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Axinellamines as Broad-Spectrum Antibacterial Agents: Scalable Synthesis and Biology

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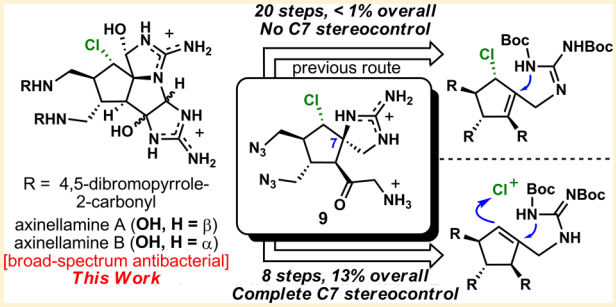
Supporting Information

ABSTRACT: Antibiotic-resistant bacteria present an ongoing challenge to both chemists and biologists as they seek novel compounds and modes of action to out-maneuver continually evolving resistance pathways, especially against Gram-negative strains. The dimeric pyrrole−imidazole alkaloids represent a unique marine natural product class with diverse primary biological activity and chemical architecture. This full account traces the strategy used to develop a second-generation route to key spirocycle **9**, culminating in a practical synthesis of the axinellamines and enabling their discovery as broad-spectrum antibacterial agents, with promising activity against both Gram-positive and Gram-negative bacteria. While their detailed mode of antibacterial action remains unclear, the axinellamines appear to cause secondary membrane destabilization and impart an aberrant cellular morphology consistent with the inhibition of normal septum formation. This study serves as a rare example of a natural product initially reported to be devoid of biological activity surfacing as an active antibacterial agent with an intriguing mode of action.



INTRODUCTION

With an estimated $45 billion in annual treatment costs in the United States, bacterial infections pose a significant burden to the public health system.[1] While ongoing efforts are made to combat pathogenic bacteria, the selection pressures that are intrinsic to therapy select for the most fit populations and drive the evolution of resistance to even the most potent antibacterials.[2] Rice has described the most dangerous multi-drug-resistant (MDR) bacteria as the "ESKAPE" pathogens.[3] While representing a minority of these pathogens, Gram-positive bacteria have been the focus of the majority of development efforts over the past two decades, especially methicillin-resistant *Staphylococcus aureus* (MRSA).[4,5] As a result, treatment options against the majority, and increasingly more dangerous,[1,6] MDR Gram-negative pathogens have become limited, in particular due to the diminishing efficacy of last-resort antibiotics: colistin (**1**, Figure 1A) and polymyxin B (**2**) (which also have significant liabilities due to nephrotoxicity and neurotoxicity).[4,7,8] These MDR pathogens can only be combated by the development of new antibiotics, preferably ones that have novel modes of action to slow the inevitable emergence of resistance. Unfortunately, no new class of antibiotics has been approved for Gram-negative bacteria in over 40 years (Figure 1B).[9] Several factors are attributed to this innovation gap: (i) low financial interest by pharmaceutical companies based on returns on investment,[10] (ii) lack of novel structures and mechanisms of action, and [11] (iii) antibacterial drug supply problems (since most successful antibiotics come from natural products or natural product-derived scaffolds).[9] The combination of natural selection and the above-mentioned factors have left the pursuit of effective antibiotics, especially those with potent activity against MDR Gram-negative pathogens, in a state of emergency.[12]

With a rich history of primary biological properties ranging from anti-infective to immunosuppressive,[13] the bioactive and architecturally complex pyrrole−imidazole alkaloids (PIAs) present a grand opportunity for both chemical synthesis and medicinal chemistry. The challenges toward a scalable production of **4**−**8**, as marine isolates (Figure 1C), are far greater compared to many terrestrially derived natural products, where the producing species can be cultivated. Aside from the traditional challenges such as low isolation yields and non-renewable sources, marine-derived agents suffer from (1) laborious sample isolation (scuba diving/ROVs),[14a] (2) precarious isolation protocols (desalting water-soluble compounds),[14b] (3) high-order aquaculture process (non-actinomycetes, symbiosis),[14a] and (4) marine microbial contamination (community-based microbes).[14c] Thus, chemical syn-

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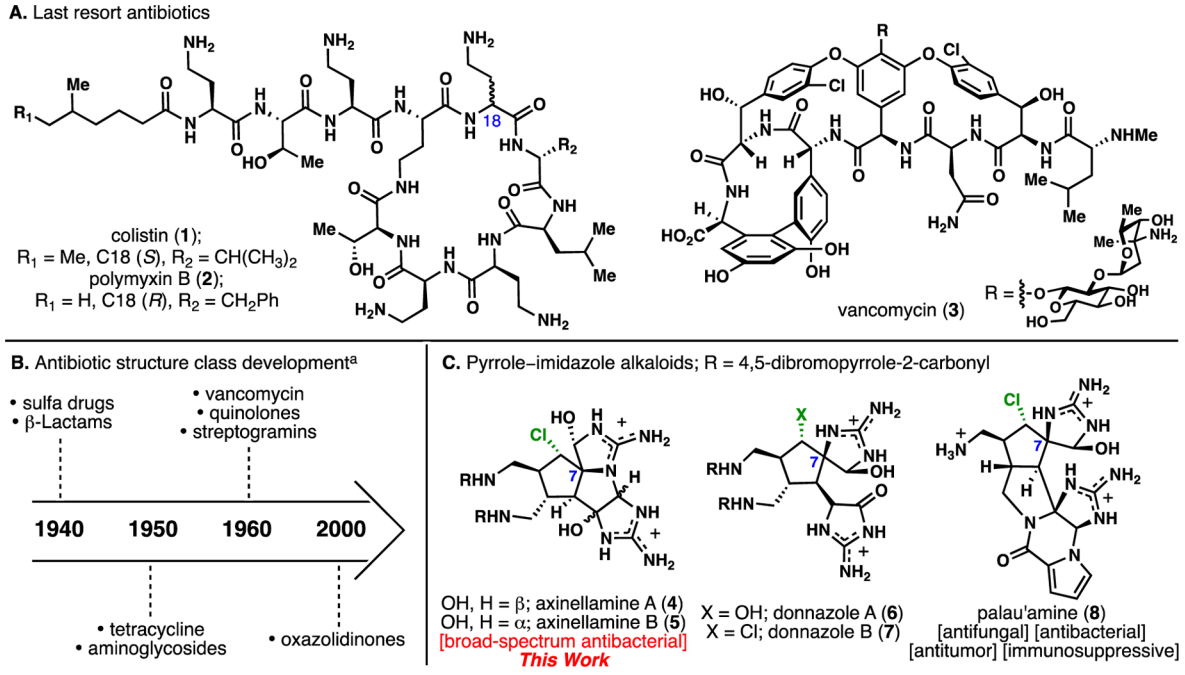

Figure 1. Antibiotic drug discovery. [a]Examples of drug class not necessarily introduced on the exact date depicted (modified from ref 11).

thesis is ideally suited to the challenge of the material supply[15] posed by such natural products.

The axinellamines (**4** and **5**), isolated by Quinn in 1999, were reported to have virtually no bioactivity (minimum inhibitory concentration (MIC) of 1 mM for axinellamine B and no bioactivity for axinellamine A against *Helicobacter pylori*).[16] A follow-up report showed limited antibiotic activity (MICs of 15−30 μM for axinellamine B against *Bacillus subtilis* only).[17]

The first section of this full account reports the biological re-evaluation of synthetic axinellamines (racemic) and the striking finding that this unique structural class in fact has promising activity against both Gram-positive and Gram-negative bacteria via an intriguing mode of action (see Table 1). The second section of this report outlines the synthetic strategy aimed at efficiently solving the stereochemical puzzle embedded in the core of these marine alkaloids. This work ultimately led to an efficient and practical synthesis of these complex marine alkaloids as well as interesting methodological developments of more broad utility.

RESULTS AND DISCUSSION

Axinellamines as Broad-Spectrum Antibacterial Agents. *Discovery.* Despite initial reports of the axinellamines' limited antibacterial activity,[16,17] we found **4** and **5** to be active against the tested bacteria (Table 1A). This activity is conserved across different Gram-positive bacteria, including against both hospital-acquired MRSA (N315) and community-acquired MRSA (USA300), and importantly Gram-negative bacteria. The inhibitory activity is, for the most part, selective for prokaryotic versus eukaryotic cells; the MIC against *Candida albicans* is at least 2- to 32-fold higher. However, the antibacterial activity of **4** and **5** is progressively decreased by the addition of 3%−50% human serum (Table 1B), suggesting that it is limited by significant protein binding. It should also be noted that **4** and **5** have limited solubility in aqueous media and precipitate significantly at concentrations approaching 60 μg/mL. Because of the identical activities of **4** and **5**, we chose to

Table 1. Minimum Inhibitory Concentrations

A. MIC (μg/mL) of Axinellamines for Gram-Positive and Gram-Negative Bacteria

Strain	Ax A (**4**)	Ax B (**5**)
E. coli K-12 MG1655	4	4
Y. pestis KIM6+	16	32
P. aeruginosa PAO1	8	8
S. aureus NCTC 8325	4	4
MRSA N315	4	4
MRSA USA300	2	2
S. epidermidis RP62A	2	2
S. pneumoniae D39	8	8
C. efficiens DSM 44549	0.5	1
E. faecalis ATCC 33186	16	8
C. albicans BWP17	32	32

B. MIC (μg/mL) of Axinellamines for *S. aureus* NCTC 8325 as a Function of Added Human Serum

	Serum (%):					
Compd	0	3.1	6.3	12.5	25	50
Ax A (**4**)	4	16	16	32	64	>64
Ax B (**5**)	4	16	32	32	64	>64

focus further characterization efforts predominantly on variant **4**. We first determined the frequency of resistance to 24 μg/mL of **4** (6× MIC) for *Escherichia coli* K-12 MG1655 and *S. aureus* NCTC 8325. The frequencies of resistance were 5×10^{-6} and 1×10^{-7}, respectively. Unexpectedly, the recovered isolates failed to grow in liquid culture supplemented with the same concentration of **4** and retained parental MICs when recharacterized using the microdilution method. Taken together, these results suggest that the susceptibility to **4** may be highly dependent on the inoculum effect.[18] To test this possibility, we repeated the MIC assays for **4** and **5** with varying *E. coli* and *S. aureus* inocula, ranging from the CLSI standard of 10^5 CFU/mL to 10^8 CFU/mL, and found that the appearance of "resistant" isolates is consistent with the high plating

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Table 2. Minimum Inhibitory Concentration (MIC, μg/mL) of Axinellamines as a Function of Bacterial Inoculation

Compd	Organism	Inoculation (CFU/mL): 10^5	10^6	10^7	10^8
Ax A (**4**)	*E. coli* K-12 MG1655	4	32	64	NT[a]
	S. aureus NCTC 8325	4	4	32	64
Ax B (**5**)	*E. coli* K-12 MG1655	4	32	64	NT[a]
	S. aureus NCTC 8325	4	4	32	64

[a]NT = not tested.

inoculations required for mutant selection on solid media (Table 2). The occurrence of "false mutants" has been observed for daptomycin, for which the occurrence of true resistance is quite low.[19,20] These results suggest that the true frequency of resistance for the axinellamines is lower than 5×10^{-6} and 1×10^{-7} for *E. coli* and *S. aureus*, respectively.

Based on their structure and broad-spectrum activity, we hypothesized that the mechanism of action of the axinellamines may be related to membrane destabilization. In order to test for outer membrane perturbation, we assayed for the cleavage of nitrocefin by periplasmic β-lactamase in *E. coli* K-12 MG1655 carrying a plasmid-encoded β-lactamase. Cleavage of nitrocefin results in a color change that can be monitored spectrophotometrically. With an intact membrane, nitrocefin is excluded from the periplasm, and thus is not cleaved by the periplasmic enzyme. As a positive control, we examined nitrocefin cleavage after addition of the lytic peptide melittin at a concentration range of 2−128 μg/mL. For compound **4**, outer membrane permeabilization was delayed compared to that for melittin (Figure 2), demonstrating that it does not efficiently induce membrane disruption, and that the disruption that does occur may be a secondary effect. Interestingly, the related marine alkaloid sceptrin has been shown to induce peptidoglycan turnover, spheroplast formation, and intracellular potassium ion release in *E. coli*,[21] consistent with membrane damage.

More recently, sceptrin has been shown to bind to MreB,[22] the actin homologue in *E. coli* which plays a key role in elongation and morphology.[23,24] Thus, we next sought to further characterize the antibacterial action of **4** by testing its effect on *E. coli* cellular morphology. After administration of 4 μg/mL of **4** (1× MIC) for 20 min, *E. coli* takes on an aberrant morphology characterized by mostly elongated and some branching forms (Figure 3). The increase in major axis length is

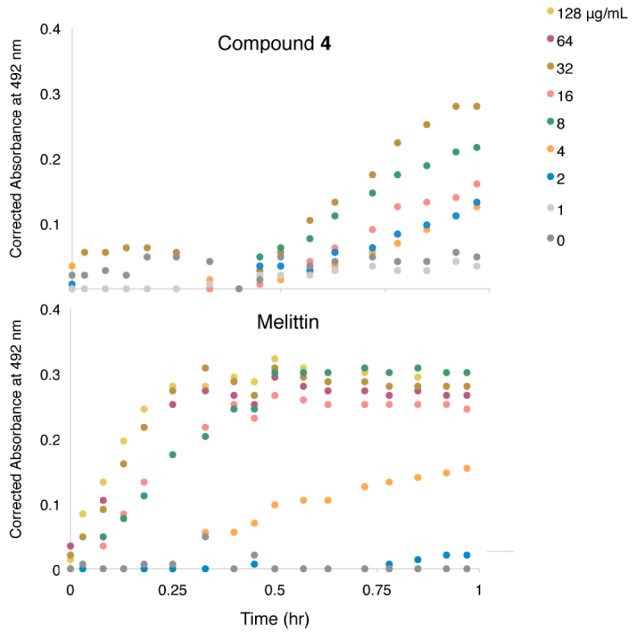

Figure 2. Representative plots showing the outer-membrane permeabilization of *E. coli* K-12 MG1655 containing pBR322 as a function of [**4**], μg/mL, using the cleavage of nitrocefin by periplasmic β-lactamase as the reporter. Melittin was used as the positive control. Other runs are shown in Supporting Information, Figure SI-1.

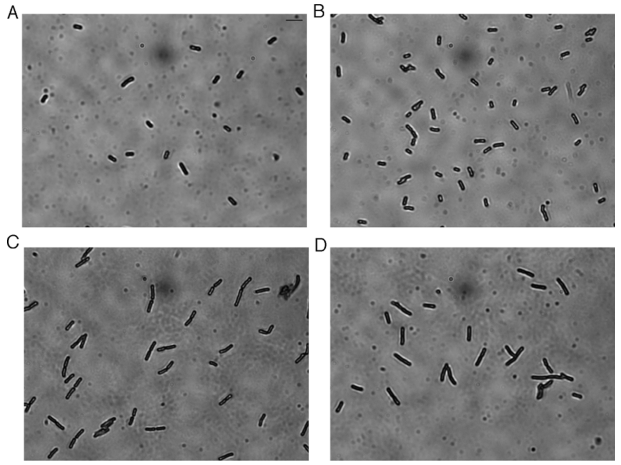

Figure 3. Cellular morphology of *E. coli* K-12 MG1655 at 1000× magnification: (A) prior to treatment, (B) treated with DMSO at 20 min, (C,D) treated with Ax A (**4**) at 20 min. The scale bar (panel A; top right) is equal to 5 μm.

Table 3. Morphological Parameters[a] (± SEM) Calculated for *E. coli* Cells Treated with Vehicle vs Axinellamine A, 20 min

Parameter	Treatment group: DMSO (n = 126)	Ax A (**4**) (n = 129)	Fold-change	p value
perimeter	135.1 ± 6.7	203.2 ± 2.9	1.50	3.4×10^{-8}
major axis	55.1 ± 2.1	86.9 ± 3.0	1.58	2.5×10^{-12}
minor axis	22.3 ± 1.1	26.0 ± 1.8	1.16	4.7×10^{-2}
aspect ratio	2.6 ± 0.1	3.8 ± 0.4	1.45	3.4×10^{-10}

[a]The parameters perimeter, major axis, and minor axis are measured in pixels (1 pixel = 0.064 μm).

significant compared to the vehicle-only control (Table 3). This phenotype is reminiscent of defects in septation. For example, deletion, mutation, or inhibition of the *fts* genes, which are essential for cell division, produces cells that grow but do not divide, resulting in a characteristic filamentous phenotype.[25,26] Interestingly, after 1 h, the axinellamine-treated cells revert to normal morphology (data not shown). A similar reversion has also been observed for sceptrin under comparable conditions.[21] The distinct cellular changes effected by compound **4** suggest that the axinellamines' antibacterial mode of action may be manifested through the inhibition of normal cellular division. It must be emphasized that the above studies required >100 mg of **4** and **5**. In the next section, the chemistry employed to enable these studies will be discussed.

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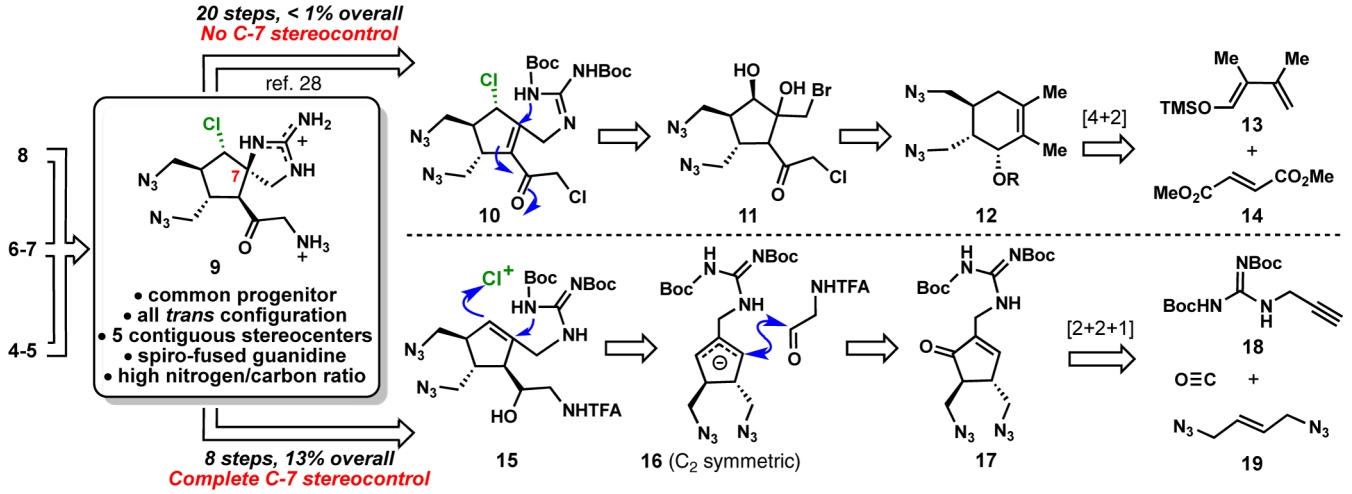
Scheme 1. (A, top) First- and (B, bottom) Second-Generation Retrosynthetic Analysis of Spiroaminoketone (9)



Retrosynthetic Analysis. Owing to their densely functionalized cores with up to eight contiguous stereocenters, high nitrogen content (ca. 1:2 N:C), and sensitive functional groups, members of the PIA class (**4**–**8**) have attained worldwide interest but only very few syntheses have been completed.[27] The high degree of molecular complexity of **4**–**8** can be largely attributed to a single densely functionalized cyclopentyl ring. Guided by the putative biosynthesis, we forged a unified strategy toward the construction of **4**–**8** from fully functionalized spiroaminoketone **9**.[28] With this divergent strategy in mind, we accomplished the first successful laboratory syntheses of **4**,[28b] **5**,[28c] and **8**[28d] on milligram-scale. Although these feasibility-era[29] syntheses provided proof of principle for the late-stage strategy, the ultimate challenge of producing these natural products on gram-scale had not been met as the synthetic routes to **4**, **5**, and **8** suffered from several inefficiencies. The majority of these inefficiencies could be traced back to the route employed to access key spiroaminoketone **9**.

The first-generation synthesis of **9** utilized a Diels–Alder/ozonolysis ring contraction strategy to construct the cyclopentyl system and a non-selective aza-Michael addition of the pendant allylic guanidine to set the challenging C7 spirocenter (Scheme 1A). The indirect approach used to access the cyclopentyl ring and the lack of C7 stereoselectivity (1:1) were significant drawbacks, not to mention numerous non-strategic redox manipulations and excessive functional group interchanges. Overall, the synthesis of **9** was accomplished in 20 steps, requiring 12 chromatographic separations to produce **9** in <1% yield from commercially available materials. Considering the poor substrate bias of enone **10** in accessing the correctly configured spirocycle, a new approach that would efficiently set the C7 stereochemistry was deemed essential.

The second-generation route[28e] envisioned (Scheme 1B) took into account the high stereochemical demand of spiroaminoketone **9** (most importantly the C7 stereochemistry) as well as its all-*trans* configuration. Thus, a revised retrosynthesis featured a speculative "chloro-guanidylation" of an allylic guanidine **15** by way of stereoselective chloronium formation (presumably due to steric clash between Cl^+ and neighboring methylene) followed by *anti* displacement of the pendant guanidine in a similar fashion to that of iodolactonizations pioneered by Corey.[30] Guided by the need for a more efficient synthesis of **9**, this key disconnection not only served as an ideal strategy for clearing the two most challenging stereocenters of the fully substituted cyclopentyl framework but also was seen as an opportunity for invention. Building from previous attempts to exploit the hidden symmetry of this class of natural products,[27a,31] we proposed allylic guanidine **15** to arise from a stereoselective condensation of a putative C_2-symmetric allylic anion (generated from the corresponding allylic chloride) with an appropriate electrophilic side-chain segment. Conversely, one can imagine the same strategic inclusion of a C_2-symmetric intermediate using a relevant nucleophile (see Scheme 2C) via an asymmetric allylic functionalization.[32] Access to either allylic anion or cation could arise from reduction of cyclopentenone **17**, which can be rapidly constructed in a single step via a Pauson–Khand [2+2+1] cycloaddition between propargyl guanidine **18**, bis-allylic azide **19**, and carbon monoxide.

Validation and Initial Forays. Halolactonizations are conducted under mild conditions and have proven to be an effective strategy to build complexity in the synthesis of many natural products.[33] Although there has been advances toward the use of unsaturated amides as the nucleophilic partner in iodolactamizations,[34] the use of guanidines is still unknown. In addition, current examples involving chlorocyclizations of amides and carbamates are limited to methods involving radical pathways.[35] To the best of our knowledge, there is only one example involving an aza-chlorospirocyclization of an unactivated sp^2 carbon in high yield,[36] and the use of an allylic guanidine as the nucleophilic counterpart is unknown. In order to validate the key transformation to our revised synthetic plan, we synthesized model allylic guanidine **20** and subjected this simple cyclopentene to a variety of chlorinating conditions (Scheme 2A). Not surprisingly, the chlorination of tri-Boc-allylic guanidine **20** was challenging. A range of chlorinating reagents was employed[35,37] (CuCl,[35a] NCS,[35b] $CrCl_2$,[37a] SDCS,[37b] *t*BuOCl,[37c] $Ca(OCl)_2$,[37d] TCIA,[37e]) but all failed to form any chlorospirocyclization product. Alternatively, we were able to achieve halospirocyclization using NBS to furnish bromospirocyclization product **21**. Although the crude NMR showed clean conversion, **21** was unstable to silica gel purification. Attempts to directly crystallize intermediate **21** from the crude reaction mixture (CH_2Cl_2, slow evaporation at room temperature) led to a crystalline urea **22**. The success of

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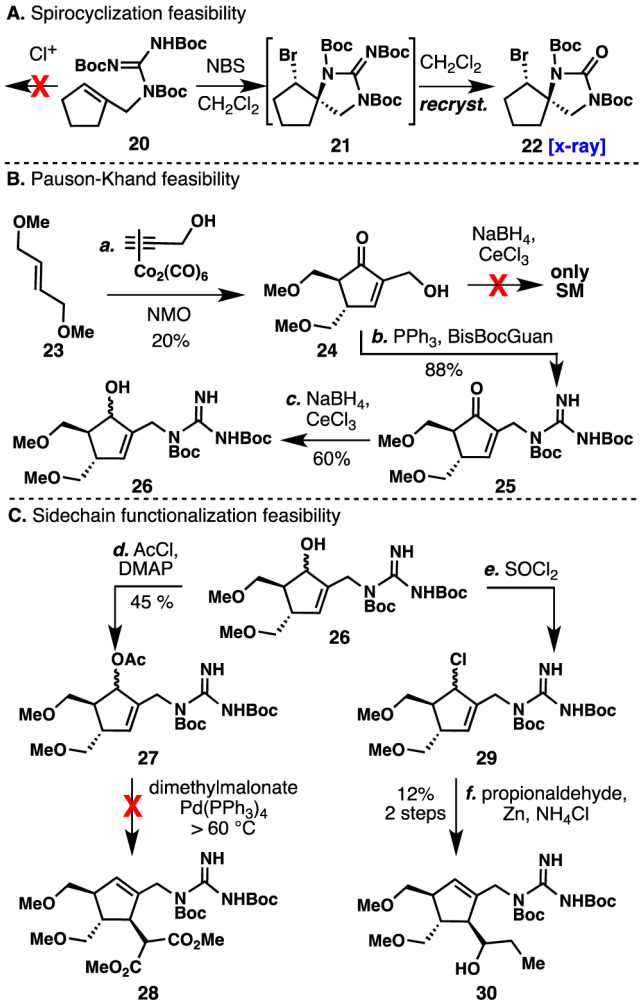
Scheme 2. Validation and Initial Feasibility Studies[a]



[a]Reagents and conditions: (a) i. propargyl alcohol (1.0 equiv), $Co_2(CO)_8$ (1.0 equiv), CH_2Cl_2, rt, 2 h; ii. (*E*)-1,4-dimethoxybut-2-ene (3.0 equiv), NMO (6.0 equiv), CH_2Cl_2, rt, 12 h. (b) PPh_3 (1.5 equiv), DIAD (1.5 equiv), bis-Boc-guanidine (1.1 equiv), THF, rt, 2 h. (c) $NaBH_4$ (4.0 equiv), $CeCl_3 \cdot 7H_2O$ (1.0 equiv), CH_3OH, 0 °C, 12 h. (d) AcCl (1.2 equiv), DMAP (10 mol%), CH_2Cl_2, rt, 12 h. (e) $SOCl_2$ (1.4 equiv), CH_2Cl_2, 0 °C, 1 h. (f) Zn (5 equiv), H_2O/NH_4Cl.

this unprecedented haloazaspirocyclization was evidence that guanidine may be a viable nucleophilic partner for this type of transformation. Also, it was believed that if a direct chlorination procedure was unsuccessful, the use of NBS could prove useful to access the desired chlorospirocycle via aziridine formation.[38]

When evaluating strategies toward construction of the cyclopentyl framework, two prerequisites had to be met (preferably in a single step): (i) proper placement of useful functionality for the generation of a C_2-symmetric synthon (Scheme 2C) that can be applied toward side-chain installation and (ii) generation of the *trans*-methylene azide functionality (**17**) that would serve as the basis for stereochemical control. The venerable Pauson–Khand (P-K) reaction met both of these criteria and pointed to an area in need of improvement since there were no known examples of non-directed intermolecular P-K cycloadditions using unactivated alkenes.[39]

Due to the intrinsic [3,3]-rearrangement of bis-allylic azide **19**[40] and the lack of reactivity displayed by propargyl guanidine **18** in the P-K cycloaddition (a variety of protecting groups under forceful conditions were explored),[41] functional group interchanges were deemed unavoidable. Thus, *trans*-bis-allylic ether (**23**) and propargyl alcohol were subjected to a variety of P-K reaction conditions, showing cyclopentenone generation only with the use of *N*-methylmorpholine *N*-oxide (NMO) (Scheme 2B).[42] Although the yield of model cyclopentenone **24** was low, to the best of our knowledge, this NMO-assisted P-K reaction was the first report of a non-directed intermolecular P-K using an unactivated alkene. Allylic alcohol **24** was found to be unreactive toward Luche conditions. Conversely, allylic guanidine **25** (derived from **24**), upon treatment with Luche conditions, afforded clean 1,2-reduction and provided alcohol **26** as a viable C_2-symmetric precursor.

The strategic inclusion of a C_2-symmetric intermediate in the synthetic design not only eliminates the regioselectivity issue during side-chain installation but also renders the stereochemistry of the corresponding allylic alcohol (**26**) irrelevant. Both electrophilic and nucleophilic side-chain substitution were explored using **27** (Scheme 2C). Although the use of palladium-catalyzed substitution is more attractive since the use of chiral ligands may render the route enantioselective, electrophilic substitution via a Barbier-type reaction was found to be more feasible (use of Pd allylation protocols only gave decomposition upon forceful conditions) and redox economical[43] (use of soft nucleophiles such as 1,3-dicarbonyls typical of Tsuji–Trost allylations would require further redox manipulation). With the successful conversion of **29** to **30**, we shifted our attention to optimization of the individual steps and finalization of the route to render it both robust and scalable.

Optimization of Key Reactions. *Diol N-Oxide-Assisted Pauson–Khand.* As discussed above, after many failed attempts, the use of tertiary amine *N*-oxides in the stoichiometric $Co_2(CO)_8$ system originally reported by Schreiber[42] was found to be a viable protocol to generate model cyclopentenone **24** from unactivated alkene **23**. Discouraged by unsuccessful cycloadditions involving other metal systems in both catalytic and stoichiometric modes and motivated by the vast amount of promoters found in the P-K literature, we redirected our screening efforts toward the optimization of the initial (20% yield; Table 4, entry 4) protocol rather than changing the transition metal.

Optimization of the stoichiometric $Co_2(CO)_8$ protocol was carried out in CH_2Cl_2 with 1.1 equiv of preformed complex (**32**), to which was directly added an excess of *trans*-alkene **23** followed by the desired additive and the oxidant (NMO as standard conditions) at room temperature (Table 4). Complex **32** is stable to silica gel chromatography but was used directly from a stock solution (control experiments with silica gel purified **32** compared to *in situ* generated **32** showed no difference in isolated yields). It was quickly realized that NMO played an enabling role in the reaction, as the use of any other oxidant (I_2, $Mn(OAc)_3$, $Co(OAc)_2$, $K_3Fe(CN)_6$, Ag_2O, $FeCl_3$, and a variety of Cu(II) salts) failed to give any desired product (only two shown in Table 4 for simplicity). Attention then shifted to screening of additives based on extensive literature precedent.[44]

The majority of known additives are Lewis bases, and it is generally accepted that "hard" Lewis bases, such as amines,[44b] ethers,[44c] and sulfides,[44d] aid in the formation of a more reactive cobalt complex by promoting CO ligand liberation. (Table 4 represents a cross-section of additives screened.) Of these, mercaptans, primary amines, and polyamines (entries 5 and 6) all had detrimental effects on product formation, giving

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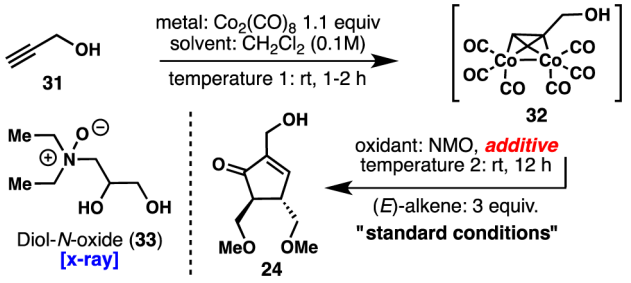
Table 4. Optimization of *N*-Oxide-Mediated Pauson–Khand Reaction



entry	change from standard conditions	yield (24)
1	**oxidant** = Silver (II)	decomp
2	**oxidant** = Iron (III) Chloride	decomp
3	**oxidant** = NMO (slow addition)	15-20%
4	**additive** = none	15-20%
5	**additive** = mercaptans: PhSH, 1,2-Ethanedithiol	decomp
6	**additive** = 1°/2° polyamines: ethylenediamine, 1,2-Diaminocyclohexane, diethylenetriamine	0% (rsm)
7	**additive** = 3° amines: dimethylethanolamine, tetramethylethylenediamine, TEA	16%
8	**additive** = 2° alkyl sulfides: nBuSMe, 2-(methylthio)ethanol	16-20%
9	**additive** = 1° alcohols: MeOH, BnOH	16-20%
10	**additive** = 1,2-Dimethoxyethane	15%
11	**additive** = unhindered diols and triols: ethylene glycol, propane-1,3-diol, glycerol	45-49%[a]
12	**additive/oxidant** = diol N-oxide (**33**)	48%

[a]Reproducible yields of 49% were obtained using 4 Å MS or BSA as a dehydrating agent. rsm = recovered starting material.

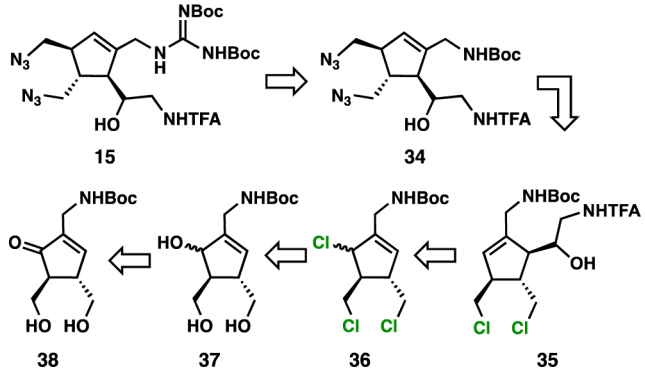
only recovered starting material or decomposition. It was found that the presence of a hydrogen donor (both amines and mercaptans, see entries 5 and 6) is not well tolerated compared to their aprotic counterparts (entries 7 and 8). Interestingly, this is not the case with alcohols (entry 9 compared to entry 10). The presence of a free hydroxyl group does not impede product formation and allows the reaction to proceed with a yield similar to to that obtained with NMO alone (entry 9 compared to entry 4).

The key breakthrough in these studies was the finding that a combination of NMO and unhindered polyols (entry 11) had a dramatic positive effect, providing the desired cyclopentenone **24** in 45–49% yield. Due to reagent practicality, ethylene glycol was selected as the polyol of choice (cost-effective and lower viscosity). Notably, ethylene glycol was evaluated as a promoter on a related catalytic P-K reaction[44c] and was shown to have no significant effects.

Hypothesizing that both ethylene glycol and NMO are involved in the formation of a more reactive cobalt complex, we designed a hybrid molecule containing components of both reagents. Thus, diol-*N*-oxide **33** was synthesized from a H_2O_2 oxidation of 3-diethylaminopropane-1,2-diol in quantitative yield. Although this new reagent proved to be an effective additive for cyclopentenone formation and could be used in reduced quantities compared to the ethylene glycol/NMO mixture, the yield was not substantially better (entry 12). After investigating the effects of oxidant and Lewis base additives on the reaction yield, we determined that an optimal combination of ethylene glycol/NMO (co-solvent and 6 equiv, respectively) or 3 equiv of **33** could be employed in the presence of 4 Å molecular sieves (MS) to reliably produce **24** in excess of 45% isolated yield.

Indium-Mediated Aqueous Barbier Reaction. In an effort to offset the synthetic inefficiency caused by the use of unavoidable concession steps (due to P-K incompatibility of **19** and **18**), a chemoselective functionalization strategy toward the construction of **15** was envisioned (Scheme 3). In the forward

Scheme 3. Chemoselective Functionalization Strategy to Chlorospirocyclization Precursor 15



sense, P-K cycloaddition with *trans*-2-butene-1,4-diol and the cobalt–alkyne complex of *N*-Boc-propargylamine could give cyclopentenone **38**. Selective 1,2-reduction of cyclopentenone **38** to yield triol **37** should be possible using Luche conditions, and conversion of triol **37** to trichloride **36** should be feasible through hydroxyl activation followed by chloride displacement. Chemoselective metal-mediated allylation of an *N*-protected α-amino aldehyde with trichloride **36** might be possible to furnish homoallylic alcohol **34**. Finally, selective *N*-alkylation of bis-chloride **35** using inorganic azide salts followed by guanidinylation using known reagents[45] should provide fully substituted chlorospirocyclization precursor **15**. In order to validate the chemoselective allylation, simplified triol **41** was targeted for synthesis and converted to the corresponding trichloride **42**.

Thus, $Co_2(CO)_8$ was used to preform a stable alkyne–cobalt complex with propyne and was subjected to our optimized ethylene glycol/NMO P-K protocol with bis-allylic TMS ether **40** to yield the corresponding bis-TMS-protected cyclopentenone. The protected cyclopentenone was found to have moderate stability to silica gel and could be deprotected in a one-pot operation with $CeCl_3$ in MeOH during the Luche reduction after an extended reaction time. With the successful production of trichloride **42** in good yield (30% overall, three steps from **39**), metal-mediated allylation protocols were evaluated using simple propionaldehyde as the model substrate (Table 5).

Initial allylation attempts using Zn in anhydrous THF were discouraging, as they yielded only a trace amount of product (entry 4). After screening a variety of metals in anhydrous THF with no success (entries 1–6), we were prompted to explore the use of aqueous media.[46] Although the use of Zn in water alone did not yield any product, the combination of Zn with aqueous NH_4Cl[47] furnished the desired homoallylic alcohol **43** in 20% yield. The yield of **43** was improved to 50% by varying the solvent choice to a biphasic system (entry 8), which effectively decreased the formation of protodemetalation byproduct **44**.

Reviewing the chemical literature involving aqueous organometallic Barbier conditions revealed two important variables: (i) the use of bimetallic systems[48] and (ii) the preferential use

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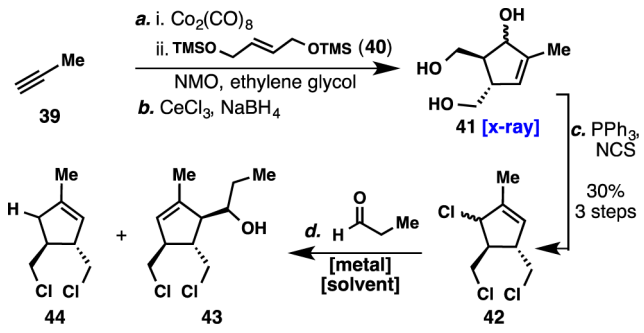
Table 5. Optimization of Chemoselective Barbier-Type Reaction[a]



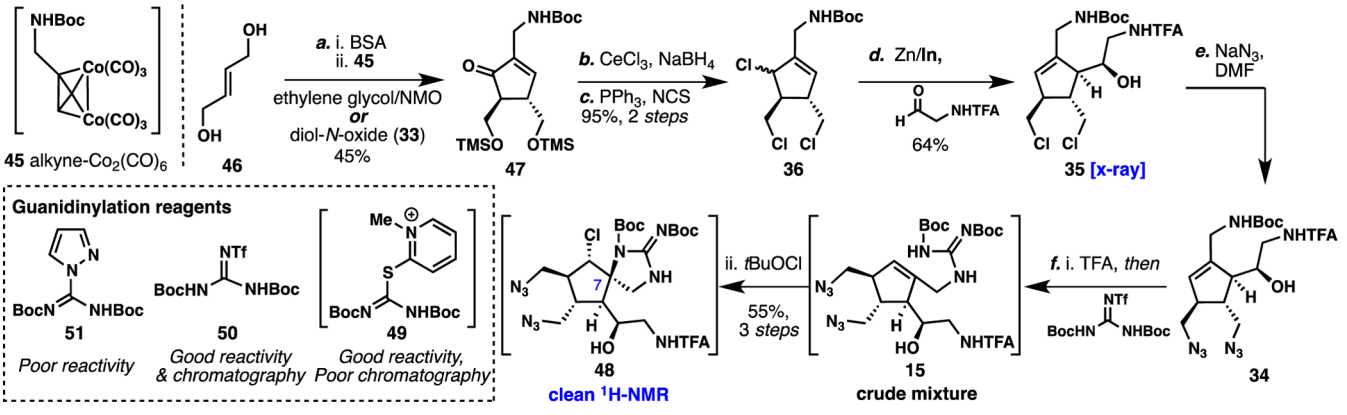
entry	metal (3-5 equiv)	solvent (0.1M)	yield (43:44)
1	Sn	THF	NR
2	Fe	THF	NR
3	In	THF	NR
4	Zn	THF	trace
5	Mg	THF	decomp.
6	Mg/I_2	THF	decomp.
7	Zn	aq. NH_4Cl	20%
8	Zn	THF/20% aq. NH_4Cl	50%
9	Mg/Zn	THF	decomp.
10	Mg/In	THF	decomp.
11	Zn/In	THF	16%
12	Zn/In	H_2O	NR
13	Zn/In	THF/ 20% aq. NH_4Cl	62% (7:1)
14	Zn/In	THF/ 6% aq. NH_4Cl	67% (9:1)
15	$InCl_3$	THF	NR
16	$InCl_3$	THF/ 6% aq. NH_4Cl	NR

[a]Reagents and conditions: (a) i. propyne (1.0 equiv), $Co(CO)_8$ (1.0 equiv), CH_2Cl_2, rt, 2 h; ii. **40** (3.0 equiv), NMO (6.0 equiv), ethylene glycol/CH_2Cl_2 (1:10, 0.1M) rt, 12 h. (b) $CeCl_3$ (1.0 equiv), $NaBH_4$ (4.0 equiv), MeOH, 0 °C, 12 h. (c) PPh_3 (3.4 equiv), NCS (3.4 equiv), THF, rt, 12 h. (d) propionaldehyde (4.0 equiv), Zn (16.0 equiv), In (2 equiv), THF/6% NH_4Cl (aq), rt, 3 h.

of indium metal for reactions involving less reactive allyl chlorides (in contrast to allyl bromides) in allylations of ketones and aldehydes.[49] Bimetallic conditions are always employed as a redox pair, where one metal serves as an "activator" by reducing the other to a reactive metal species (e.g., Sn−Al,[48a] $InCl_3$−Zn[48b]). Although the initial use of indium metal did not show promising results (entry 3), we were delighted to find that its use as a bimetallic mixture with Zn in aqueous NH_4Cl provided **43** in 62% yield (entry 13), which could be further optimized to 67% yield by decreasing the concentration of NH_4Cl (entry 14). Notably, the previously reported use of $InCl_3$−Zn mixture[48b] does not mention improved yields relative to In(0) alone. The synergistic effect on yield of a In(0)−Zn(0) combination as a non-redox pair appears to be an unrecognized phenomenon. With all of the key steps toward the construction of **15** validated and partially optimized, we directed our attention to P-K formation of the more complex cyclopentenone **38**.

Optimization and Telescoping Process of Key Spirocyclization Precursor. In order to avoid discrete protection/deprotection steps, it was surmised that mild conditions to install and remove TMS ethers could be employed en route to triol **37**. Therefore, bis-allylic alcohol **46**[50] was protected *in situ* using 7 equiv of bis(trimethylsilyl)-acetamide (BSA) in CH_2Cl_2. Using our optimized P-K conditions, alkyne−cobalt complex **45** was then added to the same solution with diol-*N*-oxide **33** as the promoter to produce cyclopentenone **47** in 45% yield (Scheme 4). As in the previous synthesis of **41**, 1,2-reduction and TMS deprotection could be carried out in a one-pot operation by extending the reaction time to 12 h. Conversion of the corresponding triol to trichloride **36** using Appel conditions was possible in 95% overall yield (from cyclopentenone **47**). Using our optimized bimetallic Zn/In conditions, side-chain installation proceeded smoothly in 64% yield, providing the desired homoallylic alcohol **35** as a single diastereomer. Alkyl chloride displacement of **35** using an excess of sodium azide in DMF (monitored by NMR for full conversion) proceeded cleanly, and after acidic workup, the crude product was of sufficient purity to be taken on directly to the next step. Boc deprotection of **34** and subsequent use of *N*,*N*′-di-Boc-*N*″-triflylguanidine **50** (Goodman's reagent) in acidic media yielded the corresponding allylic guanidine, which after evaporation of solvent and addition of CH_2Cl_2 followed by *t*BuOCl to the crude reaction mixture yielded chlorospirocyclization product **48** in 55% overall yield (three steps from **35**). To our surprise, no optimization was needed for the chlorospirocyclization of crude allylic guanidine

Scheme 4. Synthetic Route Finalization and Chlorospirocyclization of Advanced Allylic Guanidine Intermediate 15[a]



[a]Reagents and conditions: (a) i. BSA (7.0 equiv), CH_2Cl_2, 40 °C, 3h; ii. **45** (1.0 equiv), diol-*N*-oxide (3.0 equiv), CH_2Cl_2, rt, 12 h. (b) $CeCl_3$ (1.0 equiv), $NaBH_4$ (4.0 equiv), MeOH, 0 °C, 12 h. (c) PPh_3 (3.4 equiv), NCS (3.4 equiv), THF, rt, 12 h. (d) 2,2,2-Trifluoro-*N*-(2-oxoethyl)acetamide (10 equiv), zinc (16 equiv), indium (1.9 equiv), THF, NH_4Cl (6% aqueous), rt, 3 h. (e) NaN_3 (10 equiv), DMF, 85 °C, 16 h. (f) i. TFA (50% in CH_2Cl_2), rt, 23 h then TEA (5.0 equiv), **50** (1.5 equiv), rt, 12 h; ii. *t*BuOCl (2.0 equiv), CH_2Cl_2, 0 °C, 1 h.

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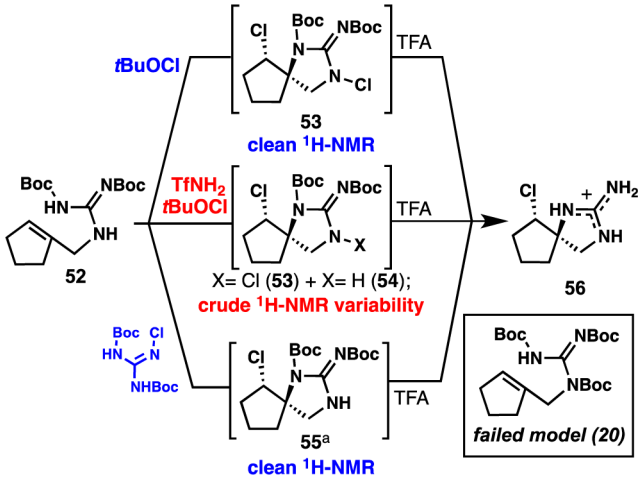
48 when using *t*BuOCl (conditions that failed using model guanidine **20**). Interestingly, although clean chlorospirocyclization was observed with crude batches of **48**, purified batches or batches using other guanidinylation reagents (**49** or **51**) varied greatly compared to crude batches (judged by NMR). Assuming from these results that the success of chlorospirocyclization was dependent on $TfNH_2$ generation from the guanidinylation step,[45] we pursued an investigation that would help understand the precise role of $TfNH_2$.

Mechanism of $TfNH_2$ in Chlorospirocyclization and Discovery of Stable *N*-Chloroguanidine 58. Although isolation of desired spirocycle **73** (see Scheme 7, below) was possible using pure batches of **48**, the yields were significantly lower compared to yields obtained from crude batches (50% and 98%, respectively). In addition, characterization of undesired products from this batch-dependent variability proved challenging. Therefore, in order to study the effect of $TfNH_2$ on the chlorospirocyclization event, a simplified model **52** displaying the correct protecting group regiochemistry was synthesized (Scheme 5). In this model system, both conditions

Scheme 5. $TfNH_2$ Effect on Model Chlorospirocyclization

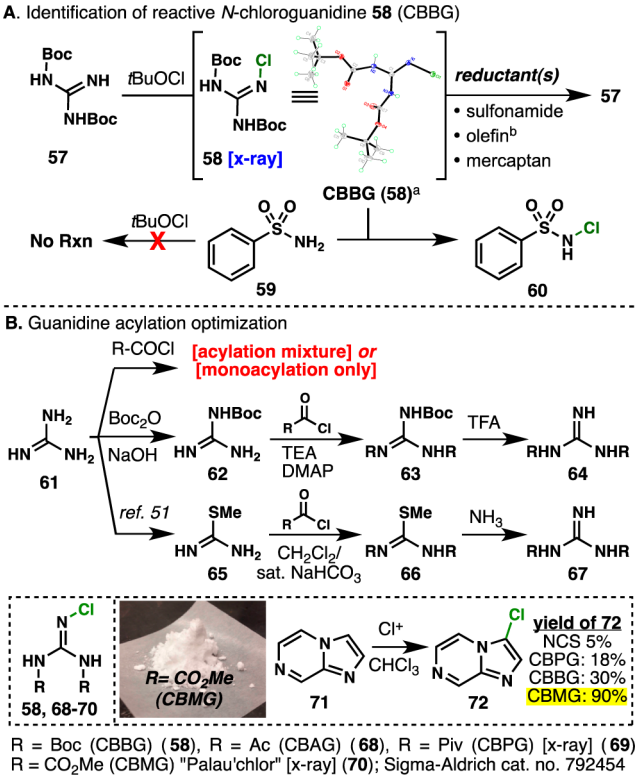


[a]Addition of 1.0 equiv of $TfNH_2$ to **53** produced **55** in quantitative yield.

using *t*BuOCl (with and without 20 mol% $TfNH_2$) showed chlorospirocyclization product formation; however, multiple intermediate products (**53**, **54**) were detected in batches using $TfNH_2$ as an additive. Interestingly, upon treatment with TFA in CH_2Cl_2, both of these reaction mixtures led to desired spirocycle **56** in comparable yields. Additionally, the use of 1.0 or 2.0 equiv of *t*BuOCl did not seem to influence chlorospirocyclization formation but did contribute to variability in intermediate (**53**, **54**) formation.

Realizing that $TfNH_2$ has an effect on intermediate formation but is not essential for attaining high yields of desired spirocycle **56**, we directed our attention to a key structural difference between our original model allylic guanidine **20** (triBoc) and revised model **52** (bisBoc). Thus, it was reasoned that *N*-chloroguanidine formation could be attributed to the success of the chlorospirocyclization event. To test this hypothesis, bis-Boc-guanidine **57** was treated with *t*BuOCl in CH_2Cl_2 at room temperature, and after only a few minutes, new product formation was observed by thin-layer chromatography (Scheme 6A). Isolation of this new product led to the characterization of chlorobis(*tert*-butoxycarbonyl)guanidine (CBBG, **58**) as a

Scheme 6. Invention of Reactive yet Stable CBMG



R = Boc (CBBG) (**58**), R = Ac (CBAG) (**68**), R = Piv (CBPG) [x-ray] (**69**)
R = CO_2Me (CBMG) "Palau'chlor" [x-ray] (**70**); Sigma-Aldrich cat. no. 792454

[a]CBBG decomposition was observed when dissolving in acetone. [b]Chlorinated products were detected from the mixture of CBBG and 2-methylallyl-4-bromobenzonate.

bench-stable white solid. To our delight, when CBBG (**58**) was used as the chlorination source in CH_2Cl_2, clean chlorospirocyclization of **52** was observed (Scheme 5B). Shockingly, when benzenesulfonamide **59** was treated with *t*BuOCl in CH_2Cl_2 at room temperature, no reaction took place, but when **59** was mixed with CBBG (**58**) under identical conditions, *N*-chlorobenzenesulfonamide **60** was formed in quantitative yield (similar results using *N*-methylbenzenesulfonamide were observed, and its product was characterized by X-ray analysis) (Scheme 6A).

In light of these results, it is proposed that the active chlorinating agent must be an *N*-chloroguanidine species and the role of $TfNH_2$ is to act as a reductant toward overchlorinated products (such as chlorination of **55**). In addition, it is reasoned that conversion of **53** and **54** to **56** is achieved via reduction from isobutene generation from Boc deprotection (2.0 equiv). In the model chlorospirocyclization of **52**, $TfNH_2$ proved to be non-essential, as both chlorination conditions (*t*BuOCl with and without $TfNH_2$) gave the desired spirocycle **56** in high yields. Interestingly, in the case of fully functionalized spirocycle precursor **15**, the absence of $TfNH_2$ or the use of an excess of $TfNH_2$ had a detrimental effect on yield.

CBBG (**58**) was further evaluated as a general chlorinating reagent by comparing its reactivity to existing reagents. With a focus on heteroaromatic chlorination, heterocycle **71** was used as a model substrate for comparing reactivity between NCS and CBBG derivatives. Systematic variation of the pendant acyl groups on the guanidine proved to be a non-trivial process, as direct acylation procedures used on unprotected guanidine **61**

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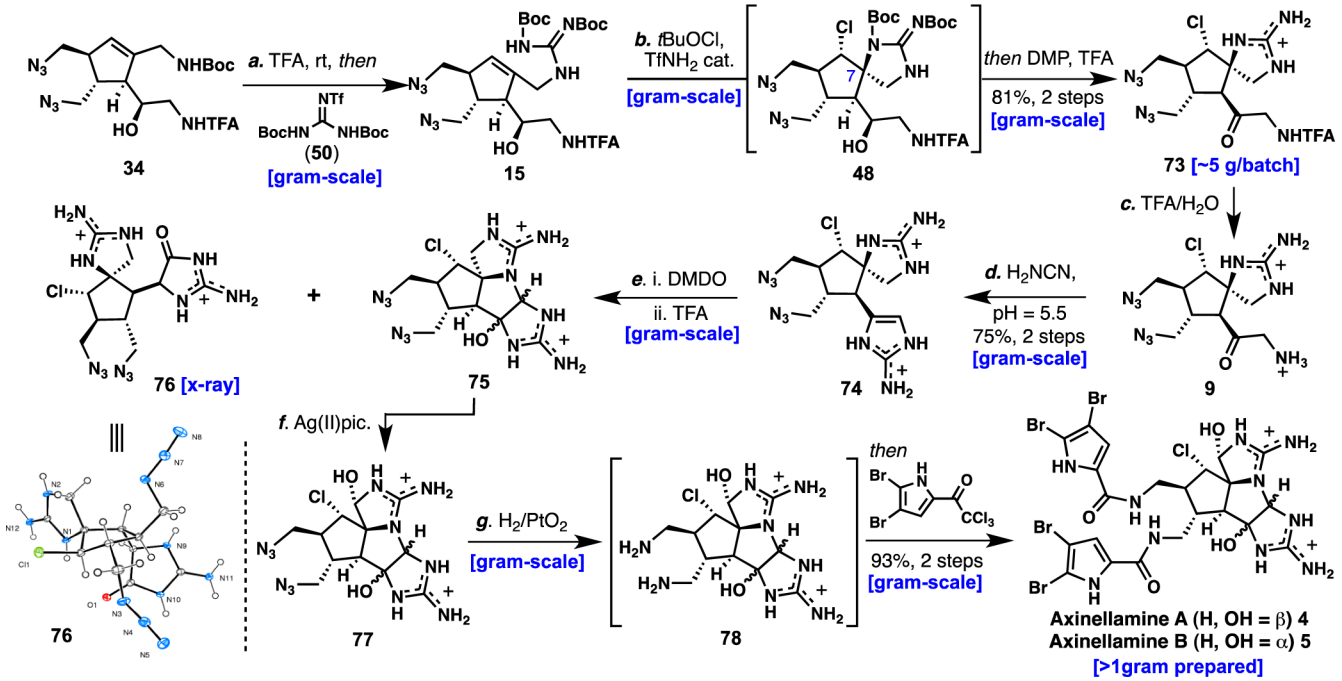
Scheme 7. Scalability of Final Synthetic Route and Reactivity of 2-Aminoimidazole toward DMDO Oxidation[a]



[a]Reagents and conditions: (a) i. TFA/CH_2Cl_2 (1:1, 0.2M), rt, 12 h; ii. CH_2Cl_2, TEA (5.0 equiv), *N,N′*-bis-Boc-*N″*-triflylguanidine (1.5 equiv). (b) i. $TfNH_2$ (0.25 equiv), CH_2Cl_2, *t*BuOCl (2.0 equiv), 0 °C, 30 min; ii. DMP (1.2 equiv), rt, 12 h. (c) H_2O/TFA (1:1, 0.2 M), 70 °C, 36 h. (d) i. AcOH; ii. $NCNH_2$ (25 equiv), 0.2 M NaOH (pH ∼5.5), 70 °C, 4 h. (e) i. TFA/CH_2Cl_2 (5:95, 0.1 M), DMDO (1.25 equiv), 0 °C, 1 h; ii. TFA/CH_2Cl_2 (1:1, 0.1 M), rt, 12 h. (f) H_2O/TFA (9:1, 0.07 M), silver(II) picolinate (2.5 equiv), rt, 2 h. (g) i. H_2O/TFA (19:1, 0.1 M), PtO_2 (0.1 equiv), H_2(g); ii. 2,3-dibromo-5-trichloroacetylpyrrole (5.0 equiv), DIPEA (4.5 equiv).

only led to acylation mixtures or monoacylation (even under forceful conditions). After extensive experimentation, two viable procedures[51] for acyl group variation on guanidine **61** were adopted, and after comparing yields of **72**, we identified chloro(bismethoxycarbonyl)guanidine (CBMG or Palau'chlor, **70**) as the optimal chlorinating reagent (Scheme 6B). Palau'chlor was found to be an effective chlorinating reagent for a variety of organic substrates and in all cases showed improved yields when compared to other known chlorinating reagents (under identical conditions).[52] Palau'chlor proved to be a stable yet reactive chlorinating reagent and has been field-tested at Bristol-Myers Squibb as well as commercialized by Sigma-Aldrich (cat. no. 792454).[52]

Finalization of Synthetic Route and Scalability. High yields of chlorospirocyclization product **48** were obtained when residual $TfNH_2$ was present in the reaction mixture (Scheme 7). Chlorospirocyclization optimization of **15** led to a process where chromatographically pure **15** is best used in combination with 20 mol% $TfNH_2$ and 2.0 equiv of *t*BuOCl in CH_2Cl_2 as the solvent. These conditions provided spirocycle **48** cleanly in near-quantitative yield (judged by NMR) with successful batch reproducibility. Oxidation of homoallylic alcohol **48** to ketone **73** using DMP was found to be compatible under acidic media and was processed in crude form using 10:1 CH_2Cl_2/TFA as solvent (81% yield from **15**). Further treatment of purified **73** with TFA in the presence of H_2O furnished spiroaminoketone **9** in a total of eight steps with only four chromatographic purifications and in 13% overall yield from **45**. In comparison to our first-generation synthesis of **9**, this route reduced the number of steps by more than half and increased the overall yield by >10-fold. Notably, each of the above-mentioned steps was successfully conducted and reproduced on gram-scale, providing 7 g of **9** by one chemist in a single batch from **45** and **46**.

Finally, in order to demonstrate the scalability of a final synthetic route to a complex PIA from spiroaminoketone **9** as well as to provide an in-depth follow-up to initial biological activity, the tetracyclic axinellamines were targeted for synthesis. Having to meet a prerequisite of >100 mg of **4** and **5** for biological collaborations, the axinellamines were prepared on gram-scale. Thus, addition of cyanamide to **9** in brine under a controlled pH (pH 5.5−5.7) led to **74** in 75% yield (2.4 g, 2 steps from **9**). Oxidation of 2-aminoimidazole **74** with DMDO followed by TFA-mediated dehydration gave tetracycle **75** as the major product in 83% yield from **9** (2 g) and ∼5 mg of tricycle **76**, presumably from DMDO oxidation followed by 1,2-shift. Tricycle **76** was characterized by X-ray analysis and was found to share the core carbon skeleton with matching oxidation to that of the donnazoles (**6** and **7**). In addition, the isolation of tricycle **76** provides evidence for the putative biogenesis of donnazoles from a common pre-axinellamine precursor.[53] Notably, the formation of tricycle **76**, having the same molecular weight as **75**, went undetected by LC-MS in smaller-scale[28b] oxidation/dehydration batches of **74**, and the X-ray analysis of **76** represents the most complex example of a polycyclic PIA intermediate to date. Tetracycle **75** was converted to 77-*α* and 77-*β* with silver(II) picolinate in 68% yield (1.4 g, 2.7:1 *β*:*α*). Conversion of the azide groups of the acylpyrrole side chains was accomplished using a one-pot operation involving PtO_2-mediated reduction followed by acylation with 2,3-dibromo-5-trichloroacetylpyrrole to provide >1 g of **4** and **5** combined in 93% yield over two steps from 77.

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CONCLUSIONS

Using an *N*-oxide/ethylene-glycol-assisted intermolecular P-K protocol to enable the use of (*E*)-alkenes in cyclopentenone generation, a synergistic bimetallic (Zn/In) Barbier-type allylation, and *N*-chloroguanidine-based chlorospirocyclization to set the two most challenging stereocenters, a robust and stereocontrolled second-generation route to key spiroaminoketone **9** has been developed (20 and 8 steps, 12 and 4 purifications, <1% overall yield and 13% overall yield, respectively). In addition to representing a formal synthesis of palau'amine (**8**) and massadines,[28c] the synthesis of spiroaminoketone **9** presented an opportunity for invention. The key chlorospirocyclization not only served as an ideal strategy for clearing the two most challenging stereocenters of the fully substituted cyclopentyl framework but also provided the chemical inspiration for CBBG (**58**). Optimization of these stable *N*-chloroguanidines led to the identification of Palau'chlor (**70**) as a reactive yet practical chlorinating reagent for both C−H and N−H chlorinations, ultimately commercialized by Sigma-Aldrich.[52] Finally, scalability of common progenitor **9** to complex members of the PIA family class has been demonstrated on multigram-scale, routinely providing ∼5 g of **73** from a single batch and final elaboration of the axinellamines on gram-scale. This enabled a re-investigation of the biological activity[16,17] of **4** and **5**, resulting in the surprising finding that they possess promising activity against a broad range of bacterial pathogens, suggesting that their scaffold has the potential for further development.[54] This article represents a case study in which the development of a scalable synthesis to a complex natural product enabled discoveries in both chemistry and biology.

ASSOCIATED CONTENT

Supporting Information

Experimental procedures, complete data acquisition, and analytical data for all new compounds, including ^{1}H and ^{13}C NMR spectra. 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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