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One-Pot Syntheses of Pseudopteroxazoles from Pseudopterosins: A Rapid Route to Non-natural Congeners with Improved Antimicrobial Activity

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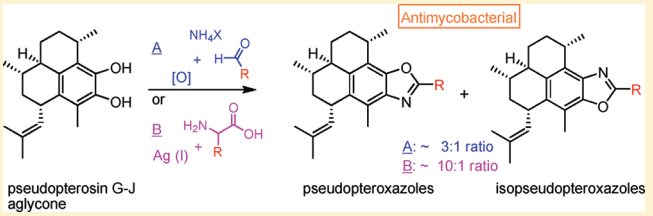
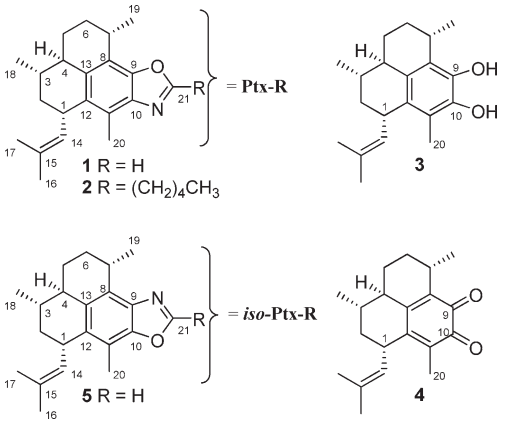
Supporting Information

ABSTRACT: Rapid one-pot methodologies to prepare pseudopteroxazole (**1**) and novel congeners from abundant natural pseudopterosins have been devised. This is highlighted here with the first synthesis of the marine natural product homopseudopteroxazole (**2**) utilizing a novel, silver(I)-mediated catechol to benzoxazole transformation. Pseudopteroxazoles and isopseudopteroxazoles exhibit potent activity against a range of important Gram-positive pathogens including *Mycobacterium* spp. and vancomycin-resistant *Enterococcus faecium*. Several non-natural pseudopteroxazoles exhibited strong activity against methicillin-resistant *Staphylococcus aureus*, thereby displaying a broader spectrum of antibiotic activity compared to pseudopteroxazole.



Natural product scaffolds (NPS) are of tremendous value in drug discovery, playing a vital role in the genesis of a significant proportion of drugs, particularly anti-infectives.[1,2] The supply of promising natural product-based lead compounds may be an issue when the natural source is limited and total synthesis is inefficient or impractical.[3] Lengthy synthetic routes to NPS may also delay or hamper medicinal chemistry efforts to generate analogues for SAR studies. An alternative to total synthesis is to utilize abundant, naturally occurring molecular architectures as starting materials for natural product-based drug discovery. Herein, we describe such methodology to rapidly supply a promising low-abundance natural product, pseudopteroxazole (**1**),[4] the related natural product homopseudopteroxazole (**2**),[5] and novel non-natural congeners including more potent antibacterial compounds.

Pseudopteroxazole is a promising antibiotic because it exhibits strong activity against *Mycobacterium tuberculosis*, the causative agent of tuberculosis, with commensurate low toxicity.[4] Interest in **1** by the synthetic community[6−8] has resulted in elegant multistep total syntheses described by the groups of Corey[9] and Harmata.[10,11] These syntheses, however, have been reported only on a low-milligram scale, and they have not led to the syntheses of congeners of **1** nor the natural product **2**. To date, medicinal chemistry investigations around **1** have not been described. An efficient route to both larger quantities and congeners of pseudopteroxazoles is therefore desirable. Using the methodologies described here, we have prepared several hundred milligrams of **1** using a one-pot synthesis.



RESULTS AND DISCUSSION

Given that **1** possesses the same backbone as the pseudopterosin G−J aglycone (**3**),[12] we rationalized that **1**, **2**, and analogues could be attainable semisynthetically from **3**. This approach seemed logical because the various pseudopterosin glycosides[13] are available in multigram quantities, constituting up to about 5% of the dry weight of the gorgonian *Pseudopterogorgia elisabethae*. In order to rapidly build a series of pseudopteroxazole congeners

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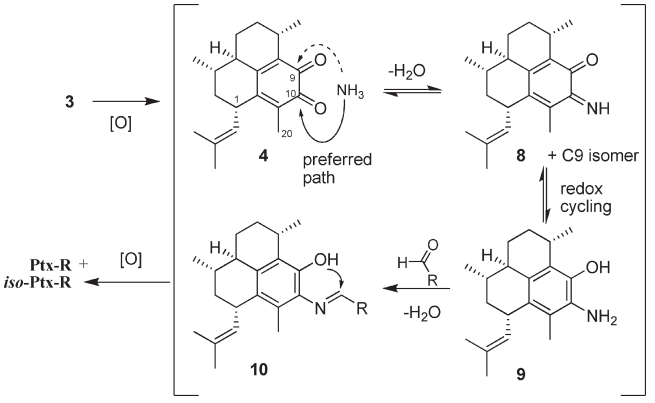
for biological evaluation, we sought chemistry that could be performed efficiently in one pot. Axiomatically, this strategy required methodology to convert the catechol in **3** into a benzoxazole; furthermore, such catechol to benzoxazole transformations will be valuable given benzoxazoles are important scaffolds in medicinal chemistry and new routes to their synthesis are desirable.[14,15]

For the required catechol to benzoxazole transformation we devised two general methods both of which proceed through oxidation of the catechol in **3** to the *ortho*-quinone **4**[16] and yield different ratios of pseudopteroxazoles and their regioisomers, "isopseudopteroxazoles" (methods A and B, Schemes 1 and 2). The titular isopseudopteroxazole (**5**) represents a benzoxazole regioisomer where the location of the N and O on the oxazole is reversed from that in **1**. In developing these procedures we sought methodology applicable to the rapid synthesis of various congeners for biological evaluation.

Method A (Table 1 and Scheme 1) was developed first and proceeds by oxidation of **3** and subsequent condensation with an ammonium salt and an aldehyde. Our second-generation procedure, method B (Scheme 2), proceeds by Ag(I)-mediated oxidation and condensation with an amino acid. In both cases the resulting pseudopteroxazoles possess different C-21 substituents depending on the nature of the aldehyde or amino acid.

Scheme 1. Method A Pseudopteroxazole Syntheses and Plausible Mechanism



These two complementary methods provide synthetic flexibility, resulting in substantial product structural diversity.

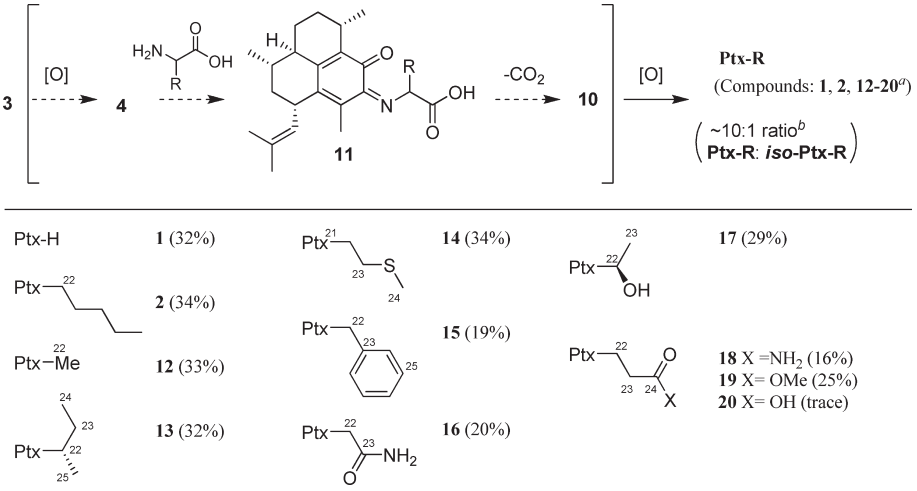
Using method A, the aglycone (**3**) is heated with a mild oxidant, an ammonium salt, and an aromatic aldehyde or paraformaldehyde, yielding pseudopteroxazoles (e.g., **1**, **6a**, **7a**) and isopseudopteroxazoles (e.g., **5**, **6b**, **7b**) in moderate yields (Scheme 1 and Table 1). The oxidant can be air or another mild oxidant, but the reaction does not proceed in the absence of an oxidant (Table 1, entry 5). Method A is based on the known reaction between an *ortho*-quinone, an ammonium salt, and an aldehyde (recent examples are given in refs 17−19).

The ratio of pseudopteroxazoles to isopseudopteroxazoles returned by method A is ~3:1. The pseudopteroxazoles are readily distinguished from the isopseudopteroxazoles by ^{1}H NMR: the chemical shifts of the C-20 methyl and the C-7 methine are relatively deshielded in the natural and *iso* pseudopteroxazoles, respectively, presumably due to diamagnetic anisotropy that is dependent on the orientation of the oxazole. The NMR data for isopseudopteroxazole (**5**) are given in Table 2. The C-7 methine and the C-20 methyl signals are observed at 3.45 and 2.33 ppm, respectively, whereas in pseudopteroxazole these signals are observed at 3.27 and 2.45 ppm. As a further example distinguishing psesudopteroxazole regioisomers, Table 2 also provides a side by side comparison of the NMR assignments for the pseudopteroxazole derivative **6a** and the isopseudopteroxazole **6b**. The C-7 methines in **6a** and **6b** are observed at 3.31 and 3.54 ppm, respectively, whereas the C-20 methyls are observed at 2.51 and 2.36 ppm, respectively (also see Supporting Information Figure S10).

A plausible mechanism for the reaction following method A is shown in Scheme 1. Initially, the aglycone **3** is oxidized to *ortho*-quinone **4**. Subsequently, nucelophilic attack of the ammonia onto one of the *ortho*-quinone carbonyls gives an imine: following the preferred pathway, attack on the less sterically hindered carbonyl gives an imine of general nature **8**, which may redox cycle to the aniline **9**. Condensation with the aldehyde yields imine **10**, which undergoes cyclization and oxidation to the generic benzoxazole product.

While method A allowed us to rapidly access pseudopteroxazole and aryl-substituted oxazole congeners, a limitation of this method is that it does not work effectively with aliphatic aldehydes (e.g., Table 1, entries 13−15). We thus developed method B as a complementary route to provide different pseudopteroxazole

Scheme 2. Method B Pseudopteroxazole Syntheses and Plausible Mechanism[a]



[a] Isolated yields after column chromatography. [b] Determined by NMR.

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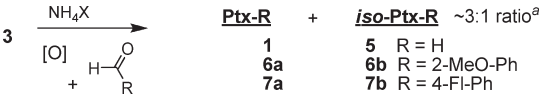
Table 1. Method A Syntheses of Pseudopteroxazoles and Selected Reaction Conditions Screened[a]



entry	X	aldehyde	equiv aldehyde	[O] (equiv)	solv	temp (°C)	time (h)	products **Ptx-R**/ ***iso*-Ptx-R** (R)	yield (%)[b]
1	OAc	paraformaldehyde	13	air	AcOH	20	24		
2	OAc	paraformaldehyde	100	air	PhMe	110	18	**1**/**5** (H)	44
3	OAc	paraformaldehyde	100	air	AcOH	118	15	**1**/**5** (H)	52
4	OAc	paraformaldehyde	100	air	AcOH	118	18	**1**/**5** (H)	41
5	OAc	paraformaldehyde	100		AcOH (degassed, N_2 atm)	118	15		
6	OAc	paraformaldehyde	100	Ag_2O (1), air	AcOH	118	4		
7	OAc	paraformaldehyde	100	$Ce(NH_4)_2(NO_3)_6$ (1)	AcOH	118	24		
8	HCO_3	paraformaldehyde	100	$Ce(NH_4)_2(NO_3)_6$ (1)	MeOH	65	24	**1**/**5** (H)	48
9	HCO_3	anisaldehyde	2.5	air	AcOH	118	3.5		
10	HCO_3	anisaldehdye	24	air	AcOH	118	15	**6a**/**6b** (2-MeO-Ph)	52
11	OAc	4-fluorobenzaldehyde	50	air	AcOH	118	16	**7a**/**7b** (4-Fl-Ph)	49
12	OAc	2-naphthaldehyde	5	air	AcOH	118	18	(*R* = naphtha)	47
13	OAc	crotonaldehyde	36	air	AcOH	118	16		
14	OAc	hexanal	2.5	air	AcOH	118	20	trace quantities of **2**	
15	OAc	hexanal	100	air	AcOH	118	20	trace quantities of **2**	

[a] Ratio determined by NMR. [b] Isolated yield after column chromatography (of both regioisomers). Where no yield is given: reaction was monitored by ELSD-LCMS and no significant conversion to the desired oxazole was detected.

congeners to address this synthetic limitation and to provide additional analogues for biological evaluation given that preliminary antimicrobial analyses indicated the aryl-substituted pseudopteroxazole derivatives **6a** and **7a** displayed no notable biological activity (Table 4).

To develop method B, we first screened reactions between the pseudopterosin aglycone **3** and glycine under a variety of conditions (Table 3). Pleasingly we found that Ag_2O effected the desired transformation to pseudopteroxazole (**1**). As far as we are aware, this is the first example of a silver(I)-mediated reaction between a catechol and an amino acid that yields a benzoxazole; however, there are a small number of literature reports of reactions between *ortho*-quinones and amino acids that yield benzoxazoles.[20−22]

In method B the aglycone (**3**) is heated with Ag_2O (or another Ag(I) salt) and an excess of an amino acid, yielding predominantly pseudopteroxazoles (e.g., **1**, **2**, **12**−**19**, Scheme 2). In the absence of a Ag(I) salt the reaction returns only trace quantities of pseudopteroxazoles (Table 3, entries 1−4). Similarly, preoxidation of the aglycone followed by filtration to remove insoluble Ag and subsequent amino acid addition gives only trace quantities of pseudopteroxazoles. Given our aim to quickly prepare and evaluate pseudopteroxazole congeners, the reaction for each amino acid has not been fully optimized. However, in general, higher yields are obtained with periodic addition of both the amino acid and the Ag_2O; presumably this attenuates Ag(I)-mediated decarboxylation of the amino acid,[23] hence the required excess of the amino acid. Silver-mediated decarboxylation presumably explains why reactions with glutamic and aspartic acid returned only trace quantities of the benzoxazole products. Nonetheless, the product formally derived from glutamic acid (i.e., **20**), was prepared by hydrolysis of **19**, which in turn was synthesized from the aglycone **3** and 2-amino-5-methoxy-5-oxopentanoic acid.

A plausible mechanism for the reaction following method B is shown in Scheme 2. As before, oxidation of the catechol **3** gives the *ortho*-quinone **4**, and subsequently attack of the nitrogen on the amino acid yields the α-carboxy imine **11**. Decarboxylation (probably Ag(I)-mediated) yields the general imine **10**, which undergoes cyclization and oxidation to the benzoxazole products. A similar mechanism for an *ortho*-quinone to benzoxazole transformation has been previously proposed.[22] In both methods A and B there is a preference for the nucleophile to attack the less hindered C-10 carbonyl; however, this preference is relatively greater in method B compared to method A, due to the greater steric bulk surrounding the nitrogenous nucleophile. Hence, the ratio of the *normal* (e.g., **1**) to *iso* (e.g., **5**) pseudopteroxazole products differs between method A (∼3:1) and method B (∼10:1). The structures of the new pseudopteroxazole derivatives were confirmed by 1D and 2D NMR analysis.

The semisynthetic pseudopteroxazoles were tested against six microorganisms (Table 4), and some of our analogues show more potent activity than pseudopteroxazole. Pseudopteroxazole (**1**), isopseudopteroxazole (**5**), and several analogues (e.g., **16**, **18**, **20**) displayed activity at a similar potency to that exhibited by the clinically used drug rifampicin against two model mycobacteria, *M. smegmatis* and *M. diernhoferi*. The compounds were also tested against vancomycin-resistant *Enterococcus faecium* (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, and *Candida albicans*. While no activity was observed against the latter two pathogens, **1**, **5**, **12**, **16**, **18**, and **20** showed strong activity against VRE, and **16**, **18**, and **20** showed strong activity against MRSA. These results reveal a further spectrum of activity possessed by pseudopteroxazoles. Effects of variation about the C-21 oxazole moiety on the biological activity are also revealed: generally, analogues with more lipophilic side chains are less active compared to the natural product. It is notable that pseudopteroxazole did not show activity against MRSA, yet

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Table 2. NMR Data for 5, 6a, and 6b[a]

	5		6a		6b	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	36.7, CH	3.93, app q (8.9)	36.4, CH	3.99, m	36.8, CH	3.95, m
2a	40.0, CH_2	2.10, m	40.1, CH_2	2.16, m	40.1, CH_2	2.11, m
2b		1.29, m		1.31, m		1.30, m
3	34.5, CH	1.26, m	34.6, CH	1.31, m	34.5, CH	1.28, m
4	44.8, CH	2.23, m	44.8, CH	2.29, m	44.9, CH	2.25, m
5a	28.1, CH_2	2.16, m	28.1, CH_2	2.19, m	28.2, CH_2	2.16, m
5b		1.04, m		1.12, m		1.08, m
6a	32.2, CH_2	2.23, m	32.4, CH_2	2.21, m	32.3, CH_2	2.23, m
6b		1.41, m		1.46, m		1.42, m
7	30.8, CH	3.45, m	30.4, CH	3.31, m	30.9, CH	3.54, m
8	131.0, C		121.7, C		130.7, C	
9	136.1,[b] C		147.6, C		138.1, C	
10	148.5, C		140.0, C		149.2, C	
11	117.3, C		126.2, C		116.9, C	
12	136.2,[b] C		134.3, C		135.4,[b] C	
13	135.3, C		135.9, C		134.9,[b] C	
14	130.5, CH	4.99, d (9.3)	131.0, CH	5.02, d (9.6)	130.8, CH	5.02, d (9.0)
15	128.9, C		128.5, C		128.6, C	
16	25.4, CH_3	1.68, s	25.4, CH_3	1.69, s	25.4, CH_3	1.69, s
17	17.6, CH_3	1.78, s	17.6, CH_3	1.80, s	17.6, CH_3	1.78, s
18	19.8, CH_3	1.06, d (6.0)	19.8, CH_3	1.07, d (6.0)	19.9, CH_3	1.06, d (6.0)
19	23.7, CH_3	1.51, d (6.8)	22.0, CH_3	1.55, d (6.6)	23.8, CH_3	1.58, d (6.6)
20	12.3, CH_3	2.33, s	13.6, CH_3	2.51, s	12.4, CH_3	2.36, s
21	150.6, CH	7.96, s	160.2, C		159.7, C	
22			117.3, C		117.6, C	
23			158.2, C		158.3, C	
24			112.1, CH	7.05, d (8.4)	112.3, CH	7.03, d (8.4)
25			132.0, CH	7.46, m	131.9, CH	7.45, m
26			120.6, CH	7.08, m	120.7, CH	7.06, m
27			131.2, CH	8.08, dd (6.6, 1.2)	131.3, CH	8.03, dd (7.2, 1.5)
28			56.0, CH_3	3.97, s	56.2, CH_3	3.95, s

[a] $CDCl_3$, 600 MHz (^{13}C: 150 MHz); assigned by COSY, HSQC, and HMBC experiments. [b] Interchangeable assignments.

three semisynthetic derivatives with relatively polar C-21 substituents showed strong activity, with IC_{50}'s in the low μg/mL range.

CONCLUSION

The catechol to benzoxazole syntheses described in this report are significant, as they provide a simple, versatile route to pseudopteroxazole and congeners for biological evaluation. The methodology makes use of a relatively abundant material (pseudopterosins G–J), and the same process should be applicable to other pseudopterosin skeletons that possess alternative backbone configurations (e.g., pseudopterosin A). Biological evaluations suggest modification of the C-21 oxazole in **1** can lead to analogues with more potent antibacterial activity. Furthermore, this work has unambiguously confirmed the structure of homopseudopteroxazole (**2**) and its skeletal relationship to the pseudopterosin G–J aglycone. Our results also tentatively support a biosynthetic route to **1**, which may proceed through condensation of **3** (or a similar pseudopterosin derivative) with glycine.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Rudolph Autopol III polarimeter. Infrared spectra were recorded using attenuated total reflectance, with samples deposited as a thin film on a Thermo Nicolet 6700 FT-IR spectrometer (Smart iTR). NMR spectra were obtained on a Bruker Avance III 600 MHz NMR spectrometer operating at 600 and 150 MHz for ^1H and ^{13}C, respectively. Chemical shifts (δ) are reported in ppm and were referenced to residual solvent signals: $CDCl_3$ (δ_H 7.26; δ_C 77.0). Coupling constants (J) are reported in Hz with the abbreviations (s) singlet, (d) doublet, (t) triplet, (q) quartet, (m) multiplet, and (app) apparent. Low-resolution (nominal mass) mass spectra were obtained using a Finnigan LXQ ion trap mass spectrometer fitted with either an APCI or ESI source: samples were typically analyzed by LCMS using either an analytical HPLC or a UPLC column, with hyphenated MS-ELSD-UV detection. High-resolution mass spectra were measured by Xiao Feng at Dalhousie Univeristy, on a Bruker microTof Focus orthogonal ESI-TOF mass spectrometer. Flash chromatography was conducted utilizing a Teledyne Combiflash Rf, with UV detection triggered fraction collection using RediSep columns.

Pseudopterosin starting materials were isolated from samples of *P. elisabethae* obtained from Victory Reef, Bahamas. The pseudopterosins

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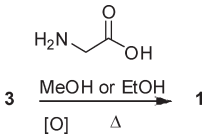
were purified by a combination of solvent extraction, liquid−liquid partitioning, and chromatography. The major pseudopterosin in the sample possesses the pseudopterosin G−J aglycone skeleton. All other starting materials, reagents, and solvents were commercially available and were generally obtained from one of the following three suppliers: VWR, Sigma-Aldrich, or Fisher Scientific.

*Pseudopterosin G−J Aglycone (**3**).* The typical procedure to prepare the aglycone is based on the literature method for the hydrolysis of pseudopterosin C.[24] A sample of pseudopterosins G−J (**3**) is refluxed, under an atmosphere of N_2, in MeOH + 1 N HCl for 2 h. After cooling, the sample is neutralized with aqueous $NaHCO_3$, and then the aglycone isolated by partitioning the sample between H_2O and EtOAc. Concentration of the organic phase yields the aglycone **3**, which may be used without further purification. Immobile brown oil; ^{1}H NMR ($CDCl_3$, 600 MHz) consistent with literature values;[12] ^{13}C NMR ($CDCl_3$, 150 MHz) δ 140.0, 139.9, 131.9, 131.4, 130.1, 128.3, 125.3, 120.4, 44.4, 40.1, 36.9, 34.0, 32.0, 28.4, 27.8, 25.4, 23.1, 19.8, 17.5, 11.9; APCIMS m/z 301 $[M + H]^+$.

Table 3. Method B Syntheses of Pseudopteroxazole and Selected Reaction Conditions Screened



entry	equivs glycine	[O] (equiv)	solvent	atm	time (h)	yield (%)[a]
1	11.4		MeOH	air	24	
2	5.0		H_2O[b]	air	0.5	trace
3	3.2	$NaIO_4$ (0.3)	H_2O/MeOH	air	3	trace
4	3.3	$NaIO_4$ (1.1)	H_2O/MeOH	air	2	trace
5	2.7	Ag_2O (0.1)	MeOH	air	18	trace
6	10.1	Ag_2O[c] (1.0)	MeOH	air	3	24
7	11.4	Ag_2O[c] (1.4)	MeOH	air	22	32
8	12.2	Ag_2O[c] (2.1)	MeOH[d]	N_2	24	minor

[a] Isolated yield after column chromatography. [b] Autoclave 121 °C, 18 PSIG. [c] Amino acid and Ag_2O were added periodically in two or more batches. [d] Degassed MeOH.

*Method A: Aldehyde Route to Pseudopteroxazole Derivatives. Synthesis of Pseudopteroxazole (**1**) and Isopseudopteroxazole (**5**) via Method A. Example 1: Utilizing Air As the Oxidant.* A sample of the aglycone (**3**, 179 mg, 0.6 mmol) was dissolved in AcOH (5 mL), and air was bubbled into the solution for 10 min. Paraformaldehyde (100 mg) and NH_4OAc (1 g) were then added, and the reaction was then refluxed for 7 h. The reaction products were then partitioned between EtOAc and H_2O, and the EtOAc-soluble material was then subjected to reversed-phase flash chromatography (43 g C-18 column) using a MeOH/H_2O gradient (40:60 to 100:0). After analysis by LCMS the fractions were recombined and concentrated *in vacuo*. One fraction was a mixture of pseudopteroxazole and isopseudopteroxazole (**1**/**5** (3:1 ratio), 95.5 mg, 0.31 mmol, 52%). The pseudopteroxazole regioisomers were separated by normal-phase flash chromatography (40 g silica column) using a MTBE/hexane gradient (0:100 to 25:75) to give pure pseudopteroxazole (**1**, 52 mg, 0.17 mmol, 29%) and isopseudopteroxazole (**5**, 12.5 mg, 0.04 mmol, 7%).

Example 2: Utilizing Ceric Ammonium Nitrate As the Oxidant. To a stirred solution of the aglycone (**3**, 214 mg, 0.71 mmol) in MeOH (10 mL) under an N_2 atmosphere was added ceric ammonium nitrate (409 mg, 0.74 mmol). The reaction was refluxed for 1 h and cooled to room temperature (rt), and then excess NH_4HCO_3 (2.4 g, 31 mmol) was added. The reaction was returned to reflux, and then paraformaldehyde was added in two batches (4 h: 500 mg; 18 h: 200 mg). After a total of 24 h at reflux the reaction products were partitioned between EtOAc and H_2O. The EtOAc-soluble material (214 mg) was purified by normal-phase flash chromatography (24 g silica column) using a MTBE/hexane gradient (0:100 for 2 min, then to 5:95 over 2 to 17 min, then to 10:90 over 17 to 22 min, 35 mL/min) to yield isopseudopteroxazole (**5**, 25 mg, 0.08 mmol, 11%) and pseudopteroxazole (**1**, 78 mg, 36%).

Pseudopteroxazole (**1**): oil; $[\alpha]^{25}_D$ +100 (*c* 0.2, $CHCl_3$); ^{1}H NMR and ^{13}C NMR consistent with literature values;[4,9,10] APCIMS m/z 310 $[M + H]^+$; HRESIMS m/z $[M + H]^+$ 310.2154 (calcd for $C_{21}H_{28}NO$, 310.2165).

Table 4. Antimicrobial Evaluation of Semisynthetic Pseudopteroxazole Congeners

compound[a]	*M. smegmatis*,[b] MIC μg/mL	*M. diernhoferi*,[c] MIC μg/mL	VRE,[d] IC_{50} μg/mL	MRSA,[e] IC_{50} μg/mL
1 (Ptx-H)	2	4	13	>128
2 (Ptx-$(CH_2)_4CH_3$)	>64	>64	>128	>128
5 (*iso*-Ptx-H)	8	8	13	>128
6a (Ptx-(2-CH_3O-Ph))	>64	>64	>128	>128
7a (Ptx-(4-F-Ph))	>64	>64	NT[f]	NT
12 (Ptx-CH_3)	8	4	2.5	>128
13 (Ptx-$CH(CH_3)CH_2CH_3$)	>64	>64	>128	>128
14 (Ptx-$(CH_2)_2SCH_3$)	16	16	>128	>128
15 (Ptx-CH_2Ph)	>64	4	>128	>128
16 (Ptx-CH_2CONH_2)	4	2	4	3
17 (Ptx-$CHOHCH_3$)	16	8	>128	>128
18 (Ptx-$(CH_2)_2CONH_2$)	4	4	7	7
19 (Ptx-$(CH_2)_2CO_2CH_3$)	>128	>128	>128	>128
20 (Ptx-$(CH_2)_2CO_2H$)	8	4	12	12
vancomycin[g]	NT	NT	NT	12.25
isonazid[g]	1	0.25	NT	NT
rifampicin[g]	4	4	8.75	NT

[a] No activity was observed against *Pseudomonas aeruginosa* or *Candida albicans*. Compounds **1** and **16** showed no activity against *Proteus vulgaris*. [b] ATCC 12051. [c] ATCC 19340. [d] Vancomycin-resistant *Enterococcus faecium* (VREF Ef 379). [e] Methicillin-resistant *Staphylococcus aureus* (ATCC 33591). [f] NT = not tested. [g] Antibiotic controls.

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Isopseudopteroxazole (**5**): oil; $[\alpha]^{25}_{D}$ +114 (*c* 1.29, $CHCl_3$); IR ν_{max} 2946, 2921, 2855, 1445, 1088 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; APCIMS *m/z* 310 $[M + H]^+$; HRESIMS *m/z* $[M + H]^+$ 310.2150 (calcd for $C_{21}H_{28}NO$, 310.2165).

*o-Anisaldehyde Derivatives (**6a** and **6b**).* A sample of pseudopterosins G–J (38 mg, 0.078 mmol) was refluxed in methanolic HCl (1.5 N, 10 mL) under N_2 for 2.5 h. The solvent was removed under a stream of nitrogen, and then NH_4HCO_3 (1 g, excess) and AcOH (4 mL) were added to the residue. After the subsequent effervescence ceased, air was bubbled into the vessel for 10 min, and then *ortho*-anisaldehyde (45 mg, 0.3 mmol) was added. The reaction was then heated at 110 °C overnight. The cooled reaction contents were partitioned between $CHCl_3$ and H_2O, and the $CHCl_3$ layer was concentrated *in vacuo* to give a residue that was purified by flash chromatography (silica, hexane/EtOAc) to give the title compounds (**6a**/**6b** (3:1 ratio), 16.7 mg, 0.040 mmol, 52%). A portion of this material was further purified by RP-HPLC (Phenomenex, phenylhexyl, 5 μm, 250 × 10 mm, 4.0 mL/min) eluted with MeOH/H_2O (isocratic 95:1) to give **6b** (eluted 14.9 to 15.6 min) and **6a** (eluted 15.6 to 16.8 min).

Compound **6a**: amorphous solid; $[\alpha]^{25}_{D}$ +76 (*c* 0.25, $CHCl_3$); IR ν_{max} 2920, 2853, 1464, 1257, 1025 cm^{-1}; ^1H and ^{13}C NMR see Table 2; APCIMS *m/z* 416 $[M + H]^+$; HRESIMS *m/z* $[M + H]^+$ 416.2568 (calcd for $C_{28}H_{34}NO_2$, 416.2584).

Compound **6b**: amorphous solid; $[\alpha]^{25}_{D}$ +72 (*c* 0.07, $CHCl_3$); IR ν_{max} 2923, 2854, 1484, 1257, 1025 cm^{-1}; ^1H and ^{13}C NMR see Table 2; APCIMS *m/z* 416 $[M + H]^+$; HRESIMS *m/z* $[M + H]^+$ 416.2569 (calcd for $C_{28}H_{34}NO_2$, 416.2584).

*p-Fluoroaldehyde Derivative (**7a**).* A solution of the aglycone (**3**, 41 mg, 0.14 mmol), NH_4OAc (1 g, excess), and 4-fluorobenzaldehyde (800 μL, excess) was refluxed in AcOH (4 mL) for 16 h. The cooled reaction mixture was subsequently partitioned between EtOAc and aqueous HCl (1 N). The organic-soluble material was purified by silica flash chromatography (hexane/EtOAc, 10:0 to 9:1 gradient) to give the mixed oxazole regioisomers (**7a**/**7b** (3:1 ratio), 27.6 mg, 0.068 mmol, 49%). A portion of the major regioisomer (**7a**) was further purified by RP-HPLC (Phenomenex, phenylhexyl, 5 μm, 250 × 10 mm, 4.0 mL/min) using isocratic MeOH (eluted across 10.0 to 10.4 min). Immobile oil; $[\alpha]^{25}_{D}$ +99 (*c* 0.14, $CHCl_3$); IR ν_{max} 2922, 2855, 1501, 841 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 8.23 (m, 2H, H-23/H-27), 7.19 (app t, 2H, *J* = 8.6 Hz, H-24/H-26), 5.00 (d, 1H, *J* = 9.4 Hz, H-14), 3.96 (m, 1H, H-1), 3.31 (m, 1H, H-7), 2.48 (s, 3H, H-20), 2.31–2.10 (m, 4H), 1.79 (s, 3H, H-17), 1.69 (s, 3H, H-16), 1.54 (d, 3H, *J* = 6.8 Hz, H-19), 1.48–1.42 (m, 1H, H-6b), 1.32–1.26 (m, 2H), 1.11 (m, 1H, H-5b), 1.06 (d, 3H, *J* = 5.7 Hz, H-18); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 164.4 (d, *J* = 251.4 Hz), 160.6, 147.9, 140.1, 136.3, 134.7, 130.8, 129.54 (d, *J* = 8.6 Hz), 128.7, 126.4, 124.3, 121.9, 115.9 (d, *J* = 22.1 Hz), 44.8, 40.1, 36.5, 34.5, 32.3, 30.4, 28.1, 25.4, 22.3, 19.8, 17.6, 13.5; APCIMS *m/z* 404 $[M + H]^+$; HRESIMS *m/z* $[M + H]^+$ 404.2384 (calcd for $C_{27}H_{31}FNO$, 404.2384).

*Method B: Amino Acid Route to Pseudopteroxazole Derivatives. Specific Example: Method B Synthesis of Pseudopteroxazole (**1**) and Isopseudopteroxazole (**5**).* A sample of **3** (33.8 mg, 0.11 mmol) and Ag_2O (18.7 mg, 0.08 mmol) was refluxed in MeOH (30 mL) for 1 h. The solution was then cooled to rt, and glycine (85 mg, 1.3 mmol) was added. The reaction mixture was refluxed for a further 19 h, with additional Ag_2O (0.08 mmol) being added 2 h into this time frame. The cooled crude reaction mixture was then filtered through Celite and dried *in vacuo*. The crude product (35 mg) was subjected to flash chromatography on a 13 g C18 column eluted with a gradient of MeOH/H_2O (from 4:6 to 1:0) to yield a mixture of pseudopteroxazole regioisomers (10:1 ratio **1**: **5** by NMR). Subsequently, the individual regioisomers were purified by flash chromatography on a 4 g silica column eluted with a gradient of MTBE/hexane (from 100% hexane to 95% hexane over 15 min) to yield **1** (11.3 mg, 0.036 mmol, 32%) and **5** (1.4 mg, 0.004 mmol, 4%). The spectroscopic data were identical to that observed for pseudopteroxazoles prepared by method A.

General Procedure for Method B. A mixture of the pseudopterosin aglycone **3** and Ag_2O (0.7 to 1 equiv) is refluxed in MeOH or EtOH for 1 h. A given amino acid (>1 equiv) is then added, and the reaction further refluxed until the starting aglycone has been consumed (monitored by TLC, LCMS, etc.). Additional batches of the amino acid and Ag_2O (0.7 to 1.4 equiv) are added periodically to drive the reaction to completion. The crude products are typically isolated by filtering the cooled crude reaction products through Celite. Purification is achieved chromatographically.

*Homopseudopteroxazole (**2**).* Homopseudopteroxazole (**2**) was synthesized from the pseudopterosin G–J aglycone (**3**, 14.2 mg, 0.047 mmol), Ag_2O (2.3 equiv), and 2-aminoheptanoic acid (3.1 equiv) following the general procedure. Purification by flash chromatography on a 4 g silica column, eluted with a gradient of MTBE/hexane (from 100% hexane to 95% hexane over 15 min), yielded homopseudopteroxazole (**2**, 6.1 mg, 0.016 mmol, 34%). Immobile oil; ^1H NMR, ^{13}C NMR, and other spectroscopic data consistent with literature values;[5] APCIMS *m/z* 380 $[M + H]^+$; HRESIMS *m/z* $[M + H]^+$ 380.2944 (calcd for $C_{26}H_{38}NO$, 380.2948).

*Alanine Product (**12**).* The pseudopteroxazole C-21 methyl derivative (**12**) was synthesized from the pseudopterosin aglycone (**3**, 9.1 mg, 0.03 mmol), Ag_2O (2.4 equiv), and alanine (2.9 equiv) following the general procedure. Purification by flash chromatography on a 4 g C18 column, eluted with a gradient of MeOH/H_2O (from 4:6 to 1:0), yielded **12** (3.2 mg, 0.01 mmol, 33%). Immobile oil; $[\alpha]^{25}_{D}$ +93 (*c* 0.38, $CHCl_3$); IR ν_{max} 2922, 2855, 1609, 1583, 1445, 1376, 1063 cm^{-1}; ^1H and ^{13}C NMR, see Tables S1 and S2; APCIMS *m/z* 324 $[M + H]^+$; HRESIMS *m/z* $[M + H]^+$ 324.2318 (calcd for $C_{22}H_{30}NO$, 324.2322).

*Asparagine Product (**16**).* The pseudopteroxazole C-21 acetamide derivative (**16**) was synthesized from the pseudopterosin aglycone (**3**, 174 mg, 0.58 mmol), Ag_2O (1.3 equiv), and asparagine (7.8 equiv) following the general procedure. Purification by flash chromatography on a 30 g diol column, eluted with a gradient of hexane/MTBE (from 0:1 to 1:0), yielded **16** (42.1 mg, 0.11 mmol, 20%). A portion of this material was further purified by RP-HPLC (Phenomenex, Gemini C18, 5 μm, 250 × 10 mm, 3.0 mL/min) eluted with MeOH/H_2O/HCO_2H (isocratic 76:24:0.1) to remove the minor regioisomer. Light orange immobile oil; $[\alpha]^{25}_{D}$ +107.3 (*c* 0.32, $CHCl_3$); IR ν_{max} 3342 (br), 3191, 2948, 2922, 2855, 1679 (CO), 1388, 1062 cm^{-1}; ^1H and ^{13}C NMR see Tables S1 and S2; APCIMS *m/z* 367 $[M + H]^+$; HRESIMS *m/z* $[M + Na]^+$ 389.2190 (calcd for $C_{23}H_{30}N_2O_2Na$, 389.2199).

*Glutamine Product (**18**).* The pseudopteroxazole propanamide derivative (**18**) was synthesized from the pseudopterosin aglycone (**3**, 51 mg, 0.17 mmol), Ag_2O (1.4 equiv), and glutamine (8 equiv) following the general procedure. Purification by flash chromatography on a 13 g C18 column, eluted with a gradient of MeOH/H_2O (from 4:6 to 1:0), yielded **18** (10.3 mg, 0.027 mmol, 16%). Orange immobile oil; $[\alpha]^{25}_{D}$ +73 (*c* 0.29, $CHCl_3$); IR ν_{max} 3346, 3198, 2923, 2855, 1672 (CO), 1444, 1374, 1064 cm^{-1}; ^1H and ^{13}C NMR see Tables S1 and S2; APCIMS *m/z* 381 $[M + H]^+$; HRESIMS *m/z* $[M + H]^+$ 381.2525 (calcd for $C_{24}H_{33}N_2O_2$, 381.2537).

*Carboxylic Acid **20**.* To a portion of the ester **19** (10 mg, 0.25 mmol) in THF (3 mL) was added a LiOH solution (12 mL, 1 N, aqueous). The mixture was stirred for 21 h at 35 °C. Dilute HCl (0.5 N) was then added to adjust the pH to ~1, and the mixture was then partitioned between EtOAc and H_2O. The combined EtOAc extracts were concentrated *in vacuo* to yield the carboxylic acid **20** (7.2 mg, 0.19 mmol, 75%). Amorphous, light yellow solid; $[\alpha]^{25}_{D}$ +73.0 (*c* 0.36, $CHCl_3$); IR ν_{max} 3000 (broad), 2921, 2856, 1714 (CO), 1576, 1443, 1167 cm^{-1}; ^1H and ^{13}C NMR, see Tables S1 and S2; APCIMS *m/z* 382 $[M + H]^+$; HRESIMS *m/z* $[M + H]^+$ 382.2360 (calcd for $C_{24}H_{32}NO_3$, 382.2377).

Antimicrobial Evaluation. Activity against *Mycobacterium smegmatis* ATCC 12051 and *Mycobacterium diernhoferi* ATCC 19340 was conducted using a microbroth dilution antibiotic susceptibility assay.

Testing was conducted in accordance with Clinical Laboratory Standards Institute testing standards (susceptibility testing of Mycobacteria, Nocardiae, and other aerobic actinomycetes; Approved Standard. M24-A Volume 23, number 18. Microbroth dilution method for antimicrobial testing of fast growing mycobacteria). Compounds were tested against each organism in triplicate. Compounds were prepared in sterile 20% DMSO and serially diluted to generate a range of eight concentrations. Replicates were performed on separate 96-well plates. Each plate contained four uninoculated contamination controls (media + 20% DMSO), four untreated controls (media + 20% DMSO + organism), and one column containing a concentration range of a control antibiotic. Control antibiotics tested included rifampicin, ciprofloxacin, and doxycycline. Growth relative to untreated control wells was assessed by visual inspection after 5 days of incubation at 30 °C.

Activity against methicillin-resistant *Staphylococcus aureus* ATCC 33591 (MRSA), vancomycin-resistant *Enterococcus faecium* Ef 379 (VRE), *Pseudomonas aeruginosa* ATCC 14210, *Proteus vulgaris* ATCC 12454, and *Candida albicans* ATCC 14035 was conducted using the microbroth dilution antibiotic susceptibility assay. Testing was conducted in accordance with Clinical Laboratory Standards Institute testing standards (Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard, Sixth Ed, M7-A6 Volume 23, number 2). Compounds were tested against each organism in triplicate. Compounds were prepared in sterile 20% DMSO and serially diluted to generate a range of eight concentrations. Each plate contained eight uninoculated contamination controls (media + 20% DMSO), eight untreated controls (media + 20% DMSO + organism), and one column containing a concentration range of a control antibiotic (vancomycin, rifampicin, gentamycin, ciprofloxacin, or nystatin, for MRSA, VRE, *P. aeruginosa*, *P. vulgaris*, and *C. albicans*, respectively). The optical density of the plate was recorded at 600 nm at time zero and then again after incubation of the plates for 22 h at 37 °C. After subtracting the time zero OD_{600} from the final reading the percentages of microorganism survival relative to vehicle control wells were calculated and the IC_{50} was determined.

■ ASSOCIATED CONTENT

Supporting Information. Details of the syntheses of compounds **13**−**15**, **17**, and **19**, ^{1}H and ^{13}C NMR spectra of new compounds (**5**, **6a**, **6b**, **7a**, **12**−**20**) and of semisynthetic pseudopteroxazole (**1**) and homopseudopteroxazole (**2**), in addition to the ^{13}C NMR spectrum of the known pseudopterosin G−J aglycone (**3**). This material is available free of charge via the Internet at http://pubs.acs.org.

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