31%
3	Sphos Pd G2	THF/H_2O	K_3PO_4	15%
4	Sphos Pd G2	THF/H_2O	KOH	64%[a]
5	Sphos Pd G2	MeCN/H_2O	K_3PO_4	trace

6	Sphos Pd G2	MeCN/H_2O	KOH	trace
7	Sphos Pd G2	H_2O	K_3PO_4	0%
8	Xphos Pd G2	THF/H_2O	KOH	21%

Table 1. Optimization of the SMC between **4** and **3**. Reagents and conditions: catalyst (10 mol%), 4 M aqueous base (8 equiv), 65 °C unless otherwise specified. (a) 64% yield at 70, 65, and 55 °C, but 21% yield at rt (b) isolated yield.

When Pd/C alone or in conjunction with the CyJohnPhos ligand was used as the catalyst, no desired coupled product was observed (not shown). However, when $PdCl_2$ and CyJohnPhos were used as the catalyst, a low yield of the coupled product without the Boc group (**5**) was obtained. Interestingly, we were not able to isolate the Boc-protected aryl-pyrrole **6** in any of the reactions. Aqueous base hydrolysis of pyrrole Boc groups has been previously observed, especially during SMC which often require heating.[12] This finding is not surprising because the pyrrole ring is able to stabilize a formal negative charge on nitrogen, aiding the decarboxylative cleavage of the Boc group without acidic activation of the nitrogen atom. This presented an advantage to us since it negates the deprotection step and makes our synthesis of **2** a two-step synthesis from commercially available starting materials, which is significantly more concise compared to the previous syntheses.

Three palladium catalysts that were available to us were investigated for their effectiveness (Table 1). The Buchwald 2nd generation precatalyst (Sphos Pd G2) was more effective than $PdCl_2$ or Xphos Pd G2 (Entries 2, 4, and 8). The stronger the aqueous base, the better yield was obtained, owing to the stability of the boronate ester group in **4**. In our hands, the SMC product, pseudilin (**5**), was obtained after purification as a white solid that turns reddish-brown after several hours at rt. Comparison of the known bromination protocol using pyridinium tribromide[7a,b,c] and molecular bromine in AcOH revealed that the latter treatment oxidatively opened the pyrrole ring to produce a carbonyl-containing product, as evidenced by the IR stretch at 1724 cm^{-1}.[13] The known protocol gratifyingly produced pentabromopseudilin **2** in 60% yield.[8,9a,9b] Our synthetic material **2** possessed virtually identical ^1H and ^{13}C NMR chemical shifts to those of the authentic sample as reported by Laatsch[14] and co-workers and the synthetic material reported by Knölker and co-workers.[8]

Freshly prepared **5** and **2** were tested against *Staphylococcus epidermidis*, a non-pathogenic relative of MRSA, using a disc diffusion assay. Both compounds were used at concentrations ranging from 50 µM to 0.05 µM to determine their relative activity against *S. epidermidis*. Compound **5** showed no activity against *S. epidermidis* growth. However, compound **2** resulted in clear zones of inhibition at 50 and 5 µM. Previously, **5** was likewise shown to have no inhibitory activity on myosin-2 while **2** showed potent inhibition through a novel allosteric binding pocket.[15] However, to the best of our knowledge, this is the first time that bromination of **5** was shown to be necessary for pentabromopseudilin's antimicrobial activity.

Conclusion

An efficient total synthesis of the antibiotic marine natural product, pentabromopseudilin **2** was achieved through SMC in 2 steps with a 38% overall yield.[16] Application of this synthesis to the biosynthetically related tricyclic natural product, 2,3,5,7-tetrabromobenzofuro[3,2-b]pyrrole is now under investigation.

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16. *Experimental Procedures:* **Materials and Methods.** The SPhos Pd G2 and XPhos Pd G2 catalysts, *N*-Boc-pyrrole-2-boronic acid MIDA ester, iodophenol, and CyJohnPhos were purchased from Sigma-Aldrich. Other reagents and solvents were obtained from various commercial sources. All solvents were deoxygenated by passing a stream of nitrogen gas immediately before use. All reactions were carried out under a nitrogen atmosphere introduced *via* evacuation by an aspirator and a nitrogen balloon. MS data were obtained on an ABSciex 3200 QTrap mass spectrometer. ^{1}H and ^{13}C NMR spectra were obtained on Bruker Avance III 500 MHz spectrometer equipped with a 5mm BBO probe in $CDCl_3$. Chemical shifts are reported relative to residual protonated chloroform (δ 7.26) for ^{1}H NMR and chloroform (δ 77.16) for ^{13}C NMR. Infrared spectra were obtained on Thermo-Fischer Nicolet iS10 FT-IR spectrometer. Thin layer chromatography analysis was performed using EMD Millipore silica gel 60 F_{254} aluminum-backed TLC plates.

Representative Protocol for synthesis of 2-phenyl-1*H*-pyrrole (5).

The small conical vial containing SPhos Pd G2 (4.5 mg, 0.0062 mmol) was charged with THF (0.5 mL). Upon stirring with a magnetic stirrer for 10 minutes at rt under N_2, 2-iodophenol (13 mg, 0.059 mmol), *N*-Boc-pyrrole-2-boronic acid MIDA ester (20 mg, 0.062 mmol), and an aqueous 4 M KOH solution (0.124 mL, 0.496 mmol) were added successively and the vial sealed with a septum lid. Immediately, the conical vial was placed in a sand bath preheated to 55 °C. While the temperature of the reaction mixture climbed to the sand bath temperature, excessive pressure was relieved twice by puncturing the septum with a needle connected to a balloon

filled with N_2. After heating and stirring overnight, the reaction mixture was cooled to rt under N_2. Upon diluting with diethyl ether, aqueous 0.5 M HCl was added to acidify the mixture. After phase separation, the aqueous layer was washed twice with diethyl ether (4 mL x2) and the combined organic layers dried over Na_2SO_4. After removal of the solvents *in vacuo*, the crude mixture was purified by a silica gel column with a gradient mobile phase ranging from 100% hexanes to 3:7 EtOAc/hexanes. After removal of the solvents *in vacuo*, a white solid (6.0 mg, 0.038 mmol, 64% yield) was obtained, which turned slightly reddish brown upon standing in air at rt. TLC R_f = 0.20 (1:4 EtOAc/hexanes), visualized by UV (spot turns purple after a while on TLC). IR (film) ν_{max} 3433 (br), 2932, 2854, 1587, 1495, 1465, 1407, 1331, 1291, 1244, 1181, 1101, 1050, 1036, 922, 817, 796, 750, 724, 658 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 9.39 (br s, 1H), 7.54 (dd, *J* = 1.6, 7.8 Hz, 1H), 7.10 (dt, *J* = 1.7, 7.7 Hz, 1H), 6.97 (dt, *J* = 1.2, 7.6 Hz, 1H), 6.91-6.90 (m, 1H), 6.85 (dd, *J* = 1.1, 8.0 Hz, 1H), 6.58-6.57 (m, 1H), 6.33-6.31 (m, 1H), 5.36 (br s, 1H) ppm. ^{13}C NMR (175 MHz, $CDCl_3$) δ 151.1, 128.8, 127.4, 127.2, 121.6, 119.8, 118.7, 116.4, 109.4, 106.4 ppm. LRMS (ESI+): calcd for $C_{10}H_{10}NO$ (M+H): 160.08, found 160.1.

Synthesis of pentabromopseudilin (2).

To a solution of pseudilin (**5**) (15 mg, 0.094 mmol) in EtOH (2.3 mL) at rt under N_2 was added pyridinium tribromide (201 mg, 0.566 mmol, 6 eq). The reaction mixture was stirred under N_2 for 3 days. Upon removal of the solvent *in vacuo*, the residues were purified by silica gel chromatography with dichloromethane/hexanes (1:1). A slightly pink-purple solid (31 mg, 0.056 mmol, 60%) was obtained upon removal of solvents *in vacuo*. TLC R_f = 0.34 (1:1 CH_2Cl_2/hexanes) and R_f = 0.65 (chloroform), visualized by UV. IR (film) ν_{max} 3473 (br), 3405 (br), 3064, 2980, 1724, 1591, 1470, 1426, 1343, 1320, 1230, 1158, 1128, 996, 976, 856, 749, 689 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 9.49 (br s, 1H), 8.11 (d, *J* = 2.2 Hz, 1H), 7.58 (d, *J* = 2.2 Hz, 1H), 6.05 (s, 1H) ppm. ^{13}C NMR (175 MHz, $CDCl_3$) δ 147.4, 133.3, 131.0, 125.2, 119.5, 113.4, 112.1, 104.0, 101.1, 99.5. ppm. LRMS (ESI-): calcd for $C_{10}H_3Br_5NO$ (M-H): 547.61, found 547.5.

Antibacterial Assay.

The Kirby-Bauer method was followed for the disk diffusion assay. Sterile filter paper disks (Fisher Scientific, Porosity: Fine, Flow Rate: Slow, cut to 7.5 mm) were embedded with 5 µL of pseudilin, pentabromopseudilin, or DMSO as a negative control. Pre-made antibiotic disks of ciprofloxacin (5 µg), chloramphenicol (30 µg), and sulfamethoxazole with trimethoprim (5 µg; all obtained from Carolina Biological Supply Company) were used as positive controls. Both pseudilin (**5**) and pentabromopseudilin (**2**) were used at the following concentrations: 50 µM, 5 µM, 0.5 µM, 0.05 µM. After adding the compounds to the filter paper disks, they were incubated overnight at 4 °C. The *Staphylococcus epidermidis* at a concentration of 1.5 x 10^8 CFU/ml was spread onto Mueller-Hinton agar plates (made from dehydrated medium from Carolina Biological Supply company and poured 4mm deep) using a glass rod. The embedded filter paper disks were added to the Mueller-Hinton agar plates and incubated at 37 °C for 21 hr. A clear zone of inhibition around the filter paper disk indicated activity against *S. epidermidis*.

Supplementary Material

The supplementary material contains copies of ^1H and ^{13}C NMR spectra of **5** and **2** (PDF).

- Two-step total synthesis of pentabromopseudilin (previously seven steps)
- MIDA boronate ester Suzuki-Miyaura coupling
- Bromination shown to be necessary for antimicrobial activity