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Antibiofilm Activity of a Diverse Oroidin Library Generated through Reductive Acylation

T. Eric Ballard, Justin J. Richards, Arianexys Aquino, Catherine S. Reed, and Christian Melander*

Department of Chemistry, North Carolina State University, 2620 Yarbrough Drive, Raleigh, North Carolina 27695

christian_melander@ncsu.edu

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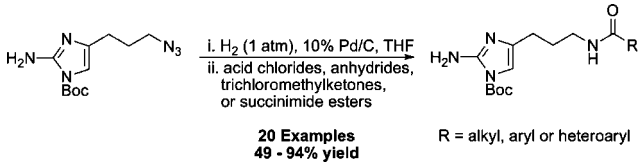

A diverse 20-compound library of analogues based on the marine alkaloid oroidin were synthesized via a reductive acylation strategy. The final target was then assayed for inhibition and dispersion activity against common proteobacteria known to form biofilms. This methodology represents a significant improvement over the generality of known methods to acylate substrates containing 2-aminoimidazoles and has the potential to have broad application to the synthesis of more advanced oroidin family members and their corresponding analogues.

Bacterial infections are now known to predominately exist in a biofilm growth state that confers both enhanced virulence and defensive properties to the bacterium.[1] Biofilms are responsible for upward of 75% of bacterial infections in the body making remediation of these infections problematic.[2] Bacteria that reside within the biofilm state also are inherently more resistant to many antibiotics and biocides that would often lead to their eradication.[2,3] Given the breadth of biofilm mediated infections, there is a significant need for new and potent antibiofilm modulators.

Oroidin (**1**) has been previously documented to have moderate biofilm inhibitory activity against PAO1 and PA14,[4] two of the most commonly studied strains of the γ-proteobacterium *Pseudomonas aeruginosa*. It was also shown that oroidin elicits its effects through a nonmicrobicidal mechanism; however, the exact mechanism of action is currently unknown and still under active investigation. Since naturally occurring biofilm modulators are scarce,[5−7] it became a goal to use oroidin as a template

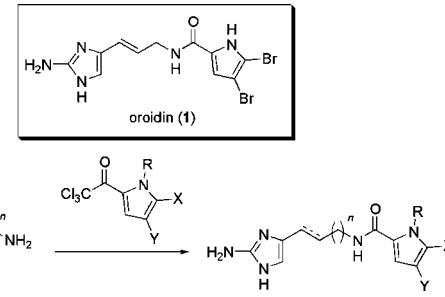

FIGURE 1. Original synthesis of oroidin analogues.

for the development of novel small molecules that control biofilm growth and maintenance (Figure 1).[4,8−13]

Numerous reports have been disclosed detailing the acylation step toward the total synthesis of oroidin with yields ranging from only 13% to 25%.[14−17] Other groups have experienced low yields on other oroidin family members incorporating the similar architecture of dibrominated pyrrole carboxamide moieties. Toward the synthesis of dimethylbromoageliferin by Ohta et al., installation of the pyrrole carboxamide proceeded in only 21% even on a methylated 2-aminoimidazole (2-AI) scaffold.[18] In the recent synthesis of preaxinellamine, Baran et al. obtained a 45% yield of the pyrrole carboxamide coupled product.[19] Additional syntheses have also suffered from the low-yielding amide bond formation step.[20−22]

We envisioned the use of a reductive *in situ* acylation reaction on the protected 2-AI azido scaffold **3** (Scheme 1) as a robust method of constructing diverse molecules with amide bond directionality identical with the natural products. Although reductive approaches to acylate 2-AI derived compounds are

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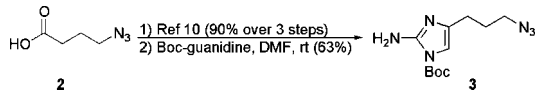
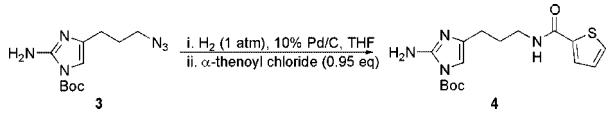
SCHEME 1. Synthesis of the Core Boc-2-AI Azide Scaffold



2 **3**

TABLE 1. Optimization of the Reductive Acylation Reaction



3 **4**

entry	temp, °C	Base	time, min	yield,[d] %
1[a]	23		20	10
2[b]	0	pyridine[c]	60	38
3[b]	−40 to 23	pyridine[c]	120	69
4[b]	−78 to 23		90	67
5[b]	−78 to 0		90	71

[a] α-Thenoyl chloride added neat. [b] α-Thenoyl chloride added dropwise in CH_2Cl_2. [c] 1.0 equiv added before α-thenoyl chloride. [d] Isolated yield.

known,[21,22] development of a generic set of reaction conditions that allow coupling to a diverse range of acylating reagents (acid chlorides, anhydrides, succinimide esters, etc.) has not been established. Furthermore, a Boc-protected 2-AI scaffold would facilitate purification and mitigate over acylation. To this end, a 20-compound oroidin analogue library was synthesized to demonstrate the applicability of this methodology. These derivatives were then assayed for their ability to inhibit and disperse the medically relevant bacterial biofilms formed by the proteobacteria *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Bordetella bronchiseptica*.

Scaffold **3** was quickly accessed from the known[23] 4-azidobutyric acid **2** by formation of the acid chloride followed by diazomethane homologation and quenching with concentrated HBr to yield the α-bromoketone in 90% yield over three steps (Scheme 1).[10] Condensation of the α-bromoketone with Boc-guanidine cleanly afforded the desired Boc-protected intermediate **3**.

Azide **3** was submitted to hydrogenation under a hydrogen atmosphere at ambient temperature in THF for 12 h. Following reduction to the amine, the Pd/C was removed by filtration and the amine was then subjected to the acylation reaction. Optimization of the acylation reaction was investigated by employing α-thenoyl chloride as the acylating agent (Table 1). The acid chloride (0.95 equiv) was first added to the amine neat at ambient temperature and the reaction was monitored by TLC analysis. Complete consumption of the starting material was evident within 20 min but only resulted in 10% of the desired product **4**. Next, the crude amine was cooled to 0 °C in the presence of pyridine. The acid chloride, diluted in CH_2Cl_2, was then added dropwise resulting in a 38% yield of **4**. Further tuning of the reaction temperature led to the optimized conditions (entry 5) which afforded a 71% yield of the desired acylated product.

Following our optimized protocol, ten additional structurally and electronically diverse acid chlorides were used as acylation coupling partners (Table 2, method A). Most notable was the overall generality of the acylation reaction with all acid chlorides, allowing for rapid incorporation of many functional groups not tolerated by previously established chemistry.

Symmetric anhydrides were next investigated for their ability to couple efficiently to the Boc-2-AI scaffold **3** (Table 2, method B). Acylating agents of this type would be advantageous in cases where the acid chloride of a desired acylating agent is either not available or less stable than its anhydride counterpart. Of the three anhydrides examined, **15** and **16** coupled in yields of >90% and the hexyl analogue **5** was isolated in 70% yield.

Acylations with various dibromopyrrole trichloromethylketones to produce Boc-2-AI congeners of previously reported dihydrooroidin analogues allowed for a direct comparison of yield improvements with our new method over known procedures (Table 2, method C).[4,13] It was quickly evident that the use of this methodology allowed for dramatic improvements in the yields of isolated products (**17**−**22**). The most remarkable improvements were seen in the reactions with the ethyl- and phenethyl-*N*-substituted dibromopyrroles (coupled products **19** and **20**) as the previous yields obtained through the original route (Figure 1) were 14% and 22%, respectively (data not shown).

With the broad generality and high yields obtained through this method, the coupling of a commercially available succinimide ester of an enantiomerically pure amino acid was investigated (Table 2, method D). The coupling reaction proceeded smoothly to afford the acylated product **23** in 88% yield. In addition to being the first example of coupling a succinimide ester to the dihydrooroidin scaffold, it was also the first example of coupling an enantiopure acylating agent.

Deprotection of all intermediates (**4**−**23**) proceeded in >95% yield with 20% TFA in methylene chloride (Table 2). After HCl salt exchange, the analogue library (**4a**−**23a**) was assayed for its ability to inhibit and disperse the three medically relevant biofilm forming bacteria *P. aeruginosa*, *A. baumannii*, and *B. bronchiseptica*.

P. aeruginosa (PA14) is a ubiquitous γ-proteobacterium that is a persistent threat to immuno-compromised patients and is also responsible for the increased morbidity and mortality of cystic fibrosis (CF) patients.[24−26] *A. baumannii* (Actb) is also an opportunistic γ-proteobacterium that has become problematic due to increased multidrug resistance and its ability to withstand harsh environmental pressures.[27−29] *B. bronchiseptica* (RB50) is a β-proteobacterium that shares the same virulence factors with *B. pertussis*, which causes whooping cough.[30,31] It has been shown that molecules incorporating a 2-AI motif have had the ability to cross bacterial class to inhibit and disperse biofilms formed by both γ- and β-proteobacteria.[11,12] Since biofilm formation is directly correlated with increased virulence of the bacterium, the above bacteria represent a significant hurdle to the medical community in terms of controlling and eradicating infections.

All library members were first screened at 100 μM for their ability to inhibit and disperse PA14, Actb, and RB50 biofilms utilizing a crystal violet reporter assay.[8,10,11,32] Upon identifica-

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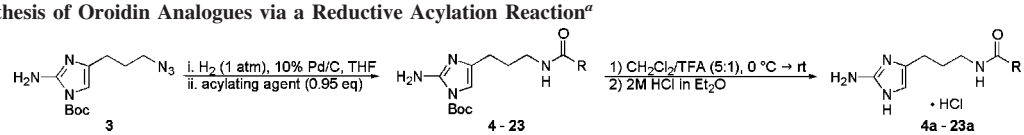
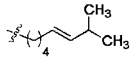
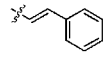
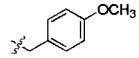
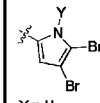
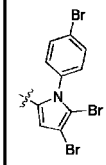
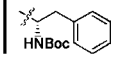
TABLE 2. Synthesis of Oroidin Analogues via a Reductive Acylation Reaction[a]



R =	Acylation Method [b]	Coupled Product (yield) [c]	Final Target
	A	4 (71%)	4a
	A	5 (73%)	5a
	A	6 (60%)	6a
	A	7 (70%)	7a
	A	8 (66%)	8a
			
Y = H	A	9 (85%)	9a
= Ph	A	10 (79%)	10a
= CF_3	A	11 (77%)	11a
= $N(CH_3)_2$	A	12 (49%)	12a
	A	13 (70%)	13a
	A	14 (51%)	14a
	B	5 (70%)	5a
	B	15 (91%)	15a
	B	16 (94%)	16a

R =	Acylation Method [b]	Coupled Product (yield) [c]	Final Target
			
Y = H	C	17 (85%)	17a
= CH_3	C	18 (89%)	18a
= CH_2CH_3	C	19 (87%)	19a
= $(CH_2)_2Ph$	C	20 (90%)	20a
	C	21 (61%)	21a
	C	22 (65%)	22a
	D	23 (88%)	23a

[a] Acylating agent added dropwise in CH_2Cl_2. [b] Method A: acid chloride. Method B: anhydride. Method C: trichloromethylketone. Method D: succinimide ester. [c] Isolated yield.

TABLE 3. Antibiofilm Activity of Selected Oroidin Analogues

analogue	PA14	Actb	RB50
	biofilm inhibition at 100 μM (%)[a]		
6a	61 ± 4	23 ± 4	42 ± 2
10a	77 ± 3	28 ± 3	45 ± 1
15a	85 ± 1	>95	79 ± 2
20a	>95	>95	95 ± 1
21a	>95	>95	>95
	biofilm dispersion at 100 μM (%)[a]		
6a	36 ± 4	22 ± 3	<5
10a	21 ± 3	27 ± 5	21 ± 2
15a	49 ± 5	29 ± 3	11 ± 3
20a	74 ± 5	70 ± 3	<5
21a	72 ± 3	>95	<5

[a] Experiments performed in triplicate or more.

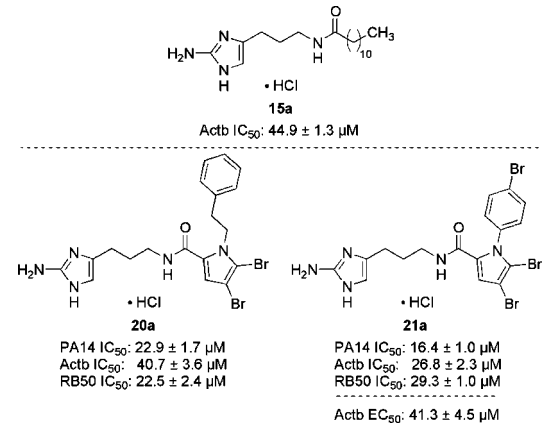


FIGURE 2. IC_{50} and EC_{50} values of the most active derivatives.

tion of compounds with greater than 95% antibiofilm activity (Supporting Information), the analogues were further evaluated to determine their respective IC_{50} (biofilm inhibition) or EC_{50} (biofilm dispersion) values. The five most active analogues are summarized in Table 3.

Following previously observed trends,[8,10−13] the analogue library was more active as biofilm inhibitors than as biofilm dispersal agents. Overall, the library displayed the greatest activity against PA14 followed by Actb and RB50. The three most active analogues (**15a**, **20a**, and **21a**) were then carried on for IC_{50} and EC_{50} value determination (Figure 2). After these experiments were performed, bacterial growth curves were run to determine if the analogues were killing planktonic bacteria or modulating biofilm growth through a nonmicrobicidal mechanism (Supporting Information). No bactericidal effects were observed for **20a** or **21a** against PA14 or Actb. However, both compounds displayed a bacteriostatic effect in the RB50 growth curves. Analogue **15a** also displayed a bacteriostatic effect during the earlier time points against Actb, but cell densities were identical at the 24-h time point.

In conclusion, we have successfully synthesized a 20-compound oroidin library utilizing a very efficient reductive acylation reaction. This methodology has allowed for the generation of new oroidin analogues employing a variety of acylating agents and has also dramatically increased acylation yields with traditional substrates such as trichloromethylketone pyrroles. Additionally, many of these new analogues are not accessible through previously known chemical pathways. The implementation of this methodology has the potential to have broad application to the synthesis of more advanced and diversely substituted 2-AI natural product analogues. Further analogue development as well as application of this methodology to more advanced structures is currently under investigation and will be presented in due course.

Experimental Section

Representative Procedure for Reductive Acylation. To a solution of anhydrous THF (4 mL) and 10% Pd/C (0.01 g) was charged compound **3** (0.10 g, 0.375 mmol). Air was removed from

the system and the reaction was back flushed with hydrogen. This process was repeated three times before setting the reaction under a hydrogen balloon at atmospheric pressure and temperature for 12 h. After that time, the reaction was filtered to remove the catalyst. The filtrate was cooled to −78 °C and α-thenoyl chloride (0.05 g, 0.356 mmol) diluted in anhydrous dichloromethane (0.50 mL) was added dropwise to this solution. The reaction was stirred at −78 °C for 30 min and then warmed to 0 °C and allowed to stir for 1 h. Upon completion of the reaction as evident by TLC analysis, the reaction was evaporated to dryness and purified by flash column chromatography (0−10% MeOH/CH_2Cl_2) to obtain the title compound **4** (0.093 g, 71%) as a white foam: mp 159−160 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.53 (t, 1H, J = 5.1 Hz), 7.72 (m, 2H), 7.12 (m, 1H), 6.57 (s, 1H), 6.39 (br s, 2H), 3.21 (dt, 2H, J = 6.6, 12.6 Hz), 2.31 (t, 2H, J = 7.2 Hz), 1.79 (tt, 2H, J = 7.2, 14.4 Hz), 1.53 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.0, 149.9, 148.9, 140.3, 138.3, 130.5, 127.8, 127.7, 105.8, 84.1, 38.7, 27.9, 27.5, 25.3; HRMS (ESI) calcd for $C_{16}H_{22}N_4O_3S$ (M^+) 350.1413, found 350.1406.

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Supporting Information Available: Complete biofilm results, growth curves for **15a**, **20a**, and **21a**, experimental procedures, and spectral data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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